UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ZOOTECNIA E ENGENHARIA DE ALIMENTOS

ALESSANDRA BRIDI

**Extracellular vesicles secreted by bovine embryos produced *in vivo* and *in vitro*: miRNAs
content and molecular effects in endometrial and luteal tissues**

Pirassununga

2021

ALESSANDRA BRIDI

**Extracellular vesicles secreted by bovine embryos produced *in vivo* and *in vitro*: miRNAs
content and molecular effects in endometrial and luteal tissues**

Versão corrigida

Tese apresentada à Faculdade de Zootecnia e Engenharia de Alimentos da Universidade de São Paulo, como parte dos requisitos para a obtenção do título de Doutor em Ciências do programa de pós-graduação em Biociência Animal.

Área de Concentração: Biociência Animal

Orientador: Prof. Dr. Felipe Perecin

Coorientador: Prof. Dr. Juliano Coelho da
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Faculdade de Zootecnia e Engenharia de Alimentos
Comitê de Ética em Pesquisa da FZEA

CERTIFICADO

Certificamos que a proposta intitulada "Vesículas extracelulares secretadas por embriões bovinos produzidos in vivo e in vitro: conteúdo de miRNAs e os efeitos moleculares sobre o endométrio e o corpo lúteo", protocolada sob o CEUA nº 1392130318, sob a responsabilidade de **Felipe Perecin e equipe; Alessandra Bridi** - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Faculdade de Zootecnia e Engenharia de Alimentos da Universidade de São Paulo - FZEA/USP (CEUA/FZEA) na reunião de 13/04/2018.

We certify that the proposal "Extracellular vesicles secreted by in vivo and in vitro produced bovine embryos: miRNA content and molecular effects on endometrium and corpus luteum", utilizing 80 Bovines (80 females), protocol number CEUA 1392130318, under the responsibility of **Felipe Perecin and team; Alessandra Bridi** - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the School of Animal Science and Food Engineering - (São Paulo University) (CEUA/FZEA) in the meeting of 04/13/2018.

Finalidade da Proposta: [Pesquisa \(Acadêmica\)](#)

Vigência da Proposta: de [05/2018](#) a [02/2021](#) Área: [Medicina Veterinária](#)

Origem: [Prefeitura do Campus da FZEA da USP](#)

Espécie: [Bovinos](#)

sexo: [Fêmeas](#)

idade: [3 a 15 anos](#)

N: [80](#)

Linhagem: [Nelore](#)

Peso: [250 a 800 kg](#)

Resumo: Embriões bovinos produzidos in vivo e in vitro possuem diferentes características metabólicas, além de diferenças no desenvolvimento embrionário e na transcrição gênica, o que consequentemente afeta as taxas de concepção e a produtividade dos rebanhos de corte. Entre os dias 8 e 17 do ciclo estral é quando ocorrem 40% das perdas embrionárias, coincidindo com a sinalização entre o conceito e o organismo materno. Vesículas extracelulares (VEs) fazem parte desta sinalização transportando miRNAs que podem atuar nas diversas células do organismo bovino. Com isso, nossa hipótese é que embriões bovinos produzidos in vivo e in vitro secretam VEs que possuem o conteúdo de miRNA diferente podendo modular diferencialmente o padrão de expressão gênica do cultivo de células luteais. Os objetivos deste projeto são: caracterizar o tamanho, a concentração e o conteúdo de miRNAs das VEs secretadas por embriões bovinos produzidos in vivo e in vitro entre o dia 7 e o dia 9 de desenvolvimento e avaliar o efeito das VEs secretadas no meio condicionado por embriões bovinos produzidos in vivo e in vitro sobre a expressão gênica do cultivo de células luteais. Os resultados obtidos mostrarão que o ambiente onde os embriões foram produzidos modifica as características morfológicas e o conteúdo de miRNAs das VEs que eles secretam, o que consequentemente altera o padrão de transcritos luteais. Desta forma, vias de sinalização associadas à comunicação materno-embrionária poderão ser identificadas, avaliando-se também o efeito da origem embrionária (in vivo ou in vitro) nestas vias.

Local do experimento: FZEA/USP Curral da Teriogenologia (Prof. Luciano Andrade Silva)

Pirassununga, 04 de fevereiro de 2019

Profa. Dra. Daniele dos Santos Martins
Coordenadora da Comissão de Ética no Uso de Animais
Faculdade de Zootecnia e Engenharia de Alimentos da
Universidade de São Paulo - FZEA/USP

Profa. Dra. Cristiane Gonçalves Titto
Vice-Coordenadora da Comissão de Ética no Uso de Animais
Faculdade de Zootecnia e Engenharia de Alimentos da
Universidade de São Paulo - FZEA/USP

CERTIFICADO

Certificamos que a proposta intitulada "Exossomos e microvesículas contendo miRNAs modulam mudanças epigenéticas durante o cultivo in vitro de gametas e embriões em bovinos", protocolada sob o CEUA nº 4166120117, sob a responsabilidade de **Juliano Coelho da Silveira** - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Faculdade de Zootecnia e Engenharia de Alimentos da Universidade de São Paulo - FZEA/USP (CEUA/FZEA) na reunião de 12/04/2017.

We certify that the proposal "Cell-secreted vesicles containing miRNAs modulate epigenetic changes during in vitro culture of bovine gametes and embryos", utilizing 50 Bovines (50 females), protocol number CEUA 4166120117, under the responsibility of **Juliano Coelho da Silveira** - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the School of Animal Science and Food Engineering - (São Paulo University) (CEUA/FZEA) in the meeting of 04/12/2017.

Finalidade da Proposta: [Pesquisa \(Acadêmica\)](#)

Vigência da Proposta: de [09/2016](#) a [08/2019](#)

Área: [Biociência Animal](#)

Origem: [Prefeitura do Campus da FZEA da USP](#)

Espécie: [Bovinos](#)

sexo: [Fêmeas](#)

idade: [3 a 10 anos](#)

N: [50](#)

Linagem: [Não definida](#)

Peso: [400 a 800 kg](#)

Resumo: O uso de técnicas de reprodução assistida é uma alternativa para solucionar problemas reprodutivos em espécies domésticas e em humanos. Todavia, existe a preocupação de que o cultivo in vitro de gametas e embriões possa gerar mudanças epigenéticas as quais podem levar à maior pré-disposição a problemas cardíacos, reprodutivos ou desenvolvimento de tumores na vida adulta. Alterações epigenéticas são reguladas por metilação do DNA, por modificações pós-traducionais de histonas ou por microRNAs (miRNA). Os miRNAs são RNAs não codificadores de proteínas que regulam a expressão gênica durante diferentes processos de diferenciação tecidual. Recentemente, vesículas secretadas por células, chamadas de exossomos e microvesículas, foram encontradas em diferentes fluidos corporais contendo material bioativo incluindo miRNAs. Estas vesículas são consideradas uma nova classe de comunicação celular com possíveis implicações em diferentes processos fisiológicos. Entretanto o seu papel na aquisição de competência dos gametas e no desenvolvimento embrionário ainda é amplamente desconhecido. A hipótese que será testada é que vesículas secretadas por células do trato reprodutivo feminino modulam mudanças epigenéticas durante o cultivo in vitro de gametas e embriões. Para testar esta hipótese vesículas serão isoladas a partir de líquido folicular ou meio de cultura de células do oviduto e adicionadas nos meios de cultura de oócitos e embriões. O primeiro objetivo é determinar a relação entre miRNAs e mudanças epigenéticas adquiridas durante a maturação de oócitos e o desenvolvimento embrionário em bovinos. O segundo objetivo é determinar os efeitos exercidos por vesículas secretadas por células do sistema reprodutivo nas taxas de maturação oocitária, fertilização e clivagem embrionária in vitro. O terceiro objetivo é determinar a função das vesículas extracelulares e miRNAs na maturação de oócitos e produção de embriões bovinos em alta e baixa tensão de oxigênio. Os resultados desse projeto ajudarão a estabelecer o papel de vesículas secretadas por células do sistema reprodutivo na determinação das características epigenéticas adquiridas durante o cultivo in vitro de oócitos e embriões. Os resultados obtidos nesse projeto permitirão o desenvolvimento de protocolos mais similares à condição fisiológica para a produção in vitro de embriões, com grande impacto na indústria de reprodução assistida em bovinos e em humanos.

Local do experimento: Faculdade de Zootecnia e Engenharia de Alimentos FZEA/USP

Pirassununga, 21 de maio de 2017

UNIVERSIDADE DE SÃO PAULO
Faculdade de Zootecnia e Engenharia de Alimentos
Comitê de Ética em Pesquisa da FZEA

Profa. Dra. Daniele dos Santos Martins
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Nome: Alessandra Bridi

Título: Extracellular vesicles secreted by bovine embryos produced *in vivo* and *in vitro*: miRNAs content and molecular effects in endometrial and luteal tissues

Tese apresentada à Faculdade de Zootecnia e Engenharia de Alimentos da Universidade de São Paulo, como parte dos requisitos para a obtenção do título de Doutor em Ciências do programa de Pós-graduação em Biociência Animal.

Área de Concentração: Biociência Animal

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DEDICATÓRIA

Dedico este trabalho aos meus pais Arcemar (*in
memorian*) e Vanilda, com todo o meu amor e
gratidão, por todo o carinho, dedicação e cuidado
que tiveram comigo.

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RESUMO

BRIDI, A. **Vesículas extracelulares secretadas por embriões bovinos produzidos *in vivo* e *in vitro*: conteúdo de miRNAs e os efeitos moleculares sobre o endométrio e o corpo lúteo.** 254 f. Tese (Doutorado) – Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga-SP, 2021.

Perfis metabólicos, padrões de expressão gênica, desenvolvimento embrionário, capacidade de estabelecer e manter gestações precoces são diferenças encontradas quando embriões bovinos produzidos *in vivo* e *in vitro* são comparados. Para que a gestação tenha sucesso é necessária uma perfeita comunicação entre o embrião e o organismo materno. Vesículas extracelulares pequenas (sEVs) fazem parte dessa comunicação e carregam moléculas bioativas, como miRNAs, que podem atuar em células-alvo dentro do sistema reprodutivo bovino. Com isso, nossa hipótese é que embriões bovinos produzidos *in vivo* e *in vitro* secretam sEVs contendo diferentes miRNAs que podem modular diferencialmente o padrão de expressão gênica endometrial e o perfil de miRNAs do corpo lúteo. Para isso, primeiramente, investigamos o conteúdo de miRNA em blastocistos eclodidos produzidos *in vivo* ou *in vitro* e, em sEVs secretadas por eles. Blastocistos eclodidos produzidos *in vivo* ou *in vitro*, no dia 9 de desenvolvimento, possuem diferentes perfis de miRNA. EVs pequenas secretadas por eles, do dia 7 até o dia 9 de desenvolvimento, contém miRNAs diferentes. Esses miRNAs diferentemente expressos são preditos por regularem vias envolvidas no desenvolvimento embrionário inicial e na receptividade endometrial. Por meio disso, as primeiras interações entre o embrião e o organismo materno podem ser modificadas, e isso pode afetar o sucesso da gestação. Em segundo lugar, investigamos as alterações no transcriptoma global do endométrio após o co-cultivo de explantes endometriais com um blastocisto produzido *in vivo* ou *in vitro* no dia 7 do desenvolvimento. Genes diferentemente expressos foram identificados no endométrio e estão associados com a presença e origem do embrião. Além disso, sEVs presentes nos meios condicionados (C.M.) por essas células embrionárias e endometriais contém diferentes miRNAs, que são preditos por modularem a via de sinalização da oxitocina. Finalmente, para compreender melhor se a comunicação entre o embrião, o endométrio e o corpo lúteo pode ser intermediada por sEVs, explantes luteais foram tratados com sEVs presentes nos C.M. por explantes endometriais cultivados sozinhos ou com um blastocisto bovino produzido *in vivo* ou *in vitro* no dia 7 do desenvolvimento. O perfil de miRNAs foi modificado nos explantes luteais tratados com essas sEVs e, nós podemos observar um efeito da presença e origem dos embriões nessas alterações. Juntos, esses resultados sugerem que sEVs intermedeiam a comunicação inicial entre o embrião, endométrio e o corpo lúteo, contribuindo para manter a viabilidade e funcionalidade luteal. Entretanto, futuros experimentos precisam ser realizados para avaliar efeitos biológicos específicos desses miRNAs carregados por essas sEVs no endométrio e no corpo lúteo. Além disso, a origem do embrião (*in vivo* ou *in vitro*) modifica as interações entre ele, o endométrio e o corpo lúteo, sugerindo fortemente que esses embriões tenham necessidades distintas para se desenvolverem.

Palavras-chave: vesículas extracelulares, comunicação, blastocisto, endométrio, corpo lúteo, microRNAs

ABSTRACT

BRIDI, A., **Extracellular vesicles secreted by bovine embryos produced *in vivo* and *in vitro*: miRNAs content and molecular effects in endometrial and luteal tissues.** 2021. 254 f. Tese (Doutorado) – Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga-SP, 2021.

Metabolic profiles, gene expression patterns, embryonic development, and the ability to establish and sustain early pregnancies are differences founded when *in vivo*- and *in vitro*- produced bovine embryos are compared. To the establishment of a successful pregnancy the perfect embryo-maternal communication is needed. Small extracellular vesicles (sEVs) are part of this crosstalk and carry bioactive molecules such as miRNAs that can act on target bovine cells within the reproductive system. Thereby, our hypothesis is that *in vivo* and *in vitro* bovine embryos secrete sEVs containing different miRNAs that can differentially modulate endometrial gene expression pattern and miRNAs profile in the corpus luteum (CL). To address this hypothesis, firstly, we investigated the miRNA content of *in vivo* and *in vitro* hatched blastocysts and in sEVs secreted by them. Day 9 *in vivo* or *in vitro* hatched blastocysts have distinct miRNA profiles. Small EVs secreted by them from day 7 up to day 9 of development contain different miRNAs. These miRNAs differently expressed are predicted to regulate pathways involved with early embryonic development and endometrial receptivity. Thereby, early embryo-maternal interactions can be modified and consequently can affect pregnancy success. Secondly, we investigated changes in the endometrial global transcriptome after the co-culture of endometrial explants with a day 7 *in vivo* or *in vitro* blastocysts. Differently expressed genes were identified in the endometrium and are associated with the embryo's presence or origin. Moreover, sEVs present in the conditioned media (C.M.) by these embryonic and endometrial cells contain different miRNAs, which are predicted to modulate the oxytocin signaling pathway. Finally, to understand if the communication among embryo, endometrium, and corpus luteum can be mediated by sEVs, luteal explants were treated with sEVs from C.M. by endometrial explants alone or cultured with a day 7 *in vivo* or *in vitro* blastocysts. MicroRNAs profile was modified in luteal explants treated with these sEVs, and we can observe an effect of the embryo presence and origin in these alterations. Together these results suggest that sEVs mediate early embryo-endometrium-corpus luteum communication, contributing to maintaining luteal viability and functionality. However, further experiments are needed to evaluate specific biological effects of these miRNAs carried by sEVs in the endometrium and in the corpus luteum. Moreover, the embryo origin (*in vivo* and *in vitro*) modify the interactions among embryo-endometrium-corpus luteum, suggesting strongly that these embryos have distinct needed to develop.

Keywords: extracellular vesicles, communication, blastocyst, endometrium, corpus luteum, microRNAs

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1. Introduction

Reproductive efficiency is one of the main factors affecting productivity. This efficiency is commonly measured through reproductive indices that include conception rate, pregnancy rate, and calving interval. The increasing use of reproductive biotechnologies contributes to accelerating the genetic advance, and consequently, the improvement of beef and dairy herds' productivity. The most used biotechnologies in Brazil are fixed-time artificial insemination (IATF), in which the embryos are produced *in vivo*; and *in vitro* embryo transfer, in which the embryos are produced through follicular aspiration and *in vitro* fertilization (OPU-IVP). However, the pregnancy rate is approximately 50.3% in IATF, and 34.0% in *in vitro* embryo transfer (SARTORI et al., 2016), suggesting that depending on the environment where the embryo is produced, pregnancy establishment can be altered.

In vitro embryo production systems involve the use of different components on culture media, which alter the embryo transcriptomic, development, metabolism, morphologic characteristics, as well as their capacity to establish the pregnancy (PLANTE; KING, 1994; HOLM; CALLESEN, 1998; HOLM et al., 1998; YOUNG et al., 2001; LONERGAN et al., 2003; RIZOS et al., 2003; MOHAN et al., 2004; CORCORAN et al., 2006; HIENDLEDER et al., 2006; PONTES et al., 2009). Thus, *in vitro* environment can modify important factors involved in the dialogue between the embryo and maternal organism, possibly causing them to communicate differently with the uterine environment.

For the pregnancy to have success, the perfect molecular dialogue between the embryo-endometrium-corpora lutea must happen. Small extracellular vesicles (sEVs) are part of this communication (RUIZ-GONZÁLEZ et al., 2015; SAADELDIN et al., 2015; BURNS et al., 2016; BRIDI et al., 2020) and carry bioactive molecules such as miRNAs (VALADI et al., 2007). These are small molecules involved in post-transcriptional regulation (FIRE et al., 1998; HWANG; MENDELL, 2006) and may play a role in modulating embryo-maternal communication during early pregnancy. MiRNAs are expressed and secreted by *in vitro*-produced embryos and can regulate important events during early embryonic development (SAADELDIN et al., 2014; MELLISHO et al., 2017; MELO-BAEZ et al., 2020). MiRNAs, present in the conditioned media by *in vitro* embryos, may be used as biological markers for embryo selection with better quality, which can improve the pregnancy rate in mammals (MELLISHO et al., 2019; DISSANAYAKE et

al., 2020). In the last decade, it was shown that miRNAs, besides acting on their origin cells, could also act via a miRNAs intercellular transport mechanism mediated by sEVs. However, there is a lack of studies comparing extracellular vesicles miRNAs content secreted by bovine embryos produced *in vivo* and *in vitro*. Based on that, in this thesis, we investigated the embryo-maternal communication mediated by sEVs.

As early as day 7 of the development, the bovine embryo is able to signal its presence to the endometrium and modulate the uterine luminal fluid composition (SPONCHIADO et al., 2017; SPONCHIADO et al., 2019). The endometrium is modulated differently by embryos of different origins (MANSOURI-ATTIA et al., 2009), suggesting that bovine embryos produced *in vivo* or *in vitro* have different needs to develop, which consequently can influence in establishment and maintenance of the pregnancy. However, which molecules contribute to pregnancy success when embryos produced by different biotechnologies are transferred remains unclear.

Besides the embryo-endometrium local communication is also important to understand how the corpus luteum receives the pregnancy signal. Corpus luteum viability is crucial for pregnancy to happen because it secretes progesterone responsible for pregnancy maintenance. In bovine, local vascularization among the uterine horn, the uterus-tubal junction, oviduct, and ovary are modified according to the stage of the estrous cycle. The ovarian artery uterine branch and uterine-ovarian arterial anastomosis are more prominent in the ipsilateral side of the corpus luteum (reviewed by (GINTHER, 1974), suggesting that the physiological function of this anastomosis possibly be: (i) to prepare the oviduct and uterine horn ipsilateral to the corpus luteum for pregnancy; and/or (ii) to maintain the highly vascularized corpus luteum. Based on that, we believe that this local vascularization allows the transit of sEVs secreted by the embryo and endometrium. These embryonic sEVs can be taken to the corpus luteum (CL) to modulate it directly, or an indirect modulation can occur (through the endometrium). This CL modulation should contribute to its maintenance and functionality, possibly resulting in pregnancy success.

Therefore, understanding the role of *in vivo* or *in vitro*- produced bovine embryos-derived sEVs in the embryo-maternal dialogue may contribute to understanding the reasons that lead to greater embryonic losses in cows receiving embryos produced *in vitro*. This thesis is organized in three chapters about bovine embryo-maternal interactions mediated by sEVs. The first chapter is a review about communication between the embryo and maternal organism through sEVs. In the second chapter, we performed a miRNA screening in bovine embryos produced from different

biotechniques (*in vivo* and *in vitro*) and sEVs secreted by them. We identified important predicted pathways that are modulated by miRNAs differently expressed and are associated with pregnancy establishment. Next, in the third chapter, 1) we studied the differences in endometrial global transcriptomic through RNAseq analysis, in endometrial explants co-cultured with a single day 7 *in vivo* or *in vitro* produced bovine embryo; 2) we analyzed the size, concentration, and miRNAs content of sEVs isolated from the conditioned medium by the endometrium and embryos produced *in vivo* or *in vitro* for 6 and 24 hours, and; 3) we analyzed the miRNAs profile in luteal explants treated with sEVs isolated from the conditioned medium by the endometrium and embryos produced *in vivo* or *in vitro*. Finally, we elaborated a general discussion and conclusion about the results obtained with the experiments of this thesis.

2. Chapter 1: Extracellular vesicles mediated early embryo–maternal interactions

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Abstract: Embryo–maternal crosstalk is an important event that involves many biological processes, which must occur perfectly for pregnancy success. This complex communication starts from the zygote stage within the oviduct and continues in the uterus up to the end of pregnancy. Small extracellular vesicles (EVs) are part of this communication and carry bioactive molecules such as proteins, lipids, mRNA, and miRNA. Small EVs are present in the oviductal and uterine fluid and have important functions during fertilization and early embryonic development. Embryonic cells are able to uptake oviductal and endometrium-derived small EVs. Conversely, embryo-derived EVs might modulate oviductal and uterine function. In this review, our aim is to demonstrate the role of extracellular vesicles modulating embryo–maternal interactions during early pregnancy.

Keywords: small extracellular vesicles; embryo; crosstalk; oviduct; endometrium; blood circulating exosomes

2.1 Introduction

In mammals, the perfect embryo–maternal communication is necessary to allow the establishment and maintenance of pregnancy. For this, oocyte maturation occurs in the preovulatory follicle, followed by the fertilization in the oviduct, and early development in the oviduct and uterus during the luteal phase (HOLM; CALLESEN, 1998). Upon ovulation, the oocyte starts its journey through the oviduct, is fertilized, and embryo development begins,

followed by its first cleavages (BETTERIDGE; FLÉCHON, 1988). Importantly, the major embryonic gene activation (EGA) occurs during the embryo passage through the oviduct (MEMILI et al., 1998). During this phase, the embryo starts to transcribe more actively, decreasing its dependency on the maternal mRNA stock. The epithelial line of the oviduct is formed by ciliated and secretory cells. These cells play roles in gamete transport, capacitation and fertilization (AL-DOSSARY et al., 2013; LI; WINUTHAYANON, 2017), and on early embryonic development (LAI et al., 1996; LOPERA-VÁSQUEZ et al., 2016), mostly by oviductal secretions (LEESE et al., 2007). Small extracellular vesicles (EVs) are present in oviductal fluid content and participate in these important reproductive events (AL-DOSSARY et al., 2013; LOPERA-VÁSQUEZ et al., 2016).

The embryo enters the uterus 4 to 5 days after oocyte fertilization, in bovine (BETTERIDGE; FLÉCHON, 1988; SPENCER, 2013). During embryonic development, the embryo reaches the blastocyst stage composed of two cell lines: the inner cell mass that will originate the embryo proper and the trophoctoderm cell monolayer, which ultimately will form the fetal adnexa/placenta (BETTERIDGE; FLÉCHON, 1988; GUILLOMOT, 1995). Fetal appendages will establish contact with the endometrium, and thus establish the interchange interface between embryo/fetus and maternal tissues. However, in order to allow embryo development and placentation, uterine epithelial and glandular cells secrete the uterine fluid that is very important for embryo nutrition because it contains proteins, lipids, amino acids, growth factors (FORDE; LONERGAN, 2012), and small EVs (NG et al., 2013; TANNETTA et al., 2014; ZHANG et al., 2017).

In most domestic mammalian species, the embryo—when in the uterus—increases the secretion of biological molecules related to the maternal recognition of pregnancy (MRP). These molecules have luteotrophic and anti-luteolytic actions, guaranteeing the corpus luteum (CL)'s capability to secrete the progesterone necessary to establish and sustain pregnancy (SPENCER; BAZER, 1995; ROBERTS et al., 2008). Additionally, different biological molecules are secreted by the embryos of several mammalian species during MRP as interferon-tau (IFNT) in ruminant (IMAKAWA et al., 1987), estradiol in pigs (BAZER et al., 1994), and chorionic gonadotropin in humans (TICCONI et al., 2007). However, recent reports indicate that the embryo is also able to secrete EVs (BURNS et al., 2016; NAKAMURA et al., 2016; MELLISHO et al., 2017), but their role in MRP is still elusive.

Therefore, in mammals, the embryos and the female tract (oviduct and endometrium) are able to secrete extracellular vesicles during the pre-implantation period. Extracellular vesicles are cell-secreted vesicles that are classified according to their size, biogenesis, and secretion, as exosomes, microvesicles, and apoptotic bodies (GYÖRGY et al., 2011). Several cellular types can secrete EVs including follicular (DA SILVEIRA et al., 2012; ANDRADE et al., 2017), oviductal, and endometrial cells (NG et al., 2013; BURNS et al., 2016; GREENING et al., 2016), as well as in vitro and in vivo produced embryos (KROPP et al., 2014; BURNS et al., 2016; NAKAMURA et al., 2016; MELLISHO et al., 2017). Extracellular vesicles carry bioactive molecules as miRNAs, mRNAs (VALADI et al., 2007), proteins (J SIMPSON et al., 2008), and lipids (SUBRA et al., 2007), which modulate various reproductive processes such as ovarian follicular development, oocyte maturation, embryonic development, maternal–embryonic communication, and the establishment of mammalian gestation. There is experimental evidence that EVs participate in intercellular communication in essential reproductive events related to the establishment of pregnancy, such as cell proliferation, crosstalk between the maternal organism and conceptus, as well as during embryonic implantation (SAADELDIN et al., 2015). In early stages of pregnancy, the communication between the conceptus and maternal organism is necessary and the evidence of the participation of EVs of maternal or embryonic origin is increasing (TANNETTA et al., 2014).

Herein, we will review the current knowledge on embryo–maternal interactions, with special emphasis on the roles of EVs during the early crosstalk between embryonic and maternal tissues.

2.2 Embryo–Maternal Interactions Mediated by Embryotropins

Embryotropins are bioactive molecules such as proteins, lipids, and miRNAs secreted from both in vivo—or in vitro—produced mammalian embryos (WYDOOGHE et al., 2017). These molecules may act in autocrine and/or paracrine ways, modulating the embryo's development (in vitro culture) and the maternal endometrial cells, respectively (WYDOOGHE et al., 2017).

In domestic ruminants, the mostly known and well-characterized embryotropin is the IFNT. IFNT is a cytokine secreted by the trophoblastic cells of the ruminant embryo and conceptus that can act in a paracrine and endocrine way. In the uterus, IFNT acts in a paracrine way, decreasing estrogen and oxytocin receptor expression in the endometrium, which is an essential step to maintaining a viable corpus luteum and producing progesterone (ROBERTS et al., 1999). Besides

antiluteolytic function, IFNT has endocrine effects, stimulating the expression of IFN-stimulated genes (ISGs) in the endometrium (AUSTIN et al., 2004), in the corpus luteum (OLIVEIRA et al., 2008; ANTONIAZZI et al., 2013; BRIDI et al., 2018), in white blood cells (HAN et al., 2006), and in the liver (MEYERHOLZ et al., 2016). The effects of IFNT secreted by the bovine embryo in the maternal organism can be detected as early as day 7 of development (SPONCHIADO et al., 2017; SPONCHIADO et al., 2019).

The IFNT function on the maternal recognition of pregnancy is well established for ruminants. There are parallels for such a response in other species. One example is the conserved response of interferon-stimulated gene 15 (*ISG15*), which is stimulated by IFNs and other cytokines. *ISG15* is up-regulated in the endometrium of ruminants (AUSTIN et al., 2004), primates (BEBINGTON et al., 1999), and mice (AUSTIN et al., 2003) during early pregnancy. However, its function during the maternal recognition of pregnancy in non-ruminant species is not well established. Mouse knockout for *Isg15* results in 50% fetal loss, which can be explained by change decidual gene expression that is functionally related to cell survival and adhesion pathways (ASHLEY et al., 2010).

Besides IFNT, day 13 bovine embryos can also secrete prostaglandins, such as prostaglandin F2 alpha (PGF), prostaglandin E2 (PGE2), and prostaglandin I2 (PGI2) (SPENCER et al., 2013). These prostaglandins act in a paracrine way in the endometrium, increasing *ISGs*' expression and function, which can be important for uterine receptivity as well as conceptus growth and development during early pregnancy (SPENCER et al., 2013). In addition, PGE2 and PGI2 can modulate blastocyst implantation, decidualization, and endometrial vascular permeability during early pregnancy in mice and rats (KENNEDY et al., 2007). In large domestic species, PGE2 is secreted by the endometrium and embryo, showing an important role as the local antiluteolytic factor (reviewed by (OTTOBRE et al., 1984)). PGE2 secreted by equine viable embryos during early embryonic development is involved with the initial oviductal transport of the embryo and opening the uterutubal papilla to allow the embryo to enter the uterus. This mechanism may explain why unfertilized oocytes or degenerate embryos are not able to gain the uterine lumen in equine (WEBER et al., 1991b; a). Additionally, the lysophosphatidic acid (LPA) is secreted by the bovine embryo and endometrium and may act as a luteotropic factor by stimulating PGE2 synthesis in stromal cells during early pregnancy (WOCLAWEK-POTOCKA et al., 2009).

Hence, embryotropins are signaling molecules acting in the maternal tissues to improve embryo–maternal recognition in mammals. Several embryotropins are secreted by different species with the same aim, which is to inform embryo presence to the mother and to maintain a functional CL, progesterone synthesis, and the maintenance of pregnancy. However, other mechanisms can be involved in the maternal recognition of pregnancy. Recently, EVs were introduced as new players in the embryo–maternal communication.

2.3 Extracellular Vesicles Derived from Female Reproductive Tract and Embryo

Extracellular vesicles (EVs) are formed by a phospholipid bilayer and secreted by different cell types (THÉRY et al., 2009). EVs were discovered approximately 40 years ago, and they were first characterized as “small trash bags”, due to the possibility of carrying cellular material to the extracellular environment (JOHNSTONE et al., 1987). Currently, these EVs have been found in different body fluids and can be divided into exosomes, microvesicles, and apoptotic bodies (GYÖRGY et al., 2011) according to their size, biosynthesis, and contents.

Exosomes are nanovesicles between 30 and 150 nm in size (MATHIVANAN et al., 2010) that carry bioactive material such as lipids (SUBRA et al., 2007), proteins (J SIMPSON et al., 2008), RNAs, and miRNAs (VALADI et al., 2007), which are able to control regulatory pathways associated with physiological or pathological functions among neighboring or distant cells through the extracellular environment (NG et al., 2013). The biogenesis of small EVs initiates with plasma membrane endocytosis, which gives rise to multivesicular bodies (MVBs). Inside MVBs intraluminal vesicles are formed, which are released out of the cell when the MVB fuses with the cell plasma membrane (THÉRY et al., 2002). The released vesicles are called exosomes (small extracellular vesicles).

Cellular communication mediated by small EVs can happen by fusion between the vesicle membrane and plasma membrane of the target cell, leading to the release of EVs contents into the target cell cytoplasm (NG et al., 2013). In addition to cell fusion, it is suggested that specific receptors present in the membrane of the target cells are involved in EVs’ endocytosis (MORELLI et al., 2004; SEGURA et al., 2007).

Small EVs have also been identified in many body fluids as follicular fluid (DA SILVEIRA et al., 2012), blood (CLEYS et al., 2014), cerebrospinal fluid (STREET et al., 2012), urine (PRUNOTTO et al., 2013), oviduct (LOPERA-VÁSQUEZ et al., 2016), and uterine fluids (NG et

al., 2013; BURNS et al., 2016). In addition to body fluids, embryos cultured in vitro can secrete small EVs in the culture medium (MELLISHO et al., 2017). There are different methods to isolate EVs from body fluids and culture medium such as ultracentrifugation, filtration, size-exclusion chromatography, polymer precipitation, immunoaffinity, and microfluidic techniques (WITWER et al., 2013). Thus, based on the research and type sample, the best isolation method can be chosen (WITWER et al., 2013). This decision is normally based on the molecule of interest since each method can yield different concentrations and purity. For example, our laboratory has used filtration and ultracentrifugation to isolate small EVs from different body fluids such as follicular fluid (ANDRADE et al., 2017; DE ÁVILA et al., 2019), polymer precipitation, and ultracentrifugation to separate small EVs from an in vitro embryo-conditioned medium (ANDRADE et al., 2019). Similarly, other studies have isolated EVs from embryos or conceptus-conditioned culture media using polymer-based precipitation (BURNS et al., 2016) and ultracentrifugation (MELLISHO et al., 2017; QU et al., 2017).

Small EVs are involved in several reproductive events related to oocyte maturation, fertilization, early embryonic development, and crosstalk between embryo and maternal organism (Table 1). One of the first studies that identified the presence of EVs associated with reproductive events used equine ovarian follicular fluid to investigate the role of extracellular vesicles during follicular development. The authors demonstrated that EVs from equine ovarian follicular fluid carried miRNAs and proteins, and were uptaken by granulosa cells in vitro and in vivo, suggesting a new cell-to-cell communication inside the ovarian follicles (DA SILVEIRA et al., 2012). Another study demonstrated that oviductal microvesicles were uptaken by oocyte cytoplasm after 72 h of maturation in bitch, demonstrating the role of EVs in improving the oocyte maturation rate (LANGE-CONSIGLIO et al., 2017). Furthermore, another study using murine oviductal EVs demonstrated that EVs carrying plasma membrane Ca^{2+} -ATPase 4 (PMCA4) were uptaken by sperm, thus inducing sperm capacitation prior to fertilization (AL-DOSSARY et al., 2013).

Table 1. Major findings about extracellular vesicles (EVs) derived from the reproductive female tract and embryos in mammals. IFNT: interferon-tau.

Topic	Findings	Reference
EVs derived from ovarian follicles	✓ Characterization of protein and miRNA content	Da Silveira et al., 2012 (DA SILVEIRA et al., 2012)
	✓ EVs from follicular fluid are uptaken by granulosa cells in vivo and in vitro	
EVs derived from in vitro produced embryos	✓ In vitro fertilization (IVF) and parthenogenetic embryos secrete EVs	Mellisho et al., 2017 (MELLISHO et al., 2017)
	✓ Small EVs from parthenogenetic porcine embryos improves cloned embryos' development	Saadeldin et al., 2014 (SAADELDIN et al., 2014)
	✓ Exposure of somatic cell nuclear transfer (SCNT) embryos to EVs from other SCNT embryos increases the blastocyst rate	Qu et al., 2017 (QU et al., 2017)
Oviductal EVs	✓ Endocytosis by oocyte cytoplasm improves maturation rate	Lange-Consiglio et al., 2017 (LANGE-CONSIGLIO et al., 2017)
	✓ Uptake of EVs by sperm cells modulate sperm capacitation	Al-Dossary et al., 2013 (AL-DOSSARY et al., 2013)
	✓ Improve in vitro embryo quality	Lopera-Vásquez et al., 2016; Almiñana et al., 2017; Lopera-Vasquez et al., 2017 (LOPERA-VÁSQUEZ et al., 2016; ALMIÑANA et al., 2017; LOPERA-VASQUEZ et al., 2017)
	✓ Increase birth rates after embryo transfer	Qu et al., 2019 (QU et al., 2019)
Embryonic and uterine EVs	✓ EVs isolated from uterine flushing are uptaken by conceptuses trophoctoderm cells	Burns et al., 2016 (BURNS et al., 2016)
	✓ Endometrial EVs containing <i>enJSRV</i> mRNA act on trophoctoderm to induce IFNT production	Ruiz-González et al., 2015 (RUIZ-GONZÁLEZ et al., 2015)
	✓ EVs derived from trophoblast cells contain IFNT that stimulates <i>ISGs</i> expression in endometrial cells	Nakamura et al., 2016; Kusama et al., 2018 (NAKAMURA et al., 2016; KUSAMA et al., 2018)
	✓ CAPG and AKR1B1 proteins are present in EVs isolated from uterine flushing	Nakamura et al., 2016 (NAKAMURA et al., 2016)
	✓ Progesterone increases the release of EVs from endometrial cells	Burns et al., 2018 (BURNS et al., 2018)

-
✓	Endometrial-derived exosomes containing miRNAs upregulated by progesterone can modulate phosphoinositide 3-kinase/Serine/threonine kinase 1 (PI3K/AKT), bone morphogenetic protein (BMP), and post-transcriptional silencing by small RNAs pathways	
✓	Exosomal miRNAs and proteins modulate the angiogenesis pathway in trophoctoderm and maternal endothelial cells	Bidarimath et al., 2017 (BIDARIMATH et al., 2017)
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2.4 Embryo–Maternal Interactions through Oviductal EVs

Oviduct used to be considered just a tubular connection between the ovary and the uterus where the oocyte and sperm passed through (BROWER; ANDERSON, 1969). However, the oviduct is composed by ciliated and secretory cells that secrete oviductal fluid (LEESE et al., 2007). Several studies demonstrated the important biological role of the oviductal fluid during sperm capacitation (POLLARD et al., 1991), fertilization (LI; WINUTHAYANON, 2017), and the outset of embryonic development (LAI et al., 1996). Additionally, oviductal fluid contents include extracellular vesicles that have an important role during oocyte fertilization (ALDOSSARY et al., 2013) and early embryonic development (LOPERA-VÁSQUEZ et al., 2016).

Recently, the functional effects of the EVs derived from the oviduct on gametes and embryos were summarized by Almiñana and Bauersachs (2019) (ALMIÑANA; BAUERSACHS, 2019). In this review, we highlight the key findings related to EVs derived from oviductal cells and their effects in early embryonic development.

EVs from bovine oviduct epithelial cells (BOEC) were used in in vitro embryo production and demonstrated to improve embryo quality based on the increase in the number in trophectoderm and total cells and survival after vitrification (LOPERA-VÁSQUEZ et al., 2016). In addition, space-specific EVs secreted from isthmus oviductal fluid were able to increase the survival rate and improve the development as well as the quality of in vitro produced blastocysts (LOPERA-VÁSQUEZ et al., 2017). In vitro embryos were able to uptake EVs derived from in vivo oviduct epithelial cells, and this communication benefits the embryo blastocyst rate, survival, and quality (ALMIÑANA et al., 2017). Moreover, EVs secreted by donor oviductal cells increase birth rates after embryo transfer in mice due to decreased apoptosis and improved cellular differentiation in embryos (QU et al., 2019). Altogether, these data show the importance of embryo–maternal interactions mediated by EVs derived from the oviduct during early embryonic development, leading to improved embryo quality and successful pregnancy.

An important problem that may occur during the passage of the zygote through the oviduct is ectopic pregnancy (EP), which occurs when the embryo after fertilization implants outside of the uterine cavity due to structural abnormalities in the fallopian tubes, for example (DELLA-GIUSTINA; DENNY, 2003; MURRAY et al., 2005). Approximately 1.5%–2% of all the pregnancies are ectopic (PISARSKA et al., 1998); 97% are in the fallopian tube (oviduct) (reviewed by (BRENNAN, 1995)). Currently, two diagnosis methods are used to detect EP: measurements of human chorionic gonadotropin (hCG) and progesterone in the serum (MURRAY et al., 2005). The serial serum hCG measurement with intervals of 48 h is needed

for the diagnostic; however, during this timecourse, tubal rupture might occur in patients, leading to possible complications in clinical status (MURRAY et al., 2005; ACOG Practice Bulletin No. 94: Medical management of ectopic pregnancy, 2008). Therefore, only these two biomarkers are not precise and efficient to detect EP. Recently, new approaches showed that circulating miRNA miR-323-3p can be associated with serum hCG and progesterone to improve EP diagnostic (ZHAO et al., 2012). Thus, the presence of miR-323-3p in serum could serve as a marker for EP. However, more studies are necessary to demonstrate if this miRNA is carried by small EVs or not, in order to become a reliable diagnostic marker.

2.5 Embryo–Maternal Interactions between Uterus and Embryo Mediated by EVs

The mammalian uterus is designed to allow sperm transport (MORTIMER et al., 2013) as well as embryonic and fetal development (ZHANG et al., 2017). In bovine, the morula stage embryo will arrive at the uterus at the uterotubal junction portion around Day 5 of embryo development (BETTERIDGE; FLÉCHON, 1988). Uterine fluid, termed histotroph in ruminants, is the result of glandular cells secretion inside of the uterine lumen (BAZER et al., 2011; FORDE; LONERGAN, 2012). This fluid is very important for the nutrition of the embryo since it contains proteins, lipids, amino acids, growth factors (FORDE; LONERGAN, 2012), and recently described extracellular vesicles that carry bioactive substances that are important for the early embryonic development (NG et al., 2013; TANNETTA et al., 2014; ZHANG et al., 2017). During early embryo development, intense crosstalk starts between the embryo and the maternal uterine environment. This communication is necessary to induce the maternal recognition of pregnancy; thus, it is important to understand the role of extracellular vesicles in the embryo–maternal interface.

During maternal recognition of the pregnancy period, EVs were isolated within the uterine flushing of ewes on day 14 of the estrus cycle were fluorescently labeled with PKH67 and observed inside conceptus trophoctoderm cells. This finding demonstrates that EVs are involved in paracrine communication between the endometrium and conceptus during the early pregnancy period (BURNS et al., 2016). In addition to that, sheep endometrial epithelium can secrete exosomes containing *ovine endogenous jaagsiekte retroviruses (enJSRV)* mRNA, which acts on trophoctoderm via toll-like receptors (TLR) to induce IFNT production (RUIZ-GONZÁLEZ et al., 2015). Trophoblast cells from the conceptus at day 15 and 17 secrete EVs containing IFNT that are able to stimulate ISGs' expression in endometrial cell culture (NAKAMURA et al., 2016; KUSAMA et al., 2018). Furthermore, macrophage-capping protein (CAPG) and aldo-keto reductase family 1, member B1 protein (AKR1B1) proteins are present

in EVs isolated from the uterine flushing of pregnant cows on days 15 and 17 of gestation (NAKAMURA et al., 2016). Besides that, EVs isolated from uterine flushing in the pre-implantation period increase the expression of apoptotic genes (*BAX*, *CASP3*, *TNFA*, and *TP53* transcripts) in endometrial cells (KUSAMA et al., 2018). In addition to that, endometrial cells treated with EVs from the post-implantation induced and increase in *vascular cell adhesion molecule 1 (VCAM)* transcript, indicating the modulation of adhesion molecules (KUSAMA et al., 2018). Furthermore, exosomes isolated from uterine flushing obtained from pregnant cows on days 17, 20, and 22 were used to treat trophoblast CT-1 cells and did not induce changes in *IFNT* and *CDX2* mRNA expression, suggesting that the pregnancy period may influence EVs' contents (KUSAMA et al., 2018). Together, this information highlights the EVs biological role during the period of maternal recognition of pregnancy, which may enhance embryo–maternal communication and consequently contribute to the maintenance of pregnancy.

Steroid hormones, such as progesterone and estradiol, can induce changes in the EVs secretion in human endometrial cells (GREENING et al., 2016). Progesterone, which is secreted by corpus luteum, is necessary to the establishment and maintenance of pregnancy and acts in the elongation and survival of the conceptus (SPENCER; BAZER, 2002). Progesterone induces myometrium relaxation and stimulates the production of mucin 1 (MUC-1), which is a protein that prevents conceptus adhesion to endometrium; thus, it can continue elongating and producing IFNT (JOHNSON et al., 2001) as well as stimulating histotroph production by endometrial glands (FORDE; LONERGAN, 2012). On day 10 to 14 of the estrous cycle in ovine, an increase in EVs secretion by endometrial luminal cells occurs, suggesting that progesterone is responsible for this event (BURNS et al., 2018). Besides that, EVs from the uterine lumen had miRNAs upregulated by progesterone that were predicted to modulate phosphoinositide 3-kinase/ Serine/threonine kinase 1 (PI3K/AKT), bone morphogenetic protein (BMP), and post-transcriptional silencing by small RNA pathways (BURNS et al., 2018). These results reinforce that progesterone is very important during the onset of pregnancy because it can modulate the endometrial function and consequently contribute to embryo development.

Extracellular vesicles are also secreted by endometrium and chorioallantoic membrane cells as well as trophoctoderm and maternal endothelial cells from sows on day 20 of pregnancy (BIDARIMATH et al., 2017). MiRNAs and proteins within EVs were able to modulate the angiogenesis pathway within trophoctoderm and maternal endothelial cells (BIDARIMATH et al., 2017). Moreover, EVs derived from the porcine trophoctoderm are uptaken by maternal endothelial cells and stimulate the cellular proliferation of these cells (BIDARIMATH et al.,

2017). Together, these results demonstrate that EVs have an important biological role in conceptus–endometrium crosstalk during the establishment of pregnancy in porcine.

Local vascularization between the uterine horn ipsilateral and the corpus luteum is more prominent during the estrous cycle luteal phase than the contralateral phase, suggesting that the oviduct and uterine could signal to CL and adjacent tissue for future pregnancy and/or luteal vascularization maintenance (GINTHER, 1974). This elevated vascularization on the ipsilateral horn could be involved with the early onset of pregnancy recognition. As an example, exosomal miRNAs were identified in serum samples of nonpregnant and pregnant mares on days 9, 11, or 13 postovulation (KLOHONATZ et al., 2016). These miRNAs were increased in nonpregnant mares and predicted to target the pathway of focal adhesion molecules (FAMs) in the endometrium (KLOHONATZ et al., 2016), which are involved in the regulation of the extracellular matrix (BURGHARDT et al., 2009). These data suggest that in pregnant mares, FAMs are normally abundant, which suggests that exosomal miRNAs are less necessary to modulate focal adhesion pathway mRNAs in the endometrium, allowing the embryo to move inside the uterus, which contributes to the maternal recognition of pregnancy (KLOHONATZ et al., 2016).

In bovine, 27 miRNAs were highly abundant in the serum small EVs of cows with embryonic mortality compared to the pregnant group on day 17 (POHLER et al., 2017). These miRNAs modulate pathways associated with many important processes such as inflammation, cell proliferation, endometriosis, cell cycle progression, contraction, infection, late-onset preeclampsia, apoptosis, differentiation, uterine leiomyoma, ovarian endometriosis, and cell viability (POHLER et al., 2017). However, in a retrospective study examining EVs isolated from the blood plasma of pregnant cows on day 21 of gestation, a low abundance of 27 miRNAs was identified in samples from initial somatic cell nuclear transfer (SCNT) embryonic loss compared with full-term SCNT pregnancies and full-term artificial insemination pregnancies. These miRNAs modulate the pathways associated with pregnancy establishment as well as cell proliferation, differentiation, apoptosis, angiogenesis, and uterus embryonic development (DE BEM et al., 2017). In addition, 29 miRNAs from serum small EVs were differently detected in the 30 days of pregnancy group compared to the normal group (MARKKANDAN et al., 2018). Different pathways involved in metabolism are modulated by these 21 up-regulated miRNAs and eight down-regulated miRNAs in pregnant cows (MARKKANDAN et al., 2018).

In Table 2, we itemized the publications about EVs carrying miRNAs in the pregnant female blood. On day 9, in pregnant mares, miRNA-29c and miRNA-101 are down-regulated in circulation exosomes (KLOHONATZ et al., 2016); however, on day 21, in bovine, these

miRNAs are more abundant (DE BEM et al., 2017). On day 17, in bovine, miRNA-15a, miRNA-15b, miRNA-101, miRNA-106b, miRNA-652, miRNA-143, and miRNA-148a presented decreased expression (POHLER et al., 2017); however, on day 21, miRNA-15a, miRNA-15b, miRNA-101, miRNA-106b, and miRNA-652 (DE BEM et al., 2017), and on day 30, miRNA-143 and miRNA-148a (MARKKANDAN et al., 2018) expression increased in the serum-derived small EVs of pregnant cows. MiRNA-148b is increased in blood exosomes on days 21 and 30 of bovine pregnancy (DE BEM et al., 2017; MARKKANDAN et al., 2018). However, on day 21, miRNA-193b is more abundant, but on day 30, miRNA-193b is down-regulated in pregnant cows (DE BEM et al., 2017; MARKKANDAN et al., 2018).

These data suggest that there is EV-mediated communication between the uterus and peripheral circulation. This communication can be direct and modulated by biological factors secreted by the embryo, or it can be indirect, where the embryo stimulates an endometrium response. Thus, extracellular vesicles can be part of this intricate mechanism improving embryo–maternal interactions and consequently pregnancy success in mammals. Moreover, these studies demonstrate the potential role of circulating exosomal miRNAs as biomarkers in early embryonic mortality or early pregnancy diagnosis.

In conclusion, small EVs carry important bioactive molecules that are involved in embryo–maternal crosstalk (Figure 1) through the modulation of important signaling pathways such as angiogenesis, apoptosis, interferon-tau, adhesion, proliferation, and cell survival. The above-mentioned studies demonstrate that EVs participate in embryo–maternal interactions during early embryonic development and the maternal recognition of pregnancy in mammals.

Table 2. The main published papers about extracellular vesicle-associated miRNAs in pregnant female blood.

Species	Period of Pregnancy	Circulating Exosomal miRNAs in Pregnant Female	Pathways Modulated by miRNAs	Reference
Equine	Day 9	Down-regulated: eca-miR-27a, eca-miR-29c, eca-miR-101, and eca-miR-486-5p	Extracellular matrix (ECM)–receptor interaction Proteoglycans in cancer TGF-beta Focal adhesion pathway	Klohonatz et al., 2016 (KLOHONATZ et al., 2016)
	Day 11	Down-regulated: eca-miR-195 Up-regulated: eca-miR-767-5p	Regulating pluripotency of stem cells Fatty acid biosynthesis FoxO Focal adhesion pathway	
	Day 13	Down-regulated: eca-miR-188-5p, eca-miR-653, eca-miR-874, and eca-miR-140-3p Up-regulated: eca-miR-30c and eca-miR-323-5p	Glutamatergic synapse Regulation of actin cytoskeleton Long-term potentiation Focal adhesion pathway	
Bovine	Day 17	Down-regulated: bta-let-7c, bta-mir-100, bta-mir-101-1, bta-mir-101-2, bta-mir-106b, bta-mir-125b-2, bta-mir-127, bta-mir-141, bta-mir-143, bta-mir-148a, bta-mir-15a, bta-mir-15b, bta-mir-16a, bta-mir-16b, bta-mir-1839, bta-mir-199a-1, bta-mir-199b, bta-mir-2415, bta-mir-25, bta-mir-331, bta-mir-339b, bta-mir-3596, bta-mir-3604-1, bta-mir-409b, bta-mir-451, bta-mir-652, bta-mir-99a	Cancer Connective tissue disorders Organismal injury and abnormalities Reproductive system disease Endocrine disorders	Pohler et al., 2017 (POHLER et al., 2017)

Bovine	Day 21	<p>Highly abundant: bta-mir-15a, bta-mir-221, bta-mir-425-5p, bta-mir-101, bta-mir-93, bta-mir-106a, bta-mir-106b, bta-mir-22-5p, bta-mir-652, bta-mir-103, bta-mir-138, bta-mir-193b, bta-let-7f, bta-let-7g, bta-mir-15b, bta-let-7e, bta-let-7a-5p, bta-let-7d, bta-mir-660, bta-mir-29a, bta-mir-30d, bta-mir-497, bta-mir-148b, bta-mir-361, bta-mir-30a-5p, bta-mir-29c, bta-mir-29d-3p</p>	<p>Wnt TGF-beta Renal cell carcinoma Melanoma Colorectal cancer Glioma p53 Pancreatic cancer; Bladder cancer Dorsoventral axis formation</p>	De Bem et al., 2017 (DE BEM et al., 2017)
Bovine	Day 30	<p>Down-regulated: bta-miR-193b, bta-miR-197, bta-miR-339a, bta-miR-326, bta-miR-484, bta-miR-486, bta-miR-423-3p, bta-miR-92a</p> <p>Up-regulated: bta-miR-146b, bta-miR-27b, bta-miR-26b, bta-miR-200a, bta-miR-450b, bta-miR-199c, bta-miR-194, bta-miR-6119-3p, bta-miR-7, bta-miR-199a-3p, bta-miR-574, bta-miR-215, bta-miR-148a, bta-let-7a-3p, bta-miR-21-5p, bta-miR-126-5p, bta-miR-148b, bta-miR-143, bta-miR-1246, bta-miR-192, bta-miR-98</p>	<p>Membrane trafficking Chromosome and associated proteins Exosome G protein-coupled receptors Transcription factors Ubiquitin system Olfactory transduction Transporters Protein kinases Cytoskeleton proteins Cell adhesion molecules and their ligands Protein phosphatase and associated proteins Peptidases Pathway in cancer Messenger RNA Biogenesis CD molecules PI3K–Akt signaling pathway Human papillomavirus infection Mitochondrial biogenesis Spliceosome</p>	Markkandan et al., 2018 (MARKKANDAN et al., 2018)

Figure 1. Embryo–maternal interactions mediated by extracellular vesicles secreted from the embryo and endometrium cells. Extracellular vesicles secreted by embryos are uptaken by endometrial cells. Extracellular vesicles (EVs) content include miRNA, mRNAs, and proteins that act by paracrine signaling. Different experiments demonstrated that embryo-derived EVs can modulate interferon-tau, apoptosis, cell proliferation, adhesion, angiogenesis, and cell survival biological pathways. Similarly, endometrium cells can secrete EVs containing proteins, miRNAs, and mRNAs. These EVs are uptaken by trophoblast cells, and their contents are predicted to regulate interferon-tau production and angiogenesis pathways. However, questions related to how embryo-derived EVs can exit the uterus and arrive in other maternal tissue cells are yet unclear (red interrogation points). Therefore, further studies are needed to understand how the factors secreted by the embryos exit the uterus and to arrive in other maternal tissue, as blood cells, to modulate biological pathways.

2.6 Conclusions

In this review, we demonstrate the biological roles of extracellular vesicles in events occurring during the onset of pregnancy and involved in the communication between the embryo and the maternal organism in different mammalian species. EVs carry important bioactive molecules that are capable of modulating key reproductive events during the early pregnancy period. Further investigations are necessary to elucidate if EVs secreted by the oviduct and endometrium as well as embryos can arrive in peripheral circulation and modulate different pathways in maternal organisms. Thus, the progression in our understanding related to this type of communication can advance the tests to detect pregnancies, abnormal pregnancies (EP), and predict pregnancy loss, as well as push the development of new technologies to modulate early embryo–maternal interactions.

2.7 References

ACOG Practice Bulletin No. 94: Medical management of ectopic pregnancy. **Obstetrics and gynecology**, v. 111, n. 6, p. 1479-85, 6/3/pubmed7/25/medline6/3/entrez 2008.

AL-DOSSARY, A. A.; STREHLER, E. E.; MARTIN-DELEON, P. A. Expression and Secretion of Plasma Membrane Ca²⁺-ATPase 4a (PMCA4a) during Murine Estrus: Association with Oviductal Exosomes and Uptake in Sperm. **PLOS ONE**, v. 8, n. 11, p. e80181, 2013.

ALMIÑANA, C.; BAUERSACHS, S. Extracellular Vesicles in the Oviduct: Progress, Challenges and Implications for the Reproductive Success. **Bioengineering (Basel, Switzerland)**, v. 6, n. 2, p. 32, 2019.

ALMIÑANA, C. et al. Oviduct extracellular vesicles protein content and their role during oviduct–embryo cross-talk. v. 154, n. 3, p. 253, 2017.

ANDRADE, G. et al. Cellular and extracellular vesicular origins of miRNAs within the bovine ovarian follicle. **Reproduction in Domestic Animals**, v. 52, n. 6, p. 1036-1045, 2017.

ANDRADE, G. M. et al. Oxygen tension modulates extracellular vesicles and its miRNA contents in bovine embryo culture medium. **Molecular Reproduction and Development**, v. 86, n. 8, p. 1067-1080, 2019.

ANTONIAZZI, A. Q. et al. Endocrine delivery of interferon tau protects the corpus luteum from prostaglandin F₂ alpha-induced luteolysis in ewes. **Biol Reprod**, v. 88, n. 6, p. 144, Jun 2013.

ASHLEY, R. L. et al. Deletion of the Isg15 Gene Results in Up-Regulation of Decidual Cell Survival Genes and Down-Regulation of Adhesion Genes: Implication for Regulation by IL-1 β . **Endocrinology**, v. 151, n. 9, p. 4527-4536, 2010.

AUSTIN, K. J. et al. Interferon-Stimulated Gene-15 (Isg15) Expression Is Up-Regulated in the Mouse Uterus in Response to the Implanting Conceptus. **Endocrinology**, v. 144, n. 7, p. 3107-3113, 2003.

AUSTIN, K. J. et al. Localization of ISG15 and Conjugated Proteins in Bovine Endometrium Using Immunohistochemistry and Electron Microscopy. **Endocrinology**, v. 145, n. 2, p. 967-975, 2004.

BAZER, F. W.; OTT, T. L.; SPENCER, T. E. Pregnancy recognition in ruminants, pigs and horses: Signals from the trophoblast. **Theriogenology**, v. 41, n. 1, p. 79-94, 1// 1994.

BAZER, F. W. et al. Uterine Histotroph and Conceptus Development: Select Nutrients and Secreted Phosphoprotein 1 Affect Mechanistic Target of Rapamycin Cell Signaling in Ewes1. **Biology of Reproduction**, v. 85, n. 6, p. 1094-1107, 2011.

BEBINGTON, C.; DOHERTY, F. J.; FLEMING, S. D. Ubiquitin cross-reactive protein gene expression is increased in decidualized endometrial stromal cells at the initiation of pregnancy. **Molecular Human Reproduction**, v. 5, n. 10, p. 966-972, October 1, 1999 1999.

BETTERIDGE, K. J.; FLÉCHON, J. E. The anatomy and physiology of pre-attachment bovine embryos. **Theriogenology**, v. 29, n. 1, p. 155-187, 1988/01/01/ 1988.

BIDARIMATH, M. et al. Extracellular vesicle mediated intercellular communication at the porcine maternal-fetal interface: A new paradigm for conceptus-endometrial cross-talk. **Scientific Reports**, v. 7, p. 40476, 01/12/online 2017.

BRENNAN, D. F. Ectopic Pregnancy—Part I: Clinical and Laboratory Diagnosis. **Academic Emergency Medicine**, v. 2, n. 12, p. 1081-1089, 1995.

BRIDI, A. et al. Parthenogenetic bovine embryos secrete type I interferon capable of stimulating ISG15 in luteal cell culture. **Animal Reproduction** v. 15, n. 4, p. 1268 - 1277, 2018.

BROWER, L. K.; ANDERSON, E. Cytological Events Associated with the Secretory Process in the Rabbit Oviduct1. **Biology of Reproduction**, v. 1, n. 2, p. 130-148, 1969.

BURGHARDT, R. C. et al. Enhanced focal adhesion assembly reflects increased mechanosensation and mechanotransduction at maternal–conceptus interface and uterine wall during ovine pregnancy. **Reproduction**, v. 137, n. 3, p. 567-582, March 1, 2009 2009.

BURNS, G. W. et al. Progesterone effects on extracellular vesicles in the sheep uterus†. **Biology of Reproduction**, v. 98, n. 5, p. 612-622, 2018.

BURNS, G. W.; BROOKS, K. E.; SPENCER, T. E. Extracellular Vesicles Originate from the Conceptus and Uterus During Early Pregnancy in Sheep. **Biology of Reproduction**, v. 94, n. 3, p. 56, 1-11, March 1, 2016 2016.

CLEYS, E. R. et al. Identification of microRNAs in exosomes isolated from serum and umbilical cord blood, as well as placentomes of gestational day 90 pregnant sheep. **Molecular Reproduction and Development**, v. 81, n. 11, p. 983-993, 2014.

DA SILVEIRA, J. C. et al. Cell-Secreted Vesicles in Equine Ovarian Follicular Fluid Contain miRNAs and Proteins: A Possible New Form of Cell Communication Within the Ovarian Follicle. **Biology of Reproduction**, v. 86, n. 3, p. 71, 1-10, March 1, 2012 2012.

DE ÁVILA, A. C. F. C. M. et al. Estrous cycle impacts miRNA content in extracellular vesicles that modulate bovine cumulus cell transcripts during in vitro maturation. **Biology of Reproduction**, 2019.

DE BEM, T. H. C. et al. Low levels of exosomal-miRNAs in maternal blood are associated with early pregnancy loss in cloned cattle. **Scientific Reports**, v. 7, n. 1, p. 14319, 2017/10/30 2017.

DELLA-GIUSTINA, D.; DENNY, M. Ectopic pregnancy. **Emergency Medicine Clinics of North America**, v. 21, n. 3, p. 565-584, 2003/08/01/ 2003.

FORDE, N.; LONERGAN, P. Transcriptomic Analysis of the Bovine Endometrium: What is Required to Establish Uterine Receptivity to Implantation in Cattle ? **Journal of Reproduction and Development**, v. 58, n. 2, p. 189-195, 2012.

GINTHER, O. J. INTERNAL REGULATION OF PHYSIOLOGICAL PROCESSES THROUGH LOCAL VENOARTERIAL PATHWAYS: A REVIEW. **JOURNAL OF ANIMAL SCIENCE**, v. 39, n. 3, p. 550 - 564, 1974.

GREENING, D. W. et al. Human Endometrial Exosomes Contain Hormone-Specific Cargo Modulating Trophoblast Adhesive Capacity: Insights into Endometrial-Embryo Interactions1. **Biology of Reproduction**, v. 94, n. 2, p. 38, 1-15-38, 1-15, 2016.

GUILLOMOT, M. Cellular interactions during implantation in domestic ruminants. **Journal of reproduction and fertility. Supplement**, v. 49, p. 39-51, 1995.

GYÖRGY, B. et al. Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles. **Cellular and Molecular Life Sciences**, v. 68, n. 16, p. 2667-2688, 2011.

HAN, H. et al. Low blood ISG15 mRNA and progesterone levels are predictive of non-pregnant dairy cows. **Journal of Endocrinology**, v. 191, n. 2, p. 505-512, November 1, 2006.

HOLM, P.; CALLESEN, H. In vivo versus in vitro produced bovine ova: similarities and differences relevant for practical application. **Reproduction Nutrition Development**, v. 38, n. 6, p. 579-594, 1998.

IMAKAWA, K. et al. Interferon-like sequence of ovine trophoblast protein secreted by embryonic trophectoderm. **Nature**, v. 330, n. 6146, p. 377-379, 11/26/print 1987.

J SIMPSON, R.; S JENSEN, S.; W E LIM, J. **Proteomic profiling of exosomes: Current perspectives**. 2008. 4083-99.

JOHNSON, G. A. et al. Muc-1, Integrin, and Osteopontin Expression During the Implantation Cascade in Sheep. **Biology of Reproduction**, v. 65, n. 3, p. 820-828, September 1, 2001 2001.

JOHNSTONE, R. M. et al. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). **The Journal of Biological Chemistry** v. 262, p. 9412-20, 1987.

KENNEDY, T.; GILLIO-MEINA, C.; PHANG, S. Prostaglandins and the initiation of blastocyst implantation and decidualization. **Reproduction**, v. 134, p. 635-643, 2007.

KLOHONATZ, K. M. et al. Circulating miRNAs as Potential Alternative Cell Signaling Associated with Maternal Recognition of Pregnancy in the Mare1. **Biology of Reproduction**, v. 95, n. 6, p. 124, 1-12-124, 1-12, 2016.

KROPP, J.; SALIH, S. M.; KHATIB, H. Expression of microRNAs in bovine and human pre-implantation embryo culture media. **Frontiers in Genetics**, v. 5, p. 91, 04/2402/17/received04/03/accepted 2014.

KUSAMA, K. et al. Intrauterine exosomes are required for bovine conceptus implantation. **Biochemical and Biophysical Research Communications**, v. 495, n. 1, p. 1370-1375, 2018/01/01/ 2018.

LAI, Y. M. et al. Insulin-like growth factor-binding proteins produced by Vero cells, human oviductal cells and human endometrial cells, and the role of insulin-like growth factor-binding protein-3 in mouse embryo co-culture systems. **Human Reproduction**, v. 11, n. 6, p. 1281-1286, 1996.

LANGE-CONSIGLIO, A. et al. Oviductal microvesicles and their effect on in vitro maturation of canine oocytes. v. 154, n. 2, p. 167, 2017.

LEESE, H. J. et al. Female reproductive tract fluids: composition, mechanism of formation and potential role in the developmental origins of health and disease. **Reproduction, Fertility and Development**, v. 20, n. 1, p. 1-8, 2007.

LI, S.; WINUTHAYANON, W. Oviduct: roles in fertilization and early embryo development. v. 232, n. 1, p. R1, 2017.

LOPERA-VÁSQUEZ, R. et al. Extracellular Vesicles from BOEC in In Vitro Embryo Development and Quality. **PLOS ONE**, v. 11, n. 2, p. e0148083, 2016.

LOPERA-VASQUEZ, R. et al. Effect of bovine oviductal extracellular vesicles on embryo development and quality in vitro. v. 153, n. 4, p. 461, 2017.

MARKKANDAN, K. et al. Profiling and identification of pregnancy-associated circulating microRNAs in dairy cattle. **Genes & Genomics**, v. 40, n. 10, p. 1111-1117, 2018/10/01 2018.

MATHIVANAN, S.; JI, H.; SIMPSON, R. J. Exosomes: Extracellular organelles important in intercellular communication. **Journal of Proteomics**, v. 73, n. 10, p. 1907-1920, 9/10/ 2010.

MELLISHO, E. A. et al. Identification and characteristics of extracellular vesicles from bovine blastocysts produced in vitro. **PLOS ONE**, v. 12, n. 5, p. e0178306, 2017.

MEMILI, E.; DOMINKO, T.; FIRST, N. L. Onset of transcription in bovine oocytes and preimplantation embryos. **Molecular Reproduction and Development**, v. 51, n. 1, p. 36-41, 1998.

MEYERHOLZ, M. M. et al. Pregnancy-Induced ISG-15 and MX-1 Gene Expression is Detected in the Liver of Holstein–Friesian Heifers During Late Peri-Implantation Period. **Reproduction in Domestic Animals**, v. 51, n. 1, p. 175-177, 2016.

MORELLI, A. E. et al. Endocytosis, intracellular sorting, and processing of exosomes by dendritic cells. **Blood**, v. 104, n. 10, p. 3257-3266, 2004.

MORTIMER, D. et al. What should it take to describe a substance or product as ‘sperm-safe’. **Human Reproduction Update**, v. 19, n. suppl 1, p. i1-i45, 2013.

MURRAY, H. et al. Diagnosis and treatment of ectopic pregnancy. **CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne**, v. 173, n. 8, p. 905-912, 2005.

NAKAMURA, K. et al. Induction of IFNT-Stimulated Genes by Conceptus-Derived Exosomes during the Attachment Period. **PLoS ONE**, v. 11, n. 6, p. e0158278, 2016.

NG, Y. H. et al. Endometrial Exosomes/Microvesicles in the Uterine Microenvironment: A New Paradigm for Embryo-Endometrial Cross Talk at Implantation. **PLoS ONE**, v. 8, n. 3, p. 2013.

OLIVEIRA, J. F. et al. Expression of Interferon (IFN)-Stimulated Genes in Extrauterine Tissues during Early Pregnancy in Sheep Is the Consequence of Endocrine IFN- τ Release from the Uterine Vein. **Endocrinology**, v. 149, n. 3, p. 1252-1259, 2008.

OTTOBRE, J. S. et al. Aspects of regulation of uterine secretion of prostaglandins during the oestrous cycle and early pregnancy. **Animal Reproduction Science**, v. 7, n. 1, p. 75-100, 1984/02/01/ 1984.

PISARSKA, M. D.; CARSON, S. A.; BUSTER, J. E. Ectopic pregnancy. **The Lancet**, v. 351, n. 9109, p. 1115-1120, 1998.

POHLER, K. G. et al. Circulating microRNA as candidates for early embryonic viability in cattle. **Molecular Reproduction and Development**, v. 84, n. 8, p. 731-743, 2017.

POLLARD, J. W. et al. Fertilizing Capacity of Bovine Sperm may be Maintained by Binding to Oviductal Epithelial Cells1. **Biology of Reproduction**, v. 44, n. 1, p. 102-107, 1991.

PRUNOTTO, M. et al. Proteomic analysis of podocyte exosome-enriched fraction from normal human urine. **Journal of Proteomics**, v. 82, p. 193-229, 2013/04/26/ 2013.

QU, P. et al. Effects of embryo-derived exosomes on the development of bovine cloned embryos. **PLOS ONE**, v. 12, n. 3, p. e0174535, 2017.

QU, P. et al. Extracellular vesicles derived from donor oviduct fluid improved birth rates after embryo transfer in mice. **Reproduction, Fertility and Development**, v. 31, n. 2, p. 324-332, 2019.

ROBERTS, R. M. et al. Interferons and the maternal–conceptus dialog in mammals. **Seminars in Cell & Developmental Biology**, v. 19, n. 2, p. 170-177, 4// 2008.

ROBERTS, R. M. et al. Trophoblast Interferons. **Placenta**, v. 20, n. 4, p. 259-264, 1999.

RUIZ-GONZÁLEZ, I. et al. Exosomes, endogenous retroviruses and toll-like receptors: pregnancy recognition in ewes. **Reproduction**, v. 149, n. 3, p. 281-291, March 1, 2015 2015.

SAAEELDIN, I. M. et al. Improvement of Cloned Embryos Development by Co-Culturing with Parthenotes: A Possible Role of Exosomes/Microvesicles for Embryos Paracrine Communication. **Cellular Reprogramming**, v. 16, n. 3, p. 223-234, 2014.

SAAEELDIN, I. M.; OH, H. J.; LEE, B. C. Embryonic–maternal cross-talk via exosomes: potential implications. **Stem Cells and Cloning : Advances and Applications**, v. 8, p. 103-107, 2015.

SEGURA, E. et al. CD8+ Dendritic Cells Use LFA-1 to Capture MHC-Peptide Complexes from Exosomes In Vivo. **The Journal of Immunology**, v. 179, n. 3, p. 1489-1496, August 1, 2007.

SPENCER, T. E. Early pregnancy: Concepts, challenges, and potential solutions. **Animal Frontiers**, v. 3, n. 4, p. 48-55, 2013.

SPENCER, T. E.; BAZER, F. W. Temporal and spatial alterations in uterine estrogen receptor and progesterone receptor gene expression during the estrous cycle and early pregnancy in the ewe. **Biology of Reproduction**, v. 53, n. 6, p. 1527-1543, December 1, 1995 1995.

SPENCER, T. E.; BAZER, F. W. Biology of progesterone action during pregnancy recognition and maintenance of pregnancy. **Front Biosci**, v. 7, n. 1-3, p. d1879-98, Sep 01 2002.

SPENCER, T. E. et al. Conceptus-derived prostaglandins regulate gene expression in the endometrium prior to pregnancy recognition in ruminants. **Reproduction**, v. 146, n. 4, p. 377-387, October 1, 2013 2013.

SPONCHIADO, M. et al. Pre-hatching embryo-dependent and -independent programming of endometrial function in cattle. **PLOS ONE**, v. 12, n. 4, p. e0175954, 2017.

SPONCHIADO, M. et al. The pre-hatching bovine embryo transforms the uterine luminal metabolite composition in vivo. **Scientific Reports**, v. 9, n. 1, p. 8354, 2019/06/07 2019.

STREET, J. M. et al. Identification and proteomic profiling of exosomes in human cerebrospinal fluid. **Journal of translational medicine**, v. 10, p. 5-5, 2012.

SUBRA, C. et al. Exosome lipidomics unravels lipid sorting at the level of multivesicular bodies. **Biochimie**, v. 89, p. 205-12, 2007.

TANNETTA, D. et al. Extracellular vesicles and reproduction-promotion of successful pregnancy. **Cell Mol Immunol**, v. 11, n. 6, p. 548-563, 11/print 2014.

THÉRY, C.; OSTROWSKI, M.; SEGURA, E. Membrane vesicles as conveyors of immune responses. **Nature Reviews Immunology**, v. 9, p. 581, 06/05/online 2009.

THÉRY, C.; ZITVOGEL, L.; AMIGORENA, S. Exosomes: composition, biogenesis and function. **Nature Reviews Immunology**, v. 2, p. 569, 08/01/online 2002.

TICCONI, C. et al. Pregnancy-Promoting Actions of HCG in Human Myometrium and Fetal Membranes. **Placenta**, v. 28, p. S137-S143, 2007/04/01/ 2007.

VALADI, H. et al. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. **Nature Cell Biology**, v. 9, p. 654–659, 2007.

WEBER, J. A. et al. Prostaglandin E2 Hastens Oviductal Transport of Equine Embryos1. **Biology of Reproduction**, v. 45, n. 4, p. 544-546, 1991a.

. Prostaglandin E2 Secretion by Oviductal Transport-Stage Equine Embryos1. **Biology of Reproduction**, v. 45, n. 4, p. 540-543, 1991b.

WITWER, K. W. et al. Standardization of sample collection, isolation and analysis methods in extracellular vesicle research. **Journal of extracellular vesicles**, v. 2, p. 10.3402/jev.v2i0.20360, 2013.

WOCLAWEK-POTOCKA, I.; BRZEZICKA, E.; SKARZYNSKI, D. J. Lysophosphatic Acid Modulates Prostaglandin Secretion in the Bovine Endometrial Cells Differently on Days 8-10 of the Estrous Cycle and Early Pregnancy. **Journal of Reproduction and Development**, v. 55, n. 4, p. 393-399, 2009.

WYDOOGHE, E. et al. Autocrine embryotropins revisited: how do embryos communicate with each other in vitro when cultured in groups? **Biological Reviews**, v. 92, n. 1, p. 505-520, 2017.

ZHANG, Y. et al. Uterine Fluid in Pregnancy: A Biological and Clinical Outlook. **Trends in Molecular Medicine**, v. 23, n. 7, p. 604-614, 2017.

ZHAO, Z. et al. Circulating MicroRNA miR-323-3p as a Biomarker of Ectopic Pregnancy. **Clinical Chemistry**, v. 58, n. 5, p. 896-905, 2012.

3. Chapter 2: Small extracellular vesicles derived from *in vivo*- or *in vitro*-produced bovine blastocysts have different miRNAs profile – implications for embryo-maternal recognition

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Abstract: *In vivo* and *in vitro*-produced bovine embryos have different metabolic profiles and differences in gene transcription patterns. These embryos also have a distinct ability to establish and sustain early pregnancies. Small extracellular vesicles (sEVs) are secreted by embryos and carry bioactive molecules, such as miRNAs. We hypothesize that *in vivo* or *in vitro*-produced bovine hatched blastocysts on day 9 and the sEVs secreted by them have different miRNA profiles. To address this hypothesis, embryos of both groups were placed in *in vitro* culture on day 7. After 48 hours, hatched embryos and hatched embryo-conditioned media (eCM) of both groups were collected. A total of 210 miRNAs were detected in embryos of both groups, of these 6 miRNAs were downregulated, while 7 miRNAs were upregulated *in vitro* group when compared to *in vivo* group. sEVs were isolated of eCM to determine miRNA profile. A total of 106 miRNAs were detected in both groups, including 14 miRNAs upregulated in sEVs from *in vivo*-eCM, and 2 miRNAs upregulated in sEVs from *in vitro*-eCM. These miRNAs express in embryos and sEVs secreted by them regulate early embryonic developmental and endometrial pathways, which can modify embryo-maternal communication during early pregnancy and consequently affect pregnancy establishment.

Keywords: small extracellular vesicles, miRNAs, bovine embryos, embryo-maternal crosstalk.

3.1 Introduction

In vivo- or *in vitro*-produced (IVP) bovine embryos have different molecular and morphological characteristics (Holm & Callesen, 1998; Holm et al., 1998; Plante & King, 1994), and dissimilarities in embryonic development (Hiendleder et al., 2006; Young et al., 2001; Holm et al., 1998). Moreover, it has been shown that different embryonic culture protocols and components can alter IVP-embryos' transcriptome, as well as their ability to establish pregnancies (Pontes et al., 2009; Corcoran et al., 2006; Mohan, Hurst, & Malayer, 2004; Lonergan et al., 2003; Rizos et al., 2003). It is consensual that the *in vitro* culture condition imposed on IVP embryos alters the transcriptional patterns, the epigenetic mechanisms controlling transcription as well as embryo's metabolism. Thus, it's reasonable to speculate that the *in vitro* culture system induces changes in embryos which can impact their development and consequently in the communication with the maternal tissues after embryo transfer.

Extracellular vesicles (EVs) can mediate the communication between the embryo and the mother. The bovine embryo and the endometrial tissue secrete small EVs (sEVs) during the pre-implantation period (Burns, Brooks, & Spencer, 2016; Ruiz-González et al., 2015; Saadeldin, Oh, & Lee, 2015). Small EVs are formed by a phospholipid bilayer inside of the endosome (Théry, Zitvogel, & Amigorena, 2002), can range between 30-150 nm in diameter (Mathivanan, Ji, & Simpson, 2010), and can carry bioactive molecules such as mRNAs, miRNAs (Valadi et al., 2007), lipids (Subra, Laulagnier, Perret, & Record, 2007) and proteins (J Simpson, S Jensen, & W E Lim, 2008). Experimental evidences suggest that sEVs participate in intercellular communications during reproductive events linked to pregnancy establishments, such as cell proliferation, crosstalk between the embryo and the maternal organism, and embryonic implantation (Burns, Brooks, & Spencer, 2016; Ruiz-González et al., 2015; Saadeldin, Oh, & Lee, 2015). Moreover, sEVs are present in the follicular fluid (da Silveira, Veeramachaneni, Winger, Carnevale, & Bouma, 2012), blood (Cleys et al., 2014), oviductal (Lopera-Vásquez et al., 2016), and uterine fluids (Burns, Brooks, & Spencer, 2016; Ng et al., 2013) of different domestic species. These nanoparticles have also been identified in the culture medium conditioned by *in vitro* produced bovine embryos (Mellisho et al., 2017; Saadeldin, Kim, Choi, & Lee, 2014). Recent studies have demonstrated that sEVs may be used as non-invasive embryo quality markers, and their concentration in culture medium was associated with the viability of bovine blastocysts produced *in vitro* (Dissanayake et al., 2020; Mellisho et al., 2019). The sEVs present in the uterine fluid are uptaken by embryonic cells and play important roles during early embryonic development (Zhang, Wang, Wang, & Duan, 2017;

Burns, Brooks, & Spencer, 2016; Ruiz-González et al., 2015; Ng et al., 2013). For example, conceptus trophectoderm cells treated with EVs isolated from day 13 cyclic ewes uterine flushing (UFs) stimulated cell proliferation and IFNT secretion (Ruiz-González et al., 2015). In addition, the signaling pathways associated with uterine receptivity in endometrial cells are modulated by sEVs secreted by the bovine and sheep conceptus (Kusama et al., 2018; Nakamura et al., 2016). IFNT was identified in EVs secreted by day 14 ovine conceptuses (Burns, Brooks, & Spencer, 2016), and in EVs isolated of the UFs of cows and ewes on day 17 of pregnancy (P17) (Kusama et al., 2018; Nakamura et al., 2016). These sEVs present in UFs increase the expression of apoptosis-related genes and interferon-stimulated genes in primary uterine endometrial epithelial cells (EECs). On the other hand, bovine IFNT-independent P17 EVs altered in EECs the expression of transcripts related to the TNF signaling pathway and inflammatory response, suggesting that P17 EVs may cause a pro-inflammatory response in the endometrium to modulate uterine receptivity, contributing to conceptus implantation (Nakamura, Kusama, Ideta, Imakawa, & Hori, 2020). Therefore, the sEVs are part of the embryo-maternal crosstalk and possibly contribute to pregnancy success. Whether the sEVs' cargo and functions are different according to the embryo's *in vivo* or *in vitro* origin is still elusive.

Small EVs can modulate target cell via its bioactive molecules (Ng et al., 2013; Segura, Guérin, Hogg, Amigorena, & Théry, 2007; Morelli et al., 2004). The miRNAs are one of the most studied biomolecules by which sEVs are predicted to act on target cells. The miRNAs regulate various reproductive processes such as embryonic development, maternal-embryonic communication, and pregnancy establishment in mammals (Tesfaye et al., 2017). In the last decade, it was shown that miRNAs, besides acting inside of cells, could also act via miRNAs secretion mechanism mediated by sEVs. The presence of miRNAs in the culture media from bovine and human embryos has already been described (Kropp & Khatib, 2015; Kropp, Salih, & Khatib, 2014). These studies demonstrate that miRNAs are expressed and secreted by *in vitro*-produced embryos, and indicate their role as potential regulators of early embryonic development. Most of the studies about miRNAs present in embryo-conditioned culture medium focused on their use as biological markers for the selection of embryos more competent to establish pregnancy (Melo-Baez et al., 2020; Mellisho et al., 2019). However, there is a lack of studies comparing the content and functions of miRNAs enclosed in sEVs secreted by bovine embryos produced *in vivo* or *in vitro*, which may contribute to progress in the understanding of embryonic-maternal communication.

Previous studies done by our group demonstrated that *in vitro*-produced bovine embryos cultured under different oxygen tensions have distinct miRNA profile at the blastocyst stage (Bomfim et al., 2017) as well as in their secreted-sEVs (Andrade et al., 2019). However, how different are these results in comparison to *in vivo*-produced embryos is still unknown. *In vivo*- or *in vitro*-derived bovine embryos have a distinct ability to establish and maintain early pregnancies (Sartori et al., 2016; Pontes et al., 2009); and most of the embryonic losses in bovine pregnancies (up to 40%) occurs between days 8 to 17 of the estrous cycle (Thatcher et al., 2001; Dunne, Diskin, & Sreenan, 2000), overlapping with early communication between the embryo and maternal organism. These pregnancy losses may occur due to communication failures between the embryo and the maternal organism after the embryo transfer, which in bovine occur on day 7 of the estrous cycle. Furthermore, up to day 9 of the development, *in vivo* and *in vitro*-produced bovine blastocysts are expected to hatch. Thus, considering that sEVs are part of the embryo-maternal crosstalk and carry bioactive molecules, we determined the miRNAs profiles in *in vivo* or *in vitro*-produced bovine hatched blastocysts on day 9 and in the sEVs secreted by them between day 7 and day 9 of development. The results of this study demonstrate that hatched blastocysts of different origins and its corresponding sEVs from *in vivo*- or *in vitro*- embryo conditioned media (eCM) have different miRNAs profile. The differently expressed miRNAs are predicted to modulate important signaling pathways involved in early embryonic development, and embryo-maternal crosstalk possibly affecting pregnancy establishment.

3.2 Materials and methods

The experimental protocol was approved by University of São Paulo Research Ethics Committee (protocol number: 1392130318). Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich/Merck Chemical Company (St. Louis, MO).

3.2.1 Experimental design

For *in vivo* embryo production, Nelore cows were superovulated, inseminated and on day 7 (day 0 = estrus) the *in vivo*-produced embryos were collected by uterine flush. For *in vitro* embryo production, Nelore cows were superovulated and the cumulus-oocyte complexes (COCs) were obtained by ovum pick-up (OPU) of the pre-ovulatory follicles. Subsequently the COCs were *in vitro*-matured, *in vitro*-fertilized and *in vitro*-cultured until day 7 of development. Semen straws from the same bull and same batch were used for insemination or *in vitro* fertilization. *In vivo*- and *in vitro*-produced blastocysts were cultured individually in 30 μ L

droplets of culture medium for 48 hours from day 7 to day 9. After this period, the hatched blastocysts of both groups were collected and snap-frozen to determine the miRNAs profile. This analysis was performed in three biological replicates per group (each replication was composed of a pool of 5 hatched blastocysts on day 9). The eCM from both groups (*in vivo*-eCM or *in vitro*-eCM) was collected and centrifuged to remove cells, cell debris, and large extracellular vesicles. Subsequently, the eCMs were centrifuged to isolate sEVs (30-150 nm). The sEVs isolated from *in vivo*-eCM or *in vitro*-eCM were characterized in terms of: (i) size and concentration of sEVs by the Nanosight equipment; and (ii) miRNA profile using RT-qPCR. These analyses were carried out in four biological replicates per group (each replication consisting in sEVs retrieved from a pool of 8 blastocysts). An additional pool of sEVs isolated from *in vitro*-eCM was used (iii) to image the sEVs by transmission electron microscopy.

