UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ZOOTECNIA E ENGENHARIA DE ALIMENTOS

NATÁLIA MARINS BASTOS

The effects of high body energy reserve on ovarian and oviductal responses in bovine females

Pirassununga

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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. Juliano Coelho da Silveira

Coorientador: Prof. Dr. Rodrigo Silva Goulart

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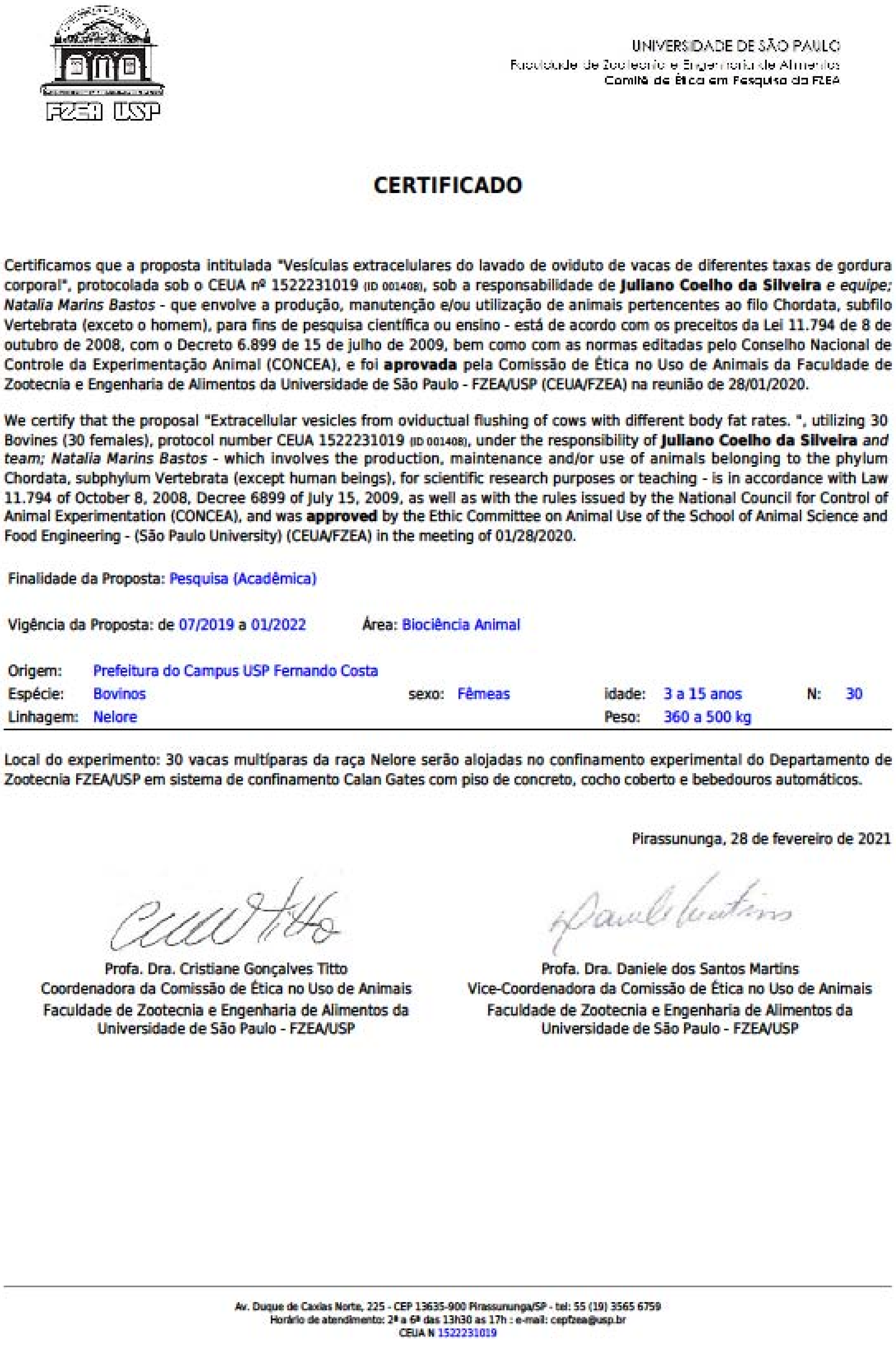

UNIVERSIDADE DE SÃO PAULO
Faculdade de Zootecnia e Engenharia de Alimentos
Comitê de Ética em Pesquisa da FZEA

CERTIFICADO

Certificamos que a proposta intitulada "Vesículas extracelulares do lavado de oviduto de vacas de diferentes taxas de gordura corporal", protocolada sob o CEUA nº 1522231019 (ID 001408), sob a responsabilidade de **Juliano Coelho da Silveira** *e equipe; Natalia Marins Bastos* - 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 28/01/2020.

We certify that the proposal "Extracellular vesicles from oviductual flushing of cows with different body fat rates. ", utilizing 30 Bovines (30 females), protocol number CEUA 1522231019 (ID 001408), under the responsibility of **Juliano Coelho da Silveira** *and team; Natalia Marins Bastos* - 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 01/28/2020.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 07/2019 a 01/2022 Área: Biociência Animal

Origem:	Prefeitura do Campus USP Fernando Costa						
Espécie:	Bovinos	sexo:	Fêmeas	idade:	3 a 15 anos	N:	30
Linhagem:	Nelore			Peso:	360 a 500 kg		

Local do experimento: 30 vacas multíparas da raça Nelore serão alojadas no confinamento experimental do Departamento de Zootecnia FZEA/USP em sistema de confinamento Calan Gates com piso de concreto, cocho coberto e bebedouros automáticos.

Pirassununga, 28 de fevereiro de 2021

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

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Folha de aprovação

Nome: Natália Marins Bastos

Título: The effects of high body energy reserve on ovarian and oviductal responses in bovine females

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.

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Dedicatória

Dedico esta tese a meus pais: Wanderli e Mariá por todo
cuidado, dedicação, amor e coragem que me deram desde
meu nascimento até o dia de hoje.
Essa conquista é nossa.

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“A abelha fazendo o mel vale o tempo que não voou”

“Amor de índio” – Beto Guedes.

Resumo

BASTOS, N. M. **Os efeitos da alta reserva energética corporal nas respostas ovarianas e ovidutais de fêmeas bovinas**. 349 f. Tese (Doutorado) – Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga – SP, 2022.

A condição metabólica controla a função reprodutiva, e a elevada reserva energética corporal (BER) pode afetar a qualidade oocitária e do embrião no início do desenvolvimento. Entretanto, os mecanismos moleculares e fisiológicos pelos quais isso ocorre ainda não são claros. O objetivo principal desta tese é avaliar o elevado BER nas respostas ovarianas e ovidutais em fêmeas bovinas. No **capítulo 1**, é apresentada uma revisão de literatura sobre a capacidade ovidutal com base em suas mudanças dinâmicas e sistêmicas durante os eventos reprodutivos, bem como a contribuição das vesículas extracelulares (EVs) neste processo. Nos capítulos 2 e 3, o mesmo modelo animal foi usado para avaliar as respostas ovarianas e ovidutais, respectivamente. Assim, vacas Nelore de um mesmo rebanho foram submetidas aleatoriamente a um período de confinamento com dois planos nutricionais com diferentes consumos de matéria seca (CMS) a fim de obter vacas com diferentes BER: vacas com alta reserva energética corporal (HBER: dieta *Ad libitum*) e moderada reserva energética corporal (MBER: vacas alimentadas com 70% da ingestão do grupo HBER). Ao final do período de alimentação, os animais foram submetidos à sincronização de estro e inseminação artificial. Os animais foram abatidos aproximadamente 120 h após a indução da ovulação. No **capítulo 2**, as vacas foram pesadas semanalmente e avaliadas quanto à espessura de gordura subcutânea e concentração sérica de insulina ao longo do período experimental. A ovulação foi analisada por ultrassom e após o abate foram coletados os tratos reprodutivos para recuperação embrionária e coleta de amostras. Complexos cumulus-oócitos (COC) e fluido folicular foram coletados de folículos ovarianos de 3-6 mm de diâmetro para análise de miRNA de células cumulus (CC) e vesículas extracelulares de fluido folicular (EV FF). Como esperado, foram observadas diferenças entre os grupos MBER e HBER para peso corporal, espessura de gordura e concentração sérica de insulina. Animais HBER apresentaram menores taxas de ovulação e recuperação de embriões em comparação com animais MBER. Diferentes miRNAs foram encontrados entre CC e EV FF dentro dos grupos, sugerindo que o BER pode influenciar a comunicação folicular. No **capítulo 3**, foram realizadas análises apenas de animais que possuíam embrião de 8 células no istmo. Foi avaliado o perfil molecular das vesículas extracelulares do lavado do oviduto (OF-EVs) e das células epiteliais luminais (OV-Cell), e análise histomorfológica no tecido para as regiões da ampola e do istmo do oviduto. O grupo HBER apresentou maior concentração em vesículas extracelulares na ampola (AMP-EVs) e maior tamanho em vesículas extracelulares no istmo (IST-EVs). Em AMP-EVs o perfil de miRNA mostrou que os miRNAs diferentemente expressos foram preditos por regular vias associadas ao crescimento celular, migração, diferenciação e metabolismo, sendo que o grupo HBER pode ser mais suscetível à modulação da insulina. Os animais MBER apresentaram maior vascularização na ampola do que HBER. Além disso, o perfil de miRNA e a expressão gênica diferencial (DEG) realizada em células epiteliais luminais da ampola (AMP-Cell) e ístmo (IST-Cell) revelaram principalmente vias relacionadas ao metabolismo da insulina. Os resultados aqui apresentados indicam que as respostas ovarianas e ovidutais são influenciadas pelo elevado BER, uma vez que a taxa de ovulação e comunicação dentro do folículo são alteradas, assim como a taxa de recuperação embrionária e ambiente/composição na ampola e istmo.

Palavras chave: Consumo de matéria seca; Insulina; miRNA; Vesículas extracelulares.

Abstract

BASTOS, N. M. **The effects of high body energy reserve on ovarian and oviductal responses in bovine females.** 349 f. Tese (Doutorado) – Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga – SP, 2022.

The metabolic status controls the reproductive function, and the elevated body energy reserve (BER) can affect oocyte and early embryo quality. However, the molecular and physiological mechanisms by which this occurs are still unclear. The main aim of this thesis is to evaluate the elevated BER in ovarian and oviductal responses in bovine females. In **chapter 1**, is presented a review about the oviductal ability based on its dynamic and systemic changes during reproductive events, as well as the extracellular vesicles (EVs) contribution in this process. In chapters 2 and 3, the same animal model was used to evaluate ovarian and oviductal responses respectively. Thus, Nellore cows from the same herd were randomly submitted to a feedlot period with two nutritional plans with different dry matter intake (DMI) in order to obtain cows with different BER: cows with high body energy reserve (HBER: *Ad libitum* diet) and moderate body energy reserve (MBER: cows fed 70% of HBER group ingestion). At the end of the feeding period, animals were submitted to estrous synchronization and artificial insemination. Animals were slaughtered approximately 120 h after ovulation induction. In **chapter 2**, cows were weekly weighted and evaluated for subcutaneous fat thickness and insulin serum concentration along the experimental period. The ovulation was analyzed by ultrasound and after slaughter the reproductive tracts were collected for embryo recovery and samples collection. Cumulus-oocyte-complexes (COC) and follicular fluid were collected from 3-6 mm in diameter ovarian follicles to perform miRNA analysis of cumulus cells (CC) and extracellular vesicles from follicular fluid (EV FF). As expected, differences were observed among MBER and HBER groups for body weight, fat thickness, and insulin serum concentration. HBER animals showed lower ovulation and embryo recovery rates compared to MBER animals. Different miRNAs were found among CC and EV FF within groups, suggesting that the BER may influence follicular communication. In **chapter 3**, analyzes were performed only from animals which had an 8-cell embryo in isthmus. It was evaluated the molecular profile of extracellular vesicles from oviductal flushing (OF-EVs) and luminal epithelial cells (OV-Cell), and histomorphological analysis in oviductal tissue for ampullary and isthmic oviductal regions. The HBER group presented higher concentration in ampullary extracellular vesicles (AMP-EVs) and larger sizer in isthmic extracellular vesicles (IST-EVs). In

AMP-EVs the miRNA profile showed that the differently expressed miRNAs were predicted to regulate pathways associated with cell growth, migration, differentiation and metabolism, being the HBER group may be more susceptible to insulin modulation. The MBER animals showed higher ampullary vascularization than HBER. Additionally, the miRNA profile and differential gene expression (DEG) performed in ampullary (AMP-Cell) and isthmic (IST-Cell) luminal epithelial cells mainly revealed pathways related with insulin metabolism. The results showed here indicates that the ovarian and oviductal responses are influenced by elevated BER once the ovulation rate and communication within the follicle is altered, as the embryo recovery rate and environment/composition at the ampulla and isthmus.

Key words: Dry matter intake; Extracellular vesicles; Insulin; miRNAs.

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1. Introduction

Processed food with excessively high caloric rates and deficient in vitamins and minerals is present in nutritional habits of modern human society (FAO, 2018). The consequences of these lifestyle are changes in body energy reserve (BER) taking to overweight and obesity which are associated with metabolic disorders such as diabetes and hypertension (CORREA; MARCINKEVAGE, 2013; CHA et al., 2021). The majority of women from developmental countries in reproductive age find themselves in this reality (KLENOV; JUNGHEIM, 2014; CHEN; XU; YAN, 2018). In this sense, metabolic impairment can alter reproductive functions, making reproductive rates unfeasible. Elevated BER in preconception is associated to altered placental function, preterm birth, macrosomia, shoulder dystocia, birth defects and stillbirth (STANG; HUFFMAN, 2016; SON et al., 2019). This indicates that the maternal metabolism dictates important reproductive rules that can act in subsequent generations. Unfortunately, these consequences are not limited to human species and in the same context, the show cattle, which represents the genetic donors used to improve cattle breeding based on its gametes (oocytes and sperm) and embryos donation through reproductive technologies are overweight (VELAZQUEZ, 2015). This is due to their long time staying on the farm as well as the high-quality food offered and low amount of exercise, induce these animals to present elevated BER. Thus, like women, these cows with high BER may present impaired reproductive functions.

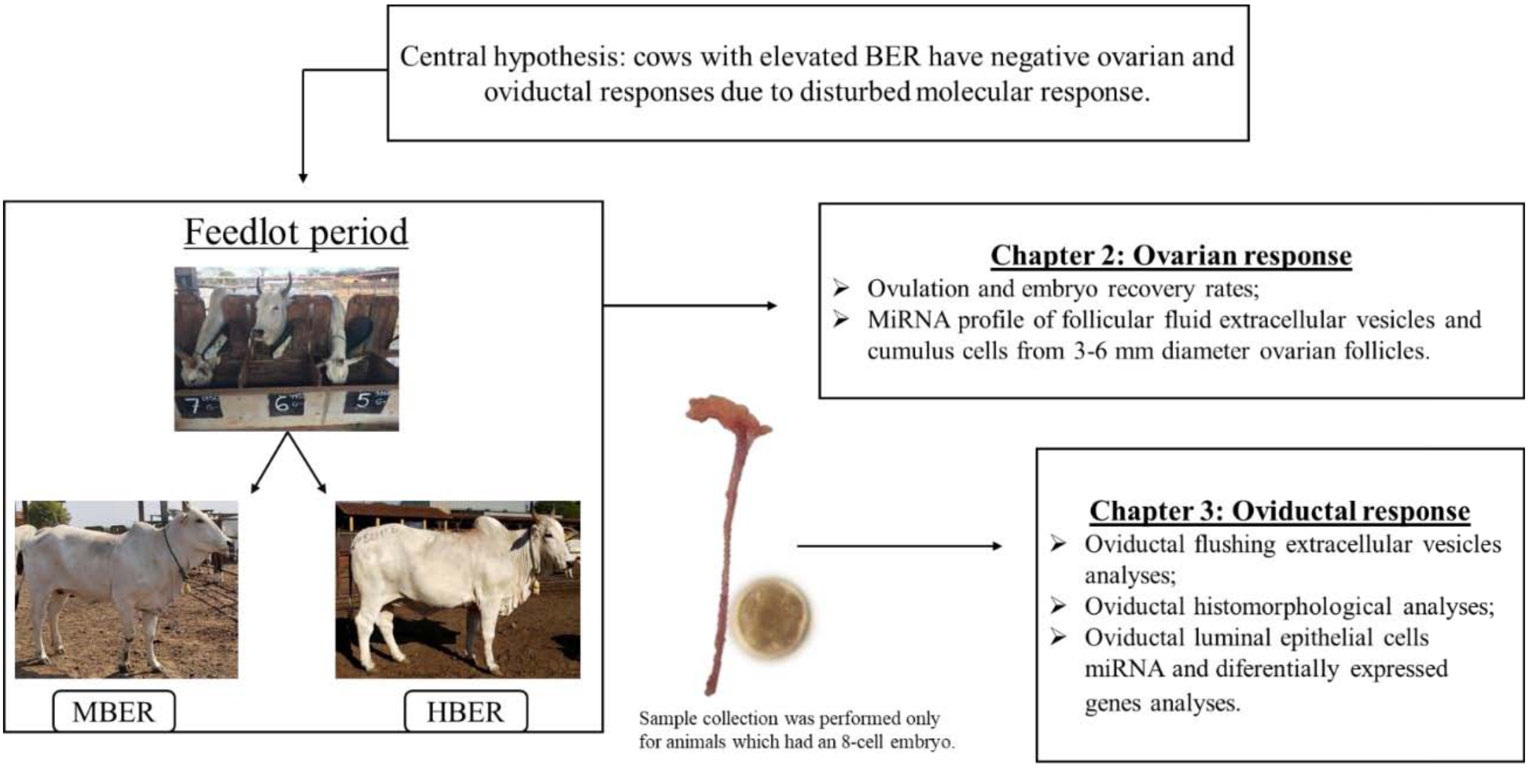
Directly or indirectly, the nutritional management affects reproductive performance. Food intake alters animal metabolism by substrates concentration and metabolic hormones, as insulin, leptin and IGF-I, acting systemically in hypothalamic-pituitary-gonadal axis modulating its response (WEBB et al., 2004; SARTORI et al., 2013; D’OCCHIO; BARUSELLI; CAMPANILE, 2019). In this scenario, changes in BER influences the animal metabolism, physiology and endocrine system impacting the ovarian function, embryonic quality and, consequently, pregnancy establishment (BOSSIS et al., 2000; SANGSRITAVONG et al., 2002; CARVALHO et al., 2014). During follicular growth, follicles undergo proliferative processes, structural reorganization and oocytes pass through morphological and biochemical alterations required for the achieve of oocyte competence (BREVINI GANDOLFI; GANDOLFI, 2001; EICHENLAUB-RITTER; PESCHKE, 2002; SU et al., 2007). These synergistically changes provides an ideal microenvironment to gametic development and to allow the mechanisms that regulates ovulation and fertilization. Once the follicle is under metabolic control, modulations in follicular environment may occur in a BER alteration. Serum metabolites from extreme BER cows can across the blood follicular barrier being able to alters the follicular and oocyte quality (HORN et al., 2022). This is due to changes in metabolic balance of reproductive tissues which negatively influences gametes and embryos quality.

After follicle ovulation, the cumulus-oocyte complex (COC) is picked up by oviductal fimbriae and important processes as final oocyte maturation, fertilization, early embryo development takes place in oviduct. The oviduct environment is composed by secretory and ciliated cells in ampullary and isthmic regions, and is responsible to generate the ideal environment to allow those processes to take place. Among them, cell communication between follicular cells-oocyte; oviduct-gametes and mother-embryo is essential and take place in the follicular and oviductal fluids composed by sugars, lipids and proteins (EDWARDS, 1972; SAINT-DIZIER et al., 2020). Another constituent of these reproductive fluids are the extracellular vesicles (EVs). These nanoparticles are biological vectors acting as mediators of cell communication that can delivery bioactive material as proteins, lipids, mRNAs and miRNAs to target cells. The miRNAs plays and important role in reproductive cycle (GROSS; KROPP; KHATIB, 2017) and can carry important molecular signals in response to environmental factors. Thus, the increase in animal BER may influence reproductive environments affecting gametes and embryo quality.

It is already known that elevated BER can affect oocyte and early embryo quality (MOLLO et al., 2017; SARTORI; SPIES; WILTBANK, 2017), however the molecular and physiological mechanisms by which this occurs are still unclear. In addition, due to the difficulty to obtain samples, little is kwon about the extent to which the oviduct is responsible for gestational failures as well as the impact of animal metabolism on the ovarian follicle and the oviduct. Once bovine can be used as biological model for human studies due to the very similar pattern of embryonic development between species, the comprehension of ovarian and oviductal response in cows with elevated BER is important. Such studies can help to elucidate the mechanisms related to altered metabolism that act in early embryonic development. Understanding the mechanisms behind these alterations can help to improve fertility rates. In this way, ***the central hypothesis of this thesis is: cows with elevated BER have negative ovarian and***

oviductal responses due to disturbed molecular response. To test this hypothesis, Nellore cows from the same herd were randomly submitted to a feedlot period with two nutritional plans with different dry matter intake (DMI) in order to obtain cows with different BER: cows with *high body energy reserve (HBER: Ad libitum diet)* and *moderate body energy reserve (MBER: cows fed 70% of HBER group ingestion)*.

This thesis is divided in three chapters being the first (Chapter 1) a literature review and the others two chapters (Chapters 2 and 3), which are studies composed by the same animal model, in order to help to understand the HBER effects on female reproductive physiology including the ovarian follicle and the early embryonic development environment at the oviduct. Thus, the samples were derived from the same experimental groups: animals with moderate and high body energy reserve (MBER and HBER, respectively; Figure 1).



Central hypothesis: cows with elevated BER have negative ovarian and oviductal responses due to disturbed molecular response.

Chapter 2: Ovarian response

- Ovulation and embryo recovery rates;
- MiRNA profile of follicular fluid extracellular vesicles and cumulus cells from 3-6 mm diameter ovarian follicles.

Chapter 3: Oviductal response

- Oviductal flushing extracellular vesicles analyses;
- Oviductal histomorphological analyses;
- Oviductal luminal epithelial cells miRNA and diferentially expressed genes analyses.

Sample collection was performed only for animals which had an 8-cell embryo.

Figure 1. Experimental design schematical representation used in reported studies which composes this thesis. To test the hypothesis, Nellre cows from a same herd were submitted to different nutritional plans during the feedlot period in order to maintain (MBER group) or elevate (HBER group) their body energy reserve. At the end of the feedlot period, the animals were submitted to estrous synchronization, artificial insemination, ovulation confirmation by ultrasound, and thereafter, slaughtered approximately 120 hours of ovulation induction. The reproductive tract was collected to obtain the samples. The oviducts ipsilateral to the corpus luteum were dissected and the isthmus portion was flushed for embryo recovery. Ovarian follicles (3-6 mm in diameter) from ipsi and contralateral ovaries were punctured to obtain cumulus-oocyte-complexes (COC) and follicular fluid to perform miRNA analysis. In **Chapter 2** was analyzed the ovarian response by ovulation and

embryo recovery rates and the ovarian follicle environment analysis. Only animals which presented an 8-cell embryo were used to perform further analysis in **Chapter 3** which evaluated the oviductal flushing extracellular vesicles, histomorphological analysis in oviductal tissue, luminal epithelial cells miRNA and differentially expressed genes analyses.

- **Chapter 1: The role of oviduct and extracellular vesicles during early embryo**.
    - Review about the oviductal ability based on its dynamic and systemic changes during reproductive events, as well as the extracellular vesicles contribution in this process.
    - Journal article published in Animal Reproduction. Accepted in March 17, 2022 (DOI: https://doi.org/10.1590/1984-3143-AR2022-0015).
- **Chapter 2: High body energy reserve influences extracellular vesicles miRNA contents within the ovarian follicle.**
    - In this study the aim was to understand the effects of altered BER on ovulation, embryo recovery and miRNA profile of extracellular vesicles present within the follicular environment in multiparous Nellore cows with different BER.
    - Article submitted to the periodic Plos One in August 8, 2022.
- **Chapter 3: The bovine oviductal environment and composition is harmfully affected by elevated body energy reserve.**
    - In this study the aim was to evaluate the effects of increased BER in the oviductal environment and composition of Nellore cows.
    - Journal article to be submitted to the periodic Biology of Reproduction.

In addition to that, at the end of this document we included a general discussion and conclusion about the major findings of both studies.

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2. Chapter 1: The role of oviduct and extracellular vesicles during early embryo development in bovine

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Abstract

The oviduct is an important reproductive structure that connects the ovary to the uterus and takes place to important events such as oocyte final maturation, fertilization and early embryonic development. Thus, gametes and embryo can be directly influenced by the oviductal microenvironment composed by epithelial cells such secretory and ciliated cells and oviductal fluid. The oviduct composition is anatomically dynamic and is under ovarian hormones control. The oviductal fluid provides protection, nourishment and transport to gametes and embryo and allows interaction to oviductal epithelial cells. All these functions together allows the oviduct to provides the ideal environment to the early reproductive events. Extracellular vesicles (EVs) are biological nanoparticles that mediates cell communication which are present at oviductal fluid and plays an important role in gametes/embryo - oviductal cells communication. This review will present the ability of the oviducts based on its dynamic and systemic changes during reproductive events, as well as the contribution of EVs in this process.

Keywords: oviductal environment, oviductal fluid, cell communication.

2.1.Introduction

In vitro embryo production (IVEP) is a very well-established reproductive biotechnology, used worldwide and capable to contribute to the increasing genetic merit and consequently improvement of different domestic species. However, the use of IVEP exposes gametes and embryos to non-physiological spatial and temporal conditions (Besenfelder et al., 2020). Furthermore, *in vivo* derived embryos still have superior quality and developmental potential than those produced *in vitro* (Rizos et al., 2002a, b). Thus, the IVEP is efficient in producing embryos, but it still does not completely mimic the physiological ovarian and oviductal environments. During the short period of early embryonic development within the oviduct, the embryo will activate its genome, thus comprising a sensitive and important period that can determine the subsequent stages of its development.

In the *in vivo* situation, the oviduct takes place to important events for the reproductive function, such as final oocyte maturation, fertilization and early embryonic development. The oviduct can provide a favorable and dynamic microenvironment for ideal functioning and development of these processes. Interestingly the oviduct of different species has similar biological properties; however, the time that the embryo resides and undergoes modifications is not the same. Bovine embryos develop within the oviduct within 4 days after ovulation (Kolle et al., 2009), 4 days in human (Aplin, 2003), 5.5 days in equine (Freeman et al., 1991) and 4 days in mice (Potts and Wilson, 1967), demonstrating the importance for studying the events that take place in the oviduct.

The oviduct fluid that bathes gametes and embryos contain substrates and co-factors that help to create the oviductal environment prepared to the embryo cleavage and development. Furthermore, present in the oviduct fluid, extracellular vesicles (EVs) are nanoparticles that mediate cell communication acting as vectors of biological information. In the oviduct, the EVs plays an important role once they interact with gametes-embryo and oviductal epithelial cells acting in this bidirectional communication. Thus, oviductal EVs can modulates the oviductal environment and influences the reproductive events that takes place there, and also the oviduct-embryo interaction in order to contribute with maternal-embryonic communication even before the recognition of pregnancy (Mazzarella et al., 2021). Therefore, this review will present the adaptability of the oviduct during reproductive events based on its dynamic and systemic changes, as well as the contribution of EVs in this process.

2.2. Composition and importance of the oviduct environment for early embryonic development

The oviduct is a small, elongated and tubular structure that connects the ovary to the uterus and is formed by a fibromuscular complex composed of layers such as mucosa, muscle and connective serosa (Besenfelder et al., 2012, 2020; Avilés et al., 2015). These layers' structure and composition depends on the three different anatomical portions that make up the oviduct: infundibulum, ampulla and isthmus (Besenfelder et al., 2012, 2020; Avilés et al., 2015). The infundibulum is responsible for capturing recently ovulated cumulus-oocyte-complexes (COCs) (Besenfelder et al., 2012; Avilés et al., 2015). The cumulus cells extracellular matrix filaments are able to adhere to the infundibulum cells glycocalyx and enter the oviduct at the ampulla region where the oocyte maturation process is completed and fertilization takes place (Kolle et al., 2009; Li and Winuthayanon, 2017). The cilia beating is responsible for creating a negative pressure and microtubule movement, which will produce a current flow that helps the COC movement throughout the oviduct towards the uterine (Olsen et al., 2018). Bad quality oocytes move faster through the oviduct by floating in oviductal lumen, demonstrating that there is function related to the movement as well as the capability of COC and oviduct to recognize themselves (Kolle et al., 2009). Female and male gametes enter at oviduct from opposite sides but oocyte and sperm meet in the ampulla (Besenfelder et al., 2020). In order to the sperm arrive at the oviduct, uterine contractibility as well as oviduct secretions play an important role directing the sperm towards the ampulla (Hawk, 1983; Suarez, 2008). Then, on isthmic region crucial processes related to pre-implantation embryo development takes place. During the time that the embryo stays at isthmus, besides the initial development, the major gene activation happens starting at the 8-cell stage (Memili and First, 2000) suggesting that this oviductal region has an important role through the subsequently embryo development at uterus.

A size comparison between the different parts of the oviduct demonstrated that the ampullary lumen is large and filled with primary and secondary folds, while the isthmic lumen is smaller and constituted only with primary folds (Besenfelder et al., 2020). The mucosa is constituted by epithelial cells that can be ciliated or secretory cells, and these cells proportion depends on the oviducts anatomical portion and the ovarian cycle stage. Endocrine mechanisms, mainly controlled by steroid hormones (estrogen and progesterone) are well known to mediate morphological, physiological and molecular changes in the oviduct (Gonella-Diaza et al., 2017; Almiñana et al., 2018; Gonella-Diaza et al., 2018). In response to the high concentrations of preovulatory estrogen (E2), the oviduct initiates morphogenic and proliferative processes in the ampulla lumen, increasing the number of secretory cells and the functional area of the epithelium (Gonella-Diaza et al., 2017). Thus, the ampulla prepares to become receptive to the COC and sperm cells. After fertilization, the number of secretory cells continuously decreases and, during the embryo first cleavages, the isthmus is mostly composed by ciliated cells (Kolle et al., 2009). COCs and embryos are immobile and must be transported through the oviductal anatomical structures by a combination of factors: waves of smooth muscle layer contraction and relaxation (longitudinal and circular), ciliary beating of epithelial cells and follicular fluid flow. These mechanisms are mostly controlled by steroid hormones and prostaglandins (Amini et al., 2015). Apparently, in a pre-ovulatory stage, E2 modulates the action of prostaglandins (PGE2 and PGF2α) in the oviduct (Lindblom et al., 1980) and is related to muscle contraction, increased frequency of ciliary beat and increased oviductal fluid volume, as it increases the number of secretory cells (Valle et al., 2007; Huang et al., 2015; Gonella-Diaza et al., 2017). Progesterone (P4) seems to have the opposite effect to E2 (Lindblom et al., 1980), once this hormone acts in the muscle relaxation and decreased frequency of ciliary beat.

The sperm, even having their own movement, must undergo morphophysiological changes to reach fertilizing capacity such as hyperactivation and acrosome reaction. In cows, the semen is ejaculated into vagina, against the cervix, where the natural selection occurs by the cervical mucus flow and only the motile sperm can advance towards the uterus (Coy et al., 2012; Li and Winuthayanon, 2017). Besides their own motility, the sperm moves through the uterus due to muscular contractions, ciliary beat and fluid flow that helps the healthy sperm to arrive at uterotubal junction (Hawk, 1983). Once in the oviduct, sperm interact with isthmic epithelial cells. The oviduct guides the sperm to the fertilization site and helps in this process, but first provides the formation of a sperm reservoir and enabling the activation of Ca^{2+} influx in the sperm in order to initiate the flagellum hyperactivation process (Miki and Clapham, 2013). Once this occurs, the sperm is able to swim against the oviduct flow to find the COC to be fertilized (Kolle et al., 2009). The follicular fluid present at the ampulla after ovulation contains progesterone

(Saint-Dizier et al., 2020) and chemoattractants that helps to bring the sperm closer to the COC within the ampullary region. Furthermore, proteins and phospholipids present in the ampulla lumen can influence the sperm fertilizing capacity inducing the acrosome reaction (Griffiths et al., 2008). Thus, the oviduct and its fluid are able to guide the sperm and provide subsidies for it to become fertile in addition to sense the presence of the sperm cells and adjust proteins and antioxidants concentration possibly reducing sperm stress. Also, the increase in E2 concentration induces the production of GPX4 (Glutathione peroxidation 4) in the oviduct, indicating an antioxidant defense mechanism for gametes and future embryonic development (Lapointe et al., 2005).

Gametic and embryo transport play an important role, since to be fertilized the COC and sperm must be at the proper time and place for fertilization, and the early development embryo must to exit from the oviduct to carry on development otherwise it can implant in the wrong place or generate an ectopic pregnancy. Furthermore, embryonic development and transport are simultaneous events under physiological conditions (Li and Winuthayanon, 2017). Embryonic movement, acts to prevent the accumulation of harmful by-products to embryonic development once allows full exposure of the embryo to the medium providing the appropriate access to nutrients and preventing metabolic stress during the first cleavages (Hu and Yu, 2018).

In addition, a recent study demonstrated that the embryo presence alters the miRNA profile of the isthmic cells generating an inflammatory type response (Mazzarella et al., 2021), suggesting that the embryo presence modulates the oviductal epithelial cells. In goats, the nutritional plan influences the ampullary epithelial cells protein profile (Fernandes et al., 2018). Furthermore, environmental factors such as the animal's energy balance also appear to have an influence on the oviduct dynamics. Therefore, the oviduct is a dynamic and adaptable structure able to respond to situations in which it is exposed and is not just a simple organ for transporting COCs and embryos.

2.3.Composition and importance of oviductal fluid

Until the establishment intimate contact between mother and embryo, embryonic development is directly influenced by secreted products by oviductal and endometrial epithelial cells (Binelli et al., 2018). Oviductal fluid, synthesized primarily by secretory epithelial cells, is also composed of transudate from the systemic circulation and supplemented by follicular fluid upon ovulation (Li and Winuthayanon, 2017; Olsen et al., 2018; Besenfelder et al., 2020). Prior to fertilization, oviductal fluid is responsible for protecting and guiding sperm and COCs (Li and Winuthayanon, 2017). After fertilization, the oviductal fluid role is responsible to nourish, protect and assist the transport of the pre-implantation embryo (Olsen et al., 2018) as well as to provide optimal pH and stable temperature (Li and Winuthayanon, 2017). Its composition is anatomically dynamic and influenced by early developing embryo presence (Rodríguez-Alonso et al., 2020a; Mazzarella et al., 2021). Interestingly, the embryo presence is capable of modulating the oviduct environment, being able to acquire components of the maternal environment.

The volume of oviductal fluid is dependent on the estrous cycle and varies among species, but in general, in mammals, the highest production is during the end of estrus and beginning of diestrus (Leese et al., 2001), when the oviduct prepares to receive gametes and embryo by increasing the number of secretory epithelial cells as well as lumen size (Leese et al., 2001; Gonella-Diaza et al., 2017). In addition, the increase in oviductal fluid volume during this period is also due to the follicular fluid coming from the recently ovulated follicle (Saint-Dizier et al., 2020). Thus, the estimated volume of the oviductal fluid in bovine is 1-3 mL per day on day one of the estrus cycle, and 0.1-0.2 mL per day at luteal phase (Kavanaugh and Killian, 1988).

Oviductal fluid is composed by substrates and cofactors linked to oocyte maturation, oocyte fertilization and early embryo development such as glucose, arginine, serum albumin, transferrin, glycoprotein, galactose, immunoglobulins, lactate, pyruvate, bicarbonate, cytokines, growth factors, amino acids, enzymes, hormones and EVs (Beier, 1974; Binelli et al., 2018; Rodríguez-Alonso et al., 2020b; Saint-Dizier et al., 2020). These constituents' concentration varies among species, estrous cycle stage and oviduct anatomical portion (Hu and Yu, 2018; Rodríguez-Alonso et al., 2020a). This is probably due to the different metabolic needs of gametes and embryo as they pass through oviduct.

Oocytes, sperm and embryos in early developmental stage, use oxidative metabolism to obtain energy. During the first cleavages, until the morula stage, embryonic mitochondria are not yet mature, so simple sugars such as pyruvate and lactate (Rodríguez-Alonso et al., 2020a) and high oxygen concentration (Hu and Yu, 2018) are essential for oxidative phosphorylation that provides energy for the first cleavages. Pyruvate is an important sugar for oxidative phosphorylation, but is mostly intended for lactate conversion. Lactate is a simple sugar that is essential for early embryonic development, which acts to protect cells at the cleavage stage from toxins, oxidative stress and infections (Hu and Yu, 2018). In cyclic and pregnant cows, the ampullary lactate concentration is higher than in the isthmus (Rodríguez-Alonso et al., 2020a). This suggests that besides oviduct adaptation to the estrous cycle and embryo presence, oviduct is able to produce lactate to help with embryo metabolism. The ATP production increases even before first cleavage, but the early embryo metabolism has low metabolic and nutrient uptake due to the minimal cell growth and membrane biosynthesis (Li and Winuthayanon, 2017; Hu and Yu, 2018). The early developing embryo has a low metabolic requirement originating the “quiet embryo” hypothesis (Leese, 2002). Leese (2002) suggests that the embryo should remain “quiet”, with reduced metabolism during early development in order to minimize the production of reactive oxygen species and other metabolic products harmful to the embryo during this vulnerable time period. Thus, is important that the oviduct can detect the embryo presence in order to regulate the right amounts of molecules to be secreted in its lumen.

As the embryo develops, mitochondrial maturation occurs and the embryo metabolism, which was previously oxidative, becomes glycolytic. Thus, the presence of glucose becomes essential for the compaction and embryonic genome activation (Li and Winuthayanon, 2017; Hu and Yu, 2018). The absence of glucose during compaction irreversibly decreases cell proliferation and increases apoptosis and oxidative stress (Jansen et al., 2008; Pantaleon et al., 2008). The concentration of glucose in the oviductal fluid is not static, that is, it varies according to oviductal need; however, during the stage of embryonic oxidative metabolism, it is essential that the concentration of this sugar stay low to avoid metabolic stress (Hu and Yu, 2018). Changes in glucose availability can accelerate or delay key events during the major genome activation shifting from oxidative to glycolytic metabolism (Harvey, 2019).

Therefore, as the gametes and embryo transit through the oviduct, the need for nutrients available in the oviductal fluid varies according to the gametic-embryonic metabolism. The oviductal fluid composition contributes to the embryo development and could modulate the maternal-embryonic communication processes even before the maternal gestation recognition. Present in oviductal fluid, the EVs acts in this modulation by mediating the bidirectional communication between oviductal epithelial cells (mother) and gametic-embryonic cells.

2.4.Role of EVs on oviductal dynamics and maternal-embryonic communication

In addition to substrates and cofactors, EVs are also present in the oviduct fluid (Al-Dossary et al., 2013) and play important roles in the oviduct dynamics environment and maternal-embryonic communication (Mazzarella et al., 2021). EVs are evolutionarily conserved mediators of cell communication (Fu et al., 2020). These biological nanoparticles use extracellular fluids to diffuse and interact with target cells in order to transfer their contents (Da Silveira et al., 2015, 2018; Gross et al., 2017) acting as vectors of biological information and being able to modify the cell function of recipient organs (Lawson et al., 2017). EVs are nanoparticles secreted by different cell types and initially classified into exosomes and microvesicles. Exosomes are small EVs originated from endosomes and have a 30-150 nm diameter; microvesicles are larger EVs, having 100-1000 nm and originated in the plasma membrane (Machtinger et al., 2016; Van Niel et al., 2018). The EVs isolated by serial centrifugation, filtered in > 0.20 µm filter and validated nanoparticle tracking analysis (*Nanotracking*), transmitted electron microscopy and specific proteins by western blot, are recently classified as small EVs, according to Minimal Information for Studies of Extracellular Vesicles guidelines (2018). Molecules present on the surface of EVs allows the interaction with target cells through their adhesion to lipids and receptor ligands, allowing their entry into cells through endocytosis or pinocytosis (Machtinger et al., 2016; Gross et al., 2017). In reproduction it was already demonstrated that EVs are able to modulate, cumulus-oocyte-complex maturation, embryonic development (Battaglia et al., 2019), as well as changes in global DNA methylation and hydroxymethylation levels of bovine embryos (Da Silveira et al., 2017).

In the oviduct, EVs were identified as key mediators components in the interaction between gametes and embryo, contributing to the pregnancy success (Almiñana and Bauersachs, 2020). The EVs that make up the oviduct fluid can have different origins: i) follicular fluid upon ovulation; ii) secreted by oviduct epithelial cells; iii) secreted by gametes; iv) secreted by the embryo (Figure 1). Although it is difficult to distinguish these EVs origins, in general they act on physiological and molecular functions influencing oocyte maturation, spermatic hyperactivation and embryonic development (Harris and Stephens, 2020). Importantly, it is possible that EVs act as fine-tuners of early reproductive events since these events can occur out of the reproductive tract. These interactions are possible due to the fact that EVs contain bioactive material such as proteins, lipids, mRNAs and miRNAs that are transferred to the target cells (Valadi et al., 2007; Da Silveira et al., 2015; Al-Dossary and Martin-DeLeon, 2016). Thus, the content of EVs in the oviduct can modulate cell function by increasing the delivery of transcripts, miRNAs and proteins, thus affecting translation of mRNAs into functional proteins (Bauersachs and Almiñana, 2020). MiRNAs are stable small non-coding RNA molecules involved in several cellular processes and indispensable for animal development, cell differentiation and homeostasis (Bartel, 2009, 2018; Gebert and Macrae, 2019). The role of miRNAs in the reproductive cycle is essential for gamete development, oocyte maturation, fertilization and early embryonic development (Hayashi et al., 2008). In the extracellular environment, miRNAs are fragile and, once inside the EVs, they are protected from degradation and can act as information vectors (Fu et al., 2020). Furthermore, EVs have powerful systemic access to the most varied and distant cells enabling miRNAs to fulfill autocrine, paracrine and endocrine signaling functions (Gross et al., 2017). Thus, EVs carrying messages including miRNAs could play an important role within the oviduct (Table 1).

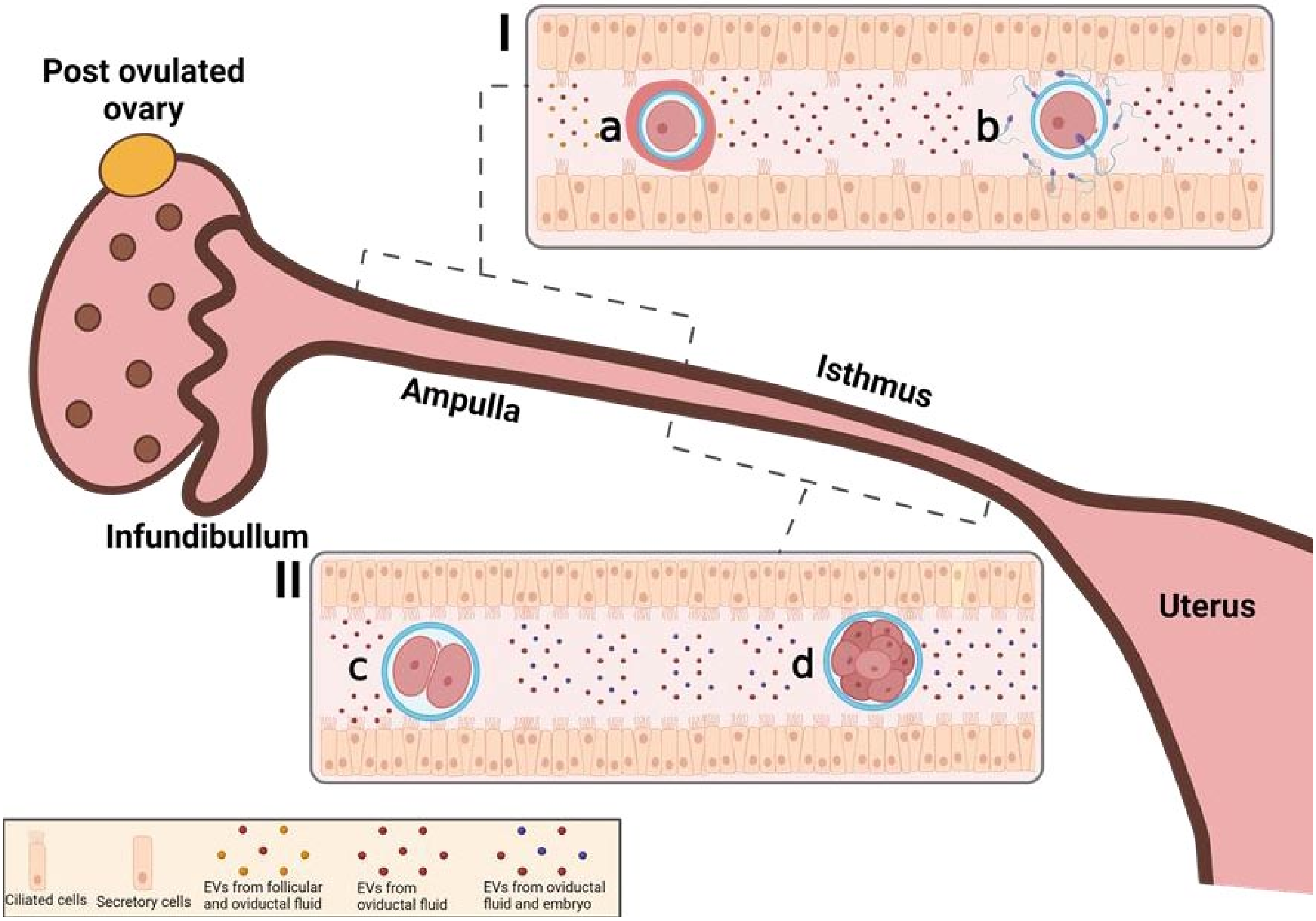

Figure 1. **Reproductive events occurring in the oviduct mediated by extracellular vesicles.** Schematic representation of female reproductive tract in post ovulation stage of estrus cycle and oviductal anatomic regions. The infundibulum is responsible for capturing recently ovulated cumulus oocyte complexes (COC). **(I)**The ampullary region is mainly composed by secretory epithelial cells which allows a high production of oviductal fluid which is also composed by follicular fluid. **(a)** Together these vesicles act at the oocyte final maturation. **(b)** At the ampulla region cumulus oocyte complexes and sperm will meet, and subsequently initiates the fertilization process. **(II)** The isthmic region is mainly composed by ciliated cells. **(c)** Once the fertilization happened, the initial embryo development occurs. **(d)** The embryo develops, activates its genome and modulates the isthmic region mediated by embryo and oviductal extracellular vesicles. This figure was Created with BioRender.com.

Table 1. The differential expression of miRNA content of oviductal extracellular vesicles and their predictive biological associated pathways.

Ovarian cycle stage[1]	**Embryo presence**	**Oviductal region**[2]	**miRNAs**	**Biological associated pathways**	**Reference**
S4 compared to other stages	No	All	miR-1291, miR-323, miR-631, miR130a, miR-433, miR-489, miR382, miR378	GnRH signaling pathway, FoxO signaling pathway, Vascular smooth muscle contraction, Signaling pathways puripotency of stem cells, Wnt signaling pathway	(HAMDI et al., 2021)
S2	Yes	Isthmus	miR-126-5p, miR129, miR-140, miR-188, miR-219, miR345-3p, miR-4523, miR-760-3p	Metabolic pathways, PI3K-Akt signaling pathway, MAPK signaling pathway, Endocytosis, Ras signaling pathway	(MAZZARELLA et al., 2021)
	No		miR-331-5p	cAMP signaling pathway, Insulin signaling pathway, Regulation of actin cytoskeleton, Wnt signaling pathway, Focal adhesion	
S1 and S4	No	All	miR-10b-5p	Pyrimidine metabolism	(ALMIÑANA et al., 2018)
			miR-423-5p	Fatty acid biosynthesis, Fatty acid metabolism	
			miR-449a	Carbon metabolism, HIF-1 signaling pathway	
			miR-375	Hippo signaling pathway, Amino sugar and nucleotide sugar metabolism	
			miR-24-3p	Fatty acid biosynthesis, Vitamin B6 metabolism, Endocytosis, Hippo signaling pathway, Bacterial invasion of epithelial cells	
			miR-148a-3p	Fatty acid biosynthesis, Steroid biosynthesis, Oocyte meiosis, Progesterone mediated oocyte maturation, FoxO signaling pathway	
			miR-429	Axon guidance, FC gamma R-mediated phagocytosis, Steroid biosynthesis, Progesterone mediated oocyte maturation, Gap junction	
			miR-34b-3p	Glycosaminoglycan degradation	
			miR-200b-3p	Ras signaling pathway, Neurotrophin signaling pathway	
			miR-92a-3p	Cell cycle, Adherens junction, Thyroid hormone signaling pathway, FoxO signaling pathway, RNA transport, Signaling pathways regulating pluripotency of stem cells	
			miR-151a-3p	Biosynthesis of unsaturated fatty acids, Fatty acid metabolism	
			miR-30d-5p	Mucin type O-, Glycan biosynthesis, Oocyte meiosis, Ubiquitin mediated proteolysis, mRNA surveillance pathway	
			miR-125b-5p	ErbB signaling pathway, Regulation of actin cytoskeleton	

[1] Ovarian cycle stage: S1: postovulatory-stage; S2: early luteal phase; S3: late luteal phase; S4: pre-ovulatory stage.
[2] Oviductal region: The total oviductal structure was flushed (ampullary and isthmic region).

In the oviduct, embryos stay and develop in a short period of time (4 – 5 days), but this can have great consequences at later stages of development (Fu et al., 2020). Zygotes and pre-implantation embryos are not in direct contact with the oviduct because they still have the zona pellucida (ZP), which is resistant to the uptake of exogenous genetic material, although the biological action of EVs allows these molecules to enter the ZP and perform functions in the embryo (Fu et al., 2020). Interestingly, the oviductal EVs miRNAs cargo are related to embryonic development, embryonic morphology and implantation (Almiñana et al., 2018). EVs originated from oviductal fluid and *in vitro* culture of bovine oviduct epithelial cell (BOEC) were able to internalize in *in vitro* produced bovine embryos, increasing production rates, prolonging embryo survival and to improving their quality and cryoprotection (Lopera-Vasquez et al., 2016; Almiñana et al., 2017). Additionally, oviductal EVs contain mRNAs associated with epigenetic DNA modifications, indicating that these biological nanoparticles can control chromatin modification and epigenetic regulation in the developing embryo (Almiñana et al., 2018). Moreover, besides the oviductal EVs addiction in IVEP did not affect the blastocyst production rates and embryo cryotolerance, the oviductal EVs were able to modulate the blastocyst phospholipid content by making it more abundant in phosphatidylcholines (PC), phosphatidylethanolamines (PE) and sphingomyelins (SM) with long-chain fatty acids (Banliat et al., 2020). This is interestingly because when analyzing the oviductal EVs lipidic content, were found that the overabundant masses in blastocysts were 100% also present in oviductal EVs (Banliat et al., 2020). This suggests that in vitro produced embryos can incorporate the lipidic EVs content which may modulate the embryonic lipidic metabolism. Thus, suggesting that EVs from the oviduct can impact bovine embryos *in vivo* and *in vitro*.

As already discussed, the oviduct is a dynamic structure capable of adapting during reproductive events (maturation, fertilization and early embryonic development) under the action of ovarian hormones. According to Rodríguez-Alonso et al. (2020a) oviductal fluid composition is anatomically dynamic and affected by embryo presence. Since the content of EVs reflects the cells of origin, changes in the epithelium cellular morphology can induce changes in the biological functions of EVs (Al-Dossary and Martin-DeLeon, 2016; Almiñana and Bauersachs, 2020). These changes could lead to changes in secretion and contents of EVs during the estrous cycle. At the estrous cycle phases (postovulatory-stage, early luteal phase, late luteal phase and pre-ovulatory stage), the oviductal fluid EVs RNA and protein contents are different and many of these molecules are related to gametic interaction and pre-implantation embryo development (Almiñana et al., 2018). Another recent study assessing the miRNA profile from oviductal and uterine fluid EVs, showed that the estrous cycle change the EV cargo (Hamdi et al., 2021). Together, this information suggests that these EVs are under hormonal control and indicates the crucial role of EVs in reproductive events. Furthermore, the isthmic EVs culture medium supplementation used in IVEP induces greater blastocysts rates (91.3%) when compared to ampullary EVs (62.2%) (Lopera-Vasquez et al., 2016). In addition, the results suggest that isthmus EVs may contribute to the normal regulation of the methylation pattern in embryos and improvement of embryonic cryopreservation, indicating that the content of EVs may be anatomically variable. However, studies analyzing the EVs content through the oviductal regions should be performed to better elucidate their function at the anatomical regions. Finally, in a recent study, although the size and concentration of EVs has not been altered, the miRNA profile from isthmus EVs of Nelore cows is altered in the presence of a single embryo (Mazzarella et al., 2021). Using a miRNAs analysis, the authors identified predicted biological pathways regulated by EVs miRNAs which were involved with the immune system suggesting that EVs can possibly mediate maternal-embryonic communication even before pregnancy recognition. Additionally, EVs from good quality embryo and degenerating embryo were supplemented to primary BOEC monolayer culture (Dissanayake et al., 2020). Genes related to interferon-τ-induced genes were upregulated at the BOEC monolayer suggesting that the embryos EVs modulates the oviduct in response to their quality (Dissanayake et al., 2020). Furthermore, it was already demonstrated that EVs can carry molecular signals in response to environmental factors such as environmental stress and body energy balance (Tesfaye et al., 2020).