3.2.2 Animals

A total of 19 Nelore cows were used in this experiment. Three cows were used both as donors of cumulus-oocyte complexes retrieved by OPU and as donors of embryos retrieved by uterine flush. Additionally, eight cows were used only as COC donors and another eight only as embryo donors. Animals were fed with *Brachiaria brizantha* pastures, supplemented with corn silage and protein salt to fulfill their maintenance requirements, and *ad libitum* access to fresh water.

3.2.3 *In vivo* embryo production

The superovulation and FTAI protocols were performed according to Baruselli et al., (2006) with modifications. Briefly, on day 0 multiparous Nelore (*Bos taurus indicus*, n = 11) cows were synchronized by i.m. applications of estradiol benzoate (2 mg; Sincrodiol®, Ourofino Saúde Animal, Cravinhos, Sao Paulo, Brazil), PGF2 α analogue (0.5 mg of sodium cloprostenol; Sincrocio®, Ourofino Saúde Animal) and insertion of an intravaginal P4-releasing device of second use (1g; Sincrogest®, Ourofino Saúde Animal). Superstimulatory treatments started on day 4 with administrations of decreasing FSH doses (100 to 133 mg; Foltropin®, Vetoquinol Saúde Animal; Mairiporã, Sao Paulo, Brazil) given twice daily up to day 7. Cows received PGF2 α analogue (0.5 mg of sodium cloprostenol; Sincrocio®, Ourofino Saúde Animal) administration on afternoon day 6. On day 8, 12 hours after P4-releasing device withdrawal, donors received GnRH analogue (0.01 mg; Sincroforte®, Ourofino Saúde Animal) and were fixed-time inseminated without estrous detection at 12 and 24 h later. Cows were

inseminated twice with frozen-thawed semen (CRV Lagoa, Sertãozinho, SP, Brazil) from a single batch of a bull of proven fertility (the same semen was used for *in vitro* fertilization).

Uterine flush for embryo recovery was carried out on day 16 (7 days after fertilization). Immediately before the flushing transrectal ultrasonography scanner was used to determine the number of CLs, and the recovery rate were determined by the ratio between the number of structures (ova and embryo) recovered and the number of CLs.

3.2.4 *In vitro* embryo production

For embryo *in vitro* production Nelore cows (n=11) were synchronized and superovulated as described previously. On day 8 of the protocol, 12 hours after P4-releasing device withdrawal, the pre-ovulatory follicles were aspirated by OPU method to obtain the cumulus-oocyte complexes (COCs) used *in vitro* embryo production.

COCs recovered were matured *in vitro* in TCM199 culture medium supplemented with 22 µg/mL sodium pyruvate, 50 µg/mL gentamicin, 0.4% BSA (bovine serum albumin), 10 ng/mL IGF-1 (insulin-like growth factor), 100 ng/mL AREG, 10^{-2} IU/mL human recombinant FSH, 50 ng/mL 17β-estradiol, 150 ng/mL progesterone and 25 mM sodium bicarbonate (Soares et al., 2017). *In vitro* maturation (IVM) was performed in 96 well plates, with the maximum 10 oocytes/cow/well with 100 µL of medium (10 µL of medium IVM/oocyte) and incubated at 38.5°C and 5% CO₂ for 22-24 h.

In vitro fertilization used to produce the *in vitro*-derived embryos was performed with the same frozen-thawed semen from a single bull used for *in vivo* embryo production. Viable sperm cell pellet was separated by percoll gradient in 45% and 90% concentration. The sperm concentration used was approximately 25×10^6 viable sperm/mL. Sperm and matured COCs were incubated in droplets of 100 µL IVF-TALP (Tyrode-lactate stock) inside of mineral oil, at 38.5°C and 5% CO₂ for 21-22 h. The fertilization medium also was supplemented with 6 mg/mL BSA, 22 µg/mL sodium pyruvate, 5.5 UI/mL heparin, 40 µL/mL PHE (2mMD-penicillamine, 1mM hypotaurine, and 245 µM epinephrine) and 50 µg/mL gentamicin.

After fertilization, presumptive zygotes were denuded by successive pipetting in TCM 199 medium modified with Hepes to remove cumulus cells and placed in groups (maximum 15 embryos/cow/droplet) in droplets (100 µL) of SOFaaci cultured medium plus 8 mg/mL BSA, 22 µg/mL sodium pyruvate, 50 µg/mL gentamicin and incubated at 38.5°C in atmosphere controlled with 5% CO₂, 5% O₂ and 90% N₂ until day 7 of embryonic development. On day 4, the cleavage rate was evaluated, and feeding was performed (50 µL of old medium were replaced by 50µL of new culture medium). On day 7, the blastocyst rate was evaluated.

3.2.5 Generation of hatched blastocysts and embryo-conditioned medium (eCM)

On day 7 embryos from both groups were cultured, individually, for 48 hours in 30 μ L of modified SOFaaci (8 mg/mL BSA, 22 μ g/mL sodium pyruvate, 50 μ g/mL gentamicin) and incubated at 38.5°C in atmosphere controlled with 5% CO₂, 5% O₂ and 90% N₂. The individual culture was performed in 96 well plates. On day 9, the hatching rate was determined by the ratio between hatched blastocyst and total number of blastocysts individually placed in culture. The blastocysts and embryo-conditioned media were collected individually. The *in vivo*- and *in vitro*-eCM were centrifuged at 16,500 xg for 30 min at 4°C to remove cells, cell debris, and large microvesicles and stored in -80°C until further processing to separate sEVs. Only eCM from hatched-blastocysts were used for further analysis.

3.2.6 Separation of sEVs from embryo-conditioned medium

The sEVs were separated from the eCM derived from *in vivo*-produced hatched blastocysts (n=32) and *in vitro*-produced hatched blastocysts (n=32). To separate the sEVs, the eCM was pooled in four biological replicates per group (each replicate formed by the eCM pooled from 8 hatched blastocysts). The initial volume was approximately 240 μ L of eCM per replicate (8 embryos x 30 μ L). The sEVs were separated as previously described by (Andrade et al., 2019), using Exoquick-TC (1:1, #EXOQ20A1, System Biosciences, CA) on 1:1 ratio (240 μ L of eCM plus 240 μ L of Exoquick) and incubated overnight at 4°C. After the incubation, samples were centrifuged at 16,500 xg for 30 min at 4°C. After that, the sEVs pellets were diluted in 2,500 μ L of PBS each, to remove the remaining Exoquick, and centrifuged again at 120,000 xg for 70 min at 4°C. Finally, the sEVs pellets were diluted in 20 μ L of PBS (1X Ca²⁺/Mg²⁺ free PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 2 mM KH₂PO₄), of which 15 μ L was used for total RNA extraction and 5 μ L for nanoparticle tracking analysis.

3.2.7 Transmission electron microscopy

Additional samples of sEVs (obtained as previously described from *in vitro* eCM) were used to image the sEVs by transmission electron microscopy. For this analysis sEVs pellets obtained from 3,000 μ L of conditioned medium by 300 *in vitro* embryos, after ultracentrifugation, were diluted in 200 μ L of fixative solution (0.1M cacodylate; 2% glutaraldehyde and 2% paraformaldehyde at pH 7.2-7.4) for 2 hours at room temperature. In the solution were added 2 mL of PBS to remove fixative solution and centrifuged to obtain

pellets of sEVs (120,000xg, 70min, 4°C). Next, sEVs pellets were diluted in 15 µL of buffer solution (1% cacodylate) and placed in a pioloform-coated copper grid for approximately 60 min and let to air dry completely at room temperature. In the grid was added into 2% of uranyl acetate for 90 seconds, the excess was removed with filter paper and then analyzed by transmission electron microscope (FEI Tecnai 20; LAB6 emission; 200 kV).

3.2.8 Nanoparticle tracking analysis

The concentration and size of sEVs were determined using 5 µL of the sEVs resuspended pellet isolated from eCM of *in vivo* or *in vitro* embryos. The initial volume was diluted 1:100 in 1X Ca₂₊/Mg₂₊ free PBS totalizing 500 µL of final volume. The sEVs analysis – using the NanoSight NS300 (NanoSight, Salisbury, UK) – was performed by capturing a minimum of 5 videos of 60 seconds each, using camera level of 13, detection threshold of 5 and controlled temperature at 38.5°C. Analyses were conducted in four biological replicates per group.

3.2.9 Total RNA extraction

Total RNA of hatched blastocysts from both groups (three biological replicates per group; n=5 blastocysts per group per replicate), and of the sEVs secreted by them and present in eCM of *in vivo* or *in vitro* groups (four biological replicates per group; n=8 eCM per group per replicate) were extracted using TRIZOL® (Invitrogen, Brazil) following the manufacturer's instruction. The aqueous phase with RNA was recovered and 1.33 µL of GlycoBlue Coprecipitant® (15µg/mL; Thermo Fisher Scientific) were added to increase RNA precipitation. RNA quality and concentration were evaluated using a NanoDrop 2000 (Thermo Fisher Scientific) and total RNA was treated with DNase I (Invitrogen; Carlsbad, CA) to avoid DNA contamination.

3.2.10 Reverse transcription and quantitative RT-PCR analysis of the microRNAs

Relative levels of 382 bovine miRNAs were evaluated by reverse transcription polymerase chain reaction (RT-PCR) in hatched blastocysts and sEVs separated from eCM of *in vivo* or *in vitro* groups. Reverse transcription of miRNAs from 200 ng of total RNA was performed using miScript II RT Kit (QIAGEN) following the manufacturer's instruction. MiScript HiFlex Buffer was used to reverse transcription of precursor and mature miRNAs.

Quantitative RT-PCR was performed using Power SYBR Green PCR Master Mix (Applied Biosystems), miScript Universal Primer (QIAGEN), 200 ng of cDNA and specific

forward primer (Supplementary Table S1). QuantStudio 6 Flex (Applied Biosystems) was used to do RT-PCR and the cycle conditions were 95°C for 15 min, followed by 45 cycles for 15 s at 94°C, 30 s at 55°C and 30 s at 70°C. This was followed by melting curve analysis to confirm the amplification of specific cDNA products. Reactions with Ct values higher than 35 and three or more melting curves were excluded. In hatched blastocysts from both groups, Ct value was normalized using the geometric mean of miR-99b, RNU43 snoRNA, and Hm/Ms/Rt U1 snRNA; and in sEVs separated from eCM of *in vivo* or *in vitro* groups, using the geometric mean of miR-99b and Hm/Ms/Rt U1 snRNA. The ΔC_t method was used to calculate the relative expression of the miRNAs individually (Schmittgen & Livak, 2008). Next, data were transformed by $2^{-\Delta C_t}$ method and used in figures. Bioinformatic analyzes were performed using miRWalk software version 3.0 to identify the pathways modulated by miRNAs differently expressed in *in vivo* or *in vitro* bovine blastocysts and in the sEVs secreted by them.

3.2.11 Statistical analysis

Hatching rates were compared by chi-square. Size and concentration of sEVs and miRNA expression data were compared using Student's *t-test* with a significance level of 5%.

3.3 Results

3.3.1 Hatching rate was different comparing *in vivo* and *in vitro*-produced bovine blastocysts

To obtain *in vivo*-produced embryos a total of 16 uterine flushes were performed in 11 cows (five cows were flushed twice). We recovered 100 ovulated structures, resulting in a recovery rate of 76.34% (100 structures/131 CLs). The blastocyst recovery rate was 77% (77 blastocysts/100 structures), and the recovery ratio was 4.81 embryos on day 7 of development per cow (77 blastocysts/16 uterine flushes). On day 7, blastocysts were individually cultured for 48 hours to generate *in vivo*-eCM. The hatching rate on day 9 was 74.03% (57 hatched blastocysts/77 total blastocysts).

To obtain *in vitro*-produced embryos we retrieved COCs (n=324) by aspiration of preovulatory follicles (n=577) from Nelore cows (n=11) in 24 OPU sessions (one cow was aspirated twice and six cows were aspirated three times). The recovery ratio was 15.88 COCs per cow (368 retrieved COCs/24 number of OPU sessions). COCs were *in vitro* matured, fertilized, and cultured in groups up to day 7. The cleavage rate was 78.40% (254 cleaved embryo/324 fertilized oocytes), and blastocyst rate was 25% (81 blastocysts/324 fertilized

oocytes). On day 7, blastocysts were individually cultured for 48 hours to generate *in vitro*-eCM. The hatching rate on day 9 was 46.91% (38 hatched blastocysts/81 total blastocysts).

Hatching rate was higher ($p=0.0005$) in *in vivo*-produced embryos than in the *in vitro* counterparts (Figure 1). Detailed information on *in vivo*- and *in vitro*-produced embryos are shown in Supplementary Tables S2A and S2B.

Figure 1. Hatching rate on day 9 of *in vivo* and *in vitro* bovine embryos. Hatching rate was higher in *in vivo* produced bovine embryos (74.03%) on day 9 than in *in vitro*-produced embryos (46.91%) ($p=0.0005$).

3.3.2 Bovine hatched blastocysts produced *in vivo* or *in vitro* have different miRNA profiles

RT-PCR analysis was performed in order to investigate the profile of 382 miRNAs in *in vivo*- or *in vitro*-produced bovine embryos on day 9 of the development. A total of 210 miRNAs were detected in *in vivo* and *in vitro* embryos. MiR-let-7g was uniquely detected in *in vitro*-produced blastocysts, and 13 miRNAs out of the 210 were differently identified between the groups (Figure 2A). In *in vivo* embryos, 6 miRNAs (miR-129, miR-132, miR-155, miR-192, miR-215, and miR-377) were upregulated (Figure 2B), while 7 miRNAs (miR-23a, miR-338, miR-34a, miR-491, miR-92b, miR-940, and miR-1271) were upregulated in *in vitro* embryos (Figure 2C). Thus, these results demonstrate that even after 48 hours of *in vitro* culture, bovine embryos of different origins have different miRNA profiles. The full list of detected miRNAs and their relative expressions are shown in Supplementary Table S3.

Figure 2. MiRNAs express in *in vivo*- or *in vitro*-produced bovine embryos on day 9 of development. (A) Venn diagram demonstrating the distribution of the 210 miRNAs present in *in vivo*- or *in vitro*- produced hatched blastocysts on day 9 of development. (B) Of these, a total of 6 miRNAs were upregulated while (C) 7 were downregulated in *in vivo* embryos compared

to *in vitro* embryos. Different letters represent significant differences ($P \leq 0.05$). Solid bars represent the group mean of $2^{-\Delta Ct}$ and error bars represent standard error of the mean (S.E.M). MiRNA = microRNA.

3.3.3 Enrichment analysis of miRNAs differentially expressed in *in vivo*- or *in vitro*-produced bovine hatched blastocysts

Bioinformatics analyzes were done to define the predicted biological pathways regulated by miRNAs differentially expressed in bovine blastocysts produced by different biotechniques on day 9 of development. In *in vivo* embryos, the 6 miRNAs upregulated are predicted to regulate 265 signaling pathways (Supplementary Table S4). Furthermore, the 7 miRNAs increased in *in vitro* embryos are predicted to modulate 310 signaling pathways (Supplementary Table S5). The first 10 enriched pathways ranked by p-value ($P \leq 0.05$) were considered (Figure 3). We identified that the miRNAs increased in *in vivo* embryos are involved in the modulation of pathways associated with cancer, Ras signaling, and regulation pluripotency of stem cells (Figure 3A), while the miRNAs upregulated in *in vitro* embryos regulated pathways that include endocytosis, regulation of actin cytoskeleton, and hippo signaling (Figure 3B). In summary, these results suggest that blastocysts' miRNAs profile vary depending on the environment where the embryos are produced, and these miRNAs can modulate important pathways associated with early embryonic development.

Figure 3. Enrichment analysis of predicted pathways modulated by miRNAs express *in vivo*- or *in vitro*-produced bovine embryos on day 9 of development. (A) Predicted pathways regulated by 6 miRNAs upregulated in *in vivo* blastocysts. (B) Pathways predicted by being modulated by 7 miRNAs more expressed in *in vitro* embryos. The left Y-axis value shows the percent of genes (%), which was calculated by the number of genes predicted to be modulated by miRNAs upregulated in each group for the respective pathway divided by the total number of genes of each pathway. Whereas the right Y-axis represents the P-value ($P \leq 0.05$) for each pathway, which was extracted from the miRWalk 3.0 software. miRNA = micro RNA.

3.3.4 *In vivo* embryos secrete less sEVs than *in vitro* embryos

To image sEVs present in the media conditioned by *in vitro*-produced bovine embryos, we used transmission electronic microscopy (TEM). Additionally, sEVs secreted in the media conditioned by *in vivo*- or *in vitro*-produced bovine embryos were characterized by nanoparticle tracking analysis. Cup-shape morphology and the characteristic size of sEVs were observed by TEM (Figure 4A and B). Furthermore, the particles separated from conditioned media had their size and concentration analyzed by NTA. *In vivo*-derived embryos secreted less particles ($1.63 \times 10^9 \pm 2.44 \times 10^8$ particles/mL; $p=0.049$) than *in vitro*-derived embryos ($3.62 \times 10^9 \pm 7.73 \times 10^8$ particles/mL; Figure 4C). However, the average size of the sEVs secreted by *in vivo* embryos (130.90 ± 11.25 nm) was similar ($p=0.6025$) to the size of sEVs secreted by *in vitro* embryos (137.95 ± 6.17 nm; Figure 4D). The average size distributions of sEVs secreted in the conditioned media by *in vivo* or *in vitro* bovine embryos were illustrated in Figure 4E. Thus, *in vivo* and *in vitro* embryos produce sEVs that resemble extracellular vesicles described in the literature. Lower concentration of sEVs was found in eCM from *in vivo* embryos compared to *in vitro* embryos.

Figure 4. SEVs characterization. (A and B) Transmission electronic microscopy images of sEVs retrieved from eCM by *in vitro*-produced bovine embryos cultured individually from day 7 up to day 9 of development. The magnification of the both electron microscopy images were 200,000x. Small EVs concentration (C) and size (D) secreted by *in vivo*- or *in vitro*-produced bovine embryos. The particles size secreted by embryos produced from different biotechnology was similar. However, sEVs particles concentration was higher in eCM by *in vitro* produced bovine embryos. (E) Nanoparticle tracking analysis based characterization of average size profiles of sEVs isolated from eCM by *in vivo*- or *in vitro*-produced bovine embryos of the day 7 up to day 9 of development. Different lowercases letters represent significant differences ($P \leq 0.05$). Solid bars represent the group mean and error bars represent standard error of the mean (S.E.M). sEVs = small extracellular vesicles; eCM = embryo conditioned media.

3.3.5 MiRNA contents in sEVs separated from conditioned media from *in vivo* or *in vitro* bovine embryos

To identify sEVs miRNA contents, the conditioned media from *in vivo*- or *in vitro*-produced bovine embryos were used to separate sEVs, which were used to obtain total RNA to investigate the levels of 382 bovine miRNAs sequences through RT-qPCR. A total of 114 miRNAs were identified in sEVs separated from eCM from *in vivo* or *in vitro* groups (Figure 5A). A total of three (miR-139, miR-380-5p and miR-660) and five (miR-10b, miR-192, miR-194, miR-542-5p and miR-708) miRNAs were uniquely detected in sEVs present in eCM from *in vivo* or *in vitro* groups, respectively, while 106 miRNAs were expressed in both groups (Figure 5A). In the sEVs derived from eCM from *in vivo* embryos a total of 14 miRNAs (miR-92b, miR-296-5p, miR-323, miR-382, miR-421, miR-541, miR-669, miR-935, miR-940, miR-1225-3p, miR-1249, miR-1281, miR-1296 and miR-1343-3p) were upregulated (Figure 5B), while 2 miRNAs (miR-494 and miR-1246) were downregulated (Figure 5C) in comparison with sEVs separated from eCM from *in vitro* embryos. Therefore, these results demonstrate that sEVs present in eCM from embryos produced in different environments (*in vivo* vs. *in vitro*) have different miRNAs content. The full list of detected miRNAs and their relative expressions are shown in Supplementary Table S6.

Figure 5. MiRNA contents identified in sEVs present in eCM from *in vivo*- or *in vitro*-produced bovine embryos. (A) Venn diagram demonstrating the distribution of the 114 miRNAs present in sEVs from eCM of *in vivo*- or *in vitro*-produced embryos. (B) A total of 14 miRNAs were downregulated while (C) two were upregulated in sEVs present in eCM from *in vitro* embryos compared to sEVs from *in vivo* embryos. Different letters represent significant differences ($P \leq 0.05$). Solid bars represent the group mean of $2^{-\Delta C_t}$ and error bars represent standard error of the mean (S.E.M). sEVs = small extracellular vesicles; miRNA = microRNA; eCM = embryo conditioned media.

3.3.6 Enrichment analysis of miRNAs differentially expressed in sEVs secreted by *in vivo* or *in vitro* embryos

In order to determine the predicted biological pathways modulated by miRNAs differently detected in sEVs separated from eCM by *in vivo* or *in vitro*-produced embryos, bioinformatics analyzes were performed using mirWalk version 3.0 software. In sEVs present

in eCM from *in vivo* embryos, 14 miRNAs were increased and are predicted to modulate 318 signaling pathways (Supplementary Table S7). Moreover, the two miRNAs increased in sEVs present in eCM from *in vitro* embryos are predicted to be involved in the regulation of 84 signaling pathways (Supplementary Table S8). For this study, we considered the first 10 enrichment pathways ranked by p-value ($P \leq 0.05$) as mainly modulated by miRNAs increased in sEVs in eCM from *in vivo* embryo group (Figure 6A). Similarly, miRNAs increased in sEVs present in eCM from *in vitro* embryo were used for bioinformatics analyses and, we considered only the enrichment pathways with p-value less than 0.05 (Figure 6B). Our results demonstrate that miRNAs carried by sEVs present in eCM from *in vivo* embryo are predicted to regulate important pathways, as Ras, MAPK, oxytocin and glycerophospholipids metabolism while miRNAs present in *in vitro* embryo-derived sEVs can be involved in regulation of lysine degradation, HIF-1, and WNT. Therefore, these miRNAs present in sEVs from conditioned media are predicted to regulate pathways important for endometrial development, which could modify embryo-maternal crosstalk during early pregnancy and consequently affect pregnancy establishment.

Figure 6. Enrichment analysis of predicted pathways modulated by sEVs miRNAs isolated in eCM by *in vivo*- or *in vitro*-produced bovine embryos of the day 7 up to day 9 of development. (A) Predicted pathways regulated by 14 sEVs miRNAs upregulated in *in vivo* blastocyst culture media. (B) Pathways predicted by sEVs miR-494 and miR-1246 upregulated in *in vitro* embryo culture media. The left Y-axis value shows the percent of genes (%), which was calculated by the number of genes predicted to be modulated by miRNAs upregulated in each group for the respective pathway divided by the total number of genes of each pathway. Whereas the right Y-axis represents the P-value ($P \leq 0.05$) for each pathway, which was extracted from the miRWalk 3.0 software. sEVs = small extracellular vesicles; miRNA = micro RNA; eCM = embryo conditioned media.

3.4 Discussion

In the present study, we verified that *in vivo*-produced blastocysts have higher hatching rates when cultured up to day 9 than *in vitro*-produced counterparts. In addition, we proposed that *in vivo*- or *in vitro*-produced bovine hatched blastocysts and sEVs secreted by these embryos have distinct miRNA profiles. We identify that bovine blastocysts produced by different biotechniques have a distinct miRNAs profile, which may modulate important pathways associated with early embryonic development. Moreover, we demonstrate that sEVs secretion was lower in *in vivo*-produced embryos compared to *in vitro* counterparts. The miRNAs profiles of sEVs were different depending on the origin of the embryos (*in vivo* versus *in vitro*). The miRNA cargo of sEVs can regulate important pathways associated with the embryo-maternal crosstalk. Thus, our results demonstrate that bovine embryos produced in different environments have distinct miRNA profiles and secrete sEVs with different miRNAs content.

The hatching rate was higher in *in vivo* embryos than *in vitro* embryos on day 9 after 48 hours of individual culture. Another study evaluated the hatching rate after different cryopreservation methods and observed that *in vitro*-produced bovine embryos (39%) had a lower hatching rate compared to *in vivo*-produced embryos (72%), regardless of the cryopreservation methods (Villamil, Lozano, Oviedo, Ongaratto, & Bó, 2012). The blastocyst capacity to hatch is one of the ways to evaluate the quality and survival of the embryo used in several studies, mainly after researches with different cryopreservation methods (Marsico, Camargo, Valente, & Sudano, 2019; Rizos et al., 2003). However, just the embryo capacity to hatch is not enough to guarantee pregnancy establishment, suggesting that there are other factors, as the early communication between embryo and endometrium that are important for pregnancy success. Here, we investigated this early communication, based on the miRNA content of sEVs secreted by the hatched embryos, which are more prone to establish successful pregnancies.

Primarily, we investigated the miRNAs profile in *in vivo*- or *in vitro*-produced bovine hatched blastocysts on day 9 of the development. Six miRNAs (miR-129, miR-132, miR-155, miR-192, miR-215, and miR-377) were decreased, while 7 miRNAs (miR-23a, miR-338, miR-34a, miR-491, miR-92b, miR-940, and miR-1271) were increased in *in vitro* embryos compared to *in vivo* embryos. Moreover, we identified 16 miRNAs differently expressed in sEVs secreted by *in vivo* or *in vitro* produced bovine embryos between day 7 up to day 9 of development. Out of the 16 differently expressed miRNAs in sEVs, 11 miRNAs were expressed in all samples of the blastocysts bovine from both groups on day 9. Additionally, out of the 16 miRNAs differently expressed in sEVs secreted from embryos of both groups, 14 miRNAs were expressed in blastocysts produced *in vivo* or *in vitro* on day 7 (Del Collado, 2017). Together, these results demonstrate that: 1) the 16 sEVs miRNAs differently expressed were transcribed by *in vivo* and *in vitro* embryos during the day 7 to day 9 period and 2) sEVs miRNA content in eCM by embryos from both groups correspond to the blastocysts' miRNA secretion. On the other hand, in the *in vitro* group, we found that the miRNAs miR 92-b and miR-940 were increased in embryos, and they were decreased in sEVs secreted by these embryos compared with *in vivo* samples. MiR-192 was uniquely expressed in sEVs secreted by *in vitro* embryos, and it was decreased in *in vitro* blastocysts on day 9. These results showed that the miRNAs expressed in sEVs secreted by *in vivo* or *in vitro* embryos do not match their cell-of-origin content, as previously mentioned by other studies (for review Mateescu et al., 2017). The RNA content, as the miRNAs, present in sEVs is originated in the parent cell, and thus any sEVs miRNAs must be also transcript at least temporarily in the source cell. However, the relative concentration of these sEVs miRNAs can differ from the cellular miRNA content (Mateescu et al., 2017; O'Brien, Breyne, Ughetto, Laurent, & Breakefield, 2020). Therefore, it seems that there is a controlled mechanism that determines the miRNA content in embryo-derived sEVs (for review Mateescu et al., 2017; O'Brien, Breyne, Ughetto, Laurent, & Breakefield, 2020), suggesting that during early embryo–maternal interactions a communication-controlled mechanism can also happen.

Furthermore, in functional enrichment analysis of the miRNAs differentially expressed in *in vivo* or *in vitro*-produced bovine embryos, we founded pathways associated with metabolism, endocytosis, as well as cell proliferation, survival, growth, migration, differentiation (pluripotency), and cytoskeletal dynamism. It is known these biological processes are important during early embryonic development and they can contribute to normal or arrest embryo development that consequently influences pregnancy success.

After, it is important to highlight that bovine embryos secrete sEVs (Andrade et al., 2019; Mellisho et al., 2017). We identified that *in vitro* embryos secrete higher amount of sEVs in the conditioned media than *in vivo* embryos from day 7 up to day 9 of development. The culture media used during this study for the individual culture of the embryos was SOF supplemented with BSA, as already described. This culture media can contain nanoparticles (NPs) derived mainly from BSA, but these particles do not have sEVs characteristic proteins (Pavani et al., 2018; Dissanayake et al., 2020). However, it has already been shown that human serum albumin can contain miRNAs (Sánchez-Ribas et al., 2019), consequently, these bioactive molecules also may be present in bovine serum albumin. Therefore, in this study, embryos were cultured in the same conditions, demonstrating that the differences found in secretion pattern are inherent to embryo-origin effect. The reasons why an embryo secretes different numbers of sEVs are not fully known. Relatively well-established factors that affect the sEVs secretion are cellular stress conditions. There are indications that embryos produced *in vitro* have signs of cell stress, such as ER stress, oxidative stress, and metabolic stress. Cellular stress conditions have already been described as inducing higher sEVs secretion (Harmati et al., 2019; Oltra et al., 2019), and therefore, should contribute to the observed differences. In addition to concentration, the sEVs size was also evaluated in the conditioned medium from day 7 up to day 9 of *in vivo* or *in vitro* bovine embryo development. We demonstrated that sEVs size was similar between the groups. Similarly, the size of EVs secreted by bovine fertilized embryos or parthenotes from day 7 up to day 9 of development was not different (Mellisho et al., 2017). Moreover, different oxygen tensions also were not able to alter the EVs size derived from conditioned media from bovine embryos with 3 or 7 days of development (Andrade et al., 2019). Our and others' results indicate that bovine embryos respond to stimuli altering the amount, but not the size, of the secreted sEVs.

Not only the concentration of sEVs, but also its cargo, differ when comparing *in vivo*- and *in vitro*-produced hatched embryos. We identified a total of 16 miRNAs expressed differently when analyzing the miRNA sEVs content in both groups; 14 miRNAs were increased (miR-92b, miR-296-5p, miR-323, miR-382, miR-421, miR-541, miR-669, miR-935, miR-940, miR-1225-3p, miR-1249, miR-1281, miR-1296 and miR-1343-3p), and two miRNAs (miR-494 and miR-1246) decreased in *in vivo* compared to *in vitro* counterparts. Approximately 15% (16/106) of the miRNAs found in both groups are differentially expressed. The reasons for this may be due to different proportions between ICM and TE, stress conditions, and different communication strategies between the embryo and the mother. *In vivo*- and *in vitro*-produced bovine embryos have different proportions between ICM and TE cells (Iwasaki,

Yoshida, Ushijima, Watanabe, & Nakahara, 1990). The differences between secretion patterns and content of sEVs secreted by ICM and TE cells are yet unclear. However, it is plausible to suppose that different cell lineages have different secretion patterns, and there is strong evidence that they are different in some studies already performed (for review (Cruz, Romero, Iglesia, & Lopes, 2018)). Also, it has been demonstrated that bovine endometrium has a different miRNA profile to establish and maintain pregnancy when one *in vivo* or *in vitro* embryo is transferred (Ponsuksili et al., 2014). Following this, we suggest that different miRNA content present in sEVs secreted by *in vivo*- or *in vitro*- produced embryos may be strategies of the embryo to modulate the endometrium in its favor, improving the pregnancy establish. On the other hand, it is possible that *in vitro* embryos are not able to communicate adequately with the endometrium, which may prejudice its development during early pregnancy, and consequently leading to pregnancy loss.

Although the classic period of maternal recognition of the pregnancy in cattle is between days 12 to 26 of pregnancy (Farin et al., 1990), there is evidence that the embryo modulates endometrial transcripts and luminal content as early as day 7 (Sponchiado et al., 2019; Sponchiado et al., 2017). This modulation probably includes the sEVs participation. In this study, we demonstrate that miRNAs present in sEVs secreted by bovine embryos are predicted to modulate oxytocin, MAPK, Ras, glycerophospholipids metabolism, lysine degradation, HIF-1, and WNT pathway as early as day 7 up to day 9 of development. All these pathways participate in the establishment of successful pregnancies in cattle.

The oxytocin signaling pathway has an important role in early pregnancy, determining pregnancy success or loss. When the female is pregnant, bioactive molecules secreted by the embryo together with progesterone are responsible to maintain the corpus luteum viability. Interferon-tau (IFNT) is among these molecules, and many studies demonstrate its endometrial effects and luteal protection functions during early pregnancy (Mathew et al., 2019; Antoniazzi et al., 2013; Bridi et al., 2018; Forde & Lonergan, 2017; Sponchiado et al., 2017; Oliveira et al., 2008; Spencer, Burghardt, Johnson, & Bazer, 2004; Binelli et al., 2001; Chami, Megevan, Ott, Bazer, & O'Neill, 1999; Roberts et al., 1989; Godkin, Bazer, & Roberts, 1984). IFNT decreases the expression of estrogen (ESR) and oxytocin receptors (OXTR) in the uterine luminal epithelium and inhibits luteolytic pulses of prostaglandin F2 alpha (PGF) (Spencer & Bazer, 1995). Moreover, IFNT actions also were demonstrated as early as day 7 of the pregnancy, in *in vivo* model, through an increase in ISGs expression in the endometrium (Sponchiado et al., 2017). Based on that, we suggest that other IFNT independent factors, such

as sEVs, can contribute to corpus luteum maintenance and consequently pregnancy establishment.

MiR-1296 is predicted to regulate OXTR mRNA as well as miR-296-5p, miR-940, and miR-1343-3p are predicted to regulate the PTGS2 (prostaglandin-endoperoxide synthase 2) transcript (MirWalk 3.0 version). These genes have an important role in the oxytocin pathway during the estrus cycle or pregnancy in ruminants. Further experiments to demonstrate the functional differences on oxytocin pathway caused by sEVs in the endometrium are still needed.

Furthermore, through the JAK-SRC-EGFR-RAS-RAF-ERK1/2-EGR-1 pathways, IFNT modulates the PGF2 α release in luminal endometrial cells by inhibiting prostaglandin transporter (PTG) (Banu, Lee, Stephen, Nithy, & Arosh, 2010). These novel JAK-SRC-EGFR-RAS-RAF-ERK1/2-EGR-1 pathways involve the components modulation of the Ras and MAPK signaling pathways, which our study they are regulated by miRNAs present in sEVs secreted by bovine embryos. Also, we demonstrate modulation in glycerophospholipids metabolism pathway that is associated with the metabolism of prostaglandins, also identified as a pathway modulated by embryos in the endometrium around day 7 (Sponchiado et al., 2017).

In non-pregnant heifers, lysine concentration decreased in uterine luminal fluid of the day 7 up to day 16 of the estrous cycle, while in pregnant heifers this concentration was not altered (Forde et al., 2014). Thus, the lysine secretion regulation by endometrial cells seems to be important during the peri-implantation period, and the miRNAs carried by sEVs secreted by the embryo may be involved in this regulation. In addition, IFNT and P4 stimulated HIF pathway genes, demonstrating that this pathway stimulates embryo development, as well as modulate important endometrial cell functions and angiogenesis, contributing to uterine receptivity establishment (Song, Kim, Bazer, & Spencer, 2008). IFNT and P4 independent factors, as the sEVs content secreted by embryos, may modulate also HIF signaling pathway in the endometrium contributing to pregnancy success. Furthermore, WNT signaling pathway genes are involved in uterine receptivity modulation and embryo implantation. Some factors of this pathway regulate trophoblast differentiation, migration, and proliferation in different mammal species (Nayeem, Arfuso, Dharmarajan, & Keelan, 2016; Pollheimer et al., 2006; Mohamed et al., 2005; Dey et al., 2004). Canonical and non-canonical WNT signaling pathways modulate ovine conceptus development during the preimplantation period, demonstrating the importance of these pathways in embryo-maternal crosstalk (Hayashi, Burghardt, Bazer, & Spencer, 2007). In our study, sEVs miRNAs secreted by embryos are predicted to regulate HIF-1 and WNT pathways, suggesting that others bioactive molecules secreted by blastocyst, as the miRNAs into sEVs, contribute to uterine receptivity establishment and in embryo-endometrial

interactions during early pregnancy. *In vivo*- or *in vitro*-produced bovine embryos modulate in different ways the endometrium (Mansouri-Attia et al., 2009). Therefore, we can suggest that embryos produced under different conditions (*in vivo* or *in vitro*) have a distinct miRNA profile and secrete sEVs with different miRNAs content because they may need distinct endometrial environments to develop.

3.5 Conclusion

Here we demonstrate that *in vivo*- or *in vitro*-derived blastocysts have different abilities to hatch and have a distinct miRNAs profiles. Moreover, we demonstrated that the sEVs secreted by these embryos carry different miRNA contents. These differentially expressed miRNAs modulate pathways associated with early embryonic development, and signaling and metabolic pathways known to be involved in the earliest interactions between the embryo and the endometrium. Thus, we speculate that the embryo-derived sEVs participate in these very early interactions, and that the differences between *in vivo* and *in vitro* embryos found in this article are, at least in part, involved in the distinct ability of these embryos to establish a successful pregnancy by modifying the initial steps of embryo-maternal crosstalk.

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Conflict of interest

The authors declare to have no conflict of interest.

3.6 References

- Andrade, G. M., Bomfim, M. M., del Collado, M., Meirelles, F. V., Perecin, F., & da Silveira, J. C. (2019). Oxygen tension modulates extracellular vesicles and its miRNA contents in bovine embryo culture medium. *Molecular Reproduction and Development*, 86(8), 1067-1080. doi:10.1002/mrd.23223

- Antoniazzi, A. Q., Webb, B. T., Romero, J. J., Ashley, R. L., Smirnova, N. P., Henkes, L. E., . . . Hansen, T. R. (2013). Endocrine delivery of interferon tau protects the corpus luteum from prostaglandin F₂ alpha-induced luteolysis in ewes. *Biol Reprod*, 88(6), 144. doi:10.1095/biolreprod.112.105684
- Banu, S. K., Lee, J., Stephen, S. D., Nithy, T. K., & Arosh, J. A. (2010). Interferon tau regulates PGF₂alpha release from the ovine endometrial epithelial cells via activation of novel JAK/EGFR/ERK/EGR-1 pathways. *Mol Endocrinol*, 24(12), 2315-2330. doi:10.1210/me.2010-0205
- Baruselli, P. S., de Sá Filho, M. F., Martins, C. M., Nasser, L. F., Nogueira, M. F. G., Barros, C. M., & Bó, G. A. (2006). Superovulation and embryo transfer in *Bos indicus* cattle. *Theriogenology*, 65(1), 77-88. doi:https://doi.org/10.1016/j.theriogenology.2005.10.006
- Binelli, M., Subramaniam, P., Diaz, T., Johnson, G. A., Hansen, T. R., Badinga, L., & Thatcher, W. W. (2001). Bovine Interferon- τ Stimulates the Janus Kinase-Signal Transducer and Activator of Transcription Pathway in Bovine Endometrial Epithelial Cells. *Biology of Reproduction*, 64(2), 654-665. doi:10.1095/biolreprod64.2.654
- Bomfim, M. M., Andrade, G. M., del Collado, M., Sangalli, J. R., Fontes, P. K., Nogueira, M. F. G., . . . Perecin, F. (2017). Antioxidant responses and deregulation of epigenetic writers and erasers link oxidative stress and DNA methylation in bovine blastocysts. *Molecular Reproduction and Development*, 0(0), 1296-1305. doi:doi:10.1002/mrd.22929
- Bridi, A., Bertolin, K., Rissi, V. B., Mujica, L. K. S., Glanzner, W. G., Macedo, M. P. d., . . . Antoniazzi, A. Q. (2018). Parthenogenetic bovine embryos secrete type I interferon capable of stimulating ISG15 in luteal cell culture. *Animal Reproduction*, 15(4), 1268 - 1277.
- Burns, G. W., Brooks, K. E., & Spencer, T. E. (2016). Extracellular Vesicles Originate from the Conceptus and Uterus During Early Pregnancy in Sheep. *Biology of Reproduction*, 94(3), 56, 51-11. doi:10.1095/biolreprod.115.134973
- Chami, O., Megevand, A., Ott, T., Bazer, F., & O'Neill, C. (1999). Platelet-activating factor may act as an endogenous pulse generator for sheep of luteolytic PGF₂ release. *American Journal of Physiology - Endocrinology And Metabolism*, 276(4), E783-E792.
- Cleys, E. R., Halleran, J. L., McWhorter, E., Hergenreder, J., Enriquez, V. A., da Silveira, J. C., . . . Bouma, G. J. (2014). Identification of microRNAs in exosomes isolated from serum and umbilical cord blood, as well as placentomes of gestational day 90 pregnant sheep. *Molecular Reproduction and Development*, 81(11), 983-993. doi:10.1002/mrd.22420
- Corcoran, D., Fair, T., Park, S., Rizos, D., Patel, O. V., Smith, G. W., . . . Lonergan, P. (2006). Suppressed expression of genes involved in transcription and translation in in vitro compared with in vivo cultured bovine embryos. *Reproduction*, 131(4), 651-660. doi:10.1530/rep.1.01015
- Cruz, L., Romero, J. A. A., Iglesia, R. P., & Lopes, M. H. (2018). Extracellular Vesicles: Decoding a New Language for Cellular Communication in Early Embryonic Development. *Frontiers in cell and developmental biology*, 6, 94-94. doi:10.3389/fcell.2018.00094
- da Silveira, J. C., Veeramachaneni, D. N. R., Winger, Q. A., Carnevale, E. M., & Bouma, G. J. (2012). Cell-Secreted Vesicles in Equine Ovarian Follicular Fluid Contain miRNAs and Proteins: A Possible New Form of Cell Communication Within the Ovarian Follicle. *Biology of Reproduction*, 86(3), 71, 71-10. doi:10.1095/biolreprod.111.093252

- Del Collado, M. (2017). Lipid metabolism and cellular stress during in vivo and in vitro oocyte maturation and embryo development in bovine. *Tesis (Doutorado em ciências) - Universidade de São Paulo*, Pirassununga, 227f, 10.11606/T.74.2017.tde-02102017-104830
- Dey, S. K., Lim, H., Das, S. K., Reese, J., Paria, B. C., Daikoku, T., & Wang, H. (2004). Molecular cues to implantation. *Endocr Rev*, 25(3), 341-373. doi:10.1210/er.2003-0020
- Dissanayake, K., Nömm, M., Lättেকivi, F., Ressaissi, Y., Godakumara, K., Lavrits, A., . . . Fazeli, A. (2020). Individually cultured bovine embryos produce extracellular vesicles that have the potential to be used as non-invasive embryo quality markers. *Theriogenology*, 149, 104-116. doi:10.1016/j.theriogenology.2020.03.008
- Dunne, L. D., Diskin, M. G., & Sreenan, J. M. (2000). Embryo and foetal loss in beef heifers between day 14 of gestation and full term. *Animal Reproduction Science*, 58(1), 39-44. doi:http://dx.doi.org/10.1016/S0378-4320(99)00088-3
- Farin, C. E., Imakawa, K., Hansen, T. R., McDonnell, J. J., Murphy, C. N., Farin, P. W., & Roberts, R. M. (1990). Expression of trophoblastic interferon genes in sheep and cattle. *Biology of Reproduction*, 43(2), 210-218. doi:10.1095/biolreprod43.2.210
- Forde, N., & Lonergan, P. (2017). Interferon-tau and fertility in ruminants. *Reproduction*, 154(5), F33-f43. doi:10.1530/rep-17-0432
- Forde, N., Simintiras, C. A., Sturmey, R., Mamo, S., Kelly, A. K., Spencer, T. E., . . . Lonergan, P. (2014). Amino Acids in the Uterine Luminal Fluid Reflects the Temporal Changes in Transporter Expression in the Endometrium and Conceptus during Early Pregnancy in Cattle. *PLoS ONE*, 9(6), e100010. doi:10.1371/journal.pone.0100010
- Godkin, J. D., Bazer, F. W., & Roberts, R. M. (1984). Ovine Trophoblast Protein 1, an Early Secreted Blastocyst Protein, Binds Specifically to Uterine Endometrium and Affects Protein Synthesis. *Endocrinology*, 114(1), 120-130. doi:doi:10.1210/endo-114-1-120
- Harmati, M., Gyukity-Sebestyen, E., Dobra, G., Janovak, L., Dekany, I., Saydam, O., . . . Buzas, K. (2019). Small extracellular vesicles convey the stress-induced adaptive responses of melanoma cells. *Scientific Reports*, 9(1), 15329-15329. doi:10.1038/s41598-019-51778-6
- Hayashi, K., Burghardt, R. C., Bazer, F. W., & Spencer, T. E. (2007). WNTs in the ovine uterus: potential regulation of periimplantation ovine conceptus development. *Endocrinology*, 148(7), 3496-3506. doi:10.1210/en.2007-0283
- Hiendleder, S., Wirtz, M., Mund, C., Klempt, M., Reichenbach, H.-D., Stojkovic, M., . . . Wolf, E. (2006). Tissue-Specific Effects of In Vitro Fertilization Procedures on Genomic Cytosine Methylation Levels in Overgrown and Normal Sized Bovine Fetuses1. *Biology of Reproduction*, 75(1), 17-23. doi:10.1095/biolreprod.105.043919
- Holm, P., & Callesen, H. (1998). In vivo versus in vitro produced bovine ova: similarities and differences relevant for practical application. *Reproduction Nutrition Development*, 38(6), 579-594.
- Holm, P., Shukri, N. N., Vajta, G., Booth, P., Bendixen, C., & Callesen, H. (1998). Developmental kinetics of the first cell cycles of bovine in vitro produced embryos in relation to their in vitro viability and sex. *Theriogenology*, 50(8), 1285-1299. doi:http://dx.doi.org/10.1016/S0093-691X(98)00227-1
- Iwasaki, S., Yoshiba, N., Ushijima, H., Watanabe, S., & Nakahara, T. (1990). Morphology and proportion of inner cell mass of bovine blastocysts fertilized in vitro and in vivo. *Journal of Reproduction and Fertility*, 90(1), 279-284. doi:10.1530/jrf.0.0900279
- J Simpson, R., S Jensen, S., & W E Lim, J. (2008). *Proteomic profiling of exosomes: Current perspectives* (Vol. 8).

- Kropp, J., & Khatib, H. (2015). Characterization of microRNA in bovine in vitro culture media associated with embryo quality and development. *Journal of Dairy Science*, 98(9), 6552-6563. doi:10.3168/jds.2015-9510
- Kropp, J., Salih, S. M., & Khatib, H. (2014). Expression of microRNAs in bovine and human pre-implantation embryo culture media. *Frontiers in Genetics*, 5, 91. doi:10.3389/fgene.2014.00091
- Kusama, K., Nakamura, K., Bai, R., Nagaoka, K., Sakurai, T., & Imakawa, K. (2018). Intrauterine exosomes are required for bovine conceptus implantation. *Biochemical and Biophysical Research Communications*, 495(1), 1370-1375. doi:https://doi.org/10.1016/j.bbrc.2017.11.176
- Lonergan, P., Rizos, D., Gutiérrez-Adán, A., Moreira, P. M., Pintado, B., de la Fuente, J., & Boland, M. P. (2003). Temporal Divergence in the Pattern of Messenger RNA Expression in Bovine Embryos Cultured from the Zygote to Blastocyst Stage In Vitro or In Vivo. *Biology of Reproduction*, 69(4), 1424-1431. doi:10.1095/biolreprod.103.018168
- Lopera-Vásquez, R., Hamdi, M., Fernandez-Fuertes, B., Maillo, V., Beltrán-Breña, P., Calle, A., . . . Rizos, D. (2016). Extracellular Vesicles from BOEC in In Vitro Embryo Development and Quality. *PLoS ONE*, 11(2), e0148083. doi:10.1371/journal.pone.0148083
- Mansouri-Attia, N., Sandra, O., Aubert, J., Degrelle, S., Everts, R. E., Giraud-Delville, C., . . . Renard, J.-P. (2009). Endometrium as an early sensor of in vitro embryo manipulation technologies. *Proceedings of the National Academy of Sciences*, 106(14), 5687-5692. doi:10.1073/pnas.0812722106
- Marsico, T. V., Camargo, J. d., Valente, R. S., & Sudano, M. J. (2019). Embryo competence and cryosurvival: Molecular and cellular features. *Animal Reproduction*, 16, 423-439. Retrieved from http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1984-31432019000300423&nrm=iso
- Mateescu, B., Kowal, E. J. K., van Balkom, B. W. M., Bartel, S., Bhattacharyya, S. N., Buzás, E. I., . . . Nolte-‘t Hoen, E. N. M. (2017). Obstacles and opportunities in the functional analysis of extracellular vesicle RNA – an ISEV position paper. *Journal of extracellular vesicles*, 6(1), 1286095. doi:10.1080/20013078.2017.1286095
- Mathew, D. J., Sánchez, J. M., Passaro, C., Charpigny, G., Behura, S. K., Spencer, T. E., & Lonergan, P. (2019). Interferon tau-dependent and independent effects of the bovine conceptus on the endometrial transcriptome†. *Biol Reprod*, 100(2), 365-380. doi:10.1093/biolre/iyoy199
- Mathivanan, S., Ji, H., & Simpson, R. J. (2010). Exosomes: Extracellular organelles important in intercellular communication. *Journal of Proteomics*, 73(10), 1907-1920. doi:http://dx.doi.org/10.1016/j.jprot.2010.06.006
- Mellisho, E. A., Briones, M. A., Velásquez, A. E., Cabezas, J., Castro, F. O., & Rodríguez-Álvarez, L. (2019). Extracellular vesicles secreted during blastulation show viability of bovine embryos. *Reproduction*, 158(6), 477-492. doi:10.1530/rep-19-0233
- Mellisho, E. A., Velásquez, A. E., Nuñez, M. J., Cabezas, J. G., Cueto, J. A., Fader, C., . . . Rodríguez-Álvarez, L. (2017). Identification and characteristics of extracellular vesicles from bovine blastocysts produced in vitro. *PLoS ONE*, 12(5), e0178306. doi:10.1371/journal.pone.0178306
- Melo-Baez, B., Wong, Y. S., Aguilera, C. J., Cabezas, J., Mançanares, A. C. F., Riadi, G., . . . Rodríguez-Alvarez, L. (2020). MicroRNAs from Extracellular Vesicles Secreted by Bovine Embryos as Early Biomarkers of Developmental Competence. *Int J Mol Sci*, 21(23). doi:10.3390/ijms21238888

- Mohamed, O. A., Jonnaert, M., Labelle-Dumais, C., Kuroda, K., Clarke, H. J., & Dufort, D. (2005). Uterine Wnt/beta-catenin signaling is required for implantation. *Proc Natl Acad Sci U S A*, *102*(24), 8579-8584. doi:10.1073/pnas.0500612102
- Mohan, M., Hurst, A. G., & Malayer, J. R. (2004). Global gene expression analysis comparing bovine blastocysts flushed on day 7 or produced in vitro. *Molecular Reproduction and Development*, *68*(3), 288-298. doi:10.1002/mrd.20086
- Morelli, A. E., Larregina, A. T., Shufesky, W. J., Sullivan, M. L. G., Stolz, D. B., Papworth, G. D., . . . Thomson, A. W. (2004). Endocytosis, intracellular sorting, and processing of exosomes by dendritic cells. *Blood*, *104*(10), 3257-3266. doi:10.1182/blood-2004-03-0824
- Nakamura, K., Kusama, K., Bai, R., Sakurai, T., Isuzugawa, K., Godkin, J. D., . . . Imakawa, K. (2016). Induction of IFNT-Stimulated Genes by Conceptus-Derived Exosomes during the Attachment Period. *PLoS ONE*, *11*(6), e0158278. doi:10.1371/journal.pone.0158278
- Nakamura, K., Kusama, K., Ideta, A., Imakawa, K., & Hori, M. (2020). IFNT-independent effects of intrauterine extracellular vesicles (EVs) in cattle. *Reproduction*, *159*(5), 503-511. doi:10.1530/rep-19-0314
- Nayeem, S. B., Arfuso, F., Dharmarajan, A., & Keelan, J. A. (2016). Role of Wnt signalling in early pregnancy. *Reproduction, Fertility and Development*, *28*(5), 525-544. doi:https://doi.org/10.1071/RD14079
- Ng, Y. H., Rome, S., Jalabert, A., Forterre, A., Singh, H., Hincks, C. L., & Salamonsen, L. A. (2013). Endometrial Exosomes/Microvesicles in the Uterine Microenvironment: A New Paradigm for Embryo-Endometrial Cross Talk at Implantation. *PLoS ONE*, *8*(3), e58502. doi:10.1371/journal.pone.0058502
- O'Brien, K., Breyne, K., Ughetto, S., Laurent, L. C., & Breakefield, X. O. (2020). RNA delivery by extracellular vesicles in mammalian cells and its applications. *Nature Reviews Molecular Cell Biology*, *21*(10), 585-606. doi:10.1038/s41580-020-0251-y
- Oliveira, J. F., Henkes, L. E., Ashley, R. L., Purcell, S. H., Smirnova, N. P., Veeramachaneni, D. N. R., . . . Hansen, T. R. (2008). Expression of Interferon (IFN)-Stimulated Genes in Extrauterine Tissues during Early Pregnancy in Sheep Is the Consequence of Endocrine IFN- τ Release from the Uterine Vein. *Endocrinology*, *149*(3), 1252-1259. doi:doi:10.1210/en.2007-0863
- Oltra, M., Vidal-Gil, L., Maisto, R., Oltra, S. S., Romero, F. J., Sancho-Pelluz, J., & Barcia, J. M. (2019). miR302a and 122 are deregulated in small extracellular vesicles from ARPE-19 cells cultured with H(2)O(2). *Scientific Reports*, *9*(1), 17954-17954. doi:10.1038/s41598-019-54373-x
- Pavani, K. C., Hendrix, A., Van Den Broeck, W., Couck, L., Szymanska, K., Lin, X., . . . Leemans, B. (2018). Isolation and Characterization of Functionally Active Extracellular Vesicles from Culture Medium Conditioned by Bovine Embryos In Vitro. *Int J Mol Sci*, *20*(1). doi:10.3390/ijms20010038
- Plante, L., & King, W. A. (1994). Light and electron microscopic analysis of bovine embryos derived by in Vitro and in Vivo fertilization. *Journal of Assisted Reproduction and Genetics*, *11*(10), 515-529. doi:10.1007/bf02216032
- Pollheimer, J., Loregger, T., Sonderegger, S., Saleh, L., Bauer, S., Bilban, M., . . . Knöfler, M. (2006). Activation of the canonical wingless/T-cell factor signaling pathway promotes invasive differentiation of human trophoblast. *Am J Pathol*, *168*(4), 1134-1147. doi:10.2353/ajpath.2006.050686
- Ponsuksili, S., Tesfaye, D., Schellander, K., Hoelker, M., Hadlich, F., Schwerin, M., & Wimmers, K. (2014). Differential Expression of miRNAs and Their Target mRNAs in Endometria Prior to Maternal Recognition of Pregnancy Associates with Endometrial

- Receptivity for In Vivo- and In Vitro-Produced Bovine Embryos1. *Biology of Reproduction*, 91(6), 135, 131-112-135, 131-112. doi:10.1095/biolreprod.114.121392
- Pontes, J. H. F., Nonato-Junior, I., Sanches, B. V., Ereno-Junior, J. C., Uvo, S., Barreiros, T. R. R., . . . Seneda, M. M. (2009). Comparison of embryo yield and pregnancy rate between in vivo and in vitro methods in the same Nelore (*Bos indicus*) donor cows. *Theriogenology*, 71(4), 690-697. doi:https://doi.org/10.1016/j.theriogenology.2008.09.031
- Rizos, D., Gutiérrez-Adán, A., Pérez-Garnelo, S., de la Fuente, J., Boland, M. P., & Lonergan, P. (2003). Bovine Embryo Culture in the Presence or Absence of Serum: Implications for Blastocyst Development, Cryotolerance, and Messenger RNA Expression1. *Biology of Reproduction*, 68(1), 236-243. doi:10.1095/biolreprod.102.007799
- Roberts, R. M., Imakawa, K., Niwano, Y., Kazemi, M., Malathy, P. V., Hansen, T. R., . . . Kronenberg, L. H. (1989). Interferon production by the preimplantation sheep embryo. *J Interferon Res*, 9(2), 175-187. doi:10.1089/jir.1989.9.175
- Ruiz-González, I., Xu, J., Wang, X., Burghardt, R. C., Dunlap, K. A., & Bazer, F. W. (2015). Exosomes, endogenous retroviruses and toll-like receptors: pregnancy recognition in ewes. *Reproduction*, 149(3), 281-291. doi:10.1530/rep-14-0538
- Saadeldin, I. M., Kim, S. J., Choi, Y. B., & Lee, B. C. (2014). Improvement of Cloned Embryos Development by Co-Culturing with Parthenotes: A Possible Role of Exosomes/Microvesicles for Embryos Paracrine Communication. *Cellular Reprogramming*, 16(3), 223-234. doi:10.1089/cell.2014.0003
- Saadeldin, I. M., Oh, H. J., & Lee, B. C. (2015). Embryonic–maternal cross-talk via exosomes: potential implications. *Stem Cells and Cloning : Advances and Applications*, 8, 103-107. doi:10.2147/SCCAA.S84991
- Sánchez-Ribas, I., Diaz-Gimeno, P., Quiñonero, A., Ojeda, M., Larreategui, Z., Ballesteros, A., & Domínguez, F. (2019). NGS Analysis of Human Embryo Culture Media Reveals miRNAs of Extra Embryonic Origin. *Reproductive Sciences*, 26(2), 214-222. doi:10.1177/1933719118766252
- Sartori, R., Prata, A., Figueiredo, A. C. S., Sanches, B., Pontes, G. C. S., Viana, J., . . . Baruselli, P. (2016). Update and overview on assisted reproductive technologies (ARTs) in Brazil. *Animal Reproduction*, 13, 300-312. doi:10.21451/1984-3143-AR873
- Schmittgen, T. D., & Livak, K. J. (2008). Analyzing real-time PCR data by the comparative CT method. *Nature Protocols*, 3(6), 1101-1108. doi:10.1038/nprot.2008.73
- Segura, E., Guérin, C., Hogg, N., Amigorena, S., & Théry, C. (2007). CD8+ Dendritic Cells Use LFA-1 to Capture MHC-Peptide Complexes from Exosomes In Vivo. *The Journal of Immunology*, 179(3), 1489-1496. doi:10.4049/jimmunol.179.3.1489
- Soares, A. C. S., Lodde, V., Barros, R. G., Price, C. A., Luciano, A. M., & Buratini, J. (2017). Steroid hormones interact with natriuretic peptide C to delay nuclear maturation, to maintain oocyte–cumulus communication and to improve the quality of <i>in vitro</i>-produced embryos in cattle. *Reproduction, Fertility and Development*, 29(11), 2217-2224. doi:https://doi.org/10.1071/RD16320
- Song, G., Kim, J., Bazer, F. W., & Spencer, T. E. (2008). Progesterone and Interferon Tau Regulate Hypoxia-Inducible Factors in the Endometrium of the Ovine Uterus. *Endocrinology*, 149(4), 1926-1934. doi:10.1210/en.2007-1530
- Spencer, T. E., & Bazer, F. W. (1995). Temporal and spatial alterations in uterine estrogen receptor and progesterone receptor gene expression during the estrous cycle and early pregnancy in the ewe. *Biology of Reproduction*, 53(6), 1527-1543. doi:10.1095/biolreprod53.6.1527

- Spencer, T. E., Burghardt, R. C., Johnson, G. A., & Bazer, F. W. (2004). Conceptus signals for establishment and maintenance of pregnancy. *Anim Reprod Sci*, 82-83, 537-550. doi:10.1016/j.anireprosci.2004.04.014
- Sponchiado, M., Gomes, N. S., Fontes, P. K., Martins, T., del Collado, M., Pastore, A. d. A., . . . Binelli, M. (2017). Pre-hatching embryo-dependent and -independent programming of endometrial function in cattle. *PLoS ONE*, 12(4), e0175954. doi:10.1371/journal.pone.0175954
- Sponchiado, M., Gonella-Diaza, A. M., Rocha, C. C., Turco, E. G. L., Pugliesi, G., Leroy, J. L. M. R., & Binelli, M. (2019). The pre-hatching bovine embryo transforms the uterine luminal metabolite composition in vivo. *Scientific Reports*, 9(1), 8354. doi:10.1038/s41598-019-44590-9
- Subra, C., Laulagnier, K., Perret, B., & Record, M. (2007). Exosome lipidomics unravels lipid sorting at the level of multivesicular bodies. *Biochimie*, 89, 205-212. doi:10.1016/j.biochi.2006.10.014
- Tesfaye, D., Salilew-Wondim, D., Gebremedhn, S., Soheli, M. M. H., Pandey, H. O., Hoelker, M., & Schellander, K. (2017). Potential role of microRNAs in mammalian female fertility. *Reproduction, Fertility and Development*, 29(1), 8-23.
- Thatcher, W. W., Guzeloglu, A., Mattos, R., Binelli, M., Hansen, T. R., & Pru, J. K. (2001). Uterine-conceptus interactions and reproductive failure in cattle. *Theriogenology*, 56(9), 1435-1450. doi:10.1016/S0093-691X(01)00645-8
- Théry, C., Zitvogel, L., & Amigorena, S. (2002). Exosomes: composition, biogenesis and function. *Nature Reviews Immunology*, 2, 569. doi:10.1038/nri855
- Valadi, H., Ekström, K., Bossios, A., Sjöstrand, M., Lee, J., & Lotvall, J. (2007). Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nature Cell Biology*, 9, 654-659.
- Villamil, P. R., Lozano, D., Oviedo, J., Ongaratto, F., & Bó, G. (2012). Developmental rates of in vivo and in vitro produced bovine embryos cryopreserved in ethylene glycol based solutions by slow freezing or solid surface vitrification. *Animal Reproduction*, 9, 86-92.
- Young, L. E., Fernandes, K., McEvoy, T. G., Butterwith, S. C., Gutierrez, C. G., Carolan, C., . . . Sinclair, K. D. (2001). Epigenetic change in IGF2R is associated with fetal overgrowth after sheep embryo culture. *Nature Genetics*, 27, 153. doi:10.1038/84769
- Zhang, Y., Wang, Q., Wang, H., & Duan, E. (2017). Uterine Fluid in Pregnancy: A Biological and Clinical Outlook. *Trends in Molecular Medicine*, 23(7), 604-614. doi:10.1016/j.molmed.2017.05.002

4. Chapter 3: *In vivo*- or *in vitro*- produced bovine embryos induce molecular alterations in the endometrium, and sEVs secreted by them modify miRNA profile in the corpus luteum

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Abstract: The first interactions between embryo, endometrium, and corpus luteum are important for pregnancy establishment and maintenance. Small EVs are part of these interactions and carry miRNAs; however, the sEVs role in this communication still deserves investigation. We hypothesize that: 1) endometrial global transcriptomic is altered by blastocyst's presence and origin (*in vivo* or *in vitro*); 2) the media conditioned by endometrial explants cultured with *in vivo* or *in vitro* embryos contain sEVs with different miRNA contents, and; 3) luteal explants treated with these sEVs have distinct miRNA profiles. For this, endometrial explants (end) were cultured in the presence or absence of a single day 7 *in vivo* (end *in vivo*) or *in vitro* (end *in vitro*) embryo for 6 or 24 hours. Endometrial explants were collect for RNAseq analysis, and the conditioned media (C.M.) were collected for sEVs isolation. After 6 and 24 hours of culture, we identified 45 and 142 differentially expressed genes (DEGs) associated with embryo presence, respectively. Associated with embryo origin were identified 391 and 156 DEGs, after 6 and 24 hours of culture, respectively. These DEGs are involved in the immune response to viruses, metabolism, water and solute transport, and hippo signaling pathways. Interestingly, ESR1 and OXTR were lower expressed end *in vivo* compared to end *in vitro* and end groups, after 6 and 24 hours of culture, respectively. MiR-199b, miR-382, miR-631, and miR-760-3p were differentially expressed in sEVs secreted by endometrial explants plus *in vivo* or *in vitro* bovine embryos, after 6 hours of culture. These miRNAs are predicted to regulate oxytocin signaling pathways. MiR-149-3p was differently expressed in luteal explants treated with sEVs from C.M. by endometrial explants plus *in vivo* or *in vitro* bovine embryos after 6 hours of culture, while miR-17-3p, miR-335, miR-340, miR-371, and miR-677 were differently expressed in luteal explants treated with sEVs from C.M. by endometrial explants plus *in vivo* or *in vitro* bovine embryos after 24 hours of culture.

PTGFR, PTGS2, and Casp3 genes are involved in the luteolytic process and can be modulated by miR-371, miR-340, and miR-17-3p (MirWalk 3.0 version), respectively. Together, these results demonstrate embryo presence and origin (*in vivo* or *in vitro*) alter endometrial genes profile; sEVs secreted by endometrium and *in vivo* or *in vitro* embryos have distinct miRNAs content, and; luteal explants treated with these sEVs have different miRNA profiles. Thus, sEVs may mediate embryo-endometrium-corpus luteum interactions, carrying miRNAs that may regulate the luteolytic process maintaining CL viable to pregnancy success.

Keywords: blastocyst, endometrium, corpus luteum, extracellular vesicles, transcriptome, miRNAs

4.1 Introduction

Pregnancy success depends on perfect communication between the embryo and maternal organism since the first stages of embryonic development. Around day 5 of the embryonic development, in the morula stage, the bovine embryo arrives at the uterotubal junction (BETTERIDGE; FLÉCHON, 1988). The bovine embryos secrete different biochemical messengers like proteins, lipids, and nucleic acid molecules (mRNA, miRNA, and DNA) denominated embryotropins (WYDOOGHE et al., 2017). Some of these molecules have receptors in the uterine environment and can stimulate the cellular and tissue response as metabolites and protein secretions, modifying the composition of the uterine fluid (WYDOOGHE et al., 2017). Among these molecules the IFNT is of paramount importance. IFNT acts inhibiting luteolytic pulses of prostaglandin F2 alpha (PGF) in the endometrium and stimulates prostaglandin E2 synthesis to maintain the corpus luteum viably and secreting progesterone (ROBERTS et al., 1999; BANU et al., 2010; ANTONIAZZI et al., 2013; SPENCER et al., 2013). Furthermore, IFNT also acts in paracrine and endocrine manner, stimulating the expression of IFN-stimulated genes (ISGs) in the endometrium (AUSTIN et al., 2004; FORDE et al., 2012; SPONCHIADO et al., 2017), and corpus luteum (OLIVEIRA et al., 2008; ANTONIAZZI et al., 2013; BRIDI et al., 2018). On the other hand, proteomic analysis of conditioned media from day 16 bovine conceptus lead to the identification of other proteins in addition to IFNT that may be involved in embryo-maternal crosstalk (FORDE et al., 2015). Moreover, the expression of interferon-tau independent genes was altered in endometrium co-cultured with day 15 IVF and IA conceptuses, demonstrating that other conceptus-derived molecules are involved in modulation of important biological pathways during pregnancy establishment (MATHEW et al., 2019).

Another embryo-derived component consists in the small extracellular vesicles (sEVs) that are involved in embryo maternal interactions as demonstrated by many studies (RUIZ-GONZÁLEZ et al., 2015; SAADELDIN et al., 2015; BURNS et al., 2016; BRIDI et al., 2020). These nanoparticles can range between 30-150 nm in diameter (MATHIVANAN et al., 2010), are formed by a phospholipid bilayer inside of the endosome (THÉRY et al., 2002), and transport important molecules such as mRNAs, miRNAs (VALADI et al., 2007), lipids (SUBRA et al., 2007) and proteins (SIMPSON et al., 2008). Bioactive molecules carried by sEVs such as miRNAs are able to modulate target cells (MORELLI et al., 2004; SEGURA et al., 2007; NG et al., 2013). The miRNAs are small non-coding RNA sequences involved in post-transcriptional regulation (FIRE et al., 1998; HWANG; MENDELL, 2006) and modulate important biological process during embryonic development, embryo-maternal interactions, and pregnancy establishment in mammals (TESFAYE et al., 2017). Together, these embryonic and maternal biochemical messengers have an important role in early communication between the embryo, endometrium, and corpus luteum, involving a multitude of reciprocal interactions, and suggesting that this molecular dialogue can imply pregnancy success. However, an inability of the early embryo in communicating its presence for a maternal organism can be responsible for pregnancy failures. In bovine, the early communication among embryos of different origins (*in vivo* or *in vitro*), endometrium, and corpus luteum after embryo transfer on Day 7 is unclear.

The *in vitro* environment is responsible to cause different morphological characteristics, metabolism and gene transcription patterns, as well as diminished survival after cryotolerance in *in vitro*-produced bovine embryos (PLANTE; KING, 1994; HOLM; CALLESEN, 1998; HOLM et al., 1998; LONERGAN et al., 2003; RIZOS et al., 2003; MOHAN et al., 2004; CORCORAN et al., 2006; PONTES et al., 2009; VILLAMIL et al., 2012). Also, it is known that the pregnancy rates are lower when bovine *in vitro* embryos are transferred to recipients (PONTES et al., 2009; SARTORI et al., 2016). Thus, it is interesting to understand if these alterations found in *in vitro*-produced embryos compared with *in vivo*-produced embryos can modify endometrial transcriptome. In addition, it is unclear if these changes can cause molecular alterations in sEVs content secreted by embryonic and endometrial cells.

On day 20 of the pregnancy, the endometrium had its gene profile modified in the presence of conceptuses produced by different biotechnology (IA, IVF, or SCNT), demonstrating that the endometrial cells can be modulated differently depend on the embryo origin (MANSOURI-ATTIA et al., 2009). However, on day 7 of the estrous cycle, when the embryo transfer is ordinarily performed, it is unclear how the endometrium responds to embryos

of different origins. It's known that on day 7 of the pregnancy, the embryo can signal its presence to the uterus in a specific-space manner and modify the metabolite composition of the uterine luminal fluid (SPONCHIADO et al., 2017; SPONCHIADO et al., 2019). Therefore, considering that *in vivo*- or *in vitro*-produced bovine embryo secret sEVs with different miRNA contents that can influence the pregnancy establishment, it is interesting to investigate how the endometrium respond to them, especially in the first hours after embryo-endometrial interaction initiation.

The pregnancy success is also dependent upon a viable and progesterone-producing corpus luteum. It has been shown that miRNAs regulate luteal cells proliferation, survival, and progesterone secretion (MAALOUF et al., 2016; MOHAMMED et al., 2017; DONADEU et al., 2020). Moreover, the miRNA profile in CL is different during formation, functionalization, and luteal regression phases over the estrous cycle (GECAL, R. M. et al., 2017). The miRNAs are involved in the regulation of CL function and survival during maternal recognition of pregnancy (MAALOUF et al., 2014). However, there is a lack of information demonstrating the sEVs roles during the early communication between the embryo, the endometrium, and the corpus luteum. Thus, it is exciting to comprehend if embryo and endometrial-derived sEVs may cause molecular alterations in the corpus luteum, contributing to maintaining its viability.

The endometrium is composed of different cell types like epithelial, stromal, endothelial and immune cells, which are modulated by steroidal hormones and embryotropins during the pregnancy in ruminants (COOKE et al., 1997; WYDOOGHE et al., 2017). Recently, endometrial explants have been used as an *ex vivo* model to study innate immune responses during the estrous cycle as well as embryo/conceptus maternal interactions (BORGES et al., 2012; PASSARO et al., 2018; MATHEW et al., 2019). The corpus luteum is composed of large luteal cells, small luteal cells, endothelial cells, and fibroblasts (PATE, 1993). Also, it secretes important hormones (progesterone and oxytocin) that modulate the endometrium during the estrous cycle and pregnancy. In comparison with 2D endometrial or luteal cell culture, endometrial and luteal explants maintain physiological cellular and extracellular structure, suggesting that this *ex vivo* model is better for short studies periods. Day 8 *in vitro*-produced blastocysts (n=20) increase ISGs expression in endometrial explants (PASSARO et al., 2019). Moreover, on day 15, IVF- or AI-derived conceptuses altered the expression of interferon- τ dependent and independent DEGs in endometrial explants, demonstrating that the communication between the conceptus and uterine environment is modified by the embryo origin (MATHEW et al., 2019). However, endometrial molecular modifications caused by a single day 7 *in vitro* or *in vivo* embryo after the embryo transfer are still elusive. Small EVs

participate in the early communication between the embryo and endometrium, as demonstrated by many studies (RUIZ-GONZÁLEZ et al., 2015; BURNS et al., 2016; NAKAMURA et al., 2016; ZHANG et al., 2017; KUSAMA et al., 2018; NAKAMURA et al., 2020), but the sEVs role secreted by them in luteal tissues is unclear. Therefore, we hypothesized that: 1) a single day 7 *in vivo* or *in vitro* bovine embryo can secrete small molecules that modify endometrial global transcriptomic response when co-cultured with endometrial explants; 2) embryo and endometrium-derived sEVs present in conditioned media have different miRNA content depending on embryo origin (*in vivo* or *in vitro*), and; 3) sEVs secreted by endometrial explants and *in vivo* or *in vitro*-produced bovine embryo alter the miRNAs profile in luteal tissue. The results obtained in this study demonstrate that only one bovine embryo produced by different biotechniques (*in vivo* or *in vitro*) modified endometrial explants' RNA profiles. Besides, sEVs secreted by endometrial explants co-cultured with *in vivo*- or *in vitro*-produced bovine embryos have different miRNA contents. Finally, miRNA profiles were distinct in luteal explants treated with these sEVs.

4.2 Materials and methods

The experimental protocol was approved by the University of São Paulo Research Ethics Committee (protocol number: 4166120117). All chemicals were purchased from Sigma-Aldrich/Merck Chemical Company (St. Louis, MO) unless otherwise stated.

4.2.1 Experimental design

This study was composed of three experiments. In *experiment 1*, in order to investigate the transcriptomic response of the endometrium to the presence of bovine embryos produced by different biotechnologies, Day 7 embryos were produced *in vivo* or *in vitro*. The endometrial explants were collected from the uterus of cows previously synchronized to be on day 7 of the estrous cycle. Endometrial explants from the same uterus (three biological replicates; n=3) were cultured with i) medium (without embryo; end); ii) or a single day 7 *in vivo*-produced bovine embryo (end *in vivo*); iii) or a single day 7 *in vitro*-produced bovine embryo (end *in vitro*). The co-cultured was performed in a 24-well culture plate with 1 mL of RPMI medium using inserts with pores of 0.4 µm size to retain the embryo directly above the endometrial surface for 6 and 24 hours. After incubation, endometrial samples were washed in PBS (1X Ca²⁺/Mg²⁺ free PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 2 mM KH₂PO₄), and snap frozen for further RNA-Seq analysis.

In *experiment 2*, to investigate the sEVs miRNAs content during early embryo-endometrium interactions, the conditioned media (C.M.) by end (n=3), end *in vivo* (n=3), and end *in vitro* (n=3) were collected for sEVs isolation. The C.M. were centrifuged to remove cells, cell debris, and large extracellular vesicles. After, the sEVs were isolated of C.M. and used: 1) to determine size and concentration by the Nanosight equipment; 2) to determine the miRNAs content using RT-PCR, and; 3) to treat luteal explants (*experiment 3*).

In *experiment 3*, to better understand the sEVs participation during early embryo-maternal communication we treated for 6 hours luteal explants with sEVs from C.M. by endometrial explants co-cultured or not with *in vivo* or *in vitro*-produced bovine embryos for 6 or 24 hours. The luteal explants (LE) were performed using ovaries from abattoir with corpus luteum in stage 2 (apex of corpus luteum red or brown) in accordance with the criteria described by IRELAND et al., 1980. The luteal explants from the same corpus luteum (three biological replicates; n=3) were treated with: 1) sEVs separated from end C.M. for 6 hours (LE end sEVs 6h); 2) sEVs separated from end *in vivo* C.M. for 6 hours (LE end *in vivo* sEVs 6h); 3) sEVs separated from end *in vitro* C.M. for 6 hours (LE end *in vitro* sEVs 6h); 4) sEVs separated from end C.M. for 24 hours (LE end sEVs 24h); 5) sEVs separated from end *in vivo* C.M. for 24 hours (LE end *in vivo* sEVs 24h), and; 6) sEVs separated from end *in vitro* C.M. for 24 hours (LE end *in vitro* sEVs 24h). After 6 hours of the treatment, luteal explants were collected, washed in PBS (1X Ca²⁺/Mg²⁺ free PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 2 mM KH₂PO₄), and used to investigate the miRNAs profile.

4.2.2 Animals

Six Nelore cows were used to *experiment 1*, three as donors of embryos, and three as uterine donors. Uterine donors were slaughtered and their uterus were used to collect the endometrial explants. Animals were fed with *Brachiaria brizantha* pastures, supplemented with protein salt, and *ad libitum* access to water.

4.2.3 In vivo and in vitro embryo production

For the *in vivo* embryo production the protocol was performed according to Baruselli, P. et. al. (2006) (BARUSELLI et al., 2006) with modifications described in Chapter 2. The intravaginal P4-releasing devices used in this synchronization protocol were Progestar® Boehringer Ingelheim (0,96g).

For *in vitro* embryo production, ovaries were obtained from slaughterhouses near Pirassununga, SP. Ovarian follicles between 3-6mm in diameter were aspirated to obtain the

cumulus-oocyte complexes (COCs) using 18-gauge needles and a 10-mL syringe. COCs presenting compact cumulus and homogenous cytoplasm were selected and, placed in *in vitro* maturation medium composed by TCM199 culture medium supplemented with 22 µg/mL sodium pyruvate, 50 µg/mL gentamicin, 0.4% BSA (bovine serum albumine), 10 ng/mL IGF-1 (insulin-like growth factor), 100 ng/mL AREG, 10^{-2} IU/mL human recombinant FSH, 50 ng/mL 17β-estradiol, 150 ng/mL progesterone, and 25mM sodium bicarbonate (SOARES et al., 2017). The COCs were incubated at 38.5°C, and 5% CO₂ for 22 hr.

In vitro fertilization was performed conform described by Bridi, et. al., 2021 (Chapter 2). The semen (STRepro, Indaiatuba, SP, Brazil) used for IVF was from the same batch of a bull with proven fertility. After fertilization, presumptive zygotes were totally denuded by vortexing in 50 µL of TCM199 medium supplemented with 22 µg/mL sodium pyruvate, 50 µg/mL gentamicin, 0.1% BSA (bovine serum albumin), for 4 minutes. The presumptive zygotes (40-45 structure per well) were cultured in 4-well culture plate with 500 µL of SOFaaci cultured medium (supplemented with 8 mg/mL BSA, 22 µg/mL sodium pyruvate, 50 µg/mL gentamicin) covered with 100 µL mineral oil and, incubated at 38.5°C in atmosphere controlled with 6% CO₂, 5% O₂, 89% N₂ until Day 7 of development. On day 3, cleavage rate was evaluated, and on day 7 blastocyst rate was analyzed.

4.2.4 Endometrial explants

Three Nelore cows were submitted to hormonal synchronization protocol for the collection of the endometrial explants. On Day -10, cows received an intravaginal P4-releasing device (1g; Repro neo®, GlobalGen vet science), i.m. applications of estradiol benzoate (2 mg; Sincrodiol®, Ourofino Saúde Animal, Cravinhos, Sao Paulo, Brazil), and PGF2α analog (0.5 mg of sodium cloprostenol; Sincrocio®; Ourofino Saúde Animal). Another PGF2α analog application (0.5 mg of sodium cloprostenol; Sincrocio®; Ourofino Saúde Animal) was performed on Day -4. P4-releasing device withdrawal and estradiol cypionate application (1 mg, E.C.P®, Zoetis – Pfizer) were made on Day -2. Seven days after the predicted day to ovulation (Day 0), one cow by biological replicate was slaughtered to collect the endometrial explants.

Endometrial explant protocol was performed according to Borges, et. al., 2012 and Passaro et al., 2018 (BORGES et al., 2012; PASSARO et al., 2018) with some modifications. Bovine uteri from previously synchronized cow have collected at university local abattoir and transported to the laboratory within 30 min to the uterus processing. The ipsilateral uterine horn to the CL was isolated, the external part was washed with 70% ethanol, and was measured as

described by Sponchiado et. al., 2017 (SPONCHIADO et al., 2017). Briefly, starting with utero-tubal junction (UTJ), the first 8cm were delimited as the anterior portion. The ipsilateral horn was opened longitudinally and the exposed endometrium washed with PBS supplemented with 100 IU/mL penicillin, 100 µg/mL streptomycin, and 0.25 µg/mL amphotericin B. The endometrial explants were collected of the cranial anterior portion denominate uterotubal junction up to the first visible caruncles using a biopsy *punch* with 4mm diameter. After, they were washed once a time in Hank's Balanced Salt Solution (HBSS) supplemented with 100 IU/mL penicillin, 100 µg/mL streptomycin, and 0.25 µg/mL amphotericin B, and twice only in HBSS. Explants were transferred to a 24-well plate so that each well contained a single explant in 1mL of Roswell Park Memorial Institute medium (RPMI 1640) supplemented with 100 IU/mL penicillin, 100 µg/mL streptomycin, and 0.25 µg/mL amphotericin B. The endometrial explants were orientated with the epithelial surface uppermost and cultured with 5% CO₂ at 38.5°C. The culture medium was changed twice time in breaks the 1h, and after the embryos were transferred to the inserts, according to the experimental design.

4.2.5 Total RNA extraction and RNA-Seq analysis

Total RNA of endometrial explants after 6 and 24 hours of co-culture with *in vivo* or *in vitro* produced embryos was extracted using miRNeasy Mini Kit (QIAGEN) following the manufacturer's instruction. The RNA total was treated with DNase I (Invitrogen; Carlsbad, CA) to avoid DNA contamination. The quantity and quality of RNA were determined using the NanoDrop 2000 (Thermo Fisher Scientific) and the Agilent Bioanalyzer. The mean RNA integrity number (RIN) $\pm$ the standard deviation was 7.82 ± 0.39 . The RNA library preparation of three replicate of endometrial explants co-cultured or not with *in vivo* or *in vitro* produced bovine embryos were performed using Illumina TruSeq Stranded mRNA Sample Prep kit. The libraries were quantified by qPCR using KAPA Library Quantification kit (Illumina; KAPA Biosystems). The sequencing was performed in 1 lane of Hiseq 2500 V4 (2x100pb).

Quality of the reads was evaluated using the FASTQC software (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc>). Samples that passed the quality filter (mean Phred score >24) and adapters removal using cutadapt within Trimgalore pipeline (MARTIN, 2011). RSUBREAD (LIAO et al., 2019) with default parameters, were used to perform reads alignment to the *Bos taurus* genome (Ensembl *Bos taurus* ARS-UCD1.2) and genes counts with feature counts (LIAO et al., 2014). Genes were considered expressed when they passed filter with more than three read counts in at least three samples. Analysis of differential gene expression was performed using DESEQ2 (LOVE et al., 2014) considering

Benjamini-Hochberg method ("BH") for adjusted p values <0.10 , and absolute log2 foldchange greater than 0.6. For the enrichment analysis, we used the R software package ClusterProfiler (YU et al., 2012) - Gene Ontology (GO) for biological process (bp), and the Kyoto Encyclopedia of Genes and Genomes (KEGG) considering BH (FDR <0.05).