Thus, EVs can act mediating the bidirectional crosstalk between mother and gametes/embryo, helping to fine tuning the oviduct and endometrium contributing to successful embryo development and implantation. However, these type of studies that analyze the role of these EVs in embryonic development are still recent (Al-Dossary et al., 2013) and compose promising lines of research, once they importance to oviductal environment and embryo development. Additionally, it is possible that EVs generate specific biological environments providing the physiological basis for oocyte maturation, fertilization and early embryonic development within the oviduct.

Despite the evidences that oviductal EVs and their contents can play important roles in oocyte maturation in the oviduct, due to the recent discovery of oviductal EVs (Al-Dossary et al., 2013) and the difficulty of obtaining oviductal samples, additional analysis are still necessary to demonstrate the action of these nanoparticles in the final oocyte maturation.

2.5.Conclusions

Anatomically the oviduct is a small structure where the embryo develops in a short period of time (4 – 5 days), furthermore this reproductive structure has a huge importance at early embryonic development and subsequently pregnancy establishment. This is due to the unique microenvironment that the oviduct provides to gametes and embryos, built under the influence of ovarian hormones, oviduct fluid composition and EVs mediation. As future perspectives, the understanding of EVs contribution to the oviductal environment fine tuning can help to better mimic the *in vitro* environment during IVEP and contribute to increase quality of *in vitro* produced embryo.

2.6.References

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3. Chapter 2: High body energy reserve influences extracellular vesicles miRNA contents within the ovarian follicle

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Short title: Body energy reserve influences intercellular communication within small ovarian follicle.

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Abstract

Aiming to evaluate the effects of increased body energy reserve (BER) in Nellore cows' reproductive efficiency, cows were fed with different nutritional plans to obtain animals with high BER (HBER; *Ad libitum* diet) and moderate BER (MBER: cows fed 70% of HBER group ingestion). To evaluate the BER, cows were weekly weighted and evaluated for subcutaneous fat thickness and insulin serum concentration along the experimental period. At the end of the experimental period, animals were submitted to estrous synchronization and artificial insemination. Animals were slaughtered approximately 120 h after ovulation induction and the reproductive tracts were collected for embryo recovery and samples collection. Cumulus-oocyte-complexes (COC) and follicular fluid were collected from 3-6 mm in diameter ovarian follicles to perform miRNA analysis of cumulus cells (CC) and extracellular vesicles from follicular fluid (EV FF). As expected, differences were observed among MBER and HBER groups for body weight, fat thickness, and insulin serum concentration. HBER animals showed lower ovulation and embryo recovery rates compared to MBER animals. Different miRNAs were found among CC and EV FF within groups, suggesting that the BER may influence follicular communication. This suggests that small follicles (3-6 mm diameter) are already under BER effects, which may be greater on later stages of follicular development.

Keywords: Dry Matter Intake; Metabolism; MiRNA; Reproductive Function; Subcutaneous Fat Thickness.

3.1. Introduction

Animal nutrition acts on the development of body tissues, homeostasis, and metabolic system, reflecting on the animal's body energy reserve (BER) and consequently directly affecting the reproductive performance of beef cows (D'OCCHIO; BARUSELLI; CAMPANILE, 2019). Animals in extreme conditions (too thin or too fat) have greater risks of metabolic changes, diseases, and reproductive problems such as dystocia, low conception rates, and prenatal/postpartum problems (ARNETT; HOLLAND; TOTUSEK, 1971; HERD; SPROTT, 1986; FUNSTON; SUMMERS; ROBERTS, 2012). Moderate BER, that is, body condition score (BCS) 5, on a 1-9 scale, or 16% of body fat, proves to be optimal for beef cows pregnancy rate (HERD; SPROTT, 1986). However, beef females with higher values than those (high BER, BCS up to 6, and body fat more than 20%) can have recurrent reproductive problems (ARNETT; HOLLAND; TOTUSEK, 1971; HERD; SPROTT, 1986). Compared to cows with adequate body conditions during the reproductive season, beef cows with high BER presented pregnancy rate 8.8% lower through fixed-time artificial insemination (FTAI) (CARVALHO et al., 2014). Once bulls and cows with high BER represent most of the show cattle and genetic donors, the effects of the altered BER on the reproductive function of these animals must be elucidated.

The plane of nutrition of the herd has a rapid and cumulative effect over time on the reproductive function of cows as it is closely linked to BER, due to its effect on follicular development, oocyte/embryonic quality, and pregnancy rate (ADAMIAK et al., 2005; HERNANDEZ-MEDRANO; CAMPBELL; WEBB, 2012; CARVALHO et al., 2014). The reason why this occurs is related to several physiological factors, including modulation of hypothalamic-pituitary-ovarian axis by hormones and metabolic substrates such as insulin (WEBB et al., 2004), which plays important roles in reproductive physiology, acting directly on ovarian follicles, oocytes, and embryo development (BOSSIS et al., 2000; BUTLER, 2000; THOMPSON, 2006; D'OCCHIO; BARUSELLI; CAMPANILE, 2019). Changes in insulin serum concentrations can alter ovarian response by modifying the follicular metabolic balance which allows changes in the local microenvironment influencing the gametic and subsequent embryonic quality (HERNANDEZ-MEDRANO; CAMPBELL; WEBB, 2012).

The follicular environment, composed by theca cells, granulosa cells, cumulus cells, oocyte, and follicular fluid (FF) is influenced by autocrine, paracrine, and endocrine factors (RODGERS; IRVING-RODGERS, 2010) and by bidirectional communication between somatic and gametic cells (DA SILVEIRA et al., 2012). FF is an exudate from the capillaries blood flow around the follicles (KNIGHT; GLISTER, 2006) that bathes and supports the communication between follicular cells. This communication process can also be mediated by extracellular vesicles (EVs) (DE ÁVILA et al., 2020). These bioactive nanoparticles are able to intermediate the cell communication by carrying lipids, proteins, mRNAs, and miRNAs that represent the partial secretory cell content to target cells (VALADI et al., 2007; DA SILVEIRA et al., 2015; AL-DOSSARY; MARTIN-DELEON, 2016). MiRNA are small RNA molecules (approximately 22 nucleotides) that do not encode proteins (non-coding RNA). Its function is related to post-transcriptional regulation(BARTEL, 2004). MiRNAs are transcribed in the nucleus and after being transferred to the cytoplasm they are processed and loaded into the RNA-induced silencing complex (RISC) to pair with the target messenger RNA in the 3'-UTR region, repressing its translation (BARTEL, 2004, 2009). Thus, intrafollicular cell communication mediated by EVs can occur by the transfer of bioactive material (DA SILVEIRA et al., 2012), such as miRNA, in response to environmental factors as metabolism (TESFAYE et al., 2020).

Although different studies have described the diet effects (YAAKUB; D O'CALLAGHAN; BOLAND, 1999), nutritional management (SANGSRITAVONG et al., 2002a; MOLLO et al., 2017) and metabolism (ADAMIAK et al., 2005; BARLETTA et al., 2017) on ovarian response, oocyte and embryonic quality, little is known about these reproductive responses after increasing BER in beef cows. Therefore, the hypothesis for this work is that the increase of BER in Nellore cows negatively influences ovulation and embryo recovery rates by changes in the miRNA profile of extracellular vesicles present within the follicular environment. Thus, the aim of this work was to understand the effects of altered BER on ovulation, embryo recovery and miRNA profile of extracellular vesicles present within the follicular environment in multiparous Nellore cows with different BER.

3.2. Material and Methods

All experimental procedures were approved by the College of Animal Science and Food Engineering, University of São Paulo (FZEA/ USP) Ethics Committee (protocol number: 1522231019). The experiment was performed at Experimental Confinement unit from the Department of Animal Science, and at the Laboratory of Morphophysiology and Molecular Development (LMMD) of the Department of Veterinary Medicine, both located at the University of São Paulo (Campus of Pirassununga, SP, Brazil).

3.2.1. Animals and nutritional management

Nellore cows from a single herd were previously evaluated by different parameters to select uniform animals. In this way, 40 Nellore cows were housed in four pens (10 x 16 m; 10 cows/pen; 16m²/cow) equipped with electronic gates (American Calan Inc., Northwood, NG, USA) that allow individual control of feed intake. The pens had concrete-floors and partial roof coverings. Cows had access to fresh ad libitum water during the entire experimental period.

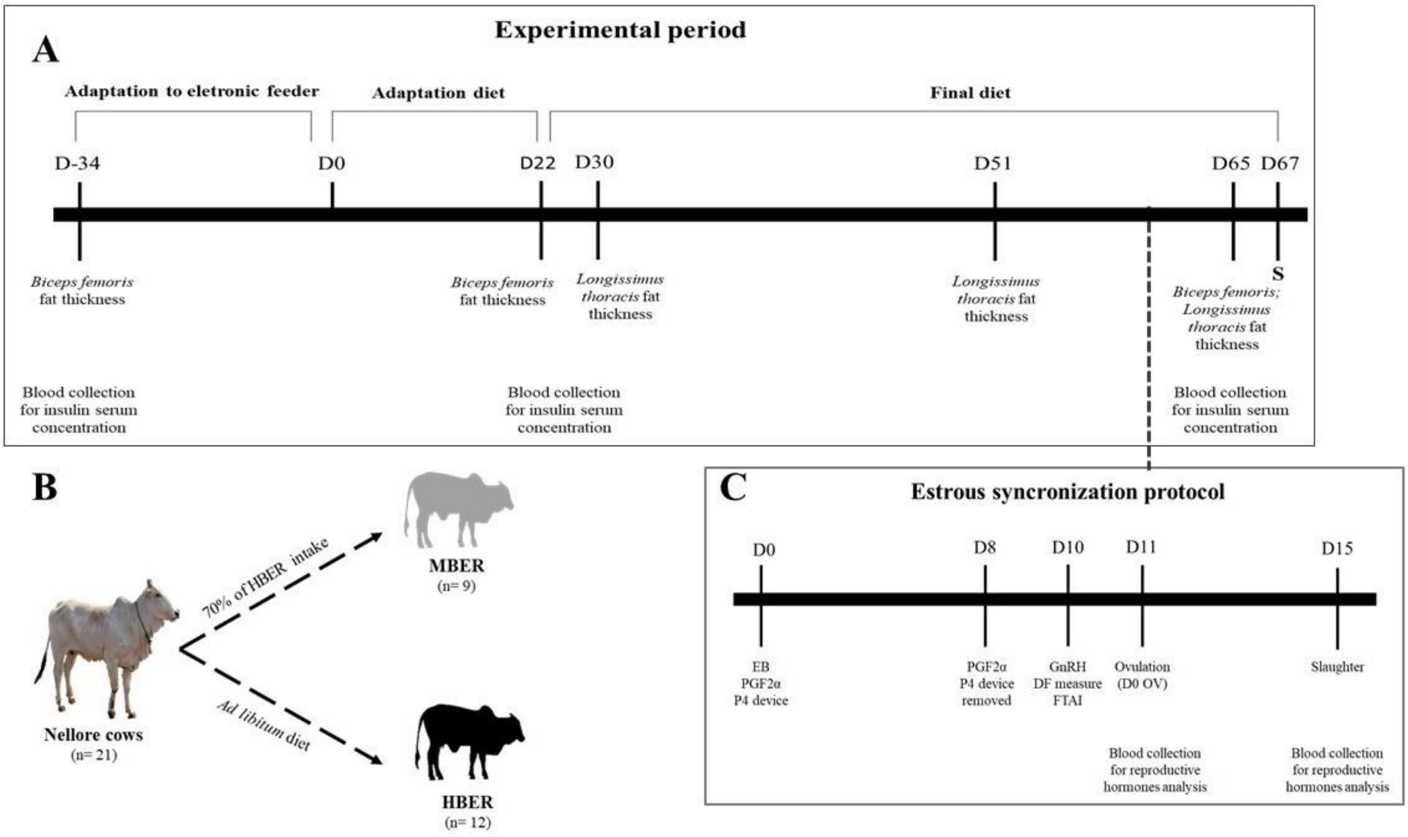


Figure 1. Schematic representation of experimental period. A. Timeline of feedlot period showing days of adaptation to electronic feeder (D-34 to D-1); adaptation diet (D0 to D21) and final diet (D22 to Slaughter (D67); S). During the feeding period the animals were submitted to: Subcutaneous fat thickness analysis of *Biceps femoris* (D-34, D22 and D65) and *Longissimus thoracis* (D30, D51 and D65); blood collection for Insulin serum concentration (D-34, D22 and D65); and weekly weighing average daily gain of each animal. **B.** Two nutritional plans were defined for cows with similar body conformations from a same herd in order to obtain cows with moderate body energy reserve (MBER) and cows with high body energy reserve (HBER) at the end of the experimental period. **C.** At the end of the experimental period, the animals were submitted to an estrous synchronization protocol (EB: estradiol benzoate, PGF2α: prostaglandin- 2α; P4: progesterone; GnRH: gonadotropin-releasing hormone), dominant follicle (DF) measure, artificial insemination (FTAI) and blood collection for reproductive hormones concentration (Day scheduled for ovulation; D0 OV and S).

Before beginning the trial, animals received, individually, free corns silage and mineral supplement ad libitum while adapting to the electronic gates (approximately 30 days; Figure 1A). Cows that did not adapt to the feeding system, were removed from the experiment. Thus, 21 Nellore multiparous (3,45 ± 0,54 number of births), non-lactating and non-pregnant cows (6.06 ± 0.54 years old and 1.44 ± 0.0083 m of withers height) were randomly divided in two groups with similar values for body weight (510.67 ± 15.55 kg) and Biceps femoris subcutaneous fat thickness (11.31 ± 1.20 mm) which represents similar values for BER, and submitted to an experimental period in order to became different in BER at the end of the feedlot period.

Thus, two nutritional plans were defined to obtain cows with moderate body energy reserve (MBER; n=9) and cows with high body energy reserve (HBER; n=12). For this, the animals belonging to the HBER treatment received ad libitum diet in order to increase body energy reserves during the experimental period that is, increasing body weight (BW) and fat thickness, while the other group (MBER) was fed 70% of the daily feed of HBER treatment (Figure 1B) to keep the BW and energy reserve throughout the experimental period.

The total feeding period had 67 days, including an adaptation period (21 days). The adaptation period consisted of 3 diets of 7 days each one (Supplementary Table 1) with increasing proportions of concentrate and corn silage ratio (40:60; 50:50 and 58:42, respectively) on DM basis, totalizing the 21 days as mentioned before. The final diet contained a 62.5:37.5 ratio of concentrate and corn silage (on DM basis; Table 1). The mineral supplement used contained Ca (208 g/kg); Co (148 mg/kg), Cu (2.7 mg/kg), S (64 g/kg), F (1.6 mg/kg), P (160 g/kg), I (141 mg/kg), Mn (2.2 mg/kg) Se (37 mg/kg), Zn (79.92 mg/kg) and sodium monensin (4000 mg/kg). Diets were formulated to meet the nutritional requirements of Nellore cows, according to Cornell Net Carbohydrate and Protein System (CNCPS; (FOX et al., 1992))

Table 1. Ingredients and chemical composition on a dry matter (DM) basis (g/kg) of final diet.

Ingredients[1]	**g/kg of DM**
Corn silage	375
Finely ground corn	510
Soybean meal	83.8
Mineral supplement	6
Limestone	9.2
Urea	6.9
Potassium chloride	5.0
Salt	4.3
Chemical composition	
Dry matter	523.5
Mineral matter	46.0
Crude protein	151.3
Ether extract	30.9
Acid detergent fiber	207.3
Neutral detergent fiber	296.7
Total digestible nutrients	775.3

[1] Ingredients: Mineral supplement: Ca (208 g/kg); Co (148 mg/kg), Cu (2.7 mg/kg), S (64 g/kg), F (1.6 mg/kg), P (160 g/kg), I (141 mg/kg), Mn (2.2 mg/kg) Se (37 mg/kg), Zn (79.92 mg/kg) and sodium monensin (4000 mg/kg)

The experimental diet was offered individually three times a day, using the following proportion: 30% of the daily offer at 8:00 am, 40% at 2:00 pm and 30% at 6:00 pm. Orts from the HBER group that should not exceed 50 g/kg were removed daily, weighted and used to determine the amount of feed to be offered on the next day based on the dry matter intake (DMI) of the previous day. Samples of the total diet were collected weekly for further chemical analysis.

In order to reach the maintenance and progression of energy reserves in MBER and HBER cows, respectively, the animals were weighted weekly to determine the BW. Analyzes of rump fat thickness and blood collection to serum concentration of insulin were performed throughout the experimental period as the HBER animals increased BW

(Figure 1A). The experimental period respected the individual animal and group performances in the experimental treatments. Thus, there was no fixed number of experimental days, thus once groups become with different BER, the experimental period has come to an end. The amount of weight that the animals gained daily during the entire experimental period was counted, obtaining the average daily gain (ADG), and after slaughter, was obtained the hot carcass weight and carcass yield.

3.2.2. Feed sample collection and chemical analysis

Diet samples were collected weekly throughout the study and dried at 105ºC for 24 h (AOAC - ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS, 1990) for dietary DM adjustments. Samples were stored at -20ºC until the end of experiment when they were grouped into composite samples, dried in a forced-air oven at 55ºC for 72 h, and ground with a 1 mm screen (Wiley mill, MA-680 Marconi Ltda, Piracicaba, SP, Brazil) for subsequent analyses. The samples were analyzed for DM in an oven at 105ºC for 24 h (AOAC, 1990), mineral matter (MM; (AOAC - ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS, 1990)), crude protein (CP) by the Kjeldahl method (KJELDAHL, 1883), ether extract (EE; (AOAC - ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS, 1990)), neutral detergent fiber (NDF; non-sequential and ash-free; (VAN SOEST; ROBERTSON; LEWIS, 1991)), acid detergent fiber (AFD) and acidic lignin as method 973.18 (VAN SOEST; ROBERTSON; LEWIS, 1991), and total digestible nutrients (TDN; (NASEM, 2016)) (Table 1). The NDF assay used a heat stable source of amylase and urea as recommended by the National Forage Testing Association (UNDERSANDER; MERTENS; THIEX, 1993). Each sample received α-amylase (Sigma A3306; Sigma-Aldrich Brazil Ltda, São Paulo, SP, Brazil) and urea separately for NDF determination. Both NDF and ADF were expressed including residual ash (Table 1).

3.2.3. Subcutaneous fat thickness analysis

Subcutaneous fat thickness was evaluated by ultrasonography (SSD 500 Micrus, Aloka Co. Ltd.) with a linear transducer of 3.5 mHz and 172 mm in length over the *Biceps femoris* (between the ilium and ischium) and also over the *Longissimus thoracis* (between the 12th and 13th rib) muscles (SANTANA et al., 2012) were collected and analyzed using the Lince® software (M&S Consultoria Agropecuária Ltda. Pirassununga, SP, Brazil). Analyzes of subcutaneous fat thickness were performed over *Biceps femoris* on days -34, 22 and 65 of the experimental periods (Figure 1A). The *Longissimus thoracis* ultrasonography analyses were performed after starting the final diet on days 30, 51, 65 (Figure1A).

3.2.4. Blood collection and biochemical analysis

Blood samples from all animals were collected from the jugular vein in silica vacutainer tubes (one collection per animal; BD, São Paulo/SP, Brazil). Blood serum was obtained by centrifugation at 1500xg for 30 min in room temperature and was used to quantify insulin, estrogen (E2), progesterone (P4).

To quantify serum insulin concentration, blood samples were collected on days -34, 22 and 65 of the experimental period (Figure 1A). Hormone concentration were performed by enzyme-linked immunosorbent assay (ELISA; Insulin Accubind ELISA, 1936, Monobind), by the Animal Physiology Laboratory of the University of São Paulo (FZEA/USP) (NASCIMENTO et al., 2015; SOUZA et al., 2019; BROLEZE et al., 2020). Intra and interassay variation coefficients were 4.40% and 6.35%, respectively.

Blood samples to quantify serum concentrations of the reproductive hormones E2 and P4 were collected after the start of the estrous synchronization protocol, on the day scheduled for ovulation (D0 OV) and on the day of slaughter (S; Figure 1C). Serum hormone measurements were performed using chemiluminescence (ADVIA Centaur ® - Siemens) by the Pasin laboratory (Santa Maria – RS) as previously used by De Ávila et al. (DE ÁVILA et al., 2020). Intra and interassay variation coefficients were 3.30% and 24.20% respectively for E2; 0.21% and 0.20%, respectively for P4.

3.2.5. Estrous synchronization protocol, artificial insemination, slaughter and embryo recovery

At the end of the experimental period, animals were submitted to estrous synchronization (sync) protocol (Figure1C). On the first day (D0 sync), cows received 2 ml of estradiol benzoate (2 mg; Sincrodiol®, Ourofino Agronegócio) intramuscularly, 2 ml of PGF2α (0.52 mg; Prostaglandin- 2α; Sincrocio®, Ourofino Agronegócio) intramuscularly and insertion of an intravaginal progesterone device (1g; Sincrogest®), Ourofino Agronegócio) which was withdrawn on the 8th day (D8 sync). Still on D8 sync, animals received 2 mL of intramuscular PGF2α at the time of removal of the intravaginal progesterone device. In D10 syn 2.5 mL of GnRH (0.0010 mg; Gonadotropin-releasing Hormone; Sincroforte®, Ourofino Agronegócio) was administered intramuscularly, the diameter of the dominant follicle (DF) was analyzed by ultrasound (MyLab Delta, Esaote, Italy), and after 12 h of GnRH administration it was performed the artificial insemination. All animals were inseminated with semen from a single bull with previously assessment confirmation of fertility. Confirmation of ovulation was performed 12 h after fixed-time artificial insemination (FTAI) by ultrasound (D0 OV).

Approximately 120 h after ovulation induction, cows were slaughtered (in FZEA/USP abattoir in accordance to the Humanitarian Slaughter Guidelines required by Brazilian laws) and the reproductive tract removed, which was immediately transported to the laboratory to obtain the samples (Supplementary Figure 1 A and B). The oviducts ipsilateral (same side) to the corpus luteum were dissected (Supplementary Figure 1 C) and the isthmus portion was flushed in the ovary-uterus direction with 1 mL of phosphate-saline solution free of calcium and magnesium (1xPBS free of Ca^{2+} and Mg^{2+}) for embryo recovery with the aid of a magnifying glass (Supplementary Figure 1 D and E).

3.2.6. Follicular aspiration

From the 21 cows used at the experimental period (MBER: n=9; HBER: n=12), only animals which presented ovulation and absence anovulatory follicle at D0 OV had the ovaries ipsi and contralateral collected (MBER: n=8; HBER: n=4) to obtain cumulus cells

(CC) and follicular fluid extracellular vesicles (FF EVs). Ovarian follicles measuring 3-6 mm in diameter (Supplementary Figure 1 F) were punctured with an 18G needle and a 10 mL syringe to obtain cumulus-oocyte-complexes (COC) and follicular fluid (FF).

Groups of five COCs were pooled from ipsi and contralateral ovarian follicles (totalizing 10 COCs per animal) and completely denuded in 1xPBS free of Ca^{2+} and Mg^{2+} in drops by pipetting. The CC obtained from COCs groups (one pool per each ipsi and contralateral ovaries per cow; MBER: n=8; HBER: n=4), were centrifuged twice at 300xg for 5 min and after having the supernatant removed, the cells were immediately frozen in liquid nitrogen for further analysis of the miRNA content. FF was collected to obtain EVs (from all aspirated follicles in ipsi and contralateral ovaries; MBER: n=8; HBER: n=4) to perform FF EV characterization and miRNA profile.

3.2.7. Isolation of small extracellular vesicles from follicular fluid

From the FF collected reported above, was obtained the FF EVs as previously described by De Ávila et al. (DE ÁVILA et al., 2020). Briefly, the samples were diluted in 500µl of 1xPBS free of Ca^{2+} and Mg^{2+} and centrifuged at 4ºC at 300xg for 10 min to remove cells, 2000xg at 4ºC for 10 min to remove cell debris, 16500xg at 4ºC for 30 min to remove larger extracellular vesicles. To obtain the pellet enriched in small extracellular vesicles (smaller than 200 nm), 800µl of the resulting supernatant were filtered through a 0.20µm pore filter (Corning, 431229) and ultracentrifuged at 119700xg by 70 min at 4ºC (Optima XE-90 Ultracetrifuge; 70 Ti rotor; Beckman Coulter). Then, the obtained pellet was washed in 1xPBS and ultracentrifuged again at 119700xg for 70 min to 4ºC. The pellet enriched with small extracellular vesicles was eluted in 50µl of 1xPBS free of Ca^{2+} and Mg^{2+} for further characterization and miRNA content analysis.

3.2.8. Characterization of small extracellular vesicles from follicular fluid

The EVs FF characterization consists of nanoparticle tracking analysis (NTA), transmission electron microscopy and qualitative protein markers by Western Blot following the Minimal Information for Studies of Extracellular Vesicles 2018 (MISEV 2018) recommendations (THÉRY et al., 2018). To perform the transmission electron microscopy and Western Blot analyses, slaughterhouse samples were collected and processed with the same protocol to avoid the lack experimental samples of EVs FF for tracking nanoparticles and miRNA content analysis.

To perform NTA analysis, after EVs FF isolation, 5µl of the eluted pellet was diluted in 995µl of 1xPBS free of Ca^{2+} and Mg^{2+}, of which 10µl were diluted again in 990µl of 1xPBS free of Ca^{2+} and Mg^{2+} for particle size and concentration evaluation using the Nanosight device (NS300; NTA 3.4 Build 3.1.45; Malvern). Five 30-second videos were taken at a controlled temperature of 38,5ºC, Camera Level 13 and the analyzes were performed considering threshold 5.

For transmission electron microscopy, after isolation by serial centrifugation, the EVs pellets were diluted in a fixative solution (0.1M Cacodylate; 2.5% Glutaraldehyde; 4% paraformaldehyde; pH 7.2 - 7.4) for 2 h to be ultracentrifuged again and resuspended in 20 µl of ultrapure milli-Q water. The analyzes were realized at the Multiuser Laboratory of Electronic Microscopy of the Department of Cellular and Molecular Biology, Faculty of Medicine of Ribeirão Preto, using a transmission electron microscope (FEI 200kV, model Tecnai 20, emitter LAB6).