4.2.6 Conditioned media from endometrial explants and *in vivo* or *in vitro*-produced bovine embryos

Endometrial explants were cultured alone (end) or co-cultured with a Day 7 *in vivo*- or *in vitro*-produced bovine embryos (end *in vivo* and end *in vitro*) for 6 or 24 hours, in RPMI supplemented with 100 IU/mL penicillin, 100 $\mu\text{g/mL}$ streptomycin, and 0.25 $\mu\text{g/mL}$ amphotericin B. The culture was performed in 24 well plates using inserts with pores of 0.4 μm size between the embryo and the endometrial explant. The C.M. was collected individually, centrifuged at 300 xg for 10 min at 4°C; 2,000 xg for 10 min at 4°C; and 16,500 xg for 30 min at 4°C to remove cells, cell debris, and large microvesicles, respectively. After, the C.M. were stored in -80°C until further processing to isolate sEVs.

4.2.7 Isolation of sEVs from conditioned media

The sEVs were separated from the C.M. derived from end, end *in vivo* and end *in vitro* cultured for 6 or 24 hours (n=3; three biological replicates in each group). To separate the sEVs, the initial volume was approximately 300 μL of C.M. to nanoparticle tracking analysis (NTA) and total RNA extraction, and 200 μL of C.M. to the treatment of luteal explants. The sEVs were separated as previously described by (ANDRADE et al., 2019) and Bridi, 2021; Chapter 2), using Exoquick-TC (1:5, #EXOQ20A1, System Biosciences, CA) on 1:5 ratio (300 μL of C.M. plus 75 μL of Exoquick to NTA and miRNA analysis; 200 μL of C.M. plus 50 μL of Exoquick to treatment of luteal explants), and incubated overnight at 4°C. After the incubation, samples were centrifuged at 16,500 xg for 30 min at 4°C. After that, the sEVs pellets were diluted in 30 μL of PBS (1X $\text{Ca}^{2+}/\text{Mg}^{2+}$ free PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 , 2 mM KH_2PO_4), of which 20 μL was used for total RNA extraction and 10 μL for NTA. To treatment of luteal explants, the sEVs pellets were diluted in 200 μL of DMEM supplemented with 0.1% BSA, 100 IU/mL penicillin, and 100 $\mu\text{g/mL}$ streptomycin.

4.2.8 Nanoparticle tracking analysis

The size and concentration of sEVs were determined using 10 μ L of the sEVs resuspended pellet isolated from C.M. by endometrial explants co-cultured or not with *in vivo* or *in vitro* embryos. The initial volume was diluted 1:50 in 1X $\text{Ca}_{2+}/\text{Mg}_{2+}$ free PBS totalizing 500 μ L of final volume. The sEVs analysis – using the NanoSight NS300 (NanoSight, Salisbury, UK) – was performed by capturing a minimum of 4 videos of 60 seconds each, using camera level of 14, detection threshold of 5 and controlled temperature at 38.5°C. Analyses were performed in three biological replicates per group.

4.2.9 Luteal explants

Ovaries with CL in stage 2 were collected in slaughterhouses located near to Pirassununga-SP region. The CL appearance, as the apex of the corpus luteum red or brown, was used to classify the CL stage, according to the criteria described by Ireland et al. 1980. The ovaries were transported in TCM 199 medium modified with Hepes supplemented with 100 IU/mL penicillin, 100 μ g/mL streptomycin, 0.25 μ g/mL amphotericin B, and maintained at a temperature of approximately 4°C. In the laboratory, the ovaries were washed with saline solution; the CL was removed from the ovary and washed with PBS supplemented with 100 IU/mL penicillin, 100 μ g/mL streptomycin. After the CL was cut in half, and the luteal explants (with approximately 10 mg each) were collected using a sterile biopsy *punch* with 4mm diameter. The luteal explants were washed three times with PBS supplemented with 100 IU/mL penicillin, 100 μ g/mL streptomycin, and placed in 96 well plates. We added the culture medium composed for DMEM (powder high glucose; Gibco) supplemented with 0.1% BSA, 100 IU/mL penicillin, 100 μ g/mL streptomycin, and incubated at 38.5°C, 5% CO₂ for one hour. After, the culture medium was removed, and sEVs diluted in DMEM (powder high glucose; Gibco) supplemented with 0.1% BSA, 100 IU/mL penicillin, 100 μ g/mL streptomycin, and used in the treatment of the luteal explants according to experimental design. The luteal explants treated with sEVs were maintained in the incubator at 38.5°C, in atmosphere controlled with 5% CO₂ for 6 hours.

4.2.10 Total RNA extraction of sEVs from conditioned media and luteal explants

Total RNA of sEVs from C.M. by endometrial explants co-cultured or not with *in vivo* or *in vitro* embryos (three biological replicates per group), and luteal explants treated with end, end *in vivo* or end *in vitro* sEVs (three biological replicates per group) were extracted using TRIZOL[®] (Invitrogen, Brazil) following the manufacturer's instruction. Only in sEVs RNA

extraction, the aqueous phase with RNA was recovered, and 1.33 μ L of GlycoBlue Coprecipitant® (15 μ g/mL; Thermo Fisher Scientific) was added to increase RNA precipitation. RNA quality and concentration were evaluated using a NanoDrop One (Thermo Fisher Scientific) and total RNA was treated with DNase I (Invitrogen; Carlsbad, CA) to avoid DNA contamination.

4.2.11 Reverse transcription and quantitative RT-PCR analysis of the microRNAs

Relative levels of 382 bovine miRNAs were evaluated by reverse transcription polymerase chain reaction (RT-PCR) in sEVs from C.M. by endometrial explants co-cultured or not with *in vivo* or *in vitro* embryos (three biological replicates per group) and luteal explants treated with end, end *in vivo* or end *in vitro* – derived sEVs. Reverse transcription of miRNAs from 100 ng of total RNA was performed using miScript II RT Kit (QIAGEN) following the manufacturer's instruction. MiScript HiFlex Buffer was used to reverse transcription of precursor and mature miRNAs.

Quantitative RT-PCR was performed using Power SYBR Green PCR Master Mix (Applied Biosystems), miScript Universal Primer (QIAGEN), 100 ng of cDNA and specific forward primer (**Supplementary Table S1**, APPENDIX A). QuantStudio 6 Flex (Applied Biosystems) was used to do RT-PCR and the cycle conditions were 95°C for 15 min, followed by 45 cycles for 15 s at 94°C, 30 s at 55°C and 30 s at 70°C. This was followed by melting curve analysis to confirm the amplification of specific cDNA products. Reactions with Ct values higher than 37 and three or more melting curves were excluded. In sEVs separated from C.M. by end, end *in vivo* and end *in vitro* groups cultured for 6 or 24 hours, Ct value was normalized using miR-99b; and in LE treated with end, end *in vivo* or end *in vitro*-derived sEVs, using the geometric mean of miR-99b, RNU43 snoRNA, and Hm/Ms/Rt U1 snRNA. The Δ Ct method was used to calculate the relative expression of the miRNAs individually (Schmittgen & Livak, 2008). Next, data were transformed by $2^{-\Delta Ct}$ method and used in figures. Bioinformatics analysis were performed using miRWalk software version 3.0 to identify the pathways modulated by miRNAs differently expressed in end, end *in vivo*, and end *in vitro*-derived sEVs 6h, and in LE treated with sEVs secreted by end, end *in vivo*, and end *in vitro* 24h.

4.2.12 Statistical analysis

Size and concentration of sEVs, and miRNA expression data were compared using ANOVA followed by Tukey with a significance level of 5%.

4.3 Results

4.3.1 *In vivo* and *in vitro* embryo production

To obtain the *in vivo*-produced embryos a total of 3 uterine flushes were performed in 3 cows (**Supplementary Table S1A**, APPENDIX B). Thirty-five ovulated structures were recovered, resulting in a recovery rate of 102.94% (35 structures/34 CLs). The blastocyst recovery rate was 97.14% (34 blastocysts/35 structures), and it was recovered 11.33 embryos on day 7 of development per cow (34 blastocysts/3 uterine flushes).

To obtain *in vitro*-produced embryos we retrieved COCs (n=451) by aspiration of 3-6 mm in diameter follicles from slaughterhouses ovaries (**Supplementary Table S1B**, APPENDIX B). The cleavage rate was 82.93% (374 cleaved embryo/451 fertilized oocytes), and the blastocyst rate was 24.83% (112 blastocysts/451 fertilized oocytes).

4.3.2 Differently expressed genes in endometrial explants exposed to only one *in vivo* or *in vitro*-produced bovine embryo

In order to identify endometrial global transcriptomic modifications caused by embryos of different origins few hours after the contact with the endometrium, *in vivo*- or *in vitro*-produced embryos were co-cultured with endometrial explants for 6 and 24 hours. The RNA profile demonstrated that endometrial explants responded differently when exposing to only one bovine embryo produced by different biotechnologies (*in vivo* or *in vitro*), as shown by the Principal Component Analysis (PCA; Figure 1).

Figure 1. Principal component analysis representing the RNA profile of endometrial explants co-cultured with only one *in vivo*- or *in vitro*-produced bovine embryos for 6 or 24 hours.

4.3.2.1 Differentially expressed genes in endometrial explants after 6 hours of the co-culture with *in vivo* or *in vitro*-produced bovine embryo

RNA-seq analyses identified 486 and 202 differentially expressed genes (DEGs) (up-or downregulated) in endometrial explants exposed for 6 hours to *in vivo* (end *in vivo*) or *in vitro* (end *in vitro*) bovine embryo, respectively, compared to control endometrium (end). ($P_{\text{adjust}} \leq 0.1$; Figure 2; **Supplementary Table S2 and S3**, APPENDIX B). The expression of 301 genes was altered in end *in vivo* group 6 hours after co-cultured, while 113 genes had their expression altered in endometrial explants co-cultured with a Day 7 *in vitro* embryo; both compared with end group (Figure 2).

Forty-five DEGs were modified in end *in vivo* and end *in vitro* groups when compared to end, suggesting that these genes are associated with blastocysts' presence independent of the embryo origin (Figure 2; **Supplementary Table S4**, APPENDIX B). The enriched KEGG pathways identified for these 45 DEGs were: 1) measles, 2) influenza A, and 3) herpes simplex infection ($P\text{-value} \leq 0.1$; **Supplementary Table S5**, APPENDIX B). Gene ontology analysis of the 45 DEGs identified 3 enriched biological processes including innate immune response, defense response to virus, and response to virus ($P\text{-value} \leq 0.1$; **Supplementary Table S5**, APPENDIX B).

Moreover, 391 genes were differentially expressed in end *in vitro* when compared to end *in vivo* group ($P_{\text{adjust}} \leq 0.1$; Figure 2; **Supplementary Table S6**, APPENDIX B). The enriched KEGG pathways analysis was executed and were identified the top 10 following pathways: 1) propanate metabolism, 2) glyoxylate and dicarboxylate metabolism, 3) tryptophan metabolism, 4) peroxisome, 5) insulin secretion, 6) complement and coagulation cascades, 7) fructose and mannose metabolism, 8) carbon metabolism, 9) hippo signaling pathway, and 10) thyroid hormone synthesis ($P_{\text{adjust}} \leq 0.05$; **Supplementary Table S7**, APPENDIX B). The top 10 enriched biological processes identified by gene ontology analysis were: 1) protein-DNA complex assembly, 2) protein-DNA complex subunit organization, 3) positive regulation of epidermis development, 4) mesenchyme morphogenesis, 5) dsRNA processing, 6) production of miRNAs involved in gene silencing by miRNA, 7) positive regulation of lipase activity, 8) production of small RNA involved in gene silencing by RNA, 9) protein hetero-oligomerization, and 10) respiratory gaseous exchange by respiratory system ($P_{\text{adjust}} \leq 0.05$; **Supplementary Table S8**, APPENDIX B).

Of these 391 genes (end *in vivo* x end *in vitro*), 209 DEGs were altered exclusively based on embryo origin (Figure 2; $P_{\text{adjust}} \leq 0.1$; **Supplementary Table S9**, APPENDIX B). Metabolic pathways, hippo signaling pathways, and peroxisome were the enriched KEGG

pathways identified ($P\text{-value} \leq 0.1$; **Supplementary Table S10**, APPENDIX B). The enriched biological process identified by gene ontology analysis were lipid homeostasis, fatty acid beta-oxidation using acyl-CoA dehydrogenase, tryptophan metabolic process, cellular response to gonadotropin stimulus, response to ATP, and seminiferous tubule development ($P\text{-value} \leq 0.1$; **Supplementary Table S10**, APPENDIX B).

Figure 2. Venn diagram (Venny 2.1 BioinfoGP) demonstrating differently expressed genes (DEGs) identified in endometrium co-cultured with a single *in vivo*- or *in vitro*-produced bovine embryo compared to control endometrium after 6 hours of culture (yellow and blue circles, respectively). The green circle illustrated the DEGs identified in end *in vivo* compared with end *in vitro* group, suggesting that 391 DEGs were altered in the endometrium by the presence of different origins embryos.

Associated with *in vivo* embryo presence, 18 IFNT-dependent genes previously identified in other studies (MATHEW et al., 2019; CHANEY et al., 2021)(IFIH1, MX2, OAS1Y, IFI44, OAS2, APOBEC3Z1, IFITM3, IFI16, IRF9, IRF8, IFIT3, IDO1, IFI44L, CCL8, BATF2, CXCL11, CXCL10, OAS1Z) were upregulated in end *in vivo* group when compared to end (Table 1). Of these, only six IFNT-dependent genes (IFIH1, MX2, OAS1Y, IFI44, OAS2, and APOBEC3Z1) were upregulated in end *in vitro* when compared to end group

(Table 1). Others important DEGs altered in endometrial explant co-cultured with *in vivo* or *in vitro* embryo, or without embryo were described in Table 1. They have a biological function known in the following pathways: sex steroid signaling, eicosanoid metabolic process, and solute and water transport.

Table 1. DEGs altered in endometrial explants co-cultured with *in vivo* or *in vitro*-produced bovine embryos associated with important biological pathways modulated during early pregnancy.

Pathways	DEGs					
	6 hours			24 hours		
	End x End <i>in vivo</i>	End x End <i>in vitro</i>	End <i>in vivo</i> x End <i>in vitro</i>	End x End <i>in vivo</i>	End x End <i>in vitro</i>	End <i>in vivo</i> x End <i>in vitro</i>
IFNT-dependent genes	<p>Up: IFITM3, IFI16, IRF9, IFIH1, IRF8, IFIT3, MX2, IDO1, OAS1Y, OAS2, IFI44L, CCL8, BATF2, CXCL11, IFI44, CXCL10, OAS1Z, APOBEC3Z1</p> <p>Down: SCRN1</p>	<p>Up: IFIH1, MX2, OAS1Y, IFI44, OAS2, APOBEC3Z1</p> <p>Down: AFAP1L1, LDB2, CD93</p>	<p>Up: CD93, IFI44L</p> <p>Down: SCRN1</p>	<p>Up: STAT1, IFI16, LGALS3BP, LGALS9, FOXS1, IRF7, TNFSF13B, IFITM3, IRF9, PLAC8B, TNFSF10, IFIH1, CCL8, IFIT2, IFITM1, SLFN11, IFIT5, IFI6, ZBP1, RSAD2, IFIT3, MX2, MX1, ISG15, IFI27, IFI44L, OAS1Y, OAS2, OAS1Z, IFI44, OAS1X</p> <p>Down: No</p>	<p>Up: STAT1, IFI16, LGALS3BP, LGALS9, FOXS1, IRF7, TNFSF13B, IFITM3, IRF9, PLAC8B, TNFSF10, IFIH1, CCL8, IFIT2, IFITM1, SLFN11, IFIT5, MAP6, IFI6, ZBP1, RSAD2, IFIT3, MX2, MX1, ISG15, IFI27, IFI44L, OAS1Y, OAS2, OAS1Z, IFI44, OAS1X</p> <p>Down: No</p>	<p>Up: No</p> <p>Down: No</p>
Sex Steroid signaling	<p>Up: No</p> <p>Down: ESR1</p>	<p>Up: No</p> <p>Down: No</p>	<p>Up: No</p> <p>Down: ESR1</p>	<p>Up: No</p> <p>Down: No</p>	<p>Up: No</p> <p>Down: No</p>	<p>Up: No</p> <p>Down: OXTR</p>
Eicosanoid Metabolic process	<p>Up: SLCO2A1</p> <p>Down: HPGD</p>	<p>Up: No</p> <p>Down: No</p>	<p>Up: No</p> <p>Down: HPGD</p>	<p>Up: No</p> <p>Down: No</p>	<p>Up: PTGER2, AKR1B1</p> <p>Down: No</p>	<p>Up: No</p> <p>Down: No</p>
Solute and water transport	<p>Up: SLC16A9, SLC36A2, SLC11A1, AQP9, AQP6</p> <p>Down: SLC17A5, SLC48A1, SLC25A35, SLC22A18, SLC6A11,</p>	<p>Up: SLC16A9, AQP6, AQP9</p> <p>Down: SLC7A8, SLC35B4, SLC8A2</p>	<p>Up: SLC6A11, AQP9, SLC9A4</p> <p>Down: No</p>	<p>Up: AQP6, AQP9</p> <p>Down: No</p>	<p>Up: SLC11A2, SLC1A1, AQP6, SLC6A19, AQP9</p> <p>Down: SLC35G2, SLC25A42, SLC25A47</p>	<p>Up: SLC11A2</p> <p>Down: SLC2A6</p>

	SLC7A8, SLC25A53, SLC35B4, SLC9A4	
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4.3.2.2 Differentially expressed genes in endometrial explants after 24 hours of the co-culture with *in vivo* or *in vitro*-produced bovine embryo

Compared to control endometrium (end), 273 DEGs were identified in end *in vivo* group and 409 in end *in vitro* group ($P \leq 0.1$; Figure 3; **Supplementary Table S11 and S12, APPENDIX B**). Of these, 142 DEGs were expressed in end *in vivo* and end *in vitro* groups, demonstrating that these DEGs may be associated with the embryo's presence in the endometrium independent of the environment where the embryo was produced (Figure 3; **Supplementary Table S13, APPENDIX B**). The enriched KEGG pathways and gene ontology analysis identified 7 pathways and 18 biologic processes, respectively ($P\text{-adjust.} \leq 0.1$; **Supplementary Table S14, APPENDIX B**). The tops five pathways identified were 1) influenza A, 2) measles, 3) herpes simplex infection, 4) hepatitis C, and 5) cytosolic DNA-sensing pathway. The tops five biological processes include: 1) defense response to virus, 2) innate immune response, 3) negative regulation of viral genome replication, 4) ISG15-protein conjugation, and 5) response to virus. Moreover, of these 142 DEGs, 31 were classified in previous studies (MATHEW et al., 2019; CHANEY et al., 2021) as IFNT-dependent genes and were up-regulated in end *in vivo* and end *in vitro* groups when compared to end group (Table 1).

Between the end *in vivo* and end *in vitro* groups, 156 genes were differentially express ($P\text{-adjust.} \leq 0.1$; Figure 3; **Supplementary Table S15, APPENDIX B**). The top five enriched pathways identified were calcium signaling pathways, ABC transporters, renin secretion, focal adhesion, and complement and coagulation cascades ($P\text{-adjust.} \leq 0.1$; **Supplementary Table S16, APPENDIX B**). The top 10 biological processes found were cellular response to stress, positive regulation of cellular catabolic process, regulation of blood coagulation, immune response-activating cell surface receptor signaling pathway, immune response-activating signal transduction, regulation of hemostasis, positive regulation of catabolic process, immune response-regulating cell surface receptor signaling pathway, regulation of coagulation, and immune response-regulating signaling pathway ($P\text{-adjust.} \leq 0.1$; **Supplementary Table S17, APPENDIX B**).

Of these 156 DEGs, 87 were exclusively altered when compared end *in vivo* with the end *in vitro* group (Figure 3; **Supplementary Table S18, APPENDIX B**), demonstrating that

they are associated with the embryo origin. The GO analyses identified steroid hormone mediated signaling pathway, and positive regulation of JNK cascade as the biological processes associated with these 87 DEGs modified between end *in vivo* and end *in vitro* groups ($P\text{-value} \leq 0.1$; **Supplementary Table S19**, APPENDIX B). However, no KEGG pathways were associated with these DEGs.

Figure 3. Venn diagram (Venny 2.1 BioinfoGP) illustrating differentially expressed genes (DEGs) identified in endometrium co-cultured with a single *in vivo* or *in vitro*-produced bovine embryo compared to end group after 24 hours of culture (yellow and blue circles, respectively). The green circle illustrated the DEGs identified in end *in vivo* compared with end *in vitro* group, suggesting that 156 DEGs were altered in the endometrium by the presence of different origins embryos.

4.3.2.3 Differentially expressed genes in endometrial explants independently of the co-culture time

The DEGs that had their expressions maintained in endometrial explants independently of the co-culture time with *in vivo* or *in vitro* embryos are described in table 2. We observed that ENSBTAG00000053654, LAMP5, AQP6, AQP9, OAS2, IFI44, MX2, OAS1Y, and LOC100138271 were express in endometrial explants exposed to *in vivo* or *in vitro* embryos

when compared with control end independently of the co-cultured time. Interestingly, these endometrial DEGs can be associated with the embryo's presence on day 7 of the development independently of the embryo's origin.

On the other hand, GATA6, ENSBTAG00000053607, ENSBTAG00000052715, ENSBTAG00000054319, and ZNF581 were altered in end *in vitro* when compared to end *in vivo* group (Table 2), suggesting that these DEGs in endometrial explants are associated with the embryo origin (*in vivo* or *in vitro*).

Table 2. DEGs that were maintained independently of the co-culture time.

Groups	DEGs
End x end <i>in vivo</i>	ZNFX1, TRIM21, HERC6, ENSBTAG00000009664, LCA5, IFI16, IRF9, SAMD9, EPSTI1, IFIT3, MX2, LOC100139670, OAS1Y, RTP4, AQP9, AQP6, OAS2, IFI44L, CCL8, IFI44, LAMP5, OVGPI, OAS1Z, LOC100138271, ENSBTAG00000053654, SGIP1, LOC104975830, SCUBE1, MASTL
End x end <i>in vitro</i>	BMX, MX2, OAS1Y, CYP4B1, IFI44, OAS2, AQP6, AQP9, OVGPI, LOC100138271, ENSBTAG00000053654, LAMP5, THSD1, MKI67, TOP2A, CENPF
End in <i>vivo</i> x end <i>in vitro</i>	GATA6, ENSBTAG00000053607, ENSBTAG00000052715, ENSBTAG00000054319, ZNF581

4.3.3 Size, concentration, and MiRNA content of sEVs isolated from conditioned media by endometrial explants co-culture or not with *in vivo* or *in vitro*-produced bovine embryo

In order to understand better the early communication between the embryo and endometrium, the conditioned media by endometrial explants co-cultured or not with *in vivo*- or *in vitro*-produced bovine embryos for 6 and 24 hours were used to isolate sEVs. These nanoparticles were analyzed about their size, concentration, and miRNA content.

4.3.3.1 Size and concentration of sEVs from conditioned media

sEVs size and concentration were analyzed by NTA. The size ($p=0.7564$) and concentration ($p=0.7609$) averages of the sEVs secreted by end (134.20 ± 2.72 nm; $3.19 \times 10^9 \pm 1.40 \times 10^8$ particles/mL), end *in vivo* (125.90 ± 9.56 nm; $2.81 \times 10^9 \pm 1.05 \times 10^9$ particles/mL), and end *in vitro* (127.97 ± 9.63 nm; $2.50 \times 10^9 \pm 3.56 \times 10^8$ particles/mL) cultured for 6 hours, were similar among the groups (Figure 4A). Similarly, the size ($p=0.8818$) and concentration ($p=0.6987$) averages of the sEVs present in conditioned media from end (121.67 ± 5.05 nm; $4.08 \times 10^9 \pm 1.51 \times 10^9$ particles/mL), end *in vivo* (124.50 ± 11.03 nm; $3.14 \times 10^9 \pm 6.72 \times 10^8$ particles/mL), and end *in vitro* (119.30 ± 3.63 nm; $2.77 \times 10^9 \pm 9.03 \times 10^8$ particles/mL) cultured

for 24 hours had no difference (Figure 4B). These results demonstrate that sEVs present in C.M. by endometrial explants co-cultured or not with *in vivo* or *in vitro* embryos had a similar size and concentration after 6 and 24 hours of culture.

Figure 4. Small EVs size and concentration secreted by end, end *in vivo*, and end *in vitro* in culture medium for 6 (A e B) and 24 (C e D) hours. The particles size and concentration secreted by endometrial explants co-cultured or not with a Day 7 *in vivo*- or *in vitro*- produced embryo for 6 or 24 hours were similar. Solid bars represent the group mean and error bars represent standard error of the mean (S.E.M). sEVs = small extracellular vesicles; C.M. = conditioned media.

4.3.3.2 sEVs miRNAs content

The sEVs separated from conditioned media from end, end *in vivo*, and end *in vitro* for 6 and 24 hours were used to evaluate the levels of 382 bovine miRNAs sequences through RT-PCR. A total of 108 miRNAs were identified in sEVs isolated from C.M. by end, end *in vivo*, and end *in vitro* for 6 hours (Figure 5A; **Supplementary Table S20**, APPENDIX B). Of these, miR-452 was uniquely detected in sEVs secreted by end group, and let-7a-3p was uniquely detected in sEVs present in end *in vitro* group. A total of 106 miRNAs were expressed in all the groups (Figure 5A), of these 4 miRNAs (miR-199b, miR-382, miR-631, and miR-760-3p) were differently expressed among the groups (Figure 5B). MiR-199b was increased in end *in vitro* – derived sEVs than end *in vivo*. Moreover, miR-382 was more expressed, and miR-760-

3p was lower expressed in end *in vitro*-derived sEVs compared with end group. MiR-631 was increased in end *in vivo*-derived sEVs than end group.

Figure 5. MiRNA content identified in sEVs present in C.M. by end, end *in vivo*, and end *in vitro* after 6 hours of culture. (A) Venn diagram demonstrating the distribution of the 108 miRNAs present in sEVs from C.M. by end, end *in vivo*, and end *in vitro* for 6 hours. (B) A total of 4 miRNAs were differently expressed among the groups. Different letters represent significant differences ($P \leq 0.05$). Solid bars represent the group mean of $2^{-\Delta C_t}$ and error bars represent standard error of the mean (S.E.M). sEVs = small extracellular vesicles; miR = microRNA; C.M. = conditioned media.

In addition, a total of 88 miRNAs were identified in sEVs present in C.M. by end, end *in vivo*, and end *in vitro* for 24 hours (**Supplementary Table S21**, APPENDIX B). MiR-184 was uniquely detected in sEVs secreted by end group. A total of 87 miRNAs were expressed in all groups, however no miRNA was differently expressed.

Interestingly, we compared sEVs miRNAs content from all groups (end, end *in vivo*, and end *in vitro*) between the co-cultured periods (6 or 24 hours), and we identified 74 miRNAs expressed in all the samples of sEVs secreted by endometrial explants independently of the culture time (**Supplementary Table S22**, APPENDIX B). Of these, 4 (miR-127, miR-331-5p, miR-615, and miR-1246) miRNAs were differently expressed comparing end 6 hours and end 24 hours sEVs; 4 (miR-130a, miR-130b, miR-656, and miR-665) miRNAs were differently expressed in sEVs present in C.M. by end *in vivo* for 6 hours and end *in vivo* for 24 hours, and; 1 (miR-767) miRNA was differently expressed between sEVs secreted by end *in vitro* for 6 hours and end *in vitro* for 24 hours.

Finally, together these results demonstrate that sEVs miRNA content was altered by: 1) embryo presence (probably due to embryo-secreted sEVs); 2) embryo origin (*in vivo* or *in vitro*), and; 3) culture time (6 or 24 hours).

4.3.3.3 Enrichment analysis of miRNAs differentially expressed in sEVs from C.M. by end, end *in vivo*, and end *in vitro* for 6 hours

To determine the predicted biological pathways modulated by miRNAs differently detected in sEVs isolated from C.M. by end, end *in vivo*, and end *in vitro* 6h, bioinformatics analysis was performed using mirWalk version 3.0 software. A total of 4 miRNAs were differently expressed (Figure 5B) and are predicted to regulate 303 signaling pathways (**Supplementary Table S23**, APPENDIX B). The first 10 enrichment pathways ranked by BH value were listed in Table 3. Our results suggest that miRNAs carried by sEVs secreted by end, end *in vivo*, and end *in vitro* 6h are predicted to regulate important pathways associated with oxytocin signaling, endocrine resistance, and metabolism. These pathways are involved in the maternal organism modulation during the pregnancy establishment.

Table 3. Top 10 predicted pathways modulated by miRNAs differentially expressed in sEVs from C.M. by end, end *in vivo*, and end *in vitro* for 6 hours ranked by BH value.

Signaling pathways	Genes predicted to be modulated by miRNAs	Number of total genes	P-value	BH
Oxytocin signaling pathway	58	152	0.0	0.0

Pathways in cancer	157	542	0.0	0.0
Phosphatidylinositol signaling system	41	99	0.0001	0.0076
Choline metabolism in cancer	41	99	0.0001	0.0076
Adrenergic signaling in cardiomyocytes	54	150	0.0002	0.0121
Endocrine resistance	38	94	0.0003	0.013
Proteoglycans in cancer	68	205	0.0003	0.013
Metabolic pathways	375	1527	0.0004	0.0152
Inositol phosphate metabolism	31	73	0.0006	0.0182
Breast cancer	52	150	0.0006	0.0182

4.3.4 MiRNAs profile in luteal explants treated with sEVs isolated from conditioned media by endometrial explants co-cultured or no with *in vivo* or *in vitro*-produced bovine embryo for 6 or 24 hours

4.3.4.1 MiRNA profile in luteal explants

In order to study the sEVs participation during the early interactions among the embryo, endometrium, and corpus luteum we analyze the profile of 382 bovine miRNAs in luteal explants treated for 6 hours with sEVs secreted by end, end *in vivo*, and end *in vitro* in culture medium for 6 or 24 hours. A total of 309 miRNAs were expressed in LE end, end *in vivo*, and end *in vitro* sEVs 6h (**Supplementary Table S24**, APPENDIX B). Of these, only miR-149-3p was differently expressed among the luteal explants groups (Figure 6A). This miRNA was decreased in LE end *in vivo* sEVs 6h than LE end sEVs 6h. Moreover, a total of 311 miRNAs were expressed in LE end, end *in vivo*, and end *in vitro* sEVs 24h (**Supplementary Table S25**, APPENDIX B). Of these, 5 miRNAs (miR-17-3p, miR-335, miR-340, miR-371, and miR-677) were differently expressed among the groups (Figure 6B). MiR-335, miR-340, and miR-677 were lower expressed in LE end *in vitro* sEVs 24h than LE end sEVs 24h. MiR-17-3p was decreased in LE end *in vivo* sEVs 24h than LE end sEVs 24h. MiR-371 was more expressed in LE end *in vitro* sEVs 24h than LE end *in vivo* sEVs 24h. Therefore, these findings suggest sEVs secreted in conditioned media by endometrial explants and embryos of different origins (*in vivo* or *in vitro*) carry different contents and were able to alter miRNAs levels in luteal explants after 6 hours of treatment.

A Luteal explant treated with sEVs from conditioned media from end, end *in vivo* and end *in vitro* for 6 hours

B Luteal explant treated with sEVs from conditioned media from end, end *in vivo* and end *in vitro* for 24 hours

Figure 6. MiRNAs differently expressed in luteal explants treated with sEVs for 6 hours. **(A)** MiR-149-3p was differently expressed in LE end, end *in vivo*, and end *in vitro* sEVs 6h. **(B)** A total of 4 miRNAs were differently expressed among the groups in LE treated with sEVs from C.M. by end, end *in vivo*, and end *in vitro* 24h. Different letters represent significant differences ($P \leq 0.05$). Solid bars represent the group mean of $2^{-\Delta C_t}$ and error bars represent standard error of the mean (S.E.M). sEVs = small extracellular vesicles; miR = microRNA; C.M. = conditioned media.

4.3.4.2 Enrichment analysis of miRNAs differentially expressed in LE treated with sEVs from C.M. by end, end *in vivo*, and end *in vitro* for 24 hours

Bioinformatics analysis were done to define the predicted biological pathways regulated by miRNAs differently expressed in LE treated with sEVs from C.M. by end, end *in vivo*, and end *in vitro* 24h. We identified 5 miRNAs differently expressed that are involved in regulating 269 signaling pathways (**Supplementary Table S26**, APPENDIX B). Of these, the top 10 enrichment pathways ranked by BH value were demonstrated in Table 4, and are involved in

the regulation of actin cytoskeleton, endocrine resistance, cell proliferation, differentiation, survival, growth, and angiogenesis. Thus, we can suggest sEVs secreted by endometrium and embryos of different origins (*in vivo* or *in vitro*) modified miRNA profiles in luteal explants, modulating important pathways associated with corpus luteum viability maintenance.

Table 4. Top 10 predicted pathways modulated by miRNAs differentially expressed in LE treated with sEVs from C.M. by end, end *in vivo*, and end *in vitro* 24h ranked by BH value.

Signaling pathways	Genes predicted to be modulated by miRNAs	Number of total genes	Pvalue	BH
Regulation of actin cytoskeleton	55	211	0.0	0.0
Proteoglycans in cancer	55	205	0.0	0.0
Endocrine resistance	30	94	0.0001	0.0054
Pancreatic cancer	26	76	0.0001	0.0054
Chronic myeloid leukemia	27	77	0.0001	0.0054
Ras signaling pathway	57	242	0.0002	0.009
Phospholipase D signaling pathway	39	152	0.0005	0.0134
Th17 cell differentiation	32	113	0.0004	0.0134
Breast cancer	39	150	0.0004	0.0134
Gastric cancer	39	153	0.0005	0.0134

4.4 Discussion

Embryo-maternal interactions are important to pregnancy establishment and maintenance in all mammal species. This study mainly aimed to enhance the understanding on the direct communication between embryos of different origins (*in vivo* or *in vitro*) and the endometrium through identification of DEGs in endometrial explants, and miRNA content of sEVs secreted by them; and on the indirect communication mediated by these sEVs in the corpus luteum investigating the miRNAs profile in luteal explants treated with them. The main findings were: 1) identification of endometrial global transcriptomic alterations caused by co-cultured of a day 7 *in vivo* or *in vitro* embryo for 6 and 24 hours; 2) identification of distinct miRNAs content in sEVs secreted by endometrial explants and *in vivo* or *in vitro*- produced bovine embryos (end, end *in vivo*, and end *in vitro*) after 6 and 24 hours of culture, and; 3) identification of different miRNA profiles in luteal explants treated with sEVs from C.M. by endometrial explants and *in vivo* or *in vitro*- produced bovine embryos. Together, these results demonstrated that the embryo presence and origin (*in vivo* or *in vitro*) caused molecular alterations in endometrial explants and sEVs content secreted by them. In addition, these sEVs

altered the miRNAs profile in luteal explants, suggesting that early communication among the embryo, endometrium, and corpus luteum can be important to guarantee pregnancy establishment.

Associated with the embryo presence, we identified 45 and 142 differentially expressed genes in endometrial explants cultured for 6 and 24 hours, respectively (Figure 3 and 4), among them are interferon-stimulated genes (ISGs) (Table 1). The pathways and biological processes that were associated with these DEGs are involved in the immune response to viruses. Similarly, other studies founded this association between embryo presence and alteration in genes related to the immune response to the virus in the endometrial cells (PASSARO et al., 2018; MATHEW et al., 2019; SPONCHIADO et al., 2020). This modulation in pathways associated with the immune response to the virus can occur because in ruminants, the IFNT, which is a type I IFN, is responsible for signaling the embryo presence to the maternal organism as early as day 7 of pregnancy (FARIN et al., 1990; ROBERTS et al., 1992; ROBERTS et al., 1999; SPONCHIADO et al., 2017; BRIDI et al., 2018). The group of type I IFN (IFN- α , IFN- β , IFN- ω) is responsible alter the ISGs expression during the viral infection in different cells type (ROBERTS et al., 1999). The expression of these ISGs also was altered in endometrial and luteal cells in early pregnancy due to IFNT secretion by trophectoderm cells (AUSTIN et al., 1996; AUSTIN et al., 2004; OLIVEIRA et al., 2008; SPONCHIADO et al., 2017; SPONCHIADO et al., 2020). Small EVs present in uterine fluid carry IFNT, and they alter ISGs expression in endometrial cells (NAKAMURA et al., 2016; KUSAMA et al., 2018), suggesting that in our study embryo-endometrial interactions also were mediated by sEVs. Moreover, ENSBTAG00000053654, LAMP5, AQP6, AQP9, OAS2, IFI44, MX2, OAS1Y, and LOC100138271 were differentially express and associated with the embryo presence independently of the co-cultured time (Table 2). OAS2, IFI44, MX2, and OAS1Y are IFNT-dependent genes and had their expression altered in endometrial explants co-cultured with bovine embryos on day 8 or on day 15 of development (MATHEW et al., 2019; PASSARO et al., 2019). Interestingly, we identified 5 DEGs (ENSBTAG00000053654, LAMP5, AQP6, AQP9, and LOC100138271) in endometrial explants that can be associated with the secretion of other bioactive molecules by the embryo, i.e IFNT- independent factors. Therefore, we can observe that only one day 7 embryo was able to modify endometrial global transcriptomic few hours after the co-culture, suggesting that early embryo-endometrial communication can start soon after embryo transfer. This interaction can occur by secretion of embryo IFNT- dependent and independent molecules and mediate by sEVs.

Endometrial explants expose to a single day 7 embryo produced *in vivo* or *in vitro*, for 6 or 24 hours, had 391 and 156 DEGs, respectively (Figure 3 and 4). These DEGs are part of pathways associated with metabolism, water and solute transport, and hippo signaling. GATA6, ENSBTAG00000053607, ENSBTAG00000052715, ENSBTAG00000054319, and ZNF581 were altered in endometrial explant co-cultured with a day 7 *in vitro* embryo when compared to end *in vivo* group. The alteration in the expression of these genes suggests as early as 6 hours after the embryo transfer is possible to observe molecular differences in endometrium caused by *in vivo* or *in vitro*-produced embryos, demonstrating that the endometrium responds quickly and differently depending on the embryo origin. These embryo-induced molecular alterations were maintained up to 24 hours of co-culture, indicating a continuous modulation of the embryo on endometrial transcriptional patterns. Moreover, the sEVs miRNAs content was different in C.M. by end *in vivo* compared to C.M. by end *in vitro*, mainly 6 hours after the co-cultured. Molecular and morphological distinct characteristics, different ability to establish and maintain the pregnancy, and sEVs secretion with distinct miRNA content are some differences found when *in vivo* and *in vitro*-produced bovine embryos are compared (PLANTE; KING, 1994; HOLM; CALLESEN, 1998; HOLM et al., 1998; LONERGAN et al., 2003; RIZOS et al., 2003; MOHAN et al., 2004; CORCORAN et al., 2006; PONTES et al., 2009; VILLAMIL et al., 2012; SARTORI et al., 2016; as also demonstrated in chapter 2). Moreover, endometrial genes profiles were altered in pregnant cows on day 20 by the presence of conceptus produced by different biotechniques (IA, IVF, or SCNT) and in endometrial explants co-cultured with on day 15 IA and IVF conceptus (MANSOURI-ATTIA et al., 2009; MATHEW et al., 2019). Thereby, we can infer that *in vivo* and *in vitro* embryos have different characteristics and can need distinct endometrial environments to develop; that is why they are able to modify endometrial genes profile and miRNAs content in sEVs from C.M. as early as on day 7 of development.

Interestingly, associated with sex steroid signaling, ESR1 was down-regulated in end *in vivo* compared to end *in vitro* and end groups after 6 hours of culture (Table 1). In addition, OXTR was lower expressed in end *in vivo* than in end *in vitro* group after 24 hours of culture (Table 2). Thus, we suggest that the embryo origin (*in vivo* or *in vitro*) can be associated with the endometrial regulation of OXTR and ESR1, two important receptors involved in luteolysis modulation (SPENCER; BAZER, 1995; CHAMI et al., 1999; MCCracken et al., 1999). In a recent study performed by our group, we identified that sEVs secreted by *in vivo* embryos had higher miR-1296 and miR-323 expression than *in vitro* embryos-derived sEVs (Bridi, et al., 2021; Chapter 2). These miRNAs, together with miR-199b, are predicted to regulate OXTR

(MirWalk 3.0 version). In this study, miR-199b was lower expressed in sEVs secreted by end *in vivo* 6h group, suggesting this occurs because it can be inhibiting OXTR expression in endometrial cells. These findings suggest miR-1296 and miR-323, more expressed in *in vivo* embryos-derived sEVs, and miR-199b may regulate OXTR expression in end *in vivo* group as early as on day 7 of pregnancy. Furthermore, miR-92b, miR-1249, miR-1281, and miR-1343-3p are predicted to modulate ESR1 expression (MirWalk 3.0 version), and they were increased in sEVs secreted by *in vivo* embryos of day 7 up to day 9 of development (as also demonstrated in chapter 2). Thus, we can suggest miRNAs, carried by sEVs secreted by the *in vivo* embryos of the day 7 up to 9 of development, act in luteolysis regulation through OXTR and ESR1 endometrial modulation and, consequently, contribute to a successful pregnancy. Besides, this higher efficiency of *in vivo* embryos in down-regulate ESR1 and OXTR in the endometrium than *in vitro* embryos few hours after the co-cultured may explain the differences in pregnancy rate. However, further experiments to demonstrate the functional regulation of these embryos-derived miRNAs carried by sEVs in the endometrium are still needed. It is known that IFNT decreases ESR1 and OXTR in the endometrium (CHAMI et al., 1999; MCCracken et al., 1999). However, IFNT mRNA expression was increased in *in vitro* embryos compared to *in vivo* embryos (LONERGAN et al., 2003). Together this information strongly indicates that other IFNT-independent molecules secreted by the embryo and carried by sEVs such as miRNAs participate in luteolysis regulation in the endometrium during early pregnancy.

The *ex vivo* model, using endometrial explants, maintains cellular and extracellular architecture, which is an advantage when compared to monolayer cells (2D culture). However, culturing explants for longer periods can be associated with cellular and molecular alterations in the tissue, such as the appearance of apoptotic cells after 24 hours of culture (BORGES et al., 2012). This is a limitation of this study, as a major effect of culture time (comparing 6 versus 24 hours) is present in our data (Figure 1).

Besides the DEGs analyzed in endometrial explants co-cultured with a day 7 *in vivo* or *in vitro*-produced bovine embryo, we also analyze sEVs miRNAs content present in C.M. by end, end *in vivo*, and end *in vitro* culture for 6 or 24 hours. We identified 4 miRNAs (miR-199b, miR-382, miR-631, and miR-760-3p) in sEVs from C.M. by end, end *in vivo*, and end *in vitro* that were differentially expressed among the groups after 6 hours of culture. It is known that a day 7 *in vivo* embryo was able to modify uterine fluid metabolites composition (SPONCHIADO et al., 2019). Moreover, changes in proteomics and sEVs miRNAs content of uterine flush were identified in superovulated pregnant cows on day 7 (KUSAMA et al., 2021). Together these findings demonstrate that the embryo presence as early as day 7 was able to alter

different components of the uterine fluid and conditioned media such as sEVs miRNA content, proteins, and metabolites, which may be necessary to embryonic development and pregnancy establishment. Interestingly, miR-199b was lower expressed in sEVs present in uterine fluid from superovulated pregnant cows on day 7 than the cyclic group (KUSAMA et al., 2021), corroborating with our results in which miR-199b was decreased in sEVs present in C.M. by the end *in vivo* than the end *in vitro* group.

Moreover, enrichment analyzes indicated that miRNAs differently expressed in sEVs from C.M. by end, end *in vivo*, and end *in vitro* culture for 6 hours are predicted to regulate oxytocin signaling pathway. MiR-199b is predicted to modulate OXTR (MirWalk 3.0 version), as previously described. OXTR was lower expressed in end *in vivo* than end *in vitro* and end groups. Thus, these data demonstrate again that sEVs miRNAs from C.M. by endometrial and embryonic cells are involved in the regulation of the oxytocin signaling pathway in the early pregnancy possibly contributing to maintain CL viability.

Small sEVs are part of the crosstalk between embryo and endometrium carrying important bioactive molecules that modulate early embryonic development and uterine receptivity (RUIZ-GONZÁLEZ et al., 2015; BURNS et al., 2016; NAKAMURA et al., 2016; ZHANG et al., 2017; KUSAMA et al., 2018; NAKAMURA et al., 2020). Furthermore, the vascularization between the ipsilateral ovary to the corpus luteum and uterine horn is altered over the estrous cycle, being more prominent in the luteal phase when compared to the contralateral local vascularization (GINTHER, 1974). This alteration in vascularization can occur to prepare the oviduct and uterine horn for future pregnancy and/or maintain luteal vascularization (GINTHER, 1974), suggesting this physiological modification could be involved in early communication between the embryo and maternal organism. Based on that, we believe that sEVs present in the uterine fluid can leave the uterus and arrive in CL, carrying molecules important to maintain the viability and functionality of the luteal cells. To better understand the embryo-maternal communication mediated by sEVs, we treated luteal explants with sEVs secreted by endometrial explants and embryos of different origins (*in vivo* or *in vitro*). Luteal explants of all groups had their miRNA profiles altered. MicroRNAs modulate proliferation, survival, progesterone secretion in luteal cells over the estrous cycle and during maternal recognition of pregnancy (MAALOUF et al., 2014; MAALOUF et al., 2016; GECAJ, R. et al., 2017; MOHAMMED et al., 2017; DONADEU et al., 2020). MiR-17-3p, miR-335, miR-340, miR-371, and miR-677 were differentially expressed among the luteal explants groups treated with sEVs from C.M. by end, end *in vivo*, and end *in vitro* for 24 hours. MiR-371, miR-340, and miR-17-3p are predicted to modulate PTGFR, PTGS2, and Casp3 mRNA,

respectively (MirWalk 3.0 version), which are genes that participate in the luteolytic process (MCCRACKEN et al., 1999; AROSH et al., 2016). Therefore, we may speculate that the luteal function is differently influenced depending on embryo origin (*in vivo* or *in vitro*) because these embryos alter endometrial transcriptional profile and sEVs content secreted in C.M. Moreover, sEVs secreted by endometrial and embryonic cells potentially regulate the luteolytic pathway in the CL. Functional modulation of the CL by the miRNAs present in sEVs secreted by endometrial and embryonic cells deserves further investigation.

4.5 Conclusion

In conclusion, the present study showed that the presence of a single day 7 blastocyst was able to induce DEGs in endometrial explants; alteration in sEVs miRNAs content secreted by endometrium and embryonic cells, and; modify miRNAs profile in luteal explants treated with these sEVs in an origin-dependent fashion (*in vivo* or *in vitro*). Interestingly, we identified endometrial DEGs modulated by the embryo presence, that remain unaltered independently of the culture time with *in vivo* and *in vitro* embryos, demonstrating that these DEGs possibly have an important role during the first embryo-maternal interactions. Moreover, ESR1 and OXTR were decreased in endometrial explants exposed to *in vivo* embryos. Differently expressed miRNAs in sEVs secreted by the end, end *in vivo*, and end *in vitro* 6h groups are predicted to modulate the oxytocin signaling pathway. To our knowledge, this is the first study to demonstrate that luteal explants had their miRNA profiles altered by sEVs secreted by *in vivo* or *in vitro* embryos and the endometrium. These differentially expressed miRNAs are predicted to modulate important genes associated with the luteolytic process. Therefore, we can propose that sEVs also mediate the first interactions between embryo, endometrium, and corpus luteum, carrying important bioactive molecules as miRNAs that may modulate luteal function to maintain the viability of CL and pregnancy successful.

4.6 References

- ANDRADE, G. M. et al. Oxygen tension modulates extracellular vesicles and its miRNA contents in bovine embryo culture medium. **Molecular Reproduction and Development**, v. 86, n. 8, p. 1067-1080, 2019.
- ANTONIAZZI, A. Q. et al. Endocrine delivery of interferon tau protects the corpus luteum from prostaglandin F2 alpha-induced luteolysis in ewes. **Biol Reprod**, v. 88, n. 6, p. 144, Jun 2013.

AROSH, J. A.; BANU, S. K.; MCCracken, J. A. Novel concepts on the role of prostaglandins on luteal maintenance and maternal recognition and establishment of pregnancy in ruminants¹. **Journal of Dairy Science**, v. 99, n. 7, p. 5926-5940, 2016/07/01/ 2016.

AUSTIN, K. J. et al. Localization of ISG15 and Conjugated Proteins in Bovine Endometrium Using Immunohistochemistry and Electron Microscopy. **Endocrinology**, v. 145, n. 2, p. 967-975, 2004.

AUSTIN, K. J. et al. Ubiquitin cross-reactive protein is released by the bovine uterus in response to interferon during early pregnancy. **Biology of Reproduction**, v. 54, n. 3, p. 600-606, March 1, 1996 1996.

BANU, S. K. et al. Interferon tau regulates PGF₂α release from the ovine endometrial epithelial cells via activation of novel JAK/EGFR/ERK/EGR-1 pathways. **Mol Endocrinol**, v. 24, n. 12, p. 2315-30, Dec 2010.

BARUSELLI, P. S. et al. Superovulation and embryo transfer in *Bos indicus* cattle. **Theriogenology**, v. 65, n. 1, p. 77-88, 2006/01/07/ 2006.

BETTERIDGE, K. J.; FLÉCHON, J. E. The anatomy and physiology of pre-attachment bovine embryos. **Theriogenology**, v. 29, n. 1, p. 155-187, 1988/01/01/ 1988.

BORGES, Á. M.; HEALEY, G. D.; SHELDON, I. M. Explants of Intact Endometrium to Model Bovine Innate Immunity and Inflammation Ex Vivo. **American Journal of Reproductive Immunology**, v. 67, n. 6, p. 526-539, 2012.

BRIDI, A. et al. Parthenogenetic bovine embryos secrete type I interferon capable of stimulating ISG15 in luteal cell culture. **Animal Reproduction** v. 15, n. 4, p. 1268 - 1277, 2018.

BRIDI, A.; PERECIN, F.; SILVEIRA, J. C. D. Extracellular Vesicles Mediated Early Embryo–Maternal Interactions. **International Journal of Molecular Sciences**, v. 21, n. 3, p. 1163, 2020.

BURNS, G. W.; BROOKS, K. E.; SPENCER, T. E. Extracellular Vesicles Originate from the Conceptus and Uterus During Early Pregnancy in Sheep. **Biology of Reproduction**, v. 94, n. 3, p. 56, 1-11, March 1, 2016 2016.

CHAMI, O. et al. Platelet-activating factor may act as an endogenous pulse generator for sheep of luteolytic PGF₂α release. **American Journal of Physiology - Endocrinology And Metabolism**, v. 276, n. 4, p. E783-E792, 1999.

CHANEY, H. L. et al. Conceptus-induced, interferon tau-dependent gene expression in bovine endometrial epithelial and stromal cells†. **Biol Reprod**, v. 104, n. 3, p. 669-683, Mar 11 2021.

COOKE, P. S. et al. Stromal estrogen receptors mediate mitogenic effects of estradiol on uterine epithelium. **Proc Natl Acad Sci U S A**, v. 94, n. 12, p. 6535-40, Jun 10 1997.

CORCORAN, D. et al. Suppressed expression of genes involved in transcription and translation in in vitro compared with in vivo cultured bovine embryos. **Reproduction**, v. 131, n. 4, p. 651-660, April 1, 2006 2006.

DONADEU, F. X. et al. Relationships between size, steroidogenesis and miRNA expression of the bovine corpus luteum. **Theriogenology**, v. 145, p. 226-230, 2020/03/15/ 2020.

FARIN, C. E. et al. Expression of trophoblastic interferon genes in sheep and cattle. **Biology of Reproduction**, v. 43, n. 2, p. 210-218, August 1, 1990 1990.

FIRE, A. et al. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. **Nature**, v. 391, n. 6669, p. 806-811, 02/19/print 1998.

FORDE, N. et al. 'Conceptualizing' the Endometrium: Identification of Conceptus-Derived Proteins During Early Pregnancy in Cattle. **Biol Reprod**, v. 92, n. 6, p. 156, Jun 2015.

FORDE, N. et al. Evidence for an early endometrial response to pregnancy in cattle: both dependent upon and independent of interferon tau. **Physiol Genomics**, v. 44, n. 16, p. 799-810, Aug 17 2012.

GECAJ, R. et al. **The Dynamics of microRNA Transcriptome in Bovine Corpus Luteum during Its Formation, Function, and Regression.** 2017. 213.

GECAJ, R. M. et al. The Dynamics of microRNA Transcriptome in Bovine Corpus Luteum during Its Formation, Function, and Regression. **Frontiers in Genetics**, v. 8, n. 213, 2017-December-15 2017.

GINTHER, O. J. Internal regulation of physiological processes through local venoarterial pathways: a review. . **Journal of Animal Science**, v. 39, n. 3, p. 550 - 564, 1974.

HOLM, P.; CALLESEN, H. In vivo versus in vitro produced bovine ova: similarities and differences relevant for practical application. **Reproduction Nutrition Development**, v. 38, n. 6, p. 579-594, 1998.

HOLM, P. et al. Developmental kinetics of the first cell cycles of bovine in vitro produced embryos in relation to their in vitro viability and sex. **Theriogenology**, v. 50, n. 8, p. 1285-1299, 1998/12/01/ 1998.

HWANG, H. W.; MENDELL, J. T. MicroRNAs in cell proliferation, cell death, and tumorigenesis. **Br J Cancer**, v. 94, n. 6, p. 776-780, 02/21/online 2006.

IRELAND, J. J.; MURPHEE, R. L.; COULSON, P. B. Accuracy of Predicting Stages of Bovine Estrous Cycle by Gross Appearance of the Corpus Luteum¹. **Journal of Dairy Science**, v. 63, n. 1, p. 155-160, 1980/01/01/ 1980.

KUSAMA, K. et al. Intrauterine exosomes are required for bovine conceptus implantation. **Biochemical and Biophysical Research Communications**, v. 495, n. 1, p. 1370-1375, 2018/01/01/ 2018.

KUSAMA, K. et al. Day 7 Embryos Change the Proteomics and Exosomal Micro-RNAs Content of Bovine Uterine Fluid: Involvement of Innate Immune Functions. **Frontiers in Genetics**, v. 12, n. 1075, 2021-June-28 2021.

LIAO, Y.; SMYTH, G. K.; SHI, W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. **Bioinformatics**, v. 30, n. 7, p. 923-30, Apr 1 2014.

LIAO, Y.; SMYTH, G. K.; SHI, W. The R package Rsubread is easier, faster, cheaper and better for alignment and quantification of RNA sequencing reads. **Nucleic Acids Research**, v. 47, n. 8, p. e47-e47, 2019.

LONERGAN, P. et al. Temporal Divergence in the Pattern of Messenger RNA Expression in Bovine Embryos Cultured from the Zygote to Blastocyst Stage In Vitro or In Vivo1. **Biology of Reproduction**, v. 69, n. 4, p. 1424-1431, 2003.

LOVE, M. I.; HUBER, W.; ANDERS, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. **Genome Biology**, v. 15, n. 12, p. 550, 2014/12/05 2014.

MAALOUF, S. W. et al. Regulating life or death: Potential role of microRNA in rescue of the corpus luteum. **Molecular and Cellular Endocrinology**, v. 398, n. 1, p. 78-88, 2014/12/01/ 2014.

MAALOUF, S. W.; SMITH, C. L.; PATE, J. L. Changes in MicroRNA Expression During Maturation of the Bovine Corpus Luteum: Regulation of Luteal Cell Proliferation and Function by MicroRNA-34a1. **Biology of Reproduction**, v. 94, n. 3, 2016.

MANSOURI-ATTIA, N. et al. Endometrium as an early sensor of in vitro embryo manipulation technologies. **Proceedings of the National Academy of Sciences**, v. 106, n. 14, p. 5687-5692, 2009.

MARTIN, M. Cutadapt removes adapter sequences from high-throughput sequencing reads. **2011**, v. 17, n. 1, p. 3, 2011-05-02 2011.

MATHEW, D. J. et al. Interferon tau-dependent and independent effects of the bovine conceptus on the endometrial transcriptome†. **Biol Reprod**, v. 100, n. 2, p. 365-380, Feb 1 2019.

MATHIVANAN, S.; JI, H.; SIMPSON, R. J. Exosomes: Extracellular organelles important in intercellular communication. **Journal of Proteomics**, v. 73, n. 10, p. 1907-1920, 9/10/ 2010.

MCCRACKEN, J. A.; CUSTER, E. E.; LAMSA, J. C. Luteolysis: A Neuroendocrine-Mediated Event. **Physiological Reviews**, v. 79, n. 2, p. 263-323, 1999.

MOHAMMED, B. T. et al. The Adequate Corpus Luteum: miR-96 Promotes Luteal Cell Survival and Progesterone Production. **The Journal of Clinical Endocrinology & Metabolism**, v. 102, n. 7, p. 2188-2198, 2017.

MOHAN, M.; HURST, A. G.; MALAYER, J. R. Global gene expression analysis comparing bovine blastocysts flushed on day 7 or produced in vitro. **Molecular Reproduction and Development**, v. 68, n. 3, p. 288-298, 2004/07/01 2004.

MORELLI, A. E. et al. Endocytosis, intracellular sorting, and processing of exosomes by dendritic cells. **Blood**, v. 104, n. 10, p. 3257-3266, 2004.

NAKAMURA, K. et al. Induction of IFNT-Stimulated Genes by Conceptus-Derived Exosomes during the Attachment Period. **PLoS ONE**, v. 11, n. 6, p. e0158278, 06/28 12/22/received 06/13/accepted 2016.

NAKAMURA, K. et al. IFNT-independent effects of intrauterine extracellular vesicles (EVs) in cattle. **Reproduction**, v. 159, n. 5, p. 503-511, 01 May. 2020 2020.

NG, Y. H. et al. Endometrial Exosomes/Microvesicles in the Uterine Microenvironment: A New Paradigm for Embryo-Endometrial Cross Talk at Implantation. **PLoS ONE**, v. 8, n. 3, p. 2013.

OLIVEIRA, J. F. et al. Expression of Interferon (IFN)-Stimulated Genes in Extrauterine Tissues during Early Pregnancy in Sheep Is the Consequence of Endocrine IFN- τ Release from the Uterine Vein. **Endocrinology**, v. 149, n. 3, p. 1252-1259, 2008.

PASSARO, C. et al. Global transcriptomic response of bovine endometrium to blastocyst-stage embryos. **Reproduction**, v. 158, n. 3, p. 223-235, Sep 2019.

PASSARO, C. et al. Blastocyst-induced changes in the bovine endometrial transcriptome. **Reproduction**, v. 156, n. 3, p. 219-229, 2018.

PATE, J. L. Isolation and culture of fully differentiated bovine luteal cells. **Methods Toxicol**, p. 360–370, 1993.

PLANTE, L.; KING, W. A. Light and electron microscopic analysis of bovine embryos derived byin Vitro andin Vivo fertilization. **Journal of Assisted Reproduction and Genetics**, v. 11, n. 10, p. 515-529, November 01 1994.

PONTES, J. H. F. et al. Comparison of embryo yield and pregnancy rate between in vivo and in vitro methods in the same Nelore (*Bos indicus*) donor cows. **Theriogenology**, v. 71, n. 4, p. 690-697, 2009/03/01/ 2009.

RIZOS, D. et al. Bovine Embryo Culture in the Presence or Absence of Serum: Implications for Blastocyst Development, Cryotolerance, and Messenger RNA Expression1. **Biology of Reproduction**, v. 68, n. 1, p. 236-243, 2003.

ROBERTS, R. M.; CROSS, J. C.; LEAMAN, D. W. Interferons as Hormones of Pregnancy*. **Endocrine Reviews**, v. 13, n. 3, p. 432-452, 1992.

ROBERTS, R. M. et al. Trophoblast Interferons. **Placenta**, v. 20, n. 4, p. 259-264, 1999.

RUIZ-GONZÁLEZ, I. et al. Exosomes, endogenous retroviruses and toll-like receptors: pregnancy recognition in ewes. **Reproduction**, v. 149, n. 3, p. 281-291, March 1, 2015 2015.

SAADELDIN, I. M.; OH, H. J.; LEE, B. C. Embryonic–maternal cross-talk via exosomes: potential implications. **Stem Cells and Cloning : Advances and Applications**, v. 8, p. 103-107, 07/07 2015.

SARTORI, R. et al. Update and overview on assisted reproductive technologies (ARTs) in Brazil. **Animal Reproduction**, v. 13, p. 300-312, 2016.

SEGURA, E. et al. CD8+ Dendritic Cells Use LFA-1 to Capture MHC-Peptide Complexes from Exosomes In Vivo. **The Journal of Immunology**, v. 179, n. 3, p. 1489-1496, August 1, 2007 2007.

SIMPSON, R. J.; JENSEN, S. S.; LIM, J. W. E. Proteomic profiling of exosomes: Current perspectives. **PROTEOMICS**, v. 8, n. 19, p. 4083-4099, 2008.

SOARES, A. C. S. et al. Steroid hormones interact with natriuretic peptide C to delay nuclear maturation, to maintain oocyte–cumulus communication and to improve the quality of *in vitro*-produced embryos in cattle. **Reproduction, Fertility and Development**, v. 29, n. 11, p. 2217-2224, 2017.

SPENCER, T. E.; BAZER, F. W. Temporal and spatial alterations in uterine estrogen receptor and progesterone receptor gene expression during the estrous cycle and early pregnancy in the ewe. **Biology of Reproduction**, v. 53, n. 6, p. 1527-1543, December 1, 1995 1995.

SPENCER, T. E. et al. Conceptus-derived prostaglandins regulate gene expression in the endometrium prior to pregnancy recognition in ruminants. **Reproduction**, v. 146, n. 4, p. 377-387, October 1, 2013 2013.

SPONCHIADO, M. et al. Pre-hatching embryo-dependent and -independent programming of endometrial function in cattle. **PLOS ONE**, v. 12, n. 4, p. e0175954, 2017.

SPONCHIADO, M. et al. The pre-hatching bovine embryo transforms the uterine luminal metabolite composition in vivo. **Scientific Reports**, v. 9, n. 1, p. 8354, 2019/06/07 2019.

SPONCHIADO, M. et al. Molecular interactions at the bovine embryo-endometrial epithelium interface. **Reproduction**, v. 160, n. 6, p. 887-903, Dec 2020.

SUBRA, C. et al. Exosome lipidomics unravels lipid sorting at the level of multivesicular bodies. **Biochimie**, v. 89, p. 205-12, 2007.

TESFAYE, D. et al. Potential role of microRNAs in mammalian female fertility. **Reproduction, Fertility and Development**, v. 29, n. 1, p. 8-23, 2017.

THÉRY, C.; ZITVOGEL, L.; AMIGORENA, S. Exosomes: composition, biogenesis and function. **Nature Reviews Immunology**, v. 2, p. 569, 08/01/online 2002.

VALADI, H. et al. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. **Nature Cell Biology**, v. 9, p. 654–659, 2007.

VILLAMIL, P. R. et al. Developmental rates of in vivo and in vitro produced bovine embryos cryopreserved in ethylene glycol based solutions by slow freezing or solid surface vitrification. **Animal reproduction**, v. 9, p. 86-92, 2012.

WYDOOGHE, E. et al. Autocrine embryotropins revisited: how do embryos communicate with each other in vitro when cultured in groups? **Biological Reviews**, v. 92, n. 1, p. 505-520, 2017.

YU, G. et al. clusterProfiler: an R package for comparing biological themes among gene clusters. **Omics**, v. 16, n. 5, p. 284-7, May 2012.

ZHANG, Y. et al. Uterine Fluid in Pregnancy: A Biological and Clinical Outlook. **Trends in Molecular Medicine**, v. 23, n. 7, p. 604-614, 2017.

5. General discussion and conclusion

This thesis aimed to deepen our knowledge on the role of sEVs mediating embryo-endometrium-corpus luteum communication. The scientific questions addressed in this thesis were: **1)** Do bovine embryos produced by different biotechniques (*in vivo* or *in vitro*) secrete sEVs that have different miRNAs content? **2)** Could molecular alterations in the endometrium and the sEVs content secreted by both in the conditioned medium be caused by embryo presence and origin (*in vivo* or *in vitro*)? **3)** Could these EVs differentially modulate corpus luteum miRNAs profile?

In chapter 1, we elaborated a review about early embryo-maternal interactions mediated by sEVs. We reviewed the literature to demonstrate the role of extracellular vesicles modulating embryo-maternal communication during early pregnancy. There is strong evidence suggesting that sEVs play an important biological role during early embryo-endometrium crosstalk, modulating embryonic development and uterine receptivity. Moreover, serum and/or plasma-derived sEVs have their content altered in pregnant cows, demonstrating that they are part of the conceptus-maternal organism communication.

In chapter 2, we demonstrate that *in vivo* and *in vitro*-produced bovine embryos secrete sEVs with different miRNAs content. Primarily, we observed that miRNA profiles were different in *in vivo* and *in vitro*-produced bovine hatched blastocysts on day 9 of development. Next, we analyzed sEVs size and concentration in conditioned media from *in vivo* and *in vitro* embryos. sEVs size was similar between the groups, while sEVs concentration was higher in the conditioned medium by *in vitro*-produced bovine blastocysts of day 7 up to day 9 of development, demonstrating for the first time that *in vivo* embryos secreted less sEVs than *in vitro* embryos. These results agree with data obtained in analyses performed by our group in collaboration with other research groups (unpublished data). Furthermore, the sEVs miRNAs content was different depending on embryo origin (*in vivo* or *in vitro*). The differentially expressed miRNAs in *in vivo* and *in vitro* blastocysts and in the sEVs secreted by them are predicted to modulate signaling pathways associated with early embryonic development, CL maintenance, and uterine receptivity. Together these results suggest that *in vivo* and *in vitro* hatched blastocysts secreted sEVs that can modify embryo-maternal communication, contributing to pregnancy establishment.

In chapter 3, using an *ex vivo* model, we aimed to interrogate the direct communication (without physical contact) between embryos of different origins and endometrium; and the indirect communication among embryo, endometrium, and corpus luteum mediated by sEVs. To simulate uterine *in vivo* environment we cultured endometrial explants alone (end), or in

combination with a single day-7 *in vivo* embryo (end *in vivo*) or a single day-7 *in vitro* embryos (end *in vitro*) for 6 or 24 hours. The embryo presence and origin altered the endometrium mRNA transcription profile. ESR1 and OXTR were decreased in endometrial explants cultured with a day-7 *in vivo* embryo, suggesting that the embryos of different origins can modulate in distinct ways the endometrium, according to their need. These results suggest a more efficient modulation of the endometrial receptors associated with the luteolytic process by *in vivo* embryos than *in vitro* derived embryos. Moreover, we speculate that these data may explain the higher rate of pregnancy generated by *in vivo* embryos compared to *in vitro* counterparts. Simulating uterine fluid, we isolated sEVs from conditioned media by endometrial explants exposed to a single day-7 *in vivo* or *in vitro* embryo. Small EVs miRNAs content was altered by embryo presence and origin in the C.M. as revealed by end, end *in vivo*, and end *in vitro* groups. After 6 hours of culture, miRNAs differentially expressed in these sEVs are predicted to regulate the oxytocin signaling pathway. This pathway is associated with CL maintenance, and consequently, pregnancy success or loss. To investigate the indirect communication among embryo, endometrium, and corpus luteum, sEVs isolated from C.M. by the end, end *in vivo*, and end *in vitro* groups were used to treat luteal explants (LE). MiRNA profiles were altered in LE treated with these sEVs. MiR-371, miR-340, and miR-17-3p are predicted to modulate PTGFR, PTGS2, and Casp3 mRNA, respectively (MirWalk 3.0 version). These predicted targets are involved in the luteolytic process. Thus, these findings suggest that sEVs miRNAs secreted by embryos of different origins and endometrial cells can modulate, in luteal cells, important targets to maintain CL viability and function. However, further experiments are needed to confirm the functional regulation mediated by these miRNAs in the corpus luteum.

In conclusion, the communication among embryo, endometrium, and corpus luteum mediated by sEVs is part of the intricate mechanisms governing reproductive success in cattle. Results here described strengths the idea that the sEVs participate in the pivotal events of early maternal recognition of pregnancy. Recently, the use of synthetic sEVs carrying specific molecules opens new paths to explore their use to understand such events. In addition, a better understanding of the sEVs biological role in embryo-endometrium-corpus luteum interaction will pave the way to modify the sEVs content in order to improve the pregnancy rate after *in vitro* embryos' transfer.

6. References

BRIDI, A.; PERECIN, F.; SILVEIRA, J. C. D. Extracellular Vesicles Mediated Early Embryo–Maternal Interactions. **International Journal of Molecular Sciences**, v. 21, n. 3, p. 1163, 2020.

BURNS, G. W.; BROOKS, K. E.; SPENCER, T. E. Extracellular Vesicles Originate from the Conceptus and Uterus During Early Pregnancy in Sheep. **Biology of Reproduction**, v. 94, n. 3, p. 56, 1-11, March 1, 2016 2016.

CORCORAN, D. et al. Suppressed expression of genes involved in transcription and translation in in vitro compared with in vivo cultured bovine embryos. **Reproduction**, v. 131, n. 4, p. 651-660, April 1, 2006 2006.

DISSANAYAKE, K. et al. Individually cultured bovine embryos produce extracellular vesicles that have the potential to be used as non-invasive embryo quality markers. **Theriogenology**, v. 149, p. 104-116, Jun 2020.

FIRE, A. et al. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. **Nature**, v. 391, n. 6669, p. 806-811, 02/19/print 1998.

GINTHER, O. J. Internal regulation of physiological processes through local venoarterial pathways: a review. . **Journal of Animal Science**, v. 39, n. 3, p. 550 - 564, 1974.

HIENDLEDER, S. et al. Tissue-Specific Effects of In Vitro Fertilization Procedures on Genomic Cytosine Methylation Levels in Overgrown and Normal Sized Bovine Fetuses1. **Biology of Reproduction**, v. 75, n. 1, p. 17-23, 2006.

HOLM, P.; CALLESEN, H. In vivo versus in vitro produced bovine ova: similarities and differences relevant for practical application. **Reproduction Nutrition Development**, v. 38, n. 6, p. 579-594, 1998.

HOLM, P. et al. Developmental kinetics of the first cell cycles of bovine in vitro produced embryos in relation to their in vitro viability and sex. **Theriogenology**, v. 50, n. 8, p. 1285-1299, 1998/12/01/ 1998.

HWANG, H. W.; MENDELL, J. T. MicroRNAs in cell proliferation, cell death, and tumorigenesis. **Br J Cancer**, v. 94, n. 6, p. 776-780, 02/21/online 2006.

LONERGAN, P. et al. Temporal Divergence in the Pattern of Messenger RNA Expression in Bovine Embryos Cultured from the Zygote to Blastocyst Stage In Vitro or In Vivo1. **Biology of Reproduction**, v. 69, n. 4, p. 1424-1431, 2003.

MANSOURI-ATTIA, N. et al. Endometrium as an early sensor of in vitro embryo manipulation technologies. **Proceedings of the National Academy of Sciences**, v. 106, n. 14, p. 5687-5692, 2009.

MELLISHO, E. A. et al. Extracellular vesicles secreted during blastulation show viability of bovine embryos. **Reproduction**, v. 158, n. 6, p. 477-492, Dec 2019.

MELLISHO, E. A. et al. Identification and characteristics of extracellular vesicles from bovine blastocysts produced in vitro. **PLOS ONE**, v. 12, n. 5, p. e0178306, 2017.

MELO-BAEZ, B. et al. MicroRNAs from Extracellular Vesicles Secreted by Bovine Embryos as Early Biomarkers of Developmental Competence. **International Journal of Molecular Sciences**, v. 21, n. 23, p. 8888, 2020.

MOHAN, M.; HURST, A. G.; MALAYER, J. R. Global gene expression analysis comparing bovine blastocysts flushed on day 7 or produced in vitro. **Molecular Reproduction and Development**, v. 68, n. 3, p. 288-298, 2004/07/01 2004.

PLANTE, L.; KING, W. A. Light and electron microscopic analysis of bovine embryos derived by in Vitro and in Vivo fertilization. **Journal of Assisted Reproduction and Genetics**, v. 11, n. 10, p. 515-529, November 01 1994.

PONTES, J. H. F. et al. Comparison of embryo yield and pregnancy rate between in vivo and in vitro methods in the same Nelore (*Bos indicus*) donor cows. **Theriogenology**, v. 71, n. 4, p. 690-697, 2009/03/01/ 2009.

RIZOS, D. et al. Bovine Embryo Culture in the Presence or Absence of Serum: Implications for Blastocyst Development, Cryotolerance, and Messenger RNA Expression1. **Biology of Reproduction**, v. 68, n. 1, p. 236-243, 2003.

RUIZ-GONZÁLEZ, I. et al. Exosomes, endogenous retroviruses and toll-like receptors: pregnancy recognition in ewes. **Reproduction**, v. 149, n. 3, p. 281-291, March 1, 2015 2015.

SAAEELDIN, I. M. et al. Improvement of Cloned Embryos Development by Co-Culturing with Parthenotes: A Possible Role of Exosomes/Microvesicles for Embryos Paracrine Communication. **Cellular Reprogramming**, v. 16, n. 3, p. 223-234, 2014.

SAAEELDIN, I. M.; OH, H. J.; LEE, B. C. Embryonic–maternal cross-talk via exosomes: potential implications. **Stem Cells and Cloning : Advances and Applications**, v. 8, p. 103-107, 07/07 2015.

SARTORI, R. et al. Update and overview on assisted reproductive technologies (ARTs) in Brazil. **Animal Reproduction**, v. 13, p. 300-312, 2016.

SPONCHIADO, M. et al. Pre-hatching embryo-dependent and -independent programming of endometrial function in cattle. **PLOS ONE**, v. 12, n. 4, p. e0175954, 2017.

SPONCHIADO, M. et al. The pre-hatching bovine embryo transforms the uterine luminal metabolite composition in vivo. **Scientific Reports**, v. 9, n. 1, p. 8354, 2019/06/07 2019.

VALADI, H. et al. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. **Nature Cell Biology**, v. 9, p. 654–659, 2007.

YOUNG, L. E. et al. Epigenetic change in IGF2R is associated with fetal overgrowth after sheep embryo culture. **Nature Genetics**, v. 27, p. 153, 02/01/online 2001.

APPENDIX A – Supplementary material of Chapter 2

Supplementary Table S1. MicroRNAs primers sequences used in quantitative RT-PCR analysis.

miRNA	Sequence	MiRNA	Sequence
bta-let-7a-3p	CTATACAATCTACTGTCTTTC	bta-miR-33b	GTGCATTGCTGTTGCATTGC
bta-miR-103	AGCAGCATTGTACAGGGCTATGA	bta-miR-340	TCCGTCTCAGTTACTTTATAGCC
bta-let-7a-5p	TGAGGTAGTAGGTTGTATAGTT	bta-miR-365-3p	TAATGCCCCATAAAAAATCCTTAT
bta-miR-105a	TCAAATGCTCAGACTCCTGTGGT	bta-miR-342	TCTCACACAGAAATCGCACCCATCT
bta-let-7b	TGAGGTAGTAGGTTGTGTGGTT	bta-miR-365-5p	AGGGACTTTTGGGGGCAGATGTG
bta-miR-105b	TCAAATGCTCAGACTCCTTGGT	bta-miR-345-3p	CCTGAAC TAGGGTCTGGAG
bta-let-7c	TGAGGTAGTAGGTTGTATGGTT	bta-miR-367	GAATTGCACTTTAGCAATGGTGA
bta-miR-106a	AAAAGTGCTTACAGTGCAGGTA	bta-miR-345-5p	GCTGACTCCTAGTCCAGTGCT
bta-let-7d	AGAGGTAGTAGGTTGCATAGTT	bta-miR-369-3p	AATAATACATGGTTGATCTTT
bta-miR-106b	TAAAGTGCTGACAGTGCAGAT	bta-miR-346	TGTCTGCCCGCATGCCTGCCTCT
bta-let-7e	TGAGGTAGGAGGTTGTATAGT	bta-miR-369-5p	ATCGACCGTGTATATTCGC
bta-miR-107	AGCAGCATTGTACAGGGCTATC	bta-miR-34a	TGGCAGTGTCTTAGCTGGTTGT
bta-let-7f	TGAGGTAGTAGATTGTATAGTT	bta-miR-370	GCCTGCTGGGGTGGAACCTGGT
bta-miR-10a	TACCCTGTAGATCCGAATTTGTG	bta-miR-34b	AGGCAGTGTAATTAGCTGATTG
bta-let-7g	TGAGGTAGTAGTTTGTACAGTT	bta-miR-371	AAGTGCCGCCATGTTTTGAGTGT
bta-miR-10b	TACCCTGTAGAACC GAATTTGTG	bta-miR-34c	AGGCAGTGTAGTTAGCTGATTG
bta-let-7i	TGAGGTAGTAGTTTGTGCTGTT	bta-miR-374a	TTATAATACAACCTGATAAGTG
bta-miR-122	TGGAGTGTGACAATGGTGTGTTG	bta-miR-361	TTATCAGAATCTCCAGGGGTAC
bta-miR-1	TGGAATGTAAAGAAGTATGTAT	bta-miR-374b	ATATAATACAACCTGCTAAGTG
bta-miR-124a	TAAGGCACGCGGTGAATGCCAAG	bta-miR-362-3p	AACACACCTATTCAAGGATTC
bta-miR-100	AACCCGTAGATCCGAAC TTGTG	bta-miR-375	TTTTGTTCGTTTCGGCTCGCGTGA
bta-miR-124b	TAAGGCACGCGGTGAATGCCAAG	bta-miR-362-5p	AATCCTTGGAACCTAGGTGTGAGT
bta-miR-101	TACAGTACTGTGATAACTGAA	bta-miR-376a	ATCATAGAGGAAAAATCCACGT
bta-miR-125a	TCCCTGAGACCCTTTAACCTGTG	bta-miR-363	ATTGCACGGTATCCATCTGCG
bta-miR-125b	TCCCTGAGACCCTAACTTGTGA	bta-miR-376b	ATCATAGAGGAAAAATCCATGTT
bta-miR-133b	TTTGGTCCCCTTCAACCAGCTA	bta-miR-376c	GTGGATATTCCTTCTATGTTTA
bta-miR-126-3p	CGTACCGTGAGTAATAATGCG	bta-miR-382	GAAGTTGTTCTGTTGGTATTCG
bta-miR-133c	ATTGTTCCATTTTACCAGC	bta-miR-376d	ATCATAGAGGAAAAATCCACAT
bta-miR-126-5p	CATTATTACTTTTGGTACGCG	bta-miR-383	AGATCAGAAGGTGATTGTGGCT
bta-miR-134	TGTGACTGGTTGACCAGAGTGG	bta-miR-376e	AACATAGAGGAAAAATCCACATT
bta-miR-127	TCGGATCCGTCTGAGCTTGGCT	bta-miR-409a	AGGTTACCCGAGCAACTTTGCAT
bta-miR-135a	TATGGCTTTTTATTCCTATGTGA	bta-miR-377	ATCACACAAAGGCAACTTTTGT
bta-miR-128	TCACAGTGAACCGTCTCTTT	bta-miR-409b	GGGGTTACCCGAGCAACATTC
bta-miR-135b	TATGGCTTTTCATTCCTATGTGA	bta-miR-378	ACTGGACTTGGAGTCAGAAGGC
bta-miR-129	CTTTTTCGCGTCTGGGCTTGCT	bta-miR-410	AATATAACACAGATGGCCTGT
bta-miR-136	ACTCCATTTGTTTTGATGATGGA	bta-miR-378b	ACTTGACTTGGAGTCAGAAGGC
bta-miR-129-3p	AAGCCCTTACCCCAAAAAGCAT	bta-miR-411a	ATAGTAGACCGTATAGCGTACG
bta-miR-137	TTATTGCTTAAGAATACGCGTAG	bta-miR-378c	ACTGGACTTGGAGTCAGAAGT
bta-miR-129-5p	CTTTTTCGCGTCTGGGCTTGCT	bta-miR-411b	TGGTCGACCATAAAACGTACGT
bta-miR-138	AGCTGGTGTGTGAATCAGGCCG	bta-miR-378d	CTGGACTTGGAGTCAGAAGACC
bta-miR-130a	CAGTGCAATGTAAAAAGGGCAT	bta-miR-411c-3p	TGTATGTCAACTGATCCACAGT

bta-miR-139	TCTACAGTGCACGTGTCTCCAGT	bta-miR-379	TGGTAGACTATGGAACGTAGG
bta-miR-130b	CAGTGCAATGATGAAAGGGCAT	bta-miR-411c-5p	GGTTGATCAGAGAACATACATT
bta-miR-140	TACCACAGGGTAGAACCACGGA	bta-miR-380-3p	TATGTAATGTGGTCCACGTCT
bta-miR-132	TAACAGTCTACAGCCATGGTCG	bta-miR-412	ACTTCACCTGGTCCACTAGCTGT
bta-miR-141	TAACACTGTCTGGTAAAGATGG	bta-miR-380-5p	TGGTTGACCATAGAACATGCGC
bta-miR-133a	TTTGGTCCCCCTCAACCAGCTG	bta-miR-421	ATCAACAGACATTAATTGGGCGC
bta-miR-142-3p	AGTGTTTCCTACTTTATGGATG	bta-miR-381	TATACAAGGGCAAGCTCTCTGT
bta-miR-142-5p	CATAAAGTAGAAAGCACTAC	bta-miR-423-3p	AAGCTCGGTCTGAGGCCCTCAGT
bta-miR-151-3p	CTAGACTGAAGTCCTTGAGG	bta-miR-423-5p	TGAGGGGCAGAGAGCGAGACTTT
bta-miR-143	TGAGATGAAGCACTGTAGCTCG	bta-miR-449c	AGGCAGTGCATCTCTAGCTGG
bta-miR-151-5p	TCGAGGAGCTCACAGTCTAGT	bta-miR-424-3p	CAAAACGTGAGGCGCTGCTAT
bta-miR-144	TACAGTATAGATGATGTACTAG	bta-miR-449d	GAAGGCTGTGTGCTGTGGAG
bta-miR-152	TCAGTGCATGACAGAACTTGGG	bta-miR-424-5p	CAGCAGCAATTCATGTTTTGA
bta-miR-145	GTCCAGTTTTCCCAGGAATCCCT	bta-miR-450a	TTTTGCGATGTGTTCTAATAT
bta-miR-153	TTGCATAGTCACAAAAGTGATC	bta-miR-425-3p	ATCGGGAATGTCGTGTCCGCC
bta-miR-146a	TGAGAACTGAATTCCATAGGTTGT	bta-miR-450b	TTTTGCAATATGTTCTGAATA
bta-miR-154a	TAGGTTATCCGTGTAGCCTTCG	bta-miR-425-5p	ATGACACGATCACTCCCGTTGA
bta-miR-146b	TGAGAACTGAATTCCATAGGCTGT	bta-miR-451	AAACCGTTACCATTACTGAGTTT
bta-miR-154b	AGAGGTCTTCCATGGTGCATTCTG	bta-miR-429	TAATACTGTCTGGTAATGCCGT
bta-miR-147	GTGTGCGGAAATGCTTCTGCTA	bta-miR-452	TGTTTGCAGAGGAACTGAGAC
bta-miR-154c	AGATATTGCACGGTTGATCTCT	bta-miR-431	TGTCTTGCAGGCCGTCATGCAGG
bta-miR-148a	TCAGTGCACTACAGAACTTTGT	bta-miR-4523	GACCGAGAGGGCCTCGGCTGT
bta-miR-155	TTAATGCTAATCGTGATAGGGGT	bta-miR-432	TCTTGAGTAGGTCAATGGGTGG
bta-miR-148b	TCAGTGCATCACAGAACTTTGT	bta-miR-453	AGGTTGTCCGTGGTGAGTTCGCA
bta-miR-15a	TAGCAGCACATAATGGTTTGT	bta-miR-433	ATCATGATGGGCTCCTCGGTGT
bta-miR-149-3p	GAGGGAGGGACGGGGGCTGTGC	bta-miR-454	TAGTGCAATATTGCTTATAGGGT
bta-miR-15b	TAGCAGCACATCATGGTTTACA	bta-miR-448	TTGCATATGTAGGATGTCCCAT
bta-miR-149-5p	TCTGGCTCCGTGTCTTCACTCCC	bta-miR-455-3p	GCAGTCCATGGGCATATACACT
bta-miR-16a	TAGCAGCACGTAAATATTGGTG	bta-miR-449a	TGGCAGTGTATTGTTAGCTGGT
bta-miR-150	TCTCCCAACCCTTGTACCAGTGT	bta-miR-455-5p	TATGTGCCTTTGGACTACATC
bta-miR-16b	TAGCAGCACGTAAATATTGGC	bta-miR-449b	AGGCAGTGTATTGTTAGCTGGC
bta-miR-17-3p	ACTGCAGTGAAGGCACTTGT	bta-miR-483	TCACTCCTCTCCTCCCGTCTT
bta-miR-188	CATCCCTTGCATGGTGGAGGGT	bta-miR-484	TCAGGCTCAGTCCCCTCCCGAT
bta-miR-17-5p	CAAAGTGCTTACAGTGCAGGTAGT	bta-miR-496	TGAGTATTACATGGCCAATCTC
bta-miR-18a	TAAGGTGCATCTAGTGCAGATA	bta-miR-485	AGAGGCTGGCCGTGATGAATTCTG
bta-miR-181a	AACATTCAACGCTGTCTGGTGAGTT	bta-miR-497	CAGCAGCACACTGTGGTTTGTA
bta-miR-18b	TAAGGTGCATCTAGTGCAGTTA	bta-miR-486	TCCTGTACTGAGCTGCCCGAG
bta-miR-181b	AACATTTCATTGCTGTCTGGTGGGTT	bta-miR-499	TTAAGACTTGCAGTGATGTTT
bta-miR-190a	TGATATGTTTGATATATTAGGT	bta-miR-487a	AATCATACAGGGACATCCAGT
bta-miR-181c	AACATTCAACCTGTCTGGTGAGTTT	bta-miR-500	TAATCCTTGCTACCTGGGTGAGA
bta-miR-190b	TGATATGTTTGATATTGGGTT	bta-miR-487b	AATCGTACAGGGTCATCCACTT
bta-miR-181d	AACATTTCATTGTTGTCTGGTGGGT	bta-miR-502a	AATGCACCTGGGCAAGGATTCA
bta-miR-191	CAACGGAATCCCAAAAGCAGCTG	bta-miR-488	TTGAAAGGCTGTTTCTTGGTC
bta-miR-182	TTTGGCAATGGTAGAACTCACACT	bta-miR-502b	AATCCACCTGGGCAAGGATTTC
bta-miR-192	CTGACCTATGAATTGACAGCCAG	bta-miR-489	GTGACATCACATATATGGCGAC
bta-miR-183	TATGGCACTGGTAGAATTCAGT	bta-miR-503-3p	GGAGTATTGTTTCTGCTGCCCGG

bta-miR-193a	GGGACTTTGTAGGCCAGTT	bta-miR-490	CAACCTGGAGGACTCCATGCTG
bta-miR-184	TGGACGGAGAACTGATAAGGGT	bta-miR-503-5p	TAGCAGCGGGAACAGTACTG
bta-miR-193a-3p	AACTGGCCTACAAAGTCCCAGT	bta-miR-491	AGTGGGGAACCCCTTCCATGAGG
bta-miR-185	TGGAGAGAAAGGCAGTTCCTGA	bta-miR-504	AGACCCTGGTCTGCACTCTGTC
bta-miR-193a-5p	TGGGTCTTTGCGGGCGAGATGA	bta-miR-493	TGAAGGTCTACTGTGTGCCAGG
bta-miR-186	CAAAGAATTCTCCTTTTGGGCT	bta-miR-505	CGTCAACACTTGCTGGTTTCCT
bta-miR-193b	AACTGGCCCAAAAGTCCCGCTTT	bta-miR-494	TGAAACATACACGGGAAACCTC
bta-miR-187	TCGTGTCTTGTGTTGCAGCCGG	bta-miR-532	CATGCCTTGAGTGTAGGACCGT
bta-miR-194	TGTAACAGCAACTCCATGTGGA	bta-miR-495	AAACAAAATGTTGCACTTCTT
bta-miR-195	TAGCAGCACAGAAATATTGGCA	bta-miR-539	GGAGAAATTATCCTTGGTGTGT
bta-miR-200c	TAATACTGCCGGGTAATGATGGA	bta-miR-541	TGGTGGGCACAGAATCCGGCCT
bta-miR-196a	TAGGTAGTTTCATGTTGTTGGG	bta-miR-582	TTACAGTTGTTCAACCACTTACT
bta-miR-202	TTCCTATGCATATACTTCTTT	bta-miR-542-5p	TCGGGGATCATCATGTCACGAG
bta-miR-196b	TAGGTAGTTTCCTGTTGTTGGGA	bta-miR-584	TGGTTTGCCTGGGACTGAG
bta-miR-204	TTCCCTTTGTCTATCCTATGCCT	bta-miR-543	AAACATTCGCGGTGCACTTCTT
bta-miR-197	TTCACCACCTTCTCCACCCAGC	bta-miR-592	ATTGTGTCAATATGCGATGATGT
bta-miR-205	TCCTTCATTCCACCGGAGTCTG	bta-miR-544a	ATTCTGCATTTTATGCAAGTTC
bta-miR-199a-3p	ACAGTAGTCTGCACATTGGTTA	bta-miR-599	GTTGTGTCAGTTTATCAAAC
bta-miR-206	TGGAATGTAAGGAAGTGTGTGG	bta-miR-544b	ATTCTGCATTTCTAACAAGTTC
bta-miR-199a-5p	CCCAGTGTTCAGACTACCTGTT	bta-miR-615	GGGGGTCCCCGGTGCTCGGATC
bta-miR-208a	ATAAGACGAGCAAAAAGCTTGT	bta-miR-545-3p	ATCAACAAACATTTATTGTGTG
bta-miR-199b	CCCAGTGTTTAGACTATCTGTTC	bta-miR-628	ATGCTGACATATTTACTAGAGG
bta-miR-208b	ATAAGACGAACAAAAGGTTTGT	bta-miR-545-5p	TCAGTAAATGTTTATTGGATG
bta-miR-199c	TACAGTAGTCTGCACATTGG	bta-miR-631	AGACCTGGCTTAGACCTCAGC
bta-miR-20a	TAAAGTGCTTATAGTGCAGGTAG	bta-miR-551a	GCGACCCAATCTTGGTTTCCA
bta-miR-19a	TGTGCAAATCTATGCAAACTGA	bta-miR-652	AATGGCGCCACTAGGGTTGTG
bta-miR-20b	CAAAGTGCTCACAGTGCAGGTA	bta-miR-551b	GGCGACCCATACTTGGTTTCAG
bta-miR-19b	TGTGCAAATCCATGCAAACTGA	bta-miR-653	GTGTTGAAACAATCTCTGTTG
bta-miR-21-3p	AACAGCAGTCGATGGGCTGTCT	bta-miR-562	AAAGCAGCTGTACCATTTAC
bta-miR-200a	TAACACTGTCTGGTAACGATGTT	bta-miR-654	TATGTCTGCTGACCATCACCTT
bta-miR-21-5p	TAGCTTATCAGACTGATGTTGACT	bta-miR-568	ATGTATAAATGTATACACAC
bta-miR-200b	TAATACTGCCTGGTAATGATG	bta-miR-655	ATAATACATGGTTAACCTCTCT
bta-miR-210	ACTGTGCGTGTGACAGCGGCTGA	bta-miR-574	TGAGTGTGTGTGTGTGAGTGTGTG
bta-miR-211	TTCCCTTTGTCTATCCTTTGCC	bta-miR-656	AATATTATACAGTCAACCTCT
bta-miR-22-5p	AGTTCTTCAGTGGCAAGCTTTA	bta-miR-658	GGCGGAGGGAAGCGGGTCCGTTGGT
bta-miR-212	ACCTTGGCTCTAGACTGCTTACT	bta-miR-758	TTTGTGACCTGGTCCACTAACC
bta-miR-221	AGCTACATTGTCTGCTGGGTTT	bta-miR-660	TACCCATTGCATATCGGAGCTG
bta-miR-214	ACAGCAGGCACAGACAGGCAGT	bta-miR-759	GCAGACTGCAAACAATTTTGAC
bta-miR-222	AGCTACATCTGGCTACTGGGT	bta-miR-664a	CAGGCTGGGGTGTGTGTGGATG
bta-miR-215	ATGACCTATGAATTGACAGACA	bta-miR-760-3p	CGGCTCTGGGTCTGTGGGGA
bta-miR-223	TGTCAGTTTGTCAAATACCCCA	bta-miR-664b	TATTCAATTATCTCCCAGCCTAC
bta-miR-216a	TAATCTCAGCTGGCAACTGTGA	bta-miR-760-5p	CCCCTCAGTCCACCAGAGCCCG
bta-miR-224	CAAGTCACTAGTGGTTCCGTTTA	bta-miR-665	ACCAGTAGGCCGAGGCCCTT
bta-miR-216b	AAATCTCTGCAGGCAAATGTGA	bta-miR-761	GCAGCAGGGTGAACTGACACA
bta-miR-23a	ATCACATTGCCAGGGATTTCCA	bta-miR-669	TGTGGGTGTGTGCATGTGCGTG
bta-miR-217	TACTGCATCAGGAAGTATTGGAT	bta-miR-763	CCAGCTGGGAGGAACCACTGGC

bta-miR-23b-3p	ATCACATTGCCAGGGATTACCAC	bta-miR-670	TCCCTGAGTATATGTGGTGAA
bta-miR-218	TTGTGCTTGATCTAACCATGTG	bta-miR-764	GGTGCTCACTCGTCCTTCT
bta-miR-23b-5p	GGGTTCCTGGCATGCTGATTT	bta-miR-671	AGGAAGCCCTGGAGGGGCTGGAG
bta-miR-219	AGAGTTGAGTCTGGACGTCCCG	bta-miR-767	TGCACCATGGTTGTCTGAGCATG
bta-miR-24	GTGCCTACTGAGCTGATATCAGT	bta-miR-677	CTCACTGATGAGCAGCTTCTGAC
bta-miR-219-3p	AGAATTGTGGCTGGACATCTG	bta-miR-769	TGAGACCTCCGGGTCTGAGCT
bta-miR-24-3p	TGGCTCAGTTCAGCAGGAACAG	bta-miR-7	TGGAAGACTAGTGATTTTGTGT
bta-miR-219-5p	TGATTGTCCAAACGCAATTCTT	bta-miR-873	GCAGGAACCTGTGAGTCTCCT
bta-miR-25	CATTGCACTTGTCTCGGTCTGA	bta-miR-708	AAGGAGCTTACAATCTAGCTGGG
bta-miR-22-3p	AAGCTGCCAGTTGAAGAACTG	bta-miR-874	CTGCCCTGGCCCGAGGGACCGA
bta-miR-26a	TTCAAGTAATCCAGGATAGGCT	bta-miR-744	TGCGGGGCTAGGGCTAACAGCA
bta-miR-26b	TTCAAGTAATTCAGGATAGGTT	bta-miR-875	TATACCTCAGTTTATCAGGTG
bta-miR-29d-3p	TAGCACCATTGAAATCGATTA	bta-miR-876	TGGATTTCTTTGTGAATCACCA
bta-miR-26c	AGCCTATCCTGGATTACTTGAA	bta-miR-98	TGAGGTAGTAAGTTGTATTGTT
bta-miR-29d-5p	TGACCGATTTCTCTGGTGTT	bta-miR-877	GTAGAGGAGATGGCGCAGGG
bta-miR-27a-3p	TTCACAGTGGCTAAGTTCCG	bta-miR-99a-3p	CAAGCTCGCTTCTATGGGT
bta-miR-29e	TAGCATCATTTGAAATCAGTGTTT	bta-miR-885	TCCATTACACTACCCTGCCTCT
bta-miR-27a-5p	AGGGCTTAGCTGCTTGAGCA	bta-miR-99a-5p	AACCCGTAGATCCGATCTTGT
bta-miR-301a	CAGTGCAATAGTATTGTCAAAGCAT	bta-miR-9-3p	ATAAAGCTAGATAACCG
bta-miR-27b	TTCACAGTGGCTAAGTTCTGC	bta-miR-99b	CACCCGTAGAACCACCTTGCG
bta-miR-301b	CAGTGCAATGATATTGTCAAAGCAT	bta-miR-9-5p	TCTTTGGTTATCTAGCTGTATG
bta-miR-28	AAGGAGCTCACAGTCTATTGAG	bta-miR-1179	AAGCATTCTTTCATTGGTTGG
bta-miR-302a	AAGTGCTTCCATGTTTTAGTGA	bta-miR-92a	TATTGCACTTGTCGGCCTGT
bta-miR-296-3p	GAGGGTTGGCGGAGGCTTTCC	bta-miR-1185	AGAGGATACCCTTTGTATGTT
bta-miR-302b	TAAGTGCTTCCATGTTTTAGTAG	bta-miR-92b	TATTGCACTCGTCCCGCCTCC
bta-miR-296-5p	GAGGGCCCCCCCCAATCCT	bta-miR-1193	TAGGTCACCCGTTTACTATC
bta-miR-302c	TAAGTGCTTCCATGTTTCAGTGG	bta-miR-93	CAAAGTGCTGTTCTGTCAGGTA
bta-miR-299	TGGTTTACCGTCCCACATACAT	bta-miR-1197	TAGGACACATGGTCTACTTCT
bta-miR-302d	TAAGTGCTTCCATGTTTTAGT	bta-miR-935	CCAGTTACCGCTTCCGCTACCGC
bta-miR-29a	CTAGCACCATCTGAAATCGGTTA	bta-miR-122	TGGAGTGTGACAATGGTGTGTTG
bta-miR-3064	TTGCCACACTGCAACACCTTACA	bta-miR-940	AAGGCTGGGCCCCGCTCCGC
bta-miR-29b	TAGCACCATTGAAATCAGTGTT	bta-miR-1224	GTGAGGACTCGGGAGGTGGAG
bta-miR-30a-5p	TGTAAACATCCTCGACTGGAAGCT	bta-miR-95	TTCAACGGGTATTTATTGAGCA
bta-miR-29c	TAGCACCATTGAAATCGGTTA	bta-miR-1225-3p	CCGAGCCCCTGTGCCGCCCCCAG
bta-miR-30b-3p	CTGGGAGGTGGATGTTTACTT	bta-miR-96	TTTGGCACTAGCACATTTTGTCT
bta-miR-30b-5p	TGTAAACATCCTACACTCAGCT	bta-miR-1246	AATGGATTTTTGGAGCAGG
bta-miR-328	CTGGCCCTCTCTGCCCTTCCGT	bta-miR-1247-3p	CGGGAACGTCGGGACTGGAGC
bta-miR-30c	TGTAAACATCCTACACTCTCAGC	bta-miR-1296	TTAGGGCCCTGGCTCCATCTCC
bta-miR-329a	AACACACCTGGTTAACCTTTTT	bta-miR-1247-5p	ACCCGTCCCGTGCCTCCCGGA
bta-miR-30d	TGTAAACATCCCCGACTGGAAGCT	bta-miR-1298	TTCATTCCGCTGTCCAGATGTA
bta-miR-329b	AGAGGTTTTCTGGGTTTCTGTTT	bta-miR-1248	ACCTTCTGTATAAGCACTGTGCTAAA
bta-miR-30e-5p	TGTAAACATCCTTGACTGGAAGCT	bta-miR-1301	TTGCAGCTGCCTAGGAGTGATTTC
bta-miR-330	GCAAAGCACACGGCTGCAGAGA	bta-miR-1249	ACGCCCTTCCCCCCTTCTTCA
bta-miR-30f	TGTAAACACCTTACACTCTCAGCT	bta-miR-1306	CCACCTCCCCTGCAAACGTCC
bta-miR-331-3p	GCCCTGGGCCTATCCTAGAA	bta-miR-1260b	ATCCCACCACTGCCACCA
bta-miR-31	AGGCAAGATGCTGGCATAGCT	bta-miR-1307	ACTCGGCGTGGCGTCGGTCGTG

bta-miR-331-5p	TCTAGGTATGGTCCCAGG	bta-miR-1271	CTTGGCACCTAGTAAGTACTCA
bta-miR-32	TATTGCACATGACTAAGTTGCAT	bta-miR-1343-3p	CTCCTGGGGCCCCGCACTCTC
bta-miR-335	TCAAGAGCAATAACGAAAAATGT	bta-miR-1277	TACGTAGATATATATGTATTTT
bta-miR-320a	AAAAGCTGGGTTGAGAGGGCGA	bta-miR-1343-5p	TGGGGAGCGGCCCCCGGGCGGG
bta-miR-338	TCCAGCATCAGTGATTTTGTTGA	bta-miR-1281	TCGCCTCCTCCTCTCCC
bta-miR-320b	AGCTGGGTTGAGAGGGTGGT	bta-miR-1388-3p	ATCTCAGGTTTGTGAGCCCGCA
bta-miR-339a	TCCCTGTCCTCCAGGAGCTCAC	bta-miR-1282	TCGTTTGCCTTTTCTGCTT
bta-miR-323	GCACATTACACGGTCGACCTCT	RNT43 snoRNA	CTTATTGACGGGCGGACAGAAAC
bta-miR-339b	TCCCTGTCCTCCAGGAGCTC	bta-miR-1284	TCTGCACAGACCCTGGCTTTTC
bta-miR-324	CGCATCCCCTAGGGCATTGGTGT	Hm/Ms/Rt T1 snRNA	CGACTGCATAATTTGTGGTAGTGG
bta-miR-33a	GTGCATTGTAGTTGCATTGCA	bta-miR-1287	TGCTGGATCAGTGGTTTGAGTC
bta-miR-326	CCTCTGGGCCCTTCCTCCAG	bta-miR-1291	TGGCCCTGACTGAAGACCTGCAGT

Supplementary Table S2A. *In vivo* embryo production data/cow/replicate.

<i>In vivo</i> embryo production data/cow/replicate					
Replicate	Cow	Recovered structures number	Number of corpora lutea	Embryos number	Number of hatched embryos
1	1	12	11	9	8
	2	3	6	3	2
	3	2	5	2	2
	4	3	3	3	2
2	5	6	6	6	5
	6	2	2	2	2
	7	6	7	6	6
	4	3	3	3	1
3	8	10	13	2	2
	9	9	11	6	1
	10	9	9	8	6
	1	11	14	10	6
4	2	5	5	2	1
	3	7	4	5	4
	7	2	5	2	2
	11	10	27	8	7
Total		100	131	77	57
Recovery rate		76,34		Hatching rate	74,03
				Embryo rate	77
Uterine flushes	16				

Supplementary Table S2B. *In vitro* embryo production data/cow/replicate.

<i>In vitro</i> embryo production data/cow/replicate							
Replicate	Cow	Retrieved COCs	Number of aspired follicles	Number of COCs placed in IVM	Number of cleaved	Embryos number	Number of hatched embryos
1	5	12	19	10	8	4	3
	7	4	10	2	0	0	0
	12	9	18	9	8	5	3
	13	14	24	13	8	3	3
	11	29	36	24	24	8	5
2	14	15	21	13	9	3	1
	15	16	30	13	9	4	2
	16	11	20	10	6	2	1
	17	13	23	13	10	2	0
	5	8	16	8	7	4	1
3	7	9	18	8	5	0	0
	11	26	42	19	15	4	3
	12	10	26	9	9	4	2
	13	6	13	6	3	1	0
	15	22	39	21	12	1	0
	17	3	9	3	2	1	1
	18	31	27	30	25	7	4
4	5	4	9	4	4	0	0
	7	9	13	6	6	2	1
	12	28	42	23	19	9	3
	13	13	26	12	9	1	0
	15	18	19	16	14	9	3
	17	3	7	3	2	0	0
	19	55	70	49	40	7	2
	Total	368	577	324	254	81	38
Cleavage rate					78,40		
Blastocyst rate					25,00		
Hatching rate					46,91		

Supplementary Table S3. The full list of detected miRNAs in *in vivo* or *in vitro* produced bovine blastocyst on day 9 of development and their relative expressions (mean and standard deviation).

MicroRNAs	<i>In vivo</i> embryos		<i>In vitro</i> embryos	
	Mean	Standard deviation	Mean	Standard deviation
bta-let-7a-3p	0.00019561	± 0.00015548	0.00024900	± 0.00009066
bta-miR-103	0.01009210	± 0.00417094	0.00910583	± 0.00032550
bta-let-7a-5p	0.00102889	± 0.00043275	0.00143327	± 0.00054595
bta-miR-105b	0.00100288	± 0.00008223	0.00072449	± 0.00024971
bta-let-7c	0.00048252	± 0.00022980	0.00107330	± 0.00072632
bta-miR-106a	0.04709207	± 0.01148939	0.04745499	± 0.01115176
bta-let-7d	0.00043310	± 0.00008868	0.00073535	± 0.00047948
bta-miR-106b	0.01294645	± 0.00332345	0.01367132	± 0.00388258
bta-miR-107	0.00051896	± 0.00010929	0.00033755	± 0.00010651
bta-miR-10b	0.00150646	± 0.00069065	0.00145925	± 0.00019955
bta-miR-101	0.00535394	± 0.00161043	0.00694170	± 0.00050929
bta-miR-125b	0.00066948	± 0.00010212	0.00125058	± 0.00088083
bta-miR-126-3p	0.00117897	± 0.00037793	0.00189008	± 0.00117035
bta-miR-126-5p	0.00030652	± 0.00008095	0.00080830	± 0.00095365
bta-miR-127	0.00593774	± 0.00048056	0.00560551	± 0.00062197
bta-miR-128	0.00031215	± 0.00017914	0.00056861	± 0.00018357
bta-miR-129	0.00125069	± 0.00019896	0.00070954	± 0.00008029
bta-miR-136	0.00036175	± 0.00012283	0.00044771	± 0.00015329
bta-miR-129-3p	0.00032713	± 0.00013142	0.00041723	± 0.00008782
bta-miR-130a	0.00305649	± 0.00042782	0.00296356	± 0.00040414
bta-miR-140	0.00119119	± 0.00040293	0.00110077	± 0.00035717
bta-miR-132	0.00043769	± 0.00004051	0.00027129	± 0.00005601
bta-miR-141	0.00255896	± 0.00073288	0.00449952	± 0.00187482
bta-miR-151-3p	0.00448077	± 0.00028478	0.00481194	± 0.00066370
bta-miR-143	0.00174398	± 0.00006346	0.00195553	± 0.00084234
bta-miR-151-5p	0.00764330	± 0.00064638	0.00848761	± 0.00093736
bta-miR-145	0.00147894	± 0.00035200	0.00136084	± 0.00048216
bta-miR-153	0.05610666	± 0.01965660	0.05223266	± 0.00535927
bta-miR-154a	0.00029886	± 0.00014023	0.00016995	± 0.00011026
bta-miR-154b	0.00513766	± 0.00144257	0.00424489	± 0.00013573
bta-miR-154c	0.00065462	± 0.00032514	0.00053684	± 0.00003369
bta-miR-148a	0.00696205	± 0.00121860	0.00791670	± 0.00100471
bta-miR-155	0.00121265	± 0.00033374	0.00059805	± 0.00003357
bta-miR-148b	0.00483798	± 0.00035306	0.00469465	± 0.00078747
bta-miR-15a	0.00385932	± 0.00005919	0.00375551	± 0.00092024
bta-miR-149-3p	0.04922071	± 0.00784011	0.05179793	± 0.01055426
bta-miR-15b	0.01440904	± 0.00206181	0.01450105	± 0.00222110
bta-miR-16a	0.01327160	± 0.00316299	0.00950138	± 0.00083544
bta-miR-16b	0.02324513	± 0.00137637	0.02122174	± 0.00041660
bta-miR-188	0.00113857	± 0.00023168	0.00094560	± 0.00024711

bta-miR-17-5p	0.00433863	±	0.00064246	0.00472199	±	0.00062064
bta-miR-18b	0.00300545	±	0.00061840	0.00350308	±	0.00050061
bta-miR-190a	0.00084565	±	0.00041701	0.00041360	±	0.00018447
bta-miR-181d	0.00024070	±	0.00007738	0.00018928	±	0.00007634
bta-miR-182	0.00097184	±	0.00058228	0.00077196	±	0.00041660
bta-miR-192	0.00230564	±	0.00021491	0.00193975	±	0.00007900
bta-miR-183	0.00037402	±	0.00010235	0.00047295	±	0.00009644
bta-miR-185	0.00175441	±	0.00008578	0.00136304	±	0.00028939
bta-miR-186	0.00197017	±	0.00047389	0.00231999	±	0.00036357
bta-miR-194	0.00933065	±	0.00182613	0.00864510	±	0.00088603
bta-miR-195	0.00297941	±	0.00033935	0.00323338	±	0.00077855
bta-miR-200c	0.00251165	±	0.00126067	0.00381747	±	0.00193768
bta-miR-196a	0.00068778	±	0.00041357	0.00086020	±	0.00022857
bta-miR-196b	0.00090327	±	0.00018963	0.00122731	±	0.00037800
bta-miR-204	0.00017352	±	0.00005133	0.00029872	±	0.00011624
bta-miR-197	0.00358963	±	0.00031443	0.00342735	±	0.00082802
bta-miR-206	0.00027029	±	0.00009212	0.00079217	±	0.00075802
bta-miR-199c	0.00044728	±	0.00010527	0.00050303	±	0.00045001
bta-miR-20a	0.05281763	±	0.01208532	0.05136144	±	0.01040648
bta-miR-19a	0.02724541	±	0.00134225	0.03178851	±	0.00301427
bta-miR-20b	0.01718959	±	0.00421337	0.01886198	±	0.00556065
bta-miR-19b	0.03088359	±	0.00811145	0.02878541	±	0.00236496
bta-miR-200a	0.00040071	±	0.00003191	0.00044770	±	0.00007153
bta-miR-21-5p	0.00349765	±	0.00035299	0.00250821	±	0.00056779
bta-miR-200b	0.00359076	±	0.00057048	0.00457025	±	0.00091226
bta-miR-210	0.00404239	±	0.00075473	0.00439971	±	0.00167650
bta-miR-211	0.00036209	±	0.00010374	0.00028389	±	0.00002633
bta-miR-22-5p	0.00064453	±	0.00032703	0.00075687	±	0.00024851
bta-miR-221	0.00166429	±	0.00043651	0.00141928	±	0.00060374
bta-miR-214	0.00146213	±	0.00036758	0.00123626	±	0.00039614
bta-miR-222	0.00553275	±	0.00073529	0.00417695	±	0.00074985
bta-miR-215	0.00263838	±	0.00010203	0.00210671	±	0.00020198
bta-miR-23a	0.00459765	±	0.00049309	0.00772201	±	0.00137072
bta-miR-23b-3p	0.00199084	±	0.00046715	0.00192428	±	0.00010974
bta-miR-24-3p	0.00360924	±	0.00093809	0.00355459	±	0.00082537
bta-miR-26a	0.01770618	±	0.00403471	0.02232273	±	0.00300735
bta-miR-26b	0.00244066	±	0.00095686	0.00316376	±	0.00044680
bta-miR-27a-3p	0.00215695	±	0.00022122	0.00284834	±	0.00074771
bta-miR-27a-5p	0.00062273	±	0.00027067	0.00030370	±	0.00025900
bta-miR-301a	0.00030854	±	0.00012196	0.00036348	±	0.00012387
bta-miR-27b	0.00395780	±	0.00065574	0.00438505	±	0.00053493
bta-miR-301b	0.00027868	±	0.00015509	0.00034682	±	0.00013722
bta-miR-28	0.00365774	±	0.00054107	0.00357201	±	0.00029156
bta-miR-302a	0.03293213	±	0.02077480	0.02256076	±	0.00093472
bta-miR-296-3p	0.00240195	±	0.00045656	0.00179624	±	0.00026934
bta-miR-302b	0.02012711	±	0.01304942	0.01582421	±	0.00167810

bta-miR-296-5p	0.00065627	±	0.00021653	0.00079796	±	0.00031106
bta-miR-302c	0.00716608	±	0.00319643	0.00419049	±	0.00049125
bta-miR-302d	0.07679770	±	0.03406295	0.04989294	±	0.00570989
bta-miR-29a	0.00092058	±	0.00055439	0.00096371	±	0.00017894
bta-miR-30a-5p	0.01114437	±	0.00315906	0.01217739	±	0.00182879
bta-miR-29c	0.00081192	±	0.00013363	0.00080222	±	0.00023858
bta-miR-30b-5p	0.00600407	±	0.00081476	0.00687607	±	0.00091466
bta-miR-328	0.00053395	±	0.00006748	0.00083731	±	0.00020635
bta-miR-30c	0.00970462	±	0.00087791	0.01134871	±	0.00181880
bta-miR-30d	0.00877024	±	0.00119509	0.01073094	±	0.00153430
bta-miR-30e-5p	0.01065254	±	0.00205347	0.01043288	±	0.00044486
bta-miR-30f	0.00477990	±	0.00085698	0.00575388	±	0.00042116
bta-miR-331-3p	0.00036629	±	0.00015009	0.00036170	±	0.00026479
bta-miR-331-5p	0.00094442	±	0.00009939	0.00117436	±	0.00028703
bta-miR-338	0.00014649	±	0.00006228	0.00031998	±	0.00001171
bta-miR-339a	0.00303374	±	0.00055775	0.00329998	±	0.00045368
bta-miR-323	16.83896434	±	4.67885491	14.95311302	±	3.15051185
bta-miR-339b	0.00369929	±	0.00035205	0.00328431	±	0.00028122
bta-miR-365-3p	0.00272882	±	0.00095141	0.00260230	±	0.00008534
bta-miR-342	0.00205019	±	0.00020501	0.00233916	±	0.00099276
bta-miR-345-3p	0.00072445	±	0.00038003	0.00072622	±	0.00031795
bta-miR-367	0.00150757	±	0.00065212	0.00098842	±	0.00045936
bta-miR-345-5p	0.00195271	±	0.00028097	0.00137496	±	0.00029049
bta-miR-369-3p	0.00069583	±	0.00009181	0.00069611	±	0.00028968
bta-miR-34a	0.00594443	±	0.00063261	0.00790877	±	0.00091545
bta-miR-34b	0.00076365	±	0.00032192	0.00148144	±	0.00028343
bta-miR-371	0.10373373	±	0.02311246	0.11028635	±	0.01167392
bta-miR-34c	0.00078199	±	0.00017951	0.00114185	±	0.00035997
bta-miR-374a	0.00322869	±	0.00031931	0.00267813	±	0.00110256
bta-miR-361	0.00167978	±	0.00029841	0.00196977	±	0.00008226
bta-miR-374b	0.00305452	±	0.00118339	0.00332398	±	0.00129550
bta-miR-362-3p	0.00058062	±	0.00014293	0.00061680	±	0.00039543
bta-miR-376c	0.00083022	±	0.00025456	0.00107374	±	0.00016896
bta-miR-382	0.00744081	±	0.00157779	0.00651686	±	0.00148894
bta-miR-376d	0.00192669	±	0.00123330	0.00194685	±	0.00033072
bta-miR-376e	0.00128342	±	0.00050704	0.00127672	±	0.00029524
bta-miR-377	0.00095733	±	0.00018161	0.00050187	±	0.00009423
bta-miR-378	0.02032867	±	0.00317408	0.02211128	±	0.00316712
bta-miR-378b	0.02100134	±	0.00213704	0.02020492	±	0.00071082
bta-miR-411a	0.00668933	±	0.00143592	0.00637365	±	0.00174038
bta-miR-378c	0.00181214	±	0.00013053	0.00209372	±	0.00020279
bta-miR-411b	0.00017086	±	0.00004729	0.00024138	±	0.00013289
bta-miR-378d	0.00096326	±	0.00008710	0.00101581	±	0.00027043
bta-miR-411c-3p	0.00107646	±	0.00075621	0.00089149	±	0.00062668
bta-miR-379	0.00395704	±	0.00035887	0.00326964	±	0.00109587
bta-miR-380-3p	0.00077534	±	0.00050409	0.00079761	±	0.00028659

bta-miR-423-3p	0.00358582	±	0.00036201	0.00335939	±	0.00076837
bta-miR-423-5p	0.00293036	±	0.00062161	0.00201775	±	0.00038435
bta-miR-449c	0.00027343	±	0.00016358	0.00049486	±	0.00033296
bta-miR-424-5p	0.00495108	±	0.00300337	0.00608501	±	0.00329812
bta-miR-450a	0.00012386	±	0.00004556	0.00009263	±	0.00001201
bta-miR-425-3p	0.02689226	±	0.00268267	0.02963997	±	0.00176939
bta-miR-425-5p	0.00124945	±	0.00069960	0.00128981	±	0.00017000
bta-miR-451	0.00041119	±	0.00039325	0.00032296	±	0.00022194
bta-miR-429	0.00174514	±	0.00098172	0.00186042	±	0.00030718
bta-miR-431	0.00113324	±	0.00024331	0.00139310	±	0.00040011
bta-miR-453	0.00055154	±	0.00020118	0.00029032	±	0.00005501
bta-miR-433	0.00326913	±	0.00184203	0.00283726	±	0.00034637
bta-miR-449a	0.00146416	±	0.00056579	0.00114448	±	0.00010467
bta-miR-449b	0.00022331	±	0.00001823	0.00026985	±	0.00020813
bta-miR-484	0.00047227	±	0.00026893	0.00054317	±	0.00033999
bta-miR-485	0.00034962	±	0.00016465	0.00022504	±	0.00006334
bta-miR-497	0.00097505	±	0.00009409	0.00078539	±	0.00016165
bta-miR-486	0.00812919	±	0.00080647	0.00624539	±	0.00136517
bta-miR-500	0.00082531	±	0.00002368	0.00070258	±	0.00028909
bta-miR-487b	0.00070760	±	0.00029205	0.00068827	±	0.00030852
bta-miR-488	0.00046082	±	0.00016900	0.00023266	±	0.00012722
bta-miR-502b	0.00064729	±	0.00030205	0.00057499	±	0.00014755
bta-miR-489	0.00207342	±	0.00111553	0.00176032	±	0.00014099
bta-miR-490	0.00053804	±	0.00014029	0.00047437	±	0.00005581
bta-miR-503-5p	0.00133925	±	0.00057386	0.00225543	±	0.00148471
bta-miR-491	0.00024697	±	0.00006636	0.00043817	±	0.00003177
bta-miR-505	0.00358274	±	0.00012161	0.00298595	±	0.00046791
bta-miR-495	0.00054312	±	0.00002139	0.00030401	±	0.00019899
bta-miR-541	0.00270182	±	0.00064275	0.00289501	±	0.00083717
bta-miR-543	0.00063471	±	0.00035640	0.00041968	±	0.00019248
bta-miR-615	2473.66153831	±	981.69037048	2269.34234938	±	199.68362655
bta-miR-545-3p	0.00026471	±	0.00010427	0.00037540	±	0.00025596
bta-miR-631	2.75143629	±	0.69296995	2.29416329	±	0.47498712
bta-miR-652	0.00165430	±	0.00064938	0.00210580	±	0.00078913
bta-miR-654	0.00279036	±	0.00100683	0.00220874	±	0.00070622
bta-miR-655	0.00035345	±	0.00015268	0.00025966	±	0.00021811
bta-miR-656	0.00121692	±	0.00057950	0.00121831	±	0.00022694
bta-miR-658	0.00026815	±	0.00008615	0.00031669	±	0.00012043
bta-miR-660	0.00065688	±	0.00025303	0.00098107	±	0.00036149
bta-miR-664a	0.00897694	±	0.00113948	0.00990049	±	0.00185751
bta-miR-760-3p	0.00095191	±	0.00046853	0.00131962	±	0.00044465
bta-miR-664b	0.00407297	±	0.00137022	0.00387398	±	0.00022907
bta-miR-760-5p	0.00884979	±	0.00256677	0.00911067	±	0.00109603
bta-miR-665	0.01763712	±	0.00383467	0.01413456	±	0.00047305
bta-miR-669	0.02632458	±	0.00192953	0.02073902	±	0.00370289
bta-miR-763	0.00174806	±	0.00043847	0.00167160	±	0.00049915

bta-miR-767	0.00156363	±	0.00047801	0.00126785	±	0.00038108
bta-miR-677	0.06026449	±	0.00427839	0.05771784	±	0.00544186
bta-miR-7	0.00316200	±	0.00069070	0.00260670	±	0.00048739
bta-miR-873	0.00011657	±	0.00006327	0.00019051	±	0.00013366
bta-miR-708	0.00064242	±	0.00022264	0.00062924	±	0.00024341
bta-miR-885	0.00053882	±	0.00022164	0.00051534	±	0.00003628
bta-miR-9-5p	0.02286100	±	0.00488922	0.02101929	±	0.00283921
bta-miR-92a	0.02809677	±	0.00315747	0.02998379	±	0.00449253
bta-miR-92b	0.01607560	±	0.00162459	0.03131500	±	0.00204155
bta-miR-93	0.01460560	±	0.00145452	0.01656063	±	0.00114749
bta-miR-940	0.11401631	±	0.03548335	0.23625264	±	0.04227800
bta-miR-1224	0.13155152	±	0.01305414	0.11876298	±	0.01361125
bta-miR-95	0.00066514	±	0.00027195	0.00061239	±	0.00044656
bta-miR-1225-3p	0.05735802	±	0.00579095	0.06792044	±	0.00384051
bta-miR-96	0.00089956	±	0.00022568	0.00102300	±	0.00008974
bta-miR-1246	6.14129601	±	0.66105241	6.38169775	±	1.16356651
bta-miR-1247-5p	0.02495384	±	0.00232240	0.02796574	±	0.00568562
bta-miR-1298	0.00010778	±	0.00001871	0.00015319	±	0.00004752
bta-miR-1248	0.04320188	±	0.01019685	0.04126168	±	0.00367106
bta-miR-1306	0.00084410	±	0.00019422	0.00085808	±	0.00016079
bta-miR-1260b	1.94269833	±	0.21186565	1.93968898	±	0.08758679
bta-miR-1307	0.03474971	±	0.00187840	0.02954807	±	0.00446303
bta-miR-1271	0.00221634	±	0.00055914	0.00367793	±	0.00054252
bta-miR-1343-3p	0.00538278	±	0.00036305	0.00629780	±	0.00101486
bta-miR-1343-5p	0.14683348	±	0.03020643	0.21358807	±	0.02855362
bta-miR-1281	0.01124480	±	0.00071436	0.01142425	±	0.00061082
bta-miR-1388-3p	0.00178329	±	0.00056731	0.00217368	±	0.00013519
bta-miR-1287	0.00019667	±	0.00005970	0.00028927	±	0.00011935
bta-miR-1291	0.01088534	±	0.00163733	0.01352003	±	0.00198020
bta-let-7g	.	±	.	0.000130961	±	3.87638E-05

Supplementary Table S4. Predicted pathways regulated by miRNAs increased in *in vivo*-produced bovine embryos.

Signaling pathways	Genes predicted to be modulated by miRNAs	Number of total genes	Pvalue	BH
bta05200 Pathways in cancer	107	542	0.0	0.0
bta04014 Ras signaling pathway	56	242	0.0001	0.0088
bta05205 Proteoglycans in cancer	50	205	0.0001	0.0088
bta04360 Axon guidance	43	178	0.0002	0.0106
bta04550 Signaling pathways regulating pluripotency of stem cells	37	142	0.0002	0.0106
bta01521 EGFR tyrosine kinase inhibitor resistance	24	80	0.0004	0.0151
bta04931 Insulin resistance	30	110	0.0004	0.0151
bta04144 Endocytosis	53	245	0.0005	0.0159
bta05214 Glioma	23	77	0.0006	0.0159
bta05215 Prostate cancer	27	98	0.0006	0.0159
bta01522 Endocrine resistance	26	94	0.0007	0.0169
bta04015 Rap1 signaling pathway	47	216	0.0009	0.0199
bta05235 PD-L1 expression and PD-1 checkpoint pathway in cancer	25	93	0.0012	0.0245
bta04514 Cell adhesion molecules (CAMs)	36	158	0.0016	0.0283
bta04810 Regulation of actin cytoskeleton	45	211	0.0016	0.0283
bta05163 Human cytomegalovirus infection	50	245	0.002	0.0331
bta05224 Breast cancer	34	150	0.0023	0.0359
bta04010 MAPK signaling pathway	57	292	0.0025	0.0363
bta04722 Neurotrophin signaling pathway	29	122	0.0026	0.0363
bta04750 Inflammatory mediator regulation of TRP channels	25	103	0.0038	0.0504
bta04066 HIF-1 signaling pathway	26	110	0.0044	0.0507
bta04151 PI3K-Akt signaling pathway	68	373	0.0044	0.0507
bta04730 Long-term depression	17	60	0.0044	0.0507
bta04910 Insulin signaling pathway	31	140	0.0046	0.0508
bta04530 Tight junction	37	177	0.0049	0.051
bta04020 Calcium signaling pathway	41	202	0.005	0.051
bta04625 C-type lectin receptor signaling pathway	25	106	0.0052	0.051
bta05212 Pancreatic cancer	19	76	0.0082	0.0776
bta05211 Renal cell carcinoma	18	71	0.0088	0.0795
bta05220 Chronic myeloid leukemia	19	77	0.0092	0.0795
bta05210 Colorectal cancer	21	89	0.0098	0.0795
bta04659 Th17 cell differentiation	25	113	0.0101	0.0795
bta04660 T cell receptor signaling pathway	24	107	0.0101	0.0795
bta04310 Wnt signaling pathway	33	162	0.0102	0.0795
bta04012 ErbB signaling pathway	20	84	0.0106	0.0803
bta05218 Melanoma	18	73	0.0111	0.0817

bta04911 Insulin secretion	20	85	0.0117	0.0829
bta04922 Glucagon signaling pathway	23	103	0.0121	0.0829
bta04921 Oxytocin signaling pathway	31	152	0.0122	0.0829
bta04923 Regulation of lipolysis in adipocytes	15	58	0.0138	0.0914
bta04919 Thyroid hormone signaling pathway	25	118	0.0156	0.1008
bta04670 Leukocyte transendothelial migration	24	113	0.0171	0.1079
bta05161 Hepatitis B	33	171	0.019	0.1171
bta05100 Bacterial invasion of epithelial cells	17	73	0.0205	0.1187
bta05165 Human papillomavirus infection	59	345	0.0206	0.1187
bta05226 Gastric cancer	30	153	0.0206	0.1187
bta04726 Serotonergic synapse	24	116	0.0217	0.1224
bta05217 Basal cell carcinoma	15	63	0.0243	0.1314
bta04070 Phosphatidylinositol signaling system	21	99	0.0246	0.1314
bta04912 GnRH signaling pathway	20	93	0.0248	0.1314
bta04024 cAMP signaling pathway	41	229	0.0265	0.1335
bta04370 VEGF signaling pathway	14	58	0.0265	0.1335
bta04713 Circadian entrainment	21	100	0.0267	0.1335
bta05160 Hepatitis C	31	164	0.0277	0.1359
bta05213 Endometrial cancer	14	59	0.0295	0.1415
bta04917 Prolactin signaling pathway	18	83	0.03	0.1415
bta04371 Apelin signaling pathway	27	140	0.0312	0.1415
bta04611 Platelet activation	24	121	0.0316	0.1415
bta05017 Spinocerebellar ataxia	20	96	0.0319	0.1415
bta04540 Gap junction	19	90	0.0323	0.1415
bta00310 Lysine degradation	15	66	0.0331	0.1415
bta04924 Renin secretion	16	72	0.0331	0.1415
bta05170 Human immunodeficiency virus 1 infection	41	234	0.0342	0.1416
bta05225 Hepatocellular carcinoma	32	174	0.0342	0.1416
bta05223 Non-small cell lung cancer	15	67	0.0364	0.1466
bta04928 Parathyroid hormone synthesis, secretion and action	21	104	0.0365	0.1466
bta05135 Yersinia infection	25	130	0.0378	0.1495
bta04961 Endocrine and other factor-regulated calcium reabsorption	12	50	0.0392	0.1497
bta05221 Acute myeloid leukemia	15	68	0.04	0.1497
bta04668 TNF signaling pathway	23	118	0.0401	0.1497
bta04935 Growth hormone synthesis, secretion and action	23	118	0.0401	0.1497
bta04970 Salivary secretion	19	93	0.0412	0.1516
bta04915 Estrogen signaling pathway	26	138	0.0418	0.1517
bta04340 Hedgehog signaling pathway	12	51	0.0437	0.1518
bta04720 Long-term potentiation	15	69	0.0439	0.1518
bta05031 Amphetamine addiction	15	69	0.0439	0.1518
bta04072 Phospholipase D signaling pathway	28	152	0.0441	0.1518

bta00601 Glycosphingolipid biosynthesis	8	29	0.0477	0.161
bta05321 Inflammatory bowel disease (IBD)	15	70	0.048	0.161
bta04916 Melanogenesis	20	102	0.0503	0.1666
bta04062 Chemokine signaling pathway	33	188	0.0511	0.1672
bta04064 NF-kappa B signaling pathway	21	109	0.0524	0.1693
bta04140 Autophagy	26	142	0.0534	0.1694
bta04330 Notch signaling pathway	12	53	0.0537	0.1694
bta04934 Cushing syndrome	28	156	0.0556	0.1706
bta04721 Synaptic vesicle cycle	16	78	0.0558	0.1706
bta04640 Hematopoietic cell lineage	21	110	0.056	0.1706
bta04926 Relaxin signaling pathway	24	130	0.0574	0.1724
bta00564 Glycerophospholipid metabolism	20	104	0.0579	0.1724
bta05219 Bladder cancer	10	42	0.0586	0.1725
bta04068 FoxO signaling pathway	24	131	0.061	0.1776
bta04710 Circadian rhythm	8	31	0.0623	0.1795
bta05167 Kaposi sarcoma-associated herpesvirus infection	35	206	0.0634	0.1804
bta05231 Choline metabolism in cancer	19	99	0.064	0.1804
bta04142 Lysosome	24	132	0.0647	0.1805
bta04962 Vasopressin-regulated water reabsorption	11	49	0.0655	0.1808
bta04350 TGF-beta signaling pathway	18	93	0.0662	0.1809
bta04977 Vitamin digestion and absorption	7	26	0.0675	0.1825
bta04071 Sphingolipid signaling pathway	22	120	0.0698	0.1868
bta04725 Cholinergic synapse	21	114	0.0725	0.1905
bta04728 Dopaminergic synapse	24	134	0.0726	0.1905
bta04913 Ovarian steroidogenesis	12	57	0.078	0.2012
bta05412 Arrhythmogenic right ventricular cardiomyopathy (ARVC)	15	76	0.0782	0.2012
bta04130 SNARE interactions in vesicular transport	8	33	0.0795	0.2026
bta04520 Adherens junction	14	70	0.0806	0.2028
bta04510 Focal adhesion	33	198	0.0826	0.2028
bta04261 Adrenergic signaling in cardiomyocytes	26	150	0.0831	0.2028
bta04933 AGE-RAGE signaling pathway in diabetic complications	19	103	0.0833	0.2028
bta04150 mTOR signaling pathway	27	157	0.0839	0.2028
bta04211 Longevity regulating pathway	17	90	0.0842	0.2028
bta04929 GnRH secretion	13	65	0.0898	0.2144
bta04141 Protein processing in endoplasmic reticulum	28	166	0.0931	0.22
bta04920 Adipocytokine signaling pathway	14	72	0.0938	0.22
bta05014 Amyotrophic lateral sclerosis (ALS)	12	60	0.1002	0.2329
bta05132 Salmonella infection	36	224	0.102	0.235
bta01210 2-Oxocarboxylic acid metabolism	5	18	0.1047	0.2392
bta04918 Thyroid hormone synthesis	14	74	0.1082	0.2451

bta00591 Linoleic acid metabolism	8	36	0.1101	0.247
bta04210 Apoptosis	24	142	0.1109	0.247
bta04914 Progesterone-mediated oocyte maturation	16	88	0.1137	0.2499
bta05166 Human T-cell leukemia virus 1 infection	37	234	0.1141	0.2499
bta00590 Arachidonic acid metabolism	15	82	0.1187	0.2578
bta04960 Aldosterone-regulated sodium reabsorption	8	37	0.1216	0.262
bta04925 Aldosterone synthesis and secretion	17	96	0.1228	0.2624
bta04976 Bile secretion	15	83	0.1264	0.2667
bta04650 Natural killer cell mediated cytotoxicity	22	131	0.1275	0.2667
bta04218 Cellular senescence	27	166	0.1278	0.2667
bta00280 Valine, leucine and isoleucine degradation	10	51	0.136	0.2816
bta04658 Th1 and Th2 cell differentiation	17	98	0.1376	0.2827
bta01212 Fatty acid metabolism	11	58	0.1409	0.2872
bta04022 cGMP-PKG signaling pathway	27	169	0.145	0.2913
bta05414 Dilated cardiomyopathy (DCM)	17	99	0.1454	0.2913
bta00760 Nicotinate and nicotinamide metabolism	8	39	0.1462	0.2913
bta05202 Transcriptional misregulation in cancer	30	191	0.1486	0.2939
bta04390 Hippo signaling pathway	25	156	0.1525	0.2969
bta04666 Fc gamma R-mediated phagocytosis	16	93	0.1525	0.2969
bta05169 Epstein-Barr virus infection	35	228	0.1541	0.2969
bta05230 Central carbon metabolism in cancer	12	66	0.1546	0.2969
bta01230 Biosynthesis of amino acids	13	73	0.1571	0.2995
bta04662 B cell receptor signaling pathway	15	87	0.1602	0.3032
bta04630 JAK-STAT signaling pathway	31	202	0.1708	0.321
bta05030 Cocaine addiction	9	48	0.1784	0.3329
bta04978 Mineral absorption	10	55	0.1822	0.3376
bta04213 Longevity regulating pathway	11	62	0.1848	0.3401
bta04971 Gastric acid secretion	13	76	0.1876	0.3429
bta04270 Vascular smooth muscle contraction	21	133	0.1923	0.349
bta04114 Oocyte meiosis	19	119	0.1948	0.3512
bta04664 Fc epsilon RI signaling pathway	12	70	0.1978	0.3542
bta00230 Purine metabolism	21	134	0.2004	0.3564
bta05145 Toxoplasmosis	18	113	0.2049	0.358
bta00020 Citrate cycle (TCA cycle)	6	30	0.2059	0.358
bta01040 Biosynthesis of unsaturated fatty acids	6	30	0.2059	0.358
bta04657 IL-17 signaling pathway	15	92	0.2083	0.358
bta05032 Morphine addiction	15	92	0.2083	0.358
bta01524 Platinum drug resistance	13	78	0.2094	0.358
bta05134 Legionellosis	10	58	0.2209	0.3752

bta05222 Small cell lung cancer	15	94	0.2292	0.3869
bta03420 Nucleotide excision repair	8	45	0.2325	0.3875
bta04216 Ferroptosis	8	45	0.2325	0.3875
bta04137 Mitophagy	11	66	0.2341	0.3877
bta05146 Amoebiasis	18	117	0.2427	0.3995
bta00561 Glycerolipid metabolism	11	67	0.2472	0.4044
bta04120 Ubiquitin mediated proteolysis	21	140	0.2526	0.4107
bta05168 Herpes simplex virus 1 infection	56	403	0.2542	0.4108
bta05033 Nicotine addiction	7	40	0.2644	0.4237
bta04380 Osteoclast differentiation	20	134	0.2654	0.4237
bta01200 Carbon metabolism	17	113	0.2767	0.4342
bta04724 Glutamatergic synapse	17	113	0.2767	0.4342
bta04727 GABAergic synapse	14	91	0.2769	0.4342
bta04215 Apoptosis	6	34	0.2823	0.4375
bta05340 Primary immunodeficiency	7	41	0.2823	0.4375
bta05410 Hypertrophic cardiomyopathy (HCM)	14	92	0.2889	0.4448
bta04217 Necroptosis	25	174	0.2904	0.4448
bta04672 Intestinal immune network for IgA production	9	56	0.2947	0.4488
bta05142 Chagas disease (American trypanosomiasis)	17	115	0.2983	0.4517
bta00030 Pentose phosphate pathway	5	28	0.3027	0.4529
bta00650 Butanoate metabolism	5	28	0.3027	0.4529
bta04152 AMPK signaling pathway	18	123	0.3042	0.4529
bta04927 Cortisol synthesis and secretion	10	65	0.3211	0.4733
bta00790 Folate biosynthesis	6	36	0.3227	0.4733
bta04060 Cytokine-cytokine receptor interaction	44	323	0.3233	0.4733
bta00062 Fatty acid elongation	5	29	0.3255	0.4739
bta00562 Inositol phosphate metabolism	11	73	0.3301	0.478
bta00630 Glyoxylate and dicarboxylate metabolism	5	30	0.3484	0.4979
bta03460 Fanconi anemia pathway	8	52	0.3493	0.4979
bta00140 Steroid hormone biosynthesis	10	67	0.3515	0.4979
bta04932 Non-alcoholic fatty liver disease (NAFLD)	22	158	0.3529	0.4979
bta05164 Influenza A	25	181	0.3532	0.4979
bta00514 Other types of O-glycan biosynthesis	7	45	0.3563	0.4996
bta05152 Tuberculosis	27	198	0.3679	0.5127
bta05162 Measles	21	152	0.3695	0.5127
bta05418 Fluid shear stress and atherosclerosis	20	145	0.3766	0.5198
bta04115 p53 signaling pathway	11	77	0.3882	0.5303
bta05133 Pertussis	11	77	0.3882	0.5303
bta00380 Tryptophan metabolism	7	47	0.3941	0.5356
bta05216 Thyroid cancer	6	40	0.405	0.5476
bta00640 Propanoate metabolism	5	33	0.4173	0.5582

bta04620 Toll-like receptor signaling pathway	15	110	0.4179	0.5582
bta00240 Pyrimidine metabolism	8	56	0.4192	0.5582
bta04061 Viral protein interaction with cytokine and cytokine receptor	13	95	0.4247	0.5627
bta00600 Sphingolipid metabolism	7	49	0.4318	0.5693
bta05206 MicroRNAs in cancer	38	292	0.435	0.5707
bta00410 beta-Alanine metabolism	5	34	0.44	0.5744
bta03320 PPAR signaling pathway	11	81	0.4469	0.5805
bta04723 Retrograde endocannabinoid signaling	20	152	0.451	0.583
bta01100 Metabolic pathways	193	1527	0.4587	0.5901
bta00513 Various types of N-glycan biosynthesis	6	43	0.4663	0.5941
bta01523 Antifolate resistance	6	43	0.4663	0.5941
bta04145 Phagosome	22	170	0.4736	0.6005
bta03022 Basal transcription factors	6	44	0.4864	0.6124
bta00565 Ether lipid metabolism	7	52	0.4876	0.6124
bta05416 Viral myocarditis	10	76	0.49	0.6125
bta04612 Antigen processing and presentation	11	85	0.5049	0.6275
bta00250 Alanine, aspartate and glutamate metabolism	5	37	0.5067	0.6275
bta05140 Leishmaniasis	10	78	0.5201	0.6411
bta04930 Type II diabetes mellitus	6	46	0.5258	0.6451
bta00620 Pyruvate metabolism	5	38	0.5283	0.6452
bta04742 Taste transduction	10	79	0.535	0.65
bta05203 Viral carcinogenesis	30	241	0.5372	0.65
bta00270 Cysteine and methionine metabolism	6	49	0.5824	0.7015
bta04714 Thermogenesis	29	239	0.5882	0.7053
bta03008 Ribosome biogenesis in eukaryotes	10	83	0.5925	0.7073
bta04080 Neuroactive ligand-receptor interaction	44	363	0.5985	0.7092
bta04979 Cholesterol metabolism	6	50	0.6005	0.7092
bta00071 Fatty acid degradation	5	42	0.6097	0.7092
bta03440 Homologous recombination	5	42	0.6097	0.7092
bta04940 Type I diabetes mellitus	7	59	0.6098	0.7092
bta04621 NOD-like receptor signaling pathway	22	184	0.6102	0.7092
bta00480 Glutathione metabolism	7	60	0.626	0.7244
bta00510 N-Glycan biosynthesis	6	52	0.6353	0.732
bta04973 Carbohydrate digestion and absorption	5	44	0.6472	0.7397
bta04974 Protein digestion and absorption	14	121	0.6476	0.7397
bta03018 RNA degradation	9	79	0.6537	0.7418
bta03040 Spliceosome	17	147	0.655	0.7418
bta04110 Cell cycle	14	123	0.6693	0.7545
bta04260 Cardiac muscle contraction	10	89	0.6719	0.7545
bta03050 Proteasome	5	46	0.6823	0.7629

bta04975 Fat digestion and absorption	5	48	0.715	0.7928
bta05332 Graft-versus-host disease	5	48	0.715	0.7928
bta04972 Pancreatic secretion	11	102	0.7218	0.797
bta04623 Cytosolic DNA-sensing pathway	7	67	0.7277	0.8002
bta00520 Amino sugar and nucleotide sugar metabolism	5	50	0.7452	0.816
bta03013 RNA transport	19	178	0.7735	0.8435
bta05010 Alzheimer disease	19	180	0.7877	0.8555
bta00010 Glycolysis Gluconeogenesis	6	64	0.8031	0.8687
bta05330 Allograft rejection	5	56	0.8214	0.8839
bta05016 Huntington disease	29	274	0.8239	0.8839
bta03015 mRNA surveillance pathway	9	95	0.8284	0.8852
bta05144 Malaria	5	59	0.852	0.9067
bta04512 ECM-receptor interaction	8	89	0.8563	0.9077
bta02010 ABC transporters	5	60	0.8612	0.9085
bta05034 Alcoholism	23	229	0.8639	0.9085
bta04622 RIG-I-like receptor signaling pathway	9	102	0.8792	0.9209
bta04146 Peroxisome	7	84	0.8901	0.9261
bta05323 Rheumatoid arthritis	9	104	0.8912	0.9261
bta00970 Aminoacyl-tRNA biosynthesis	5	66	0.9066	0.938
bta05204 Chemical carcinogenesis	6	77	0.9097	0.938
bta05320 Autoimmune thyroid disease	5	71	0.934	0.9593
bta05012 Parkinson disease	12	150	0.9536	0.9722
bta00983 Drug metabolism	5	76	0.9539	0.9722
bta04610 Complement and coagulation cascades	5	92	0.9864	0.9986
bta05150 Staphylococcus aureus infection	6	105	0.9873	0.9986
bta00190 Oxidative phosphorylation	7	140	0.9978	1.0
bta03010 Ribosome	5	160	1.0	1.0
bta04740 Olfactory transduction	27	1145	1.0	1.0

Supplementary Table S5. Predicted pathways modulated by miRNAs increased in *in vitro*-produced bovine embryos.

Signaling pathways	Genes predicted to be modulated by miRNAs	Number of total genes	Pvalue	BH
bta04144 Endocytosis	102	245	0.0	0.0
bta04810 Regulation of actin cytoskeleton	89	211	0.0001	0.0103
bta05205 Proteoglycans in cancer	86	205	0.0001	0.0103
bta04072 Phospholipase D signaling pathway	66	152	0.0003	0.0186
bta04390 Hippo signaling pathway	68	156	0.0003	0.0186
bta04014 Ras signaling pathway	94	242	0.0005	0.0258
bta04360 Axon guidance	73	178	0.0006	0.0266
bta05100 Bacterial invasion of epithelial cells	37	73	0.0008	0.0279
bta04934 Cushing syndrome	65	156	0.0009	0.0279
bta05200 Pathways in cancer	183	542	0.0009	0.0279
bta04012 ErbB signaling pathway	40	84	0.0012	0.031
bta04910 Insulin signaling pathway	59	140	0.0012	0.031
bta04140 Autophagy	59	142	0.0015	0.0358
bta04310 Wnt signaling pathway	64	162	0.0026	0.0576
bta01100 Metabolic pathways	457	1527	0.0028	0.0579
bta04550 Signaling pathways regulating pluripotency of stem cells	57	142	0.0032	0.062
bta04722 Neurotrophin signaling pathway	50	122	0.004	0.0707
bta01521 EGFR tyrosine kinase inhibitor resistance	36	80	0.0042	0.0707
bta04010 MAPK signaling pathway	102	292	0.0046	0.0707
bta05231 Choline metabolism in cancer	42	99	0.0048	0.0707
bta04068 FoxO signaling pathway	52	131	0.0055	0.0707
bta04261 Adrenergic signaling in cardiomyocytes	58	150	0.0055	0.0707
bta04520 Adherens junction	32	70	0.0055	0.0707
bta01522 Endocrine resistance	40	94	0.0056	0.0707
bta04710 Circadian rhythm	18	31	0.0057	0.0707
bta04931 Insulin resistance	45	110	0.0061	0.0712
bta00564 Glycerophospholipid metabolism	43	104	0.0062	0.0712
bta04070 Phosphatidylinositol signaling system	41	99	0.0073	0.0756
bta04666 Fc gamma R-mediated phagocytosis	39	93	0.0074	0.0756
bta04137 Mitophagy	30	66	0.0075	0.0756
bta04218 Cellular senescence	62	166	0.0077	0.0756
bta05224 Breast cancer	57	150	0.0078	0.0756
bta04024 cAMP signaling pathway	81	229	0.0081	0.0761
bta05212 Pancreatic cancer	33	76	0.0088	0.0802
bta05214 Glioma	33	77	0.0102	0.0903
bta04916 Melanogenesis	41	102	0.0106	0.0913

bta04015 Rap1 signaling pathway	76	216	0.0109	0.0913
bta04392 Hippo signaling pathway	16	29	0.0125	0.098
bta04062 Chemokine signaling pathway	67	188	0.0129	0.098
bta05163 Human cytomegalovirus infection	84	245	0.0129	0.098
bta04152 AMPK signaling pathway	47	123	0.0133	0.098
bta00600 Sphingolipid metabolism	23	49	0.0134	0.098
bta04071 Sphingolipid signaling pathway	46	120	0.0136	0.098
bta04150 mTOR signaling pathway	57	157	0.0154	0.1078
bta05211 Renal cell carcinoma	30	71	0.0158	0.1078
bta04925 Aldosterone synthesis and secretion	38	96	0.016	0.1078
bta04921 Oxytocin signaling pathway	55	152	0.0179	0.1181
bta04022 cGMP-PKG signaling pathway	60	169	0.0186	0.1201
bta04664 Fc epsilon RI signaling pathway	29	70	0.021	0.1275
bta05225 Hepatocellular carcinoma	61	174	0.0214	0.1275
bta04919 Thyroid hormone signaling pathway	44	118	0.0217	0.1275
bta04935 Growth hormone synthesis, secretion and action	44	118	0.0217	0.1275
bta04370 VEGF signaling pathway	25	58	0.0218	0.1275
bta04020 Calcium signaling pathway	69	202	0.0233	0.1336
bta04350 TGF-beta signaling pathway	36	93	0.0237	0.1336
bta05170 Human immunodeficiency virus 1 infection	78	234	0.0256	0.1365
bta05226 Gastric cancer	54	153	0.0262	0.1365
bta04660 T cell receptor signaling pathway	40	107	0.0267	0.1365
bta05135 Yersinia infection	47	130	0.0268	0.1365
bta04371 Apelin signaling pathway	50	140	0.027	0.1365
bta04920 Adipocytokine signaling pathway	29	72	0.0272	0.1365
bta04530 Tight junction	61	177	0.0273	0.1365
bta05215 Prostate cancer	37	98	0.0288	0.1417
bta00760 Nicotinate and nicotinamide metabolism	18	39	0.0297	0.1439
bta05210 Colorectal cancer	34	89	0.031	0.1465
bta05418 Fluid shear stress and atherosclerosis	51	145	0.0312	0.1465
bta00910 Nitrogen metabolism	10	17	0.0331	0.1509
bta05132 Salmonella infection	74	224	0.0331	0.1509
bta04510 Focal adhesion	66	198	0.0368	0.1653
bta04911 Insulin secretion	32	85	0.0406	0.1786
bta04668 TNF signaling pathway	42	118	0.0409	0.1786
bta00310 Lysine degradation	26	66	0.042	0.1808
bta00562 Inositol phosphate metabolism	28	73	0.045	0.1834
bta04340 Hedgehog signaling pathway	21	51	0.0462	0.1834
bta04976 Bile secretion	31	83	0.0464	0.1834
bta04912 GnRH signaling pathway	34	93	0.0473	0.1834
bta05223 Non-small cell lung cancer	26	67	0.0473	0.1834

bta05235 PD-L1 expression and PD-1 checkpoint pathway in cancer	34	93	0.0473	0.1834
bta04926 Relaxin signaling pathway	45	130	0.0484	0.1834
bta04130 SNARE interactions in vesicular transport	15	33	0.0485	0.1834
bta04115 p53 signaling pathway	29	77	0.0491	0.1834
bta04713 Circadian entrainment	36	100	0.0491	0.1834
bta05220 Chronic myeloid leukemia	29	77	0.0491	0.1834
bta04728 Dopaminergic synapse	46	134	0.051	0.1882
bta04146 Peroxisome	31	84	0.0516	0.1882
bta04611 Platelet activation	42	121	0.0533	0.1921
bta01524 Platinum drug resistance	29	78	0.0546	0.1946
bta04142 Lysosome	45	132	0.0571	0.2011
bta04924 Renin secretion	27	72	0.0578	0.2013
bta04270 Vascular smooth muscle contraction	45	133	0.0618	0.2109
bta04971 Gastric acid secretion	28	76	0.0625	0.2109
bta04625 C-type lectin receptor signaling pathway	37	106	0.0626	0.2109
bta04659 Th17 cell differentiation	39	113	0.0639	0.213
bta04750 Inflammatory mediator regulation of TRP channels	36	103	0.0647	0.2134
bta00230 Purine metabolism	45	134	0.0667	0.217
bta04211 Longevity regulating pathway	32	90	0.0672	0.217
bta00561 Glycerolipid metabolism	25	67	0.0683	0.2183
bta01212 Fatty acid metabolism	22	58	0.0746	0.2319
bta04923 Regulation of lipolysis in adipocytes	22	58	0.0746	0.2319
bta05219 Bladder cancer	17	42	0.0748	0.2319
bta04721 Synaptic vesicle cycle	28	78	0.0765	0.2348
bta04927 Cortisol synthesis and secretion	24	65	0.0785	0.2375
bta00670 One carbon pool by folate	9	18	0.0789	0.2375
bta04064 NF-kappa B signaling pathway	37	109	0.0807	0.2405
bta04670 Leukocyte transendothelial migration	38	113	0.0845	0.2495
bta00240 Pyrimidine metabolism	21	56	0.0861	0.2518
bta04917 Prolactin signaling pathway	29	83	0.0894	0.2575
bta05162 Measles	49	152	0.0897	0.2575
bta04961 Endocrine and other factor-regulated calcium reabsorption	19	50	0.0914	0.2593
bta05166 Human T-cell leukemia virus 1 infection	72	234	0.0922	0.2593
bta00512 Mucin type O-glycan biosynthesis	13	31	0.0932	0.2593
bta04928 Parathyroid hormone synthesis, secretion and action	35	104	0.0937	0.2593
bta05167 Kaposi sarcoma-associated herpesvirus infection	64	206	0.0952	0.2612
bta05161 Hepatitis B	54	171	0.098	0.2665
bta04727 GABAergic synapse	31	91	0.0994	0.2679
bta05032 Morphine addiction	31	92	0.108	0.2886

bta00514 Other types of O-glycan biosynthesis	17	45	0.1094	0.2899
bta04141 Protein processing in endoplasmic reticulum	52	166	0.1113	0.2924
bta05017 Spinocerebellar ataxia	32	96	0.113	0.2935
bta04933 AGE-RAGE signaling pathway in diabetic complications	34	103	0.1136	0.2935
bta00533 Glycosaminoglycan biosynthesis	7	14	0.1146	0.2936
bta05031 Amphetamine addiction	24	69	0.117	0.2973
bta05169 Epstein-Barr virus infection	69	228	0.118	0.2974
bta05412 Arrhythmogenic right ventricular cardiomyopathy (ARVC)	26	76	0.119	0.2975
bta05230 Central carbon metabolism in cancer	23	66	0.1214	0.3011
bta05217 Basal cell carcinoma	22	63	0.1259	0.3098
bta01040 Biosynthesis of unsaturated fatty acids	12	30	0.1272	0.3105
bta05014 Amyotrophic lateral sclerosis (ALS)	21	60	0.1307	0.3165
bta04151 PI3K-Akt signaling pathway	108	373	0.1317	0.3165
bta04913 Ovarian steroidogenesis	20	57	0.1357	0.3235
bta00900 Terpenoid backbone biosynthesis	9	21	0.1367	0.3235
bta05165 Human papillomavirus infection	100	345	0.1393	0.3271
bta00410 beta-Alanine metabolism	13	34	0.1408	0.3282
bta05410 Hypertrophic cardiomyopathy (HCM)	30	92	0.1419	0.3283
bta04922 Glucagon signaling pathway	33	103	0.1468	0.3371
bta04216 Ferroptosis	16	45	0.1583	0.3608
bta04964 Proximal tubule bicarbonate reclamation	9	22	0.1598	0.3614
bta05142 Chagas disease (American trypanosomiasis)	36	115	0.1609	0.3614
bta04114 Oocyte meiosis	37	119	0.1653	0.3687
bta00270 Cysteine and methionine metabolism	17	49	0.1683	0.37
bta04962 Vasopressin-regulated water reabsorption	17	49	0.1683	0.37
bta04658 Th1 and Th2 cell differentiation	31	98	0.1696	0.3703
bta00062 Fatty acid elongation	11	29	0.1714	0.3703
bta05133 Pertussis	25	77	0.172	0.3703
bta04915 Estrogen signaling pathway	42	138	0.1757	0.3726
bta04061 Viral protein interaction with cytokine and cytokine receptor	30	95	0.1761	0.3726
bta05145 Toxoplasmosis	35	113	0.1778	0.3726
bta04614 Renin-angiotensin system	10	26	0.1779	0.3726
bta04210 Apoptosis	43	142	0.1796	0.3737
bta05414 Dilated cardiomyopathy (DCM)	31	99	0.1814	0.3749
bta00260 Glycine, serine and threonine metabolism	15	43	0.1828	0.3753
bta00515 Mannose type O-glycan biosynthesis	9	23	0.1846	0.3765
bta03410 Base excision repair	12	33	0.186	0.3769
bta04725 Cholinergic synapse	35	114	0.1891	0.3807

bta00100 Steroid biosynthesis	8	20	0.1913	0.3826
bta04514 Cell adhesion molecules (CAMs)	47	158	0.194	0.3855
bta04120 Ubiquitin mediated proteolysis	42	140	0.1963	0.3876
bta04960 Aldosterone-regulated sodium reabsorption	13	37	0.199	0.3904
bta04744 Phototransduction	10	27	0.2022	0.3942
bta04540 Gap junction	28	90	0.2036	0.3945
bta04215 Apoptosis	12	34	0.208	0.4005
bta04978 Mineral absorption	18	55	0.2119	0.4055
bta05218 Melanoma	23	73	0.2165	0.4113
bta05160 Hepatitis C	48	164	0.2176	0.4113
bta05213 Endometrial cancer	19	59	0.2203	0.4139
bta00071 Fatty acid degradation	14	42	0.2319	0.4331
bta05020 Prion diseases	11	32	0.2423	0.4498
bta04970 Salivary secretion	28	93	0.2462	0.4543
bta03013 RNA transport	51	178	0.2481	0.4547
bta00531 Glycosaminoglycan degradation	8	22	0.2506	0.4547
bta04979 Cholesterol metabolism	16	50	0.2517	0.4547
bta05221 Acute myeloid leukemia	21	68	0.2528	0.4547
bta00601 Glycosphingolipid biosynthesis	10	29	0.2544	0.4547
bta04110 Cell cycle	36	123	0.2552	0.4547
bta05222 Small cell lung cancer	28	94	0.2612	0.4615
bta05152 Tuberculosis	56	198	0.2621	0.4615
bta00770 Pantothenate and CoA biosynthesis	7	19	0.2635	0.4615
bta05202 Transcriptional misregulation in cancer	54	191	0.2673	0.4641
bta04136 Autophagy	11	33	0.268	0.4641
bta04724 Glutamatergic synapse	33	113	0.2698	0.4647
bta03015 mRNA surveillance pathway	28	95	0.2766	0.4737
bta04640 Hematopoietic cell lineage	32	110	0.2798	0.4754
bta00340 Histidine metabolism	8	23	0.2822	0.4754
bta03060 Protein export	8	23	0.2822	0.4754
bta00330 Arginine and proline metabolism	15	48	0.2856	0.4786
bta00565 Ether lipid metabolism	16	52	0.2939	0.4898
bta04380 Osteoclast differentiation	38	134	0.3027	0.5018
bta04730 Long-term depression	18	60	0.3085	0.5087
bta00603 Glycosphingolipid biosynthesis	6	17	0.3164	0.519
bta04930 Type II diabetes mellitus	14	46	0.3234	0.5277
bta04662 B cell receptor signaling pathway	25	87	0.3283	0.5328
bta04066 HIF-1 signaling pathway	31	110	0.3362	0.54
bta04620 Toll-like receptor signaling pathway	31	110	0.3362	0.54
bta00513 Various types of N-glycan biosynthesis	13	43	0.34	0.5433
bta00380 Tryptophan metabolism	14	47	0.3476	0.5526
bta00591 Linoleic acid metabolism	11	36	0.3494	0.5526
bta03320 PPAR signaling pathway	23	81	0.3554	0.5572

bta00061 Fatty acid biosynthesis	6	18	0.3559	0.5572
bta00592 alpha-Linolenic acid metabolism	9	29	0.3595	0.56
bta04726 Serotonergic synapse	32	116	0.371	0.571
bta04975 Fat digestion and absorption	14	48	0.3721	0.571
bta05030 Cocaine addiction	14	48	0.3721	0.571
bta03460 Fanconi anemia pathway	15	52	0.3782	0.5775
bta04672 Intestinal immune network for IgA production	16	56	0.3837	0.5817
bta05340 Primary immunodeficiency	12	41	0.3847	0.5817
bta04972 Pancreatic secretion	28	102	0.3916	0.5893
bta00620 Pyruvate metabolism	11	38	0.4057	0.606
bta03430 Mismatch repair	7	23	0.4066	0.606
bta04929 GnRH secretion	18	65	0.415	0.6123
bta04914 Progesterone-mediated oocyte maturation	24	88	0.4151	0.6123
bta04657 IL-17 signaling pathway	25	92	0.418	0.6123
bta04720 Long-term potentiation	19	69	0.4187	0.6123
bta00604 Glycosphingolipid biosynthesis	5	16	0.4255	0.6193
bta04145 Phagosome	45	170	0.4292	0.6217
bta04213 Longevity regulating pathway	17	62	0.4335	0.6247
bta00532 Glycosaminoglycan biosynthesis	6	20	0.4353	0.6247
bta04918 Thyroid hormone synthesis	20	74	0.4427	0.6297
bta00534 Glycosaminoglycan biosynthesis	7	24	0.4428	0.6297
bta00280 Valine, leucine and isoleucine degradation	14	51	0.4466	0.6322
bta00650 Butanoate metabolism	8	28	0.449	0.6327
bta04621 NOD-like receptor signaling pathway	48	184	0.46	0.6452
bta04932 Non-alcoholic fatty liver disease (NAFLD)	41	158	0.4788	0.6686
bta03420 Nucleotide excision repair	12	45	0.4923	0.6844
bta04330 Notch signaling pathway	14	53	0.4959	0.6863
bta00730 Thiamine metabolism	5	18	0.5113	0.7013
bta01210 2-Oxocarboxylic acid metabolism	5	18	0.5113	0.7013
bta00630 Glyoxylate and dicarboxylate metabolism	8	30	0.515	0.7033
bta00520 Amino sugar and nucleotide sugar metabolism	13	50	0.5195	0.7063
bta05321 Inflammatory bowel disease (IBD)	18	70	0.5231	0.7081
bta00140 Steroid hormone biosynthesis	17	67	0.5442	0.7306
bta03018 RNA degradation	20	79	0.5444	0.7306
bta00220 Arginine biosynthesis	5	19	0.5522	0.7379
bta05416 Viral myocarditis	19	76	0.5645	0.7505
bta00480 Glutathione metabolism	15	60	0.567	0.7505
bta00510 N-Glycan biosynthesis	13	52	0.5689	0.7505
bta04973 Carbohydrate digestion and absorption	11	44	0.5716	0.7508
bta05164 Influenza A	45	181	0.5758	0.7518
bta00500 Starch and sucrose metabolism	8	32	0.5781	0.7518

bta04966 Collecting duct acid secretion	7	28	0.5814	0.7518
bta04260 Cardiac muscle contraction	22	89	0.582	0.7518
bta01230 Biosynthesis of amino acids	18	73	0.5855	0.7531
bta03440 Homologous recombination	10	42	0.6267	0.8028
bta00982 Drug metabolism	15	63	0.6325	0.8069
bta00020 Citrate cycle (TCA cycle)	7	30	0.644	0.8182
bta01523 Antifolate resistance	10	43	0.6521	0.8224
bta04950 Maturity onset diabetes of the young	6	26	0.6526	0.8224
bta00350 Tyrosine metabolism	9	39	0.6579	0.8257
bta00590 Arachidonic acid metabolism	19	82	0.6778	0.845
bta05140 Leishmaniasis	18	78	0.6811	0.845
bta05216 Thyroid cancer	9	40	0.6832	0.845
bta05206 MicroRNAs in cancer	70	292	0.6842	0.845
bta04217 Necroptosis	41	174	0.6906	0.8495
bta03008 Ribosome biogenesis in eukaryotes	19	83	0.6951	0.8498
bta00360 Phenylalanine metabolism	5	23	0.6963	0.8498
bta05134 Legionellosis	13	58	0.7024	0.8513
bta05146 Amoebiasis	27	117	0.703	0.8513
bta00250 Alanine, aspartate and glutamate metabolism	8	37	0.7162	0.8639
bta04650 Natural killer cell mediated cytotoxicity	30	131	0.7228	0.8678
bta00640 Propanoate metabolism	7	33	0.7267	0.8678
bta04612 Antigen processing and presentation	19	85	0.7278	0.8678
bta00051 Fructose and mannose metabolism	7	34	0.7512	0.89
bta05168 Herpes simplex virus 1 infection	95	403	0.7537	0.89
bta00053 Ascorbate and aldarate metabolism	5	25	0.7551	0.89
bta01200 Carbon metabolism	25	113	0.7627	0.8956
bta04723 Retrograde endocannabinoid signaling	34	152	0.7721	0.9032
bta00563 Glycosylphosphatidylinositol (GPI)-anchor biosynthesis	5	26	0.781	0.9102
bta00040 Pentose and glucuronate interconversions	6	31	0.788	0.912
bta04080 Neuroactive ligand-receptor interaction	84	363	0.7898	0.912
bta03030 DNA replication	7	36	0.7952	0.912
bta04940 Type I diabetes mellitus	12	59	0.7992	0.912
bta05144 Malaria	12	59	0.7992	0.912
bta04060 Cytokine-cytokine receptor interaction	74	323	0.8002	0.912
bta00010 Glycolysis Gluconeogenesis	13	64	0.8072	0.9166
bta04742 Taste transduction	16	79	0.8285	0.9368
bta05310 Asthma	7	38	0.833	0.9368
bta04512 ECM-receptor interaction	18	89	0.841	0.9368
bta05332 Graft-versus-host disease	9	48	0.8418	0.9368
bta03020 RNA polymerase	5	29	0.8462	0.9368

bta00983 Drug metabolism	15	76	0.8468	0.9368
bta00980 Metabolism of xenobiotics by cytochrome P450	13	67	0.8482	0.9368
bta05143 African trypanosomiasis	8	44	0.8528	0.9368
bta05323 Rheumatoid arthritis	21	104	0.8575	0.9368
bta04714 Thermogenesis	52	239	0.8582	0.9368
bta05204 Chemical carcinogenesis	15	77	0.8582	0.9368
bta00860 Porphyrin and chlorophyll metabolism	7	40	0.8651	0.9368
bta05033 Nicotine addiction	7	40	0.8651	0.9368
bta05010 Alzheimer disease	38	180	0.8673	0.9368
bta00052 Galactose metabolism	5	31	0.88	0.9472
bta05330 Allograft rejection	10	56	0.8842	0.9484
bta04630 JAK-STAT signaling pathway	42	202	0.8956	0.9574
bta05203 Viral carcinogenesis	50	241	0.9149	0.9715
bta04622 RIG-I-like receptor signaling pathway	19	102	0.9151	0.9715
bta03050 Proteasome	7	46	0.9322	0.9863
bta02010 ABC transporters	9	60	0.9554	0.9991
bta05150 Staphylococcus aureus infection	18	105	0.9555	0.9991
bta00970 Aminoacyl-tRNA biosynthesis	10	66	0.9592	0.9991
bta04974 Protein digestion and absorption	21	121	0.9609	0.9991
bta05034 Alcoholism	44	229	0.9632	0.9991
bta04623 Cytosolic DNA-sensing pathway	10	67	0.9636	0.9991
bta03040 Spliceosome	25	147	0.9777	1.0
bta05016 Huntington disease	52	274	0.9782	1.0
bta03022 Basal transcription factors	5	44	0.9803	1.0
bta00830 Retinol metabolism	8	64	0.9861	1.0
bta04610 Complement and coagulation cascades	13	92	0.987	1.0
bta05320 Autoimmune thyroid disease	9	71	0.9883	1.0
bta05012 Parkinson disease	22	150	0.9958	1.0
bta00190 Oxidative phosphorylation	18	140	0.9988	1.0
bta03010 Ribosome	17	160	1.0	1.0
bta04740 Olfactory transduction	45	1145	1.0	1.0
bta05322 Systemic lupus erythematosus	17	182	1.0	1.0

Supplementary Table S6. The full list of detected miRNAs in sEVs secreted by *in vivo*- or *in vitro*- produced bovine embryos and their relative expressions (mean and standard deviation).