To verify the presence of specific EVs proteins in FF by Western Blot, after EVs isolation protocol, samples were resuspended in 20 uL of lysis buffer (RIPA), stored on ice under constant agitation. The samples were applied on SDS-PAGE polyacrylamine gel 6% and 15% for better representation of the proteins, and the run was performed at 100 V for approximately 120 min prior to the wet transfer to nitrocellulose membrane at 80 V for 120 min. After the transfer, membranes were kept 1 h in blocking solution (3% nonfat dry milk in TBST – Tris buffered saline with Tween-20), prior to the incubation with primary antibodies overnight at 4ºC. The specific vesicle proteins studied were ALIX (rabbit, sab4200476, Sigma-Aldrich, St. Louis, MO, USA), CD9 (mouse, sc-13118, Santa Cruz, CA, USA) and the non-vesicular protein control was GRP78 (protein from endoplasmic reticulum; mouse, sc-376768, Santa Cruz) (THÉRY et al., 2018). After the incubation period, the membrane was washed three times in TBST for 5 min and maintained for 1 h in anti-rabbit (A0545sc-2357 Sigma-Aldrich, St. Louis, MO, USA) and anti-mouse (#7076S, Cell Signaling Technology) secondary antibody. Then, the secondary antibodies were removed, the membrane washed and exposed to the developing solution (170-560, Clarity Western ECL), for analyzes carried out by the ChemiDoc MP Image System (Bio-RAd, Hercules, CA, USA).

3.2.9. Total RNA extraction and miRNA analysis

Total RNA content from CCs and EVs FF (MBER: n=8; HBER: n=4) was extracted using Qiazol (QIAGEN) according to the manufacturer's instructions, adding 1.33µl of GlycoBlue coprecipitator (Thermo Fisher Scientific) to the aqueous phase before RNA precipitation (DA SILVEIRA et al., 2018). RNA quantity and quality were analyzed by spectrometry (NanoDrop 2000, Thermo Fisher Scientific; absorbance ratio 260/280nm) and treated with DNaseI (Invitrogen; Carlsbad, CA) according to the manufacturer's instructions. After extraction, the RNA was stored at -80ºC until use.

The total RNA was reverse transcribed into cDNA using the miScript II RT kit (QIAGEN), using the miScript HiSpec Buffer to obtain mature miRNAs in CC and miScript HiFlex Buffer to obtain mature and precursor miRNAs in FF EVs. CC reactions contained 100 ng of total RNA, while for EVs FF reactions were performed with 200 ng of total RNA. Both were performed in a thermocycler (Life Technology) at 37ºC for 60 min, followed by 95ºC for 5 min. The RT-PCR reactions to quantify the transcripts were performed according to Da Silveira et al. (DA SILVEIRA et al., 2018), using at least 0.2 ng of cDNA and 1µl of 10 mM forward primers obtained based on the mature bovine miRNAs sequences available in the mirBase software (http://www.mirbase.org). The temperatures and times used were 95ºC for 15 min followed by 45 cycles of 94ºC for 15 seconds, 55ºC for 30 seconds and 70ºC for 30 seconds. For each sample (CC and FF EV) and group (MBER: n=8; HBER: n=4), the expression analysis of 383 bovine miRNAs was performed using the same forward sequences as published before (DA SILVEIRA et al., 2018; BRIDI et al., 2021). The miRNAs were considered present when they presented a cycle threshold (CT) lower than 37 in all biological repetitions and an appropriate melting curve in more than 50% of samples; the miRNAs were considered exclusive when were expressed in all samples versus none samples. CT data generated by amplification were normalized using bta-miR-99b as previously published (DA SILVEIRA et al., 2017). The miRNAs differently expressed between groups were evaluated by miRWalk software version 3.0 in order to identify predicted regulated pathways. Predicted pathways were considered significant when adjusted *P*-value < 0.05.

3.2.10. Statistical analysis

The animal's BER (BW and fat thickness), and hormone levels (insulin, E2 and P4) data were analyzed using a model including the fixed effects of the BER class (MBER and HBER), the time class (days in experimental period), and the interaction between BER and time. When significant effects were found (*P*<0.05), the least-square means were compared using the Tukey test. The heigh of withers, DMI, ADG, carcass traits (weight and yield), response to the estrous synchronization protocol, EVs size and concentration and miRNA data were analyzed using the fixed effect of the body energy reserve. The means were adjusted by the least-squares method and compared using the probability of difference using the Student's *t* test. The reproductive analyzes (ovulation, and embryo recovery rate) were analyzed using the chi-square test. All the analyses utilized the program "JMP" (7.01 version, Statistical Analysis Software Institute, SAS® Inc., Cary, NC). A significant difference was declared when *P*<0.05.

3.3. Results

3.3.1. Feedlot performance and carcass traits

In order to establish two experimental groups, 21 cows were divided into moderated and high BER (MBER: n=9; HBER: n=12), animals with similar BER and wither height, were submitted to different DMI according to the experimental groups (MBER and HBER) presenting different DMI (*P*<0.0001) and ADG (*P*<0.0001) until the end of the experimental period, being HBER with higher values than MBER animals (Supplementary Table 2). As expected, similar cow's BW was observed at the beginning of the study (D-34; MBER: 498.00 kg ± 24.49 kg; HBER: 520.17 ± 22.32 kg, *P* = 0.4947, Figure 2A); however, at the end of the experimental period the HBER group was 121.22

kg heavier than MBER group (at D67: MBER: 503.11 kg ± 17.66; HBER: 624.33 ± 23.25 kg, *P* = 0.0009). At slaughter (D67), animals with different body energy reserves had different hot carcass weight (MBER: *P* = 0.002) and carcass yield (*P* = 0.0331; Supplementary Table 2).

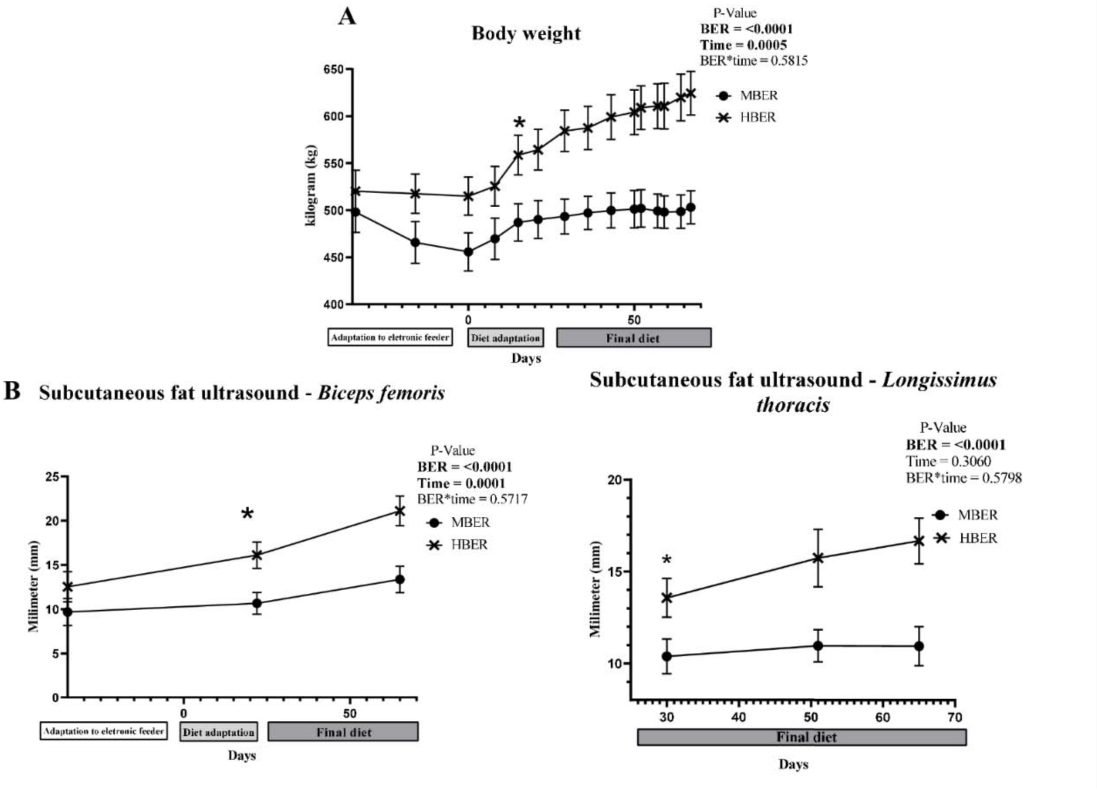


Figure 2. Body weight and subcutaneous fat ultrasound of Nellore cows during the experimental period. A. Evolution of body weight of cows with different energy reserves. **B.** Analysis of subcutaneous fat thickness of *Biceps femoris* and *Longissimus thoracis* measured by carcass ultrasound of cows with different energy reserves. Mean standard error for body energy reserve, time and body energy reserve and time interaction. P-values are on the right top of the Figures. *Beginning and prevalence of statistical differences ($P<0.05$).

Biceps femoris fat thickness measurements by ultrasound were similar at the beginning of the study for the two groups of cows (MBER: 9.68 ± 1.53 mm; HBER: 12.53 ± 1.72 mm, *P*=0.2478). However, at the end of the feedlot period (D67), cows from HBER group had superior *Biceps femoris* fat thickness (21.11 ± 1.68 mm) in comparison with MBER (13.35 ± 1.51 mm, *P* = 0.0037, Figure 2B). For the *Longissimus thoracis,* the analyzes performed by ultrasound after the beginning of the finishing diet (MBER: 10.38 ± 0.94 mm; HBER: 13.57 ± 1.05 mm, *P* = 0.0425) and the end of the experimental period (MBER: 10.94 mm ± 1, 06; HBER: 16.67 ± 1.24 mm, *P* = 0.0034, Figure 2B) demonstrated different values for animals with different body energy reserves (Supplementary Figure 2).

3.3.2. Hormone analysis

The serum concentration of Insulin was analyzed in animals with different BER (MBER: n=9; HBER: n=12) along the experimental period. There was an interaction between BER and time for serum insulin concentrations (*P* = 0.0178, Figure 3A) indicating that BER across the time was responsible for the increased insulin serum concentration.

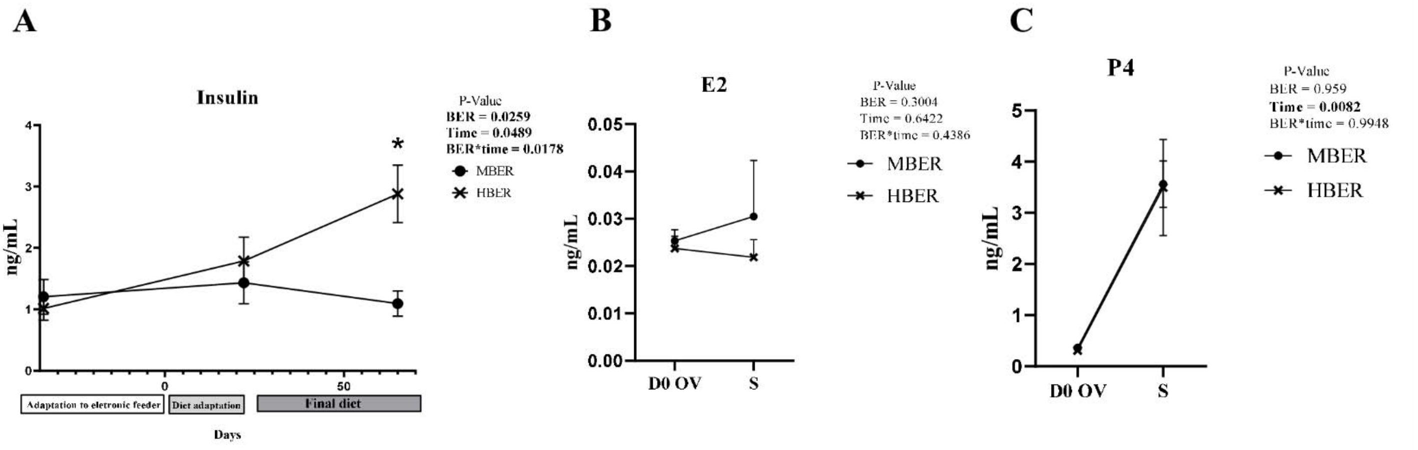


Figure 3. Serum insulin and reproductive hormones levels of Nellore cows during the experimental period. **A.** Insulin concentration. **B.** E2 concentration. **C.** P4 concentration. Standard error of the mean for body energy reserve, time and body energy reserve and time interaction. P-values are on the right top of the Figures *Statistical difference (*P*<0.05). D0 OV: day scheduled for ovulation. S: day of slaughter.

There was no difference after the estrous synchronization protocol for estrogen (*P* = 0.3004, Figure 3B) and progesterone (*P* = 0.9590, Figure 3C) levels between animals with different BER (MBER: n=9; HBER: n=12). However, the serum concentration of progesterone varied over time, between the day of ovulation (D0 OV) and slaughter (*P* = 0.0082).

3.3.3. Response to estrous synchronization protocol, ovulation and embryo recovery rates

The diameter of the dominant follicle did not differ in animals with different BER (table 2). Additionally, we evaluated the presence of anovulatory follicle at D0 OV, which was similar between groups (Table 2). However, MBER animals have higher ovulation rates (MBER: 9/9; HBER: 7/12, *P* = 0.0094, Table 2,) when compared to HBER animals. Based on the animals in which ovulation was confirmed, the embryo recovery rate of the MBER (7/9) and HBER (3/7) animals was calculated, which was higher (*P* = 0.0121; Table 2) for the MBER group.

Table 2. Least squares mean, standard error of mean and probability of response to the estrus synchronization protocol and reproductive rates of cows with different body energy reserve.

Reproductive analyses[1]	**Body energy reserve**[3]		**P- value**[4]
	MBER	**HBER**	
DF, mm	14.33 ± 0.79	13.92 ± 1.06	0.7696
Reproductive rates[2]			
Anovulatory follicle at D0 OV, %	11.11	33.33	0.2211
Ovulation, %	100	58.33	**0.0094**
Embryo recovery rate %	77.78	42.86	**0.0121**

[1]Response to the estrus synchronization protocol: DF: Dominant follicle diameter. [2]Reproductive rates: Anovulatory follicle at D0 OV: Anovulatory follicle at the day scheduled for ovulation. [3]Body energy reserve: MBER: Cows with moderated body energy reserve; HBER: Cows with high body energy reserve; [4]P-value: P value between animals with different body energy reserve.

3.3.4. Extracellular vesicles characterization

The size and concentration of particles obtained by isolation were evaluated by NTA (Supplementary Figure 3 A) and, there were no differences for size (MBER: 159.45 ± 3.57 nm; HBER: 168.27 ± 5.58 nm, *P* = 0.1969, Supplementary Figure 3 B) and particle concentration (MBER: 1.00 x 10^{12} ± 1.30 particles/mL x 10^{11}; HBER: 1.39 x 10^{12} ± 2.48 x 10^{11} particles/mL, $P = 0.6032$, Supplementary Figure 3 B). The Western blot analysis allowed to verify the presence of characteristic proteins of EVs (Supplementary Figure 3 C). ALIX and CD9 proteins were found in EV lysate, while endoplasmic reticulum marker proteins (GRP78) was only present in follicular cells. Transmission electron microscopy images demonstrate the presence of small extracellular vesicles in the follicular fluid (Supplementary Figure 3D). These results demonstrate the efficiency of the EV FF isolation protocol.

3.3.5. Cumulus cells and follicular fluid extracellular vesicles miRNA content

To understand the influence of BER on the follicular environment, the profile of 383 bovine miRNAs was analyzed in CC and EV FF only from ovulated animals with no anovulatory follicle at D0 OV (MBER: n=8; HBER: n=4; Supplementary Tables 3 and 4). The mean repeatability of the samples in CC was 0.77 and 0.81 for the MBER and HBER groups, respectively, while in the EV FF it was 0.82 and 0.84 for MBER and HBER groups, respectively. A total of 40 mature miRNAs common between groups were detected in CC samples with no statistical differences. Similarly, the EV FF analysis demonstrated a total of 264 mature and precursor miRNAs common among groups, which only the bta-miR-489 miRNA was increased in the MBER group (Figure 4). However, aiming to evaluate the communication between follicular compartments, the relationship between the miRNA contents in CC and EV FF within each experimental group, we performed a miRNA comparison among samples for each BER category. Thus, for MBER group (Figure 5A), there are 6 exclusives miRNA were detected in EV FF (bta-miR-181c, bta-miR-193a-3p, bta-miR-193b, bta-miR-29d-3p, bta-miR-1248 and bta-miR-1249; Supplementary Table 5) and 79 miRNAs commonly detected between CC and EVs, in which two of them are up regulated in CC (bta-miR-27a-5p and bta-miR-323) and 60 up regulated in EV FF (Supplementary Table 6). Comparing the CC miRNA content with EV FF for HBER group (Figure 5B), there were 42 exclusives miRNA detected in EV FF compared to CC (Supplementary Table 7) and 46 miRNAs commonly detected between CC and EVs, in which one (bta-miR-27a-5p) is up regulated in CC and 21 are up regulated In EVs (Supplementary Table 8).

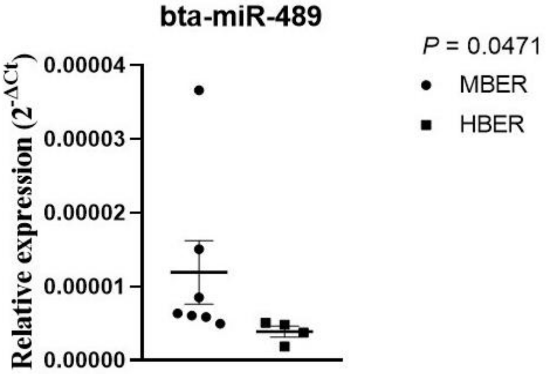
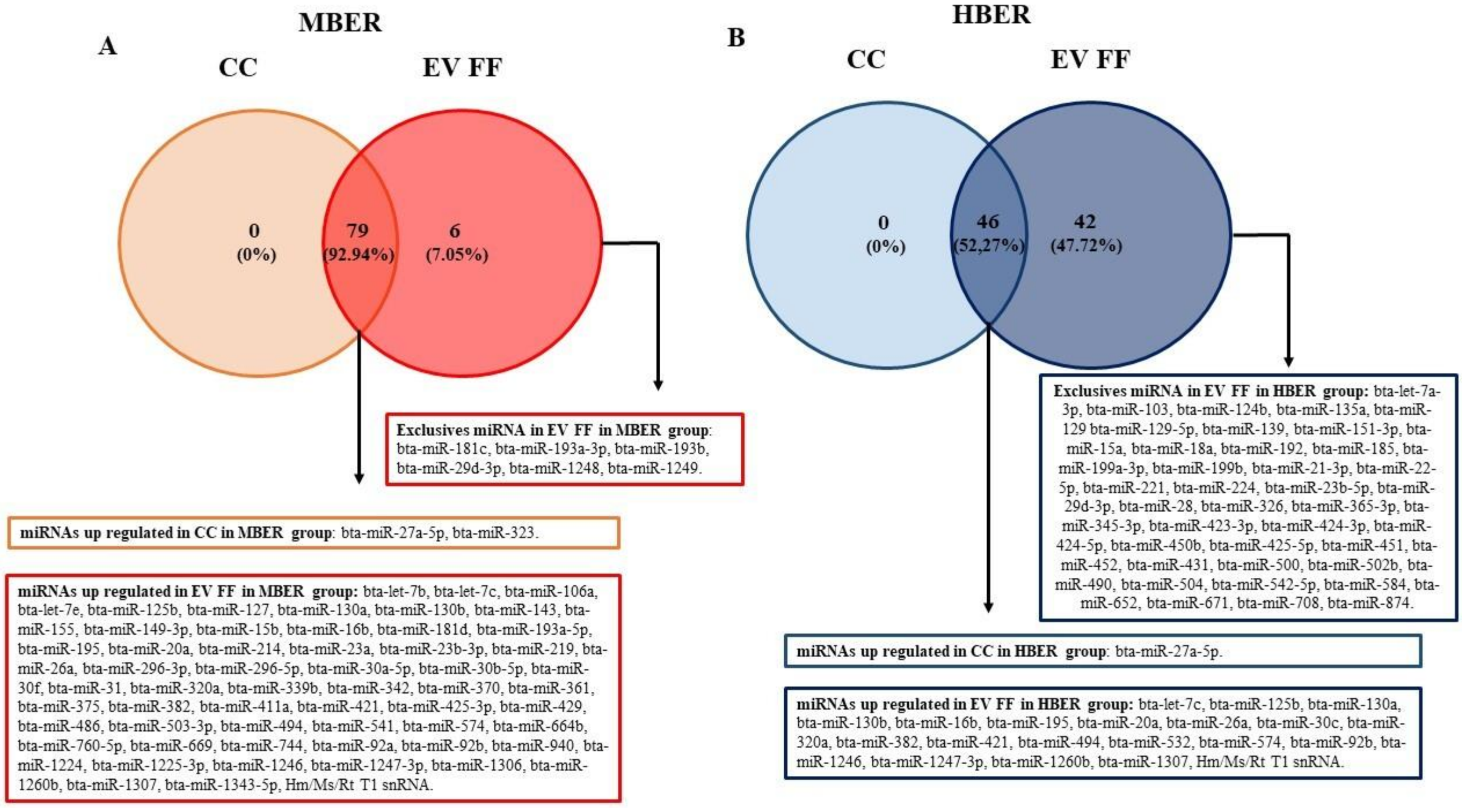


Figure 4. Relative expression of bta-miR-489 which is higher in the follicular fluid extracellular vesicles in MBER group. Mean ± standard error of the mean. P-value is in bold on the right top of the Figureure.



Exclusives miRNA in EV FF in MBER group: bta-miR-181c, bta-miR-193a-3p, bta-miR-193b, bta-miR-29d-3p, bta-miR-1248, bta-miR-1249.

miRNAs up regulated in CC in MBER group: bta-miR-27a-5p, bta-miR-323.

miRNAs up regulated in EV FF in MBER group: bta-let-7b, bta-let-7c, bta-miR-106a, bta-let-7e, bta-miR-125b, bta-miR-127, bta-miR-130a, bta-miR-130b, bta-miR-143, bta-miR-155, bta-miR-149-3p, bta-miR-15b, bta-miR-16b, bta-miR-181d, bta-miR-193a-5p, bta-miR-195, bta-miR-20a, bta-miR-214, bta-miR-23a, bta-miR-23b-3p, bta-miR-219, bta-miR-26a, bta-miR-296-3p, bta-miR-296-5p, bta-miR-30a-5p, bta-miR-30b-5p, bta-miR-30f, bta-miR-31, bta-miR-320a, bta-miR-339b, bta-miR-342, bta-miR-370, bta-miR-361, bta-miR-375, bta-miR-382, bta-miR-411a, bta-miR-421, bta-miR-425-3p, bta-miR-429, bta-miR-486, bta-miR-503-3p, bta-miR-494, bta-miR-541, bta-miR-574, bta-miR-664b, bta-miR-760-5p, bta-miR-669, bta-miR-744, bta-miR-92a, bta-miR-92b, bta-miR-940, bta-miR-1224, bta-miR-1225-3p, bta-miR-1246, bta-miR-1247-3p, bta-miR-1306, bta-miR-1260b, bta-miR-1307, bta-miR-1343-5p, Hm/Ms/Rt T1 snRNA.

Exclusives miRNA in EV FF in HBER group: bta-let-7a-3p, bta-miR-103, bta-miR-124b, bta-miR-135a, bta-miR-129 bta-miR-129-5p, bta-miR-139, bta-miR-151-3p, bta-miR-15a, bta-miR-18a, bta-miR-192, bta-miR-185, bta-miR-199a-3p, bta-miR-199b, bta-miR-21-3p, bta-miR-22-5p, bta-miR-221, bta-miR-224, bta-miR-23b-5p, bta-miR-29d-3p, bta-miR-28, bta-miR-326, bta-miR-365-3p, bta-miR-345-3p, bta-miR-423-3p, bta-miR-424-3p, bta-miR-424-5p, bta-miR-450b, bta-miR-425-5p, bta-miR-451, bta-miR-452, bta-miR-431, bta-miR-500, bta-miR-502b, bta-miR-490, bta-miR-504, bta-miR-542-5p, bta-miR-584, bta-miR-652, bta-miR-671, bta-miR-708, bta-miR-874.

miRNAs up regulated in CC in HBER group: bta-miR-27a-5p.

miRNAs up regulated in EV FF in HBER group: bta-let-7c, bta-miR-125b, bta-miR-130a, bta-miR-130b, bta-miR-16b, bta-miR-195, bta-miR-20a, bta-miR-26a, bta-miR-30c, bta-miR-320a, bta-miR-382, bta-miR-421, bta-miR-494, bta-miR-532, bta-miR-574, bta-miR-92b, bta-miR-1246, bta-miR-1247-3p, bta-miR-1260b, bta-miR-1307, Hm/Ms/Rt T1 snRNA.

Figure 5. Total numbers of miRNAs detected in cumulus cells (CC) and follicular fluid extracellular vesicles (EV FF) from 3-6 mm follicles from cows with different body energy reserve. **A.** Venn diagram demonstrating a total of 79 miRNAs commonly detected between CC and EV FF for MBER group, which 2 were up regulated in CC samples and 60 up regulated in EV FF and, 6 exclusively detected miRNAs in EV FF but not in CC. **B.** Venn diagram demonstrating a total of 46 miRNAs commonly detected between CC and EV FF for HBER group, only 1 was up regulated in CC samples and 21 up regulated in EVs FF and, a total of 42 exclusively detected miRNAs in EV FF but not in CC.

3.3.6. Enrichment analysis of miRNA differentially expressed in cumulus cells and small extracellular vesicles from follicular fluid

To determine the predicted biological functions regulated by miRNAs differently expressed in CC and EV FF we performed bioinformatics analysis for the exclusively detected miRNAs in EV FF compared to CC for MBER group. The analysis revealed a total of 275 predicted pathways (Supplementary Table 9), being 26 significant in which 10 of them, with the highest percent of genes predicted to be modulate by those miRNAs are represented in Figure 6A. These miRNAs are predicted to regulate pathways like Proteoglycans in cancer, Endocrine resistance, Adherens junction, HIF-1 signaling pathways and Circadian rhythm. For the differently expressed miRNAs in CC compared to EV FF, the up regulated miRNAs in CC are predicted to modulate Adherens junction, Axon guidance, Pathways in cancer and Neurotrophin signaling pathway (Figure 6B), while the up regulated miRNAs in EVs are predicted to modulate Metabolic pathways (Supplementary Table 10).