MicroRNAs	<i>In vivo</i> embryos-derived sEVs			<i>In vitro</i> embryos-derived sEVs		
	Mean	Standard deviation		Mean	Standard deviation	
bta-miR-103	0.000586	±	0.000199	0.000800	±	0.000494
bta-let-7a-5p	0.000597	±	0.000299	0.001305	±	0.001960
bta-let-7b	0.000764	±	0.000437	0.000680	±	0.000569
bta-miR-106a	0.001835	±	0.000610	0.001464	±	0.000649
bta-let-7d	0.000453	±	0.000248	0.000681	±	0.000904
bta-miR-124a	0.001132	±	0.001184	0.000423	±	0.000239
bta-miR-124b	0.001197	±	0.000983	0.000457	±	0.000171
bta-miR-125b	0.006457	±	0.012056	0.001638	±	0.001968
bta-miR-126-3p	0.003546	±	0.001542	0.002820	±	0.003925
bta-miR-134	0.000921	±	0.000573	0.000299	±	0.000178
bta-miR-127	0.003512	±	0.002953	0.001814	±	0.000559
bta-miR-130a	0.002753	±	0.001171	0.002306	±	0.000625
bta-miR-130b	0.004252	±	0.001209	0.003208	±	0.000784
bta-miR-132	0.000759	±	0.000420	0.000549	±	0.000629
bta-miR-133a	0.001802	±	0.001940	0.055883	±	0.062776
bta-miR-143	0.001276	±	0.000684	0.000829	±	0.000334
bta-miR-151-5p	0.000323	±	0.000034	0.000453	±	0.000411
bta-miR-145	0.000854	±	0.000427	0.000599	±	0.000341
bta-miR-154b	0.011714	±	0.007087	0.005112	±	0.002964
bta-miR-149-3p	0.006274	±	0.001818	0.008300	±	0.003290
bta-miR-149-5p	0.003700	±	0.002646	0.001946	±	0.002286
bta-miR-16a	0.000768	±	0.000729	0.000956	±	0.001200
bta-miR-16b	0.001683	±	0.000814	0.001654	±	0.001387
bta-miR-188	0.001241	±	0.001364	0.000578	±	0.000198
bta-miR-191	0.001046	±	0.000490	38.060764	±	52.763954
bta-miR-193a-5p	0.001295	±	0.000550	0.001085	±	0.000589
bta-miR-187	0.002892	±	0.000924	0.001473	±	0.000696
bta-miR-195	0.000942	±	0.000355	0.000734	±	0.000542
bta-miR-197	0.001375	±	0.001235	0.000417	±	0.000018
bta-miR-20a	0.001224	±	0.000533	0.001520	±	0.000889
bta-miR-19b	0.000723	±	0.000540	0.000788	±	0.000655
bta-miR-22-5p	0.002260	±	0.001319	0.001667	±	0.001429
bta-miR-221	0.001325	±	0.001227	0.000289	±	0.000075
bta-miR-223	0.001538	±	0.001239	0.000367	±	0.000256
bta-miR-24-3p	0.000647	±	0.000231	0.001653	±	0.002715
bta-miR-27a-3p	0.000868	±	0.000676	0.001023	±	0.001084
bta-miR-296-3p	0.000416	±	0.000190	0.000274	±	0.000037
bta-miR-296-5p	0.120279	±	0.046548	0.050901	±	0.024758
bta-miR-302d	0.000835	±	0.000636	0.001939	±	0.001947
bta-miR-30c	0.000925	±	0.000803	0.001352	±	0.002161
bta-miR-30d	0.000493	±	0.000439	0.000774	±	0.000831

bta-miR-31	0.001934	±	0.002864	0.000331	±	0.000182
bta-miR-331-5p	0.001906	±	0.000666	0.001003	±	0.000447
bta-miR-320a	0.014192	±	0.001898	0.009096	±	0.003223
bta-miR-320b	0.000680	±	0.000426	0.000501	±	0.000360
bta-miR-323	76.110919	±	15.900141	49.365376	±	13.483477
bta-miR-339b	0.000692	±	0.000466	0.000509	±	0.000217
bta-miR-346	0.000808	±	0.000260	0.000701	±	0.000503
bta-miR-34a	0.004215	±	0.007071	0.001047	±	0.000178
bta-miR-371	0.003807	±	0.001739	0.004410	±	0.000382
bta-miR-375	0.001278	±	0.000613	0.001271	±	0.000881
bta-miR-382	0.013143	±	0.006093	0.004392	±	0.002390
bta-miR-378	0.000923	±	0.000604	0.003303	±	0.004825
bta-miR-378b	0.000764	±	0.000315	0.002101	±	0.002985
bta-miR-411a	0.001978	±	0.001179	0.000961	±	0.000665
bta-miR-421	0.015915	±	0.007015	0.004543	±	0.002804
bta-miR-425-3p	0.074573	±	0.039170	0.025913	±	0.031862
bta-miR-429	0.011796	±	0.003282	0.008212	±	0.002086
bta-miR-4523	0.000924	±	0.000500	0.000292	±	0.000101
bta-miR-453	0.000876	±	0.000516	0.000642	±	0.000158
bta-miR-433	0.006909	±	0.001501	0.005360	±	0.002032
bta-miR-486	0.009768	±	0.002539	0.006232	±	0.001105
bta-miR-489	0.002935	±	0.001725	0.001864	±	0.000870
bta-miR-503-3p	0.002551	±	0.000357	0.001999	±	0.000328
bta-miR-505	0.001835	±	0.002380	0.000459	±	0.000246
bta-miR-494	0.154410	±	0.047682	0.290558	±	0.074545
bta-miR-541	0.009415	±	0.001014	0.005943	±	0.001551
bta-miR-584	0.003513	±	0.001554	0.002089	±	0.000602
bta-miR-592	0.000863	±	0.000235	0.000748	±	0.000358
bta-miR-615	9657.234069	±	2790.750557	5774.502872	±	1149.620898
bta-miR-631	15.411154	±	3.327767	11.097141	±	3.996591
bta-miR-574	0.178750	±	0.104524	0.100773	±	0.031565
bta-miR-656	0.004395	±	0.002464	0.002485	±	0.000881
bta-miR-664a	0.000923	±	0.000326	0.000460	±	0.000065
bta-miR-760-3p	0.001571	±	0.000685	0.000670	±	0.000354
bta-miR-760-5p	0.000913	±	0.000256	0.001637	±	0.000864
bta-miR-665	0.010005	±	0.003382	0.004168	±	0.004188
bta-miR-669	0.001051	±	0.000454	0.000371	±	0.000133
bta-miR-763	0.001258	±	0.001020	0.000423	±	0.000206
bta-miR-767	0.002696	±	0.000809	0.002609	±	0.000921
bta-miR-677	0.000951	±	0.000411	0.002177	±	0.000967
bta-miR-874	0.001465	±	0.000926	0.000741	±	0.000296
bta-miR-877	0.003291	±	0.000667	0.002822	±	0.000556
bta-miR-92a	0.002198	±	0.000583	0.002750	±	0.000590
bta-miR-92b	0.058183	±	0.023234	0.021875	±	0.017230
bta-miR-93	0.000310	±	0.000061	0.000474	±	0.000168
bta-miR-935	0.002310	±	0.000511	0.000845	±	0.000356

bta-miR-940	2.202809	±	0.825598	0.681601	±	0.516959
bta-miR-1224	0.024754	±	0.007270	0.026613	±	0.010549
bta-miR-1225-3p	0.635795	±	0.354712	0.225509	±	0.178973
bta-miR-1246	0.200657	±	0.059137	0.346751	±	0.089120
bta-miR-1247-3p	0.001275	±	0.000370	0.001476	±	0.000662
bta-miR-1296	0.001354	±	0.000399	0.000594	±	0.000210
bta-miR-1247-5p	0.003330	±	0.001316	0.002887	±	0.001518
bta-miR-1249	0.005254	±	0.001091	0.001487	±	0.000620
bta-miR-1260b	0.101209	±	0.025976	0.319705	±	0.500956
bta-miR-1307	0.003584	±	0.000802	0.004423	±	0.001633
bta-miR-1343-3p	0.004225	±	0.001125	0.001884	±	0.001076
bta-miR-1343-5p	0.012216	±	0.005154	0.013692	±	0.003160
bta-miR-1281	0.197537	±	0.084138	0.079172	±	0.046905
bta-miR-1388-3p	0.002129	±	0.000916	0.001116	±	0.000857
bta-miR-1282	0.002504	±	0.001435	0.001675	±	0.000488
RNT43 snoRNA	0.005506	±	0.005186	0.005640	±	0.001990
bta-miR-1287	0.000481	±	0.000190	0.000470	±	0.000355
bta-miR-139	0.000258	±	0.000052	.	±	.
bta-miR-380-5p	0.000720	±	0.000785	.	±	.
bta-miR-660	0.000453	±	0.000219	.	±	.
bta-miR-10b	.	±	.	0.000392	±	0.000168
bta-miR-192	.	±	.	0.000270	±	0.000095
bta-miR-194	.	±	.	0.000196	±	0.000017
bta-miR-542-5p	.	±	.	0.000263	±	0.000112
bta-miR-708	.	±	.	0.000422	±	0.000263

Supplementary Table S7. Predicted pathways regulated by miRNAs increased in sEVs secreted by *in vivo*-produced bovine embryos.

Signaling pathways	Genes predicted to be modulated by miRNAs	Number of total genes	Pvalue	BH
bta04014 Ras signaling pathway	148	242	0.0	0.0
bta04360 Axon guidance	109	178	0.0	0.0
bta04010 MAPK signaling pathway	158	292	0.0001	0.0064
bta04142 Lysosome	84	132	0.0001	0.0064
bta04144 Endocytosis	137	245	0.0001	0.0064
bta05205 Proteoglycans in cancer	117	205	0.0002	0.0106
bta00564 Glycerophospholipid metabolism	68	104	0.0003	0.0136
bta05212 Pancreatic cancer	53	76	0.0004	0.0141
bta04810 Regulation of actin cytoskeleton	117	211	0.0004	0.0141
bta04921 Oxytocin signaling pathway	89	152	0.0005	0.0159
bta01100 Metabolic pathways	664	1527	0.0007	0.0202
bta05200 Pathways in cancer	256	542	0.0011	0.0292
bta04012 ErbB signaling pathway	53	84	0.0019	0.0389
bta04062 Chemokine signaling pathway	101	188	0.0019	0.0389
bta04919 Thyroid hormone signaling pathway	69	118	0.002	0.0389
bta05231 Choline metabolism in cancer	60	99	0.0021	0.0389
bta04514 Cell adhesion molecules (CAMs)	87	158	0.0022	0.0389
bta04015 Rap1 signaling pathway	113	216	0.0022	0.0389
bta05214 Glioma	49	77	0.0025	0.0418
bta05220 Chronic myeloid leukemia	48	77	0.0036	0.0496
bta04310 Wnt signaling pathway	87	162	0.0037	0.0496
bta01522 Endocrine resistance	56	94	0.0037	0.0496
bta04068 FoxO signaling pathway	73	131	0.0038	0.0496
bta04910 Insulin signaling pathway	77	140	0.0039	0.0496
bta04668 TNF signaling pathway	67	118	0.0039	0.0496
bta04072 Phospholipase D signaling pathway	82	152	0.0043	0.0522
bta04666 Fc gamma R-mediated phagocytosis	55	93	0.0045	0.0522
bta04520 Adherens junction	44	70	0.0046	0.0522
bta04071 Sphingolipid signaling pathway	67	120	0.0051	0.0549
bta04371 Apelin signaling pathway	76	140	0.0052	0.0549
bta05032 Morphine addiction	54	92	0.0054	0.0549
bta05223 Non-small cell lung cancer	42	67	0.0058	0.0549
bta04070 Phosphatidylinositol signaling system	57	99	0.0058	0.0549
bta04150 mTOR signaling pathway	83	157	0.006	0.0549
bta04928 Parathyroid hormone synthesis, secretion and action	59	104	0.0064	0.0549
bta04140 Autophagy	76	142	0.0066	0.0549
bta04024 cAMP signaling pathway	114	229	0.0066	0.0549

bta04920 Adipocytokine signaling pathway	44	72	0.0067	0.0549
bta04120 Ubiquitin mediated proteolysis	75	140	0.0068	0.0549
bta05215 Prostate cancer	56	98	0.0069	0.0549
bta04750 Inflammatory mediator regulation of TRP channels	58	103	0.0076	0.0572
bta00562 Inositol phosphate metabolism	44	73	0.0079	0.0572
bta04611 Platelet activation	66	121	0.0079	0.0572
bta04931 Insulin resistance	61	110	0.008	0.0572
bta05211 Renal cell carcinoma	43	71	0.0081	0.0572
bta01521 EGFR tyrosine kinase inhibitor resistance	47	80	0.0087	0.0601
bta04722 Neurotrophin signaling pathway	66	122	0.009	0.0609
bta04625 C-type lectin receptor signaling pathway	58	106	0.0115	0.0748
bta04270 Vascular smooth muscle contraction	70	133	0.0116	0.0748
bta04390 Hippo signaling pathway	80	156	0.0118	0.0748
bta04912 GnRH signaling pathway	52	93	0.012	0.0748
bta04971 Gastric acid secretion	44	76	0.013	0.078
bta05166 Human T-cell leukemia virus 1 infection	113	234	0.013	0.078
bta04066 HIF-1 signaling pathway	59	110	0.0145	0.0854
bta04530 Tight junction	88	177	0.0153	0.0865
bta05132 Salmonella infection	108	224	0.0153	0.0865
bta04710 Circadian rhythm	22	31	0.0155	0.0865
bta05235 PD-L1 expression and PD-1 checkpoint pathway in cancer	51	93	0.0164	0.0898
bta04727 GABAergic synapse	50	91	0.0169	0.0898
bta04064 NF-kappa B signaling pathway	58	109	0.017	0.0898
bta04022 cGMP-PKG signaling pathway	84	169	0.0174	0.0898
bta04660 T cell receptor signaling pathway	57	107	0.0176	0.0898
bta04152 AMPK signaling pathway	64	123	0.0178	0.0898
bta04510 Focal adhesion	96	198	0.0188	0.0934
bta04934 Cushing syndrome	78	156	0.0192	0.0939
bta05161 Hepatitis B	84	171	0.0212	0.0995
bta05224 Breast cancer	75	150	0.0213	0.0995
bta00600 Sphingolipid metabolism	30	49	0.0216	0.0995
bta04380 Osteoclast differentiation	68	134	0.0218	0.0995
bta05163 Human cytomegalovirus infection	115	245	0.0219	0.0995
bta04216 Ferroptosis	28	45	0.0225	0.1007
bta05226 Gastric cancer	76	153	0.0228	0.1007
bta04370 VEGF signaling pathway	34	58	0.0233	0.1014
bta05210 Colorectal cancer	48	89	0.0236	0.1014
bta04721 Synaptic vesicle cycle	43	78	0.0242	0.1026
bta05167 Kaposi sarcoma-associated herpesvirus infection	98	206	0.0251	0.1041
bta04911 Insulin secretion	46	85	0.0252	0.1041

bta04961 Endocrine and other factor-regulated calcium reabsorption	30	50	0.0258	0.1052
bta05160 Hepatitis C	80	164	0.0267	0.1069
bta04261 Adrenergic signaling in cardiomyocytes	74	150	0.0269	0.1069
bta05225 Hepatocellular carcinoma	84	174	0.0281	0.1097
bta05219 Bladder cancer	26	42	0.0283	0.1097
bta04916 Melanogenesis	53	102	0.0291	0.1115
bta05218 Melanoma	40	73	0.0307	0.1156
bta04210 Apoptosis	70	142	0.0309	0.1156
bta04218 Cellular senescence	80	166	0.0321	0.1177
bta04925 Aldosterone synthesis and secretion	50	96	0.0322	0.1177
bta05418 Fluid shear stress and atherosclerosis	71	145	0.0329	0.1189
bta05170 Human immunodeficiency virus 1 infection	108	234	0.0345	0.1233
bta04670 Leukocyte transendothelial migration	57	113	0.0356	0.1258
bta04664 Fc epsilon RI signaling pathway	38	70	0.0377	0.1299
bta04926 Relaxin signaling pathway	64	130	0.038	0.1299
bta04972 Pancreatic secretion	52	102	0.038	0.1299
bta04137 Mitophagy	36	66	0.0403	0.1363
bta04933 AGE-RAGE signaling pathway in diabetic complications	52	103	0.0425	0.1411
bta05165 Human papillomavirus infection	152	345	0.0426	0.1411
bta04962 Vasopressin-regulated water reabsorption	28	49	0.0451	0.145
bta05410 Hypertrophic cardiomyopathy (HCM)	47	92	0.0454	0.145
bta04659 Th17 cell differentiation	56	113	0.0456	0.145
bta05414 Dilated cardiomyopathy (DCM)	50	99	0.0456	0.145
bta04211 Longevity regulating pathway	46	90	0.047	0.1468
bta05020 Prion diseases	20	32	0.0471	0.1468
bta04020 Calcium signaling pathway	93	202	0.0479	0.1479
bta04550 Signaling pathways regulating pluripotency of stem cells	68	142	0.0485	0.1483
bta04713 Circadian entrainment	50	100	0.0509	0.1522
bta04964 Proximal tubule bicarbonate reclamation	15	22	0.051	0.1522
bta00670 One carbon pool by folate	13	18	0.0512	0.1522
bta05221 Acute myeloid leukemia	36	68	0.0528	0.1555
bta05230 Central carbon metabolism in cancer	35	66	0.0548	0.1599
bta04728 Dopaminergic synapse	64	134	0.0558	0.1613
bta05135 Yersinia infection	62	130	0.0599	0.1688
bta04720 Long-term potentiation	36	69	0.06	0.1688
bta00592 alpha-Linolenic acid metabolism	18	29	0.06	0.1688
bta05412 Arrhythmogenic right ventricular cardiomyopathy (ARVC)	39	76	0.0608	0.169
bta04976 Bile secretion	42	83	0.0611	0.169
bta04725 Cholinergic synapse	55	114	0.0638	0.1749

bta00230 Purine metabolism	63	134	0.0691	0.1873
bta04922 Glucagon signaling pathway	50	103	0.0695	0.1873
bta00565 Ether lipid metabolism	28	52	0.0707	0.1889
bta04966 Collecting duct acid secretion	17	28	0.0747	0.198
bta04960 Aldosterone-regulated sodium reabsorption	21	37	0.0783	0.2058
bta05031 Amphetamine addiction	35	69	0.0796	0.2075
bta04730 Long-term depression	31	60	0.0822	0.2121
bta00561 Glycerolipid metabolism	34	67	0.0827	0.2121
bta04340 Hedgehog signaling pathway	27	51	0.0842	0.2127
bta01212 Fatty acid metabolism	30	58	0.0855	0.2127
bta04923 Regulation of lipolysis in adipocytes	30	58	0.0855	0.2127
bta04151 PI3K-Akt signaling pathway	158	373	0.0856	0.2127
bta05017 Spinocerebellar ataxia	46	96	0.0889	0.2191
bta04724 Glutamatergic synapse	53	113	0.0906	0.2216
bta04662 B cell receptor signaling pathway	42	87	0.0938	0.2262
bta00514 Other types of O-glycan biosynthesis	24	45	0.0946	0.2262
bta00591 Linoleic acid metabolism	20	36	0.0952	0.2262
bta04726 Serotonergic synapse	54	116	0.0953	0.2262
bta05169 Epstein-Barr virus infection	99	228	0.1024	0.2412
bta04978 Mineral absorption	28	55	0.1048	0.2449
bta04350 TGF-beta signaling pathway	44	93	0.1055	0.2449
bta05014 Amyotrophic lateral sclerosis (ALS)	30	60	0.109	0.2498
bta04330 Notch signaling pathway	27	53	0.1092	0.2498
bta04935 Growth hormone synthesis, secretion and action	54	118	0.1127	0.256
bta05222 Small cell lung cancer	44	94	0.1158	0.2612
bta00270 Cysteine and methionine metabolism	25	49	0.1185	0.2654
bta04114 Oocyte meiosis	54	119	0.1222	0.2717
bta05100 Bacterial invasion of epithelial cells	35	73	0.1234	0.2725
bta04540 Gap junction	42	90	0.125	0.2741
bta05213 Endometrial cancer	29	59	0.1273	0.2773
bta03420 Nucleotide excision repair	23	45	0.1289	0.2781
bta04914 Progesterone-mediated oocyte maturation	41	88	0.1299	0.2781
bta04392 Hippo signaling pathway	16	29	0.1303	0.2781
bta05142 Chagas disease (American trypanosomiasis)	52	115	0.1313	0.2784
bta04213 Longevity regulating pathway	30	62	0.1362	0.2868
bta00533 Glycosaminoglycan biosynthesis	9	14	0.1408	0.2946
bta04924 Renin secretion	34	72	0.1419	0.2949
bta04930 Type II diabetes mellitus	23	46	0.146	0.3005
bta00515 Mannose type O-glycan biosynthesis	13	23	0.1474	0.3005
bta00340 Histidine metabolism	13	23	0.1474	0.3005

bta05162 Measles	66	152	0.1541	0.3118
bta03015 mRNA surveillance pathway	43	95	0.1559	0.3118
bta04061 Viral protein interaction with cytokine and cytokine receptor	43	95	0.1559	0.3118
bta03320 PPAR signaling pathway	37	81	0.166	0.3299
bta04130 SNARE interactions in vesicular transport	17	33	0.1676	0.331
bta04146 Peroxisome	38	84	0.1739	0.3414
bta00531 Glycosaminoglycan degradation	12	22	0.1821	0.3525
bta04975 Fat digestion and absorption	23	48	0.1838	0.3525
bta04929 GnRH secretion	30	65	0.184	0.3525
bta04927 Cortisol synthesis and secretion	30	65	0.184	0.3525
bta00100 Steroid biosynthesis	11	20	0.1908	0.3621
bta04215 Apoptosis	17	34	0.1913	0.3621
bta04744 Phototransduction	14	27	0.1928	0.3628
bta00240 Pyrimidine metabolism	26	56	0.1982	0.3698
bta04217 Necroptosis	73	174	0.1994	0.3698
bta04973 Carbohydrate digestion and absorption	21	44	0.2012	0.3698
bta05143 African trypanosomiasis	21	44	0.2012	0.3698
bta01040 Biosynthesis of unsaturated fatty acids	15	30	0.2106	0.3829
bta00071 Fatty acid degradation	20	42	0.2107	0.3829
bta00510 N-Glycan biosynthesis	24	52	0.2163	0.3881
bta05202 Transcriptional misregulation in cancer	79	191	0.2176	0.3881
bta04913 Ovarian steroidogenesis	26	57	0.2181	0.3881
bta04657 IL-17 signaling pathway	40	92	0.2204	0.3881
bta05216 Thyroid cancer	19	40	0.2208	0.3881
bta04115 p53 signaling pathway	34	77	0.2209	0.3881
bta04141 Protein processing in endoplasmic reticulum	69	166	0.2249	0.393
bta04979 Cholesterol metabolism	23	50	0.2262	0.3931
bta04658 Th1 and Th2 cell differentiation	42	98	0.2358	0.4005
bta00220 Arginine biosynthesis	10	19	0.236	0.4005
bta00770 Pantothenate and CoA biosynthesis	10	19	0.236	0.4005
bta00330 Arginine and proline metabolism	22	48	0.2367	0.4005
bta01524 Platinum drug resistance	34	78	0.2389	0.4005
bta05217 Basal cell carcinoma	28	63	0.2393	0.4005
bta01230 Biosynthesis of amino acids	32	73	0.2393	0.4005
bta00062 Fatty acid elongation	14	29	0.2515	0.4187
bta00760 Nicotinate and nicotinamide metabolism	18	39	0.2581	0.4267
bta00511 Other glycan degradation	11	22	0.259	0.4267
bta05144 Malaria	26	59	0.2606	0.4272
bta05145 Toxoplasmosis	47	113	0.2724	0.4442
bta05321 Inflammatory bowel disease (IBD)	30	70	0.28	0.4538
bta04970 Salivary secretion	39	93	0.2811	0.4538

bta05164 Influenza A	73	181	0.2837	0.4556
bta04917 Prolactin signaling pathway	35	83	0.2854	0.4561
bta04621 NOD-like receptor signaling pathway	74	184	0.2888	0.4568
bta00730 Thiamine metabolism	9	18	0.2902	0.4568
bta00061 Fatty acid biosynthesis	9	18	0.2902	0.4568
bta00360 Phenylalanine metabolism	11	23	0.2958	0.4634
bta00650 Butanoate metabolism	13	28	0.2986	0.4648
bta04915 Estrogen signaling pathway	56	138	0.3001	0.4648
bta05152 Tuberculosis	79	198	0.3011	0.4648
bta00310 Lysine degradation	28	66	0.3037	0.4666
bta00604 Glycosphingolipid biosynthesis	8	16	0.3084	0.4695
bta04620 Toll-like receptor signaling pathway	45	110	0.3086	0.4695
bta04918 Thyroid hormone synthesis	31	74	0.3106	0.4701
bta04650 Natural killer cell mediated cytotoxicity	53	131	0.3126	0.4701
bta00900 Terpenoid backbone biosynthesis	10	21	0.3134	0.4701
bta05133 Pertussis	32	77	0.3197	0.4773
bta00410 beta-Alanine metabolism	15	34	0.3317	0.4928
bta00601 Glycosphingolipid biosynthesis	13	29	0.3332	0.4928
bta00380 Tryptophan metabolism	20	47	0.3398	0.4974
bta05146 Amoebiasis	47	117	0.3401	0.4974
bta05323 Rheumatoid arthritis	42	104	0.341	0.4974
bta04145 Phagosome	67	170	0.3486	0.5062
bta04110 Cell cycle	49	123	0.3534	0.5085
bta00910 Nitrogen metabolism	8	17	0.3547	0.5085
bta04723 Retrograde endocannabinoid signaling	60	152	0.355	0.5085
bta05033 Nicotine addiction	17	40	0.3601	0.5135
bta00020 Citrate cycle (TCA cycle)	13	30	0.3686	0.5233
bta00513 Various types of N-glycan biosynthesis	18	43	0.3728	0.5269
bta00532 Glycosaminoglycan biosynthesis	9	20	0.3765	0.5298
bta04122 Sulfur relay system	5	10	0.3795	0.5316
bta04136 Autophagy	14	33	0.3837	0.5352
bta04623 Cytosolic DNA-sensing pathway	27	67	0.3876	0.5382
bta03030 DNA replication	15	36	0.3975	0.5496
bta01210 2-Oxocarboxylic acid metabolism	8	18	0.4016	0.5523
bta00512 Mucin type O-glycan biosynthesis	13	31	0.4045	0.5523
bta04640 Hematopoietic cell lineage	43	110	0.4047	0.5523
bta04512 ECM-receptor interaction	35	89	0.4085	0.5551
bta01200 Carbon metabolism	44	113	0.4112	0.5553
bta00563 Glycosylphosphatidylinositol (GPI)-anchor biosynthesis	11	26	0.4121	0.5553
bta00051 Fructose and mannose metabolism	14	34	0.4182	0.5611
bta03018 RNA degradation	31	79	0.4216	0.5633

bta03020 RNA polymerase	12	29	0.427	0.5681
bta00534 Glycosaminoglycan biosynthesis	10	24	0.4369	0.5789
bta00010 Glycolysis Gluconeogenesis	25	64	0.4445	0.5865
bta04932 Non-alcoholic fatty liver disease (NAFLD)	60	158	0.4508	0.5912
bta03008 Ribosome biogenesis in eukaryotes	32	83	0.4518	0.5912
bta04260 Cardiac muscle contraction	34	89	0.4659	0.6072
bta03013 RNA transport	67	178	0.4693	0.6091
bta05340 Primary immunodeficiency	16	41	0.4746	0.6133
bta03410 Base excision repair	13	33	0.4764	0.6133
bta03460 Fanconi anemia pathway	20	52	0.482	0.618
bta03022 Basal transcription factors	17	44	0.4844	0.6186
bta00590 Arachidonic acid metabolism	31	82	0.4899	0.6231
bta03040 Spliceosome	55	147	0.4918	0.6231
bta00350 Tyrosine metabolism	15	39	0.4972	0.6274
bta00052 Galactose metabolism	12	31	0.5017	0.6306
bta03430 Mismatch repair	9	23	0.5074	0.6352
bta04950 Maturity onset diabetes of the young	10	26	0.5187	0.6468
bta00250 Alanine, aspartate and glutamate metabolism	14	37	0.5212	0.6474
bta05030 Cocaine addiction	18	48	0.5234	0.6476
bta05203 Viral carcinogenesis	88	241	0.5537	0.6782
bta04060 Cytokine-cytokine receptor interaction	118	323	0.5541	0.6782
bta00620 Pyruvate metabolism	14	38	0.5545	0.6782
bta03450 Non-homologous end-joining	5	13	0.5575	0.6793
bta00630 Glyoxylate and dicarboxylate metabolism	11	30	0.5662	0.6872
bta05134 Legionellosis	21	58	0.572	0.6909
bta00640 Propanoate metabolism	12	33	0.5736	0.6909
bta05140 Leishmaniasis	28	78	0.586	0.7032
bta04940 Type I diabetes mellitus	21	59	0.5981	0.715
bta05416 Viral myocarditis	27	76	0.6038	0.7191
bta00120 Primary bile acid biosynthesis	6	17	0.6155	0.7276
bta00603 Glycosphingolipid biosynthesis	6	17	0.6155	0.7276
bta00260 Glycine, serine and threonine metabolism	15	43	0.6231	0.7295
bta01523 Antifolate resistance	15	43	0.6231	0.7295
bta03060 Protein export	8	23	0.6243	0.7295
bta04614 Renin-angiotensin system	9	26	0.6286	0.7295
bta04977 Vitamin digestion and absorption	9	26	0.6286	0.7295
bta00450 Selenocompound metabolism	6	18	0.6607	0.764
bta00790 Folate biosynthesis	12	36	0.6723	0.7746
bta00280 Valine, leucine and isoleucine degradation	17	51	0.685	0.7864
bta00480 Glutathione metabolism	20	60	0.6927	0.7924
bta00053 Ascorbate and aldarate metabolism	8	25	0.6997	0.7975

bta04630 JAK-STAT signaling pathway	69	202	0.7287	0.8249
bta00500 Starch and sucrose metabolism	10	32	0.7307	0.8249
bta00520 Amino sugar and nucleotide sugar metabolism	16	50	0.7324	0.8249
bta04080 Neuroactive ligand-receptor interaction	126	363	0.7341	0.8249
bta05332 Graft-versus-host disease	15	48	0.7552	0.8456
bta04714 Thermogenesis	81	239	0.7613	0.8495
bta00140 Steroid hormone biosynthesis	21	67	0.7773	0.8643
bta05016 Huntington disease	92	274	0.796	0.882
bta00830 Retinol metabolism	19	64	0.8298	0.9162
bta04610 Complement and coagulation cascades	28	92	0.8394	0.9236
bta00860 Porphyrin and chlorophyll metabolism	11	40	0.8472	0.929
bta05330 Allograft rejection	16	56	0.8501	0.929
bta04742 Taste transduction	23	79	0.8675	0.9441
bta00030 Pentose phosphate pathway	7	28	0.8699	0.9441
bta05168 Herpes simplex virus 1 infection	133	403	0.8735	0.9448
bta05204 Chemical carcinogenesis	22	77	0.8805	0.9466
bta03440 Homologous recombination	11	42	0.8811	0.9466
bta04974 Protein digestion and absorption	36	121	0.89	0.9512
bta04672 Intestinal immune network for IgA production	15	56	0.8944	0.9512
bta00970 Aminoacyl-tRNA biosynthesis	18	66	0.8986	0.9512
bta00982 Drug metabolism	17	63	0.9008	0.9512
bta05010 Alzheimer disease	55	180	0.9025	0.9512
bta00983 Drug metabolism	21	76	0.9033	0.9512
bta04622 RIG-I-like receptor signaling pathway	29	102	0.9111	0.9562
bta00040 Pentose and glucuronate interconversions	7	31	0.919	0.9613
bta04612 Antigen processing and presentation	23	85	0.9262	0.9635
bta05206 MicroRNAs in cancer	91	292	0.9271	0.9635
bta03050 Proteasome	11	46	0.9306	0.9639
bta02010 ABC transporters	15	60	0.9348	0.9648
bta00980 Metabolism of xenobiotics by cytochrome P450	17	67	0.9375	0.9648
bta05034 Alcoholism	67	229	0.9592	0.984
bta05310 Asthma	7	38	0.9761	0.9981
bta05320 Autoimmune thyroid disease	15	71	0.9856	1.0
bta00190 Oxidative phosphorylation	35	140	0.9861	1.0
bta05012 Parkinson disease	33	150	0.9981	1.0
bta05150 Staphylococcus aureus infection	19	105	0.9993	1.0
bta03010 Ribosome	26	160	1.0	1.0
bta04740 Olfactory transduction	56	1145	1.0	1.0
bta05322 Systemic lupus erythematosus	25	182	1.0	1.0

Supplementary Table S8. Predicted pathways modulated by miRNAs increased in sEVs secreted by *in vitro*-produced bovine embryos.

Signaling pathways	Genes predicted to be modulated by miRNAs	Number of total genes	Pvalue	BH
bta05231 Choline metabolism in cancer	9	99	0.0029	0.2436
bta04360 Axon guidance	12	178	0.0065	0.273
bta05205 Proteoglycans in cancer	12	205	0.017	0.3116
bta04310 Wnt signaling pathway	10	162	0.0203	0.3116
bta04514 Cell adhesion molecules (CAMs)	9	158	0.0401	0.3116
bta04066 HIF-1 signaling pathway	7	110	0.0411	0.3116
bta00310 Lysine degradation	5	66	0.0439	0.3116
bta04724 Glutamatergic synapse	7	113	0.046	0.3116
bta04144 Endocytosis	12	245	0.0509	0.3116
bta04520 Adherens junction	5	70	0.0533	0.3116
bta04550 Signaling pathways regulating pluripotency of stem cells	8	142	0.0533	0.3116
bta04114 Oocyte meiosis	7	119	0.0569	0.3116
bta04924 Renin secretion	5	72	0.0584	0.3116
bta04974 Protein digestion and absorption	7	121	0.0609	0.3116
bta05412 Arrhythmogenic right ventricular cardiomyopathy (ARVC)	5	76	0.0695	0.3116
bta05212 Pancreatic cancer	5	76	0.0695	0.3116
bta05220 Chronic myeloid leukemia	5	77	0.0724	0.3116
bta05226 Gastric cancer	8	153	0.0738	0.3116
bta04750 Inflammatory mediator regulation of TRP channels	6	103	0.0769	0.3116
bta00564 Glycerophospholipid metabolism	6	104	0.0796	0.3116
bta04934 Cushing syndrome	8	156	0.0801	0.3116
bta04810 Regulation of actin cytoskeleton	10	211	0.0816	0.3116
bta04660 T cell receptor signaling pathway	6	107	0.0878	0.3134
bta04728 Dopaminergic synapse	7	134	0.0906	0.3134
bta05200 Pathways in cancer	21	542	0.0957	0.3134
bta05202 Transcriptional misregulation in cancer	9	191	0.0971	0.3134
bta05161 Hepatitis B	8	171	0.116	0.3134
bta05169 Epstein-Barr virus infection	10	228	0.1166	0.3134
bta04727 GABAergic synapse	5	91	0.12	0.3134
bta05032 Morphine addiction	5	92	0.1239	0.3134
bta05410 Hypertrophic cardiomyopathy (HCM)	5	92	0.1239	0.3134
bta04350 TGF-beta signaling pathway	5	93	0.1278	0.3134
bta04666 Fc gamma R-mediated phagocytosis	5	93	0.1278	0.3134
bta05235 PD-L1 expression and PD-1 checkpoint pathway in cancer	5	93	0.1278	0.3134

bta05222 Small cell lung cancer	5	94	0.1318	0.3134
bta04261 Adrenergic signaling in cardiomyocytes	7	150	0.1363	0.3134
bta04921 Oxytocin signaling pathway	7	152	0.1427	0.3134
bta05215 Prostate cancer	5	98	0.1482	0.3134
bta05414 Dilated cardiomyopathy (DCM)	5	99	0.1524	0.3134
bta04070 Phosphatidylinositol signaling system	5	99	0.1524	0.3134
bta04390 Hippo signaling pathway	7	156	0.1559	0.3134
bta04713 Circadian entrainment	5	100	0.1567	0.3134
bta04916 Melanogenesis	5	102	0.1654	0.3226
bta04068 FoxO signaling pathway	6	131	0.169	0.3226
bta04064 NF-kappa B signaling pathway	5	109	0.1972	0.3594
bta04620 Toll-like receptor signaling pathway	5	110	0.2019	0.3594
bta04010 MAPK signaling pathway	11	292	0.2036	0.3594
bta04120 Ubiquitin mediated proteolysis	6	140	0.2054	0.3594
bta05170 Human immunodeficiency virus 1 infection	9	234	0.2162	0.3706
bta05225 Hepatocellular carcinoma	7	174	0.2214	0.372
bta04668 TNF signaling pathway	5	118	0.2408	0.3966
bta05224 Breast cancer	6	150	0.2486	0.399
bta04723 Retrograde endocannabinoid signaling	6	152	0.2576	0.399
bta04072 Phospholipase D signaling pathway	6	152	0.2576	0.399
bta05165 Human papillomavirus infection	12	345	0.2646	0.399
bta04152 AMPK signaling pathway	5	123	0.266	0.399
bta04024 cAMP signaling pathway	8	229	0.3131	0.4469
bta04510 Focal adhesion	7	198	0.3199	0.4469
bta00230 Purine metabolism	5	134	0.3233	0.4469
bta04380 Osteoclast differentiation	5	134	0.3233	0.4469
bta01100 Metabolic pathways	46	1527	0.3245	0.4469
bta05166 Human T-cell leukemia virus 1 infection	8	234	0.3329	0.451
bta04371 Apelin signaling pathway	5	140	0.355	0.4733
bta04014 Ras signaling pathway	8	242	0.3648	0.4788
bta04530 Tight junction	6	177	0.3743	0.4837
bta04621 NOD-like receptor signaling pathway	6	184	0.4075	0.5168
bta04060 Cytokine-cytokine receptor interaction	10	323	0.4122	0.5168
bta05132 Salmonella infection	7	224	0.4324	0.5341
bta04218 Cellular senescence	5	166	0.491	0.5892
bta04141 Protein processing in endoplasmic reticulum	5	166	0.491	0.5892
bta05167 Kaposi sarcoma-associated herpesvirus infection	6	206	0.51	0.6034
bta04217 Necroptosis	5	174	0.5309	0.6194
bta04080 Neuroactive ligand-receptor interaction	10	363	0.5509	0.6292

bta04015 Rap1 signaling pathway	6	216	0.5543	0.6292
bta04151 PI3K-Akt signaling pathway	10	373	0.5836	0.6536
bta05152 Tuberculosis	5	198	0.6407	0.7079
bta04630 JAK-STAT signaling pathway	5	202	0.6573	0.7079
bta04020 Calcium signaling pathway	5	202	0.6573	0.7079
bta05163 Human cytomegalovirus infection	6	245	0.6705	0.7129
bta05016 Huntington disease	6	274	0.7651	0.8034
bta05203 Viral carcinogenesis	5	241	0.7923	0.8216
bta05206 MicroRNAs in cancer	5	292	0.9011	0.9231
bta05168 Herpes simplex virus 1 infection	7	403	0.9227	0.9338
bta04740 Olfactory transduction	5	1145	1.0	1.0

APPENDIX B – Supplementary material of Chapter 3

Supplementary Table S1A. *In vivo* embryo production data/cow/replicate.

<i>In vivo</i> embryo production data/cow/replicate				
Replicate	Cow/id	Recovered structures number	Number of corpora lutea	Embryos number
1	1	13	11	12
2	2	9	8	9
3	3	13	15	13
Uterine flushes	3	35	34	34
Recovery rate	102.94			
Embryo rate	97.14			
Embryos on day 7 of development recovered/cow	11.33			

Supplementary Table S1B. *In vitro* embryo production data/replicate.

<i>In vitro</i> embryo production data/replicate			
Replicate	Number of COCs placed in IVM	Number of clevaged	Embryos number
1	150	119	31
2	151	128	41
3	150	127	40
Total	451	374	112
Cleavage rate	82.93		
Blastocyst rate	24.83		

Supplementary Table S2. Differentially expressed genes in endometrial explants exposed for 6 hours to a Day 7 *in vivo* bovine embryo compared to end group. (end x end *in vivo*)

Gene symbol	Log2FoldChange	p-adjusted	Group
ZFP3	-0.50134623338703	0.0996958266354157	end in vivo 6h
TRMT6	-0.501425368412534	0.0996958266354157	end in vivo 6h
COPRS	-0.515261024816315	0.0996958266354157	end in vivo 6h
CTSS	-0.516748615918333	0.0996958266354157	end in vivo 6h
MRPS18C	-0.517394320924977	0.0996958266354157	end in vivo 6h
ENSBTAG00000049323	-0.518743987781298	0.0996958266354157	end in vivo 6h
ASF1A	-0.519631807998242	0.0996958266354157	end in vivo 6h
ENSBTAG00000043575	-0.524163253738047	0.0996958266354157	end in vivo 6h
THAP1	-0.524257023990246	0.0996958266354157	end in vivo 6h
ZNHIT6	-0.535485089765577	0.0996958266354157	end in vivo 6h
ACTR6	-0.53769956520964	0.0996958266354157	end in vivo 6h
STK26	-0.539558311238667	0.0996958266354157	end in vivo 6h
ZNFX1	-0.547003978167962	0.0996958266354157	end in vivo 6h
TM2D3	-0.551949721213191	0.0996958266354157	end in vivo 6h
ENSBTAG00000051325	-0.552544793440863	0.0996958266354157	end in vivo 6h
TMEM178A	-0.556265264752753	0.0996958266354157	end in vivo 6h
MRPS30	-0.558029164081749	0.0996958266354157	end in vivo 6h
LSM1	-0.559209912828006	0.0996958266354157	end in vivo 6h
ENSBTAG00000054082	-0.559358930396712	0.0996958266354157	end in vivo 6h
ISCA2	-0.561433369107545	0.0996958266354157	end in vivo 6h
SP110	-0.563805545464151	0.0996958266354157	end in vivo 6h
SERPINE2	-0.564503737177079	0.0996958266354157	end in vivo 6h
TRIM21	-0.570726603727602	0.0996958266354157	end in vivo 6h
ALG10	-0.572795250199026	0.0996958266354157	end in vivo 6h
IFITM3	-0.578942364119344	0.0996958266354157	end in vivo 6h
TRIM34	-0.584196125872308	0.0996958266354157	end in vivo 6h
THAP2	-0.58891947062399	0.0996958266354157	end in vivo 6h
TMEM185B	-0.591931879938303	0.0996958266354157	end in vivo 6h
PARP8	-0.596474345504013	0.0996958266354157	end in vivo 6h
ENSBTAG00000043565	-0.599060118550863	0.0996958266354157	end in vivo 6h
HERC6	-0.608251682940256	0.0996958266354157	end in vivo 6h
ENSBTAG00000043551	-0.613536737380418	0.0996958266354157	end in vivo 6h
ENSBTAG00000048456	-0.61833200263245	0.0996958266354157	end in vivo 6h
HSPA12A	-0.622860147420483	0.0996958266354157	end in vivo 6h
ERAP1	-0.626058134014255	0.0996958266354157	end in vivo 6h
SLC16A9	-0.630505996097637	0.0996958266354157	end in vivo 6h
FAM3B	-0.631900125355403	0.0996958266354157	end in vivo 6h
TLR4	-0.633800225619169	0.0996958266354157	end in vivo 6h
WASF1	-0.648686063704059	0.0996958266354157	end in vivo 6h
ENSBTAG00000038900	-0.652663995095801	0.0996958266354157	end in vivo 6h
CCBE1	-0.658944216039209	0.0996958266354157	end in vivo 6h
ENSBTAG00000043552	-0.664448831543389	0.0996958266354157	end in vivo 6h
OXLD1	-0.673365755670155	0.0996958266354157	end in vivo 6h

ENSBTAG0000009664	-0.674740484192637	0.0996958266354157	end in vivo 6h
LCA5	-0.676643985781567	0.0996958266354157	end in vivo 6h
IFI16	-0.678285063940627	0.0996958266354157	end in vivo 6h
ENSBTAG00000043572	-0.682640059342516	0.0996958266354157	end in vivo 6h
SERTAD1	-0.687057448836492	0.0815295500703684	end in vivo 6h
SP140L	-0.688116492773784	0.0996958266354157	end in vivo 6h
ENSBTAG00000047133	-0.688662707673057	0.0996958266354157	end in vivo 6h
C3	-0.699928002971114	0.0996958266354157	end in vivo 6h
HNF1A	-0.710272154544817	0.0996958266354157	end in vivo 6h
SGK1	-0.715170779312291	0.0996958266354157	end in vivo 6h
IRF9	-0.718437201939643	0.0996958266354157	end in vivo 6h
ELOVL4	-0.722007745905788	0.0996958266354157	end in vivo 6h
CP	-0.735576170523197	0.0996958266354157	end in vivo 6h
KCNJ15	-0.736597813214665	0.0996958266354157	end in vivo 6h
TLR3	-0.760420173238922	0.0996958266354157	end in vivo 6h
UMPS	-0.771277274175232	0.0996958266354157	end in vivo 6h
CDO1	-0.773440647497919	0.0996958266354157	end in vivo 6h
ENSBTAG00000043544	-0.775678503892056	0.0996958266354157	end in vivo 6h
CITED2	-0.776642225867526	0.0996958266354157	end in vivo 6h
FAM169A	-0.783852932438584	0.0996958266354157	end in vivo 6h
CASP4	-0.784414771824998	0.0996958266354157	end in vivo 6h
GSTM1	-0.791738337080379	0.0996958266354157	end in vivo 6h
EDNRA	-0.801696834806059	0.0996958266354157	end in vivo 6h
ENSBTAG00000048992	-0.803610367013836	0.0996958266354157	end in vivo 6h
ENSBTAG00000048741	-0.821219831059203	0.0996958266354157	end in vivo 6h
FST	-0.822852431902338	0.0996958266354157	end in vivo 6h
THOC6	-0.840997979397497	0.0996958266354157	end in vivo 6h
ENSBTAG00000053607	-0.854542569906083	0.0996958266354157	end in vivo 6h
TMEM100	-0.85555632229438	0.0996958266354157	end in vivo 6h
IFIH1	-0.857133969246908	0.0996958266354157	end in vivo 6h
ENSBTAG00000054230	-0.863068027714114	0.0996958266354157	end in vivo 6h
RRAD	-0.87495285842803	0.0996958266354157	end in vivo 6h
ENSBTAG00000050139	-0.878925060929489	0.0996958266354157	end in vivo 6h
CYP3A4	-0.879042784207617	0.0996958266354157	end in vivo 6h
C1QC	-0.880089813951956	0.0996958266354157	end in vivo 6h
PI15	-0.890562795585883	0.0996958266354157	end in vivo 6h
IL15RA	-0.893474815514358	0.0996958266354157	end in vivo 6h
STAR	-0.908160593225335	0.0996958266354157	end in vivo 6h
ENSBTAG00000052221	-0.910128249997564	0.0996958266354157	end in vivo 6h
ENSBTAG00000010030	-0.917868521097991	0.0996958266354157	end in vivo 6h
FBXO5	-0.939259456888937	0.0996958266354157	end in vivo 6h
ENSBTAG00000049612	-0.953385428149817	0.0997366936593684	end in vivo 6h
KHDRBS3	-0.954649590397557	0.0388312058722952	end in vivo 6h
IRF8	-0.955911920026682	0.0996958266354157	end in vivo 6h
SLC36A2	-0.957045385390056	0.0996958266354157	end in vivo 6h
LOC100852077	-0.958048522402935	0.0996958266354157	end in vivo 6h

PARP9	-0.968336107270983	0.0996958266354157	end in vivo 6h
SLCO2A1	-0.968810906066413	0.0996958266354157	end in vivo 6h
SAMD9	-0.969104000688561	0.0996958266354157	end in vivo 6h
CIDEB	-0.979508330566007	0.0996958266354157	end in vivo 6h
BARD1	-1.00652325328592	0.0996958266354157	end in vivo 6h
ENSBTAG00000043414	-1.00705433062324	0.0996958266354157	end in vivo 6h
ENSBTAG00000048433	-1.0145928496071	0.0996958266354157	end in vivo 6h
FNDC4	-1.0156968166039	0.0996958266354157	end in vivo 6h
LOC511531	-1.02824394900978	0.0996958266354157	end in vivo 6h
TRPC2	-1.03497597587779	0.0996958266354157	end in vivo 6h
ENSBTAG00000050108	-1.04025243839708	0.0996958266354157	end in vivo 6h
APOL3	-1.05383886799363	0.0996958266354157	end in vivo 6h
EPSTI1	-1.07422642518674	0.0996958266354157	end in vivo 6h
ITGAL	-1.08458088123319	0.0996958266354157	end in vivo 6h
UBA7	-1.11389078718717	0.0996958266354157	end in vivo 6h
IFIT3	-1.11730420519563	0.0996958266354157	end in vivo 6h
SPI1	-1.13222046434693	0.0996958266354157	end in vivo 6h
ADM5	-1.1512455583241	0.0996958266354157	end in vivo 6h
SPP1	-1.15154063996924	0.0996958266354157	end in vivo 6h
TMEM262	-1.16743166734682	0.0996958266354157	end in vivo 6h
BHLHA15	-1.17106194244379	0.0996958266354157	end in vivo 6h
ENSBTAG00000051798	-1.17219406222498	0.0996958266354157	end in vivo 6h
TPBG	-1.17975816030513	0.0996958266354157	end in vivo 6h
CDK5R1	-1.22687279422301	0.0996958266354157	end in vivo 6h
ENSBTAG00000054738	-1.23831211609668	0.0996958266354157	end in vivo 6h
MX2	-1.23902859406873	0.0996958266354157	end in vivo 6h
PLSCR1	-1.24176390668036	0.0996958266354157	end in vivo 6h
LOC100139670	-1.24426963113924	0.0996958266354157	end in vivo 6h
LTB4R	-1.2457223896804	0.0996958266354157	end in vivo 6h
RRM2	-1.27357936346215	0.0996958266354157	end in vivo 6h
CCDC141	-1.28730860497848	0.0996958266354157	end in vivo 6h
IDO1	-1.28827940000317	0.0996958266354157	end in vivo 6h
MIR12028	-1.33721649117167	0.0996958266354157	end in vivo 6h
NFAM1	-1.33829702042087	0.0996958266354157	end in vivo 6h
MIR677	-1.377091860656	0.0996958266354157	end in vivo 6h
CD80	-1.38090693214427	0.0996958266354157	end in vivo 6h
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LOC781710	-1.42690163548059	0.0996958266354157	end in vivo 6h
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CHI3L1	-1.44117058633801	0.0996958266354157	end in vivo 6h
OAS1Y	-1.45204984254885	0.0996958266354157	end in vivo 6h
RTP4	-1.48465518363405	0.0996958266354157	end in vivo 6h
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AQP9	-1.57437502630048	0.0996958266354157	end in vivo 6h
IRX5	-1.59066762618766	0.0996958266354157	end in vivo 6h
SPAM1	-1.5994352055541	0.0996958266354157	end in vivo 6h
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PDCD1LG2	-1.67571539126293	0.0996958266354157	end in vivo 6h
TRPM5	-1.73302260105703	0.0996958266354157	end in vivo 6h
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AQP6	-1.73405855514802	0.0996958266354157	end in vivo 6h
CLEC5A	-1.73644307684873	0.0996958266354157	end in vivo 6h
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LOC507055	-1.83605298565648	0.0996958266354157	end in vivo 6h
EGR4	-1.83980142242679	0.0996958266354157	end in vivo 6h
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IFI44L	-1.85708134950312	0.0996958266354157	end in vivo 6h
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SAA2	-1.88973411985232	0.0996958266354157	end in vivo 6h
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ATP10B	-2.08048547607265	0.0996958266354157	end in vivo 6h
HAL	-2.25273618802615	0.0996958266354157	end in vivo 6h
LOC513659	-2.27012867193012	0.0996958266354157	end in vivo 6h
GPR87	-2.30858311233839	0.0996958266354157	end in vivo 6h
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LOC512867	-2.40245339899134	0.0996958266354157	end in vivo 6h
CXCL11	-2.43391357641158	0.0996958266354157	end in vivo 6h
GSTA2	-2.43922740193556	0.0996958266354157	end in vivo 6h
GDAP1L1	-2.47796583194827	0.0996958266354157	end in vivo 6h
GALNT5	-2.48580252753753	0.0996958266354157	end in vivo 6h
IFI44	-2.51310155179125	0.0920117533211989	end in vivo 6h
ART3	-2.51475798663152	0.0996958266354157	end in vivo 6h
LPO	-2.56843807585216	0.0996958266354157	end in vivo 6h
TPPA	-2.60775530847287	0.0996958266354157	end in vivo 6h
LOC112446726	-2.63291235991921	0.0996958266354157	end in vivo 6h
GPR34	-2.66471092748742	0.0996958266354157	end in vivo 6h
LY86	-2.67800766302676	0.0996958266354157	end in vivo 6h
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HAO1	-2.73396043728445	0.0996958266354157	end in vivo 6h
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FRMD7	-2.77807285235902	0.0996958266354157	end in vivo 6h
CXCL10	-2.78814545532633	0.0996958266354157	end in vivo 6h
OVGP1	-2.8064166088805	0.0815295500703684	end in vivo 6h
LOC112446894	-2.81408956874904	0.0996958266354157	end in vivo 6h
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ADRB1	-3.01203485237013	0.0996958266354157	end in vivo 6h
SMCO1	-3.01910022088896	0.0996958266354157	end in vivo 6h
TREM1	-3.06085552476483	0.0996958266354157	end in vivo 6h
LOC100139209	-3.10148487210828	0.0996958266354157	end in vivo 6h
ZBPB	-3.10336785814962	0.0996958266354157	end in vivo 6h
LOC112446120	-3.14029077889012	0.0996958266354157	end in vivo 6h
S100A8	-3.3636273682539	0.0996958266354157	end in vivo 6h
VSIG1	-3.41888232753691	0.0996958266354157	end in vivo 6h
OAS1Z	-3.65494227789834	0.0996958266354157	end in vivo 6h
CD209	-3.78578460437563	0.0996958266354157	end in vivo 6h
APOBEC3Z1	-3.80643988173728	0.0996958266354157	end in vivo 6h
KCTD19	-3.86572643245648	0.0996958266354157	end in vivo 6h
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SCP2	0.505088635752832	0.0996958266354157	end 6h
RCN1	0.506410156728758	0.0996958266354157	end 6h
PIKFYVE	0.510030807120866	0.0996958266354157	end 6h
RC3H1	0.510974349938614	0.0996958266354157	end 6h
FMNL2	0.516180831902832	0.0996958266354157	end 6h
ABCB1	0.517140968519958	0.0996958266354157	end 6h
LDLRAD4	0.517840836606146	0.0996958266354157	end 6h
S100B	0.51838210793585	0.0996958266354157	end 6h
AAK1	0.518900930224567	0.0996958266354157	end 6h
RSPRY1	0.520212005991998	0.0567495207127449	end 6h
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KIAA1549	0.522982868969814	0.0996958266354157	end 6h
PFKFB2	0.523580961405776	0.0996958266354157	end 6h
CAT	0.525690845132356	0.0996958266354157	end 6h
PODXL	0.526078355329435	0.0996958266354157	end 6h
CRY2	0.529712312147239	0.0996958266354157	end 6h
CCDC106	0.529921966328399	0.0996958266354157	end 6h
TIMM21	0.530567697883899	0.0996958266354157	end 6h
TBC1D5	0.531243985682117	0.0996958266354157	end 6h

SLC48A1	0.532322569529298	0.0996958266354157	end 6h
JARID2	0.533099049382646	0.0996958266354157	end 6h
CLOCK	0.533161227668555	0.0996958266354157	end 6h
FARP2	0.535697162390608	0.0996958266354157	end 6h
PER1	0.536166737753632	0.0996958266354157	end 6h
RHOF	0.53726576957836	0.0996958266354157	end 6h
NDE1	0.54262786983234	0.0996958266354157	end 6h
TMEM120B	0.543816879513335	0.0996958266354157	end 6h
STXBP4	0.544490581388271	0.0996958266354157	end 6h
ARHGEF15	0.544855083350159	0.0996958266354157	end 6h
GCA	0.545212649747636	0.0997366936593684	end 6h
METTL27	0.545970797886806	0.0996958266354157	end 6h
ITPR2	0.548452931042548	0.0996958266354157	end 6h
TRIM16	0.550386839131425	0.0996958266354157	end 6h
ELOVL5	0.550418383863822	0.0996958266354157	end 6h
MITF	0.553909169784979	0.0996958266354157	end 6h
TLE2	0.555539667359129	0.0920117533211989	end 6h
LIMS2	0.556229309873392	0.0996958266354157	end 6h
LOC101904339	0.561417075940806	0.0996958266354157	end 6h
ASRGL1	0.563129637069884	0.0996958266354157	end 6h
GRHL1	0.563255444126899	0.0996958266354157	end 6h
ENSBTAG00000053410	0.563811746076935	0.0996958266354157	end 6h
GGT5	0.567172791207255	0.0996958266354157	end 6h
ENPP6	0.570808043837854	0.0996958266354157	end 6h
PARP1	0.571420429788424	0.0996958266354157	end 6h
ENSBTAG00000033166	0.573162674679603	0.0996958266354157	end 6h
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PATZ1	0.576551336171848	0.0996958266354157	end 6h
PKD1L2	0.578074212872271	0.0996958266354157	end 6h
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PDXP	0.57874814341177	0.0996958266354157	end 6h
SORBS1	0.579051358140956	0.0996958266354157	end 6h
SOCS3	0.579819473259034	0.0996958266354157	end 6h
PLEKHH1	0.579863347512941	0.0996958266354157	end 6h
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FBXO44	0.580770429491943	0.0996958266354157	end 6h
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NHS	0.583222038535948	0.0996958266354157	end 6h
HOXA5	0.59640782619458	0.0996958266354157	end 6h
RNF43	0.596930182793952	0.0996958266354157	end 6h
SUGP2	0.597978226124872	0.0996958266354157	end 6h
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MPV17L2	0.598110971243396	0.0996958266354157	end 6h
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SETX	0.606089825254818	0.0996958266354157	end 6h
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PLLP	0.62554011315203	0.0996958266354157	end 6h
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PBX3	0.630728104665526	0.0996958266354157	end 6h
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ESR1	0.63440226966673	0.0996958266354157	end 6h
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NR4A1	0.643238447163356	0.0999538251962677	end 6h
PIDD1	0.64436710679006	0.0996958266354157	end 6h
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RANBP17	0.648508243499364	0.0996958266354157	end 6h
DGCR6L	0.6492704425753	0.0996958266354157	end 6h
BCKDHB	0.65171497063866	0.0996958266354157	end 6h
PCCA	0.652110100063088	0.0996958266354157	end 6h
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SH3TC1	0.661006214235546	0.0996958266354157	end 6h
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FKBP5	0.671859148486978	0.0996958266354157	end 6h
ERC2	0.67330145258048	0.0996958266354157	end 6h
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MAGIX	0.682755937208498	0.0996958266354157	end 6h
DUSP19	0.683067081552649	0.0996958266354157	end 6h
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TBC1D30	0.688040692770396	0.0996958266354157	end 6h
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SYK	0.700743375005573	0.0996958266354157	end 6h

PRKN	0.703007576635448	0.0996958266354157	end 6h
RMND1	0.713086206592602	0.0996958266354157	end 6h
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MGARP	0.744782626971971	0.0996958266354157	end 6h
EXOC3L4	0.749293676316042	0.0996958266354157	end 6h
RASGEF1A	0.750553216924216	0.0996958266354157	end 6h
STX10	0.753254219000654	0.0996958266354157	end 6h
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SFXN5	0.7638399589879	0.0996958266354157	end 6h
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MCM10	0.768625044575906	0.0996958266354157	end 6h
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FGFBP1	0.806233724304346	0.0996958266354157	end 6h
CIT	0.807582073381703	0.0996958266354157	end 6h
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TST	0.881185244368155	0.0996958266354157	end 6h
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WIPF3	1.00715992292023	0.0996958266354157	end 6h
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CPED1	1.01345037452701	0.0996958266354157	end 6h
SGIP1	1.01590280744318	0.0996958266354157	end 6h
GJB5	1.01787758068971	0.0996958266354157	end 6h
SPOCK2	1.02855046388809	0.0996958266354157	end 6h
NFIA	1.0304612435204	0.0996958266354157	end 6h
DNAJA4	1.03199147145281	0.0996958266354157	end 6h
CENPF	1.04708925394659	0.0996958266354157	end 6h
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SCUBE1	1.09272511047292	0.0996958266354157	end 6h
LOC100297513	1.09560629458388	0.0996958266354157	end 6h
ENSBTAG00000048617	1.10124917562507	0.0996958266354157	end 6h
ADCYAP1R1	1.10602537420711	0.0996958266354157	end 6h
FREM1	1.1185661202909	0.0996958266354157	end 6h
ACTN2	1.12866193219898	0.0996958266354157	end 6h
ENSBTAG00000054035	1.12975564751111	0.0996958266354157	end 6h
KCNC3	1.13035325942013	0.0996958266354157	end 6h
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SLC6A11	1.13117698348229	0.0996958266354157	end 6h
CREB3L4	1.13702768076203	0.0996958266354157	end 6h
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GJB1	1.14449354324021	0.0996958266354157	end 6h
EHHADH	1.15109291367483	0.0996958266354157	end 6h
GLYATL2	1.15416709355062	0.0996958266354157	end 6h
ADA	1.15531097127493	0.0996958266354157	end 6h
ENSBTAG00000054238	1.17782496266175	0.0996958266354157	end 6h
GJB4	1.17876820322354	0.0996958266354157	end 6h
SLC25A53	1.19623115655648	0.0996958266354157	end 6h
ABCA7	1.19732743753596	0.0996958266354157	end 6h
CCNB1	1.23297528896661	0.0996958266354157	end 6h

ENSBTAG00000048857	1.23336665761332	0.0996958266354157	end 6h
ENSBTAG00000053707	1.23829090257431	0.0996958266354157	end 6h
IL31RA	1.24243464522188	0.0996958266354157	end 6h
RADIL	1.24663964870529	0.0996958266354157	end 6h
ENSBTAG00000048395	1.24676238524324	0.0996958266354157	end 6h
TROAP	1.25722417412643	0.0996958266354157	end 6h
GAT	1.25742007628234	0.0996958266354157	end 6h
PCLAF	1.27445412305492	0.0996958266354157	end 6h
AMZ1	1.27646247337769	0.0996958266354157	end 6h
ENSBTAG00000052097	1.29684244864472	0.0996958266354157	end 6h
IL17B	1.30316024648481	0.0996958266354157	end 6h
LOC617475	1.313437862173	0.0996958266354157	end 6h
TRPM3	1.31491035657283	0.0996958266354157	end 6h
FMO2	1.3214128750046	0.0996958266354157	end 6h
FCGR2A	1.32508475165314	0.0996958266354157	end 6h
NCKAP5	1.33111294201552	0.0996958266354157	end 6h
NOL4	1.33336886290347	0.0996958266354157	end 6h
MASTL	1.33445035854777	0.0996958266354157	end 6h
DMTN	1.34288281065119	0.0996958266354157	end 6h
ENSBTAG00000051802	1.34445310515683	0.0996958266354157	end 6h
SHCBP1	1.36608098999483	0.0996958266354157	end 6h
ENSBTAG00000048956	1.43952699837054	0.0678639341400746	end 6h
ENSBTAG00000048609	1.47442499184864	0.0996958266354157	end 6h
COL13A1	1.47462798685274	0.0996958266354157	end 6h
SLC35B4	1.50242538277287	0.0996958266354157	end 6h
FBXO24	1.51906069027496	0.0996958266354157	end 6h
CYP7A1	1.59508703088097	0.0996958266354157	end 6h
HIF3A	1.60323187248657	0.0996958266354157	end 6h
ENSBTAG00000009268	1.62118696762063	0.0996958266354157	end 6h
ENSBTAG00000051627	1.64730599041192	0.0996958266354157	end 6h
IZUMO1	1.68170451639049	0.0996958266354157	end 6h
SOX6	1.68998259337593	0.0567495207127449	end 6h
LOC100848288	1.70535370780978	0.0996958266354157	end 6h
ENSBTAG00000039937	1.71015250926151	0.0996958266354157	end 6h
AR	1.78353524740268	0.0678639341400746	end 6h
ENSBTAG00000035171	1.79256688111855	0.0996958266354157	end 6h
ENSBTAG00000053812	1.92669348322318	0.0996958266354157	end 6h
MYBPH	1.99364636121342	0.0996958266354157	end 6h
LOC527981	2.02281837302965	0.0996958266354157	end 6h
PBK	2.18333341478685	0.0996958266354157	end 6h
NOS1	2.18654176262385	0.0996958266354157	end 6h
SCN8A	2.19158609683266	0.0996958266354157	end 6h
ENSBTAG00000051077	2.23921141800828	0.0996958266354157	end 6h
LOC112441717	2.2597675849478	0.0996958266354157	end 6h
MIR2384	2.37288791264818	0.0996958266354157	end 6h
ENSBTAG00000052885	2.45315447796295	0.0996958266354157	end 6h

ZAN	2.5753301060954	0.0996958266354157	end 6h
SOHLH2	2.58485179056951	0.0996958266354157	end 6h
ENSBTAG00000053802	2.61629978484344	0.0996958266354157	end 6h
PLAG1	2.64231672806678	0.0996958266354157	end 6h
CSTA	2.73463922484679	0.0996958266354157	end 6h
ENSBTAG00000049959	2.75941844070742	0.0996958266354157	end 6h
ENSBTAG00000049837	2.7626982995678	0.0996958266354157	end 6h
BTNL9	2.78581363211803	0.0996958266354157	end 6h
WNT10A	2.82485863731276	0.0996958266354157	end 6h
ENSBTAG00000048573	2.90832855437945	0.0996958266354157	end 6h
HOXC8	2.92191348531842	0.0996958266354157	end 6h
LOC112442111	2.93197459031014	0.0996958266354157	end 6h
ENSBTAG00000051096	3.06415320306053	0.0996958266354157	end 6h
ENSBTAG00000048179	3.14630816554253	0.0996958266354157	end 6h
KCNH5	3.21844701934456	0.0996958266354157	end 6h
ENSBTAG00000052972	3.22042655412341	0.0996958266354157	end 6h
MIR1584	3.27513581977414	0.0996958266354157	end 6h
EPHA8	3.40402336422102	0.0996958266354157	end 6h
ENSBTAG00000048878	3.40956442582171	0.0996958266354157	end 6h
LOC112443645	3.4769666832681	0.0996958266354157	end 6h
KRT6C	3.51811829854415	0.0996958266354157	end 6h
LOC781679	3.62848331786484	0.0996958266354157	end 6h
FMO1	3.6670371476152	0.0996958266354157	end 6h
ENSBTAG00000048547	3.69136974036496	0.0996958266354157	end 6h
SP5	3.70093188991819	0.0996958266354157	end 6h
LOC112446111	3.72286461848177	0.0996958266354157	end 6h
ENSBTAG00000051484	3.902309667949	0.0996958266354157	end 6h
SLC9A4	4.50990126858629	0.0996958266354157	end 6h
ENSBTAG00000049386	4.62751945260049	0.0996958266354157	end 6h

Supplementary Table S3. Differentially expressed genes in endometrial explants exposed for 6 hours to a Day 7 *in vitro* bovine embryo compared to end group. (end x end *in vitro*)

Gene symbol	Log2FoldChange	p-adjusted	Group
ENSBTAG00000048699	-0.536390339485885	0.0989219787793112	end in vitro 6h
TRIM34	-0.593218931842952	0.0989219787793112	end in vitro 6h
ENSBTAG00000050071	-0.600200179620808	0.0989219787793112	end in vitro 6h
IFIH1	-0.617693407111478	0.0989219787793112	end in vitro 6h
LPAR3	-0.617778189644391	0.0989219787793112	end in vitro 6h
ENSBTAG00000040051	-0.654686722003645	0.0989219787793112	end in vitro 6h
SLC16A9	-0.686946364024871	0.0989219787793112	end in vitro 6h
ENSBTAG00000054933	-0.693589302369964	0.0989219787793112	end in vitro 6h
C18H16orf46	-0.735031339416807	0.0989219787793112	end in vitro 6h
LOC112445242	-0.74768607404839	0.0989219787793112	end in vitro 6h
BMX	-0.79850608730819	0.0989219787793112	end in vitro 6h
LOC101906101	-0.859862901926141	0.0989219787793112	end in vitro 6h
PROX1	-0.878084325910717	0.0989219787793112	end in vitro 6h
GPNMB	-0.904809565520762	0.0989219787793112	end in vitro 6h
ENSBTAG00000006562	-0.9102156934975	0.0989219787793112	end in vitro 6h
MX2	-0.928929837825352	0.0989219787793112	end in vitro 6h
H3F3C	-0.935035181228099	0.0989219787793112	end in vitro 6h
KCNJ15	-0.958004556959884	0.0989219787793112	end in vitro 6h
FAM228B	-1.02295941987211	0.0989219787793112	end in vitro 6h
ENSBTAG00000054738	-1.05390346071212	0.0989219787793112	end in vitro 6h
OAS1Y	-1.05583658231142	0.0989219787793112	end in vitro 6h
IL1RL1	-1.06196980592127	0.0989219787793112	end in vitro 6h
LOC112448905	-1.08073464562829	0.0989219787793112	end in vitro 6h
ENSBTAG00000035660	-1.11125606863014	0.0989219787793112	end in vitro 6h
ENSBTAG00000054999	-1.14811976690936	0.0989219787793112	end in vitro 6h
EBI3	-1.15006200348372	0.0989219787793112	end in vitro 6h
NTF4	-1.1759894288307	0.0989219787793112	end in vitro 6h
BHLHA15	-1.21035937393572	0.0989219787793112	end in vitro 6h
ENSBTAG00000051950	-1.27354580052086	0.0989219787793112	end in vitro 6h
ENSBTAG00000055172	-1.31383091249336	0.0989219787793112	end in vitro 6h
ENSBTAG00000051963	-1.40085897095174	0.0989219787793112	end in vitro 6h
ENSBTAG00000055058	-1.42856715170902	0.0989219787793112	end in vitro 6h
CLEC5A	-1.57445557898518	0.0989219787793112	end in vitro 6h
CYP4B1	-1.61577388306241	0.0989219787793112	end in vitro 6h
ADGRG2	-1.62376773789639	0.0989219787793112	end in vitro 6h
NLRC4	-1.63453730484735	0.0989652213009968	end in vitro 6h
PDCD1LG2	-1.70192156859022	0.0989219787793112	end in vitro 6h
KCNK3	-1.73241747078597	0.0989219787793112	end in vitro 6h
LOC407163	-1.74250528068096	0.0989219787793112	end in vitro 6h
RASAL3	-1.81496169042456	0.0989219787793112	end in vitro 6h
UBASH3A	-1.83911875365564	0.0989219787793112	end in vitro 6h
MSLN	-1.8564613187143	0.0989219787793112	end in vitro 6h
ENSBTAG00000017248	-1.86146180110371	0.0989219787793112	end in vitro 6h

IFI44	-1.94538569470956	0.0989219787793112	end in vitro 6h
OAS2	-1.98699865111941	0.0989219787793112	end in vitro 6h
ENSBTAG00000052434	-2.00969874884537	0.0989219787793112	end in vitro 6h
LOC512867	-2.05292871832896	0.0989219787793112	end in vitro 6h
LOC785479	-2.15207339341348	0.0989219787793112	end in vitro 6h
ENSBTAG00000054671	-2.16693882988111	0.0989219787793112	end in vitro 6h
GSTA2	-2.26090331839607	0.0989219787793112	end in vitro 6h
ENSBTAG00000036102	-2.40446101442042	0.0989219787793112	end in vitro 6h
ENSBTAG00000020813	-2.42171375675748	0.0989219787793112	end in vitro 6h
TPPA	-2.51464604769375	0.0989219787793112	end in vitro 6h
CD84	-2.53884025446853	0.0989219787793112	end in vitro 6h
ATP10B	-2.58018986557902	0.0989219787793112	end in vitro 6h
AQP6	-2.59109470122415	0.0989219787793112	end in vitro 6h
KLRA1	-2.59476467541086	0.0989219787793112	end in vitro 6h
ENSBTAG00000052745	-2.63532296002366	0.0989219787793112	end in vitro 6h
AQP9	-2.79584028522063	0.0305618087348887	end in vitro 6h
ENSBTAG00000025908	-2.81339317208243	0.0989219787793112	end in vitro 6h
LOC112446120	-2.82227887504545	0.0989219787793112	end in vitro 6h
GNRH1	-2.85853310860407	0.0989219787793112	end in vitro 6h
ENSBTAG00000052892	-2.90288634187723	0.0989219787793112	end in vitro 6h
LOC531557	-2.94437847756298	0.0989219787793112	end in vitro 6h
ENSBTAG00000050476	-2.95123686773197	0.0989219787793112	end in vitro 6h
SPINK1	-2.95920017324538	0.0989219787793112	end in vitro 6h
ENSBTAG00000051177	-2.97864165175272	0.0989219787793112	end in vitro 6h
KCTD19	-3.02033393526063	0.0989219787793112	end in vitro 6h
ENSBTAG00000053912	-3.0224534884356	0.0989219787793112	end in vitro 6h
SMCO1	-3.10631298054619	0.0989219787793112	end in vitro 6h
MIR2385	-3.13667403249498	0.0989219787793112	end in vitro 6h
LY86	-3.15151249860914	0.0989219787793112	end in vitro 6h
NFASC	-3.15169660930037	0.0989219787793112	end in vitro 6h
LOC112446726	-3.15683371949596	0.0989219787793112	end in vitro 6h
ENSBTAG00000051928	-3.23974801540584	0.0989219787793112	end in vitro 6h
OVGP1	-3.31379371575653	0.0185005139056895	end in vitro 6h
GPR34	-3.41191383976185	0.0989219787793112	end in vitro 6h
MT3	-3.78423030917405	0.0989219787793112	end in vitro 6h
APOBEC3Z1	-3.99020758707822	0.0989219787793112	end in vitro 6h
LOC100138271	-4.41514508834527	0.0185005139056895	end in vitro 6h
ENSBTAG00000053654	-4.61319089337899	0.0305618087348887	end in vitro 6h
LAMP5	-4.88188218221693	0.0305618087348887	end in vitro 6h
LOC112445778	-9.14976981524071	0.0989219787793112	end in vitro 6h
PPP1R16B	0.505911461763396	0.0989219787793112	end 6h
B3GNT9	0.508594684039226	0.0990816175046942	end 6h
EIF2B1	0.515688763970804	0.0992083154306656	end 6h
RBPMS2	0.518407567005889	0.0989219787793112	end 6h
THSD1	0.519762570899938	0.0989219787793112	end 6h
AFAP1L1	0.540687448139178	0.0989219787793112	end 6h

LPAR1	0.55454402365386	0.0989219787793112	end 6h
ESAM	0.555472787297921	0.0989219787793112	end 6h
NHS	0.565074125106561	0.0989219787793112	end 6h
H2AFY2	0.565144466903521	0.0989219787793112	end 6h
ENSBTAG00000048411	0.574210336062318	0.0989219787793112	end 6h
HELQ	0.59919464986152	0.0989219787793112	end 6h
ENPP6	0.620495670971686	0.0989219787793112	end 6h
TIE1	0.642353667274825	0.0989219787793112	end 6h
ARHGEF6	0.667378115860417	0.0989219787793112	end 6h
RASSF2	0.684413085182517	0.0989652213009968	end 6h
KCNAB2	0.693953064846574	0.0989219787793112	end 6h
LDB2	0.71551606104949	0.0989219787793112	end 6h
ZNF383	0.729176860589442	0.0989219787793112	end 6h
ABCA7	0.756562856316866	0.0989219787793112	end 6h
SLC7A8	0.775071153900058	0.0989219787793112	end 6h
TRIP13	0.784193411392524	0.0989219787793112	end 6h
HEYL	0.79092740201471	0.0989219787793112	end 6h
ENSBTAG00000054744	0.816072972753933	0.0989219787793112	end 6h
BMP6	0.824378085225342	0.0989219787793112	end 6h
AJAP1	0.831793911934166	0.0989652213009968	end 6h
GNA14	0.838618956652753	0.0989219787793112	end 6h
STAG3	0.865244217491227	0.0989219787793112	end 6h
ENSBTAG00000022427	0.883675989798377	0.0989219787793112	end 6h
PODN	0.915002396606543	0.0989219787793112	end 6h
NAP1L5	0.915429595997013	0.0989219787793112	end 6h
ALX3	0.923596606168835	0.0989219787793112	end 6h
HAND2	0.924228338942474	0.0989219787793112	end 6h
CD93	0.930897431793886	0.0989219787793112	end 6h
ENSBTAG00000053294	0.951205869092961	0.0989219787793112	end 6h
NREP	0.962791900046989	0.0989219787793112	end 6h
GPIHBP1	0.963767391961451	0.0989652213009968	end 6h
NOVA2	0.967113031495939	0.0989219787793112	end 6h
TMEM130	0.967167237505286	0.0989219787793112	end 6h
ENSBTAG00000048660	0.967209386520605	0.0989219787793112	end 6h
STEAP1	0.995263503783244	0.0989219787793112	end 6h
ENSBTAG00000055293	1.0012215285533	0.0989219787793112	end 6h
ALX1	1.0483466833093	0.0989219787793112	end 6h
DIPK2B	1.055404511815	0.0989219787793112	end 6h
MKI67	1.07535981241976	0.0989219787793112	end 6h
ZGRF1	1.08516526865308	0.0989219787793112	end 6h
COL13A1	1.0889483395752	0.0989219787793112	end 6h
KIF22	1.1402466280301	0.0989219787793112	end 6h
ENSBTAG00000048617	1.14493178079975	0.0989219787793112	end 6h
PPP2R2C	1.21316013507512	0.0989219787793112	end 6h
REG4	1.22809272916679	0.0989219787793112	end 6h
C2H2orf72	1.2632759528093	0.0989652213009968	end 6h

TOP2A	1.26594368590531	0.0989219787793112	end 6h
ALB	1.29639689416453	0.0989219787793112	end 6h
LOR	1.30064941644662	0.0989219787793112	end 6h
LOC617475	1.30395300126614	0.0989219787793112	end 6h
ZNF319	1.31149763055986	0.0989219787793112	end 6h
P2RY1	1.32440566299309	0.0989219787793112	end 6h
POLR3G	1.34522569473804	0.0989219787793112	end 6h
BLOC1S3	1.38494871813189	0.0989219787793112	end 6h
LOC100297056	1.40481105283915	0.0989219787793112	end 6h
CENPF	1.4160379934507	0.0989219787793112	end 6h
ENSBTAG00000052913	1.42113081335206	0.0989219787793112	end 6h
ENSBTAG00000029954	1.46300229942105	0.0989652213009968	end 6h
ALX4	1.47359959731881	0.0989219787793112	end 6h
ESCO2	1.48275923920988	0.0989219787793112	end 6h
HJURP	1.48440462312301	0.0989219787793112	end 6h
BUB1	1.49922944178129	0.0989219787793112	end 6h
SHCBP1	1.50310130918213	0.0989219787793112	end 6h
TMEM255A	1.50464930877882	0.0989219787793112	end 6h
ENSBTAG00000053054	1.56868634850241	0.0989219787793112	end 6h
DLGAP5	1.5996275121506	0.0989219787793112	end 6h
ENSBTAG00000049104	1.65997872187441	0.0989219787793112	end 6h
PADI1	1.70979451048839	0.0989219787793112	end 6h
HOPX	1.74925288942266	0.0989219787793112	end 6h
KNL1	1.75466876766697	0.0989219787793112	end 6h
TFEC	1.81566918409179	0.0989219787793112	end 6h
ENSBTAG00000052642	1.86608506361094	0.0989219787793112	end 6h
FAM217B	1.88537621279142	0.0989219787793112	end 6h
ARHGAP11A	1.89252339023015	0.0989219787793112	end 6h
SLC35B4	1.9049404003212	0.0989219787793112	end 6h
SGO1	1.92656263675594	0.0989219787793112	end 6h
ENSBTAG00000052715	1.92963680251408	0.0989219787793112	end 6h
SIX3	1.93396862507677	0.0989652213009968	end 6h
FGD2	1.93974523642014	0.0989219787793112	end 6h
ENSBTAG00000003645	1.94279629892393	0.0989652213009968	end 6h
MMRN1	2.05559378756589	0.0989219787793112	end 6h
ENSBTAG00000053553	2.07914977117401	0.0989219787793112	end 6h
LOC101907883	2.07958275330262	0.0989219787793112	end 6h
ENSBTAG00000045601	2.19219062875811	0.0989219787793112	end 6h
TRAPPC3L	2.21141602784579	0.0989219787793112	end 6h
ENSBTAG00000051077	2.22221188979791	0.0989219787793112	end 6h
REELD1	2.24703095062043	0.0990816175046942	end 6h
ENSBTAG00000053773	2.30413072653228	0.0989219787793112	end 6h
DACT3	2.3937605832051	0.0989219787793112	end 6h
GALNT14	2.5069908397227	0.0989219787793112	end 6h
CELF4	2.55514361848825	0.0989219787793112	end 6h
ENSBTAG00000053802	2.66199645101998	0.0989219787793112	end 6h

ALDH1A2	2.6758537896194	0.0989219787793112	end 6h
ENSBTAG00000000719	2.69822323229859	0.0989219787793112	end 6h
ENSBTAG00000053523	2.76714657712572	0.0989219787793112	end 6h
SLC8A2	2.76796783055173	0.0989219787793112	end 6h
ENSBTAG00000054557	2.7732979945672	0.0989219787793112	end 6h
ENSBTAG00000050695	2.93147367279195	0.0989219787793112	end 6h
HOXC8	2.93618877145793	0.0989219787793112	end 6h
H1FOO	2.98352978409921	0.0989219787793112	end 6h
FAM228A	3.23460558288559	0.0989219787793112	end 6h
GLP1R	3.24708402898181	0.0989219787793112	end 6h
FAM209A	3.49078481493665	0.0989219787793112	end 6h
ENSBTAG00000052740	3.49934582895567	0.0989219787793112	end 6h
MYBPHL	3.51475653066913	0.0989219787793112	end 6h
ENSBTAG00000000853	3.52231294571579	0.0989219787793112	end 6h
GPR50	3.53404343256906	0.0989219787793112	end 6h
ENSBTAG00000053916	3.71187702596845	0.0989219787793112	end 6h
PAG2	3.72442528395509	0.0989219787793112	end 6h
ENSBTAG00000052066	3.92641018804374	0.0989219787793112	end 6h
STXBP5L	3.97128603794009	0.0989219787793112	end 6h
KCNJ4	4.10747388507027	0.0989219787793112	end 6h
ENSBTAG00000053126	4.51618876964591	0.0989219787793112	end 6h

Supplementary Table S4. Forty-five DEGs in end *in vivo* and end *in vitro* groups compared to end group.

Gene symbol
TRIM34
IFIH1
SLC16A9
MX2
KCNJ15
ENSBTAG00000054738
OAS1Y
ENSBTAG00000035660
BHLHA15
CLEC5A
ADGRG2
PDCD1LG2
KCNK3
IFI44
OAS2
LOC512867
GSTA2
TTPA
ATP10B
AQP6
ENSBTAG00000025908
LOC112446120
KCTD19
SMCO1
LY86
LOC112446726
OVGP1
GPR34
APOBEC3Z1
LOC100138271
ENSBTAG00000053654
NHS
ENPP6
ABCA7
SLC7A8
PODN
COL13A1
ENSBTAG00000048617
LOC617475
CENPF
SHCBP1
SLC35B4
ENSBTAG00000051077
ENSBTAG00000053802
HOXC8

Supplementary Table S5. The enriched pathways and biological processes associated with the 45 DEGs in end *in vivo* and end *in vitro* groups compared to end group.

Pathways	P-Value
Measles	0.017
Influenza A	0.025
Herpes simplex infection	0.03
Biological processes	P-Value
Innate immune response	0.0086
Defense response to virus	0.023
Response to virus	0.08

Supplementary Table S6. Differentially expressed genes in end *in vitro* compared to end *in vivo* 6h (end *in vitro* x end *in vivo*).