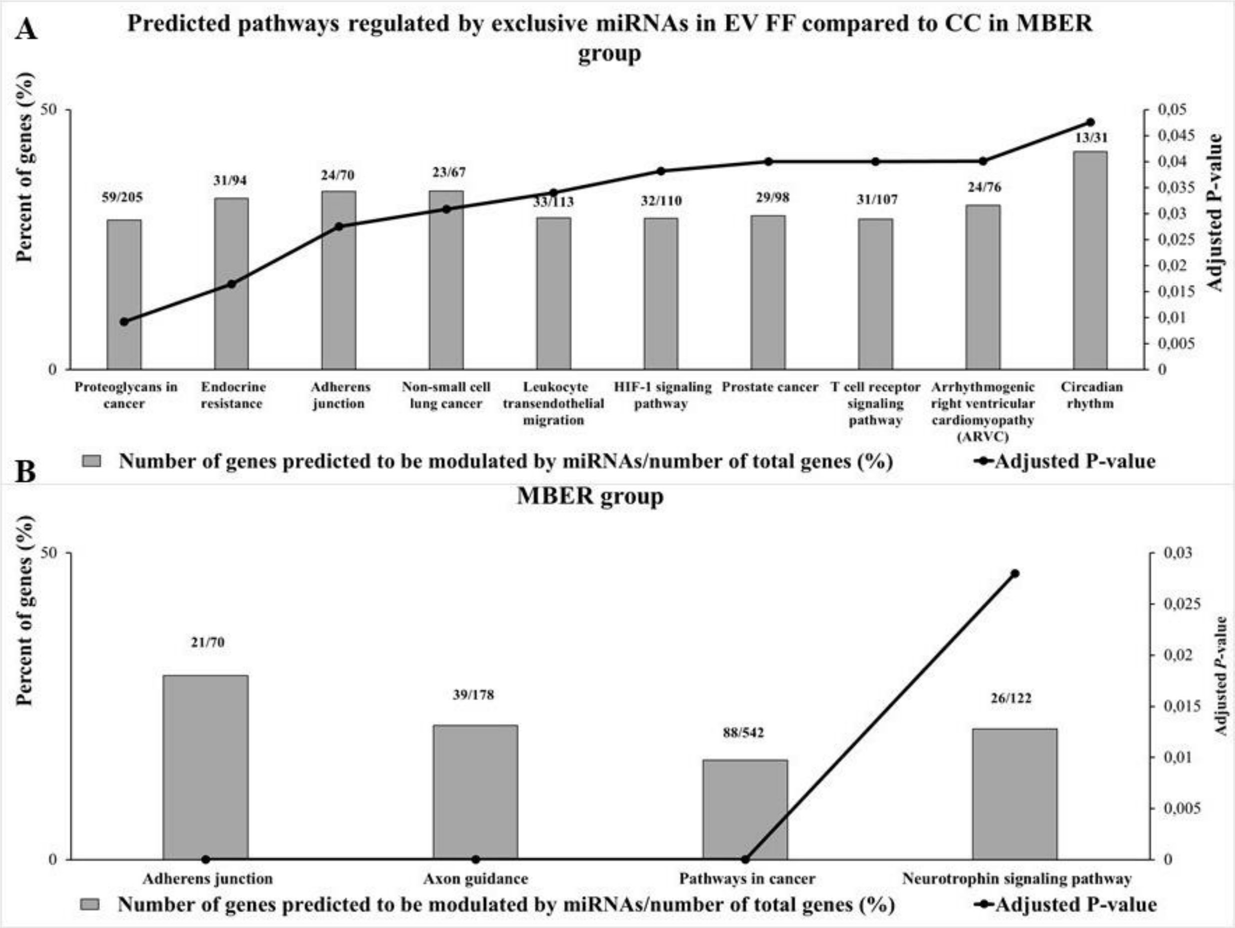


Figure 6. Enrichment analysis performed in miRWalk 3.0 software of predicted pathways modulated by exclusives or up-regulated miRNAs of cumulus cells (CC) and follicular fluid extracellular vesicles (EV FF) from 3-6 mm follicles from cows with moderated body energy reserve (MBER). A. The 10 predicted pathways with highest percent of genes predicted to be modulated by exclusives miRNAs in EV FF compared to CC in MBER group. **B.** The predicted pathways modulated by miRNAs up regulated in CC compared to EV FF in MBER group. The Y-axis in left represents the percent of genes (%) predicted to be modulated by miRNAs and the Y-axis in right shows the adjusted *P*-value (Adjusted *P*-value < 0.05).

For HBER group, the exclusive detected miRNAs in EV FF compared to CC are predicted to regulate 321 predicted pathways (Supplementary Table 11) which 8 are significant and are represented in Figure 7A. For the differently expressed miRNAs in CC compared to EV FF, the up regulated miRNAs in CC are predicted to modulate 320 signaling pathways (Supplementary Table 12) which 57 are significant and the 10 with the highest percent of genes predicted to be modulated by those miRNAs are represented in Figure 7B. Our results demonstrated that exclusives miRNAs in EV FF compared to CC are predicted to regulate Metabolic pathways, Endocytosis, Regulation of actin on cytoskeleton, Ras and MAPK signaling pathways, while the miRNAs up regulated in EV

FF compared to CC are predicted to regulate Metabolic pathways, MAPK, Ras, Wnt, and Insulin signaling pathways.

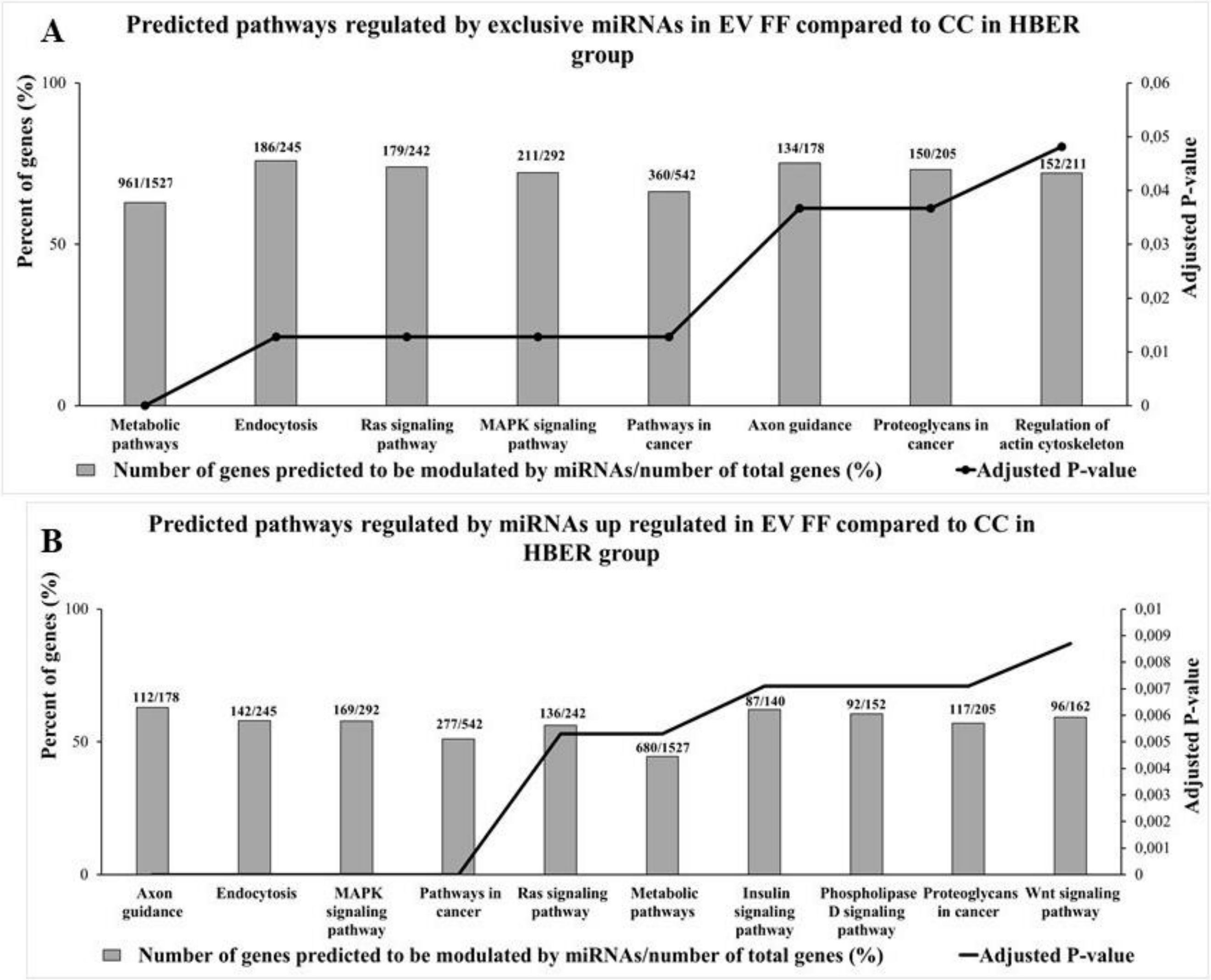


Figure 7. Enrichment analysis performed in miRWalk 3.0 software of predicted pathways modulated by exclusives or up-regulated miRNAs of cumulus cells (CC) and follicular fluid extracellular vesicles (EV FF) from 3-6 mm follicles from cows with high body energy reserve (HBER). A. The predicted pathways modulated by exclusives miRNAs in EV FF compared to CC in MBER group. **B.** The 10 predicted pathways with highest percent of genes predicted to be modulated by miRNAs up regulated in EV FF compared to CC in HBER group. The Y-axis in left represents the percent of genes (%) predicted to be modulated by miRNAs and the Y-axis in right shows the adjusted *P*-value (Adjusted *P*-value < 0.05).

3.4. Discussion

Due to the importance of body condition for the reproductive response, this study aimed to understand the effects of altered BER on ovulation, embryo recovery and miRNA profile of extracellular vesicles present within the follicular environment in multiparous Nellore cows with different BER. Thus, two different nutritional plans were applied in which animals in HBER treatment had higher values for DMI and ADG changing the animal's BW and subcutaneous fat thickness. Thus, despite the homogeneity between the groups at the beginning of the experiment (D-34), we demonstrated that at the end of the experimental period (S), animals differed in BER with HBER animals having higher BER than MBER animals.

Importantly, most of the studies involving the effects of nutrition and metabolism on reproductive function were performed in dairy cattle with negative energy balance, during the postpartum period, or submitted to a multiple ovulation protocol (WILTBANK et al., 2006, 2014; BARLETTA et al., 2017). Thus, the correct comparison between studies is essential for a better comprehension of the nutritional effects on reproductive function. The present work evaluated multiparous, non-lactating and non-pregnant Nellore cows (beef cattle), whose DMI was obtained daily and individually for a long period (67 days). Other studies aiming to understand the effects of nutritional management and metabolism on reproductive efficiency also validated the experimental model in beef cattle with different DMI-inducing changes in BER (ADAMIAK et al., 2005; BASTOS et al., 2009; MOLLO et al., 2017). In ruminants, the glucose is obtained by ruminal volatile fatty acids and, despite these animals having a low peripheral glucose concentration, glucose acts as the main substrate for cell metabolism (KOSTER; OPSOMER, 2013). However, by increasing the BER, the high fatty acids (FA) availability stimulated the FA oxidation to become the main substrate for cellular metabolism (HUE et al., 2009) which allows for higher availability of glucose and subsequently insulin concentrations. A state whereby a normal concentration of insulin induces a decreased biological response in the insulin-sensitive tissues is defined as insulin resistance (KOSTER; OPSOMER, 2013). Before getting to this metabolic condition, the insulin concentration is increased by pancreatic β cells, promoting hyperinsulinemia (KOSTER; OPSOMER, 2013). Also, basal hyperinsulinemia in cattle is a result not of a reduced insulin body catabolism, but a higher glucose-induced insulin secretion rate which is potentiated in estrus (MCCANN; REIMERS, 1985a, 1985b, 1986). Evaluating cows with moderated BER, in which DMI was high, Adamiak et al. (ADAMIAK et al., 2005) observed that nutritional plan increased the blood insulin concentration and promoted a hyperinsulinemia condition.

Our data corroborate this work suggesting that HBER cows may have a hyperinsulinemia condition due to high insulin levels.

High BER is associated with increased hepatic blood flow and, consequently, steroid metabolism (HUNTINGTON, 1990; WILTBANK et al., 2006). Despite the evidences that BER influences steroids metabolization, in the present work, differences in BER do not alter E2 and P4 serum concentration. Additionally, P4 concentrations were different between days because the P4 concentration was increased following ovulation, with the presence of an active corpus luteum, which is the main structure responsible for P4 synthesis (IRELAND; MURPHEE; COULSON, 1980). Despite we have no data from liver metabolism and clearance, the ovarian hormones serum concentration may have no statistical differences between BER due to higher metabolic liver clearance of E2 and P4 (SANGSRITAVONG et al., 2002b; BATISTA et al., 2020). Thus, our results suggest that the effects observed in the reproductive efficiency are not due to altered reproductive steroid hormones and probably due to other effects caused by the elevated BER such as alterations within the follicular environment.

Cow nutrition influences ovarian follicles’ selection and development, and changing body energy condition may alter the follicular environment and gametic/embryonic quality (LEROY et al., 2004; HERNANDEZ-MEDRANO; CAMPBELL; WEBB, 2012). The increase in BER by the high DMI promotes lower response to the multiple ovulation protocol, subsequently lower ovulation rates (SIDDIQUI et al., 2002; MOLLO et al., 2017) and increased propensity of ovarian cysts (SIDDIQUI et al., 2002). However, it is important to consider that the ovarian response and embryo quality is dependent on the animal's initial BER. High DMI is beneficial for early embryonic development in animals with low BER values, but the opposite occurs for animals with moderate BER (ADAMIAK et al., 2005). In this work, the nutritional plan aimed to increase animals’ BER, in this way, our data corroborate with Adamiak et al. (ADAMIAK et al., 2005) which observed that high DMI in moderated BER cows has a negative and cumulative effect on ovarian response and embryo quality. In addition, it is important to mention that embryo recovery rate was calculated taking into account only ovulated animals. That is, HBER animals had lower ovulation rate than MBER animals, and the embryo recovery rate was even lower.

The high DMI and change in BER can alter serum concentration of important hormones. Our data revealed that elevated BER increased serum insulin concentration but did not influence serum concentrations of E2 and P4. In the present work with the aim to evaluate the influences of the changing BER in the follicular environment, the EVs FF size and concentration were evaluated as well as the profile of bovine miRNA in CC and EVs FF. The BER did not induce changes in EVs FF size and concentration; however, the bta-miR-489 decreased in the EVs FF of HBER cows. This miRNA is widely associated with several types of cancer since its function is related to cell proliferation inhibition and apoptosis induction by PI3K/AKT pathway inactivation (ZHENG; CHEN, 2020). One of the actions of miR-489 is to regulate apoptosis by targeting 3'-UTR of IGF-I, which inhibits its expression (TANG et al., 2020). Also, glucose fluctuations reduce miR-489 expression (FU et al., 2021). In this way, this miRNA function appear to be related to glucose metabolism. Despite that this study did not evaluate the bta-miR-489 targets and its application on biological pathways. Our data suggests that bta-miR-489 decreased levels are induced by HBER influence on follicular environment, which can be connected with increased levels of serum insulin in HBER cows. Similar studies evaluating EVs FF miRNA content observed metabolic influences on follicular environment. A total of 16 miRNAs in EVs FF were differently expressed in cows with negative and positive energy balance predicting to target biological pathways as TGF-beta signaling, apoptosis, cell cycle and FoxO signaling (HAILAY et al., 2019). In humans, 18 EVs FF miRNAs were associated with high body mass index which are related with PI3K/AKT signaling, adipocytokine signaling, AMPK signaling other important pathways to folicular development (MARTINEZ et al., 2019).

Otherwise, when comparing the miRNA profile of CC and EV FF within treatments we observed that there are several distinct miRNAs among samples, suggesting that BER can influence follicular communication mediated by EVs. It is important to consider that during the estrous cycle, follicles go through different sizes and phases impacting their biochemical characteristic (ADAMS et al., 2008). These follicular changes are highly influenced by the bidirectional communication between follicular cells impacting gamete quality. The EVs within the follicular fluid, are one of the main communication agents, actively interact with granulosa cells and CCs, sending bioactive molecules and, thus, assisting in the oocyte's process of acquiring competence (DA SILVEIRA et al., 2012; ANDRADE et al., 2017; DE ÁVILA et al., 2020). In high BER animals, the elevated

DMI appears to affect later follicular developmental stages as well as early embryonic development (MOLLO et al., 2017; SARTORI; SPIES; WILTBANK, 2017), suggesting an influence in communication between follicular compartments as early as 3 - 6 mm follicular diameter. Based on our data, the functional enrichment analysis for miRNAs differentially expressed between CC and EV FF in each group, we found predicted pathways related to cell proliferation, survival, growth and metabolism, which are biological processes associated with the follicular development. Furthermore, the HBER cows showed higher serum insulin concentration than MBER cows, and also the miRNAs up-regulated in EV FF compared to CC in HBER that are predicted to downregulate the insulin signaling pathway. Cows with extreme BER had significant differences in serum metabolomic profile that may cross the blood follicular barrier influencing the follicle and oocyte quality (HORN et al., 2022). This suggest that the elevated serum concentration of insulin in HBER cows may play a role on follicular development, which can be partially mediated by altered miRNAs contents in EVs FF.

We also identified that miRNAs exclusively detected in EV FF compared to CC, are different between MBER and HBER group as well as their predicted biological function. While miRNAs in EVs in MBER group are predicted to regulated pathways as Endocrine resistance, Adherens junction and HIF-1 signaling pathway, the pathways predicted by exclusives EV miRNA in HBER groups are Metabolic pathways, Ras and MAPK signaling pathways. Additionally, the number of miRNAs exclusively or differentially expressed in EVs FF was higher than CC in both groups. It is important to highlight that we used different buffers for miRNAs profile analysis by RT- PCR, being HiSpec for CC and HiFlex for EV FF miScript (miScript II RT kit; QIAGEN). The HiSpec buffer is indicated to evaluate the mature miRNAs, that is, the miRNA that has being processed by DROSHA and DICER and is ready to be loaded into the RISC complex (BARTEL, 2009, 2018; VAN GOETHEM et al., 2017). While, the HiFlex buffer promotes the cDNA conversion of the precursor and mature miRNAs, being able to detected a larger amount of miRNAs, since it also evaluates the miRNAs that have not yet been fully processed and are preferentially secreted by the cells (BARTEL, 2009, 2018; VAN GOETHEM et al., 2017). Additionally, in HBER group, 47.72% (42/88) of the miRNAs are exclusive to EVs FF, while in MBER group the similarity between CC and EVs FF is greater, since only 7.05% (6/79) of the miRNAs are exclusive to EV FF, suggesting an active secretion of molecules by the HBER follicular cells. It is plausible to suppose that it occurs because the EVs FF origin may be from other follicular cell types, such as granulosa cells, which due to the BER effect, may have metabolic alterations. Granulosa cells are influenced by high BER leading to increased apoptosis processes and altered mitochondrial metabolic function (WU et al., 2015; KORDUS et al., 2020) suggesting that molecules secreted by them may be different. In this way, the BER can influence indirectly the follicular environment by changing the communication mediated by EVs between the different follicular compartments in 3-6 mm follicles.

3.5. Conclusion

Taken together, our data reveal that changing BER promotes a hyperinsulinemia condition, decreases ovulation rates and decreases embryo recovery rates. Additionally, our data suggests that elevated BER can modify EVs miRNAs contents within follicles between 3-6 mm, which are commonly used for commercial ovum pick-up (OPU). This suggests that in later stages the follicular development and oocyte quality can possibly be altered. Additionally, more studies exploiting the BER effect should be performed in other reproductive compartments, such as the oviduct, the subsequent structure where fertilization and early embryo development takes place. Thus, three important areas of research need to be further evaluated in the future, being the regulation of ovulation, fertilization environment, and early embryo development in high body energy reserve beef cows.

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4. Chapter 3: The bovine oviductal environment and composition is harmfully affected by elevated body energy reserve

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Abstract

Aiming to analyze the high body energy reserve (BER) effects within oviductal environment and composition, Nellore cows were fed in two different nutritional plans to obtain animals with moderate BER (MBER) and high BER (HBER). After obtaining the groups with different BER, all animals were submitted to estrus synchronization, artificial insemination and 120 hours after ovulation induction, cows were slaughtered, the reproductive tract removed and the ipsilateral oviduct to the corpus luteum were collected and dissected. Analyzes were performed only from animals which had an 8-cell embryo in isthmus evaluating the molecular profile of extracellular vesicles from oviductal flushing (OF-EVs) and luminal epithelial cells (OV-Cell), and histomorphological analysis in oviductal tissue for ampullary and isthmic oviductal regions. The HBER group presented higher concentration in ampullary extracellular vesicles (AMP-EVs) and larger sizer in isthmic extracellular vesicles (IST-EVs). In AMP-EVs the miRNA profile showed that the differently expressed miRNAs were predicted to regulate pathways associated with cell growth, migration, differentiation and metabolism, being the HBER group may be more susceptible to insulin modulation. The MBER animals showed higher ampullary vascularization than HBER. Additionally, the miRNA profile and differential gene expression (DEG) performed in ampullary (AMP-Cell) and isthmic (IST-Cell) luminal epithelial cells mainly revealed pathways related with insulin metabolism. Thus, the elevated BER may leads an oviductal insulin resistance affecting the normal functioning and, probably, the embryo metabolism during early development impacting gestational rates in these animals.

Key words : Ampulla, isthmus, miRNA, extracellular vesicles, metabolism.

4.1. Introduction

Until pregnancy establishment, gametes and embryo go through several molecular, biochemical and morphological processes within the ovary and the oviduct until reaching the uterus for further development. Despite being a small anatomic structure, the oviduct has high relevance in the reproductive function due to the dynamic and unique microenvironment important to reproductive events as final oocyte maturation, fertilization and early embryonic development (AVILÉS; COY; RIZOS, 2015). The oviduct is composed by three different anatomical regions: infundibulum, ampulla and isthmus, and three cellular layers: mucosa, muscle and serosa (EDDY; PAUERSTEIN, 1980; AVILÉS; COY; RIZOS, 2015). The mucosa layer has ciliated and secretory cells (KOLLE et al., 2009; AVILÉS; COY; RIZOS, 2015) that together with the oviductal fluid allows gametic-embryonic transport and nourishment comprising the oviductal microenvironment (LI; WINUTHAYANON, 2017; BINELLI et al., 2018; BASTOS et al., 2022).

Substrates and cofactors such as glucose, galactose, lactate, pyruvate, growth factors, amino acids, glycoproteins, serum albumin, enzymes and hormones (BEIER, 1974; BINELLI et al., 2018; RODRÍGUEZ-ALONSO et al., 2020; SAINT-DIZIER et al., 2020) are components of the oviductal fluid produced by secretory epithelial cells, which is a transudate from systemic circulation in addition to follicular fluid resulting from ovulation (LI; WINUTHAYANON, 2017; BESENFELDER; BREM; HAVLICEK, 2020). Additionally, the small extracellular vesicles (EVs) are present in the oviductal fluid (AL-DOSSARY; STREHLER; MARTIN-DELEON, 2013) and enables bidirectional cell communication between mother (oviductal epithelial cells) and gametic-embryonic cells. EVs are biological nanoparticles that uses extracellular fluids to diffuse and interact with target cells by transferring their content (MACHTINGER; LAURENT; BACCARELLI, 2016; GROSS; KROPP; KHATIB, 2017) and acting as biological vectors modulating the receptor cells functions, increasing the delivery of transcripts, micro RNAs (miRNAs) and proteins which may affect the messenger RNA (mRNA) translation in target cells (LAWSON et al., 2017; ALMIÑANA; BAUERSACHS, 2020). This is due to the fact that EVs contain bioactive material as proteins, lipids, mRNAs, miRNAs which partially represent the secretory cell content (DA SILVEIRA et al., 2015; AL-DOSSARY; MARTIN-DELEON, 2016). Thus, EVs can participate in the gametic-embryonic development, having important roles in these process (DA SILVEIRA et al., 2015, 2018; ANDRADE et al., 2017; DE ÁVILA et al., 2019). Since EVs carrying messages as miRNA have powerful systemic access to varied and distant cells acting in autocrine, paracrine and endocrine signaling functions (GROSS; KROPP; KHATIB, 2017), they may operate as fine-tuners in reproductive events such early embryo development.

As it is already known, all the oviduct structure is under ovarian cyclicity. Hormonal changes across the estrous cycle provides alterations in histomorphology and epithelial cellular composition in ampulla and isthmus (KOLLE et al., 2009; GONELLA-DIAZA et al., 2017), and modifications in EVs cargo from oviductal flushing (ALMIÑANA et al., 2018; HAMDI et al., 2021). In addition, the embryo presence modulates not only the oviductal epithelial cells molecular profile, but also the oviductal flushing and EVs content (MAILLO et al., 2016; RODRÍGUEZ-ALONSO et al., 2020; MAZZARELLA et al., 2021). This indicates that external (ovarian cyclicity) and internal (embryo presence) processes modulates the oviductal microenvironment response (BINELLI et al., 2018). Associated with external processes, nutritional management influences the body energy reserves (BER) which acts on animal metabolism, physiology and endocrine system (WILTBANK et al., 2014; SARTORI; SPIES; WILTBANK, 2017; D’OCCHIO; BARUSELLI; CAMPANILE, 2019). So, the BER can modulates ovarian function, embryonic quality and further pregnancy establishment (SANGSRITAVONG et al., 2002; ADAMIAK et al., 2005). Compared to moderated BER animals, cows with elevated BER have a higher reproductive failure rate and metabolic diseases risks (HERD; SPROTT, 1986; CARVALHO et al., 2014) suggesting that reproductive deficiency may be related to other anatomical structures besides the ovary. In a recent work performed by our group, cows high HBER presented lower ovulation rate and even lower embryo recovery rate possibly due to a hyperinsulinic condition (Bastos et al., 2022, data unpublished). Inconsistently, high genetic merit cows used as donors of oocytes and embryos are usually animals with high BER and present reproductive problems. Once bovine can be biological model to human studies due to similar embryo development characteristics and, in modern human society obesity and overweigh are increasingly present in women of reproductive active age, studies aiming to understand the effects of high BER on the oviductal microenvironment and composition should be carried out to increase our understand about possible consequences in the subsequent pregnancy stages. In this way, the hypothesis for this work is that the increase of BER alters the oviductal environment and composition providing a harmful place to embryo development.

4.2. Material and methods

All experimental procedures were approved by the University of São Paulo Research Ethics Committee (protocol number: 1522231019). The experiment was performed Laboratory of Morphophysiology and Molecular Development (LMMD) of the Department of Veterinary Medicine, both located at University of São Paulo (Campus of Pirassununga, SP, Brazil).

4.2.1. Animal model and sample collection

Nellore cows (n=21) were randomly divided in two experimental groups and submitted to two different nutritional plans aiming to have animals with moderated (MBER, n=9) and high (HBER; n=12) body energy reserve (BER) as previously described at Bastos et al., 2022, unpublished data. After obtaining the groups with different BER, all animals were submitted to estrus synchronization, artificial insemination with semen from a single bull and, approximately 120 hours after ovulation induction, cows were slaughtered and the reproductive tract removed was immediately transported to the laboratory (Bastos et al., 2022, unpublished data). The ipsilateral oviducts to the corpus luteum were collected, dissected and oviductal portions of ampulla and isthmus were previously separated through the ampullary-isthmic junction before oviductal flushing (Bastos et al., 2022, data unpublished; MAZZARELLA et al., 2021). From the 21 cows (MBER: n=9; HBER: n=12) a total of 6 animals presented an 8-cell embryo within the flushing, based on that sample collection was performed in MBER: n=3 and HBER: n=3. Oviductal tissue, oviductal luminal epithelial cells and oviductal flushing were collected for histomorfological and molecular analysis, and extracellular vesicles isolation, respectively, for ampullary and isthmic oviductal regions (Figure 1).

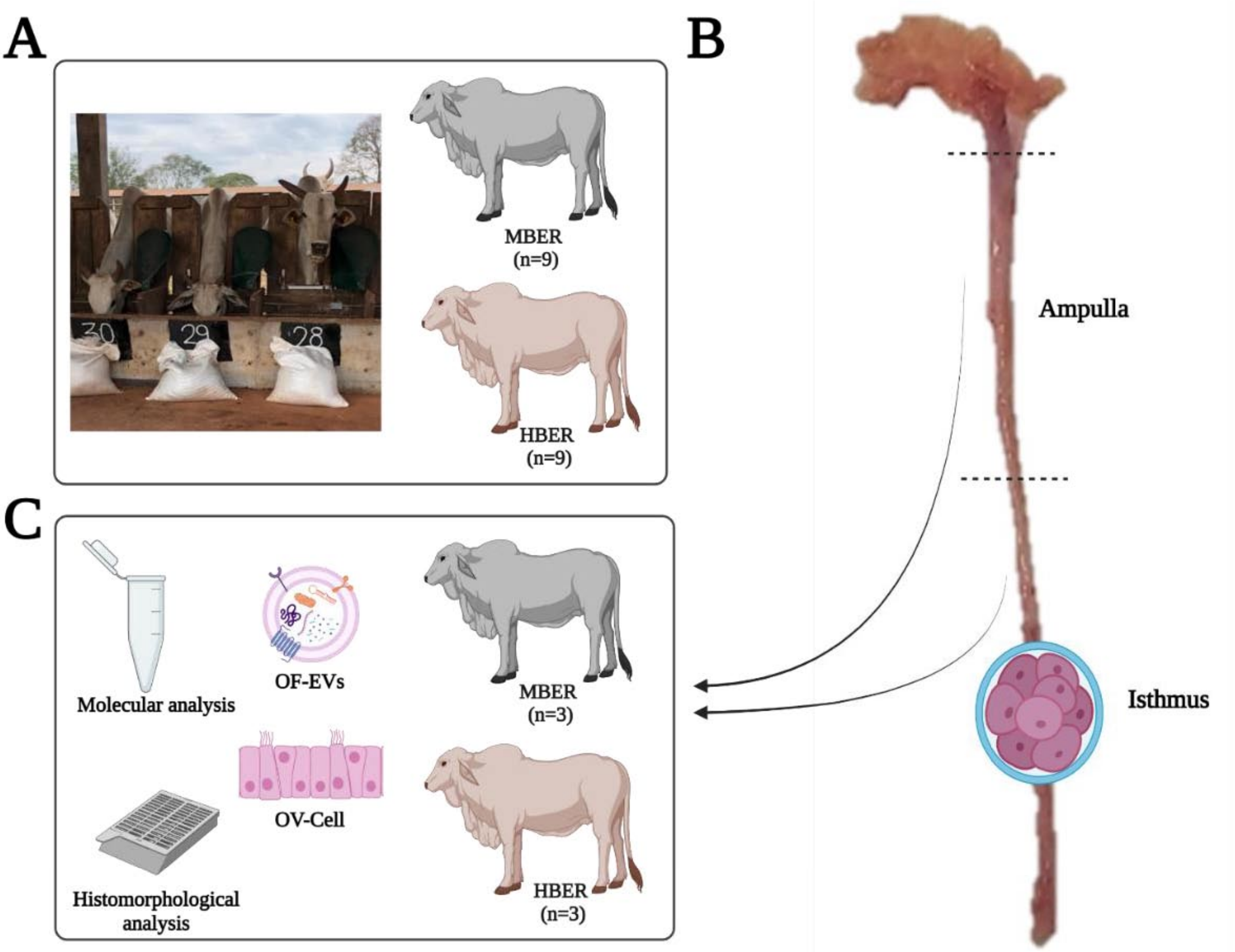


Figure 1. Schematic representation of sample collects. **A.** Nellore cows from a same herd was previously submitted at a feedlot period with two nutritional plans in order to obtain cows with moderate body energy reserve (MBER; n=9) and high body energy reserve (HBER; n=12). **B.** After obtaining the two well-defined groups, the animals were submitted to estrus synchronization, slaughter and the ipsilateral oviducts to the corpus luteum were collected, dissected and the ampullary-isthmic junction were separated and the sample collection was performed only for animals which had an 8-cell embryo. **C.** Oviductal samples were collected for analysis in MBER (n=3) and HBER (n=3). It was performed molecular analysis in oviductal flushing extracellular vesicles (OF-EVs) and oviductal cells (OV-Cell) and also histomorfological analysis in oviductal tissue. This image was created with Biorender.com.

4.2.2. Isolation of small extracellular vesicles from oviductal flushing

After obtaining the oviducts, ampullary and isthmic portions were individually flushed with 1mL of phosphate-saline solution calcium and magnesium free (1xPBS) and used later to obtain oviductal small extracellular vesicles (OF-EVs) as previously described by Mazzarella et al. (2021). Briefly, samples were centrifuged at 4ºC at 300xg for 10 minutes to remove cells, 2000xg for 10 minutes to remove cell debris, 16500xg for 30 minutes to remove larger extracellular vesicles. To obtain a pellet enriched in small extracellular vesicles (smaller than 200 nm), 200 µl of the resulting supernatant were filtered through a 0.20µm pore filter (Corning) and ultracentrifuged at 119700xg by 70 minutes at 4ºC (Optima XE-90 Ultracetrifuge; 70 Ti rotor; Beckman Coulter). Then, the obtained pellet was washed in 1xPBS and ultracentrifuged again at 119700xg for 70 minutes to 4ºC. The pellet enriched with small extracellular vesicles was eluted in 20 µl of 1xPBS for further characterization and miRNA content analysis.

4.2.2.1. Characterization of small extracellular vesicles from oviductal flushing

The OF-EV characterization consisted of nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM) and Western Blot. To perform the TEM and Western Blot analyses, slaughterhouse samples were used to avoid the waste of OF-EV from the experimental samples which were used for NTA and miRNA content analysis.

4.2.2.1.1. Nanoparticle tracking analysis

The OF-EV ampullary (AMP-EV) and isthmic (IST-EV) small extracellular vesicles were isolated from 200 µl of oviductal isthmic flushing. Upon the AMP-EV and IST-EV isolation, 5 µl of the eluted pellet for the both oviductal portions were diluted in 495µl of 1xPBS each and used for particle size and concentration evaluation using Nanosight device (NS300; NTA 3.4 Build 3.1.45; Malvern). Five 30-second videos were taken at a controlled temperature of 38,5ºC, Camera Level 13 considering threshold of 5. Data from size and concentration of each video was considered for statistical analysis.

4.2.2.1.2. Transmission electron microscopy

After isolation by serial centrifugation, the EVs pellets were diluted in fixative solution (0.1M Cacodylate; 2.5% Glutaraldehyde; 4% paraformaldehyde; pH 7.2 - 7.4) for two hours before being ultracentrifuged again and resuspended in 20 µl of ultrapure milli-Q water. The analyzes were realized at the Multiuser Laboratory of Electronic Microscopy of the Department of Cellular and Molecular Biology, Faculty of Medicine of Ribeirão Preto, using a transmission electron microscope (FEI 200kV, model Tecnai 20, emitter LAB6).

4.2.2.1.3. Western blotting analysis

After the EV isolation protocol, samples were resuspended in 20 uL of lysis buffer (RIPA), stored on ice under constant agitation. The samples were applied on SDS-PAGE polyacrylamine gel 6% and 15% for better representation of the proteins, and the run was performed at 100 V for approximately 120 minutes prior to the wet transfer to nitrocellulose membrane at 80 V for 120 minutes. After the transfer, membranes were kept 1 hour in blocking solution (3% non-fat dry milk in TBST – Tris buffered saline with Tween-20), prior to the incubation with primary antibodies overnight at 4ºC. The specific vesicle proteins studied were ALIX (0.3 µg/mL; rabbit, sab4200476, Sigma-Aldrich, St. Louis, MO, USA), CD9 (0.6 µg/mL; mouse, sc-13118, Santa Cruz, CA, USA) and the non-vesicular protein control was GRP78 (protein from endoplasmic reticulum; 0.6 µg/mL; mouse, sc-376768, Santa Cruz). After the incubation period, the membrane was washed three times in TBST for 5 minutes and maintained for 1 hour in anti-rabbit (A0545sc-2357 Sigma-Aldrich, St. Louis, MO, USA) and anti-mouse (#7076S, Cell Signaling Technology) secondary antibody. Then, the secondary antibodies were removed, the membrane washed and exposed to the developing solution (170-560, Clarity Western ECL), for analyzes carried out by the ChemiDoc MP Image System (Bio-RAd, Hercules, CA, USA).

4.2.3. Histomorfological analysis

After the oviductal flushing, tissue samples from ampulla and isthmus were collected and fixed in 10% buffered formalin for 48 hours. Then, samples were routinely processed for histology. Four-µm cross-sections were obtained from each sample. Digital images from the ampullary and isthmic sections were obtained using a microscope coupled to a digital camera (Leica DM500 and Leica ICC50HD, Leica Microsystems; Heerbrugg, Switzerland).

The Masson's trichrome stain was used to identify collagen fibers in blue. The mucosa total area and the collagen area were measured to calculate the collagen rate (collagen area/total area). These values are presented in percentage (%).

To determine the vascularization, blood vessels were detected using immunohistochemistry (rabbit polyclonal anti-von Willebrand factor antibody (vWF), Abcam, code ab6994) at 40x magnification for both oviductal regions. The mucosal area without the lining epithelium was obtained and the positive vessels for vWF were counted for vascularization determination (vessels/mm^2).

The luminal epithelial perimeter (mm) was obtained by drawing the luminal epithelial lining by the "trace-wand" tool of the Image ProPlus software (version 4.5 for Windows, Media Cybernetics; Silver Spring, MD, USA), as previously described by Gonella-Diaza (2017) in the slides used to analyze the collagen rate.

4.2.4. Luminal epithelial cells collection for molecular analysis

The oviductal luminal epithelial cells (OV-Cell) from each ampulla (AMP-Cell) and isthmus (IST-Cell) were obtained by squeezing the tissue with a sterile glass slide (MAZZARELLA et al., 2021). The cells were immediately frozen in liquid nitrogen for further molecular analyzes.

4.2.5. Total RNA extraction

Total RNA content from OF-EV and OV-Cell were extracted using miRNeasy Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions (DE ÁVILA et al., 2020). RNA quantity and quality were analyzed by spectrometry (NanoDrop 2000, Thermo Fisher Scientific; absorbance ratio 260/280nm) and treated with DNaseI (Invitrogen; Carlsbad, CA) according to the manufacturer's instructions (DE ÁVILA et al., 2020). After extraction, the RNA was stored at -80ºC until use.

4.2.6. MiRNA analysis

The total RNA was transformed into cDNA using the miScript II RT kit (QIAGEN), using the miScript HiSpec Buffer to obtain mature miRNAs in OV-Cell and miScript HiFlex Buffer to obtain mature and precursor miRNAs in OF-EVs. OV-Cell reactions contained 100 ng of total RNA, while for OF-EVs reactions were performed with 200 ng of total RNA. Both were performed in a thermocycler (Life Technology) at 37ºC for 60 minutes, followed by 95ºC for 5 minutes. The RT-PCR reactions to quantify the transcripts were performed according to Da Silveira et al. (2018), using at least 0.2 ng of cDNA and 1µl of forward primers obtained based on the mature bovine miRNAs sequences available in the mirBase software (http://www.mirbase.org). The temperatures and times used were 95ºC for 15 minutes followed by 45 cycles of 94ºC for 15 seconds, 55ºC for 30 seconds and 70ºC for 30 seconds. For each sample, the expression analysis of 383 bovine miRNAs was performed (DA SILVEIRA et al., 2018). The miRNAs were considered present when they presented a cycle threshold (CT) lower than 37 in all biological repetitions and an appropriate melting curve. CT data generated by amplification were normalized using geometric mean of bta-miR-99b, Hm/Ms/Rt T1 snRNA and RNT43 snoRNA for OV-Cell, and bta-miR-99 for OF-EVs (DA SILVEIRA et al., 2017). The miRNAs differently expressed between groups were evaluated by miRWalk software version 3.0 and it was identified the predicted regulated pathways. Predicted pathways were considered significant when BH (Benjamini – Hochberg) adjusted $P < 0.05$.

4.2.7. RNA library preparation and sequencing

Sequencing libraries of OV-Cell were prepared using the TruSeq Stranded mRNA Kit (Illumina). In brief, Poly-A RNAs were captured using poly-T oligo attached magnetic beads. Poly-A RNAs were then fragmented, subjected to double-strand cDNA synthesis, ligated with dual-index adapters, PCR enriched and purified to create the final cDNA library. A High Sensitivity DNA Kit (Agilent) was used to confirm library length (~300 bp) and lack of dimers. Finally, libraries were quantified by quantitative PCR using the KAPA Library Quantification Kit (KAPA), pooled and the final library concentration adjusted at 1 nM based on Qubit dsDNA HS Assay Kit (ThermoFisher Scientific). Sequencing was performed on the NextSeq 550 instrument (Illumina) using 1.8 pM of pooled libraries and the NextSeq 500/550 High Output Kit v2.5 (75 cycles).

The quality of the reads was assessed using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/).The 76 bp reads were mapped using Star (DOBIN et al., 2013), and identification and quantification were performed using ARS-UCD1.2 (Ensembl and NCBI) as a reference genome using the featureCounts implemented in Rsuberead package (LIAO; SMYTH; SHI, 2014; LIAO; SMYTH, 2019) for gene count. Once the genes were identified, differential expression analysis was performed between groups using DESEq2 (LOVE; HUBER; ANDERS, 2014) considering a padj<0,01 and an absolute log2Folchange >0.5. Additionally, we considered genes as different expressed if they were exclusive, expressed in one group (expressed in all samples from the same group) and not expressed in the other group (zero counts in all samples from the same group) within comparison and using the function filterByExpr from edgeR package (ROBINSON; MCCARTHY; SMYTH, 2010). We estimate the hub genes using CeTF (BIAGI et al., 2021) based on RIF—Regulatory Impact Factor and PCIT—Partial Correlation and Information Theory (REVERTER; CHAN, 2008; REVERTER et al., 2010). Gene ontology analysis were performed using clusterProfiler (YU et al., 2012) and pathways explored using Pathview (LUO; BROUWER, 2013). Data were visualized using R software, in which we primarily observed the classification, intensity, and difference in expression between groups.

4.2.8. Statistical analysis

The OF-EVs size and concentration, miRNA expression of OF-EVs and OV-Cells and histomorphological analysis data was analyzed using the fixed effect of the body energy reserve which the means were adjusted by the least squares method and compared using the probability of difference using the Student *t* test. All the analyses utilized the program “JMP” (7.01 version, Statistical Analysis Software Institute, SAS® Inc., Cary, NC). A significant difference was declared when $P<0.05$.

4.3. Results

4.3.1. Characterization of small extracellular vesicles from oviductal flushing

After obtaining oviductal flushing, to confirm the EV isolation protocol AMP-EV and IST-EV were isolated and characterized. The size and concentration were performed by NTA analyses. For ampulla, there is no difference for size (MBER: 151.41 ± 6.60 nm; HBER: 153.53 ± 4.47 nm, $P= 0.7929$, Figure 2A) but, the AMP-EV concentration is higher in HBER animals (MBER: $6.30 \times 10^{9} \pm 4.80 \times 10^{8}$ particles/mL; HBER: $9.50 \times 10^{9} \pm 8.54 \times 10^{8}$ particles/mL, $P= 0.0028$, Figure 2A). For isthmus, the HBER IST-EV presented larger sizes (MBER: 116.67 ± 2.16 nm; HBER: 140.05 ± 8.50 nm, $P= 0.0126$, Figure 2A) and there is no difference for particle concentration (MBER: $5.15 \times 10^{9} \pm 7.68 \times 10^{8}$ particles/mL; HBER: $4.29 \times 10^{9} \pm 8.50 \times 10^{8}$ particles/mL, $P= 0.3373$, Figure 2A). The Western blotting analyses allowed the specific proteins verification from OF-EV. The ALIX and CD9 proteins were found in the OF-EV lysate, while the endoplasmic reticulum marker proteins (GRP78) were found only in oviductal cells confirming the isolation protocol (Figure 2B). The transmission electron microscopy images showed the presence of OF-EV (Figure 2C).

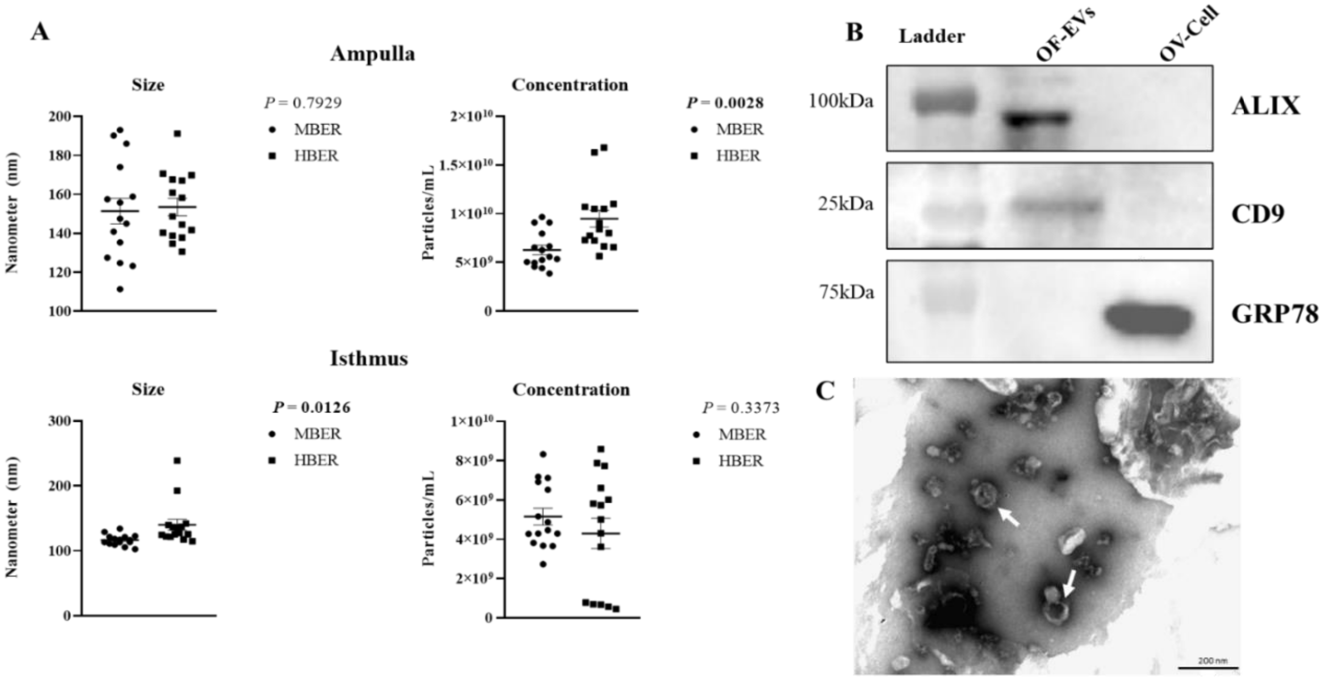
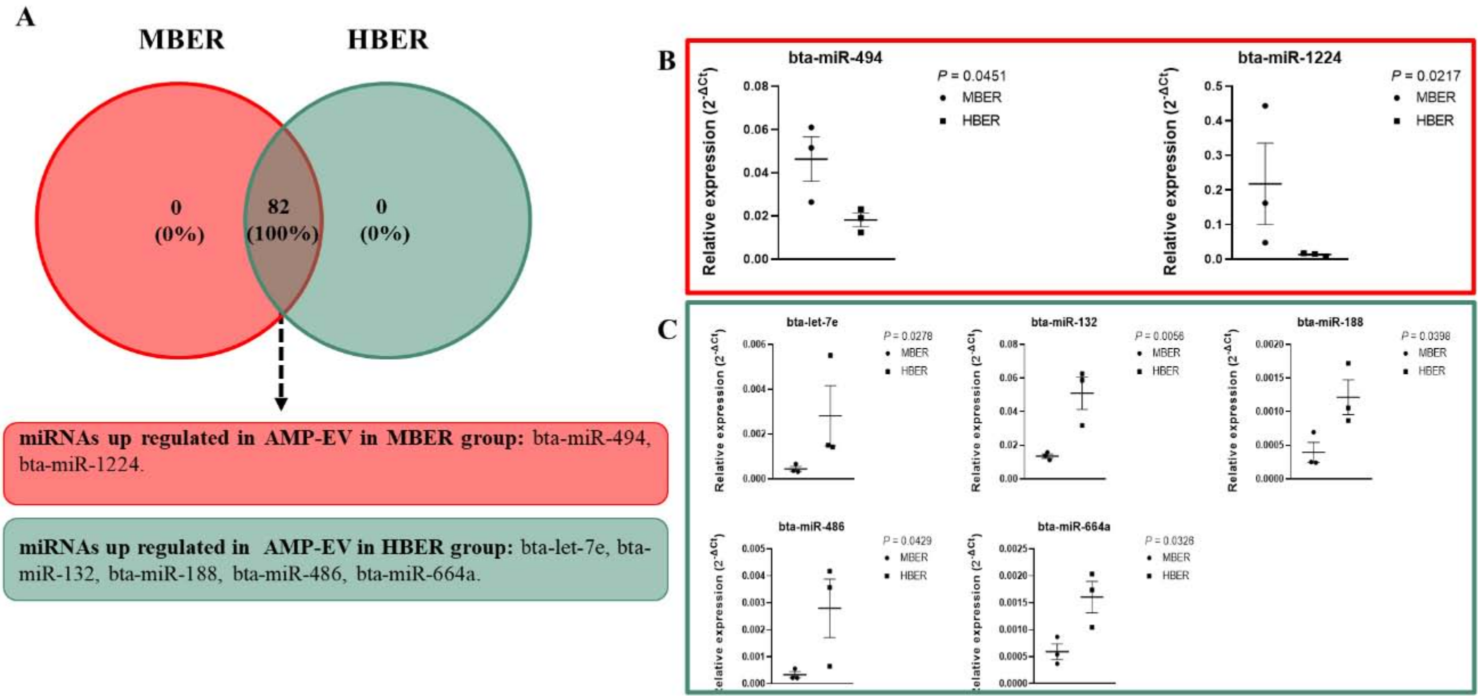


Figure 2. Oviductal flushing extracellular vesicles characterization from cows with different body energy reserve. **A.** Extracellular vesicles size and concentration in ampulla and isthmus analyzed by NTA. Mean ± standard error. P-value is on the right top of the figure. **B.** Western blotting analysis demonstrates the presence of characteristic vesicles proteins (ALIX and CD9) and the absence of cell-specific proteins (GRP78) in oviductal flushing. Western blot images were cropped from the original images available in the Supplementary Figure 1. **C.** Transmission electron microscopy images shows (white arrows) the extracellular vesicles presence in oviductal flushing.

4.3.2. MiRNA analyses of small extracellular vesicles from oviductal flushing

In order to understand the BER influences on oviductal environment, the OF-EV miRNA content was performed analyzing a 383 custom miRNA profile (Supplementary tables 1 and 2). The AMP-EV miRNA content presented mean repeatability of 0.76 and 0.75 for MBER and HBER, respectively. There were 82 commons miRNAs between groups (Figure 3A; Supplementary Table 3) in which two of them are up regulated in MBER animals (bta-miR-494, bta-miR-1224, Figure 3A and B) and five are up regulated in HBER animals (bta-let-7e, bta-miR-132, bta-miR-188, bta-miR-486, bta-miR-664a; Figura 3A and C). For IST-EV, the mean repeatability of samples was 0.80 and 0.85 for MBER and HBER, respectively. There were 150 common miRNAs between groups; however, we were not able to identify statistical differences in expression of these miRNAs (Supplementary Table 4).



miRNAs up regulated in AMP-EV in MBER group: bta-miR-494, bta-miR-1224.

miRNAs up regulated in AMP-EV in HBER group: bta-let-7e, bta-miR-132, bta-miR-188, bta-miR-486, bta-miR-664a.

Figure 3. miRNAs expression in extracellular vesicles from ampullary flushing (AMP-EVs) from cows with different body energy reserve. **A.** Venn diagram demonstrating the 82 common miRNAs between groups which 2 were up regulated in MBER group and 5 up regulated in HBER group. **B.** Relative expression of up regulated miRNAs in AMP-EVs in MBER group. **C.** Relative expression of up regulated miRNAs in AMP-EVs in HBER group. Mean ± standard error. P-value is on the right top of the figure.