Gene symbol	Log2FoldChange	p-adjusted	Group
ZFP3	-0.507093904916661	0.0998512019030394	end in vivo 6h
LPAR1	-0.509293546097938	0.0998512019030394	end in vivo 6h
ENSBTAG00000006530	-0.513630986204479	0.0998512019030394	end in vivo 6h
ENSBTAG00000055000	-0.517003487430468	0.0998512019030394	end in vivo 6h
THOC6	-0.519196820631132	0.0998512019030394	end in vivo 6h
SDC2	-0.520847279897534	0.0998512019030394	end in vivo 6h
ENSBTAG00000049842	-0.521984660578501	0.0998512019030394	end in vivo 6h
TMEM178A	-0.530734611090861	0.0998512019030394	end in vivo 6h
B3GNT9	-0.533205286036798	0.0998512019030394	end in vivo 6h
LOC112442392	-0.535541806648904	0.0998512019030394	end in vivo 6h
DEGS1	-0.538256664327881	0.0998512019030394	end in vivo 6h
TFAP4	-0.544488446004232	0.0998512019030394	end in vivo 6h
ENSBTAG00000048734	-0.545757782024005	0.0998512019030394	end in vivo 6h
AMMECR1	-0.554363559039564	0.0998512019030394	end in vivo 6h
WDR77	-0.555043552080437	0.0998512019030394	end in vivo 6h
ENSBTAG00000052354	-0.570140099074543	0.0998512019030394	end in vivo 6h
TIE1	-0.577522771765924	0.0998512019030394	end in vivo 6h
ENSBTAG00000004939	-0.588108102784653	0.0998512019030394	end in vivo 6h
ECE2	-0.593836607087824	0.0998512019030394	end in vivo 6h
ENSBTAG00000046928	-0.596759691060424	0.0998512019030394	end in vivo 6h
TMEM126B	-0.597568596954739	0.0998512019030394	end in vivo 6h
ENSBTAG00000043566	-0.606206385997223	0.0998512019030394	end in vivo 6h
PPP1R14B	-0.615583466621559	0.0998512019030394	end in vivo 6h
SEMA6B	-0.616757956449214	0.0998512019030394	end in vivo 6h
ENSBTAG00000048992	-0.620528576105083	0.0998512019030394	end in vivo 6h
MON1A	-0.620807560932725	0.0998512019030394	end in vivo 6h
EIF2B1	-0.62329137154055	0.0998512019030394	end in vivo 6h
NCOA7	-0.631014400636671	0.0998512019030394	end in vivo 6h
BMP6	-0.63117407097501	0.0998512019030394	end in vivo 6h
HCLS1	-0.638618127451931	0.0998512019030394	end in vivo 6h
ENSBTAG00000043552	-0.642452279127281	0.0998512019030394	end in vivo 6h
ENSBTAG00000025146	-0.648596027273777	0.0998512019030394	end in vivo 6h
TMEM185B	-0.671230733776299	0.0998512019030394	end in vivo 6h
TRPC2	-0.677930986460627	0.0998512019030394	end in vivo 6h
ENSBTAG00000043544	-0.686431216045787	0.0998512019030394	end in vivo 6h
ZNF382	-0.687551134276738	0.0998512019030394	end in vivo 6h
LXN	-0.687972940692	0.0998512019030394	end in vivo 6h
CHST2	-0.690483944506678	0.0998512019030394	end in vivo 6h
PCDH11X	-0.707907251363654	0.0998512019030394	end in vivo 6h
UMPS	-0.70929972722574	0.0998512019030394	end in vivo 6h
THSD1	-0.711080420190144	0.0998512019030394	end in vivo 6h
CCDC71L	-0.712119953477133	0.0998512019030394	end in vivo 6h
ENSBTAG00000048939	-0.724975221032934	0.0998512019030394	end in vivo 6h

DNAJC30	-0.729379473437409	0.0998512019030394	end in vivo 6h
KHDRBS3	-0.739311199585376	0.0998512019030394	end in vivo 6h
CD93	-0.740532217236409	0.0998512019030394	end in vivo 6h
ENSBTAG00000010030	-0.747738407300134	0.0998512019030394	end in vivo 6h
MSRB3	-0.75066340791528	0.0998512019030394	end in vivo 6h
ENSBTAG00000038900	-0.752986663144096	0.0998512019030394	end in vivo 6h
LCMT2	-0.761357833181563	0.0998512019030394	end in vivo 6h
ENSBTAG00000005559	-0.762453647437877	0.0998512019030394	end in vivo 6h
GSTM1	-0.769266824247816	0.0998512019030394	end in vivo 6h
ZNF467	-0.775610508169149	0.0998512019030394	end in vivo 6h
C1QC	-0.789533699499025	0.0998512019030394	end in vivo 6h
FBXO5	-0.800577074080533	0.0998512019030394	end in vivo 6h
ENSBTAG00000048671	-0.802035751635633	0.0998512019030394	end in vivo 6h
LOC530348	-0.806740262881751	0.0998512019030394	end in vivo 6h
LYSMD2	-0.80754394268739	0.0998512019030394	end in vivo 6h
CLDN2	-0.813350788839941	0.0998512019030394	end in vivo 6h
ENSBTAG00000049530	-0.816422174607197	0.0998512019030394	end in vivo 6h
FST	-0.834557341558816	0.0998512019030394	end in vivo 6h
ALX3	-0.851308836407931	0.0998512019030394	end in vivo 6h
PII5	-0.860612434689243	0.0998512019030394	end in vivo 6h
ENSBTAG00000054744	-0.863820480732242	0.0998512019030394	end in vivo 6h
TMEM100	-0.877096877595957	0.0998512019030394	end in vivo 6h
LRFN1	-0.886188349186196	0.0998512019030394	end in vivo 6h
C1QB	-0.893265812135002	0.0998512019030394	end in vivo 6h
PRKRA	-0.900604801855902	0.0998512019030394	end in vivo 6h
LOC786974	-0.91114708786075	0.0998512019030394	end in vivo 6h
GPRC5B	-0.915892439293221	0.0998512019030394	end in vivo 6h
B3GALT6	-0.918632737466324	0.0998512019030394	end in vivo 6h
ENSBTAG00000025814	-0.939023497291723	0.0998512019030394	end in vivo 6h
NCBP2AS2	-0.971629212923814	0.0998512019030394	end in vivo 6h
C5AR1	-0.972086178395224	0.0998512019030394	end in vivo 6h
LOC524181	-0.986445746213933	0.0998512019030394	end in vivo 6h
FBP2	-0.986513252084208	0.0998512019030394	end in vivo 6h
GATA6	-0.996210275434555	0.0998512019030394	end in vivo 6h
ENSBTAG00000049718	-1.05368252479156	0.0998512019030394	end in vivo 6h
ENSBTAG00000052981	-1.07352282025127	0.0998512019030394	end in vivo 6h
ENSBTAG00000052913	-1.07415575969846	0.0998512019030394	end in vivo 6h
ENSBTAG00000053607	-1.10899015665526	0.0998512019030394	end in vivo 6h
IFI44L	-1.11683646973188	0.0998512019030394	end in vivo 6h
GUCA1A	-1.13902989282997	0.0998512019030394	end in vivo 6h
KCNJ11	-1.14074269657923	0.0998512019030394	end in vivo 6h
SNX10	-1.14885573920762	0.0998512019030394	end in vivo 6h
ENSBTAG00000055293	-1.15986875334719	0.0998512019030394	end in vivo 6h
C2	-1.24115215473994	0.0998512019030394	end in vivo 6h
C2H2orf72	-1.26159231753488	0.0998512019030394	end in vivo 6h
ESCO2	-1.26314560891275	0.0998512019030394	end in vivo 6h

CYP3A5	-1.33269752991791	0.0998512019030394	end in vivo 6h
GPIHBP1	-1.33477370950502	0.0998512019030394	end in vivo 6h
CDT1	-1.35186054755054	0.0998512019030394	end in vivo 6h
ENSBTAG00000048433	-1.36451191932209	0.0998512019030394	end in vivo 6h
C19H17orf67	-1.36912914805532	0.0998512019030394	end in vivo 6h
ASF1B	-1.40347283457383	0.0998512019030394	end in vivo 6h
ENSBTAG00000053054	-1.42006768619308	0.0998512019030394	end in vivo 6h
LOC100297056	-1.4211303829609	0.0998512019030394	end in vivo 6h
CD80	-1.45240748406063	0.0998512019030394	end in vivo 6h
NINJ2	-1.49297207982556	0.0998512019030394	end in vivo 6h
TRPC3	-1.49880507403949	0.0998512019030394	end in vivo 6h
POLR3G	-1.51978080994044	0.0998512019030394	end in vivo 6h
MELTF	-1.5397765267432	0.0998512019030394	end in vivo 6h
KIF18A	-1.56210022945767	0.0998512019030394	end in vivo 6h
DNASE1L3	-1.56486991109945	0.0998512019030394	end in vivo 6h
PADII	-1.60475652239848	0.0998512019030394	end in vivo 6h
S100A8	-1.61191494644836	0.0998512019030394	end in vivo 6h
RRM2	-1.62705175636064	0.0998512019030394	end in vivo 6h
ENSBTAG00000050108	-1.74696184187435	0.0998512019030394	end in vivo 6h
ENSBTAG00000052386	-1.74984189174977	0.0998512019030394	end in vivo 6h
ENSBTAG00000029954	-1.77081626372015	0.0998512019030394	end in vivo 6h
ATP1B4	-1.77169393622793	0.0998512019030394	end in vivo 6h
TFEC	-1.80518351676954	0.0998512019030394	end in vivo 6h
ENSBTAG00000052715	-1.8187070924151	0.0998512019030394	end in vivo 6h
HTR7	-1.9059695472619	0.0998512019030394	end in vivo 6h
LOC100335729	-1.98442382783042	0.0998512019030394	end in vivo 6h
ENSBTAG00000045601	-2.01301040673428	0.0998512019030394	end in vivo 6h
NTN3	-2.05923539356003	0.0998512019030394	end in vivo 6h
LDHC	-2.0995381569243	0.0998512019030394	end in vivo 6h
LOC100847469	-2.14736536606514	0.0998512019030394	end in vivo 6h
LOC512541	-2.22420895531924	0.0998512019030394	end in vivo 6h
ENSBTAG00000051502	-2.2971105231949	0.0998512019030394	end in vivo 6h
ENSBTAG00000050985	-2.53044624810383	0.0998512019030394	end in vivo 6h
PPP1R3D	-2.53094381314588	0.0998512019030394	end in vivo 6h
OLFM1	-2.61081822749156	0.0998512019030394	end in vivo 6h
LPO	-2.6392621312338	0.0998512019030394	end in vivo 6h
REELD1	-2.67836057760636	0.0998512019030394	end in vivo 6h
DACT3	-2.73341140250254	0.0998512019030394	end in vivo 6h
ENSBTAG00000050536	-2.74122349554133	0.0998512019030394	end in vivo 6h
ZBTB32	-2.81715459852286	0.0998512019030394	end in vivo 6h
GLP1R	-2.83546556227143	0.0998512019030394	end in vivo 6h
ENSBTAG00000050205	-2.87249441295936	0.0998512019030394	end in vivo 6h
ENSBTAG00000054558	-2.88229587205358	0.0998512019030394	end in vivo 6h
MYBPHL	-2.88871755083408	0.0998512019030394	end in vivo 6h
PNPLA1	-2.9733796692171	0.0998512019030394	end in vivo 6h
LOC101904668	-2.99122665712875	0.0998512019030394	end in vivo 6h

ENSBTAG00000016306	-3.01753650356808	0.0998512019030394	end in vivo 6h
FLT3	-3.06812129970275	0.0998512019030394	end in vivo 6h
ENSBTAG00000010970	-3.08272525011935	0.0998512019030394	end in vivo 6h
CLCA4	-3.13447676775038	0.0998512019030394	end in vivo 6h
ENSBTAG00000051483	-3.1351394693257	0.0998512019030394	end in vivo 6h
ENSBTAG00000053126	-3.14544080536486	0.0998512019030394	end in vivo 6h
ENSBTAG00000052740	-3.17226160783961	0.0998512019030394	end in vivo 6h
STXBP5L	-3.23315666863731	0.0998512019030394	end in vivo 6h
KCNJ4	-3.23335904552605	0.0998512019030394	end in vivo 6h
ENSBTAG00000007280	-3.41232514324786	0.0998512019030394	end in vivo 6h
ENSBTAG00000000853	-3.4195734934087	0.0998512019030394	end in vivo 6h
SPACA5	-3.47643342446974	0.0998512019030394	end in vivo 6h
SFTPD	-3.52107972093449	0.0998512019030394	end in vivo 6h
TLE2	0.500671547173789	0.0998512019030394	end in vitro 6h
CYB5B	0.502147935628032	0.0998512019030394	end in vitro 6h
ZFYVE19	0.502943315479258	0.0998512019030394	end in vitro 6h
PREX2	0.504990291421001	0.0998512019030394	end in vitro 6h
RC3H1	0.507683401113805	0.0998512019030394	end in vitro 6h
PATZ1	0.510676595175926	0.0998512019030394	end in vitro 6h
ACOX2	0.512123751395999	0.0998512019030394	end in vitro 6h
SCRN1	0.512612826629259	0.0998512019030394	end in vitro 6h
CAT	0.514201126149024	0.0998512019030394	end in vitro 6h
PRR15L	0.515503628410903	0.0998512019030394	end in vitro 6h
AREG	0.521216925768857	0.0998512019030394	end in vitro 6h
ZMIZ2	0.526999592374779	0.0998512019030394	end in vitro 6h
AXIN2	0.527541474918978	0.0998512019030394	end in vitro 6h
PEX10	0.529022544571176	0.0998512019030394	end in vitro 6h
RWDD4	0.529328844346298	0.0998512019030394	end in vitro 6h
SORBS1	0.533500986039824	0.0998512019030394	end in vitro 6h
FBXO2	0.534273535783004	0.0998512019030394	end in vitro 6h
WBP1	0.536068389740334	0.0998512019030394	end in vitro 6h
ENSBTAG00000039823	0.536497287209934	0.0998512019030394	end in vitro 6h
CATSPERG	0.537936170743187	0.0998512019030394	end in vitro 6h
TBC1D5	0.538636638792851	0.0998512019030394	end in vitro 6h
FMN1	0.539427791126268	0.0998512019030394	end in vitro 6h
FKBP5	0.539488949878598	0.0998512019030394	end in vitro 6h
FBXO44	0.540399016174748	0.0998512019030394	end in vitro 6h
ASRGL1	0.541583010675819	0.0998512019030394	end in vitro 6h
TUBB4A	0.541970636075378	0.0998512019030394	end in vitro 6h
GCA	0.546093291734941	0.0998512019030394	end in vitro 6h
SNTB2	0.546387542943319	0.0998512019030394	end in vitro 6h
SYNE3	0.549686191802579	0.0998512019030394	end in vitro 6h
ZNF827	0.552337416428352	0.0998512019030394	end in vitro 6h
BCKDHB	0.553936740658275	0.0998512019030394	end in vitro 6h
PER1	0.554584437490656	0.0998512019030394	end in vitro 6h
SLX4IP	0.556283728361234	0.0998512019030394	end in vitro 6h

GAS2L2	0.559151340730437	0.0998512019030394	end in vitro 6h
CLOCK	0.560652106416725	0.0998512019030394	end in vitro 6h
METTTL27	0.561975880779933	0.0998512019030394	end in vitro 6h
HACL1	0.563808886648722	0.0998512019030394	end in vitro 6h
NEIL2	0.564154713872057	0.0998512019030394	end in vitro 6h
STX10	0.564549293253243	0.0998512019030394	end in vitro 6h
ENSBTAG00000050189	0.565237971539479	0.0998512019030394	end in vitro 6h
ENSBTAG00000053410	0.565821146026445	0.0998512019030394	end in vitro 6h
ING2	0.56589750210204	0.0998512019030394	end in vitro 6h
LOC511229	0.568542077165369	0.0998512019030394	end in vitro 6h
ATXN3	0.569914180652546	0.0998512019030394	end in vitro 6h
ACADS	0.573100406863223	0.0998512019030394	end in vitro 6h
XRCC3	0.579209851690722	0.0998512019030394	end in vitro 6h
ENSBTAG00000017162	0.579338189513035	0.0998512019030394	end in vitro 6h
MAP3K14	0.579735694321974	0.0998512019030394	end in vitro 6h
PDIA2	0.581435917842316	0.0998512019030394	end in vitro 6h
CDON	0.582308150266184	0.0998512019030394	end in vitro 6h
MORN4	0.583896752010639	0.0998512019030394	end in vitro 6h
PLIN3	0.584481531135319	0.0998512019030394	end in vitro 6h
GPR146	0.58521168997204	0.0998512019030394	end in vitro 6h
ESR1	0.585673142762193	0.0998512019030394	end in vitro 6h
WHRN	0.585744142131536	0.0998512019030394	end in vitro 6h
ENSBTAG00000021594	0.586629676564413	0.0998512019030394	end in vitro 6h
WDR76	0.590316732515578	0.0998512019030394	end in vitro 6h
ENSBTAG00000052297	0.591019555102048	0.0998512019030394	end in vitro 6h
DHDH	0.591577659852121	0.0998512019030394	end in vitro 6h
SH3TC1	0.592526799198184	0.0998512019030394	end in vitro 6h
ENSBTAG00000007382	0.593346490536871	0.0998512019030394	end in vitro 6h
PLEKHH1	0.596004038650721	0.0998512019030394	end in vitro 6h
SUGP2	0.597428154446031	0.0998512019030394	end in vitro 6h
TBC1D30	0.597810564045622	0.0998512019030394	end in vitro 6h
KIAA1549	0.597815493670139	0.0998512019030394	end in vitro 6h
ZMYND12	0.59993784112742	0.0998512019030394	end in vitro 6h
HOXB9	0.601564224456223	0.0998512019030394	end in vitro 6h
GCDH	0.603108665509453	0.0998512019030394	end in vitro 6h
SSBP3	0.606900865951663	0.0998512019030394	end in vitro 6h
SNAPC5	0.609945587727481	0.0998512019030394	end in vitro 6h
KRT10	0.614007646607875	0.0998512019030394	end in vitro 6h
PCBD1	0.614354389471574	0.0998512019030394	end in vitro 6h
ENSBTAG00000054319	0.615156107699332	0.0998512019030394	end in vitro 6h
PFKFB2	0.617784583828031	0.0998512019030394	end in vitro 6h
MPV17L2	0.61920133649613	0.0998512019030394	end in vitro 6h
ENSBTAG00000043574	0.622403675115267	0.0998512019030394	end in vitro 6h
PLA2G4B	0.624698496955828	0.0998512019030394	end in vitro 6h
GTSE1	0.625919326949535	0.0998512019030394	end in vitro 6h
GGCT	0.634523587312788	0.0998512019030394	end in vitro 6h

ZSWIM7	0.634550650509478	0.0998512019030394	end in vitro 6h
ALKBH6	0.636661306585822	0.0998512019030394	end in vitro 6h
LOC101904339	0.636832215836611	0.0998512019030394	end in vitro 6h
TPM2	0.637820173312797	0.0998512019030394	end in vitro 6h
MOCOS	0.644185687721834	0.0998512019030394	end in vitro 6h
CHAF1A	0.654944355677038	0.0998512019030394	end in vitro 6h
ENSBTAG00000033680	0.657676257341687	0.0998512019030394	end in vitro 6h
SPAG8	0.663222565366906	0.0998512019030394	end in vitro 6h
RTTN	0.667187042257631	0.0998512019030394	end in vitro 6h
CD320	0.669816877808436	0.0998512019030394	end in vitro 6h
CENPH	0.669922697705935	0.0998512019030394	end in vitro 6h
PWWP3B	0.673055446201357	0.0998512019030394	end in vitro 6h
TCTEX1D1	0.674494996020818	0.0998512019030394	end in vitro 6h
ENSBTAG00000051364	0.677075060958967	0.0998512019030394	end in vitro 6h
RGS11	0.689392914465959	0.0998512019030394	end in vitro 6h
TSC22D3	0.698001279755903	0.0998512019030394	end in vitro 6h
MYH11	0.699476630070055	0.0998512019030394	end in vitro 6h
DGCR6L	0.699955273262353	0.0998512019030394	end in vitro 6h
GMPPB	0.701722219620131	0.0998512019030394	end in vitro 6h
TYSND1	0.707294634822751	0.0998512019030394	end in vitro 6h
NAT14	0.720826540547683	0.0998512019030394	end in vitro 6h
RMND1	0.721359105566073	0.0998512019030394	end in vitro 6h
PAX8	0.722917583512369	0.0998512019030394	end in vitro 6h
VMAC	0.725566964984876	0.0998512019030394	end in vitro 6h
WIF1	0.726951815471995	0.0998512019030394	end in vitro 6h
LRP2	0.72977786662608	0.0998512019030394	end in vitro 6h
BPIFB1	0.736224825097978	0.0998512019030394	end in vitro 6h
SYCE2	0.746388645476117	0.0998512019030394	end in vitro 6h
ZCWPW1	0.752351752049112	0.0998512019030394	end in vitro 6h
DUSP19	0.756001683719975	0.0998512019030394	end in vitro 6h
HMCN1	0.757554934675508	0.0998512019030394	end in vitro 6h
EXOC3L4	0.760969416382826	0.0998512019030394	end in vitro 6h
HPGD	0.76546870581442	0.0998512019030394	end in vitro 6h
PLEKHM3	0.76739048772053	0.0998512019030394	end in vitro 6h
PCCA	0.77395706679826	0.0998512019030394	end in vitro 6h
FAT2	0.77984932588042	0.0998512019030394	end in vitro 6h
BCL7A	0.791654089921923	0.0998512019030394	end in vitro 6h
THNSL1	0.794848074512867	0.0998512019030394	end in vitro 6h
ERC2	0.798719070845249	0.0998512019030394	end in vitro 6h
LOC112448904	0.828056425043133	0.0998512019030394	end in vitro 6h
SFXN5	0.828550241873909	0.0998512019030394	end in vitro 6h
LOC112441895	0.828775467924308	0.0998512019030394	end in vitro 6h
ENSBTAG00000049517	0.846563852704836	0.0998512019030394	end in vitro 6h
ENSBTAG00000049635	0.849180734970894	0.0998512019030394	end in vitro 6h
POLE	0.852721331778912	0.0998512019030394	end in vitro 6h
LBH	0.857080765301651	0.0998512019030394	end in vitro 6h

LOC783399	0.859046057499293	0.0998512019030394	end in vitro 6h
FREM1	0.860544182750536	0.0998512019030394	end in vitro 6h
LOC104975830	0.868846862801275	0.0998512019030394	end in vitro 6h
CES4A	0.877692004336236	0.0998512019030394	end in vitro 6h
ENSBTAG00000047761	0.880724332113911	0.0998512019030394	end in vitro 6h
ENSBTAG00000054933	0.884766770740868	0.0998512019030394	end in vitro 6h
TPCN1	0.886062076201508	0.0998512019030394	end in vitro 6h
MRPS36	0.887808786045789	0.0998512019030394	end in vitro 6h
NFIA	0.889075277590967	0.0998512019030394	end in vitro 6h
RAB3A	0.89759607752779	0.0998512019030394	end in vitro 6h
ENSBTAG00000003593	0.919109641991987	0.0998512019030394	end in vitro 6h
ENSBTAG00000002009	0.927001622846234	0.0998512019030394	end in vitro 6h
ENSBTAG00000050237	0.927001622846234	0.0998512019030394	end in vitro 6h
PPM1K	0.93978610701461	0.0998512019030394	end in vitro 6h
PROX1	0.948236834165321	0.0998512019030394	end in vitro 6h
MIR1248-1	0.956546879774704	0.0998512019030394	end in vitro 6h
UBE3D	0.957439818666295	0.0998512019030394	end in vitro 6h
SEMA3E	0.957783224035357	0.0998512019030394	end in vitro 6h
SCUBE1	0.967862962191015	0.0998512019030394	end in vitro 6h
ETNK2	0.990139548951107	0.0998512019030394	end in vitro 6h
ENSBTAG00000011928	1.0007415235094	0.0998512019030394	end in vitro 6h
WIPF3	1.00320862503899	0.0998512019030394	end in vitro 6h
CCNB1	1.00701454152853	0.0998512019030394	end in vitro 6h
FMO2	1.01097354845262	0.0998512019030394	end in vitro 6h
ACSS2	1.01318559848758	0.0998512019030394	end in vitro 6h
ACTG2	1.02196828603302	0.0998512019030394	end in vitro 6h
CREB3L4	1.03383194542341	0.0998512019030394	end in vitro 6h
PDLIM3	1.03590332959433	0.0998512019030394	end in vitro 6h
ENSBTAG00000040473	1.04732866850435	0.0998512019030394	end in vitro 6h
ADAM11	1.05430875164518	0.0998512019030394	end in vitro 6h
ENSBTAG00000054238	1.06270937271849	0.0998512019030394	end in vitro 6h
ENSBTAG00000048395	1.0665765471556	0.0998512019030394	end in vitro 6h
TRPM3	1.07579408420935	0.0998512019030394	end in vitro 6h
BRINP2	1.07935260725638	0.0998512019030394	end in vitro 6h
LOC514658	1.09752520901793	0.0998512019030394	end in vitro 6h
ENSBTAG00000051963	1.09915574451203	0.0998512019030394	end in vitro 6h
ENSBTAG00000006562	1.11598793158843	0.0998512019030394	end in vitro 6h
ACMSD	1.12135393990676	0.0998512019030394	end in vitro 6h
ADA	1.1328632779443	0.0998512019030394	end in vitro 6h
HIF3A	1.13355808105256	0.0998512019030394	end in vitro 6h
ENSBTAG00000048956	1.13444888770012	0.0998512019030394	end in vitro 6h
SOX6	1.13639390999234	0.0998512019030394	end in vitro 6h
SLC6A11	1.13987125004491	0.0998512019030394	end in vitro 6h
AR	1.14769297804149	0.0998512019030394	end in vitro 6h
LOC508455	1.16832179630215	0.0998512019030394	end in vitro 6h
ZNF581	1.21751412702419	0.0998512019030394	end in vitro 6h

LOC112448315	1.21899175747092	0.0998512019030394	end in vitro 6h
AQP9	1.22146525892015	0.0998512019030394	end in vitro 6h
LOC112444950	1.22296315407807	0.0998512019030394	end in vitro 6h
ENSBTAG00000052265	1.24030114119145	0.0998512019030394	end in vitro 6h
AFMID	1.24387555874924	0.0998512019030394	end in vitro 6h
ENSBTAG00000053269	1.24553638802164	0.0998512019030394	end in vitro 6h
LOC112445055	1.28436796451188	0.0998512019030394	end in vitro 6h
H3F3C	1.295937720028	0.0998512019030394	end in vitro 6h
IL31RA	1.30992666565126	0.0998512019030394	end in vitro 6h
ENSBTAG00000051328	1.31412018748078	0.0998512019030394	end in vitro 6h
ENSBTAG00000051802	1.37942933229535	0.0998512019030394	end in vitro 6h
ENSBTAG00000055058	1.40088374112524	0.0998512019030394	end in vitro 6h
ENSBTAG00000054820	1.41157588066578	0.0998512019030394	end in vitro 6h
ENSBTAG00000053368	1.43868749970879	0.0998512019030394	end in vitro 6h
C4H7orf31	1.52072465580153	0.0998512019030394	end in vitro 6h
FBXO24	1.54760547395541	0.0998512019030394	end in vitro 6h
ENSBTAG00000021359	1.56823753494708	0.0998512019030394	end in vitro 6h
FRMD1	1.57397658603291	0.0998512019030394	end in vitro 6h
CYS1	1.58053776502985	0.0998512019030394	end in vitro 6h
LOC112441905	1.66300057550576	0.0998512019030394	end in vitro 6h
MYT1L	1.66520341198874	0.0998512019030394	end in vitro 6h
TNS4	1.67486482619401	0.0998512019030394	end in vitro 6h
ENSBTAG00000054946	1.69075872603549	0.0998512019030394	end in vitro 6h
BCL2L15	1.71648664223318	0.0998512019030394	end in vitro 6h
LOC112443614	1.74715192079698	0.0998512019030394	end in vitro 6h
MIR2384	1.77836022676446	0.0998512019030394	end in vitro 6h
TERB1	1.78153227297863	0.0998512019030394	end in vitro 6h
ELAVL3	1.81070678123653	0.0998512019030394	end in vitro 6h
ENSBTAG00000053254	1.88139235775802	0.0998512019030394	end in vitro 6h
LOC112442430	1.91985727785853	0.0998512019030394	end in vitro 6h
ENSBTAG00000052133	1.91985727785853	0.0998512019030394	end in vitro 6h
LOC112443674	1.93877566593344	0.0998512019030394	end in vitro 6h
KRT222	1.95071509478595	0.0998512019030394	end in vitro 6h
LOC112445778	10.1681057488513	0.0998512019030394	end in vitro 6h
THRSP	2.03595715760718	0.0998512019030394	end in vitro 6h
ENSBTAG00000015395	2.11767607410446	0.0998512019030394	end in vitro 6h
LAMP5	2.12215653422249	0.0998512019030394	end in vitro 6h
CRB2	2.14519661036963	0.0998512019030394	end in vitro 6h
RSPO1	2.15608589985389	0.0998512019030394	end in vitro 6h
ARRDC5	2.15789078589069	0.0998512019030394	end in vitro 6h
ANK1	2.18645652727998	0.0998512019030394	end in vitro 6h
BTNL9	2.19286764657343	0.0998512019030394	end in vitro 6h
TP53TG5	2.20122175890974	0.0998512019030394	end in vitro 6h
ASB2	2.20803611334029	0.0998512019030394	end in vitro 6h
ZAN	2.22088810764207	0.0998512019030394	end in vitro 6h
PBK	2.34707422428738	0.0998512019030394	end in vitro 6h

ENSBTAG00000046888	2.49088624705089	0.0998512019030394	end in vitro 6h
KLRA1	2.62480128408172	0.0998512019030394	end in vitro 6h
ENSBTAG00000052885	2.63490970261607	0.0998512019030394	end in vitro 6h
MIR1584	2.68401862475562	0.0998512019030394	end in vitro 6h
C11H9orf50	2.6916359536349	0.0998512019030394	end in vitro 6h
CSTA	2.72632239207566	0.0998512019030394	end in vitro 6h
WNT10A	2.74609089769722	0.0998512019030394	end in vitro 6h
SPHKAP	2.74962659652549	0.0998512019030394	end in vitro 6h
LOC785479	2.80543722316981	0.0998512019030394	end in vitro 6h
KRT6C	2.84595189912671	0.0998512019030394	end in vitro 6h
LOC781679	2.99512487358669	0.0998512019030394	end in vitro 6h
LDLOC1	3.06117964287603	0.0998512019030394	end in vitro 6h
ENSBTAG00000048573	3.17247749054383	0.0998512019030394	end in vitro 6h
ENSBTAG00000051266	3.17706297054072	0.0998512019030394	end in vitro 6h
ENSBTAG00000052892	3.18845226898073	0.0998512019030394	end in vitro 6h
MIR2370	3.20681106606998	0.0998512019030394	end in vitro 6h
NFASC	3.20824536462597	0.0998512019030394	end in vitro 6h
ENSBTAG00000048179	3.31059616401082	0.0998512019030394	end in vitro 6h
SP5	3.4006360772851	0.0998512019030394	end in vitro 6h
CEL	3.41552919586016	0.0998512019030394	end in vitro 6h
ENSBTAG00000052972	3.43834746880751	0.0998512019030394	end in vitro 6h
ENSBTAG00000054637	3.44170247926116	0.0998512019030394	end in vitro 6h
ENSBTAG00000050849	3.65492985111647	0.0998512019030394	end in vitro 6h
VSIG4	3.66412975135117	0.0998512019030394	end in vitro 6h
LOC112443645	3.79850791300946	0.0998512019030394	end in vitro 6h
SLC9A4	4.02162421156451	0.0998512019030394	end in vitro 6h

Supplementary Table S7. The enriched pathways associated with the 391 DEGs in end *in vitro* compared to end *in vivo* group 6h.

Pathways	p-adjusted
Propanoate metabolism	6.09074216083391e-05
Glyoxylate and dicarboxylate metabolism	0.000575926589811241
Tryptophan metabolism	0.00341601555600572
Peroxisome	0.00468044271963701
Insulin secretion	0.00492225410624365
Complement and coagulation cascades	0.00718560755837956
Fructose and mannose metabolism	0.00959677118578191
Carbon metabolism	0.0158267313906887
Hippo signaling pathway	0.0160394927139551
Thyroid hormone synthesis	0.0170694457929933
Valine, leucine and isoleucine degradation	0.0284921551109227
Glutathione metabolism	0.0448838645095424
Ovarian steroidogenesis	0.0486188818276198
Glycolysis / Gluconeogenesis	0.0505419553106351
Fatty acid elongation	0.0543146575969096
Metabolism of xenobiotics by cytochrome P450	0.0565300082617624
Biosynthesis of unsaturated fatty acids	0.0577113596697447
Circadian rhythm	0.0611800698804995
Wnt signaling pathway	0.0680370928882739
Base excision repair	0.0683239066785448
Drug metabolism - other enzymes	0.0763877038523676
Pertussis	0.0811678746420246
p53 signaling pathway	0.0860749065256212
Chemical carcinogenesis	0.0860749065256212
Folate biosynthesis	0.0912425350527727

Supplementary Table S8. The enriched biological process associated with the 391 DEGs in end *in vitro* compared to end *in vivo* group 6h.

Biological process	p-adjusted
Protein-DNA complex assembly	0.000825023987731581
Protein-DNA complex subunit organization	0.00224607491631628
Positive regulation of epidermis development	0.00860969329164272
Mesenchyme morphogenesis	0.0104253324491263
dsRNA processing	0.0123944561218642
Production of miRNAs involved in gene silencing by miRNA	0.0123944561218642
Positive regulation of lipase activity	0.0123944561218642
Production of small RNA involved in gene silencing by RNA	0.0123944561218642
Protein heterooligomerization	0.014512382890326
Respiratory gaseous exchange by respiratory system	0.0167745310618647
Ribonucleoside monophosphate biosynthetic process	0.0167745310618647
Regulation of lipase activity	0.0167745310618647
Complement activation, classical pathway	0.0191764168364688
Regulation of epidermis development	0.0191764168364688
Nucleoside monophosphate biosynthetic process	0.0217136525034737
Humoral immune response	0.0222241620486878
DNA-templated transcription, initiation	0.0243819446687386
Cellular oxidant detoxification	0.0251223569904421
Detoxification	0.029834276265688
Cellular detoxification	0.029834276265688
Nucleosome assembly	0.0300949860725578
Humoral immune response mediated by circulating immunoglobulin	0.0331316045669252
Ribonucleoside monophosphate metabolic process	0.0362830147311438
Monocarboxylic acid catabolic process	0.0362830147311438
Positive regulation of intrinsic apoptotic signaling pathway	0.0362830147311438
Activation of immune response	0.0367930613849038
Complement activation	0.039545369194143
Gene silencing by miRNA	0.0429149048775947
Posttranscriptional gene silencing	0.046387941423188
Chromatin assembly	0.046387941423188
Post-transcriptional gene silencing by RNA	0.046387941423188
Nuclear chromatin	0.0168002701761018
Cell junction	0.0420568654757697
Collagen trimer	0.0463185093885092
Lyase activity	0.00363261877000352
Oxidoreductase activity, acting on the CH-CH group of donors	0.00420999961659242
Antioxidant activity	0.00476218233601758
Peroxidase activity	0.00869086480534801
Oxidoreductase activity, acting on peroxide as acceptor	0.0146487246785065
ATPase binding	0.0274307942011758
Identical protein binding	0.038962936489131

Hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds	0.0399124803740147
Flavin adenine dinucleotide binding	0.0433127166366264

Supplementary Table S9. Two hundred nine DEGs altered exclusively based on embryo origin (*in vivo* or *in vitro*).

Gene symbol			
ENSBTAG00000006530	SNX10	HACL1	UBE3D
ENSBTAG000000055000	C2	NEIL2	ETNK2
SDC2	CYP3A5	ING2	ACTG2
ENSBTAG000000049842	CDT1	ATXN3	PDLIM3
LOC112442392	C19H17orf67	ACADS	ENSBTAG000000040473
DEGS1	ASF1B	MAP3K14	ADAM11
TFAP4	NINJ2	PDIA2	LOC514658
ENSBTAG000000048734	TRPC3	PLIN3	ACMSD
AMMECR1	MELTF	WHRN	LOC508455
WDR77	KIF18A	WDR76	ZNF581
ENSBTAG000000052354	DNASE1L3	ENSBTAG000000052297	LOC112448315
ENSBTAG00000004939	ENSBTAG000000052386	DHDH	LOC112444950
ECE2	ATP1B4	ZMYND12	ENSBTAG000000052265
ENSBTAG000000046928	LOC100335729	HOXB9	ENSBTAG000000053269
TMEM126B	NTN3	GCDH	LOC112445055
ENSBTAG000000043566	LDHC	SSBP3	ENSBTAG000000051328
PPP1R14B	LOC100847469	KRT10	ENSBTAG000000054820
SEMA6B	ENSBTAG000000051502	ENSBTAG000000054319	ENSBTAG000000053368
MON1A	ENSBTAG000000050985	ENSBTAG000000043574	C4H7orf31
NCOA7	PPP1R3D	PLA2G4B	FRMD1
HCLS1	OLFM1	GGCT	CYS1
ENSBTAG000000025146	ENSBTAG000000050536	ZSWIM7	LOC112441905
ZNF382	ZBTB32	ALKBH6	MYT1L
LXN	ENSBTAG000000050205	TPM2	TNS4
CHST2	ENSBTAG000000054558	CHAF1A	ENSBTAG000000054946
PCDH11X	PNPLA1	ENSBTAG000000033680	BCL2L15
CCDC71L	LOC101904668	SPAG8	LOC112443614
ENSBTAG000000048939	ENSBTAG000000016306	RTTN	TERB1
DNAJC30	CLCA4	CD320	ELAVL3
MSRB3	ENSBTAG000000051483	PWHP3B	ENSBTAG000000053254
LCMT2	ENSBTAG000000007280	TCTEX1D1	LOC112442430
ENSBTAG00000005559	SPACA5	ENSBTAG000000051364	ENSBTAG000000052133
ZNF467	SFTPD	GMPPB	LOC112443674
ENSBTAG000000048671	CYB5B	TYSND1	KRT222
LOC530348	ZFYVE19	PAX8	THRSP
LYSMD2	PREX2	VMAC	ENSBTAG000000051395
CLDN2	ACOX2	SYCE2	CRB2
ENSBTAG000000049530	PRR15L	ZCWPW1	RSPO1
LRFN1	AREG	PLEKHM3	ARRDC5
C1QB	ZMIZ2	FAT2	ANK1
PRKRA	AXIN2	BCL7A	TP53TG5
LOC786974	PEX10	LOC112448904	ASB2
GPRC5B	RWDD4	LOC112441895	ENSBTAG000000046888
B3GALT6	WBP1	ENSBTAG000000049517	C11H9orf50
ENSBTAG000000025814	ENSBTAG000000039823	ENSBTAG000000049635	SPHKAP
NCBP2AS2	CATSPERG	POLE	LDOC1
LOC524181	FMN1	LOC783399	ENSBTAG000000051266
FBP2	TUBB4A	ENSBTAG000000047761	MIR2370
GATA6	SNTB2	ENSBTAG000000003593	CEL
ENSBTAG000000049718	ZNF827	ENSBTAG000000002009	ENSBTAG000000054637
ENSBTAG000000052981	SLX4IP	ENSBTAG000000050237	ENSBTAG000000050849
GUCA1A	GAS2L2	MIR1248-1	VSIG4
KCNJ11			

Supplementary Table S10. The enriched pathways and biological processes associated with the 209 DEGs in end *in vivo* compared to end *in vitro* 6h groups.

Pathways	P-Value
Metabolic pathways	0.01
Hippo signaling pathway	0.06
Peroxisome	0.089
Biological processes	P-Value
Lipid homeostasis	0.00098
Fatty acid beta-oxidation using acyl-CoA dehydrogenase	0.0058
Tryptophan metabolic process	0.014
Cellular response to gonadotropin stimulus	0.027
Response to ATP	0.066
Seminiferous tubule development	0.066

Supplementary Table S11. Differentially expressed genes in endometrial explants exposed for 24 hours to a Day 7 *in vivo* bovine embryo compared to end group (end *in vivo* x end).

Gene symbol	Log2FoldChange	p-adjusted	Group
FAM199X	-0.5008036840721	0.0980072518979004	end 24h
TAB3	-0.503677705342072	0.0862611958868408	end 24h
LIMD1	-0.510626953528417	0.097644430690686	end 24h
WDR7	-0.518579547685535	0.0958295810397293	end 24h
TMEM88	-0.520473522623076	0.0980072518979004	end 24h
ZNF287	-0.526942215275653	0.0980072518979004	end 24h
ENSBTAG00000049736	-0.540244932783188	0.097644430690686	end 24h
PLAGL2	-0.543078113631877	0.0980072518979004	end 24h
PDIA2	-0.552965951110243	0.0982151753137068	end 24h
LOC574091	-0.566247236282492	0.0966761530575656	end 24h
E2F3	-0.5726090662815	0.0862611958868408	end 24h
USP49	-0.59480653709987	0.097644430690686	end 24h
ENSBTAG00000004061	-0.600506107266198	0.097644430690686	end 24h
LTBP1	-0.606149448765578	0.097644430690686	end 24h
PIGH	-0.629346276001299	0.0650984770833342	end 24h
RIPOR3	-0.632693687301073	0.097644430690686	end 24h
MIR421	-0.645261157748804	0.0958295810397293	end 24h
BDKRB2	-0.673215688021897	0.097644430690686	end 24h
ZNF362	-0.680605094176399	0.086219765126255	end 24h
ERI2	-0.685107538595277	0.0958295810397293	end 24h
KLHL25	-0.688702249419695	0.0958295810397293	end 24h
KLF9	-0.69899793508773	0.0982151753137068	end 24h
INPP5J	-0.718476117754743	0.097644430690686	end 24h
NFKBIE	-0.723730166261826	0.0991023735927394	end 24h
ENSBTAG000000052673	-0.733918797220134	0.0966761530575656	end 24h
TPM2	-0.737318915105152	0.0958295810397293	end 24h
ERICH5	-0.739385297085674	0.0966761530575656	end 24h
MN1	-0.745228145222288	0.0980072518979004	end 24h
TTC23	-0.746090991592353	0.0991023735927394	end 24h
LOC100336941	-0.761344521032333	0.0966761530575656	end 24h
PTGDS	-0.765413967963286	0.097644430690686	end 24h
ENSBTAG000000017169	-0.78136659691848	0.0991023735927394	end 24h
C3	-0.789005288096256	0.0958295810397293	end 24h
ENSBTAG000000054084	-0.791629500363261	0.051704465285298	end 24h
SCUBE1	-0.844060896057283	0.0958295810397293	end 24h
LOC112448164	-0.848390158250058	0.0980072518979004	end 24h
BACH2	-0.854331612336922	0.0982151753137068	end 24h
MAFB	-0.904992760643911	0.097644430690686	end 24h
SEMA3A	-0.912685122070222	0.097644430690686	end 24h
ENSBTAG000000047783	-0.918579514676906	0.0862611958868408	end 24h
MIR6501	-0.930522742440558	0.0678296399435172	end 24h
FAT3	-0.935446827413285	0.097644430690686	end 24h
ANTXR1	-0.944575800372623	0.097644430690686	end 24h

ENSBTAG00000052707	-0.961994747735471	0.0991023735927394	end 24h
LOC104975830	-0.964064730576805	0.0966761530575656	end 24h
ENSBTAG00000052981	-0.980698680740023	0.0980072518979004	end 24h
ENSBTAG00000038978	-0.992262744331598	0.0958295810397293	end 24h
ENSBTAG00000049420	-1.00739577885017	0.0966761530575656	end 24h
MRVII	-1.01056810113738	0.0958295810397293	end 24h
TSPAN11	-1.01156423309223	0.0966761530575656	end 24h
IL1RL1	-1.01944248439696	0.0955971410862411	end 24h
CHRD1	-1.04254672285552	0.0815346852516651	end 24h
C1R	-1.05073683145814	0.051704465285298	end 24h
APOD	-1.08941655433809	0.097644430690686	end 24h
C1S	-1.09351490929153	0.0689421133200788	end 24h
CENPE	-1.10662674186762	0.0994762522483149	end 24h
ENSBTAG00000054952	-1.12097071569859	0.0966761530575656	end 24h
MIR4657	-1.12685013498425	0.0980072518979004	end 24h
LAIR1	-1.12771807505666	0.097644430690686	end 24h
PLCXD2	-1.13404243564937	0.0958295810397293	end 24h
MFAP4	-1.22740339043027	0.097644430690686	end 24h
TNMD	-1.23322787530545	0.0966761530575656	end 24h
USP13	-1.25777888474049	0.097644430690686	end 24h
SGIP1	-1.26066779167269	0.0958295810397293	end 24h
HDX	-1.26106529792493	0.0966761530575656	end 24h
ENSBTAG00000052755	-1.32824484833149	0.097644430690686	end 24h
ENSBTAG00000039086	-1.33930419401583	0.097644430690686	end 24h
KIAA0040	-1.36362510404939	0.0958295810397293	end 24h
ENSBTAG00000050793	-1.3782992147552	0.097644430690686	end 24h
GIMAP7	-1.41059566044834	0.0989271078392587	end 24h
FAM124A	-1.42068847470146	0.097644430690686	end 24h
MYOC	-1.44173761658171	0.0728279616547219	end 24h
ENSBTAG00000053553	-1.5105114784037	0.0987381452387176	end 24h
ITGBL1	-1.53079568653505	0.0966761530575656	end 24h
ENSBTAG00000049522	-1.53255060018868	0.0966761530575656	end 24h
HASPIN	-1.60949736435454	0.097644430690686	end 24h
WNT6	-1.6109168708452	0.0966761530575656	end 24h
PTN	-1.61334193485335	0.0444927705732098	end 24h
ABCA9	-1.65008302017833	0.0958295810397293	end 24h
TMEM108	-1.74006085464608	0.0980072518979004	end 24h
ENSBTAG00000054991	-1.77589390747674	0.097644430690686	end 24h
ENSBTAG00000030834	-1.77589390747674	0.097644430690686	end 24h
CNN1	-1.78864567816096	0.0958295810397293	end 24h
TP53TG5	-1.83810778002455	0.0966761530575656	end 24h
GLT8D2	-1.86959947582273	0.0958295810397293	end 24h
LOC112444948	-1.90918417189628	0.0966761530575656	end 24h
SCN2B	-1.95107793778068	0.0505566801081562	end 24h
ENSBTAG00000053984	-1.96665738501436	0.0385607352631587	end 24h
ENSBTAG00000033143	-1.98466310687615	0.0452851066917413	end 24h

LOC784768	-2.06368295180724	0.0296728934355361	end 24h
C7	-2.06953142765754	0.0261117918535394	end 24h
ENSBTAG00000052686	-2.07784287485797	0.0987381452387176	end 24h
ENSBTAG00000051894	-2.20567517793447	0.0958295810397293	end 24h
PJVK	-2.25680198221304	0.097644430690686	end 24h
ENSBTAG00000050503	-2.54765533963326	0.0958295810397293	end 24h
ENSBTAG00000052715	-2.66294763825803	0.0931124037010343	end 24h
ENSBTAG00000049667	-2.70955281477472	0.0543686327505632	end 24h
CXCL9	-2.72621763682582	0.0977829610983259	end 24h
CCDC116	-2.94827986673877	0.097644430690686	end 24h
MIR2422	-2.95606424396863	0.0966761530575656	end 24h
GLRB	-2.97646855443585	0.0862611958868408	end 24h
ARHGAP11A	-3.06586218642508	0.0678296399435172	end 24h
NR1I2	-3.07171494651528	0.0505566801081562	end 24h
ENSBTAG00000008365	-3.12814665884188	0.0958295810397293	end 24h
ENSBTAG00000049267	-3.23258527311188	0.097644430690686	end 24h
ENSBTAG00000050960	-3.26150675194352	0.097644430690686	end 24h
LOC617833	-3.27433460409857	0.0966761530575656	end 24h
CD300E	-3.31525462261549	0.0808073989513422	end 24h
LOC515276	-3.51960461617113	0.0958295810397293	end 24h
PLP1	-3.5239460740938	0.0958295810397293	end 24h
CLCA2	-3.56990485962337	0.0670792525012017	end 24h
MIR10168	-3.73227706150175	0.0958295810397293	end 24h
ENSBTAG00000052291	-4.40778300849466	0.0119290044476591	end 24h
PLSCR2	0.5010154265984	0.0749317637527643	end in vivo 24h
TRIM14	0.502174101315828	0.0994762522483149	end in vivo 24h
AKAP14	0.507037506142572	0.0980072518979004	end in vivo 24h
ARHGEF3	0.510940291632907	0.0958295810397293	end in vivo 24h
CMTR1	0.515591827198713	0.0777310463193032	end in vivo 24h
TTC21A	0.538407255285233	0.0958295810397293	end in vivo 24h
ENSBTAG00000031711	0.539501701280927	0.0966761530575656	end in vivo 24h
ASPN	0.547171302386676	0.097644430690686	end in vivo 24h
TRPC6	0.567637610436669	0.0981424469118083	end in vivo 24h
ENSBTAG00000017162	0.575383452229448	0.0958295810397293	end in vivo 24h
GCK	0.584166288833141	0.097644430690686	end in vivo 24h
PARP12	0.590102840893043	0.0966761530575656	end in vivo 24h
PSMF1	0.592867241902734	0.0266874343986317	end in vivo 24h
SP140L	0.627726299682035	0.097644430690686	end in vivo 24h
MIR2887-2	0.630574643698572	0.0980072518979004	end in vivo 24h
SASS6	0.635648154996982	0.0958295810397293	end in vivo 24h
ATG4A	0.642555259377778	0.0966761530575656	end in vivo 24h
STAT1	0.64779568253433	0.0966761530575656	end in vivo 24h
ENSBTAG00000050116	0.656749856791846	0.0980072518979004	end in vivo 24h
CDHR4	0.657661019899415	0.099031447405403	end in vivo 24h
TREX1	0.671198704103483	0.0815346852516651	end in vivo 24h
PML	0.675382997463044	0.0148265513953222	end in vivo 24h

FGFR3	0.681555499436741	0.0966761530575656	end in vivo 24h
RBM43	0.686059356835673	0.0862611958868408	end in vivo 24h
CMTR2	0.69665551108349	0.0881141761400201	end in vivo 24h
ENSBTAG00000046316	0.699496096427639	0.0351406437672646	end in vivo 24h
TRIM21	0.702505737860281	0.086219765126255	end in vivo 24h
FBXO15	0.714529303425689	0.0867603389534366	end in vivo 24h
IFI16	0.734056785844163	0.0851409067431691	end in vivo 24h
TM6SF2	0.750727097889368	0.097644430690686	end in vivo 24h
STAT4	0.751812100831876	0.0966761530575656	end in vivo 24h
PGGHG	0.755714123989751	0.00788596507107868	end in vivo 24h
TDRD7	0.756677221746139	0.0650984770833342	end in vivo 24h
LCA5	0.799748330144852	0.0958295810397293	end in vivo 24h
ENSBTAG00000047133	0.807095762555427	0.0670792525012017	end in vivo 24h
PWWP3B	0.821262101655017	0.0966761530575656	end in vivo 24h
EYA2	0.822538276697525	0.097644430690686	end in vivo 24h
UBE2L6	0.824506054216649	0.0815346852516651	end in vivo 24h
ERAS	0.836824911476262	0.0958295810397293	end in vivo 24h
ASB13	0.846708860093548	0.0966761530575656	end in vivo 24h
LGALS3BP	0.858532662615588	0.0359511498632278	end in vivo 24h
ENSBTAG00000048525	0.865095530491904	0.0982151753137068	end in vivo 24h
LOC522334	0.871629940611861	0.0966761530575656	end in vivo 24h
NUPR2	0.891444829373222	0.0966761530575656	end in vivo 24h
ADAR	0.904864325929151	0.0237489984494368	end in vivo 24h
ENSBTAG00000009664	0.914806778250028	0.00574900705212091	end in vivo 24h
TRIM5	0.933221256313318	0.0701220297247157	end in vivo 24h
HSPBAP1	0.94160044229598	0.0165972314252308	end in vivo 24h
PDE7A	0.948537018620943	0.0991023735927394	end in vivo 24h
LOC112444252	0.962170728683527	0.0958295810397293	end in vivo 24h
LGALS9	0.968956290261622	0.0161213470131835	end in vivo 24h
LOC112444847	0.994030913835104	0.030365706329473	end in vivo 24h
ADAP2	0.994979596982904	0.097644430690686	end in vivo 24h
ENSBTAG00000025642	1.00043980795066	0.0980072518979004	end in vivo 24h
LOC616948	1.013271588643	0.00682068750283813	end in vivo 24h
LOC112441484	1.04133511307664	0.00225928444038194	end in vivo 24h
ENSBTAG00000021359	1.04490925740616	0.097644430690686	end in vivo 24h
SP110	1.05789988680721	0.00288603867871853	end in vivo 24h
IFITM3	1.07999699479032	0.00157780131615453	end in vivo 24h
HERC5	1.13335754232372	0.000174482237238585	end in vivo 24h
IRF7	1.14552261208998	9.56058509608786e-05	end in vivo 24h
ENSBTAG00000003743	1.15182058178485	0.0366403797241709	end in vivo 24h
TNFSF10	1.17051684439454	0.0565799072531962	end in vivo 24h
CGAS	1.17934835274975	0.00162672422698264	end in vivo 24h
GBP4	1.18006302306405	0.0966761530575656	end in vivo 24h
PNPT1	1.20457551217809	0.000361310544200357	end in vivo 24h
TNFSF18	1.2148976506933	0.0966761530575656	end in vivo 24h
TNFSF13B	1.22081999320401	0.0777310463193032	end in vivo 24h

IFITM1	1.22730524109915	0.0678296399435172	end in vivo 24h
PARP10	1.23276143814472	0.00311278313848807	end in vivo 24h
IRF9	1.25918388044654	0.000158908263791369	end in vivo 24h
MASTL	1.26688207441063	0.0966761530575656	end in vivo 24h
DHX58	1.30258829493309	0.000196876414963161	end in vivo 24h
ENSBTAG00000053071	1.34056435438087	0.0911176669950088	end in vivo 24h
ENSBTAG00000052346	1.35703207393295	0.0958295810397293	end in vivo 24h
UPB1	1.37498836671456	0.0966761530575656	end in vivo 24h
ZNFX1	1.38293816801088	4.19760396098402e-05	end in vivo 24h
IFIH1	1.44520234818268	5.28159528462469e-05	end in vivo 24h
DTX3L	1.54672563328402	0.000192281984470455	end in vivo 24h
TRIM34	1.54972841985397	2.4943839643397e-05	end in vivo 24h
LOC788334	1.55798819435927	0.0966761530575656	end in vivo 24h
XAF1	1.56979853180466	2.86378109435113e-06	end in vivo 24h
PLAC8B	1.5918305913595	0.0749317637527643	end in vivo 24h
FOXS1	1.62451769681807	0.000431271551885497	end in vivo 24h
ENSBTAG00000053834	1.62921768615406	0.0958295810397293	end in vivo 24h
KRT5	1.6381033841655	0.0966761530575656	end in vivo 24h
LOC516599	1.66797010846922	0.0199775274645143	end in vivo 24h
ENSBTAG00000050415	1.6825041606918	0.097644430690686	end in vivo 24h
IFIT5	1.7082101577212	3.29654515828562e-06	end in vivo 24h
ENSBTAG00000034176	1.76945047341997	0.0890490403653338	end in vivo 24h
IZUMO4	1.78741407895587	0.0728279616547219	end in vivo 24h
ENSBTAG00000052434	1.79955511449476	0.097644430690686	end in vivo 24h
PARP9	1.82626298845742	6.84982326789348e-06	end in vivo 24h
ENSBTAG00000044417	1.83144954394872	0.0966761530575656	end in vivo 24h
HERC6	1.88409429126921	4.11226296687011e-06	end in vivo 24h
SULT6B1	1.89568612590228	0.0115999891139915	end in vivo 24h
LOC618409	1.89778413163309	1.56791145519112e-05	end in vivo 24h
LOC112447603	1.90459046627796	0.097644430690686	end in vivo 24h
EIF2AK2	1.90565814014887	5.50267141622244e-07	end in vivo 24h
CCL8	1.9422909209108	0.0815346852516651	end in vivo 24h
CMPK2	1.94908259054891	5.56978022183923e-06	end in vivo 24h
SLFN11	1.96821368883297	0.0958295810397293	end in vivo 24h
PARP14	1.97036816451369	4.55324161061179e-05	end in vivo 24h
LOC618737	1.97898538163526	0.00429608906310752	end in vivo 24h
IFI6	2.00315844452566	1.33161411653278e-05	end in vivo 24h
HBQ1	2.06257298392278	0.097644430690686	end in vivo 24h
IFIT2	2.0775403064568	0.00192935383211062	end in vivo 24h
FAM13C	2.13353791637682	0.0966761530575656	end in vivo 24h
CYP4B1	2.16758369749869	0.0155425565882918	end in vivo 24h
EPSTI1	2.22813823154905	2.08903308661669e-06	end in vivo 24h
RTP4	2.28711931614274	0.000776006603524603	end in vivo 24h
LOC509283	2.29204917814245	2.40331953677463e-07	end in vivo 24h
ENSBTAG00000014397	2.29336297468135	0.0966761530575656	end in vivo 24h
REC8	2.32419579785446	0.00174439416026288	end in vivo 24h

LOC112449173	2.35032098414566	0.0980072518979004	end in vivo 24h
SAMD9	2.44461936016147	2.47810891526702e-05	end in vivo 24h
ZBP1	2.456439301433	0.00034206529532986	end in vivo 24h
AQP6	2.60300330108677	0.0464313872397556	end in vivo 24h
ENSBTAG00000048969	2.79464192480757	0.097644430690686	end in vivo 24h
ENSBTAG00000048506	2.81548041009217	0.0925474965856696	end in vivo 24h
TRNP1	2.82535946694174	0.0966761530575656	end in vivo 24h
DDX58	2.85422602616564	1.5070930494173e-12	end in vivo 24h
LIPA	2.94686912895563	6.61268612619531e-10	end in vivo 24h
ENSBTAG00000048754	2.98729745632298	0.0966761530575656	end in vivo 24h
KLHL14	3.00724766994758	0.0966761530575656	end in vivo 24h
CNGA1	3.04449814419656	0.097644430690686	end in vivo 24h
PITX1	3.09734476372599	0.0966761530575656	end in vivo 24h
ENSBTAG00000053990	3.10547211888456	0.0980072518979004	end in vivo 24h
SYP	3.11310399762815	0.097644430690686	end in vivo 24h
AQP9	3.14611714656689	0.00288603867871853	end in vivo 24h
MX2	3.15745288619644	6.18110507381584e-08	end in vivo 24h
IFIT3	3.16530018055259	2.72030467322937e-09	end in vivo 24h
LOC112443528	3.2323410295961	0.024507159307168	end in vivo 24h
LOC112446495	3.31020971474295	0.0966761530575656	end in vivo 24h
GCSAM	3.35078931559029	0.0958295810397293	end in vivo 24h
UBA7	3.35246089074157	4.49592357191601e-11	end in vivo 24h
MIR7864	3.36472552495069	0.0958295810397293	end in vivo 24h
ENSBTAG00000048403	3.38071472230162	0.0958295810397293	end in vivo 24h
RSAD2	3.3936868332837	5.83887823773202e-11	end in vivo 24h
IFI27	3.44168202629003	1.40420210473056e-09	end in vivo 24h
ENSBTAG00000052462	3.57413744159295	0.0670792525012017	end in vivo 24h
CASR	3.61569798658008	0.0187829843645512	end in vivo 24h
LOC100298356	3.6301417799942	1.93604161090868e-09	end in vivo 24h
LOC781679	3.64165151405333	0.0958295810397293	end in vivo 24h
MX1	3.64520573052688	7.01757392511177e-13	end in vivo 24h
RMRP	3.8085689558826	0.0867603389534366	end in vivo 24h
LOC100139670	3.85316083869292	4.35675529931706e-08	end in vivo 24h
ISG15	4.05059762014679	7.01757392511177e-13	end in vivo 24h
USP18	4.11900182947823	2.14047583515576e-09	end in vivo 24h
LOC112441507	4.25379502294275	1.41350281083359e-08	end in vivo 24h
OVGP1	4.31534026692822	9.99375881550273e-06	end in vivo 24h
IFI44L	4.47466738690244	1.661515704988e-12	end in vivo 24h
OAS2	4.47717821413273	9.03706187476145e-07	end in vivo 24h
OAS1Y	4.47977804667055	6.42418689732262e-15	end in vivo 24h
IFI44	4.6416754125861	5.2433798296213e-10	end in vivo 24h
OAS1Z	4.73517706495986	0.015247884062708	end in vivo 24h
LOC100138271	4.86959189209384	0.000120339481249787	end in vivo 24h
ENSBTAG00000053654	5.14759573499637	0.00109226244905067	end in vivo 24h
LAMP5	5.29699416021477	0.00163620112811687	end in vivo 24h
OAS1X	5.54449451848811	9.03706187476145e-07	end in vivo 24h

Supplementary Table S12. Differentially expressed genes in endometrial explants exposed for 24 hours to a Day 7 *in vitro* bovine embryo compared to end group (end *in vitro* x end).

Gene symbol	Log2FoldChange	p-adjusted	Group
ST3GAL3	-0.504643841808457	0.0851952386850649	end 24h
PDXP	-0.514114783775296	0.0998005819816256	end 24h
ENSBTAG00000003219	-0.518173466506162	0.0998005819816256	end 24h
MIF	-0.52205897010142	0.0998005819816256	end 24h
ENSBTAG00000052325	-0.525131856231742	0.0991326892771778	end 24h
CPE	-0.527009269812389	0.0998005819816256	end 24h
LY96	-0.530599536296425	0.0411290017151602	end 24h
MACROD1	-0.533005161160848	0.0998005819816256	end 24h
ENSBTAG00000051246	-0.540425933921043	0.0998005819816256	end 24h
NINJ1	-0.544177233516748	0.0998005819816256	end 24h
THRA	-0.551179347846466	0.0998005819816256	end 24h
PAQR6	-0.558201075178039	0.0998005819816256	end 24h
RBPMS	-0.566876174801767	0.0998005819816256	end 24h
PIM1	-0.575481111072557	0.0998005819816256	end 24h
FGF1	-0.607642803622832	0.0998005819816256	end 24h
RGS21	-0.617343693578006	0.0998005819816256	end 24h
PDIA2	-0.617957094840126	0.0998005819816256	end 24h
SERPINC1	-0.619191790538024	0.0998005819816256	end 24h
TPM2	-0.621010520249489	0.0998005819816256	end 24h
LTBP1	-0.621123382856357	0.0998005819816256	end 24h
COX4I2	-0.623417067058524	0.0998005819816256	end 24h
JUNB	-0.624782797177977	0.0998005819816256	end 24h
WDR88	-0.634253072931024	0.0998005819816256	end 24h
MYH11	-0.639022804249867	0.0998005819816256	end 24h
PXMP2	-0.644657771022639	0.0998005819816256	end 24h
OLFM2	-0.644737280159921	0.0998005819816256	end 24h
ENSBTAG00000040051	-0.648471119896124	0.0998005819816256	end 24h
SLC35G2	-0.654171530265391	0.0998005819816256	end 24h
RAC2	-0.672585872537196	0.0998005819816256	end 24h
ACTA2	-0.67805856751254	0.0998005819816256	end 24h
PARD6G	-0.685205443119161	0.0998005819816256	end 24h
ACAD11	-0.696352100461253	0.0998005819816256	end 24h
BTBD19	-0.697299062066221	0.0998005819816256	end 24h
MYOZ1	-0.70673170279697	0.0998005819816256	end 24h
TCTEX1D1	-0.710842691733354	0.0998005819816256	end 24h
TEX26	-0.71920393447917	0.0998005819816256	end 24h
PLEKHF1	-0.724531416804422	0.0998005819816256	end 24h
SNX22	-0.73481444934572	0.0998005819816256	end 24h
MPP2	-0.736553512398623	0.0998005819816256	end 24h
ENSBTAG00000021821	-0.746514138163458	0.0998005819816256	end 24h
NCAPH	-0.765355068052523	0.0998005819816256	end 24h
ENSBTAG00000054319	-0.770723784151162	0.0998005819816256	end 24h
TWIST2	-0.775228231472198	0.0998005819816256	end 24h

MIR1842	-0.779391646419812	0.0998005819816256	end 24h
ENSBTAG00000053164	-0.80336792741	0.0998005819816256	end 24h
SCUBE1	-0.804768734919033	0.0998005819816256	end 24h
PAH	-0.809694425760592	0.0998005819816256	end 24h
ASIP	-0.823438222187455	0.0998005819816256	end 24h
IGF2	-0.827401619486954	0.0998005819816256	end 24h
DIPK1B	-0.830862942034419	0.0998005819816256	end 24h
ARMH4	-0.837802838901572	0.0998005819816256	end 24h
APOE	-0.842757513370185	0.0998005819816256	end 24h
TMEM81	-0.846427436293002	0.0998005819816256	end 24h
C1R	-0.861195890083214	0.0998005819816256	end 24h
CTSK	-0.867314622015201	0.0998005819816256	end 24h
C3H1orf216	-0.870988435738402	0.0998005819816256	end 24h
CTH	-0.872978888782525	0.0998005819816256	end 24h
EPHB2	-0.873524911301716	0.0998005819816256	end 24h
ENSBTAG00000054055	-0.88910521119047	0.0998005819816256	end 24h
TTC23	-0.89039175570241	0.0998005819816256	end 24h
ENSBTAG00000053607	-0.903311744230557	0.0998005819816256	end 24h
ERICH5	-0.908772145452417	0.0994729926365114	end 24h
RTN2	-0.9119713817302	0.0998005819816256	end 24h
STAC	-0.936938692742387	0.0998005819816256	end 24h
ZBTB26	-0.940020375523874	0.0998005819816256	end 24h
GATA6	-0.945549282232584	0.0998005819816256	end 24h
AUNIP	-0.947018142380184	0.0998005819816256	end 24h
SLC25A42	-0.954385966278112	0.0998005819816256	end 24h
INPP5J	-0.957128208014952	0.0983840718117054	end 24h
SOD2	-0.965010412771249	0.0998005819816256	end 24h
PORCN	-0.969745011756032	0.0998005819816256	end 24h
DIO2	-0.99158718001206	0.0998005819816256	end 24h
RIPOR3	-0.993688677993994	0.0424895311828145	end 24h
GFRA3	-1.00054404684355	0.0998005819816256	end 24h
ENSBTAG00000054952	-1.0184398226067	0.0998005819816256	end 24h
SERPING1	-1.01887674237447	0.0998005819816256	end 24h
RGMA	-1.02376127077551	0.0994729926365114	end 24h
ENSBTAG00000050493	-1.03411179090789	0.0998005819816256	end 24h
SH3BGR	-1.03574622362951	0.0931111678157584	end 24h
TMEM176B	-1.08169753222635	0.0998005819816256	end 24h
PTGDS	-1.08177967301255	0.077659017651976	end 24h
LOC536097	-1.12502348234238	0.0998005819816256	end 24h
C7	-1.14355926079848	0.0998005819816256	end 24h
CCL28	-1.16429535581001	0.0998005819816256	end 24h
ENSBTAG00000053984	-1.16469041269956	0.0998005819816256	end 24h
ADCY5	-1.19584672011162	0.0998005819816256	end 24h
SH2D7	-1.20877070023397	0.0998005819816256	end 24h
LOC104975830	-1.21022574616037	0.0900676341799666	end 24h
C1S	-1.21122817136018	0.0551357722732922	end 24h

IL1RL1	-1.23564196687733	0.0664032626801801	end 24h
BMERB1	-1.2432438305262	0.0998005819816256	end 24h
CYP3A24	-1.24925303799347	0.0998005819816256	end 24h
KIAA0040	-1.27946573352416	0.0998005819816256	end 24h
LOC112446756	-1.29739595966621	0.0998005819816256	end 24h
ADAM11	-1.31108025083825	0.0998005819816256	end 24h
LOC507550	-1.31346272040896	0.0998005819816256	end 24h
ENSBTAG00000050858	-1.33586790201778	0.0998005819816256	end 24h
MKI67	-1.35938260595111	0.0998005819816256	end 24h
CENPF	-1.38139938107828	0.0998005819816256	end 24h
MIR11974	-1.38778466331423	0.0998005819816256	end 24h
ENSBTAG00000048854	-1.40643420835144	0.0998005819816256	end 24h
MYOC	-1.42069954759421	0.097886132492853	end 24h
TAGLN	-1.43114867286276	0.0998005819816256	end 24h
IGF1	-1.43388950469017	0.0998005819816256	end 24h
WNT6	-1.43832428630447	0.0998005819816256	end 24h
ZNF581	-1.44800495033526	0.0998005819816256	end 24h
LOC518080	-1.52371659336852	0.091452255441423	end 24h
BEST2	-1.52855734899612	0.0998005819816256	end 24h
CXCL2	-1.53211262105339	0.0998005819816256	end 24h
TOP2A	-1.58363778267117	0.0764107130688876	end 24h
OVOL3	-1.60987580532897	0.0998005819816256	end 24h
ENSBTAG00000004692	-1.64705409304127	0.0998005819816256	end 24h
KCNJ10	-1.66401149663061	0.0998005819816256	end 24h
BBOF1	-1.68885549190486	0.0998005819816256	end 24h
FRMD1	-1.69457500056971	0.0766118964350028	end 24h
PTN	-1.70884647176549	0.0430360812720903	end 24h
KIF20A	-1.72268906215136	0.0998005819816256	end 24h
PLK5	-1.78323715540061	0.0998005819816256	end 24h
ENSBTAG00000033289	-1.81644795375711	0.0998005819816256	end 24h
MYL6B	-1.84736437208232	0.0998005819816256	end 24h
ENSBTAG00000033143	-1.86388256710816	0.0826625019335975	end 24h
PLA2R1	-1.89462322642283	0.0998005819816256	end 24h
SLC25A47	-1.95735629966125	0.0998005819816256	end 24h
NOG	-1.98508982070475	0.0998005819816256	end 24h
PLA2G4D	-2.03213016272442	0.0998005819816256	end 24h
FAM83A	-2.05468164047294	0.0998005819816256	end 24h
MEOX1	-2.11578879007896	0.0900676341799666	end 24h
NCF2	-2.22697272243385	0.0998005819816256	end 24h
ENSBTAG00000050274	-2.24901597366323	0.0998005819816256	end 24h
ITGBL1	-2.25306993330146	0.0451651210748485	end 24h
GLRB	-2.2810044060476	0.0998005819816256	end 24h
ENSBTAG00000052616	-2.28227412250567	0.0983840718117054	end 24h
CNN1	-2.3980431237225	0.05083177873652	end 24h
GLT8D2	-2.41161902171221	0.0851952386850649	end 24h
ENSBTAG00000050685	-2.42121190843226	0.0998005819816256	end 24h

OVCH2	-2.58184558690704	0.0998005819816256	end 24h
EGR4	-2.64253634541196	0.0998005819816256	end 24h
ENSBTAG00000051814	-2.65510386692729	0.0998005819816256	end 24h
MIR12042	-2.66711806303945	0.0998005819816256	end 24h
SPATA46	-2.67548496367494	0.0998005819816256	end 24h
SCN2A	-2.8085850945832	0.0998005819816256	end 24h
ENSBTAG00000049564	-2.87383071742921	0.0998005819816256	end 24h
LOC112442440	-3.02367373844269	0.0998005819816256	end 24h
ENSBTAG00000024125	-3.03289931181071	0.0430360812720903	end 24h
LOC112443281	-3.13034970316954	0.0998005819816256	end 24h
CDK5R2	-3.14454755230691	0.0998005819816256	end 24h
ENSBTAG00000049505	-3.18862941571092	0.0998005819816256	end 24h
SRPK3	-3.30720440645545	0.08469684426157	end 24h
GFRA1	-3.39376140180444	0.0998005819816256	end 24h
MIR2370	-3.41471582480745	0.0998005819816256	end 24h
PLXNA4	-3.62209786083209	0.0998005819816256	end 24h
ENSBTAG00000051203	-3.62508681210406	0.0998005819816256	end 24h
ENSBTAG00000053220	-3.62660362593116	0.0998005819816256	end 24h
CDSN	-3.67133948054479	0.0991326892771778	end 24h
DEPDC1B	-3.85715795900147	0.0998005819816256	end 24h
ABCB10	0.500026898164424	0.0998005819816256	end in vitro 24h
RESF1	0.501565282324012	0.0998005819816256	end in vitro 24h
CMTR1	0.508985054144944	0.0983840718117054	end in vitro 24h
LOC526621	0.517014949846755	0.0998005819816256	end in vitro 24h
FILIP1	0.521041834370147	0.0998005819816256	end in vitro 24h
THSD1	0.525615412747094	0.0998005819816256	end in vitro 24h
PPCS	0.527704331200634	0.0994484919486131	end in vitro 24h
DMXL2	0.532626651840995	0.0998005819816256	end in vitro 24h
ENSBTAG00000035572	0.535304194987069	0.0998005819816256	end in vitro 24h
ASPN	0.536479465652458	0.0998005819816256	end in vitro 24h
EMCN	0.540606416824134	0.0998005819816256	end in vitro 24h
ANKRD49	0.541855805131479	0.0998005819816256	end in vitro 24h
NOTCH4	0.552322412763951	0.0998005819816256	end in vitro 24h
GPT2	0.552438379015103	0.0998005819816256	end in vitro 24h
PTPRB	0.55871355175664	0.0550620760699035	end in vitro 24h
GNB5	0.558724576484433	0.0998005819816256	end in vitro 24h
ARHGEF3	0.560907959574925	0.0998005819816256	end in vitro 24h
ENSBTAG00000017162	0.563189174027945	0.0998005819816256	end in vitro 24h
AGRN	0.571925087747771	0.0983840718117054	end in vitro 24h
USHBP1	0.579260178275461	0.0998005819816256	end in vitro 24h
IDH1	0.584543346821963	0.0998005819816256	end in vitro 24h
SHE	0.586487463736852	0.0998005819816256	end in vitro 24h
CIP2A	0.588093346893414	0.0998005819816256	end in vitro 24h
TREX1	0.588222005744384	0.0998005819816256	end in vitro 24h
GRAMD1C	0.609378369706668	0.0998005819816256	end in vitro 24h
SPTBN5	0.610991991636679	0.0998005819816256	end in vitro 24h

ZNF398	0.618822467128143	0.0991326892771778	end in vitro 24h
CLEC1A	0.620281859816424	0.0991326892771778	end in vitro 24h
CHD5	0.632044969563601	0.0998005819816256	end in vitro 24h
TMLHE	0.632659385080684	0.0998005819816256	end in vitro 24h
BLVRA	0.635142688828217	0.0845147067145942	end in vitro 24h
DTHD1	0.641519152847072	0.0998005819816256	end in vitro 24h
USP40	0.644986950439815	0.0998005819816256	end in vitro 24h
SOAT1	0.652151216435766	0.0998005819816256	end in vitro 24h
KITLG	0.653024673916759	0.0964398033284861	end in vitro 24h
SP140L	0.653202185302207	0.0998005819816256	end in vitro 24h
SMIM24	0.6631144656361	0.0998005819816256	end in vitro 24h
EXOC3L1	0.668085914189904	0.0998005819816256	end in vitro 24h
PLSCR2	0.674720978998795	0.0164032504555216	end in vitro 24h
FBXO15	0.677616495144618	0.0998005819816256	end in vitro 24h
LCA5	0.684387251308174	0.0998005819816256	end in vitro 24h
FAM3B	0.686236103284042	0.0998005819816256	end in vitro 24h
ERC2	0.68725706299742	0.0998005819816256	end in vitro 24h
UBE2L6	0.689457260014009	0.0998005819816256	end in vitro 24h
PTGER2	0.691503691659022	0.0998005819816256	end in vitro 24h
MS4A8	0.694992760211142	0.0998005819816256	end in vitro 24h
ENSBTAG00000050116	0.702223630292327	0.0998005819816256	end in vitro 24h
ARSH	0.70765270555128	0.0998005819816256	end in vitro 24h
TDRD7	0.70907571754218	0.0983840718117054	end in vitro 24h
CMTR2	0.710427502792771	0.0998005819816256	end in vitro 24h
STAT1	0.724812440993595	0.0998005819816256	end in vitro 24h
FUT10	0.725036238009981	0.0998005819816256	end in vitro 24h
ENSBTAG00000050348	0.735917796042792	0.0998005819816256	end in vitro 24h
PPARGC1A	0.736015366652047	0.0998005819816256	end in vitro 24h
SSUH2	0.736676262615658	0.0998005819816256	end in vitro 24h
KDR	0.73837038561375	0.010384864658106	end in vitro 24h
PSMF1	0.739732870985472	0.00515238044323876	end in vitro 24h
CCDC162P	0.742037584227834	0.097886132492853	end in vitro 24h
BMX	0.744560125744537	0.0998005819816256	end in vitro 24h
SASS6	0.748747911416136	0.0845147067145942	end in vitro 24h
TRIM14	0.751556417934189	0.0733606285081307	end in vitro 24h
NME6	0.753450237959598	0.0998005819816256	end in vitro 24h
PML	0.7602076095732	0.00634788978347833	end in vitro 24h
HES2	0.777510519494652	0.0344752262987527	end in vitro 24h
ENSBTAG00000054585	0.783196780221913	0.0998005819816256	end in vitro 24h
STON2	0.783275759385583	0.0998005819816256	end in vitro 24h
ZCCHC2	0.786275256446192	0.0831158417739846	end in vitro 24h
ENSBTAG00000047133	0.790613596035866	0.0926716213847106	end in vitro 24h
RYR1	0.790920332303965	0.0998005819816256	end in vitro 24h
STPG4	0.807052723339174	0.0998005819816256	end in vitro 24h
PWWP3B	0.815243240040459	0.0998005819816256	end in vitro 24h
OTULINL	0.824537503766805	0.0998005819816256	end in vitro 24h

IFI16	0.850997727345705	0.0629482959078287	end in vitro 24h
ENSBTAG00000049270	0.851386444392571	0.0998005819816256	end in vitro 24h
LOC112444252	0.866012657337284	0.0998005819816256	end in vitro 24h
TRIM5	0.876844290187471	0.0998005819816256	end in vitro 24h
LOC112444966	0.879177932703375	0.0998005819816256	end in vitro 24h
KCNRG	0.883441421862725	0.0998005819816256	end in vitro 24h
PDE6C	0.894933087147367	0.0998005819816256	end in vitro 24h
PGGHG	0.900387158131007	0.00148544528855564	end in vitro 24h
LGALS3BP	0.905346046042079	0.0347440043755736	end in vitro 24h
RBM43	0.916045296215219	0.0265382517975584	end in vitro 24h
GRM8	0.920647857095331	0.0998005819816256	end in vitro 24h
SLC11A2	0.921447950378337	0.0766118964350028	end in vitro 24h
ENSBTAG00000050110	0.925903533491168	0.0513876432182897	end in vitro 24h
PRSS22	0.942219053484467	0.0998005819816256	end in vitro 24h
HERC1	0.942513254327875	0.0998005819816256	end in vitro 24h
REEP5	0.943807930536232	0.0991326892771778	end in vitro 24h
AKR1B1	0.944361497133224	0.0998005819816256	end in vitro 24h
ENSBTAG00000006872	0.95945629999258	0.0938902217249739	end in vitro 24h
HSPBAP1	0.97772834047815	0.0169312666086623	end in vitro 24h
FAM3D	0.979336506151507	0.0998005819816256	end in vitro 24h
HERC5	0.981581660781705	0.00235897422372575	end in vitro 24h
MT1E	0.986283102140647	0.0998005819816256	end in vitro 24h
ENSBTAG00000054597	0.99800671325163	0.0998005819816256	end in vitro 24h
ACMSD	1.00763322502367	0.0998005819816256	end in vitro 24h
ENSBTAG00000051491	1.01117574912755	0.0998005819816256	end in vitro 24h
ACSF2	1.01838901534259	0.0998005819816256	end in vitro 24h
PARS2	1.01933445592755	0.0939257314606472	end in vitro 24h
LOC112441484	1.0204122496307	0.00439816667712649	end in vitro 24h
PARP10	1.02911052236313	0.0265382517975584	end in vitro 24h
ADAR	1.03623840480835	0.0102230639621295	end in vitro 24h
ENSBTAG00000038823	1.03866426614121	0.0998005819816256	end in vitro 24h
ENSBTAG00000055208	1.03905751299805	0.0983840718117054	end in vitro 24h
ENSBTAG00000021359	1.04582666881258	0.0998005819816256	end in vitro 24h
EYA2	1.04626426133138	0.0991326892771778	end in vitro 24h
ENSBTAG00000051890	1.05553622400777	0.0998005819816256	end in vitro 24h
LOC616948	1.05587388671676	0.00634788978347833	end in vitro 24h
TINAGL1	1.06538705900045	0.0880486696241827	end in vitro 24h
ENSBTAG00000009664	1.07360290192135	0.0011791579274595	end in vitro 24h
LGALS9	1.0811484214209	0.00799685928385498	end in vitro 24h
SP110	1.09681200441947	0.00289949162760814	end in vitro 24h
FOXS1	1.09770691141086	0.0430360812720903	end in vitro 24h
IRF7	1.10228216335046	0.000282260457308562	end in vitro 24h
CPVL	1.10719898359539	0.0998005819816256	end in vitro 24h
LOC514658	1.10743653522612	0.098035350297084	end in vitro 24h
CHAD	1.11377998309019	0.0998005819816256	end in vitro 24h
SLC1A1	1.13471945634027	0.0998005819816256	end in vitro 24h

ZBTB8B	1.15327434149985	0.0998005819816256	end in vitro 24h
TRIM21	1.15938071669996	0.00311362305899146	end in vitro 24h
ADAP2	1.16660803187346	0.0998005819816256	end in vitro 24h
HOXD8	1.16697528233198	0.0939257314606472	end in vitro 24h
TNFSF13B	1.17012407543748	0.0998005819816256	end in vitro 24h
IFITM3	1.17342808784498	0.000705014351406101	end in vitro 24h
IRF9	1.17436415445472	0.000751174206133926	end in vitro 24h
RN7SL1	1.18836922357084	0.0998005819816256	end in vitro 24h
PLAC8B	1.20293827899164	0.0998005819816256	end in vitro 24h
ENSBTAG00000048251	1.20422490345497	0.0998005819816256	end in vitro 24h
RAD21L1	1.21463817260372	0.0252006510484804	end in vitro 24h
MYO7A	1.22321112487073	0.0998005819816256	end in vitro 24h
PIGZ	1.22592345764791	0.0998005819816256	end in vitro 24h
LOC508459	1.23593855463204	0.0998005819816256	end in vitro 24h
ELOVL2	1.24108943258843	0.0998005819816256	end in vitro 24h
BAIAP2L2	1.2446091969532	0.0998005819816256	end in vitro 24h
LOC512464	1.24553972431835	0.0682778948350908	end in vitro 24h
GLYATL2	1.25078030459173	0.0998005819816256	end in vitro 24h
TNFSF10	1.25697502755686	0.05083177873652	end in vitro 24h
CAMK1G	1.25704670830635	0.0998005819816256	end in vitro 24h
ZBTB3	1.2574511358649	0.0998005819816256	end in vitro 24h
TXNDC16	1.26133378884851	0.0998005819816256	end in vitro 24h
LOC112444847	1.26377523344788	0.00515238044323876	end in vitro 24h
IFIH1	1.30360173520045	0.00051663608792458	end in vitro 24h
TIFA	1.30473626900691	0.0998005819816256	end in vitro 24h
F8A1	1.3098624175629	0.0998005819816256	end in vitro 24h
PNPT1	1.32960649991982	9.66932707322007e-05	end in vitro 24h
ATP6V0A4	1.36394051708171	0.0998005819816256	end in vitro 24h
GBP4	1.36819572614567	0.0998005819816256	end in vitro 24h
ENSBTAG00000053834	1.41612378950403	0.0998005819816256	end in vitro 24h
CGAS	1.41692642421312	0.000125730615453172	end in vitro 24h
ENSBTAG00000003743	1.42447652056204	0.00854597353628007	end in vitro 24h
LOC515755	1.42876735542649	0.0998005819816256	end in vitro 24h
TNFSF18	1.4354851575712	0.0983840718117054	end in vitro 24h
DHX58	1.43713273747616	4.97047501547922e-05	end in vitro 24h
TMEM213	1.43998700774859	0.0998005819816256	end in vitro 24h
ENSBTAG000000034176	1.44753930581125	0.0998005819816256	end in vitro 24h
KIF28	1.46146816485105	0.0998005819816256	end in vitro 24h
ZNFX1	1.46309945397271	1.85595629946483e-05	end in vitro 24h
ENSBTAG00000011305	1.4957177512879	0.0998005819816256	end in vitro 24h
ENSBTAG00000053071	1.50048963970653	0.0851952386850649	end in vitro 24h
NRAP	1.50707978282799	0.0998005819816256	end in vitro 24h
CCL8	1.50954856551739	0.0998005819816256	end in vitro 24h
CYP39A1	1.5149423188837	0.0998005819816256	end in vitro 24h
LOC112441481	1.53237698551371	0.0998005819816256	end in vitro 24h
IFIT2	1.56389757715797	0.0419630022928805	end in vitro 24h

ENSBTAG00000052365	1.59805143172678	0.0998005819816256	end in vitro 24h
CHST4	1.60526217330239	0.0991326892771778	end in vitro 24h
IFITM1	1.61170480338259	0.0162249141061552	end in vitro 24h
TRIM34	1.64014323733043	1.11632038665218e-05	end in vitro 24h
DTX3L	1.67383841679855	6.61957302728398e-05	end in vitro 24h
BCL2L14	1.68408774371769	0.0998005819816256	end in vitro 24h
XAF1	1.69052021776865	5.47865309189618e-07	end in vitro 24h
SLFN11	1.69892707298398	0.0998005819816256	end in vitro 24h
LOC618409	1.70748329359078	0.000216421524477978	end in vitro 24h
ENSBTAG00000044417	1.70938525542287	0.0998005819816256	end in vitro 24h
GPLD1	1.72559133234498	0.0998005819816256	end in vitro 24h
IFIT5	1.72604978661222	3.41578665986167e-06	end in vitro 24h
MAP2	1.75341391634705	0.0998005819816256	end in vitro 24h
HCRT1	1.77301544969304	0.0355521712988124	end in vitro 24h
RHCG	1.83135771619029	0.0998005819816256	end in vitro 24h
LOC112449395	1.83788125857378	0.0998005819816256	end in vitro 24h
LOC516849	1.88380128736144	0.0998005819816256	end in vitro 24h
GSTA1	1.88508281341734	0.0998005819816256	end in vitro 24h
LOC507055	1.89156068822863	0.0998005819816256	end in vitro 24h
CMPK2	1.90200893486805	1.46956652462454e-05	end in vitro 24h
MPP4	1.90270950633239	0.0998005819816256	end in vitro 24h
EIF2AK2	1.9195165119199	6.14056573356406e-07	end in vitro 24h
HERC6	1.93336596773227	2.99163221335989e-06	end in vitro 24h
PARP9	1.93455717898745	2.34965367726547e-06	end in vitro 24h
CYP4B1	1.93504124376935	0.0494232731115492	end in vitro 24h
PROM2	1.95066721276265	0.0998005819816256	end in vitro 24h
PARP14	1.98698891728702	5.74664011555855e-05	end in vitro 24h
AOX4	1.99890397125238	0.0998005819816256	end in vitro 24h
SULT6B1	2.03100937335731	0.00799685928385498	end in vitro 24h
LOC516599	2.15422745727879	0.00173260926550692	end in vitro 24h
MAP6	2.17966141135643	0.0998005819816256	end in vitro 24h
REC8	2.18760438025902	0.00573388976982955	end in vitro 24h
IFI6	2.20413917170975	1.87480168694897e-06	end in vitro 24h
OBSCN	2.26338211958998	0.0998005819816256	end in vitro 24h
LOC112448163	2.27400882513578	0.0998005819816256	end in vitro 24h
ENSBTAG00000015493	2.29039071907699	0.0281735936643607	end in vitro 24h
LOC618737	2.3038815418878	0.000877592279293573	end in vitro 24h
ENSBTAG00000054065	2.38331954304357	0.0998005819816256	end in vitro 24h
KLK11	2.38372571613452	0.0998005819816256	end in vitro 24h
LYZ1	2.38434004456124	0.0998005819816256	end in vitro 24h
EPSTI1	2.38519836886187	4.61073542344961e-07	end in vitro 24h
ENSBTAG00000053758	2.39063176824816	0.0998005819816256	end in vitro 24h
AQP6	2.43280216779662	0.0851952386850649	end in vitro 24h
ATF7IP2	2.47281375262362	0.0998005819816256	end in vitro 24h
SAMD9	2.48296804791291	2.61621355994865e-05	end in vitro 24h
ENSBTAG00000028273	2.49252960104022	0.0998005819816256	end in vitro 24h

LOC509283	2.55682227597352	1.06362085273432e-08	end in vitro 24h
RTP4	2.59468578071556	0.000138370058590749	end in vitro 24h
ENSBTAG00000048969	2.68868468984548	0.0998005819816256	end in vitro 24h
LIPA	2.70632052248219	2.50673161530263e-08	end in vitro 24h
ZBP1	2.77260975705969	5.42391612313349e-05	end in vitro 24h
LOC112447584	2.84255947427582	0.0998005819816256	end in vitro 24h
LOC516378	2.88833972273138	0.0998005819816256	end in vitro 24h
GCSAM	2.89022364262745	0.0998005819816256	end in vitro 24h
CASR	2.89570456156577	0.0939257314606472	end in vitro 24h
SLC6A19	2.89637811631184	0.0998005819816256	end in vitro 24h
ENSBTAG00000049474	2.90152085224112	0.0998005819816256	end in vitro 24h
DDX58	2.91709934182411	8.36750020244728e-13	end in vitro 24h
ENSBTAG00000054829	2.93471949859884	0.0109012695286692	end in vitro 24h
ENSBTAG00000052664	2.98107258622544	0.0998005819816256	end in vitro 24h
RSAD2	2.99864976197812	1.68908266822623e-08	end in vitro 24h
IFIT3	3.01911467341586	2.6441477877549e-08	end in vitro 24h
MIR7864	3.16921992095004	0.0998005819816256	end in vitro 24h
MX2	3.17610664188013	7.81649337518787e-08	end in vitro 24h
AQP9	3.17638531464773	0.00396467750738902	end in vitro 24h
ZDBF2	3.18311269848545	0.0998005819816256	end in vitro 24h
ENSBTAG00000044010	3.23435379514462	0.0851952386850649	end in vitro 24h
ENSBTAG00000053990	3.23840576180229	0.0998005819816256	end in vitro 24h
SYP	3.27034618417457	0.0998005819816256	end in vitro 24h
UBA7	3.28635573539949	1.68679841131173e-10	end in vitro 24h
TRNP1	3.5078520789034	0.0838122126034444	end in vitro 24h
ENSBTAG00000049455	3.52929128794597	0.0998005819816256	end in vitro 24h
LOC100298356	3.63208189149268	3.62771853978582e-09	end in vitro 24h
MX1	3.66021029976998	8.36750020244728e-13	end in vitro 24h
LOC100139670	3.67234102593583	3.31038093414889e-07	end in vitro 24h
USP18	3.96246484300117	1.71598176123102e-08	end in vitro 24h
ISG15	3.99913540810302	1.43928517641622e-12	end in vitro 24h
IFI27	4.01855984587436	1.43928517641622e-12	end in vitro 24h
LOC112441507	4.24463737663794	2.50673161530263e-08	end in vitro 24h
OVGP1	4.3465358115968	1.24897300466831e-05	end in vitro 24h
PITX1	4.37028606898824	0.0364436725523335	end in vitro 24h
IFI44L	4.45956401528267	2.71488876210663e-12	end in vitro 24h
OAS1Y	4.53845521487028	3.81365592625986e-15	end in vitro 24h
OAS2	4.58739838463634	6.28651840667947e-07	end in vitro 24h
OAS1Z	4.65734915926922	0.0245977088983486	end in vitro 24h
ENSBTAG00000053654	4.67600687685433	0.00527376355054472	end in vitro 24h
LOC100138271	4.69615924490477	0.00033475454745336	end in vitro 24h
IFI44	4.78006266701291	1.97006891941375e-10	end in vitro 24h
LAMP5	5.38830773075666	0.00195524366109376	end in vitro 24h
OAS1X	5.61625040511872	8.38168462991437e-07	end in vitro 24h

Supplementary Table S13. One hundred and forty-two DEGs in end *in vivo* and end *in vitro* groups compared to end group.