4.3.3. *Enrichment analysis of miRNA differentially expressed in small extracellular vesicles from oviductal flushing*

To determine the predicted biological functions regulated by miRNAs differently expressed in OF-EVs, we performed the bioinformatics analysis. In AMP-EVs, from the 283 predicted pathways (Supplementary Table 5) as regulated by the two miRNAs up regulated in MBER group, 51 are significant in which 10 of them, with highest percent of genes predicted to be modulated by those miRNAs are represented in figure 4A. For the five miRNAs up regulated in HBER group, there is 313 total predicted pathways (Supplementary Table 6) of which 42 are significant and 10 selected pathways with highest percent of genes predicted to be modulated by those miRNAs are represented in figure 4B. Our results demonstrate that miRNA AMP-EVs cargo in MBER group are predicted to regulate pathways like Glycerophospholipid metabolism, Regulation of actin cytoskeleton, GnRH and TGF-beta signaling pathways, while the miRNA present in AMP-EVs in HBER group can be involved in Endocytosis, Insulin resistance, Hippo and ErbB signaling pathway and cell-to-cell interaction pathways.

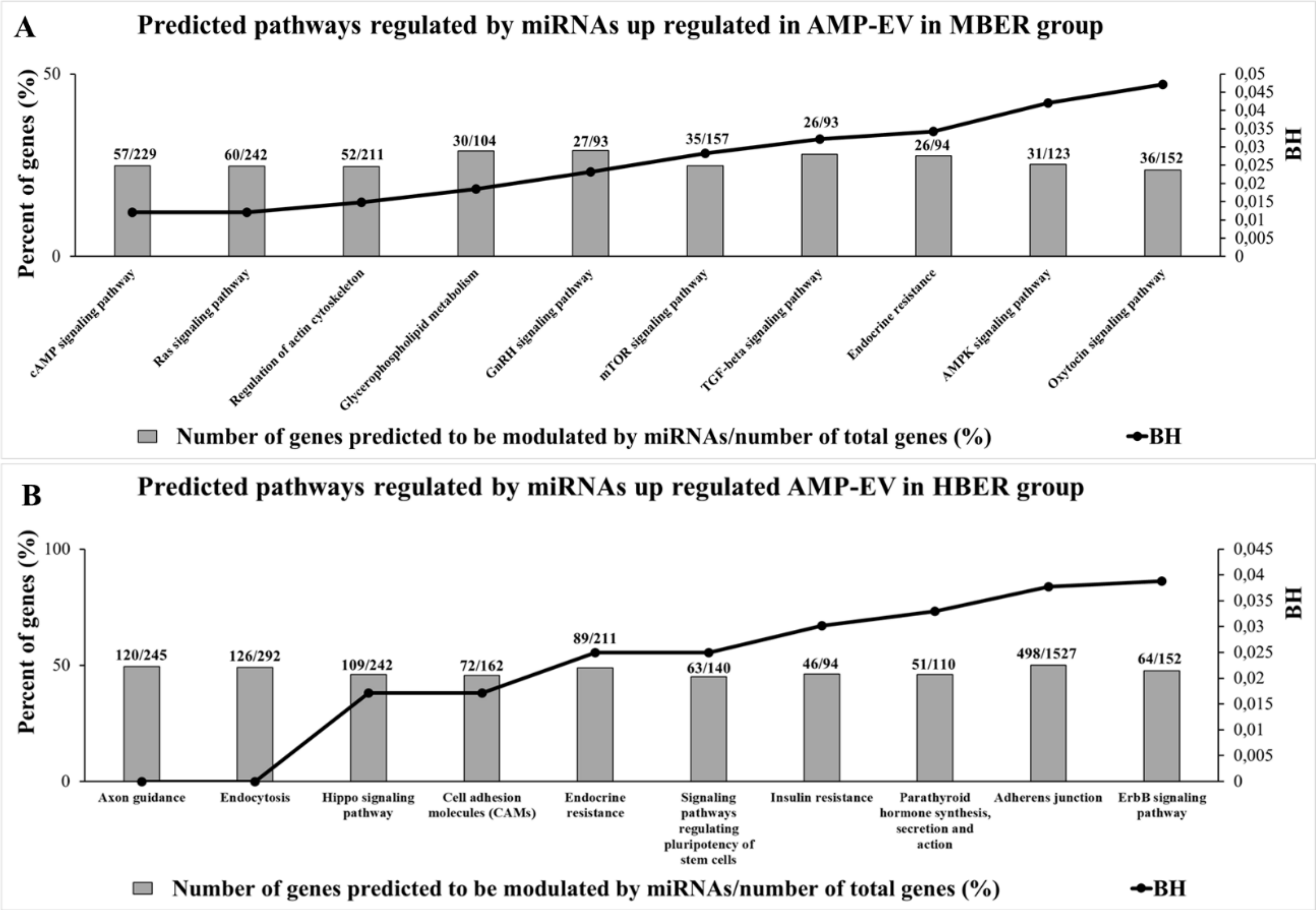


Figure 4. Enrichment analysis performed in miRWalk 3.0 software of predicted pathways modulated by miRNAs differentially expressed in small extracellular vesicles from ampullary oviductal flushing (AMP-EVs). **A.** The 10 predicted pathways with highest percent of genes predicted to be modulated by miRNAs up regulated in AMP-EVs in MBER group. **B.** The 10 predicted pathways with highest percent of genes predicted to be modulated by miRNAs up regulated in AMP-EVs in HBER group. The Y-axis in left represents the percent of genes (%) predicted to be modulated by miRNAs and the Y-axis in right shows the BH (BH < 0.05).

4.3.4. Histomorfological analysis

The histomorphological analyses were performed to obtain collagen rate (Figure 5A), vascularization (Figure 5B) and luminal perimeter (Figure 5C) of ampullary (Figure 5D) and isthmic (Figure 5E) oviductal regions. In ampulla, there were no differences between groups for collagen rate and luminal perimeter, however, MBER animals had higher ampullary vascularization values than HBER animals (*P* = 0.0351; figure 5D). The collagen rate, vascularization and luminal perimeter did not differ between groups at the isthmus portion.

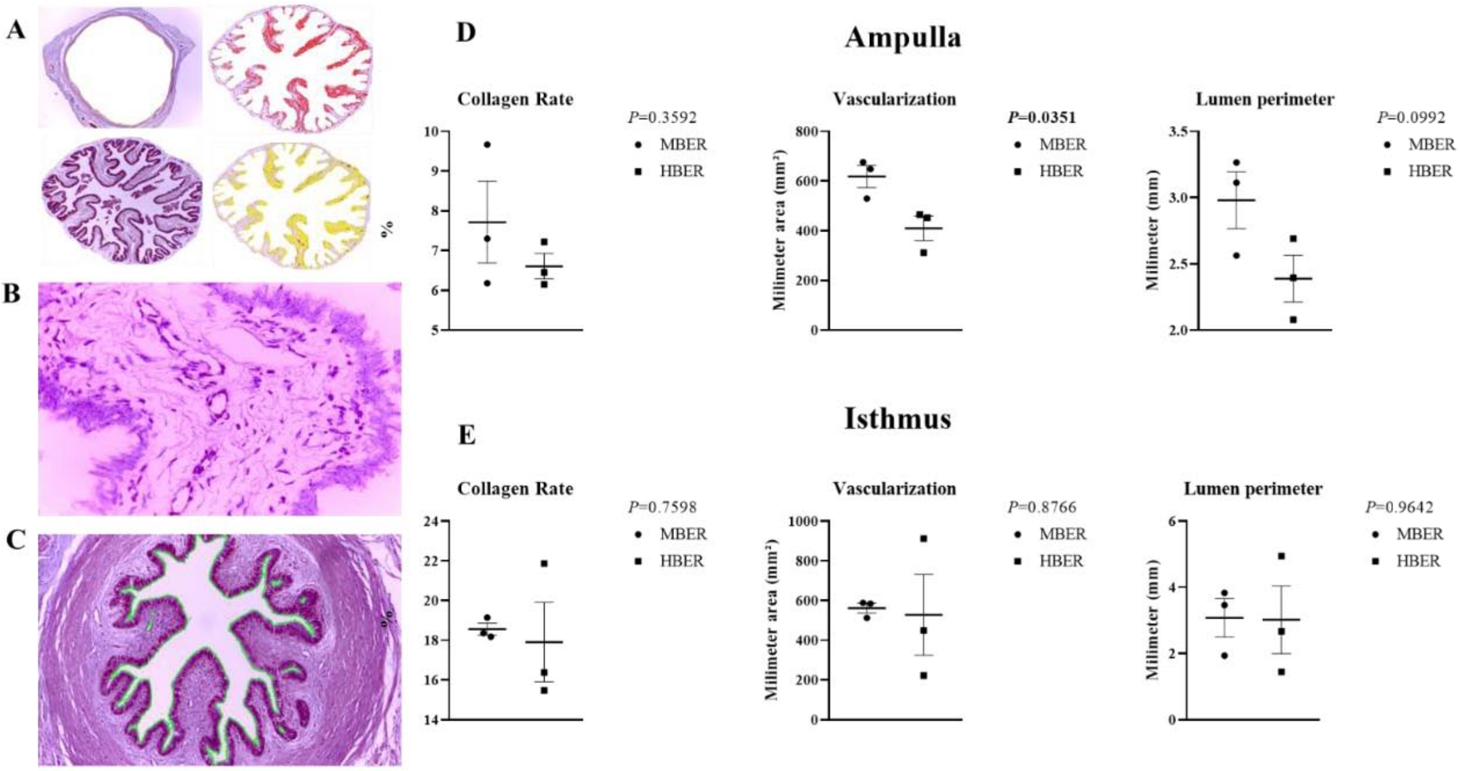


Figure 5. Oviductal histomorphological analysis to obtain **A.** collagen rate, by color segmentation of the image; **B.** vascularization (blood vessels/mm²) and **C.** luminal perimeter (micrometer; µm) from cows with different body energy reserve. **D.** Histomorphological analysis in ampullary region. **E.** Histomorphological analysis in isthmic region. Mean ± standard error. P-value is on the right top of the figure.

4.3.5. MiRNA analyses of oviductal epithelial cells

In order to understand the BER influences on oviductal epithelial cells, the OV-Cell miRNA contents we performed the analysis of 383 miRNAs in each sample (Supplementary 7 and 8). The AMP-Cell miRNA contents presented mean repeatability of 0.90 and 0.90 for MBER and HBER, respectively. There was a total of 210 common miRNAs between groups (Figure 6A; Supplementary Table 9) and 10 exclusive miRNA presents only in HBER animals (bta-miR-133b, bta-miR-193b, bta-miR-196b, bta-miR-21-3p, bta-miR-212, bta-miR-411c-3p, bta-miR-431, bta-miR-432, bta-miR-658, bta-miR-1193; Figure 6A). Among the common miRNAs between the groups, a total of eight miRNAs were up regulated in HBER animals (bta-miR-100, bta-miR-101, bta-miR-190a, bta-miR-19b, bta-miR-30b-5p, bta-miR-30e-5p, bta-miR-425-5p, bta-miR-99a-5p, Figure 6A and B) compared to MBER animals.

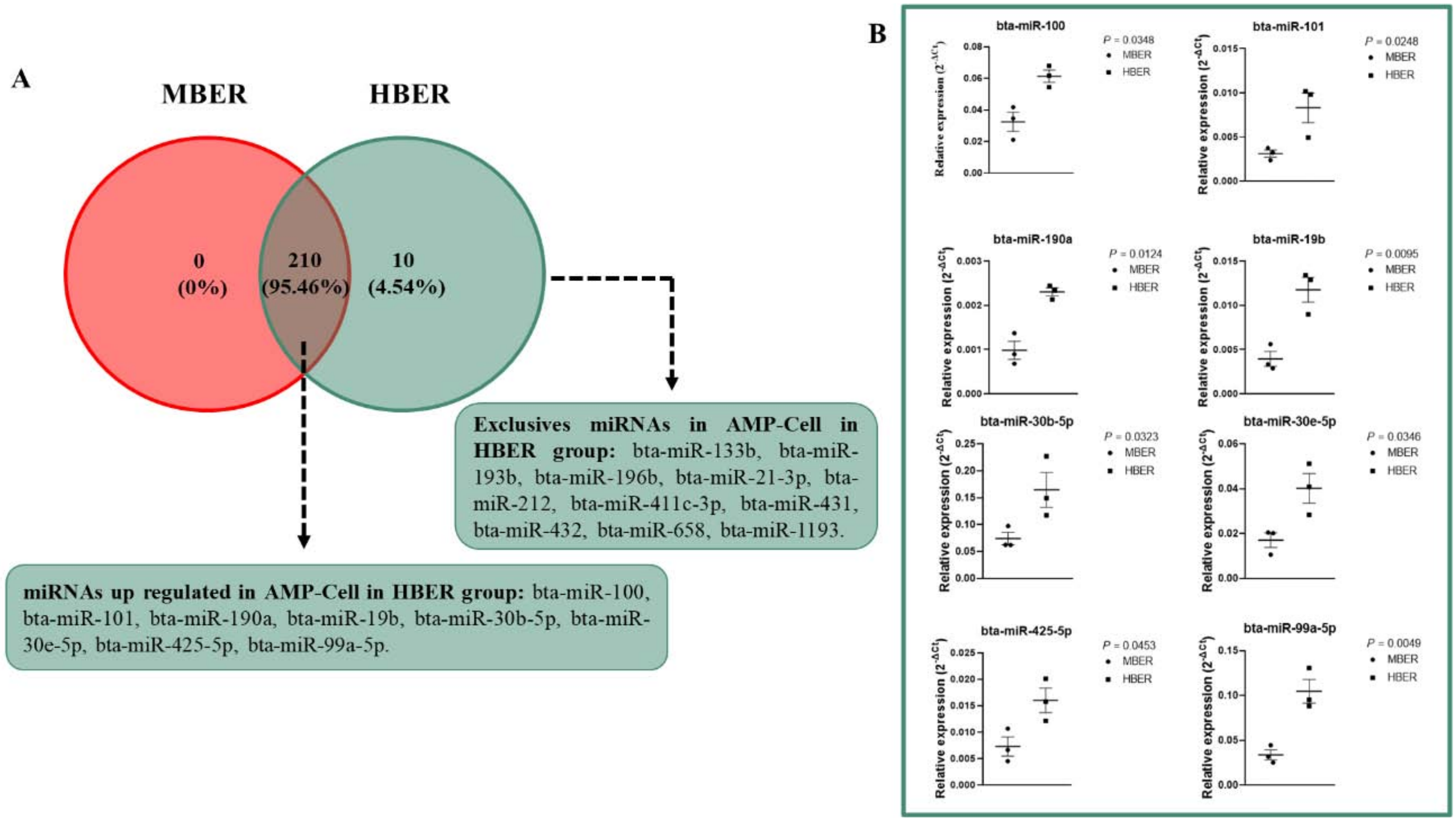


Figure 6. miRNAs expression in ampullary luminal epithelial cells (AMP-Cell) from cows with different body energy reserve. **A.** Venn diagram demonstrating the 210 common miRNAs between groups and 10 exclusives miRNAs in HBER group. Of the 210 common miRNAs, 8 were up regulated in HBER group **B.** Relative expression of up regulated miRNAs in AMP-Cell in HBER group. Mean ± standard error. P-value is on the right top of the figure.

For IST-Cell, the mean repeatability of samples were 0.80 and 0.85 for MBER and HBER, respectively. There were a total of 242 common miRNAs between groups (Figure 7A; Supplementary Table 10) and one exclusive miRNA (bta-miR-138) present only in HBER animals. Among the common miRNAs between groups, a total of 13 were up regulated in HBER animals (bta-miR-106a, bta-miR-101, bta-miR-148a, bta-miR-18b, bta-miR-192, bta-miR-186, bta-miR-20b, bta-miR-210, bta-miR-28, bta-miR-296-5p, bta-miR-30a-5p, bta-miR-365-5p, bta-miR-1271, Figure 7A and B) compared to MBER animals.

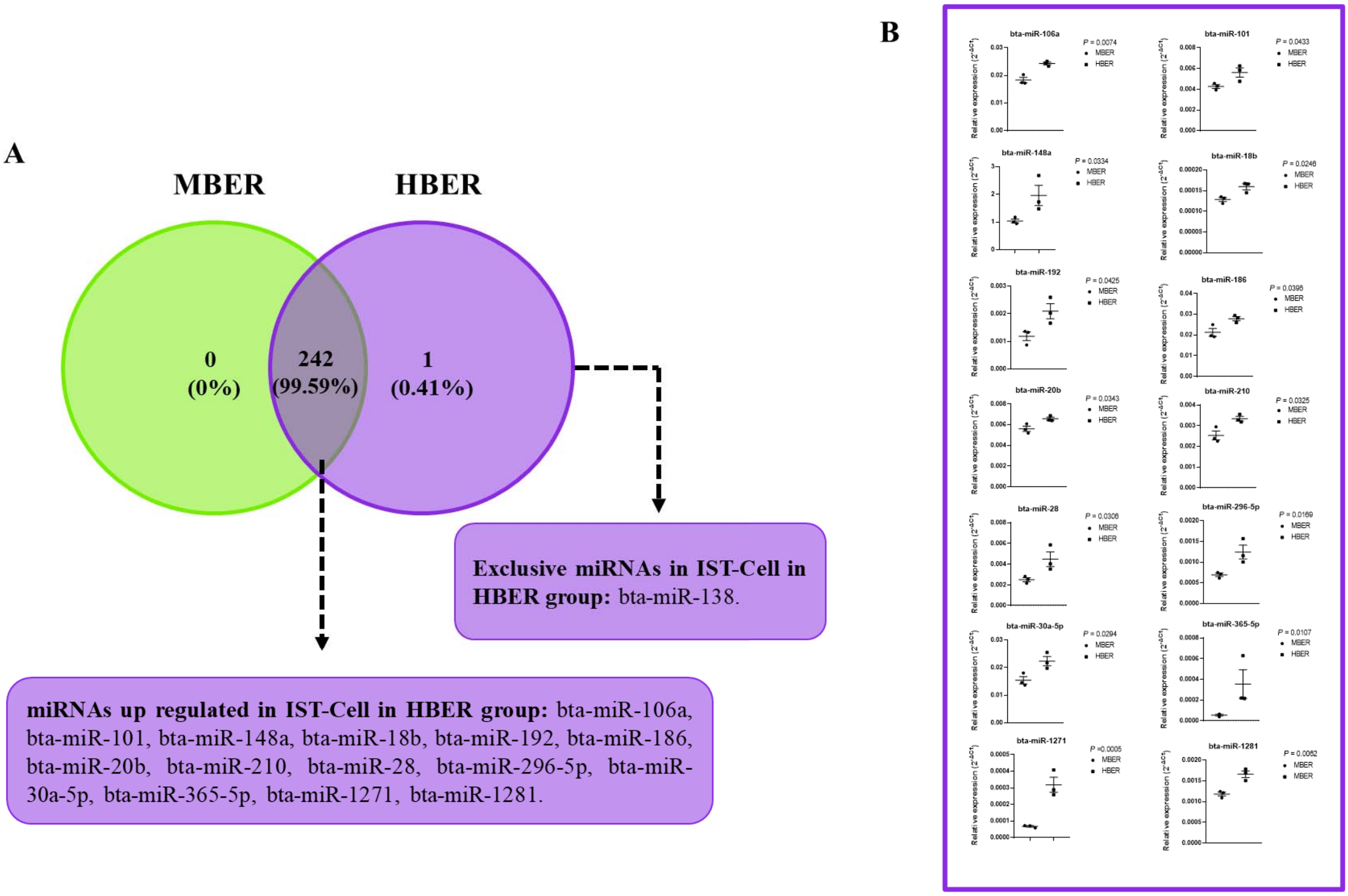


Figure 7. miRNAs expression in isthmic luminal epithelial cells (IST-Cell) from cows with different body energy reserve. **A.** Venn diagram demonstrating the 242 common miRNAs between groups and 1 exclusive miRNAs in HBER group. Of the 243 common miRNAs, 14 were up regulated in HBER group **B.** Relative expression of up regulated miRNAs in IST-Cell in HBER group. Mean ± standard error. P-value is on the right top of the figure.

4.3.6. *Enrichment analysis of miRNA differentially expressed in oviductal luminal epithelial cells*

To determine the predicted biological functions regulated by miRNAs differently expressed in OV-Cell, we performed the bioinformatics analysis using the unique as well as the differently expressed miRNAs. In AMP-Cell, there were total of 315 predictive pathways as regulated by the 10 exclusive miRNAs in HBER group (Supplementary Table 11), in which 16 were significantly different according to BH values, and 10 were selected as pathways with highest percent of genes predicted to be modulated by those miRNAs (Figure 8A). From the 199 predicted pathways as regulated by the 8 miRNAs up regulated in HBER group (Supplementary Table 12), 19 were significantly different according to BH values and 10 of them, with highest percent of genes predicted to be modulated by those miRNAs (Figure 8B). Our results demonstrate that the exclusive miRNAs in HBER AMP-Cell are predicted to regulate pathways like Glucagon, Insulin and Oxytocin signaling pathways, while the up regulated miRNAs in HBER AMP-Cell are predicted to be involved in Proteoglycans in cancer, ErbB and VEGF signaling pathways.

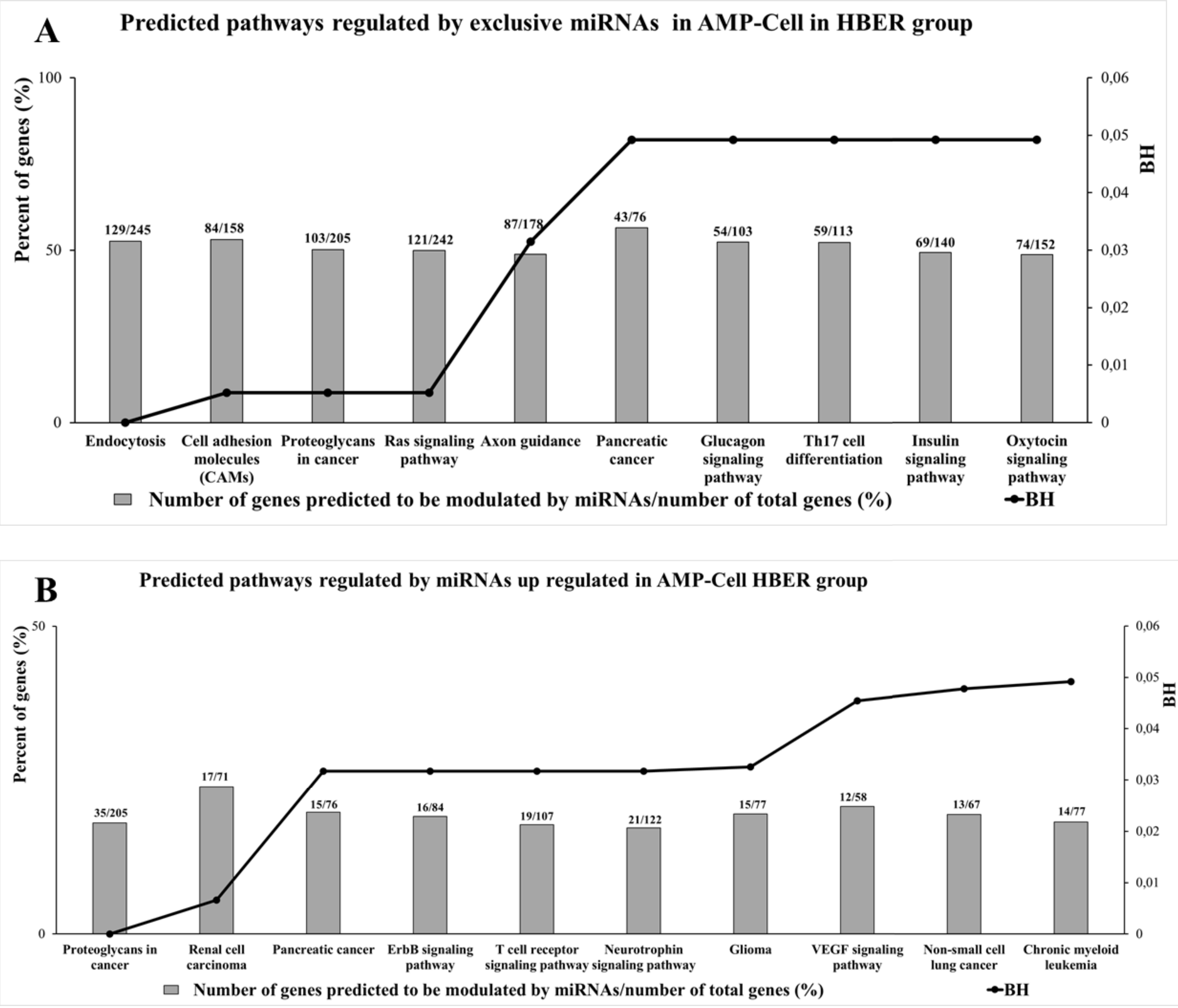


Figure 8. Enrichment analysis performed in miRWalk 3.0 software of predicted pathways modulated by miRNAs exclusives and differentially expressed in ampullary luminal epithelial cells (AMP-Cell) from HBER group. **A.** The 10 predicted pathways with highest percent of genes predicted to be modulated by exclusives miRNAs in AMP-Cell in HBER group. **B.** The 10 predicted pathways with highest percent of genes predicted to be modulated by miRNAs up regulated AMP-Cells in HBER group. The Y-axis in left represents the percent of genes (%) predicted to be modulated by miRNAs and the Y-axis in right shows the BH (BH < 0.05).

The 14 miRNAs up regulated in HBER IST-Cell are predicted to regulate a total of 314 pathways (Supplementary Table 13), in which 48 are significantly different according to BH values and 10 with highest percent of genes predicted to be modulated by those miRNAs (Figure 9). The up regulated miRNAs in HBER IST-Cell were related with Endocrine resistance, Insulin resistance and Insulin signaling pathway.