Gene symbol			
PDIA2	TDRD7	IFIH1	LOC509283
TPM2	CMTR2	PNPT1	RTP4
LTBP1	STAT1	GBP4	ENSBTAG00000048969
SCUBE1	PSMF1	ENSBTAG00000053834	LIPA
C1R	SASS6	CGAS	ZBP1
TTC23	TRIM14	ENSBTAG00000003743	GCSAM
ERICH5	PML	TNFSF18	CASR
INPP5J	ENSBTAG00000047133	DHX58	DDX58
RIPOR3	PWWP3B	ENSBTAG00000034176	RSAD2
ENSBTAG00000054952	IFI16	ZNFX1	IFIT3
PTGDS	LOC112444252	ENSBTAG00000053071	MIR7864
C7	TRIM5	CCL8	MX2
ENSBTAG00000053984	PGGHG	IFIT2	AQP9
LOC104975830	LGALS3BP	IFITM1	ENSBTAG00000053990
C1S	RBM43	TRIM34	SYP
IL1RL1	HSPBAP1	DTX3L	UBA7
KIAA0040	HERC5	XAF1	TRNP1
MYOC	LOC112441484	SLFN11	LOC100298356
WNT6	PARP10	LOC618409	MX1
PTN	ADAR	ENSBTAG00000044417	LOC100139670
ENSBTAG00000033143	ENSBTAG00000021359	IFIT5	USP18
ITGBL1	EYA2	CMPK2	ISG15
GLRB	LOC616948	EIF2AK2	IFI27
CNN1	ENSBTAG00000009664	HERC6	LOC112441507
GLT8D2	LGALS9	PARP9	OVGP1
CMTR1	SP110	CYP4B1	PITX1
ASPN	FOXS1	PARP14	IFI44L
ARHGEF3	IRF7	SULT6B1	OAS1Y
ENSBTAG00000017162	TRIM21	LOC516599	OAS2
TREX1	ADAP2	REC8	OAS1Z
SP140L	TNFSF13B	IFI6	ENSBTAG00000053654
PLSCR2	IFITM3	LOC618737	LOC100138271
FBXO15	IRF9	EPSTI1	IFI44
LCA5	PLAC8B	AQP6	LAMP5
UBE2L6	TNFSF10	SAMD9	OAS1X
ENSBTAG00000050116	LOC112444847		

Supplementary Table S14. The enriched pathways and biological processes associated with the 142 DEGs in end *in vivo* and end *in vitro* groups compared to end group.

Pathways	p-adjusted
Influenza A	0.0000000002
Measles	0.0000000039
Herpes simplex infection	0.0000010000
Hepatitis C	0.0000073000
Cytosolic DNA-sensing pathway	0.0002300000
RIG-I-like receptor signaling pathway	0.0080000000
Hepatitis B	0.4300000000
Complement and coagulation cascades	0.5000000000
Systemic lupus erythematosus	0.5200000000
Biological processes	P-adjusted
Defense response to virus	0.0000000000
Innate immune response	0.0000000020
Negative regulation of viral genome replication	0.0000003600
ISG15-protein conjugation	0.0002400000
Response to virus	0.0006400000
Modification-dependent protein catabolic process	0.0260000000
Response to interferon-alpha	0.0340000000
Positive regulation of interferon-alpha production	0.0550000000
Positive regulation of interferon-beta production	0.2800000000
Immune response	0.4700000000
Organelle fission	0.4700000000
Negative regulation of viral life cycle	0.6400000000
Positive regulation of inflammatory response	0.8900000000
Double-strand break repair	0.8900000000
Autophagy	0.8900000000
Response to type I interferon	0.8900000000
7-methylguanosine mRNA capping	0.8900000000
Cellular response to interferon-alpha	0.9800000000
Complement activation	1.0000000000
Cellular response to interferon-beta	1.0000000000
Negative regulation of viral transcription	1.0000000000
Response to biotic stimulus	1.0000000000
Negative regulation of viral release from host cell	1.0000000000
Cellular water homeostasis	1.0000000000
Complement activation, classical pathway	1.0000000000

Supplementary Table S15. Differentially expressed genes in end *in vitro* compared to end *in vivo* 24h (end *in vitro* x end *in vivo*).

Gene symbol	Log2FoldChange	p-adjusted	Group
ENSBTAG00000048538	-0.504335418761473	0.0996116752400896	end in vivo 24h
IFNLR1	-0.586439065650221	0.0996116752400896	end in vivo 24h
MIR6535	-0.610920828104664	0.0996116752400896	end in vivo 24h
SERPINC1	-0.614252671833156	0.0996116752400896	end in vivo 24h
MCOLN3	-0.618410479747556	0.0996116752400896	end in vivo 24h
MACROD1	-0.620292980172721	0.0996116752400896	end in vivo 24h
ENSBTAG00000054319	-0.626263933664781	0.0996116752400896	end in vivo 24h
ENSBTAG00000047132	-0.627040600499866	0.0996116752400896	end in vivo 24h
MGARP	-0.636341959700351	0.0996116752400896	end in vivo 24h
LOC112446645	-0.653235346935436	0.0996116752400896	end in vivo 24h
BTBD19	-0.667455331343315	0.0996116752400896	end in vivo 24h
LCMT2	-0.690995065313056	0.0996116752400896	end in vivo 24h
ENSBTAG00000030490	-0.698949661653709	0.0996116752400896	end in vivo 24h
WDR88	-0.728279325671509	0.0996116752400896	end in vivo 24h
ENSBTAG00000055247	-0.76582087752126	0.0996116752400896	end in vivo 24h
ANXA9	-0.801581943372945	0.0996116752400896	end in vivo 24h
ENSBTAG00000007199	-0.803587605612285	0.0996116752400896	end in vivo 24h
GATA6	-0.805623430909454	0.0996116752400896	end in vivo 24h
CTH	-0.837972142144949	0.0996116752400896	end in vivo 24h
ARMH4	-0.85372205617012	0.0996116752400896	end in vivo 24h
ENSBTAG00000053607	-0.89024090916802	0.0996116752400896	end in vivo 24h
ENSBTAG00000034844	-0.899621617317623	0.0996116752400896	end in vivo 24h
RAB40B	-0.919558089809923	0.0996116752400896	end in vivo 24h
PORCN	-0.958746853868665	0.0996116752400896	end in vivo 24h
ENSBTAG00000051171	-0.990714732588596	0.0996116752400896	end in vivo 24h
TCF19	-0.991337973594777	0.0996116752400896	end in vivo 24h
LOC783399	-1.00163230405831	0.0996116752400896	end in vivo 24h
PCLAF	-1.0565585266966	0.0996116752400896	end in vivo 24h
ENSBTAG00000049489	-1.11229068535131	0.0996116752400896	end in vivo 24h
SLC2A6	-1.11748401767176	0.0996116752400896	end in vivo 24h
NUPR2	-1.1655916799247	0.0996116752400896	end in vivo 24h
ENSBTAG00000053164	-1.18910888351113	0.0996116752400896	end in vivo 24h
ENSBTAG00000053850	-1.19019050603655	0.0996116752400896	end in vivo 24h
KRT23	-1.23064184442439	0.0996116752400896	end in vivo 24h
LOC518080	-1.24184832431198	0.0996116752400896	end in vivo 24h
ZNF581	-1.24411055257187	0.0996116752400896	end in vivo 24h
ENSBTAG00000048854	-1.28121783559575	0.0996116752400896	end in vivo 24h
ADAM32	-1.3251889146806	0.0996116752400896	end in vivo 24h
ATP6V1G3	-1.46273095315045	0.0996116752400896	end in vivo 24h
LOC112445176	-1.47574037883796	0.0996116752400896	end in vivo 24h
ENSBTAG00000050921	-1.47865645402719	0.0996116752400896	end in vivo 24h
GPC5	-1.61831081796866	0.0996116752400896	end in vivo 24h
PLK5	-1.71452466187352	0.0996116752400896	end in vivo 24h

KRT5	-1.91245676956721	0.0996116752400896	end in vivo 24h
LOC112443250	-1.92526010435773	0.0996116752400896	end in vivo 24h
ENSBTAG00000033289	-2.00824789837459	0.0996116752400896	end in vivo 24h
LOC112442874	-2.08403592483641	0.0996116752400896	end in vivo 24h
KRT6B	-2.1107003172991	0.0996116752400896	end in vivo 24h
PLA2R1	-2.34574153672079	0.0996116752400896	end in vivo 24h
ENSBTAG00000024125	-2.39383225652477	0.0996116752400896	end in vivo 24h
ENSBTAG00000051814	-2.61907416655002	0.0996116752400896	end in vivo 24h
ENSBTAG00000052616	-2.68400750264227	0.0996116752400896	end in vivo 24h
LOC112443528	-2.72755564033726	0.0996116752400896	end in vivo 24h
SRPK3	-2.75230532968788	0.0996116752400896	end in vivo 24h
ENSBTAG00000050274	-2.87075044297288	0.0996116752400896	end in vivo 24h
MIR12050	-3.02543196438655	0.0996116752400896	end in vivo 24h
GFRA1	-3.68613435229155	0.0996116752400896	end in vivo 24h
CDSN	-3.8937059083625	0.0996116752400896	end in vivo 24h
ENSBTAG00000048699	0.513967052649104	0.0996116752400896	end in vitro 24h
PRR5L	0.514089760283036	0.0996116752400896	end in vitro 24h
SOGA1	0.524353725142835	0.0996116752400896	end in vitro 24h
TAB3	0.531973324817817	0.0996116752400896	end in vitro 24h
INSYN2A	0.532484710401205	0.0996116752400896	end in vitro 24h
ENSBTAG00000052735	0.556023449983286	0.0996116752400896	end in vitro 24h
KDR	0.56644549074712	0.0996116752400896	end in vitro 24h
ZNF287	0.566660510373507	0.0996116752400896	end in vitro 24h
LOC100296627	0.592195215772817	0.0996116752400896	end in vitro 24h
HES2	0.593722510807544	0.0996116752400896	end in vitro 24h
INAFM1	0.618019072618434	0.0996116752400896	end in vitro 24h
SHE	0.629490036155255	0.0996116752400896	end in vitro 24h
ENSBTAG00000052361	0.63969268576741	0.0996116752400896	end in vitro 24h
COL4A5	0.641455977515002	0.0996116752400896	end in vitro 24h
ENSBTAG00000010813	0.653583715037126	0.0996116752400896	end in vitro 24h
SLC11A2	0.654117106043545	0.0996116752400896	end in vitro 24h
ENSBTAG00000054084	0.6566195095165	0.0996116752400896	end in vitro 24h
ZNF362	0.656933727513765	0.0996116752400896	end in vitro 24h
ENSBTAG00000013879	0.659464584647326	0.0996116752400896	end in vitro 24h
C8H9orf85	0.660162467448682	0.0996116752400896	end in vitro 24h
LMBRD2	0.666287109749656	0.0996116752400896	end in vitro 24h
PROS1	0.689006178309593	0.0996116752400896	end in vitro 24h
ENSBTAG00000038916	0.692447634911033	0.0996116752400896	end in vitro 24h
MIR421	0.698827850063644	0.0996116752400896	end in vitro 24h
RASSF2	0.706060688372749	0.0996116752400896	end in vitro 24h
TCF4	0.728275201404577	0.0996116752400896	end in vitro 24h
OTULINL	0.734497388822377	0.0996116752400896	end in vitro 24h
RAD21L1	0.762382259554515	0.0996116752400896	end in vitro 24h
ENSBTAG00000047783	0.771205986693782	0.0996116752400896	end in vitro 24h
ZNF423	0.777373688169604	0.0996116752400896	end in vitro 24h
ENSBTAG00000038084	0.784336511798983	0.0996116752400896	end in vitro 24h

ZNF621	0.787806834141783	0.0996116752400896	end in vitro 24h
OXTR	0.788515573444422	0.0996116752400896	end in vitro 24h
THNSL2	0.798266035500545	0.0996116752400896	end in vitro 24h
STAG3	0.807002192886821	0.0996116752400896	end in vitro 24h
FKBPL	0.811371271093471	0.0996116752400896	end in vitro 24h
EXTL2	0.817128789300325	0.0996116752400896	end in vitro 24h
BMP7	0.818116052973617	0.0996116752400896	end in vitro 24h
TINAGL1	0.822754737751002	0.0996116752400896	end in vitro 24h
ZFP64	0.86286390427355	0.0996116752400896	end in vitro 24h
ENSBTAG00000040559	0.877326489427999	0.0996116752400896	end in vitro 24h
NR3C2	0.888004582886881	0.0996116752400896	end in vitro 24h
LOC112448601	0.926701858919404	0.0996116752400896	end in vitro 24h
MIR6501	0.956550943436155	0.0996116752400896	end in vitro 24h
PRSS22	1.00240254581398	0.0996116752400896	end in vitro 24h
ENSBTAG00000052204	1.01060256916572	0.0996116752400896	end in vitro 24h
LOC112446876	1.06558661942181	0.0996116752400896	end in vitro 24h
ENSBTAG00000051491	1.08589446682867	0.0996116752400896	end in vitro 24h
ENSBTAG00000053385	1.09111841172865	0.0996116752400896	end in vitro 24h
SGIP1	1.11697021105812	0.0996116752400896	end in vitro 24h
STPG4	1.11813696555076	0.0996116752400896	end in vitro 24h
ANG2	1.14816248329016	0.0996116752400896	end in vitro 24h
ENSBTAG00000052981	1.22754892705625	0.0996116752400896	end in vitro 24h
LOC532100	1.24473020654272	0.0996116752400896	end in vitro 24h
LOC508459	1.31296516401149	0.0996116752400896	end in vitro 24h
FLT4	1.32366513554265	0.0996116752400896	end in vitro 24h
MIR4657	1.32955471708309	0.0996116752400896	end in vitro 24h
SCGN	1.33193420572013	0.0996116752400896	end in vitro 24h
ENSBTAG00000054363	1.35782336728092	0.0996116752400896	end in vitro 24h
LOC515755	1.39019759458153	0.0996116752400896	end in vitro 24h
LOC112446462	1.40210806278925	0.0996116752400896	end in vitro 24h
KLRB1	1.46491668477315	0.0996116752400896	end in vitro 24h
ENSBTAG00000051909	1.51782124772754	0.0996116752400896	end in vitro 24h
ENSBTAG00000048323	1.51898464681153	0.0996116752400896	end in vitro 24h
ENSBTAG00000015493	1.5205328136988	0.0996116752400896	end in vitro 24h
USP13	1.59282477886394	0.0996116752400896	end in vitro 24h
ENSBTAG00000054829	1.59594804614005	0.0996116752400896	end in vitro 24h
LOC784768	1.5984680977076	0.0996116752400896	end in vitro 24h
ENSBTAG00000050910	1.62639713643178	0.0996116752400896	end in vitro 24h
ENSBTAG00000054028	1.65084567193679	0.0996116752400896	end in vitro 24h
GPLD1	1.65461754808228	0.0996116752400896	end in vitro 24h
ENSBTAG00000049104	1.8081888322193	0.0996116752400896	end in vitro 24h
RHCG	1.8209270418161	0.0996116752400896	end in vitro 24h
LOC787611	1.85605225526958	0.0996116752400896	end in vitro 24h
HIGD1B	1.86586200361838	0.0996116752400896	end in vitro 24h
ENSBTAG00000038534	1.88210793461358	0.0996116752400896	end in vitro 24h
ADAM20	1.99491547733385	0.0996116752400896	end in vitro 24h

GPR87	1.99839712984677	0.0996116752400896	end in vitro 24h
ENSBTAG00000052119	2.32324118714916	0.0996116752400896	end in vitro 24h
LOC516378	2.38809177205221	0.0996116752400896	end in vitro 24h
PJVK	2.48533188843875	0.0996116752400896	end in vitro 24h
ARHGAP11A	2.49521924159718	0.0996116752400896	end in vitro 24h
GAS1	2.56429463118599	0.0996116752400896	end in vitro 24h
CD300E	2.58591663674268	0.0996116752400896	end in vitro 24h
ENSBTAG00000049945	2.60728448533092	0.0996116752400896	end in vitro 24h
CLCA2	2.60919404265974	0.0996116752400896	end in vitro 24h
ENSBTAG00000052715	2.61935684775827	0.0996116752400896	end in vitro 24h
CD79A	2.76886245443008	0.0996116752400896	end in vitro 24h
LOC112447620	2.82272408383836	0.0996116752400896	end in vitro 24h
NR1I2	3.09917999603869	0.0996116752400896	end in vitro 24h
ENSBTAG00000047569	3.25140091082839	0.0996116752400896	end in vitro 24h
LOC617833	3.28518879478122	0.0996116752400896	end in vitro 24h
ENSBTAG00000008365	3.35896435953931	0.0996116752400896	end in vitro 24h
PLP1	3.53447222380905	0.0996116752400896	end in vitro 24h
MIR10168	3.5704660519713	0.0996116752400896	end in vitro 24h
ENSBTAG00000049267	3.72191915229306	0.0996116752400896	end in vitro 24h
CCDC116	4.13628750206378	0.0996116752400896	end in vitro 24h
ENSBTAG00000052291	4.13736212108658	0.0996116752400896	end in vitro 24h

Supplementary Table S16. The enriched pathways associated with the 156 DEGs in end *in vitro* compared to end *in vivo* group 24h.

Pathways	p-adjusted
Calcium signaling pathway	0.0130442913646641
ABC transporters	0.0256195102935754
Renin secretion	0.0292222650558509
Focal adhesion	0.0367816350551302
Complement and coagulation cascades	0.0454643069952879
Pancreatic secretion	0.0536419191638068

Supplementary Table S17. The biological processes associated with the 156 DEGs in end *in vitro* compared to end *in vivo* group 24h.

Biological processes	p-adjusted
Cellular response to stress	0.000466748523216494
Positive regulation of cellular catabolic process	0.00230111649854755
Regulation of blood coagulation	0.00304477461161547
Immune response-activating cell surface receptor signaling pathway	0.0033413156334446
Immune response-activating signal transduction	0.0033413156334446
Regulation of hemostasis	0.0033413156334446
Positive regulation of catabolic process	0.00379176921414755
Immune response-regulating cell surface receptor signaling pathway	0.00397332752424496
Regulation of coagulation	0.00397332752424496
Immune response-regulating signaling pathway	0.00430860451352288
Cellular response to DNA damage stimulus	0.0057302352138254
Regulation of wound healing	0.00617386670021687
Regulation of response to wounding	0.00700634826827278
Negative regulation of cell population proliferation	0.00763906949587921
Positive regulation of protein catabolic process	0.00929698113668965
Cell population proliferation	0.00987374809627253
Cellular response to peptide hormone stimulus	0.0113388850172123
Regulation of cellular catabolic process	0.0137698290916232
Regulation of response to stress	0.0150190021175211
Cellular response to peptide	0.0159568992520279
Positive regulation of response to stimulus	0.0171272773193434
Activation of immune response	0.0172186475689006
Blood coagulation	0.0172186475689006
Hemostasis	0.0172186475689006
Coagulation	0.017865140608093
Response to peptide hormone	0.0198660931369017
Cellular response to oxidative stress	0.0205532926381761
Positive regulation of proteolysis	0.0205532926381761
Regulation of catabolic process	0.0252253805242786
Response to peptide	0.0264030515706479
Cellular response to chemical stress	0.0271770925118047
Response to organonitrogen compound	0.0273048971739263
Positive regulation of protein transport	0.0303650133916926
Positive regulation of transport	0.0303692517794289
Regulation of protein catabolic process	0.0336960630627282
Positive regulation of secretion	0.0336960630627282
Positive regulation of establishment of protein localization	0.0345506215933538
Response to nitrogen compound	0.0390665926308234
Response to Ph	0.0393951100216781
Regulation of triglyceride biosynthetic process	0.0393951100216781
Astrocyte development	0.0393951100216781
Membrane protein proteolysis	0.0393951100216781
Plasma lipoprotein particle clearance	0.0393951100216781

Secondary metabolite biosynthetic process	0.0393951100216781
Positive regulation of peptide hormone secretion	0.0393951100216781
Regulation of cellular response to insulin stimulus	0.0393951100216781
Ameboidal-type cell migration	0.0416894105698073
Sulfur amino acid metabolic process	0.0432529609854468
Insulin receptor signaling pathway	0.0432529609854468
Long-chain fatty acid transport	0.0432529609854468
Positive regulation of dephosphorylation	0.0432529609854468
Protein K63-linked deubiquitination	0.0432529609854468
Protein K48-linked deubiquitination	0.0432529609854468
Positive regulation of triglyceride metabolic process	0.0432529609854468
Wound healing	0.0454528308976673
GPI anchor metabolic process	0.0470961760495532
Cell aging	0.0470961760495532
Triglyceride biosynthetic process	0.0470961760495532
Neutral lipid catabolic process	0.0470961760495532
Acylglycerol catabolic process	0.0470961760495532
Phosphatidylcholine metabolic process	0.0470961760495532
Positive regulation of mRNA catabolic process	0.0470961760495532
Response to oxidative stress	0.0493395708061692
Intermediate filament	0.0097783560594836
Intermediate filament cytoskeleton	0.0115243394640754
Apical plasma membrane	0.0197514133321091
Apical part of cell	0.0284629930622495
Protein-lipid complex	0.0399322404174228
Plasma lipoprotein particle	0.0399322404174228
Myelin sheath	0.0399322404174228
Lipoprotein particle	0.0399322404174228
Multivesicular body	0.0496790467236879
Ubiquitin binding	0.036596869832346
Ubiquitin-like protein binding	0.039861791806324

Supplementary Table S18. Eighty-seven DEGs altered exclusively based on embryo origin (*in vivo* or *in vitro*).

Gene symbol		
ENSBTAG00000048538	KRT6B	ENSBTAG00000040559
IFNLR1	MIR12050	NR3C2
MIR6535	ENSBTAG00000048699	LOC112448601
MCOLN3	PRR5L	ENSBTAG00000052204
ENSBTAG00000047132	SOGA1	LOC112446876
MGARP	INSYN2A	ENSBTAG00000053385
LOC112446645	ENSBTAG00000052735	ANG2
LCMT2	LOC100296627	LOC532100
ENSBTAG00000030490	INAFM1	FLT4
ENSBTAG00000055247	ENSBTAG00000052361	SCGN
ANXA9	COL4A5	ENSBTAG00000054363
ENSBTAG00000007199	ENSBTAG00000010813	LOC112446462
ENSBTAG00000034844	ENSBTAG00000013879	KLRB1
RAB40B	C8H9orf85	ENSBTAG00000051909
ENSBTAG00000051171	LMBRD2	ENSBTAG00000048323
TCF19	PROS1	ENSBTAG00000050910
LOC783399	ENSBTAG00000038916	ENSBTAG00000054028
PCLAF	RASSF2	ENSBTAG00000049104
ENSBTAG00000049489	TCF4	LOC787611
SLC2A6	ZNF423	HIGD1B
ENSBTAG00000053850	ENSBTAG00000038084	ENSBTAG00000038534
KRT23	ZNF621	ADAM20
ADAM32	OXTR	GPR87
ATP6V1G3	THNSL2	ENSBTAG00000052119
LOC112445176	STAG3	GAS1
ENSBTAG00000050921	FKBPL	ENSBTAG00000049945
GPC5	EXTL2	CD79A
LOC112443250	BMP7	LOC112447620
LOC112442874	ZFP64	ENSBTAG00000047569

Supplementary Table S19. The biological processes associated with the 87 DEGs in end *in vivo* compared to end *in vitro* 24h groups.

Biological processes	p-value
Steroid hormone mediated signaling pathway	0.0370
Positive regulation of JNK cascade	0.0960

Supplementary Table S20. MicroRNAs identified in sEVs isolated from C.M. by end, end *in vivo*, and end *in vitro* for 6 hours.

MicroRNAs	End C.M. sEVs 6h			End <i>in vivo</i> C.M. sEVs 6h			End <i>in vitro</i> C.M. sEVs 6h		
	Mean	Standard deviation		Mean	Standard deviation		Mean	Standard deviation	
bta-miR-103	0.00614	±	0.00575	0.00185	±	0.00149	0.00800	±	0.00855
bta-let-7a-5p	0.05557	±	0.04031	0.03386	±	0.02499	0.10631	±	0.08782
bta-let-7b	0.17544	±	0.20548	0.14496	±	0.17596	0.32787	±	0.26036
bta-let-7c	0.17047	±	0.19691	0.11008	±	0.11170	0.27200	±	0.22981
bta-let-7d	0.01629	±	0.01095	0.01070	±	0.00590	0.03812	±	0.03151
bta-let-7e	0.10311	±	0.06486	0.07050	±	0.04800	0.19782	±	0.14746
bta-let-7f	0.01467	±	0.00681	0.01046	±	0.00603	0.02548	±	0.01792
bta-miR-10a	0.00934	±	0.01240	0.00490	±	0.00593	0.01694	±	0.01486
bta-miR-100	0.00972	±	0.01399	0.00712	±	0.00901	0.02212	±	0.02377
bta-miR-126-3p	0.01135	±	0.01562	0.00603	±	0.00672	0.01355	±	0.01446
bta-miR-126-5p	0.00885	±	0.01418	0.00515	±	0.00649	0.00792	±	0.01116
bta-miR-127	0.00232	±	0.00051	0.00260	±	0.00170	0.00188	±	0.00067
bta-miR-130a	0.00368	±	0.00022	0.00368	±	0.00015	0.00352	±	0.00031
bta-miR-130b	0.00519	±	0.00124	0.00552	±	0.00021	0.00574	±	0.00147
bta-miR-132	0.00068	±	0.00035	0.00151	±	0.00166	0.00067	±	0.00045
bta-miR-143	0.00519	±	0.00716	0.00310	±	0.00110	0.00746	±	0.00875
bta-miR-145	0.00994	±	0.01223	0.00112	±	0.00091	0.01897	±	0.01604
bta-miR-154b	0.00868	±	0.00038	0.01137	±	0.00446	0.01756	±	0.01095
bta-miR-148a	0.01315	±	0.00917	0.00253	±	0.00161	0.04129	±	0.03499
bta-miR-148b	0.00910	±	0.00678	0.00279	±	0.00090	0.02184	±	0.01861
bta-miR-149-3p	0.85120	±	0.31448	1.03681	±	1.05576	0.74050	±	0.67315
bta-miR-16a	0.01827	±	0.02460	0.00801	±	0.00907	0.02753	±	0.03560
bta-miR-16b	0.03622	±	0.04484	0.01019	±	0.01205	0.05313	±	0.06351
bta-miR-188	0.00130	±	0.00091	0.00107	±	0.00115	0.00116	±	0.00099
bta-miR-17-5p	0.00040	±	0.00028	0.00034	±	0.00019	0.00071	±	0.00047
bta-miR-181d	0.00237	±	0.00267	0.00194	±	0.00183	0.00271	±	0.00247
bta-miR-187	0.00408	±	0.00073	0.00441	±	0.00295	0.00333	±	0.00038
bta-miR-195	0.03398	±	0.03932	0.01111	±	0.01048	0.05425	±	0.06366
bta-miR-200c	0.01790	±	0.02458	0.01259	±	0.01497	0.04825	±	0.04318
bta-miR-196a	0.00583	±	0.00521	0.00208	±	0.00042	0.00567	±	0.00590
bta-miR-196b	0.00600	±	0.00538	0.00181	±	0.00107	0.00800	±	0.00682
bta-miR-197	0.00116	±	0.00146	0.00060	±	0.00024	0.00650	±	0.00564
bta-miR-199a-3p	0.03326	±	0.04721	0.01838	±	0.02790	0.06756	±	0.07425
bta-miR-199b	0.00494	±	0.00292	0.00111	±	0.00013	0.01587	±	0.01326
bta-miR-199c	0.07311	±	0.10383	0.03045	±	0.04163	0.11693	±	0.13037
bta-miR-20a	0.00562	±	0.00452	0.00209	±	0.00160	0.01555	±	0.01325
bta-miR-200b	0.09996	±	0.07644	0.06442	±	0.05628	0.25454	±	0.22134
bta-miR-210	0.00228	±	0.00069	0.00174	±	0.00136	0.00192	±	0.00153
bta-miR-22-5p	0.00159	±	0.00118	0.00109	±	0.00018	0.00088	±	0.00034

bta-miR-23a	0.04614	±	0.06275	0.02305	±	0.03126	0.06049	±	0.05990
bta-miR-24-3p	0.01300	±	0.01423	0.00239	±	0.00142	0.04265	±	0.03657
bta-miR-22-3p	1668275.55271	±	81273.34495	1785969.05067	±	286863.55114	1576152.64269	±	358518.38268
bta-miR-26a	0.04311	±	0.05636	0.01901	±	0.01673	0.09191	±	0.08549
bta-miR-26b	0.01706	±	0.02065	0.00796	±	0.00731	0.04193	±	0.03621
bta-miR-29d-3p	0.00568	±	0.00706	0.00197	±	0.00137	0.01021	±	0.00869
bta-miR-27a-3p	0.00434	±	0.00690	0.00088	±	0.00113	0.00967	±	0.01328
bta-miR-27a-5p	0.00033	±	0.00033	0.00066	±	0.00036	0.00048	±	0.00009
bta-miR-296-3p	0.00213	±	0.00091	0.00237	±	0.00129	0.00197	±	0.00044
bta-miR-296-5p	0.00227	±	0.00156	0.00122	±	0.00023	0.00084	±	0.00025
bta-miR-29a	0.02670	±	0.03078	0.00824	±	0.00735	0.04087	±	0.03568
bta-miR-29c	0.02476	±	0.02701	0.00890	±	0.00812	0.04377	±	0.03759
bta-miR-328	0.00316	±	0.00047	0.00205	±	0.00112	0.00236	±	0.00131
bta-miR-30c	0.00093	±	0.00113	0.00072	±	0.00022	0.00460	±	0.00642
bta-miR-331-5p	0.00112	±	0.00032	0.00154	±	0.00056	0.00215	±	0.00082
bta-miR-320a	0.03351	±	0.02274	0.02598	±	0.01561	0.03825	±	0.02011
bta-miR-320b	0.00144	±	0.00031	0.00211	±	0.00153	0.00174	±	0.00139
bta-miR-323	34.92466	±	2.81654	37.42019	±	2.15220	36.00241	±	0.30805
bta-miR-339b	0.00038	±	0.00009	0.00111	±	0.00067	0.00125	±	0.00072
bta-miR-33b	0.00069	±	0.00029	0.00102	±	0.00041	0.00063	±	0.00016
bta-miR-345-3p	0.00320	±	0.00053	0.00208	±	0.00063	0.00266	±	0.00164
bta-miR-346	0.00241	±	0.00077	0.00051	±	0.00032	0.00274	±	0.00204
bta-miR-375	0.01174	±	0.01653	0.00508	±	0.00509	0.01700	±	0.01390
bta-miR-382	0.00884	±	0.00303	0.00993	±	0.00077	0.01631	±	0.00269
bta-miR-411a	0.00495	±	0.00645	0.00236	±	0.00136	0.00610	±	0.00730
bta-miR-411b	0.00096	±	0.00046	0.00096	±	0.00075	0.00098	±	0.00042
bta-miR-378d	0.00042	±	0.00003	0.00033	±	0.00010	0.00054	±	0.00015
bta-miR-421	0.00271	±	0.00022	0.00311	±	0.00401	0.00136	±	0.00099
bta-miR-423-5p	0.01131	±	0.01127	0.00927	±	0.00809	0.01963	±	0.01719
bta-miR-449d	0.00090	±	0.00062	0.00100	±	0.00036	0.00107	±	0.00074
bta-miR-424-5p	0.01222	±	0.01801	0.00389	±	0.00427	0.02101	±	0.02184
bta-miR-425-3p	0.01599	±	0.00725	0.01575	±	0.01271	0.01412	±	0.00336
bta-miR-4523	0.00035	±	0.00026	0.12381	±	0.21354	0.00031	±	0.00010
bta-miR-453	0.00071	±	0.00037	0.00163	±	0.00097	0.00090	±	0.00032
bta-miR-433	0.00651	±	0.00278	0.00887	±	0.00008	0.01149	±	0.00396
bta-miR-489	0.00120	±	0.00043	0.00183	±	0.00019	0.00239	±	0.00112
bta-miR-493	0.00234	±	0.00152	0.00201	±	0.00089	0.00270	±	0.00105
bta-miR-541	0.00329	±	0.00042	0.00446	±	0.00153	0.00457	±	0.00049
bta-miR-584	0.00166	±	0.00074	0.00126	±	0.00127	0.00105	±	0.00078
bta-miR-615	7212.09446	±	447.52621	10492.59793	±	947.65320	8378.23898	±	3586.98505
bta-miR-631	13.94727	±	0.62762	15.41813	±	0.60039	15.35972	±	0.52067
bta-miR-574	0.12312	±	0.02023	0.11905	±	0.04354	0.13181	±	0.08320
bta-miR-656	0.00332	±	0.00109	0.00315	±	0.00033	0.00450	±	0.00268
bta-miR-760-3p	0.00228	±	0.00077	0.00145	±	0.00072	0.00062	±	0.00026

bta-miR-760-5p	0.00244	±	0.00067	0.00110	±	0.00062	0.00251	±	0.00131
bta-miR-665	0.00526	±	0.00180	0.00457	±	0.00239	0.00547	±	0.00169
bta-miR-669	0.00300	±	0.00060	0.00354	±	0.00154	0.00213	±	0.00098
bta-miR-767	0.00300	±	0.00023	0.00344	±	0.00110	0.00408	±	0.00033
bta-miR-877	0.02976	±	0.00340	0.02500	±	0.01669	0.02657	±	0.01143
bta-miR-99a-5p	0.01572	±	0.02192	0.00760	±	0.00986	0.03500	±	0.04107
bta-miR-92b	0.03280	±	0.01813	0.02695	±	0.00935	0.02849	±	0.00828
bta-miR-940	0.06027	±	0.01331	0.04204	±	0.02405	0.04457	±	0.01881
bta-miR-1224	0.23795	±	0.00976	0.27180	±	0.20425	0.23971	±	0.17050
bta-miR-1225-3p	0.04865	±	0.01482	0.04813	±	0.03876	0.05188	±	0.02880
bta-miR-1246	3.54409	±	2.57456	2.20406	±	2.03141	6.28244	±	5.04695
bta-miR-1247-3p	0.00338	±	0.00028	0.00430	±	0.00300	0.00283	±	0.00129
bta-miR-1247-5p	0.06857	±	0.03621	0.05772	±	0.05389	0.03829	±	0.01823
bta-miR-1260b	0.17501	±	0.18262	0.88147	±	1.29550	0.09739	±	0.05446
bta-miR-1307	0.01653	±	0.00130	0.01858	±	0.01119	0.01795	±	0.00918
bta-miR-1343-3p	0.00388	±	0.00234	0.00301	±	0.00183	0.00414	±	0.00033
bta-miR-1343-5p	0.20435	±	0.01533	0.20556	±	0.11531	0.23986	±	0.13578
bta-miR-1281	0.00622	±	0.00321	0.00539	±	0.00285	0.00518	±	0.00177
bta-miR-1388-3p	0.00040	±	0.00018	0.00081	±	0.00055	0.00151	±	0.00044
bta-miR-1282	0.00074	±	0.00064	0.00056	±	0.00015	0.00052	±	0.00034
RNT43 snoRNA	0.00868	±	0.00326	0.01086	±	0.00709	0.00685	±	0.00374
Hm/Ms/Rt T1 snRNA	4.25832	±	3.45421	2.45843	±	2.11083	8.60303	±	9.43096
bta-let-7a-3p	.	±	.	.	±	.	0.00040	±	0.00006
bta-miR-452	0.00022	±	0.00009	.	±	.	.	±	.

Supplementary Table S21. MicroRNAs identified in sEVs isolated from C.M. by end, end *in vivo*, and end *in vitro* for 24 hours.

MicroRNAs	End C.M. sEVs 24h			End <i>in vivo</i> C.M. sEVs 24h			End <i>in vitro</i> C.M. sEVs 24h		
	Mean	Standard deviation		Mean	Standard deviation		Mean	Standard deviation	
bta-let-7a-5p	0.02035	± 0.01862		0.01575	± 0.01473		0.01148	± 0.01525	
bta-let-7b	0.34453	± 0.31722		0.18941	± 0.26761		0.08843	± 0.09788	
bta-let-7c	0.22182	± 0.18742		0.11354	± 0.14748		0.05891	± 0.07534	
bta-miR-106a	0.02252	± 0.02351		0.00713	± 0.00918		0.00271	± 0.00210	
bta-let-7e	0.09883	± 0.04602		0.05978	± 0.06844		0.04810	± 0.05687	
bta-miR-125b	0.13475	± 0.21090		0.07288	± 0.12338		0.01681	± 0.02023	
bta-miR-126-3p	0.02461	± 0.03673		0.01636	± 0.02134		0.00867	± 0.00685	
bta-miR-126-5p	0.03693	± 0.06079		0.01792	± 0.02916		0.00430	± 0.00481	
bta-miR-127	0.00143	± 0.00011		0.00173	± 0.00067		0.00162	± 0.00056	
bta-miR-130a	0.00425	± 0.00047		0.00486	± 0.00080		0.00389	± 0.00064	
bta-miR-130b	0.00624	± 0.00186		0.00751	± 0.00073		0.00597	± 0.00182	
bta-miR-143	0.01329	± 0.01807		0.00843	± 0.01178		0.00223	± 0.00134	
bta-miR-151-5p	0.00857	± 0.00640		0.00630	± 0.00868		0.00330	± 0.00247	
bta-miR-152	0.00280	± 0.00190		0.00287	± 0.00428		0.00043	± 0.00012	
bta-miR-145	0.04991	± 0.06913		0.02292	± 0.03700		0.00292	± 0.00146	
bta-miR-154b	0.01558	± 0.01065		0.01197	± 0.00216		0.00831	± 0.00317	
bta-miR-148a	0.05797	± 0.05973		0.02648	± 0.03810		0.01090	± 0.00954	
bta-miR-148b	0.03182	± 0.03001		0.01688	± 0.02453		0.00806	± 0.00601	
bta-miR-149-3p	0.82394	± 1.15753		0.51498	± 0.55913		1.01322	± 1.48347	
bta-miR-16a	0.05259	± 0.08439		0.03218	± 0.05363		0.00622	± 0.00593	
bta-miR-16b	0.15094	± 0.24203		0.08778	± 0.14838		0.01235	± 0.01276	
bta-miR-181d	0.00847	± 0.01241		0.00245	± 0.00339		0.00133	± 0.00168	
bta-miR-193a-5p	0.00448	± 0.00401		0.00473	± 0.00632		0.00184	± 0.00178	
bta-miR-187	0.00513	± 0.00556		0.00269	± 0.00081		0.00344	± 0.00502	
bta-miR-195	0.10085	± 0.15064		0.06195	± 0.10199		0.01750	± 0.01487	
bta-miR-200c	0.08168	± 0.10840		0.04834	± 0.07923		0.01500	± 0.01190	
bta-miR-196b	0.00902	± 0.00726		0.00421	± 0.00478		0.00265	± 0.00215	
bta-miR-199a-3p	0.05859	± 0.06537		0.02915	± 0.04000		0.00839	± 0.00921	
bta-miR-199c	0.11540	± 0.12433		0.06383	± 0.08395		0.02309	± 0.01542	
bta-miR-20a	0.03001	± 0.03888		0.01232	± 0.01889		0.00380	± 0.00421	
bta-miR-200b	0.34757	± 0.31792		0.19707	± 0.28068		0.12763	± 0.13868	
bta-miR-211	0.00927	± 0.01311		0.01077	± 0.01735		0.00178	± 0.00149	
bta-miR-23a	0.21583	± 0.32326		0.09951	± 0.16174		0.02492	± 0.01841	
bta-miR-24-3p	0.11414	± 0.15910		0.05552	± 0.09024		0.01580	± 0.01047	
bta-miR-22-3p	1951886.87667	± 202801.00111		1881959.62893	± 483378.32353		2268880.07039	± 212571.26739	
bta-miR-26a	0.09798	± 0.08000		0.05950	± 0.07717		0.04022	± 0.05299	
bta-miR-26b	0.02767	± 0.02020		0.01777	± 0.02253		0.01466	± 0.02153	
bta-miR-29c	0.02797	± 0.04086		0.01370	± 0.02193		0.00335	± 0.00196	
bta-miR-328	0.00372	± 0.00029		0.00261	± 0.00165		0.00374	± 0.00521	

bta-miR-331-5p	0.00199 ± 0.00027	0.00260 ± 0.00197	0.00161 ± 0.00082
bta-miR-320a	0.04583 ± 0.04046	0.02232 ± 0.01964	0.01489 ± 0.01281
bta-miR-323	33.38266 ± 1.65212	34.01427 ± 1.14187	33.46532 ± 5.93417
bta-miR-339b	0.00093 ± 0.00075	0.00169 ± 0.00019	0.00082 ± 0.00067
bta-miR-33b	0.00087 ± 0.00016	0.00062 ± 0.00024	0.00070 ± 0.00015
bta-miR-34b	0.01415 ± 0.01588	0.00817 ± 0.01195	0.00161 ± 0.00101
bta-miR-375	0.02517 ± 0.03877	0.01539 ± 0.02069	0.00260 ± 0.00144
bta-miR-382	0.02129 ± 0.01423	0.01604 ± 0.00929	0.01232 ± 0.00505
bta-miR-411a	0.00699 ± 0.00792	0.00515 ± 0.00495	0.00277 ± 0.00079
bta-miR-411b	0.00065 ± 0.00028	0.00072 ± 0.00036	0.00058 ± 0.00040
bta-miR-378d	0.00168 ± 0.00240	0.00049 ± 0.00028	0.00034 ± 0.00016
bta-miR-421	0.00602 ± 0.00756	0.00183 ± 0.00179	0.00057 ± 0.00043
bta-miR-423-3p	0.00981 ± 0.01035	0.00364 ± 0.00404	0.00242 ± 0.00177
bta-miR-423-5p	0.02439 ± 0.02034	0.01024 ± 0.00908	0.00907 ± 0.01303
bta-miR-425-3p	0.01125 ± 0.00843	0.00389 ± 0.00221	0.00617 ± 0.00762
bta-miR-429	0.05052 ± 0.03250	0.02621 ± 0.01324	0.02605 ± 0.02174
bta-miR-453	0.00105 ± 0.00078	0.00165 ± 0.00083	0.00069 ± 0.00034
bta-miR-433	0.01345 ± 0.00761	0.00921 ± 0.00210	0.00832 ± 0.00197
bta-miR-489	0.00172 ± 0.00052	0.00148 ± 0.00036	0.00161 ± 0.00077
bta-miR-503-3p	0.00611 ± 0.00465	0.00310 ± 0.00194	0.00386 ± 0.00453
bta-miR-491	0.00480 ± 0.00720	0.00279 ± 0.00348	0.00118 ± 0.00137
bta-miR-494	0.04195 ± 0.02162	0.03111 ± 0.00747	0.03743 ± 0.03170
bta-miR-541	0.00514 ± 0.00245	0.00607 ± 0.00199	0.00430 ± 0.00065
bta-miR-615	10244.61861 ± 884.09988	13939.42530 ± 2977.00757	12983.04943 ± 2228.28895
bta-miR-631	14.99871 ± 1.20474	14.09367 ± 0.56829	14.23063 ± 0.90841
bta-miR-574	0.11977 ± 0.11777	0.09161 ± 0.03673	0.11450 ± 0.12286
bta-miR-656	0.00637 ± 0.00599	0.00400 ± 0.00040	0.00405 ± 0.00180
bta-miR-760-3p	0.00256 ± 0.00179	0.00161 ± 0.00058	0.00163 ± 0.00189
bta-miR-760-5p	0.00664 ± 0.00834	0.00213 ± 0.00190	0.00139 ± 0.00138
bta-miR-665	0.00239 ± 0.00228	0.00126 ± 0.00027	0.00226 ± 0.00311
bta-miR-767	0.00312 ± 0.00074	0.00362 ± 0.00044	0.00352 ± 0.00012
bta-miR-874	0.00200 ± 0.00175	0.00190 ± 0.00174	0.00270 ± 0.00354
bta-miR-877	0.02399 ± 0.02954	0.01600 ± 0.01433	0.02588 ± 0.03640
bta-miR-92b	0.03608 ± 0.02378	0.02314 ± 0.01741	0.02353 ± 0.01175
bta-miR-940	0.05372 ± 0.05704	0.03546 ± 0.02415	0.05255 ± 0.06279
bta-miR-1224	0.07081 ± 0.08744	0.05620 ± 0.04558	0.11494 ± 0.16972
bta-miR-1225-3p	0.04147 ± 0.05764	0.02715 ± 0.02921	0.04689 ± 0.06619
bta-miR-1246	32.45329 ± 25.51170	12.25743 ± 18.10035	9.89943 ± 5.09335
bta-miR-1247-3p	0.00741 ± 0.00483	0.00301 ± 0.00190	0.00414 ± 0.00334
bta-miR-1247-5p	0.04747 ± 0.06873	0.02740 ± 0.01502	0.05310 ± 0.06975
bta-miR-1260b	0.05543 ± 0.04138	0.04903 ± 0.03510	0.12330 ± 0.17550
bta-miR-1307	0.00756 ± 0.00816	0.00450 ± 0.00336	0.00586 ± 0.00777
bta-miR-1343-5p	0.14211 ± 0.19561	0.08807 ± 0.06700	0.19309 ± 0.27423
bta-miR-1281	0.00879 ± 0.01012	0.00362 ± 0.00117	0.00261 ± 0.00316

bta-miR-1388-3p	0.00185	±	0.00120	0.00219	±	0.00156	0.00081	±	0.00034
RNT43 snoRNA	0.01397	±	0.01404	0.01183	±	0.01101	0.01263	±	0.01043
Hm/Ms/Rt T1 snRNA	15.19422	±	13.98857	6.40439	±	9.09812	4.51572	±	4.47241
bta-miR-184	0.00170	±	0.00247	.	±	.	.	±	.

Supplementary Table S22. MicroRNAs expressed in all the samples of sEVs secreted by endometrial explants independently of the culture time.

MicroRNAs		
bta-let-7a-5p	bta-miR-200b	bta-miR-615
bta-let-7b	bta-miR-23a	bta-miR-631
bta-let-7c	bta-miR-24-3p	bta-miR-574
bta-let-7e	bta-miR-22-3p	bta-miR-656
bta-miR-126-3p	bta-miR-26a	bta-miR-760-3p
bta-miR-126-5p	bta-miR-26b	bta-miR-760-5p
bta-miR-127	bta-miR-29c	bta-miR-665
bta-miR-130a	bta-miR-328	bta-miR-767
bta-miR-130b	bta-miR-331-5p	bta-miR-877
bta-miR-143	bta-miR-320a	bta-miR-92b
bta-miR-145	bta-miR-323	bta-miR-940
bta-miR-154b	bta-miR-339b	bta-miR-1224
bta-miR-148a	bta-miR-33b	bta-miR-1225-3p
bta-miR-148b	bta-miR-375	bta-miR-1246
bta-miR-149-3p	bta-miR-382	bta-miR-1247-3p
bta-miR-16a	bta-miR-411a	bta-miR-1247-5p
bta-miR-16b	bta-miR-411b	bta-miR-1260b
bta-miR-181d	bta-miR-378d	bta-miR-1307
bta-miR-187	bta-miR-421	bta-miR-1343-5p
bta-miR-195	bta-miR-423-5p	bta-miR-1281
bta-miR-200c	bta-miR-425-3p	bta-miR-1388-3p
bta-miR-196b	bta-miR-453	RNT43 snoRNA
bta-miR-199a-3p	bta-miR-433	Hm/Ms/Rt T1
bta-miR-199c	bta-miR-489	snRNA
bta-miR-20a	bta-miR-541	bta-miR-99b

Supplementary Table S23. The predicted biological pathways modulated by miRNAs differently detected in sEVs isolated from C.M. by end, end *in vivo*, and end *in vitro* 6h.

Signaling pathways	Genes predicted to be modulated by miRNAs	Number of total genes	Pvalue	BH
bta04921 Oxytocin signaling pathway	58	152	0.0	0.0
bta05200 Pathways in cancer	157	542	0.0	0.0
bta04070 Phosphatidylinositol signaling system	41	99	0.0001	0.0076
bta05231 Choline metabolism in cancer	41	99	0.0001	0.0076
bta04261 Adrenergic signaling in cardiomyocytes	54	150	0.0002	0.0121
bta01522 Endocrine resistance	38	94	0.0003	0.013
bta05205 Proteoglycans in cancer	68	205	0.0003	0.013
bta01100 Metabolic pathways	375	1527	0.0004	0.0152
bta00562 Inositol phosphate metabolism	31	73	0.0006	0.0182
bta05224 Breast cancer	52	150	0.0006	0.0182
bta04144 Endocytosis	76	245	0.0007	0.0193
bta04934 Cushing syndrome	53	156	0.0008	0.0202
bta05412 Arrhythmogenic right ventricular cardiomyopathy (ARVC)	31	76	0.0009	0.021
bta04014 Ras signaling pathway	74	242	0.0011	0.0238
bta05165 Human papillomavirus infection	99	345	0.0012	0.0242
bta05410 Hypertrophic cardiomyopathy (HCM)	35	92	0.0013	0.0246
bta04728 Dopaminergic synapse	46	134	0.0014	0.025
bta04012 ErbB signaling pathway	32	84	0.0019	0.0251
bta04071 Sphingolipid signaling pathway	42	120	0.0017	0.0251
bta04310 Wnt signaling pathway	53	162	0.0016	0.0251
bta04514 Cell adhesion molecules (CAMs)	51	158	0.0024	0.0251
bta04668 TNF signaling pathway	41	118	0.0021	0.0251
bta04910 Insulin signaling pathway	47	140	0.0018	0.0251
bta04912 GnRH signaling pathway	34	93	0.0024	0.0251
bta04916 Melanogenesis	37	102	0.0018	0.0251
bta04928 Parathyroid hormone synthesis, secretion and action	37	104	0.0024	0.0251
bta05210 Colorectal cancer	33	89	0.0023	0.0251
bta05214 Glioma	30	77	0.002	0.0251
bta05226 Gastric cancer	50	153	0.0021	0.0251
bta04072 Phospholipase D signaling pathway	49	152	0.0029	0.0283
bta04924 Renin secretion	28	72	0.0028	0.0283
bta04024 cAMP signaling pathway	68	229	0.0031	0.0294
bta04066 HIF-1 signaling pathway	38	110	0.0032	0.0294
bta00564 Glycerophospholipid metabolism	36	104	0.0039	0.0311
bta04520 Adherens junction	27	70	0.0036	0.0311
bta04660 T cell receptor signaling pathway	37	107	0.0035	0.0311
bta04810 Regulation of actin cytoskeleton	63	211	0.0038	0.0311
bta05017 Spinocerebellar ataxia	34	96	0.0037	0.0311

bta04371 Apelin signaling pathway	45	140	0.0044	0.0342
bta05225 Hepatocellular carcinoma	53	174	0.0053	0.0399
bta05414 Dilated cardiomyopathy (DCM)	34	99	0.0054	0.0399
bta04710 Circadian rhythm	15	31	0.0056	0.0404
bta04068 FoxO signaling pathway	42	131	0.0059	0.0416
bta04713 Circadian entrainment	34	100	0.0061	0.042
bta05223 Non-small cell lung cancer	25	67	0.0069	0.0465
bta04020 Calcium signaling pathway	59	202	0.0071	0.0468
bta04360 Axon guidance	53	178	0.0076	0.0485
bta04625 C-type lectin receptor signaling pathway	35	106	0.0078	0.0485
bta04725 Cholinergic synapse	37	114	0.0079	0.0485
bta04935 Growth hormone synthesis, secretion and action	38	118	0.008	0.0485
bta04130 SNARE interactions in vesicular transport	15	33	0.0086	0.0511
bta04723 Retrograde endocannabinoid signaling	46	152	0.0096	0.054
bta05032 Morphine addiction	31	92	0.0093	0.054
bta05167 Kaposi sarcoma-associated herpesvirus infection	59	206	0.0098	0.054
bta05220 Chronic myeloid leukemia	27	77	0.0097	0.054
bta04664 Fc epsilon RI signaling pathway	25	70	0.0105	0.0568
bta04270 Vascular smooth muscle contraction	41	133	0.0108	0.0574
bta04140 Autophagy	43	142	0.0117	0.0591
bta04722 Neurotrophin signaling pathway	38	122	0.012	0.0591
bta04919 Thyroid hormone signaling pathway	37	118	0.0121	0.0591
bta04931 Insulin resistance	35	110	0.0121	0.0591
bta05215 Prostate cancer	32	98	0.0119	0.0591
bta04151 PI3K-Akt signaling pathway	97	373	0.0123	0.0592
bta04730 Long-term depression	22	60	0.0125	0.0592
bta05163 Human cytomegalovirus infection	67	245	0.0141	0.0647
bta05212 Pancreatic cancer	26	76	0.014	0.0647
bta04726 Serotonergic synapse	36	116	0.0148	0.0659
bta04961 Endocrine and other factor-regulated calcium reabsorption	19	50	0.0146	0.0659
bta01230 Biosynthesis of amino acids	25	73	0.0155	0.0681
bta04010 MAPK signaling pathway	77	292	0.0179	0.0775
bta04390 Hippo signaling pathway	45	156	0.0193	0.0824
bta04120 Ubiquitin mediated proteolysis	41	140	0.0204	0.0847
bta04215 Apoptosis	14	34	0.0204	0.0847
bta04150 mTOR signaling pathway	45	157	0.021	0.086
bta04925 Aldosterone synthesis and secretion	30	96	0.0225	0.0897
bta04971 Gastric acid secretion	25	76	0.0223	0.0897
bta04062 Chemokine signaling pathway	52	188	0.0232	0.0913
bta04666 Fc gamma R-mediated phagocytosis	29	93	0.0251	0.0946
bta04915 Estrogen signaling pathway	40	138	0.0246	0.0946
bta04926 Relaxin signaling pathway	38	130	0.0253	0.0946
bta05100 Bacterial invasion of epithelial cells	24	73	0.0249	0.0946

bta05132 Salmonella infection	60	224	0.0258	0.0949
bta05160 Hepatitis C	46	164	0.026	0.0949
bta04370 VEGF signaling pathway	20	58	0.0267	0.0963
bta05222 Small cell lung cancer	29	94	0.0277	0.0987
bta04727 GABAergic synapse	28	91	0.0308	0.1085
bta00230 Purine metabolism	38	134	0.0352	0.1147
bta04510 Focal adhesion	53	198	0.0343	0.1147
bta04550 Signaling pathways regulating pluripotency of stem cells	40	142	0.0339	0.1147
bta04611 Platelet activation	35	121	0.0341	0.1147
bta04659 Th17 cell differentiation	33	113	0.0352	0.1147
bta05169 Epstein-Barr virus infection	60	228	0.0332	0.1147
bta05221 Acute myeloid leukemia	22	68	0.0348	0.1147
bta00270 Cysteine and methionine metabolism	17	49	0.0373	0.1193
bta04022 cGMP-PKG signaling pathway	46	169	0.0374	0.1193
bta04911 Insulin secretion	26	85	0.0382	0.1203
bta05031 Amphetamine addiction	22	69	0.0389	0.1203
bta05218 Melanoma	23	73	0.0388	0.1203
bta04658 Th1 and Th2 cell differentiation	29	98	0.0407	0.1233
bta05219 Bladder cancer	15	42	0.0407	0.1233
bta00512 Mucin type O-glycan biosynthesis	12	31	0.0419	0.1252
bta04721 Synaptic vesicle cycle	24	78	0.0429	0.1252
bta04750 Inflammatory mediator regulation of TRP channels	30	103	0.0438	0.1252
bta04922 Glucagon signaling pathway	30	103	0.0438	0.1252
bta04933 AGE-RAGE signaling pathway in diabetic complications	30	103	0.0438	0.1252
bta05230 Central carbon metabolism in cancer	21	66	0.0435	0.1252
bta00280 Valine, leucine and isoleucine degradation	17	51	0.0482	0.1351
bta05213 Endometrial cancer	19	59	0.0486	0.1351
bta05217 Basal cell carcinoma	20	63	0.0486	0.1351
bta05145 Toxoplasmosis	32	113	0.0501	0.138
bta01521 EGFR tyrosine kinase inhibitor resistance	24	80	0.0523	0.1408
bta05033 Nicotine addiction	14	40	0.0525	0.1408
bta05216 Thyroid cancer	14	40	0.0525	0.1408
bta00592 alpha-Linolenic acid metabolism	11	29	0.0552	0.1467
bta04152 AMPK signaling pathway	34	123	0.0562	0.1481
bta05133 Pertussis	23	77	0.0583	0.1523
bta04720 Long-term potentiation	21	69	0.0597	0.1543
bta05340 Primary immunodeficiency	14	41	0.0601	0.1543
bta00514 Other types of O-glycan biosynthesis	15	45	0.0608	0.1548
bta04530 Tight junction	46	177	0.0632	0.1596
bta00770 Pantothenate and CoA biosynthesis	8	19	0.0647	0.162
bta04662 B cell receptor signaling pathway	25	87	0.0677	0.1678
bta04923 Regulation of lipolysis in adipocytes	18	58	0.0681	0.1678

bta04917 Prolactin signaling pathway	24	83	0.069	0.1686
bta05211 Renal cell carcinoma	21	71	0.0726	0.176
bta04015 Rap1 signaling pathway	54	216	0.0762	0.1818
bta05166 Human T-cell leukemia virus 1 infection	58	234	0.0761	0.1818
bta04114 Oocyte meiosis	32	119	0.0791	0.1872
bta05142 Chagas disease (American trypanosomiasis)	31	115	0.0811	0.1905
bta05014 Amyotrophic lateral sclerosis (ALS)	18	60	0.0837	0.1951
bta05020 Prion diseases	11	32	0.0863	0.1996
bta05162 Measles	39	152	0.0911	0.2091
bta00600 Sphingolipid metabolism	15	49	0.0966	0.2179
bta04670 Leukocyte transendothelial migration	30	113	0.0958	0.2179
bta05161 Hepatitis B	43	171	0.0971	0.2179
bta04960 Aldosterone-regulated sodium reabsorption	12	37	0.0988	0.2201
bta01210 2-Oxocarboxylic acid metabolism	7	18	0.105	0.2322
bta05202 Transcriptional misregulation in cancer	47	191	0.1076	0.2363
bta04930 Type II diabetes mellitus	14	46	0.1089	0.2372
bta05235 PD-L1 expression and PD-1 checkpoint pathway in cancer	25	93	0.1096	0.2372
bta00410 beta-Alanine metabolism	11	34	0.112	0.2373
bta01040 Biosynthesis of unsaturated fatty acids	10	30	0.112	0.2373
bta04972 Pancreatic secretion	27	102	0.1115	0.2373
bta04340 Hedgehog signaling pathway	15	51	0.1186	0.2478
bta04380 Osteoclast differentiation	34	134	0.1182	0.2478
bta00010 Glycolysis Gluconeogenesis	18	64	0.1216	0.2506
bta04218 Cellular senescence	41	166	0.121	0.2506
bta00360 Phenylalanine metabolism	8	23	0.1267	0.2576
bta00515 Mannose type O-glycan biosynthesis	8	23	0.1267	0.2576
bta04744 Phototransduction	9	27	0.1275	0.2576
bta04929 GnRH secretion	18	65	0.1324	0.2657
bta00310 Lysine degradation	18	66	0.1437	0.2833
bta04137 Mitophagy	18	66	0.1437	0.2833
bta04914 Progesterone-mediated oocyte maturation	23	88	0.144	0.2833
bta03420 Nucleotide excision repair	13	45	0.1506	0.287
bta04146 Peroxisome	22	84	0.1484	0.287
bta04216 Ferroptosis	13	45	0.1506	0.287
bta04350 TGF-beta signaling pathway	24	93	0.1495	0.287
bta04962 Vasopressin-regulated water reabsorption	14	49	0.1469	0.287
bta04141 Protein processing in endoplasmic reticulum	40	166	0.1528	0.2894
bta04512 ECM-receptor interaction	23	89	0.1542	0.2902
bta00561 Glycerolipid metabolism	18	67	0.1556	0.291
bta05135 Yersinia infection	32	130	0.1579	0.2935

bta04211 Longevity regulating pathway	23	90	0.1648	0.3026
bta04540 Gap junction	23	90	0.1648	0.3026
bta00603 Glycosphingolipid biosynthesis	6	17	0.1668	0.3045
bta05170 Human immunodeficiency virus 1 infection	54	234	0.1737	0.3152
bta01524 Platinum drug resistance	20	78	0.1817	0.3276
bta04210 Apoptosis	34	142	0.1831	0.3276
bta04620 Toll-like receptor signaling pathway	27	110	0.1849	0.3276
bta04640 Hematopoietic cell lineage	27	110	0.1849	0.3276
bta04614 Renin-angiotensin system	8	26	0.1877	0.3287
bta04927 Cortisol synthesis and secretion	17	65	0.187	0.3287
bta00565 Ether lipid metabolism	14	52	0.1912	0.331
bta04964 Proximal tubule bicarbonate reclamation	7	22	0.1911	0.331
bta04970 Salivary secretion	23	93	0.199	0.3407
bta05164 Influenza A	42	181	0.1986	0.3407
bta00052 Galactose metabolism	9	31	0.2045	0.3481
bta04260 Cardiac muscle contraction	22	89	0.2059	0.3485
bta04213 Longevity regulating pathway	16	62	0.2083	0.3506
bta00340 Histidine metabolism	7	23	0.2161	0.3598
bta01212 Fatty acid metabolism	15	58	0.2158	0.3598
bta04724 Glutamatergic synapse	27	113	0.2177	0.3605
bta00220 Arginine biosynthesis	6	19	0.2211	0.3641
bta04920 Adipocytokine signaling pathway	18	72	0.2225	0.3644
bta04110 Cell cycle	29	123	0.2255	0.3673
bta04080 Neuroactive ligand-receptor interaction	79	363	0.2396	0.3882
bta00532 Glycosaminoglycan biosynthesis	6	20	0.2502	0.3998
bta03440 Homologous recombination	11	42	0.2507	0.3998
bta04976 Bile secretion	20	83	0.2486	0.3998
bta04918 Thyroid hormone synthesis	18	74	0.2523	0.4002
bta00062 Fatty acid elongation	8	29	0.2586	0.4039
bta00601 Glycosphingolipid biosynthesis	8	29	0.2586	0.4039
bta04672 Intestinal immune network for IgA production	14	56	0.2585	0.4039
bta05418 Fluid shear stress and atherosclerosis	33	145	0.2607	0.4051
bta00510 N-Glycan biosynthesis	13	52	0.269	0.4159
bta00260 Glycine, serine and threonine metabolism	11	43	0.2714	0.4174
bta01200 Carbon metabolism	26	113	0.2744	0.4199
bta00330 Arginine and proline metabolism	12	48	0.2803	0.4204
bta00900 Terpenoid backbone biosynthesis	6	21	0.2803	0.4204
bta04913 Ovarian steroidogenesis	14	57	0.2765	0.4204
bta05030 Cocaine addiction	12	48	0.2803	0.4204
bta03320 PPAR signaling pathway	19	81	0.2884	0.4263
bta04061 Viral protein interaction with cytokine and cytokine receptor	22	95	0.2865	0.4263
bta04330 Notch signaling pathway	13	53	0.2881	0.4263

bta00910 Nitrogen metabolism	5	17	0.2921	0.4282
bta05143 African trypanosomiasis	11	44	0.2925	0.4282
bta00563 Glycosylphosphatidylinositol (GPI)-anchor biosynthesis	7	26	0.2968	0.4323
bta04115 p53 signaling pathway	18	77	0.2996	0.4323
bta04932 Non-alcoholic fatty liver disease (NAFLD)	35	158	0.2991	0.4323
bta04657 IL-17 signaling pathway	21	92	0.312	0.448
bta00591 Linoleic acid metabolism	9	36	0.3201	0.4532
bta03030 DNA replication	9	36	0.3201	0.4532
bta04621 NOD-like receptor signaling pathway	40	184	0.3195	0.4532
bta00670 One carbon pool by folate	5	18	0.3269	0.4593
bta04978 Mineral absorption	13	55	0.3274	0.4593
bta04714 Thermogenesis	51	239	0.3339	0.4662
bta00240 Pyrimidine metabolism	13	56	0.3475	0.483
bta00030 Pentose phosphate pathway	7	28	0.3538	0.4851
bta00650 Butanoate metabolism	7	28	0.3538	0.4851
bta04966 Collecting duct acid secretion	7	28	0.3538	0.4851
bta05152 Tuberculosis	42	198	0.3651	0.4983
bta00513 Various types of N-glycan biosynthesis	10	43	0.3756	0.5103
bta04975 Fat digestion and absorption	11	48	0.3805	0.5147
bta00051 Fructose and mannose metabolism	8	34	0.3895	0.5245
bta05146 Amoebiasis	25	117	0.3956	0.5304
bta04142 Lysosome	28	132	0.3991	0.5327
bta03018 RNA degradation	17	79	0.4165	0.5535
bta00520 Amino sugar and nucleotide sugar metabolism	11	50	0.4255	0.563
bta02010 ABC transporters	13	60	0.4293	0.5656
bta05321 Inflammatory bowel disease (IBD)	15	70	0.4321	0.5668
bta03015 mRNA surveillance pathway	20	95	0.4366	0.5684
bta04950 Maturity onset diabetes of the young	6	26	0.4371	0.5684
bta04145 Phagosome	35	170	0.4414	0.5694
bta05010 Alzheimer disease	37	180	0.4416	0.5694
bta00380 Tryptophan metabolism	10	47	0.4703	0.5987
bta00590 Arachidonic acid metabolism	17	82	0.4699	0.5987
bta04623 Cytosolic DNA-sensing pathway	14	67	0.4703	0.5987
bta04217 Necroptosis	35	174	0.4902	0.6215
bta01523 Antifolate resistance	9	43	0.4948	0.6247
bta00640 Propanoate metabolism	7	33	0.4974	0.6254
bta03430 Mismatch repair	5	23	0.5008	0.627
bta05144 Malaria	12	59	0.5127	0.6393
bta00760 Nicotinate and nicotinamide metabolism	8	39	0.5219	0.6428
bta04630 JAK-STAT signaling pathway	40	202	0.5212	0.6428
bta04973 Carbohydrate digestion and absorption	9	44	0.5191	0.6428
bta03020 RNA polymerase	6	29	0.529	0.6464

bta05016 Huntington disease	54	274	0.5291	0.6464
bta00534 Glycosaminoglycan biosynthesis	5	24	0.5338	0.6496
bta05416 Viral myocarditis	15	76	0.5449	0.6604
bta00630 Glyoxylate and dicarboxylate metabolism	6	30	0.5582	0.6738
bta00053 Ascorbate and aldarate metabolism	5	25	0.5657	0.6786
bta03050 Proteasome	9	46	0.5666	0.6786
bta00790 Folate biosynthesis	7	36	0.5787	0.6848
bta03008 Ribosome biogenesis in eukaryotes	16	83	0.5773	0.6848
bta04064 NF-kappa B signaling pathway	21	109	0.578	0.6848
bta05323 Rheumatoid arthritis	20	104	0.5808	0.6848
bta00071 Fatty acid degradation	8	42	0.5963	0.6952
bta04650 Natural killer cell mediated cytotoxicity	25	131	0.5956	0.6952
bta04977 Vitamin digestion and absorption	5	26	0.5965	0.6952
bta00250 Alanine, aspartate and glutamate metabolism	7	37	0.6043	0.7015
bta05203 Viral carcinogenesis	46	241	0.6077	0.7028
bta00500 Starch and sucrose metabolism	6	32	0.614	0.7074
bta05310 Asthma	7	38	0.6291	0.722
bta03410 Base excision repair	6	33	0.6403	0.7294
bta04136 Autophagy	6	33	0.6403	0.7294
bta04060 Cytokine-cytokine receptor interaction	61	323	0.6449	0.7319
bta00350 Tyrosine metabolism	7	39	0.653	0.7383
bta00860 Porphyrin and chlorophyll metabolism	7	40	0.6759	0.7613
bta04742 Taste transduction	14	79	0.6875	0.7715
bta03013 RNA transport	32	178	0.7139	0.7982
bta00983 Drug metabolism	13	76	0.7259	0.8086
bta03022 Basal transcription factors	7	44	0.7577	0.841
bta04612 Antigen processing and presentation	14	85	0.7736	0.8555
bta03460 Fanconi anemia pathway	8	52	0.7939	0.8747
bta05012 Parkinson disease	25	150	0.8101	0.8893
bta05140 Leishmaniasis	12	78	0.8276	0.9053
bta00140 Steroid hormone biosynthesis	10	67	0.8365	0.9117
bta00620 Pyruvate metabolism	5	38	0.8586	0.9299
bta00982 Drug metabolism	9	63	0.8593	0.9299
bta05134 Legionellosis	8	58	0.8716	0.9398
bta00480 Glutathione metabolism	8	60	0.8914	0.951
bta00970 Aminoacyl-tRNA biosynthesis	9	66	0.889	0.951
bta04622 RIG-I-like receptor signaling pathway	15	102	0.8858	0.951
bta00190 Oxidative phosphorylation	21	140	0.9025	0.9595
bta05330 Allograft rejection	7	56	0.9123	0.9665
bta04979 Cholesterol metabolism	6	50	0.9172	0.9683
bta04940 Type I diabetes mellitus	7	59	0.9339	0.9696
bta05034 Alcoholism	35	229	0.9344	0.9696
bta05168 Herpes simplex virus 1 infection	66	403	0.9284	0.9696

bta05204 Chemical carcinogenesis	10	77	0.9245	0.9696
bta05206 MicroRNAs in cancer	46	292	0.9337	0.9696
bta00980 Metabolism of xenobiotics by cytochrome P450	8	67	0.9418	0.9706
bta05150 Staphylococcus aureus infection	14	105	0.9394	0.9706
bta05332 Graft-versus-host disease	5	48	0.9506	0.9764
bta00830 Retinol metabolism	7	64	0.9596	0.9823
bta04974 Protein digestion and absorption	15	121	0.9711	0.9907
bta03040 Spliceosome	18	147	0.9828	0.9959
bta05320 Autoimmune thyroid disease	7	71	0.9805	0.9959
bta03010 Ribosome	9	160	1.0	1.0
bta04610 Complement and coagulation cascades	6	92	0.9994	1.0
bta04740 Olfactory transduction	35	1145	1.0	1.0
bta05322 Systemic lupus erythematosus	16	182	0.9998	1.0

Supplementary Table S24. MicroRNAs expressed in LE treated with sEVs secreted by end, end *in vivo*, and end *in vitro* 6h.

MicroRNAs	LE			LE			LE		
	End sEVs 6h			End <i>in vivo</i> sEVs 6h			End <i>in vitro</i> sEVs 6h		
	Mean	Standard deviation		Mean	Standard deviation		Mean	Standard deviation	
bta-let-7a-3p	0.00079	± 0.00031		0.00064	± 0.00029		0.0009	± 0.00054	
bta-miR-103	0.01193	± 0.00484		0.01179	± 0.00270		0.0121	± 0.00373	
bta-let-7a-5p	0.33653	± 0.09879		0.37428	± 0.05585		0.3621	± 0.05887	
bta-miR-105a	0.00009	± 0.00004		0.00013	± 0.00007		0.0001	± 0.00006	
bta-let-7b	0.43933	± 0.08221		0.41261	± 0.03938		0.4187	± 0.08737	
bta-miR-105b	0.00054	± 0.00021		0.00039	± 0.00015		0.0005	± 0.00017	
bta-let-7c	0.52206	± 0.06130		0.49373	± 0.07481		0.5059	± 0.07960	
bta-miR-106a	0.00230	± 0.00117		0.00216	± 0.00099		0.0024	± 0.00128	
bta-let-7d	0.13165	± 0.03865		0.17003	± 0.01624		0.1452	± 0.01574	
bta-let-7e	0.47698	± 0.08902		0.51003	± 0.03163		0.4377	± 0.03213	
bta-miR-107	0.00081	± 0.00020		0.00087	± 0.00018		0.0009	± 0.00028	
bta-let-7f	0.05499	± 0.02374		0.05737	± 0.01286		0.0585	± 0.02450	
bta-miR-10a	0.00447	± 0.00163		0.00435	± 0.00125		0.0051	± 0.00196	
bta-let-7g	0.01630	± 0.00366		0.01873	± 0.00357		0.0178	± 0.00431	
bta-miR-10b	0.00407	± 0.00114		0.00367	± 0.00090		0.0046	± 0.00199	
bta-let-7i	0.01905	± 0.01021		0.01750	± 0.00763		0.0201	± 0.01314	
bta-miR-122	0.00003	± 0.00001		0.00003	± 0.00002		0.0000	± 0.00003	
bta-miR-124a	0.00026	± 0.00009		0.00033	± 0.00019		0.0004	± 0.00015	
bta-miR-100	0.00615	± 0.00254		0.00655	± 0.00180		0.0077	± 0.00273	
bta-miR-124b	0.00026	± 0.00006		0.00040	± 0.00014		0.0003	± 0.00009	
bta-miR-101	0.00016	± 0.00001		0.00015	± 0.00003		0.0001	± 0.00004	
bta-miR-125a	0.01489	± 0.00247		0.01755	± 0.00112		0.0182	± 0.00448	
bta-miR-125b	0.04762	± 0.00169		0.05343	± 0.00519		0.0592	± 0.01445	
bta-miR-126-3p	0.05981	± 0.00964		0.06133	± 0.01301		0.0628	± 0.01825	
bta-miR-133c	0.00001	± 0.00001		0.00002	± 0.00001		0.0000	± 0.00001	
bta-miR-126-5p	0.21106	± 0.07186		0.20555	± 0.06900		0.2441	± 0.12776	
bta-miR-134	0.00020	± 0.00006		0.00023	± 0.00009		0.0002	± 0.00008	
bta-miR-127	0.00434	± 0.00118		0.00569	± 0.00130		0.0049	± 0.00163	
bta-miR-135a	0.00001	± 0.00001		0.00001	± 0.00001		0.0000	± 0.00001	
bta-miR-128	0.00241	± 0.00034		0.00234	± 0.00025		0.0022	± 0.00071	
bta-miR-135b	0.00001	± 0.00001		0.00001	± 0.00001		0.0000	± 0.00000	
bta-miR-129	0.00073	± 0.00034		0.00096	± 0.00031		0.0009	± 0.00037	
bta-miR-136	0.00009	± 0.00004		0.00009	± 0.00004		0.0001	± 0.00007	
bta-miR-129-5p	0.00075	± 0.00032		0.00099	± 0.00035		0.0008	± 0.00031	
bta-miR-138	0.00032	± 0.00005		0.00043	± 0.00015		0.0004	± 0.00016	
bta-miR-130a	0.00123	± 0.00077		0.00099	± 0.00052		0.0011	± 0.00065	
bta-miR-139	0.00905	± 0.00314		0.00709	± 0.00134		0.0082	± 0.00367	
bta-miR-130b	0.01584	± 0.00261		0.01898	± 0.00514		0.0178	± 0.00468	
bta-miR-140	0.00212	± 0.00061		0.00202	± 0.00030		0.0026	± 0.00094	

bta-miR-132	0.00640	±	0.00215	0.00566	±	0.00166	0.0069	±	0.00358
bta-miR-141	0.00010	±	0.00007	0.00016	±	0.00009	0.0002	±	0.00010
bta-miR-133a	0.00052	±	0.00020	0.00072	±	0.00030	0.0007	±	0.00025
bta-miR-142-5p	0.00002	±	0.00001	0.00004	±	0.00001	0.0000	±	0.00001
bta-miR-151-3p	0.00389	±	0.00184	0.00354	±	0.00140	0.0044	±	0.00261
bta-miR-143	0.00161	±	0.00052	0.00137	±	0.00027	0.0017	±	0.00052
bta-miR-151-5p	0.02336	±	0.00347	0.02319	±	0.00283	0.0234	±	0.00467
bta-miR-152	0.00083	±	0.00015	0.00087	±	0.00011	0.0009	±	0.00020
bta-miR-153	0.00000	±	0.00000	0.00000	±	0.00000	0.0000	±	0.00000
bta-miR-146a	0.00007	±	0.00003	0.00005	±	0.00002	0.0001	±	0.00003
bta-miR-154a	0.00002	±	0.00000	0.00004	±	0.00002	0.0000	±	0.00000
bta-miR-146b	0.00010	±	0.00003	0.00006	±	0.00001	0.0001	±	0.00003
bta-miR-154b	0.00074	±	0.00040	0.00073	±	0.00034	0.0008	±	0.00063
bta-miR-147	0.00007	±	0.00002	0.00008	±	0.00001	0.0001	±	0.00001
bta-miR-154c	0.00029	±	0.00019	0.00027	±	0.00017	0.0003	±	0.00024
bta-miR-148a	0.00612	±	0.00149	0.00548	±	0.00080	0.0066	±	0.00222
bta-miR-155	0.00321	±	0.00079	0.00294	±	0.00070	0.0027	±	0.00179
bta-miR-148b	0.00445	±	0.00089	0.00429	±	0.00096	0.0046	±	0.00183
bta-miR-15a	0.00422	±	0.00025	0.00457	±	0.00030	0.0045	±	0.00082
bta-miR-149-3p	0.49394	±	0.02969	0.41855	±	0.02031	0.4723	±	0.02163
bta-miR-15b	0.01353	±	0.00156	0.01393	±	0.00032	0.0121	±	0.00106
bta-miR-149-5p	0.00095	±	0.00023	0.00095	±	0.00025	0.0009	±	0.00032
bta-miR-16a	0.02053	±	0.00709	0.02042	±	0.00558	0.0219	±	0.00875
bta-miR-150	0.00041	±	0.00012	0.00040	±	0.00012	0.0004	±	0.00014
bta-miR-16b	0.03261	±	0.00369	0.03291	±	0.00720	0.0364	±	0.01295
bta-miR-17-3p	0.00010	±	0.00003	0.00012	±	0.00004	0.0001	±	0.00002
bta-miR-188	0.00080	±	0.00011	0.00086	±	0.00006	0.0008	±	0.00009
bta-miR-17-5p	0.00021	±	0.00007	0.00027	±	0.00012	0.0002	±	0.00005
bta-miR-18a	0.00008	±	0.00004	0.00008	±	0.00005	0.0001	±	0.00003
bta-miR-181a	0.00231	±	0.00166	0.00197	±	0.00120	0.0024	±	0.00179
bta-miR-18b	0.00003	±	0.00002	0.00002	±	0.00001	0.0000	±	0.00001
bta-miR-181b	0.00679	±	0.00350	0.00609	±	0.00269	0.0072	±	0.00425
bta-miR-181c	0.00079	±	0.00065	0.00075	±	0.00047	0.0008	±	0.00063
bta-miR-190b	0.00002	±	0.00002	0.00002	±	0.00000	0.0000	±	0.00001
bta-miR-181d	0.01260	±	0.00555	0.01212	±	0.00515	0.0135	±	0.00852
bta-miR-191	0.00295	±	0.00031	0.00362	±	0.00053	0.0033	±	0.00012
bta-miR-182	0.00012	±	0.00010	0.00013	±	0.00011	0.0001	±	0.00013
bta-miR-192	0.00025	±	0.00004	0.00029	±	0.00006	0.0003	±	0.00005
bta-miR-193a	0.00001	±	0.00001	0.00001	±	0.00001	0.0000	±	0.00001
bta-miR-184	0.00001	±	0.00001	0.00002	±	0.00001	0.0000	±	0.00001
bta-miR-185	0.00112	±	0.00033	0.00087	±	0.00019	0.0010	±	0.00038
bta-miR-193a-5p	0.00626	±	0.00153	0.00609	±	0.00120	0.0067	±	0.00212
bta-miR-186	0.00341	±	0.00175	0.00254	±	0.00130	0.0032	±	0.00237
bta-miR-193b	0.00061	±	0.00017	0.00052	±	0.00008	0.0006	±	0.00021

bta-miR-187	0.00218	±	0.00020	0.00181	±	0.00074	0.0023	±	0.00041
bta-miR-195	0.01692	±	0.00032	0.01990	±	0.00344	0.0199	±	0.00411
bta-miR-200c	0.00005	±	0.00002	0.00006	±	0.00002	0.0001	±	0.00001
bta-miR-196a	0.00026	±	0.00014	0.00036	±	0.00024	0.0003	±	0.00018
bta-miR-202	0.00249	±	0.00170	0.00251	±	0.00175	0.0021	±	0.00123
bta-miR-196b	0.00018	±	0.00013	0.00021	±	0.00016	0.0002	±	0.00012
bta-miR-204	0.00288	±	0.00146	0.00314	±	0.00100	0.0035	±	0.00115
bta-miR-197	0.00436	±	0.00036	0.00464	±	0.00108	0.0049	±	0.00031
bta-miR-205	0.00023	±	0.00005	0.00028	±	0.00004	0.0003	±	0.00008
bta-miR-199a-3p	0.00696	±	0.00375	0.00602	±	0.00266	0.0085	±	0.00448
bta-miR-206	0.00026	±	0.00021	0.00037	±	0.00014	0.0004	±	0.00020
bta-miR-199a-5p	0.00347	±	0.00118	0.00354	±	0.00108	0.0046	±	0.00170
bta-miR-208a	0.00002	±	0.00001	0.00002	±	0.00001	0.0000	±	0.00001
bta-miR-199b	0.00170	±	0.00077	0.00164	±	0.00058	0.0022	±	0.00088
bta-miR-199c	0.01218	±	0.00590	0.01066	±	0.00512	0.0152	±	0.00849
bta-miR-20a	0.00262	±	0.00145	0.00223	±	0.00102	0.0027	±	0.00156
bta-miR-19a	0.00022	±	0.00005	0.00023	±	0.00005	0.0002	±	0.00002
bta-miR-20b	0.00132	±	0.00062	0.00129	±	0.00055	0.0014	±	0.00061
bta-miR-19b	0.00019	±	0.00004	0.00020	±	0.00001	0.0002	±	0.00002
bta-miR-21-5p	0.00152	±	0.00095	0.00163	±	0.00098	0.0022	±	0.00145
bta-miR-200b	0.00023	±	0.00015	0.00027	±	0.00019	0.0003	±	0.00015
bta-miR-210	0.01313	±	0.00288	0.01102	±	0.00069	0.0124	±	0.00301
bta-miR-211	0.00355	±	0.00066	0.00336	±	0.00095	0.0031	±	0.00040
bta-miR-22-5p	0.00239	±	0.00081	0.00214	±	0.00085	0.0023	±	0.00087
bta-miR-212	0.00006	±	0.00002	0.00011	±	0.00002	0.0001	±	0.00002
bta-miR-221	0.00538	±	0.00096	0.00523	±	0.00035	0.0057	±	0.00076
bta-miR-214	0.00809	±	0.00137	0.00896	±	0.00091	0.0099	±	0.00211
bta-miR-222	0.00627	±	0.00222	0.00501	±	0.00087	0.0056	±	0.00105
bta-miR-215	0.00010	±	0.00007	0.00011	±	0.00004	0.0001	±	0.00006
bta-miR-223	0.00029	±	0.00010	0.00025	±	0.00007	0.0003	±	0.00008
bta-miR-216a	0.00017	±	0.00015	0.00027	±	0.00017	0.0002	±	0.00013
bta-miR-224	0.00041	±	0.00036	0.00029	±	0.00022	0.0004	±	0.00032
bta-miR-216b	0.00003	±	0.00001	0.00005	±	0.00003	0.0001	±	0.00005
bta-miR-23a	0.15258	±	0.03517	0.15822	±	0.02589	0.1759	±	0.03599
bta-miR-217	0.00018	±	0.00004	0.00016	±	0.00002	0.0002	±	0.00004
bta-miR-23b-3p	0.03010	±	0.00646	0.02908	±	0.00297	0.0311	±	0.00463
bta-miR-218	0.00031	±	0.00018	0.00027	±	0.00015	0.0004	±	0.00020
bta-miR-23b-5p	0.00006	±	0.00001	0.00011	±	0.00003	0.0001	±	0.00006
bta-miR-219	0.00114	±	0.00006	0.00123	±	0.00020	0.0013	±	0.00019
bta-miR-24	0.00005	±	0.00001	0.00006	±	0.00001	0.0001	±	0.00002
bta-miR-219-3p	0.00042	±	0.00022	0.00063	±	0.00037	0.0006	±	0.00029
bta-miR-24-3p	0.00857	±	0.00035	0.00902	±	0.00031	0.0102	±	0.00154
bta-miR-219-5p	0.00000	±	0.00000	0.00000	±	0.00000	0.0000	±	0.00000
bta-miR-25	0.01881	±	0.00784	0.01611	±	0.00525	0.0190	±	0.01007

bta-miR-22-3p	8843.25356	±	3107.83857	9388.42010	±	2931.38930	10995.1325	±	1609.17431
bta-miR-26a	0.02967	±	0.00806	0.03802	±	0.00890	0.0383	±	0.00969
bta-miR-26b	0.00624	±	0.00231	0.00621	±	0.00067	0.0066	±	0.00279
bta-miR-29d-3p	0.00105	±	0.00034	0.00089	±	0.00027	0.0010	±	0.00041
bta-miR-29d-5p	0.00036	±	0.00017	0.00032	±	0.00012	0.0003	±	0.00009
bta-miR-27a-3p	0.00824	±	0.00130	0.00796	±	0.00075	0.0079	±	0.00180
bta-miR-29e	0.00006	±	0.00000	0.00006	±	0.00001	0.0001	±	0.00001
bta-miR-27a-5p	0.00113	±	0.00015	0.00143	±	0.00032	0.0012	±	0.00034
bta-miR-301a	0.00020	±	0.00007	0.00022	±	0.00007	0.0002	±	0.00010
bta-miR-27b	0.01543	±	0.00174	0.01752	±	0.00183	0.0171	±	0.00275
bta-miR-301b	0.00017	±	0.00007	0.00018	±	0.00008	0.0002	±	0.00009
bta-miR-28	0.00191	±	0.00060	0.00210	±	0.00056	0.0022	±	0.00089
bta-miR-302a	0.00004	±	0.00001	0.00005	±	0.00002	0.0001	±	0.00002
bta-miR-296-3p	0.00490	±	0.00038	0.00469	±	0.00010	0.0055	±	0.00081
bta-miR-296-5p	0.00078	±	0.00022	0.00084	±	0.00019	0.0009	±	0.00027
bta-miR-302c	0.00003	±	0.00002	0.00004	±	0.00002	0.0000	±	0.00002
bta-miR-299	0.00036	±	0.00018	0.00037	±	0.00019	0.0004	±	0.00014
bta-miR-29a	0.02427	±	0.00444	0.02214	±	0.00447	0.0238	±	0.00625
bta-miR-3064	0.00021	±	0.00017	0.00044	±	0.00018	0.0003	±	0.00025
bta-miR-29b	0.00047	±	0.00005	0.00059	±	0.00016	0.0005	±	0.00018
bta-miR-30a-5p	0.00460	±	0.00174	0.00356	±	0.00123	0.0040	±	0.00209
bta-miR-29c	0.02222	±	0.00411	0.01995	±	0.00389	0.0244	±	0.00648
bta-miR-30b-5p	0.00694	±	0.00238	0.00584	±	0.00147	0.0073	±	0.00345
bta-miR-328	0.00376	±	0.00043	0.00385	±	0.00064	0.0041	±	0.00049
bta-miR-30c	0.04702	±	0.02126	0.03363	±	0.01123	0.0458	±	0.03033
bta-miR-329a	0.00003	±	0.00002	0.00003	±	0.00001	0.0000	±	0.00004
bta-miR-30d	0.00448	±	0.00168	0.00365	±	0.00108	0.0040	±	0.00211
bta-miR-329b	0.00004	±	0.00002	0.00004	±	0.00002	0.0000	±	0.00003
bta-miR-30e-5p	0.00433	±	0.00164	0.00359	±	0.00125	0.0039	±	0.00219
bta-miR-330	0.00127	±	0.00036	0.00168	±	0.00044	0.0017	±	0.00055
bta-miR-30f	0.01987	±	0.00853	0.01471	±	0.00510	0.0202	±	0.01308
bta-miR-331-3p	0.00182	±	0.00015	0.00178	±	0.00042	0.0019	±	0.00043
bta-miR-31	0.00163	±	0.00050	0.00184	±	0.00075	0.0017	±	0.00052
bta-miR-335	0.00001	±	0.00000	0.00001	±	0.00001	0.0000	±	0.00001
bta-miR-320a	0.05739	±	0.01250	0.05333	±	0.00878	0.0591	±	0.01898
bta-miR-338	0.00027	±	0.00006	0.00026	±	0.00008	0.0003	±	0.00007
bta-miR-320b	0.00369	±	0.00161	0.00551	±	0.00159	0.0044	±	0.00212
bta-miR-339a	0.00353	±	0.00028	0.00378	±	0.00044	0.0037	±	0.00025
bta-miR-323	0.10359	±	0.02323	0.10148	±	0.02523	0.1181	±	0.03291
bta-miR-33a	0.00007	±	0.00002	0.00005	±	0.00002	0.0000	±	0.00001
bta-miR-326	0.00214	±	0.00031	0.00248	±	0.00063	0.0026	±	0.00045
bta-miR-33b	0.00002	±	0.00001	0.00003	±	0.00001	0.0000	±	0.00001
bta-miR-340	0.00002	±	0.00001	0.00003	±	0.00001	0.0000	±	0.00000
bta-miR-365-3p	0.00536	±	0.00093	0.00564	±	0.00079	0.0054	±	0.00075

bta-miR-342	0.00121	±	0.00073	0.00103	±	0.00035	0.0015	±	0.00065
bta-miR-365-5p	0.00152	±	0.00092	0.00251	±	0.00132	0.0018	±	0.00087
bta-miR-345-3p	0.00148	±	0.00029	0.00128	±	0.00009	0.0014	±	0.00030
bta-miR-369-3p	0.00013	±	0.00009	0.00009	±	0.00005	0.0001	±	0.00013
bta-miR-346	0.00773	±	0.00197	0.00903	±	0.00229	0.0096	±	0.00297
bta-miR-369-5p	0.00009	±	0.00004	0.00009	±	0.00005	0.0001	±	0.00008
bta-miR-34a	0.00701	±	0.00140	0.00670	±	0.00094	0.0067	±	0.00179
bta-miR-370	0.00077	±	0.00030	0.00095	±	0.00039	0.0009	±	0.00031
bta-miR-34b	0.00001	±	0.00000	0.00001	±	0.00001	0.0000	±	0.00001
bta-miR-34c	0.00002	±	0.00001	0.00002	±	0.00000	0.0000	±	0.00001
bta-miR-374a	0.00119	±	0.00002	0.00117	±	0.00047	0.0016	±	0.00088
bta-miR-361	0.00131	±	0.00041	0.00087	±	0.00036	0.0011	±	0.00067
bta-miR-374b	0.00855	±	0.00386	0.00879	±	0.00354	0.0095	±	0.00524
bta-miR-375	0.00044	±	0.00008	0.00038	±	0.00009	0.0004	±	0.00009
bta-miR-362-5p	0.00027	±	0.00010	0.00029	±	0.00009	0.0003	±	0.00009
bta-miR-363	0.00008	±	0.00005	0.00010	±	0.00003	0.0001	±	0.00001
bta-miR-376b	0.00001	±	0.00000	0.00002	±	0.00000	0.0000	±	0.00001
bta-miR-376c	0.00007	±	0.00005	0.00009	±	0.00005	0.0001	±	0.00003
bta-miR-382	0.00207	±	0.00139	0.00164	±	0.00070	0.0022	±	0.00162
bta-miR-376d	0.00009	±	0.00005	0.00009	±	0.00005	0.0001	±	0.00008
bta-miR-383	0.00023	±	0.00006	0.00040	±	0.00013	0.0004	±	0.00018
bta-miR-376e	0.00004	±	0.00002	0.00006	±	0.00004	0.0001	±	0.00003
bta-miR-409a	0.00021	±	0.00011	0.00020	±	0.00008	0.0002	±	0.00016
bta-miR-409b	0.00002	±	0.00000	0.00002	±	0.00000	0.0000	±	0.00000
bta-miR-410	0.00021	±	0.00005	0.00024	±	0.00005	0.0003	±	0.00008
bta-miR-378b	0.00163	±	0.00050	0.00165	±	0.00051	0.0018	±	0.00104
bta-miR-411a	0.00131	±	0.00064	0.00148	±	0.00065	0.0014	±	0.00068
bta-miR-411b	0.00008	±	0.00002	0.00007	±	0.00002	0.0001	±	0.00001
bta-miR-378d	0.00043	±	0.00011	0.00050	±	0.00018	0.0005	±	0.00010
bta-miR-379	0.00141	±	0.00076	0.00147	±	0.00072	0.0014	±	0.00061
bta-miR-411c-5p	0.00041	±	0.00026	0.00033	±	0.00022	0.0004	±	0.00038
bta-miR-380-5p	0.00014	±	0.00003	0.00010	±	0.00002	0.0002	±	0.00004
bta-miR-421	0.00178	±	0.00062	0.00173	±	0.00048	0.0019	±	0.00091
bta-miR-381	0.00025	±	0.00005	0.00023	±	0.00005	0.0003	±	0.00007
bta-miR-423-5p	0.01105	±	0.00231	0.00923	±	0.00157	0.0106	±	0.00275
bta-miR-424-3p	0.00247	±	0.00050	0.00284	±	0.00053	0.0026	±	0.00054
bta-miR-449d	0.00310	±	0.00132	0.00398	±	0.00171	0.0036	±	0.00131
bta-miR-424-5p	0.04298	±	0.00822	0.04753	±	0.01357	0.0441	±	0.01577
bta-miR-450a	0.00049	±	0.00009	0.00048	±	0.00011	0.0005	±	0.00011
bta-miR-425-3p	0.13713	±	0.00564	0.11301	±	0.02165	0.1458	±	0.03259
bta-miR-450b	0.00014	±	0.00003	0.00014	±	0.00003	0.0002	±	0.00004
bta-miR-425-5p	0.00393	±	0.00187	0.00346	±	0.00150	0.0045	±	0.00317
bta-miR-451	0.01771	±	0.01314	0.02337	±	0.02309	0.0241	±	0.02053
bta-miR-429	0.00003	±	0.00001	0.00005	±	0.00002	0.0000	±	0.00000

bta-miR-452	0.00017 ± 0.00008	0.00024 ± 0.00010	0.0002 ± 0.00008
bta-miR-431	0.00085 ± 0.00043	0.00140 ± 0.00069	0.0013 ± 0.00078
bta-miR-4523	0.00036 ± 0.00016	0.00047 ± 0.00018	0.0004 ± 0.00019
bta-miR-432	0.00098 ± 0.00047	0.00093 ± 0.00030	0.0010 ± 0.00046
bta-miR-454	0.00053 ± 0.00046	0.00051 ± 0.00044	0.0005 ± 0.00041
bta-miR-448	0.00001 ± 0.00000	0.00001 ± 0.00001	0.0000 ± 0.00001
bta-miR-455-3p	0.00032 ± 0.00003	0.00036 ± 0.00008	0.0003 ± 0.00011
bta-miR-449a	0.00023 ± 0.00015	0.00039 ± 0.00014	0.0003 ± 0.00020
bta-miR-449b	0.00011 ± 0.00006	0.00013 ± 0.00004	0.0002 ± 0.00011
bta-miR-483	0.00122 ± 0.00017	0.00107 ± 0.00014	0.0015 ± 0.00038
bta-miR-484	0.00169 ± 0.00048	0.00180 ± 0.00026	0.0021 ± 0.00094
bta-miR-496	0.00006 ± 0.00002	0.00008 ± 0.00001	0.0001 ± 0.00001
bta-miR-497	0.00160 ± 0.00020	0.00166 ± 0.00033	0.0016 ± 0.00032
bta-miR-499	0.00001 ± 0.00000	0.00002 ± 0.00001	0.0000 ± 0.00001
bta-miR-500	0.00085 ± 0.00029	0.00083 ± 0.00022	0.0010 ± 0.00042
bta-miR-502a	0.00013 ± 0.00003	0.00018 ± 0.00006	0.0002 ± 0.00005
bta-miR-488	0.00003 ± 0.00000	0.00005 ± 0.00002	0.0000 ± 0.00003
bta-miR-489	0.00012 ± 0.00005	0.00016 ± 0.00007	0.0001 ± 0.00004
bta-miR-503-3p	0.00796 ± 0.00093	0.00786 ± 0.00040	0.0078 ± 0.00094
bta-miR-490	0.00049 ± 0.00029	0.00067 ± 0.00035	0.0007 ± 0.00031
bta-miR-503-5p	0.00335 ± 0.00125	0.00358 ± 0.00133	0.0029 ± 0.00116
bta-miR-491	0.00102 ± 0.00011	0.00094 ± 0.00017	0.0010 ± 0.00050
bta-miR-504	0.00019 ± 0.00008	0.00027 ± 0.00009	0.0003 ± 0.00015
bta-miR-493	0.00532 ± 0.00332	0.00718 ± 0.00248	0.0064 ± 0.00283
bta-miR-494	10.90066 ± 5.79087	13.90975 ± 4.14385	12.5196 ± 7.13801
bta-miR-495	0.00055 ± 0.00043	0.00033 ± 0.00022	0.0005 ± 0.00044
bta-miR-541	0.00091 ± 0.00024	0.00107 ± 0.00035	0.0010 ± 0.00021
bta-miR-582	0.00001 ± 0.00001	0.00001 ± 0.00000	0.0000 ± 0.00000
bta-miR-542-5p	0.00031 ± 0.00008	0.00029 ± 0.00006	0.0003 ± 0.00011
bta-miR-584	0.00140 ± 0.00065	0.00182 ± 0.00073	0.0017 ± 0.00078
bta-miR-543	0.00051 ± 0.00036	0.00042 ± 0.00016	0.0006 ± 0.00041
bta-miR-544b	0.00006 ± 0.00004	0.00009 ± 0.00006	0.0001 ± 0.00005
bta-miR-545-3p	0.00001 ± 0.00000	0.00001 ± 0.00001	0.0000 ± 0.00001
bta-miR-628	0.00031 ± 0.00005	0.00026 ± 0.00002	0.0003 ± 0.00006
bta-miR-631	0.05128 ± 0.01115	0.04933 ± 0.00714	0.0587 ± 0.01331
bta-miR-551a	0.00001 ± 0.00000	0.00002 ± 0.00001	0.0000 ± 0.00001
bta-miR-652	0.01236 ± 0.00387	0.01133 ± 0.00370	0.0114 ± 0.00512
bta-miR-551b	0.00001 ± 0.00000	0.00001 ± 0.00001	0.0000 ± 0.00001
bta-miR-562	0.00001 ± 0.00000	0.00003 ± 0.00001	0.0000 ± 0.00001
bta-miR-654	0.00038 ± 0.00014	0.00050 ± 0.00025	0.0005 ± 0.00027
bta-miR-568	0.00000 ± 0.00000	0.00000 ± 0.00000	0.0000 ± 0.00000
bta-miR-655	0.00017 ± 0.00014	0.00011 ± 0.00007	0.0002 ± 0.00019
bta-miR-574	0.15672 ± 0.10137	0.23084 ± 0.06937	0.2073 ± 0.12612
bta-miR-656	0.00002 ± 0.00001	0.00002 ± 0.00001	0.0000 ± 0.00001

bta-miR-758	0.00003	±	0.00002	0.00003	±	0.00002	0.0000	±	0.00002
bta-miR-660	0.00291	±	0.00121	0.00283	±	0.00104	0.0030	±	0.00164
bta-miR-664a	0.00680	±	0.00289	0.00984	±	0.00348	0.0088	±	0.00392
bta-miR-760-3p	0.00232	±	0.00053	0.00280	±	0.00035	0.0028	±	0.00004
bta-miR-664b	0.04109	±	0.00724	0.04027	±	0.00754	0.0421	±	0.00613
bta-miR-760-5p	0.03754	±	0.00589	0.04242	±	0.00324	0.0435	±	0.00883
bta-miR-665	0.09086	±	0.01523	0.06413	±	0.01681	0.0791	±	0.03294
bta-miR-763	0.00073	±	0.00036	0.00104	±	0.00042	0.0009	±	0.00050
bta-miR-670	0.00002	±	0.00000	0.00004	±	0.00003	0.0000	±	0.00001
bta-miR-764	0.00012	±	0.00005	0.00018	±	0.00010	0.0001	±	0.00008
bta-miR-671	0.00046	±	0.00010	0.00042	±	0.00010	0.0005	±	0.00010
bta-miR-767	0.00010	±	0.00004	0.00012	±	0.00003	0.0001	±	0.00003
bta-miR-677	0.02185	±	0.00304	0.02455	±	0.00169	0.0238	±	0.00086
bta-miR-7	0.00020	±	0.00010	0.00017	±	0.00007	0.0002	±	0.00012
bta-miR-708	0.00319	±	0.00178	0.00293	±	0.00151	0.0035	±	0.00266
bta-miR-874	0.00756	±	0.00028	0.00794	±	0.00085	0.0081	±	0.00041
bta-miR-744	0.00095	±	0.00013	0.00109	±	0.00003	0.0011	±	0.00021
bta-miR-98	0.00184	±	0.00091	0.00184	±	0.00065	0.0019	±	0.00092
bta-miR-99a-3p	0.00040	±	0.00016	0.00028	±	0.00006	0.0004	±	0.00022
bta-miR-885	0.00055	±	0.00013	0.00058	±	0.00016	0.0007	±	0.00019
bta-miR-99a-5p	0.00936	±	0.00276	0.01069	±	0.00233	0.0116	±	0.00204
bta-miR-9-5p	0.00004	±	0.00002	0.00004	±	0.00002	0.0000	±	0.00001
bta-miR-92a	0.04850	±	0.01048	0.04285	±	0.00537	0.0463	±	0.01059
bta-miR-92b	0.03067	±	0.00438	0.02905	±	0.00608	0.0318	±	0.00687
bta-miR-1193	0.00003	±	0.00001	0.00003	±	0.00001	0.0000	±	0.00001
bta-miR-93	0.00453	±	0.00066	0.00525	±	0.00081	0.0056	±	0.00019
bta-miR-935	0.00108	±	0.00011	0.00117	±	0.00014	0.0012	±	0.00006
bta-miR-940	0.08070	±	0.01657	0.07038	±	0.00181	0.0782	±	0.01569
bta-miR-1224	1.13902	±	0.63683	1.61560	±	0.48777	1.4203	±	0.68703
bta-miR-1225-3p	0.39884	±	0.03123	0.38442	±	0.01286	0.3997	±	0.03484
bta-miR-1246	18.35792	±	0.87176	17.74285	±	1.53890	18.5146	±	1.49217
bta-miR-1296	0.00400	±	0.00114	0.00470	±	0.00153	0.0047	±	0.00141
bta-miR-1247-5p	0.09880	±	0.01694	0.08609	±	0.01140	0.0931	±	0.02195
bta-miR-1298	0.00001	±	0.00001	0.00002	±	0.00001	0.0000	±	0.00001
bta-miR-1248	0.05149	±	0.02195	0.07584	±	0.02932	0.0670	±	0.04149
bta-miR-1301	0.00006	±	0.00002	0.00010	±	0.00003	0.0001	±	0.00007
bta-miR-1249	0.00050	±	0.00005	0.00051	±	0.00004	0.0005	±	0.00012
bta-miR-1306	0.00169	±	0.00019	0.00182	±	0.00033	0.0018	±	0.00023
bta-miR-1260b	0.30763	±	0.16576	0.29998	±	0.16128	0.2936	±	0.10148
bta-miR-1307	0.38756	±	0.05928	0.38770	±	0.05004	0.3857	±	0.00930
bta-miR-1271	0.00029	±	0.00006	0.00032	±	0.00010	0.0003	±	0.00008
bta-miR-1343-3p	0.03149	±	0.00383	0.02555	±	0.00181	0.0301	±	0.00588
bta-miR-1343-5p	0.77561	±	0.18359	0.59447	±	0.07976	0.7146	±	0.21692
bta-miR-1281	0.00932	±	0.00093	0.00866	±	0.00119	0.0113	±	0.00120

bta-miR-1284	0.00010	±	0.00006	0.00017	±	0.00010	0.0002	±	0.00008
bta-miR-1287	0.00005	±	0.00004	0.00007	±	0.00005	0.0001	±	0.00006
bta-miR-1291	0.01412	±	0.00407	0.01625	±	0.00815	0.0196	±	0.00958

Supplementary Table S25. MicroRNAs expressed in LE treated with sEVs secreted by end, end *in vivo*, and end *in vitro* 24h.

MicroRNAs	LE			LE			LE		
	End sEVs 24h			End <i>in vivo</i> sEVs 24h			End <i>in vitro</i> sEVs 24h		
	Mean	Standard deviation		Mean	Standard deviation		Mean	Standard deviation	
bta-let-7a-3p	0.00059	± 0.00025		0.00087	± 0.00051		0.00054	± 0.00036	
bta-miR-103	0.00996	± 0.00173		0.00968	± 0.00153		0.01041	± 0.00397	
bta-let-7a-5p	0.35013	± 0.03597		0.29340	± 0.08723		0.35173	± 0.07746	
bta-miR-105a	0.00016	± 0.00007		0.00009	± 0.00009		0.00010	± 0.00004	
bta-let-7b	0.41989	± 0.03725		0.44537	± 0.07596		0.45387	± 0.09331	
bta-miR-105b	0.00045	± 0.00028		0.00075	± 0.00058		0.00079	± 0.00092	
bta-let-7c	0.50213	± 0.04102		0.46728	± 0.10244		0.46578	± 0.07886	
bta-miR-106a	0.00198	± 0.00091		0.00212	± 0.00089		0.00191	± 0.00142	
bta-let-7d	0.15116	± 0.01265		0.10949	± 0.02060		0.13910	± 0.02104	
bta-let-7e	0.48715	± 0.05722		0.37712	± 0.06148		0.41965	± 0.08455	
bta-miR-107	0.00079	± 0.00011		0.00061	± 0.00017		0.00079	± 0.00030	
bta-let-7f	0.05918	± 0.01498		0.04553	± 0.01023		0.05707	± 0.02953	
bta-miR-10a	0.00385	± 0.00069		0.00398	± 0.00141		0.00232	± 0.00077	
bta-let-7g	0.01529	± 0.00309		0.01306	± 0.00125		0.01328	± 0.00488	
bta-miR-10b	0.00341	± 0.00059		0.00332	± 0.00067		0.00237	± 0.00104	
bta-let-7i	0.01794	± 0.00796		0.01699	± 0.00884		0.01579	± 0.00971	
bta-miR-124a	0.00034	± 0.00013		0.00025	± 0.00017		0.00028	± 0.00013	
bta-miR-100	0.00548	± 0.00032		0.00447	± 0.00137		0.00364	± 0.00080	
bta-miR-124b	0.00036	± 0.00013		0.00024	± 0.00020		0.00030	± 0.00012	
bta-miR-101	0.00011	± 0.00002		0.00013	± 0.00001		0.00009	± 0.00002	
bta-miR-125a	0.01689	± 0.00413		0.01336	± 0.00541		0.01624	± 0.00476	
bta-miR-125b	0.05236	± 0.01111		0.04294	± 0.00840		0.04461	± 0.01370	
bta-miR-133b	0.00004	± 0.00001		0.00002	± 0.00001		0.00002	± 0.00001	
bta-miR-126-3p	0.05185	± 0.00355		0.04519	± 0.00448		0.04037	± 0.00602	
bta-miR-133c	0.00003	± 0.00001		0.00001	± 0.00001		0.00002	± 0.00000	
bta-miR-126-5p	0.16808	± 0.04012		0.19954	± 0.08073		0.11084	± 0.07583	
bta-miR-134	0.00026	± 0.00009		0.00018	± 0.00011		0.00021	± 0.00009	
bta-miR-127	0.00590	± 0.00235		0.00325	± 0.00165		0.00482	± 0.00214	
bta-miR-128	0.00203	± 0.00014		0.00227	± 0.00065		0.00182	± 0.00045	
bta-miR-129	0.00101	± 0.00032		0.00078	± 0.00039		0.00088	± 0.00024	
bta-miR-136	0.00006	± 0.00003		0.00008	± 0.00006		0.00005	± 0.00002	
bta-miR-129-5p	0.00109	± 0.00038		0.00073	± 0.00039		0.00086	± 0.00030	
bta-miR-130a	0.00091	± 0.00051		0.00114	± 0.00057		0.00084	± 0.00065	
bta-miR-139	0.00780	± 0.00247		0.00936	± 0.00319		0.00791	± 0.00246	
bta-miR-130b	0.02098	± 0.00509		0.01686	± 0.00723		0.01946	± 0.00762	
bta-miR-140	0.00201	± 0.00048		0.00263	± 0.00090		0.00210	± 0.00073	
bta-miR-132	0.00483	± 0.00143		0.00620	± 0.00222		0.00538	± 0.00347	
bta-miR-141	0.00015	± 0.00006		0.00007	± 0.00007		0.00018	± 0.00008	
bta-miR-133a	0.00071	± 0.00031		0.00050	± 0.00040		0.00045	± 0.00020	

bta-miR-142-5p	0.00004	±	0.00000	0.00002	±	0.00001	0.00003	±	0.00002
bta-miR-151-3p	0.00344	±	0.00128	0.00378	±	0.00174	0.00268	±	0.00178
bta-miR-143	0.00127	±	0.00031	0.00156	±	0.00070	0.00144	±	0.00046
bta-miR-151-5p	0.02278	±	0.00286	0.02397	±	0.00423	0.02284	±	0.00720
bta-miR-152	0.00084	±	0.00020	0.00093	±	0.00023	0.00076	±	0.00032
bta-miR-146a	0.00005	±	0.00000	0.00006	±	0.00003	0.00004	±	0.00001
bta-miR-154a	0.00003	±	0.00001	0.00002	±	0.00001	0.00004	±	0.00002
bta-miR-154b	0.00074	±	0.00045	0.00089	±	0.00057	0.00094	±	0.00067
bta-miR-147	0.00010	±	0.00002	0.00007	±	0.00002	0.00008	±	0.00003
bta-miR-154c	0.00024	±	0.00021	0.00026	±	0.00019	0.00021	±	0.00021
bta-miR-148a	0.00488	±	0.00030	0.00615	±	0.00129	0.00458	±	0.00220
bta-miR-155	0.00265	±	0.00158	0.00358	±	0.00145	0.00366	±	0.00183
bta-miR-148b	0.00385	±	0.00063	0.00402	±	0.00082	0.00299	±	0.00104
bta-miR-15a	0.00373	±	0.00054	0.00337	±	0.00032	0.00286	±	0.00091
bta-miR-149-3p	0.42739	±	0.09643	0.47161	±	0.19512	0.40768	±	0.05244
bta-miR-15b	0.01213	±	0.00189	0.01001	±	0.00189	0.01238	±	0.00238
bta-miR-149-5p	0.00106	±	0.00011	0.00092	±	0.00022	0.00072	±	0.00020
bta-miR-16a	0.01523	±	0.00338	0.01670	±	0.00532	0.01247	±	0.00487
bta-miR-150	0.00045	±	0.00007	0.00040	±	0.00003	0.00038	±	0.00009
bta-miR-16b	0.02611	±	0.00438	0.02618	±	0.00780	0.02144	±	0.00716
bta-miR-17-3p	0.00014	±	0.00003	0.00007	±	0.00002	0.00011	±	0.00002
bta-miR-17-5p	0.00019	±	0.00004	0.00017	±	0.00003	0.00017	±	0.00005
bta-miR-18a	0.00008	±	0.00004	0.00008	±	0.00003	0.00006	±	0.00002
bta-miR-181a	0.00167	±	0.00122	0.00167	±	0.00107	0.00159	±	0.00136
bta-miR-18b	0.00002	±	0.00001	0.00003	±	0.00002	0.00003	±	0.00002
bta-miR-181b	0.00583	±	0.00375	0.00693	±	0.00380	0.00662	±	0.00451
bta-miR-181c	0.00066	±	0.00049	0.00065	±	0.00044	0.00055	±	0.00043
bta-miR-190b	0.00003	±	0.00002	0.00002	±	0.00001	0.00003	±	0.00000
bta-miR-181d	0.01207	±	0.00731	0.01265	±	0.00583	0.01348	±	0.00831
bta-miR-191	0.00314	±	0.00051	0.00272	±	0.00091	0.00267	±	0.00037
bta-miR-182	0.00010	±	0.00007	0.00012	±	0.00008	0.00012	±	0.00011
bta-miR-192	0.00028	±	0.00008	0.00021	±	0.00009	0.00024	±	0.00002
bta-miR-183	0.00025	±	0.00013	0.00023	±	0.00018	0.00027	±	0.00028
bta-miR-193a	0.00002	±	0.00001	0.00001	±	0.00001	0.00001	±	0.00001
bta-miR-184	0.00003	±	0.00002	0.00003	±	0.00002	0.00004	±	0.00003
bta-miR-185	0.00094	±	0.00027	0.00128	±	0.00051	0.00103	±	0.00056
bta-miR-193a-5p	0.00590	±	0.00112	0.00663	±	0.00162	0.00655	±	0.00231
bta-miR-186	0.00281	±	0.00186	0.00445	±	0.00280	0.00336	±	0.00294
bta-miR-187	0.00260	±	0.00020	0.00203	±	0.00069	0.00248	±	0.00073
bta-miR-194	0.00018	±	0.00002	0.00015	±	0.00002	0.00018	±	0.00005
bta-miR-195	0.01543	±	0.00277	0.01497	±	0.00123	0.01250	±	0.00377
bta-miR-196a	0.00050	±	0.00001	0.00039	±	0.00025	0.00037	±	0.00021
bta-miR-202	0.00251	±	0.00078	0.00195	±	0.00123	0.00112	±	0.00066
bta-miR-196b	0.00032	±	0.00006	0.00025	±	0.00015	0.00022	±	0.00012

bta-miR-204	0.00292	±	0.00061	0.00264	±	0.00099	0.00218	±	0.00038
bta-miR-197	0.00530	±	0.00048	0.00423	±	0.00074	0.00443	±	0.00088
bta-miR-205	0.00029	±	0.00003	0.00024	±	0.00006	0.00027	±	0.00006
bta-miR-199a-3p	0.00477	±	0.00172	0.00653	±	0.00436	0.00438	±	0.00255
bta-miR-206	0.00037	±	0.00018	0.00026	±	0.00021	0.00032	±	0.00014
bta-miR-199a-5p	0.00352	±	0.00091	0.00303	±	0.00097	0.00290	±	0.00120
bta-miR-208a	0.00002	±	0.00001	0.00003	±	0.00001	0.00003	±	0.00000
bta-miR-199b	0.00173	±	0.00046	0.00159	±	0.00068	0.00159	±	0.00059
bta-miR-199c	0.00876	±	0.00354	0.01255	±	0.00859	0.00846	±	0.00592
bta-miR-20a	0.00206	±	0.00092	0.00234	±	0.00120	0.00197	±	0.00125
bta-miR-19a	0.00020	±	0.00005	0.00014	±	0.00002	0.00013	±	0.00000
bta-miR-20b	0.00117	±	0.00030	0.00116	±	0.00047	0.00101	±	0.00060
bta-miR-19b	0.00021	±	0.00004	0.00021	±	0.00003	0.00016	±	0.00003
bta-miR-21-3p	0.00032	±	0.00008	0.00023	±	0.00011	0.00030	±	0.00012
bta-miR-21-5p	0.00110	±	0.00072	0.00125	±	0.00108	0.00087	±	0.00062
bta-miR-200b	0.00027	±	0.00017	0.00022	±	0.00012	0.00023	±	0.00019
bta-miR-210	0.01231	±	0.00266	0.01199	±	0.00232	0.01155	±	0.00288
bta-miR-211	0.00417	±	0.00178	0.00352	±	0.00193	0.00379	±	0.00183
bta-miR-22-5p	0.00162	±	0.00042	0.00214	±	0.00098	0.00144	±	0.00071
bta-miR-212	0.00009	±	0.00005	0.00006	±	0.00004	0.00007	±	0.00002
bta-miR-221	0.00506	±	0.00007	0.00446	±	0.00089	0.00407	±	0.00157
bta-miR-214	0.00931	±	0.00097	0.00870	±	0.00157	0.00858	±	0.00052
bta-miR-222	0.00502	±	0.00045	0.00537	±	0.00052	0.00515	±	0.00159
bta-miR-215	0.00009	±	0.00005	0.00011	±	0.00006	0.00010	±	0.00010
bta-miR-223	0.00030	±	0.00006	0.00023	±	0.00006	0.00019	±	0.00004
bta-miR-216a	0.00024	±	0.00015	0.00015	±	0.00018	0.00022	±	0.00017
bta-miR-224	0.00028	±	0.00020	0.00038	±	0.00031	0.00026	±	0.00019
bta-miR-23a	0.14544	±	0.01377	0.14714	±	0.02929	0.13142	±	0.04355
bta-miR-217	0.00020	±	0.00003	0.00017	±	0.00005	0.00026	±	0.00004
bta-miR-23b-3p	0.02926	±	0.00425	0.02267	±	0.00466	0.02488	±	0.00463
bta-miR-218	0.00024	±	0.00011	0.00025	±	0.00014	0.00019	±	0.00016
bta-miR-219	0.00120	±	0.00014	0.00113	±	0.00011	0.00121	±	0.00002
bta-miR-24	0.00005	±	0.00001	0.00005	±	0.00002	0.00003	±	0.00001
bta-miR-24-3p	0.00870	±	0.00050	0.00897	±	0.00117	0.00676	±	0.00200
bta-miR-219-5p	0.00001	±	0.00000	0.00001	±	0.00001	0.00001	±	0.00001
bta-miR-25	0.01558	±	0.00590	0.01982	±	0.00904	0.01582	±	0.00954
bta-miR-22-3p	8495.66050	±	319.58930	9534.48154	±	3722.36377	13685.12568	±	612.10993
bta-miR-26a	0.02716	±	0.00356	0.03189	±	0.00864	0.02609	±	0.00756
bta-miR-26b	0.00483	±	0.00129	0.00483	±	0.00145	0.00387	±	0.00211
bta-miR-29d-3p	0.00079	±	0.00011	0.00075	±	0.00010	0.00083	±	0.00034
bta-miR-29d-5p	0.00024	±	0.00000	0.00029	±	0.00012	0.00032	±	0.00016
bta-miR-27a-3p	0.00777	±	0.00048	0.00807	±	0.00275	0.00683	±	0.00166
bta-miR-29e	0.00005	±	0.00001	0.00005	±	0.00002	0.00003	±	0.00001
bta-miR-27a-5p	0.00135	±	0.00024	0.00090	±	0.00036	0.00108	±	0.00010

bta-miR-301a	0.00024	±	0.00008	0.00023	±	0.00012	0.00031	±	0.00019
bta-miR-27b	0.01613	±	0.00187	0.01475	±	0.00255	0.01390	±	0.00328
bta-miR-301b	0.00020	±	0.00004	0.00021	±	0.00010	0.00027	±	0.00013
bta-miR-28	0.00171	±	0.00047	0.00169	±	0.00051	0.00177	±	0.00057
bta-miR-302a	0.00008	±	0.00005	0.00007	±	0.00002	0.00009	±	0.00006
bta-miR-296-3p	0.00512	±	0.00051	0.00537	±	0.00065	0.00523	±	0.00079
bta-miR-296-5p	0.00099	±	0.00031	0.00069	±	0.00034	0.00074	±	0.00022
bta-miR-302c	0.00005	±	0.00002	0.00003	±	0.00003	0.00006	±	0.00003
bta-miR-299	0.00030	±	0.00013	0.00029	±	0.00014	0.00031	±	0.00015
bta-miR-302d	0.00003	±	0.00002	0.00002	±	0.00002	0.00002	±	0.00002
bta-miR-29a	0.02136	±	0.00433	0.02076	±	0.00409	0.02162	±	0.00798
bta-miR-3064	0.00039	±	0.00029	0.00027	±	0.00035	0.00032	±	0.00023
bta-miR-29b	0.00037	±	0.00006	0.00027	±	0.00005	0.00032	±	0.00013
bta-miR-30a-5p	0.00362	±	0.00141	0.00489	±	0.00217	0.00406	±	0.00260
bta-miR-29c	0.01944	±	0.00484	0.01960	±	0.00340	0.01900	±	0.00438
bta-miR-30b-3p	0.00028	±	0.00004	0.00023	±	0.00003	0.00033	±	0.00008
bta-miR-30b-5p	0.00618	±	0.00202	0.00837	±	0.00318	0.00622	±	0.00384
bta-miR-328	0.00386	±	0.00045	0.00358	±	0.00003	0.00340	±	0.00015
bta-miR-30c	0.03975	±	0.02364	0.05689	±	0.02819	0.04677	±	0.03331
bta-miR-329a	0.00003	±	0.00001	0.00003	±	0.00001	0.00003	±	0.00002
bta-miR-30d	0.00353	±	0.00129	0.00486	±	0.00214	0.00402	±	0.00260
bta-miR-329b	0.00005	±	0.00001	0.00004	±	0.00003	0.00005	±	0.00001
bta-miR-30e-5p	0.00349	±	0.00146	0.00462	±	0.00192	0.00401	±	0.00250
bta-miR-330	0.00161	±	0.00038	0.00117	±	0.00071	0.00135	±	0.00038
bta-miR-30f	0.01848	±	0.00907	0.02268	±	0.01047	0.01921	±	0.01330
bta-miR-331-3p	0.00188	±	0.00042	0.00157	±	0.00010	0.00194	±	0.00020
bta-miR-31	0.00131	±	0.00032	0.00142	±	0.00045	0.00153	±	0.00070
bta-miR-32	0.00000	±	0.00000	0.00001	±	0.00000	0.00000	±	0.00000
bta-miR-335	0.00001	±	0.00000	0.00001	±	0.00000	0.00000	±	0.00000
bta-miR-320a	0.05649	±	0.01673	0.07068	±	0.02211	0.06855	±	0.02110
bta-miR-338	0.00022	±	0.00001	0.00018	±	0.00009	0.00015	±	0.00006
bta-miR-320b	0.00515	±	0.00171	0.00369	±	0.00222	0.00567	±	0.00156
bta-miR-339a	0.00372	±	0.00017	0.00374	±	0.00063	0.00388	±	0.00070
bta-miR-323	0.10845	±	0.02985	0.13297	±	0.05397	0.19361	±	0.06799
bta-miR-33a	0.00004	±	0.00001	0.00005	±	0.00001	0.00003	±	0.00000
bta-miR-326	0.00266	±	0.00014	0.00206	±	0.00056	0.00231	±	0.00061
bta-miR-340	0.00002	±	0.00000	0.00002	±	0.00000	0.00001	±	0.00000
bta-miR-365-3p	0.00510	±	0.00013	0.00429	±	0.00067	0.00389	±	0.00130
bta-miR-342	0.00120	±	0.00063	0.00135	±	0.00052	0.00108	±	0.00058
bta-miR-365-5p	0.00231	±	0.00099	0.00142	±	0.00135	0.00188	±	0.00127
bta-miR-345-3p	0.00145	±	0.00017	0.00149	±	0.00024	0.00147	±	0.00033
bta-miR-367	0.00001	±	0.00000	0.00000	±	0.00000	0.00001	±	0.00000
bta-miR-369-3p	0.00008	±	0.00006	0.00011	±	0.00007	0.00008	±	0.00007
bta-miR-346	0.00984	±	0.00366	0.00583	±	0.00322	0.00775	±	0.00347

bta-miR-369-5p	0.00006 ± 0.00003	0.00007 ± 0.00004	0.00007 ± 0.00005
bta-miR-34a	0.00775 ± 0.00204	0.00668 ± 0.00276	0.00784 ± 0.00451
bta-miR-370	0.00103 ± 0.00045	0.00066 ± 0.00053	0.00087 ± 0.00042
bta-miR-34b	0.00003 ± 0.00001	0.00002 ± 0.00001	0.00003 ± 0.00002
bta-miR-371	0.00004 ± 0.00000	0.00004 ± 0.00001	0.00006 ± 0.00000
bta-miR-34c	0.00002 ± 0.00001	0.00002 ± 0.00000	0.00002 ± 0.00002
bta-miR-374a	0.00088 ± 0.00036	0.00086 ± 0.00049	0.00075 ± 0.00039
bta-miR-361	0.00085 ± 0.00034	0.00148 ± 0.00085	0.00079 ± 0.00052
bta-miR-374b	0.00704 ± 0.00232	0.00694 ± 0.00363	0.00456 ± 0.00266
bta-miR-375	0.00045 ± 0.00006	0.00039 ± 0.00008	0.00044 ± 0.00011
bta-miR-376a	0.00004 ± 0.00003	0.00004 ± 0.00003	0.00004 ± 0.00005
bta-miR-363	0.00008 ± 0.00000	0.00006 ± 0.00002	0.00008 ± 0.00002
bta-miR-376c	0.00008 ± 0.00005	0.00008 ± 0.00004	0.00007 ± 0.00006
bta-miR-382	0.00180 ± 0.00100	0.00210 ± 0.00133	0.00219 ± 0.00188
bta-miR-383	0.00038 ± 0.00014	0.00026 ± 0.00021	0.00030 ± 0.00016
bta-miR-376e	0.00004 ± 0.00003	0.00004 ± 0.00002	0.00004 ± 0.00003
bta-miR-409a	0.00015 ± 0.00009	0.00015 ± 0.00009	0.00013 ± 0.00008
bta-miR-409b	0.00002 ± 0.00001	0.00002 ± 0.00001	0.00002 ± 0.00001
bta-miR-378	0.00188 ± 0.00057	0.00194 ± 0.00050	0.00200 ± 0.00092
bta-miR-378b	0.00164 ± 0.00076	0.00170 ± 0.00048	0.00169 ± 0.00087
bta-miR-411a	0.00107 ± 0.00055	0.00118 ± 0.00077	0.00122 ± 0.00085
bta-miR-411b	0.00009 ± 0.00002	0.00008 ± 0.00003	0.00007 ± 0.00002
bta-miR-378d	0.00067 ± 0.00003	0.00053 ± 0.00017	0.00074 ± 0.00024
bta-miR-411c-3p	0.00006 ± 0.00001	0.00007 ± 0.00002	0.00007 ± 0.00004
bta-miR-379	0.00111 ± 0.00059	0.00094 ± 0.00050	0.00099 ± 0.00050
bta-miR-411c-5p	0.00029 ± 0.00028	0.00042 ± 0.00032	0.00036 ± 0.00037
bta-miR-412	0.00005 ± 0.00002	0.00003 ± 0.00002	0.00004 ± 0.00001
bta-miR-380-5p	0.00013 ± 0.00002	0.00011 ± 0.00004	0.00014 ± 0.00006
bta-miR-421	0.00176 ± 0.00088	0.00210 ± 0.00115	0.00192 ± 0.00109
bta-miR-381	0.00030 ± 0.00001	0.00028 ± 0.00007	0.00024 ± 0.00005
bta-miR-423-5p	0.00924 ± 0.00218	0.01144 ± 0.00294	0.00972 ± 0.00317
bta-miR-449c	0.00020 ± 0.00008	0.00011 ± 0.00006	0.00016 ± 0.00005
bta-miR-424-3p	0.00224 ± 0.00031	0.00260 ± 0.00056	0.00236 ± 0.00075
bta-miR-449d	0.00435 ± 0.00110	0.00265 ± 0.00182	0.00442 ± 0.00241
bta-miR-424-5p	0.03506 ± 0.00708	0.04293 ± 0.01543	0.03332 ± 0.01825
bta-miR-450a	0.00045 ± 0.00002	0.00039 ± 0.00009	0.00031 ± 0.00013
bta-miR-425-3p	0.13737 ± 0.02197	0.11287 ± 0.00969	0.12107 ± 0.01330
bta-miR-450b	0.00012 ± 0.00003	0.00013 ± 0.00004	0.00008 ± 0.00003
bta-miR-425-5p	0.00301 ± 0.00177	0.00377 ± 0.00196	0.00272 ± 0.00225
bta-miR-451	0.00840 ± 0.00776	0.01838 ± 0.01369	0.00752 ± 0.00725
bta-miR-429	0.00006 ± 0.00001	0.00006 ± 0.00003	0.00008 ± 0.00004
bta-miR-452	0.00027 ± 0.00011	0.00017 ± 0.00013	0.00016 ± 0.00007
bta-miR-431	0.00134 ± 0.00061	0.00079 ± 0.00070	0.00106 ± 0.00064
bta-miR-4523	0.00051 ± 0.00018	0.00033 ± 0.00024	0.00041 ± 0.00026

bta-miR-432	0.00102	±	0.00057	0.00110	±	0.00048	0.00118	±	0.00052
bta-miR-453	0.00031	±	0.00003	0.00028	±	0.00012	0.00029	±	0.00009
bta-miR-433	0.00152	±	0.00069	0.00142	±	0.00047	0.00144	±	0.00065
bta-miR-454	0.00047	±	0.00039	0.00048	±	0.00036	0.00049	±	0.00047
bta-miR-448	0.00001	±	0.00000	0.00001	±	0.00000	0.00001	±	0.00001
bta-miR-455-5p	0.00015	±	0.00004	0.00016	±	0.00005	0.00013	±	0.00007
bta-miR-449b	0.00021	±	0.00008	0.00010	±	0.00009	0.00018	±	0.00006
bta-miR-483	0.00134	±	0.00020	0.00138	±	0.00035	0.00118	±	0.00031
bta-miR-484	0.00172	±	0.00039	0.00193	±	0.00058	0.00177	±	0.00083
bta-miR-497	0.00143	±	0.00011	0.00132	±	0.00024	0.00124	±	0.00039
bta-miR-500	0.00074	±	0.00015	0.00078	±	0.00017	0.00073	±	0.00032
bta-miR-502a	0.00018	±	0.00005	0.00014	±	0.00006	0.00014	±	0.00004
bta-miR-488	0.00005	±	0.00002	0.00005	±	0.00003	0.00004	±	0.00002
bta-miR-489	0.00017	±	0.00007	0.00010	±	0.00003	0.00017	±	0.00006
bta-miR-503-3p	0.00735	±	0.00050	0.00831	±	0.00178	0.00764	±	0.00065
bta-miR-490	0.00073	±	0.00036	0.00048	±	0.00046	0.00056	±	0.00030
bta-miR-503-5p	0.00238	±	0.00080	0.00323	±	0.00147	0.00256	±	0.00121
bta-miR-491	0.00092	±	0.00002	0.00091	±	0.00020	0.00059	±	0.00023
bta-miR-504	0.00028	±	0.00011	0.00018	±	0.00015	0.00024	±	0.00014
bta-miR-493	0.00832	±	0.00227	0.00525	±	0.00444	0.00773	±	0.00221
bta-miR-505	0.00955	±	0.00107	0.00911	±	0.00235	0.01024	±	0.00289
bta-miR-494	12.81458	±	8.13532	7.60215	±	6.24410	10.30318	±	6.74203
bta-miR-495	0.00036	±	0.00033	0.00056	±	0.00038	0.00052	±	0.00057
bta-miR-541	0.00129	±	0.00001	0.00097	±	0.00028	0.00116	±	0.00011
bta-miR-542-5p	0.00023	±	0.00008	0.00034	±	0.00012	0.00025	±	0.00009
bta-miR-584	0.00197	±	0.00063	0.00132	±	0.00088	0.00168	±	0.00062
bta-miR-543	0.00035	±	0.00025	0.00056	±	0.00035	0.00041	±	0.00037
bta-miR-544a	0.00003	±	0.00002	0.00001	±	0.00001	0.00002	±	0.00001
bta-miR-628	0.00031	±	0.00003	0.00030	±	0.00002	0.00023	±	0.00013
bta-miR-545-5p	0.00003	±	0.00001	0.00003	±	0.00001	0.00003	±	0.00002
bta-miR-631	0.05194	±	0.00958	0.06939	±	0.02371	0.07860	±	0.02381
bta-miR-652	0.01091	±	0.00264	0.01280	±	0.00356	0.01186	±	0.00754
bta-miR-654	0.00043	±	0.00023	0.00035	±	0.00030	0.00046	±	0.00027
bta-miR-568	0.00000	±	0.00000	0.00000	±	0.00000	0.00000	±	0.00000
bta-miR-655	0.00013	±	0.00009	0.00019	±	0.00011	0.00017	±	0.00019
bta-miR-574	0.23047	±	0.14175	0.15782	±	0.17197	0.25196	±	0.19881
bta-miR-656	0.00002	±	0.00001	0.00002	±	0.00002	0.00003	±	0.00002
bta-miR-758	0.00003	±	0.00001	0.00002	±	0.00002	0.00004	±	0.00001
bta-miR-660	0.00210	±	0.00067	0.00293	±	0.00156	0.00202	±	0.00113
bta-miR-664a	0.00985	±	0.00309	0.00746	±	0.00614	0.00972	±	0.00582
bta-miR-760-3p	0.00280	±	0.00026	0.00254	±	0.00024	0.00315	±	0.00075
bta-miR-664b	0.04080	±	0.00714	0.04284	±	0.01229	0.03758	±	0.00848
bta-miR-760-5p	0.03887	±	0.00668	0.04055	±	0.00448	0.03619	±	0.00947
bta-miR-665	0.07572	±	0.02296	0.07488	±	0.02170	0.07551	±	0.02945

bta-miR-761	0.00014	±	0.00003	0.00010	±	0.00007	0.00015	±	0.00004
bta-miR-669	0.02187	±	0.01283	0.01514	±	0.01575	0.02028	±	0.01745
bta-miR-763	0.00099	±	0.00054	0.00073	±	0.00066	0.00092	±	0.00065
bta-miR-764	0.00016	±	0.00004	0.00012	±	0.00006	0.00014	±	0.00004
bta-miR-671	0.00048	±	0.00003	0.00050	±	0.00009	0.00050	±	0.00011
bta-miR-767	0.00014	±	0.00006	0.00011	±	0.00006	0.00012	±	0.00005
bta-miR-677	0.02261	±	0.00289	0.01871	±	0.00422	0.01474	±	0.00045
bta-miR-769	0.00028	±	0.00010	0.00021	±	0.00011	0.00029	±	0.00009
bta-miR-7	0.00015	±	0.00004	0.00018	±	0.00010	0.00019	±	0.00017
bta-miR-873	0.00016	±	0.00002	0.00013	±	0.00002	0.00016	±	0.00006
bta-miR-708	0.00222	±	0.00161	0.00263	±	0.00158	0.00230	±	0.00153
bta-miR-874	0.00774	±	0.00005	0.00809	±	0.00090	0.00873	±	0.00093
bta-miR-875	0.00001	±	0.00001	0.00001	±	0.00001	0.00000	±	0.00000
bta-miR-876	0.00002	±	0.00000	0.00001	±	0.00001	0.00002	±	0.00001
bta-miR-98	0.00176	±	0.00063	0.00147	±	0.00065	0.00194	±	0.00115
bta-miR-877	0.01620	±	0.00040	0.01591	±	0.00238	0.01662	±	0.00294
bta-miR-99a-3p	0.00033	±	0.00009	0.00049	±	0.00024	0.00031	±	0.00012
bta-miR-885	0.00065	±	0.00007	0.00054	±	0.00023	0.00061	±	0.00013
bta-miR-99a-5p	0.00968	±	0.00075	0.00823	±	0.00196	0.00674	±	0.00162
bta-miR-99b	0.01170	±	0.00137	0.01283	±	0.00245	0.01321	±	0.00306
bta-miR-9-5p	0.00004	±	0.00002	0.00003	±	0.00002	0.00003	±	0.00001
bta-miR-1179	0.00001	±	0.00001	0.00001	±	0.00001	0.00002	±	0.00000
bta-miR-92a	0.04185	±	0.00697	0.05069	±	0.01160	0.04282	±	0.01272
bta-miR-92b	0.03177	±	0.00105	0.03281	±	0.00333	0.03087	±	0.01005
bta-miR-1193	0.00003	±	0.00001	0.00003	±	0.00002	0.00004	±	0.00002
bta-miR-93	0.00534	±	0.00025	0.00477	±	0.00115	0.00451	±	0.00093
bta-miR-935	0.00127	±	0.00007	0.00124	±	0.00014	0.00115	±	0.00025
bta-miR-122	0.00005	±	0.00001	0.00003	±	0.00003	0.00003	±	0.00001
bta-miR-940	0.06690	±	0.01334	0.08000	±	0.02351	0.06267	±	0.01222
bta-miR-1224	1.63671	±	0.89506	0.87945	±	0.77921	1.33473	±	0.63456
bta-miR-95	0.00020	±	0.00006	0.00018	±	0.00008	0.00013	±	0.00005
bta-miR-1225-3p	0.37063	±	0.03937	0.35912	±	0.05816	0.35766	±	0.04802
bta-miR-96	0.00007	±	0.00002	0.00004	±	0.00003	0.00004	±	0.00001
bta-miR-1246	18.58720	±	1.64730	20.20525	±	1.41742	18.75171	±	2.50240
bta-miR-1296	0.00496	±	0.00122	0.00348	±	0.00171	0.00429	±	0.00145
bta-miR-1247-5p	0.08679	±	0.00769	0.09340	±	0.02037	0.08374	±	0.01979
bta-miR-1248	0.06821	±	0.03460	0.05329	±	0.04585	0.04377	±	0.02445
bta-miR-1301	0.00010	±	0.00006	0.00008	±	0.00009	0.00010	±	0.00007
bta-miR-1249	0.00054	±	0.00009	0.00051	±	0.00012	0.00049	±	0.00011
bta-miR-1306	0.00175	±	0.00024	0.00162	±	0.00011	0.00167	±	0.00016
bta-miR-1260b	0.18478	±	0.04381	0.27769	±	0.10869	0.25820	±	0.08955
bta-miR-1307	0.40497	±	0.10331	0.34038	±	0.06829	0.36084	±	0.03741
bta-miR-1271	0.00031	±	0.00001	0.00029	±	0.00007	0.00029	±	0.00003
bta-miR-1343-3p	0.02900	±	0.00533	0.02860	±	0.00562	0.02942	±	0.00587

bta-miR-1343-5p	0.59491	±	0.19023	0.85187	±	0.35507	0.68346	±	0.26289
bta-miR-1281	0.01047	±	0.00076	0.00953	±	0.00107	0.01007	±	0.00302
bta-miR-1282	0.00006	±	0.00003	0.00003	±	0.00004	0.00005	±	0.00002
bta-miR-1284	0.00016	±	0.00007	0.00011	±	0.00008	0.00014	±	0.00008
bta-miR-1287	0.00010	±	0.00005	0.00008	±	0.00009	0.00009	±	0.00008
bta-miR-1291	0.02052	±	0.00963	0.01742	±	0.01406	0.01430	±	0.00833

Supplementary Table S26. The predicted biological pathways regulated by miRNAs differently expressed in LE treated with sEVs from C.M. from end, end *in vivo*, and end *in vitro* 24h.

Signaling pathways	Genes predicted to be modulated by miRNAs	Number of total genes	Pvalue	BH
bta04810 Regulation of actin cytoskeleton	55	211	0.0	0.0
bta05205 Proteoglycans in cancer	55	205	0.0	0.0
bta01522 Endocrine resistance	30	94	0.0001	0.0054
bta05212 Pancreatic cancer	26	76	0.0001	0.0054
bta05220 Chronic myeloid leukemia	27	77	0.0001	0.0054
bta04014 Ras signaling pathway	57	242	0.0002	0.009
bta04072 Phospholipase D signaling pathway	39	152	0.0005	0.0134
bta04659 Th17 cell differentiation	32	113	0.0004	0.0134
bta05224 Breast cancer	39	150	0.0004	0.0134
bta05226 Gastric cancer	39	153	0.0005	0.0134
bta04010 MAPK signaling pathway	63	292	0.0008	0.0143
bta04012 ErbB signaling pathway	25	84	0.0008	0.0143
bta04144 Endocytosis	55	245	0.0007	0.0143
bta04928 Parathyroid hormone synthesis, secretion and action	29	104	0.0008	0.0143
bta05214 Glioma	24	77	0.0006	0.0143
bta05219 Bladder cancer	16	42	0.0009	0.0151
bta00230 Purine metabolism	34	134	0.0012	0.019
bta05200 Pathways in cancer	102	542	0.0017	0.0254
bta05161 Hepatitis B	40	171	0.0018	0.0255
bta04722 Neurotrophin signaling pathway	31	122	0.0019	0.0256
bta05225 Hepatocellular carcinoma	40	174	0.0023	0.0295
bta05160 Hepatitis C	38	164	0.0026	0.0318
bta05218 Melanoma	21	73	0.0028	0.0325
bta05231 Choline metabolism in cancer	26	99	0.0029	0.0325
bta01521 EGFR tyrosine kinase inhibitor resistance	22	80	0.0036	0.0387
bta04660 T cell receptor signaling pathway	27	107	0.0038	0.0393
bta04022 cGMP-PKG signaling pathway	38	169	0.004	0.0399
bta05135 Yersinia infection	31	130	0.0042	0.0403
bta00564 Glycerophospholipid metabolism	26	104	0.0049	0.042
bta04140 Autophagy	33	142	0.0046	0.042
bta05223 Non-small cell lung cancer	19	67	0.005	0.042
bta05235 PD-L1 expression and PD-1 checkpoint pathway in cancer	24	93	0.0048	0.042
bta04917 Prolactin signaling pathway	22	83	0.0052	0.0424
bta05210 Colorectal cancer	23	89	0.0056	0.0443
bta04330 Notch signaling pathway	16	53	0.0058	0.0446
bta04015 Rap1 signaling pathway	45	216	0.0063	0.0471
bta04921 Oxytocin signaling pathway	34	152	0.0066	0.048
bta04530 Tight junction	38	177	0.0075	0.0531

bta04658 Th1 and Th2 cell differentiation	24	98	0.0082	0.0566
bta04310 Wnt signaling pathway	35	162	0.0092	0.0604
bta04914 Progesterone-mediated oocyte maturation	22	88	0.0091	0.0604
bta04520 Adherens junction	18	70	0.0136	0.0851
bta04933 AGE-RAGE signaling pathway in diabetic complications	24	103	0.0133	0.0851
bta04024 cAMP signaling pathway	45	229	0.0146	0.0885
bta05014 Amyotrophic lateral sclerosis (ALS)	16	60	0.0148	0.0885
bta01040 Biosynthesis of unsaturated fatty acids	10	30	0.0156	0.0908
bta04910 Insulin signaling pathway	30	140	0.0162	0.0908
bta05230 Central carbon metabolism in cancer	17	66	0.016	0.0908
bta01100 Metabolic pathways	240	1527	0.0174	0.0934
bta04625 C-type lectin receptor signaling pathway	24	106	0.0175	0.0934
bta04950 Maturity onset diabetes of the young	9	26	0.0177	0.0934
bta04724 Glutamatergic synapse	25	113	0.0194	0.1004
bta04650 Natural killer cell mediated cytotoxicity	28	131	0.02	0.1015
bta04510 Focal adhesion	39	198	0.0208	0.1036
bta04071 Sphingolipid signaling pathway	26	120	0.0213	0.1042
bta01212 Fatty acid metabolism	15	58	0.022	0.1057
bta05167 Kaposi sarcoma-associated herpesvirus infection	40	206	0.0232	0.1095
bta05215 Prostate cancer	22	98	0.0237	0.1099
bta04912 GnRH signaling pathway	21	93	0.0254	0.1158
bta05414 Dilated cardiomyopathy (DCM)	22	99	0.0259	0.1161
bta04137 Mitophagy	16	66	0.0289	0.1196
bta04218 Cellular senescence	33	166	0.0281	0.1196
bta04360 Axon guidance	35	178	0.0276	0.1196
bta04730 Long-term depression	15	60	0.0275	0.1196
bta04935 Growth hormone synthesis, secretion and action	25	118	0.0288	0.1196
bta00062 Fatty acid elongation	9	29	0.0296	0.1206
bta04068 FoxO signaling pathway	27	131	0.0311	0.1249
bta04142 Lysosome	27	132	0.0333	0.1298
bta05100 Bacterial invasion of epithelial cells	17	73	0.0331	0.1298
bta04340 Hedgehog signaling pathway	13	51	0.0342	0.1314
bta04611 Platelet activation	25	121	0.036	0.1345
bta04930 Type II diabetes mellitus	12	46	0.036	0.1345
bta04380 Osteoclast differentiation	27	134	0.0382	0.1374
bta05166 Human T-cell leukemia virus 1 infection	43	234	0.0381	0.1374
bta05410 Hypertrophic cardiomyopathy (HCM)	20	92	0.0383	0.1374
bta04720 Long-term potentiation	16	69	0.039	0.138
bta04350 TGF-beta signaling pathway	20	93	0.0415	0.145
bta05321 Inflammatory bowel disease (IBD)	16	70	0.0428	0.1476
bta00500 Starch and sucrose metabolism	9	32	0.0462	0.1573
bta05163 Human cytomegalovirus infection	44	245	0.0475	0.1597

bta04261 Adrenergic signaling in cardiomyocytes	29	150	0.0483	0.1604
bta04915 Estrogen signaling pathway	27	138	0.0496	0.1627
bta04020 Calcium signaling pathway	37	202	0.0523	0.1665
bta04721 Synaptic vesicle cycle	17	78	0.0518	0.1665
bta04978 Mineral absorption	13	55	0.0526	0.1665
bta04514 Cell adhesion molecules (CAMs)	30	158	0.0535	0.1673
bta04727 GABAergic synapse	19	91	0.0565	0.1739
bta04728 Dopaminergic synapse	26	134	0.0569	0.1739
bta00240 Pyrimidine metabolism	13	56	0.0581	0.1756
bta04215 Apoptosis	9	34	0.0601	0.1796
bta05032 Morphine addiction	19	92	0.0609	0.18
bta04392 Hippo signaling pathway	8	29	0.0628	0.1828
bta04550 Signaling pathways regulating pluripotency of stem cells	27	142	0.0632	0.1828
bta04926 Relaxin signaling pathway	25	130	0.0653	0.1869
bta04934 Cushing syndrome	29	156	0.0682	0.1931
bta04114 Oocyte meiosis	23	119	0.0716	0.1945
bta05169 Epstein-Barr virus infection	40	228	0.0715	0.1945
bta05222 Small cell lung cancer	19	94	0.0705	0.1945
bta05412 Arrhythmogenic right ventricular cardiomyopathy (ARVC)	16	76	0.0715	0.1945
bta04270 Vascular smooth muscle contraction	25	133	0.0779	0.2015
bta04723 Retrograde endocannabinoid signaling	28	152	0.0775	0.2015
bta05133 Pertussis	16	77	0.0773	0.2015
bta05162 Measles	28	152	0.0775	0.2015
bta05418 Fluid shear stress and atherosclerosis	27	145	0.0749	0.2015
bta05202 Transcriptional misregulation in cancer	34	191	0.0788	0.2019
bta05165 Human papillomavirus infection	57	345	0.0839	0.2129
bta04151 PI3K-Akt signaling pathway	61	373	0.0868	0.2179
bta04726 Serotonergic synapse	22	116	0.0875	0.2179
bta04911 Insulin secretion	17	85	0.0888	0.2181
bta04931 Insulin resistance	21	110	0.0892	0.2181
bta04110 Cell cycle	23	123	0.091	0.2205
bta04390 Hippo signaling pathway	28	156	0.0954	0.2291
bta04070 Phosphatidylinositol signaling system	19	99	0.0984	0.2322
bta04668 TNF signaling pathway	22	118	0.0984	0.2322
bta04130 SNARE interactions in vesicular transport	8	33	0.1026	0.24
bta00760 Nicotinate and nicotinamide metabolism	9	39	0.1055	0.2405
bta04713 Circadian entrainment	19	100	0.1048	0.2405
bta05132 Salmonella infection	38	224	0.1055	0.2405
bta05145 Toxoplasmosis	21	113	0.1066	0.241
bta00280 Valine, leucine and isoleucine degradation	11	51	0.1077	0.2414
bta04664 Fc epsilon RI signaling pathway	14	70	0.1142	0.2539
bta04923 Regulation of lipolysis in adipocytes	12	58	0.1167	0.2573

bta04925 Aldosterone synthesis and secretion	18	96	0.1209	0.2644
bta04540 Gap junction	17	90	0.1236	0.266
bta05211 Renal cell carcinoma	14	71	0.1226	0.266
bta04146 Peroxisome	16	84	0.1262	0.2673
bta04922 Glucagon signaling pathway	19	103	0.1252	0.2673
bta04152 AMPK signaling pathway	22	123	0.1294	0.2698
bta04620 Toll-like receptor signaling pathway	20	110	0.1292	0.2698
bta04150 mTOR signaling pathway	27	157	0.1366	0.2827
bta03030 DNA replication	8	36	0.1398	0.2871
bta04666 Fc gamma R-mediated phagocytosis	17	93	0.1477	0.301
bta00512 Mucin type O-glycan biosynthesis	7	31	0.153	0.3049
bta04670 Leukocyte transendothelial migration	20	113	0.1515	0.3049
bta04710 Circadian rhythm	7	31	0.153	0.3049
bta04371 Apelin signaling pathway	24	140	0.1558	0.3082
bta03040 Spliceosome	25	147	0.1582	0.3106
bta05164 Influenza A	30	181	0.161	0.3138
bta00100 Steroid biosynthesis	5	20	0.1637	0.3168
bta03022 Basal transcription factors	9	44	0.1659	0.3188
bta04512 ECM-receptor interaction	16	89	0.1701	0.32
bta04916 Melanogenesis	18	102	0.1691	0.32
bta05206 MicroRNAs in cancer	46	292	0.1698	0.32
bta04064 NF-kappa B signaling pathway	19	109	0.1727	0.3226
bta05017 Spinocerebellar ataxia	17	96	0.1743	0.3234
bta03410 Base excision repair	7	33	0.1851	0.3387
bta04370 VEGF signaling pathway	11	58	0.1843	0.3387
bta00510 N-Glycan biosynthesis	10	52	0.1891	0.3414
bta00565 Ether lipid metabolism	10	52	0.1891	0.3414
bta01524 Platinum drug resistance	14	78	0.191	0.3425
bta05170 Human immunodeficiency virus 1 infection	37	234	0.1933	0.3444
bta05033 Nicotine addiction	8	40	0.1982	0.3485
bta05213 Endometrial cancer	11	59	0.1969	0.3485
bta00310 Lysine degradation	12	66	0.2033	0.3551
bta04062 Chemokine signaling pathway	30	188	0.2086	0.362
bta04725 Cholinergic synapse	19	114	0.2184	0.3766
bta00592 alpha-Linolenic acid metabolism	6	29	0.2244	0.3836
bta04210 Apoptosis	23	142	0.2253	0.3836
bta05142 Chagas disease (American trypanosomiasis)	19	115	0.2281	0.3859
bta04972 Pancreatic secretion	17	102	0.234	0.3934
bta00534 Glycosaminoglycan biosynthesis	5	24	0.2509	0.4151
bta04211 Longevity regulating pathway	15	90	0.2515	0.4151
bta04976 Bile secretion	14	83	0.2488	0.4151
bta00250 Alanine, aspartate and glutamate metabolism	7	37	0.2561	0.4175
bta04960 Aldosterone-regulated sodium reabsorption	7	37	0.2561	0.4175
bta04919 Thyroid hormone signaling pathway	19	118	0.2584	0.4187

bta04217 Necroptosis	27	174	0.2624	0.4227
bta04120 Ubiquitin mediated proteolysis	22	140	0.2706	0.4318
bta05140 Leishmaniasis	13	78	0.2713	0.4318
bta04657 IL-17 signaling pathway	15	92	0.2754	0.4358
bta00514 Other types of O-glycan biosynthesis	8	45	0.2821	0.4364
bta04924 Renin secretion	12	72	0.2823	0.4364
bta04927 Cortisol synthesis and secretion	11	65	0.2794	0.4364
bta04929 GnRH secretion	11	65	0.2794	0.4364
bta05020 Prion diseases	6	32	0.2862	0.4399
bta04662 B cell receptor signaling pathway	14	87	0.2991	0.4571
bta00480 Glutathione metabolism	10	60	0.3069	0.4644
bta00561 Glycerolipid metabolism	11	67	0.309	0.4644
bta00640 Propanoate metabolism	6	33	0.3076	0.4644
bta05216 Thyroid cancer	7	40	0.3138	0.469
bta00590 Arachidonic acid metabolism	13	82	0.3252	0.4754
bta04640 Hematopoietic cell lineage	17	110	0.3243	0.4754
bta04750 Inflammatory mediator regulation of TRP channels	16	103	0.3248	0.4754
bta05221 Acute myeloid leukemia	11	68	0.3241	0.4754
bta04971 Gastric acid secretion	12	76	0.3394	0.4935
bta00650 Butanoate metabolism	5	28	0.3465	0.5011
bta05152 Tuberculosis	29	198	0.3487	0.5016
bta04115 p53 signaling pathway	12	77	0.354	0.5054
bta05217 Basal cell carcinoma	10	63	0.3551	0.5054
bta00513 Various types of N-glycan biosynthesis	7	43	0.3735	0.526
bta04961 Endocrine and other factor-regulated calcium reabsorption	8	50	0.3733	0.526
bta04080 Neuroactive ligand-receptor interaction	51	363	0.387	0.5422
bta03015 mRNA surveillance pathway	14	95	0.4064	0.5646
bta05146 Amoebiasis	17	117	0.4093	0.5646
bta05168 Herpes simplex virus 1 infection	56	403	0.4086	0.5646
bta03420 Nucleotide excision repair	7	45	0.4136	0.5676
bta04918 Thyroid hormone synthesis	11	74	0.4169	0.5693
bta00052 Galactose metabolism	5	31	0.4197	0.5702
bta04260 Cardiac muscle contraction	13	89	0.424	0.5731
bta05416 Viral myocarditis	11	76	0.448	0.6026
bta05031 Amphetamine addiction	10	69	0.4535	0.6069
bta04213 Longevity regulating pathway	9	62	0.4595	0.6119
bta04970 Salivary secretion	13	93	0.4808	0.6351
bta05340 Primary immunodeficiency	6	41	0.4816	0.6351
bta00010 Glycolysis Gluconeogenesis	9	64	0.4938	0.6394
bta00410 beta-Alanine metabolism	5	34	0.4913	0.6394
bta00600 Sphingolipid metabolism	7	49	0.4928	0.6394
bta03018 RNA degradation	11	79	0.4944	0.6394
bta04913 Ovarian steroidogenesis	8	57	0.5024	0.6436
bta04920 Adipocytokine signaling pathway	10	72	0.5021	0.6436

bta00591 Linoleic acid metabolism	5	36	0.5371	0.684
bta04932 Non-alcoholic fatty liver disease (NAFLD)	21	158	0.5391	0.684
bta04216 Ferroptosis	6	45	0.5641	0.7124
bta00620 Pyruvate metabolism	5	38	0.581	0.7303
bta04630 JAK-STAT signaling pathway	26	202	0.5962	0.7459
bta00330 Arginine and proline metabolism	6	48	0.6215	0.7604
bta04141 Protein processing in endoplasmic reticulum	21	166	0.6219	0.7604
bta04672 Intestinal immune network for IgA production	7	56	0.6215	0.7604
bta05030 Cocaine addiction	6	48	0.6215	0.7604
bta05332 Graft-versus-host disease	6	48	0.6215	0.7604
bta00562 Inositol phosphate metabolism	9	73	0.6378	0.7728
bta01230 Biosynthesis of amino acids	9	73	0.6378	0.7728
bta04621 NOD-like receptor signaling pathway	23	184	0.6448	0.7743
bta04974 Protein digestion and absorption	15	121	0.643	0.7743
bta00520 Amino sugar and nucleotide sugar metabolism	6	50	0.6573	0.7858
bta00071 Fatty acid degradation	5	42	0.6614	0.7872
bta04940 Type I diabetes mellitus	7	59	0.6707	0.7948
bta00260 Glycine, serine and threonine metabolism	5	43	0.6798	0.7951
bta01523 Antifolate resistance	5	43	0.6798	0.7951
bta04610 Complement and coagulation cascades	11	92	0.6776	0.7951
bta02010 ABC transporters	7	60	0.6862	0.7991
bta04066 HIF-1 signaling pathway	13	110	0.6991	0.8052
bta04622 RIG-I-like receptor signaling pathway	12	102	0.7004	0.8052
bta04973 Carbohydrate digestion and absorption	5	44	0.6976	0.8052
bta00982 Drug metabolism	7	63	0.7297	0.8334
bta01200 Carbon metabolism	13	113	0.7312	0.8334
bta00380 Tryptophan metabolism	5	47	0.7467	0.844
bta03320 PPAR signaling pathway	9	81	0.7443	0.844
bta05330 Allograft rejection	6	56	0.7512	0.8455
bta04975 Fat digestion and absorption	5	48	0.7617	0.8537
bta03008 Ribosome biogenesis in eukaryotes	9	83	0.7672	0.8563
bta00140 Steroid hormone biosynthesis	7	67	0.781	0.861
bta04962 Vasopressin-regulated water reabsorption	5	49	0.776	0.861
bta05134 Legionellosis	6	58	0.778	0.861
bta05204 Chemical carcinogenesis	8	77	0.7955	0.8734
bta04061 Viral protein interaction with cytokine and cytokine receptor	10	95	0.8023	0.8773
bta04714 Thermogenesis	27	239	0.8149	0.885
bta04742 Taste transduction	8	79	0.8159	0.885
bta03013 RNA transport	19	178	0.8477	0.9158
bta00980 Metabolism of xenobiotics by cytochrome P450	6	67	0.8719	0.9292
bta00983 Drug metabolism	7	76	0.8692	0.9292

bta05203 Viral carcinogenesis	26	241	0.8677	0.9292
bta05323 Rheumatoid arthritis	10	104	0.8739	0.9292
bta05144 Malaria	5	59	0.8847	0.9369
bta05320 Autoimmune thyroid disease	6	71	0.9015	0.951
bta00970 Aminoacyl-tRNA biosynthesis	5	66	0.9306	0.9717
bta04612 Antigen processing and presentation	7	85	0.9258	0.9717
bta04623 Cytosolic DNA-sensing pathway	5	67	0.9356	0.9717
bta05010 Alzheimer disease	17	180	0.9334	0.9717
bta04060 Cytokine-cytokine receptor interaction	33	323	0.9394	0.9719
bta05016 Huntington disease	27	274	0.9465	0.9755
bta04145 Phagosome	15	170	0.9559	0.9814
bta05034 Alcoholism	20	229	0.9764	0.9987
bta00190 Oxidative phosphorylation	7	140	0.9989	1.0
bta03010 Ribosome	8	160	0.9994	1.0
bta04740 Olfactory transduction	18	1145	1.0	1.0
bta05012 Parkinson disease	9	150	0.9968	1.0
bta05150 Staphylococcus aureus infection	5	105	0.9973	1.0
bta05322 Systemic lupus erythematosus	5	182	1.0	1.0