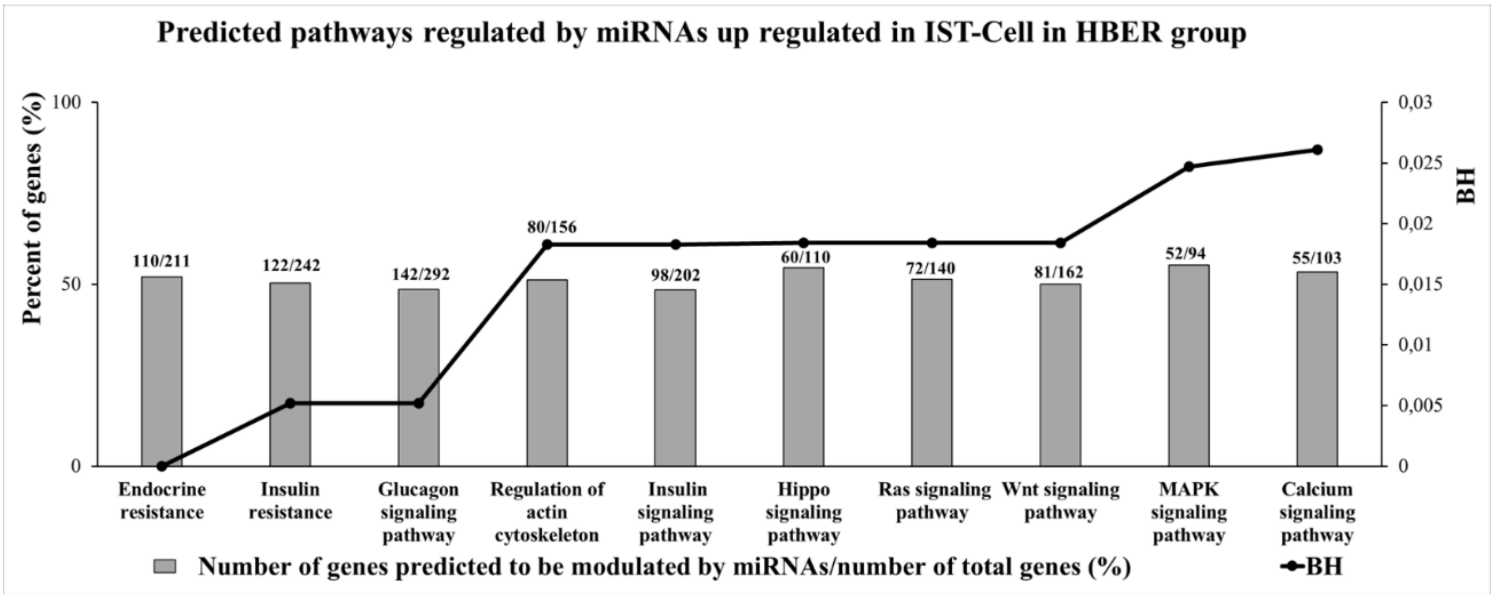


Figure 9. Enrichment analysis performed in miRWalk 3.0 software of predicted pathways modulated by miRNAs differentially expressed in isthmic luminal epithelial cells (IST-Cell) from HBER group. The 10 predicted pathways with highest percent of genes predicted to be modulated by miRNAs up regulated IST-Cells in HBER group. The Y-axis in left represents the percent of genes (%) predicted to be modulated by miRNAs and the Y-axis in right shows the BH (BH < 0.05).

4.3.7. Differentially expressed genes in oviductal epithelial cells

With the aim of understand the BER influences on the RNA profile and biological processes, the RNAseq technique was performed in OV-Cells. We evaluated differently expressed genes (DEGs) between contrasts, and our results demonstrate a differential RNA profile in OV-Cells from cows with different BER. Briefly, in AMP-Cell, there were 11 DEGs in which 6 were up regulated in MBER group (ENSBTAG00000049543, PDYN, EPPK1, NQO2, SCG5 and IQCE; Figure 10A, red) and 5 up regulated in HBER group (KRT80, TPMT, SFMBT2, MFAP2 and CA10; Figure 10A, green). Heatmap analysis demonstrate the differences within samples within each group (Figure 10b). These DEGs are involved in biological pathways as Neuroactive ligand-receptor, Cytokine-cytokine receptor interaction, Glycine, serine and threonine metabolism, Cysteine and methionine metabolism, Hedgehog signaling pathway and Wnt signaling pathway (Figure 10C).

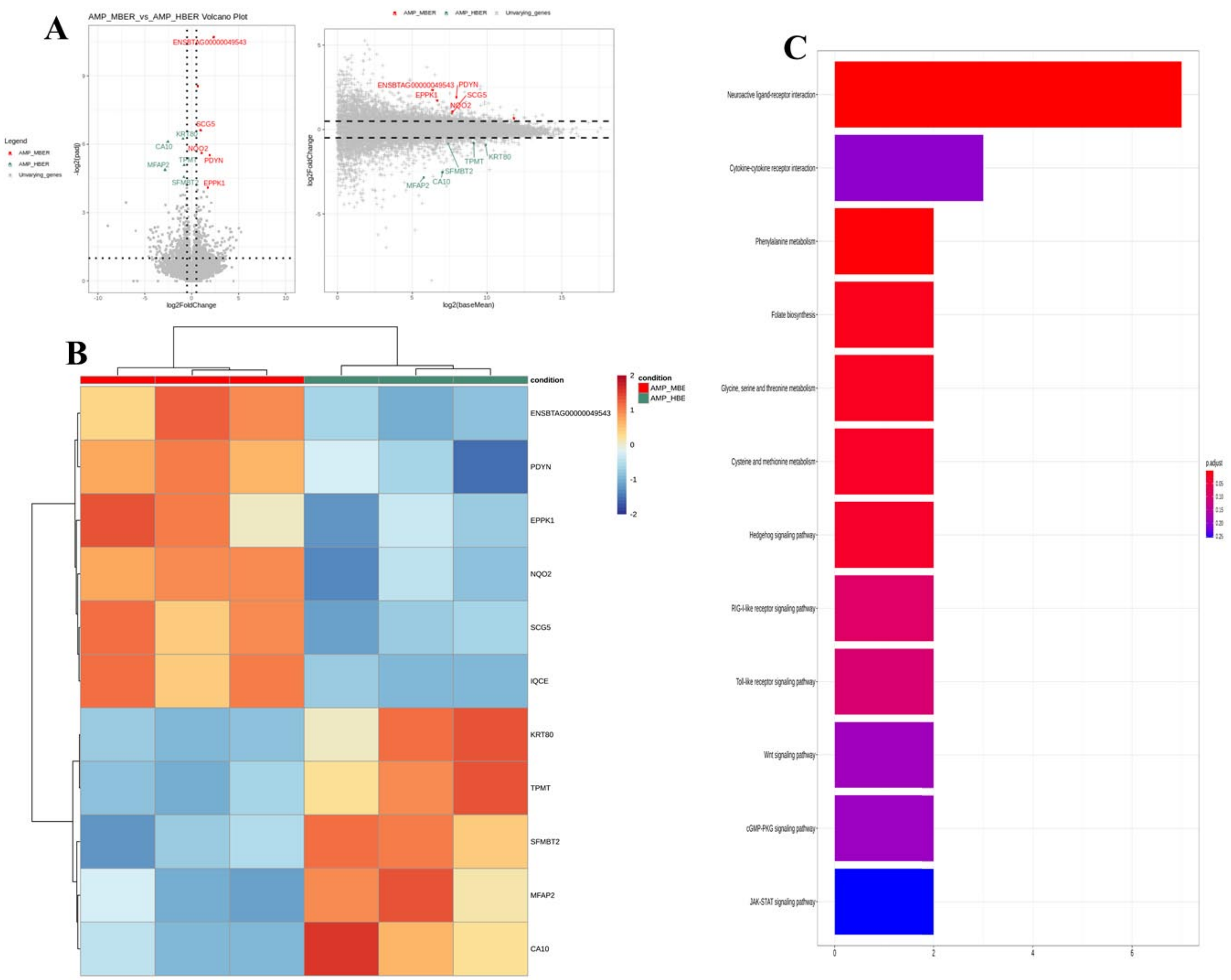


Figure 10. Differential gene expression (DEG) in ampullary luminal epithelial cells (AMP-Cell) from cows with different body energy reserve. **A.** Volcano and smir plot representing the variation in DEGs in AMP-Cell for MBER and HBER group **B.** Heatmap showing the variation in the DEGs in AMP-Cell for MBER and HBER group. **C.** Biological pathways affected by DEGs in AMP-Cell.

In IST-Cell, there were a total of 17 DEGs in which 8 were up regulated in MBER group (LOC112444164, BCL2L14, CLDC, D2HGDH, TBC1D8, VCAN, NTS, ALAD; figure 11A, green), and 9 up regulated in HBER group (LOC78706, ENSBTAG00000049543, TMEM246, VNN2, LOC112447728, EPPK1, ADH6, AIM2, PDYN; Figure 11A, purple). Heatmap analysis demonstrate the differences within samples within each group (figure 11B). These DEGs are involved in biological pathways as Neuroactive ligand-receptor interaction, Calcium signaling pathway, cAMP signaling pathway, Vitamin digestion and absorption, Alanine, aspartate and glutamate metabolism, Carbohydrate digestion and absorption, Estrogen signaling pathway and Vascular smooth muscle contraction (Figure 11C).

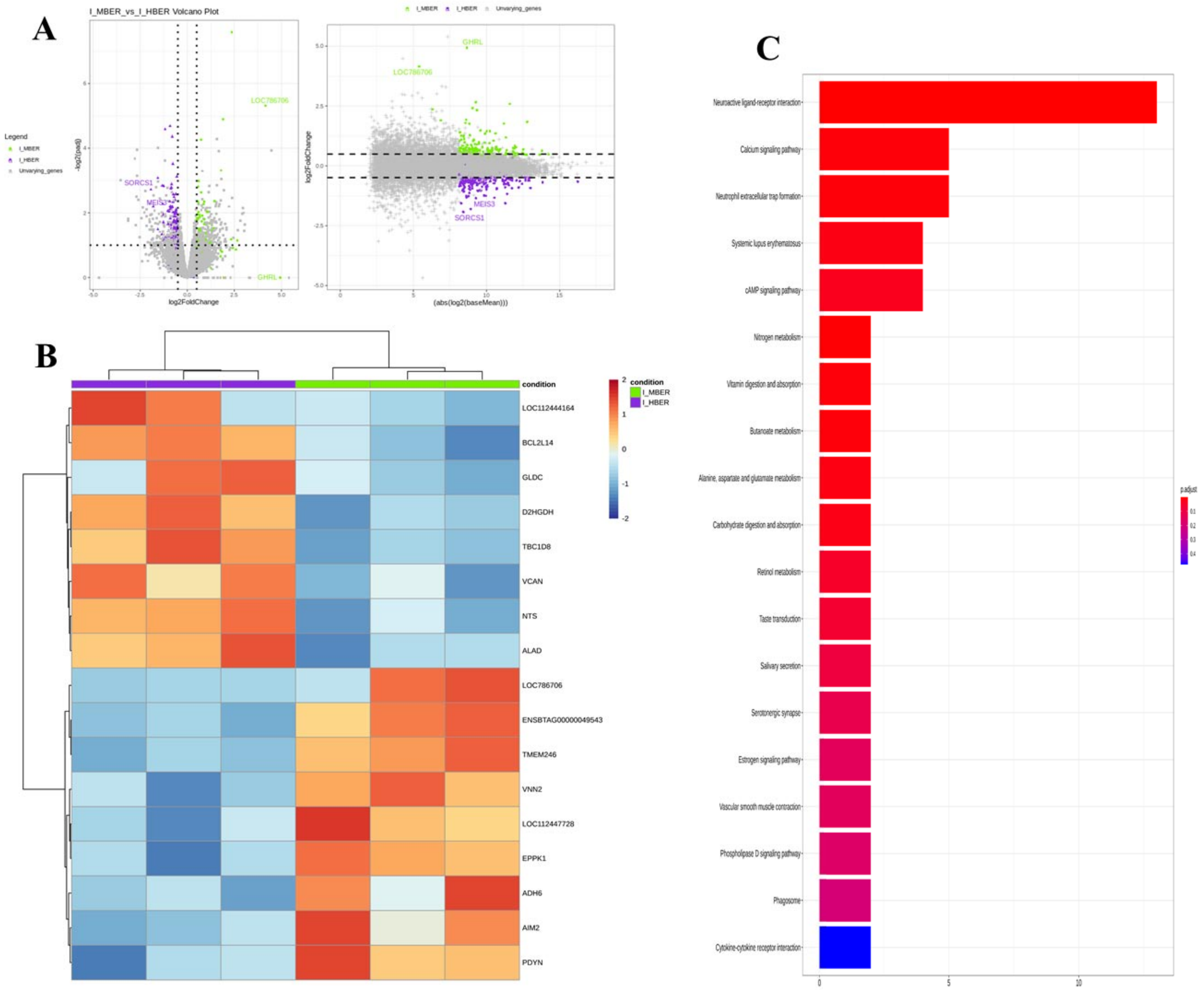


Figure 11. Differential gene expression (DEG) in isthmic luminal epithelial cells (IST-Cell) from cows with different body energy reserve. **A.** Volcano and smir plot representing the variation in DEGs in IST-Cell for MBER and HBER group **B.** Heatmap showing the variation in the DEGs in IST-Cell for MBER and HBER group. **C.** Biological pathways affected by DEGs in IST-Cell.

4.4. Discussion

The genetic donors' cattle which can have high BER, frequently suffer with reproductive problems. In another context, due to the modern human lifestyle, the number of women in childbearing age overweighted or obese has significantly increased over the years representing an important problem to be solve (FAO, 2018). This metabolic condition affects women’s health and reproductive function which may negatively affect the fetal outcomes (STANG; HUFFMAN, 2016; CHA et al., 2021). However, the biological and molecular causes that make metabolism and, consequently, the BER responsible for reproductive disorders and the consequences for embryo development in animals and humans are not yet fully elucidated. Once the oviduct is a dynamic structure that, under ovarian hormones control, provides the ideal microenvironment for fertilization and embryo development, this work aimed to evaluate the oviduct environment composition as well as epithelial cells from cows with different BER. To our knowledge this is the first study connecting BER with oviductal effects as well as the evaluation of the different oviduct regions (ampulla and isthmus). In order to do that, we submitted Nellore cows from a same herd to a feedlot period in order to obtain cows with moderated BER and elevated BER. Once the animals had different BER, the cows were submitted to estrus synchronization, slaughter and the ipsilateral oviducts to the corpus luteum were collected, dissected, and only from animals which had an 8-cell embryo the samples were collected. Thus, we evaluated the oviductal (ampulla and isthmus) environment through oviductal flushing extracellular vesicles and oviductal composition by oviductal epithelial cells molecular analysis aiming the prediction of biological pathways and the DEG genes study.

The oviductal fluid that bathes the oviductal lumen allows the oviduct to provide a unique microenvironment to gametes acquire fertilization competence as well as the ideal conditions to initial embryo development. The EVs, which composes the oviductal fluid, are key mediators to the oviductal dynamism and communication between mother and embryo suggesting their important role in reproductive function. In the present work with the aim to evaluate the BER influences in oviductal environment, the OF-EVs size and concentration was evaluated as well as their miRNA contents. We found a BER influence in AMP-EVs concentration and IST-EVs size. Cells under stress conditions as oxidative and metabolic stress secretes higher EVs amounts (HARMATI et al., 2019; OLTRA et al., 2019). Women embryos produced i*n vitro* with positive pregnancy outcomes secreted fewer EVs compared to non-pregnant women embryos (ABU-HALIMA et al., 2017). The authors also observed that EVs secreted from non-pregnant women embryos were bigger that EVs from positive pregnancy outcomes (ABU-HALIMA et al., 2017). The EVs size is negatively correlated with embryo quality, which increased EVs size is related to poorly embryo quality (GARDINER et al., 2013). This suggests that the HBER may propitiated a stressful ampullary environment and also influenced in embryonic quality in isthmus, where an 8-cell embryo was found. Additionally, the BER induced changes in AMP-EVs miRNA content and no alteration in miRNAs of IST-EV. The oviductal anatomic structure is composed by three different regions: infundibulum, ampulla and isthmus. Despite the physiologic importance to reproductive functions of the oviduct the knowledge about the EVs cargo from the different oviductal regions is still limited. Importantly, previous studies have demonstrated that the estrous cycle (ALMIÑANA et al., 2018; HAMDI et al., 2021) and embryo presence (MAZZARELLA et al., 2021) are capable to modulate the miRNA cargo from oviductal EVs. Our data shows that the BER changes the miRNA cargo in AMP-EVs suggesting that the metabolism modulates the ampullary environment. The functional enrichment analysis of differentially expressed miRNAs in AMP-EVs, were predicted to regulate pathways related with cell growth, migration, differentiation and metabolism. The up regulated miRNA in HBER AMP-EVs (bta-miR-664a) is predicted to regulate transcripts of INSR and GLUT4 (MirWalk 3.0 version), which have important roles in the insulin signaling and insulin resistance pathways. Thus, there are indications that the insulin may have an important role modulating metabolic processes in the ampulla from HBER animals. The oviductal EVs analysis from isthmus region did not found any differences between the miRNAs contents of these EVs. Comparing the miRNA content from non-pregnant and pregnant cows, 1 miRNA up regulated was found in non-pregnant isthmic EVs while 8 miRNAs up regulated were found in the presence of one embryo, suggesting that the embryo presence tightly modulates de isthmic environment (MAZZARELLA et al., 2021). Once the bovine embryo also secretes EVs (ANDRADE et al., 2019; BRIDI et al., 2021), in our study the 8-cell stage embryo presence in both groups may masked a metabolic effect coming from BER in isthmus. However, EVs are composed by many other biological molecules that were not analyzed in this study and it is possible that their profile is altered by BER.

In women and other animals' species, metabolic problems like high DMI, elevated BER and hyperinsulinemia are related with a pathological state that can cause remodeling in many biological tissues (TESAURO; CARDILLO, 2011; WILLIAMS; KANG; WASSERMAN, 2015; SON et al., 2019). The mainly alteration related with metabolic problems is vascular disfunction affecting peripheric vascular resistance and substrates delivery (TESAURO; CARDILLO, 2011). This is probably due to the extracellular matrix, which acts as a physical barrier for metabolites diffusion and can remodel itself in diverse situations. In response to elevated BER and in a hyperinsulinemia condition, the extracellular matrix can remodel itself and accumulate collagen deposition to increasing physical barriers to glucose, insulin and fatty acids transport and decreasing their vascular delivery (WILLIAMS; KANG; WASSERMAN, 2015). It is already known that under hormonal influence and embryonic presence, the extracellular matrix of the oviduct and endometrium undergoes to molecular changes to assist embryo development and implantation (BURGHARDT et al., 2002; SCOLARI et al., 2017; GONELLA-DIAZA et al., 2018). Additionally, maternal obesity impaired placental angiogenesis and blood vessel density, promoting hypoxia, hypoglycemia and hypoinsulinemia in fetus (WALLACE et al., 2000, 2003; HAYES et al., 2012; SON et al., 2019). However, data showing the BER effects through oviductal histomorphology is still limited. Our data reveled that the elevated BER impaired the ampullary vascularization. In this way, even without changing the collagen rate, our results suggests that the oviduct may be regulating the substrates delivery by vascularization, due to its dynamic capacity, in an attempt to adequate the metabolites concentration. Our results also demonstrate that luminal perimeter was not affected by the BER, in accordance with previously demonstrated that this variable is under ovarian hormones control (GONELLA-DIAZA et al., 2017).

The cells molecular profile which composes the oviductal epithelia became an interesting type of sample to understand the possible ways that the metabolism is affecting this tissue. It is already known that the oviductal epithelial cells molecular profile can be altered through the estrous cycle (BAUERSACHS et al., 2004), by ovarian hormones (GONELLA-DIAZA et al., 2018), embryo presence (MAILLO et al., 2016; MAZZARELLA et al., 2021) and also lactation (GEGENFURTNER et al., 2019; LOCATELLI et al., 2019). Additionally, the nutritional plan influenced the ampullary epithelial cells protein profile in goats (FERNANDES et al., 2018). In this way, we evaluated the miRNA content and DEGs in OV-Cell to investigate the BER effect on oviductal epithelial cells molecular architecture. When analyzing the OV-Cell miRNA profile, we observed that BER changed the miRNA expression in ampulla and isthmus. There were a total of 10 miRNAs which expression was exclusively in HBER AMP-Cell. The predicted pathways related to those miRNAs are mainly associated to metabolism, such as Glucagon and Insulin signaling pathways. Interestingly, the exclusively detected bta-miR-21-3p and bta-miR-432 are predicted to regulate the INSR transcript, in addition to that miR-432 is also predicted to regulate GLUT4 transcript (MirWalk 3.0 version), suggesting that the HBER may regulate the AMP-Cell insulin metabolism. Once our data revealed that, simultaneously in HBER ampulla, the miRNA EV content may be involved with insulin resistance, we suggest that insulin have an important role in ampulla of HBER animals. Briefly, according to the literature, insulin is important for maintain glucose homeostasis and regulate carbohydrate, lipid and protein metabolism influencing these macronutrients stores (BOUGLÉ; ANNANE, 2009; NISWENDER, 2011). Thus, insulin signaling regulates important biological processes such as synthesis and uptake of glucose, gluconeogenesis, lipid metabolism, protein synthesis, cell growth and differentiation (TANIGUCHI; EMANUELLI; KAHN, 2006; BOUCHER; KLEINRIDDERS; KAHN, 2014). Furthermore, there is a total of 8 miRNA which expression is elevated in HBER group that are predicted to regulate pathways related to cell proliferation, differentiation and vascularization. The VEGF signaling pathway is associated with vascular development in which its family members are glycoproteins known to regulates vasculogenesis and angiogenesis processes in embryo development and pathological conditions (STACKER; ACHEN, 1999). In bovine oviduct, the VEGF property appears to be related to vascular permeability, epithelial cell secretion and motility providing the ideal supply of factors and nutrients and gametes/embryo transport, acting as a fine-regulator of the oviductal environment (GABLER et al., 1999; WIJAYAGUNAWARDANE; KODITHUWAKKU; YAMAMOTO, 2005). However, the VEGF function can be modulated by IGF-I and insulin, being the last more relevant (KONDO et al., 2003). In endothelial cells, insulin is able to suppress VEGF expression (KONDO et al., 2003), and the increase in BER has a strong positive correlation with high VEGF serum levels proteins (SILHA et al., 2005). This suggests that the association between low VEGF expression and high VEGF family proteins may indicate an insulin resistance condition. Additionally, this data corroborates our histomorphology result that showed lower vascularization in HBER ampulla. Thus, we speculate that insulin have the main role in ampullary physiology in HBER animals.

In IST-Cell, there were 14 miRNAs up regulated HBER that are predicted to regulate metabolic pathways, as endocrine resistance, insulin resistance, insulin and glucagon signaling. The bta-miR-20b and bta-miR-28 are predicted to regulate INSR transcript, while bta-miR-30a-5p and bta-miR-365-5p are predicted to regulate GLUT4 transcript (MirWalk 3.0 version). This suggests that regulation of insulin effects may have an important role in HBER isthmus cells. Also, the bta-miR-138 present only in HBER IST-Cell, is related with proliferation inhibition (HRDLIČKOVÁ et al., 2014), apoptosis induction (CHAKRABARTI; BANIK; RAY, 2013), inflammation (ZHANG et al., 2019) and its expression can be under hormonal regulation in testis, ovary and adrenal cells (HU et al., 2013). Despite these well-established functions, studies reports that the miR-138 may be related to alterations in cell glycolysis (CHEN et al., 2016; ZHU et al., 2017). Additionally, miR-138 was upregulated in diabetic nephropathy clinical patient samples (LIU et al., 2021) and data relates its expression to carcinogenesis due to elevated BER (OLIVO-MARSTON et al., 2014). It is important to emphasize that the isthmus samples collected in this study had a single 8-cells embryo presence. At this development stage, dramatic changes take place within the embryo. Once the major gene activation arises at 8-cell embryo (MEMILI; FIRST, 2000), metabolically events support embryonic development. Thus, simultaneously, the early embryo that prefer pyruvate for oxidative phosphorylation, slowly transits to develop glycolytic metabolism as the mitochondria becomes mature (SINCLAIR; ROOKE; MCEVOY, 2003; HARVEY, 2019). Since those are important ultrastructural changes, that can interfere in later development stages, the metabolic status of the developing embryo and the environment around this embryo may have important contributions to the following steps.

To increase our understanding about the elevated BER effects in OV-Cells, we used RNAseq analysis to identify DEGs. Previous studies identified a significant number of DEGs modulated by ovarian hormones (GONELLA-DIAZA et al., 2018) and embryo presence (MAILLO et al., 2016). Our data revealed 11 and 17 DEGs in AMP-Cell and IST-Cell, respectively, suggesting that BER has a slight effect in DEGs on OV-Cells. However, the impact and effects of this must be considered. In AMP-Cells, the biological pathways affected by DEGs are related to molecular response to the oviductal environment, as cell metabolism, proliferation and development. According to ampullary physiological role, after ovulation, this oviductal structure prepares itself generating an extremely secretory environment (GONELLA-DIAZA et al., 2017). Given the time when samples were collected (120 hours after ovulation induction), we expect that the ampulla changes its cellular constitution decreasing the secretory cells and increasing ciliated cells numbers and activities (KOLLE et al., 2009). However, under high BER, the natural physiological responses to these processes may be altered due to changes in the epithelium molecular response. Similar to AMP-Cell samples, the DEGs identified in isthmus samples are involved in biological pathways associated with molecular response focused in cell metabolism. In IST-Cell, the DEGs analysis, suggested that carbohydrate digestion and absorption pathway is affected by BER possibly leading to alteration in sugar metabolism caused mainly by insulin as already pointed by miRNA analysis, at an important developmental period for embryonic metabolism transition (THOMPSON et al., 1996). In addition, for IST-Cell, the DEGs influences pathways related to embryo transport as Estrogen signaling pathway and Vascular smooth muscle contraction. Besides to all the important functions in reproductive tract controlled by ovarian hormones, the oviduct is responsible for the gametes and embryo transport through smooth muscle contractions, ciliated cells beating, and oviduct fluid flow (BASTOS et al., 2022). Thus, estrogen can induce muscle contractions, faster ciliary beat and increase in oviductal fluid in order to advance the transport (VALLE et al., 2007; GONELLA-DIAZA et al., 2017). However, these oviductal functions must be orchestrated along with the embryo development, otherwise, the lack of synchrony between oviduct and embryo may result in ectopic pregnancy or failure in embryo development (KOLLE et al., 2009; OLSEN et al., 2018; BASTOS et al., 2022). Thus, we speculate that even with a small number of DEGs in oviductal epithelial cells under different BER, these genes might have a considerate impact in the normal physiological function of the oviduct. Therefore, our data reveal that the elevated BER may alter the oviductal metabolism, which possibly leads a local insulin resistance, affecting the normal functioning and, probably, the embryo metabolism during early development, impacting gestational rates in these animals.

4.5. Conclusion

The elevated BER alters the oviductal environment and constitutions which may compromise the oviductal physiological function. This may impair the normal embryo development and later pregnancy stages. Other studies should be performed to enhance our knowledge about the effects induced by altered BER at the oviduct and embryo levels; importantly, using other approaches as metabolomics and proteomics to determine the consequences in metabolism and proteomic profile.

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5. General discussion and conclusion

The increase in livestock efficiency is based on several factors, among which the herd reproductive efficiency that has a significant importance. This is important since it can help to solve the "17 Sustainable Development Goals" objectives as zero hunger, zero deforestation since the increase in reproductive efficiency mean more animal protein and less deforestation. Reproductive biotechnologies such as artificial insemination (AI), *in vitro* embryo production (IVEP) and embryo transfer (ET) are tools capable to optimize the productive and reproductive potential of males and females. Such techniques are greatly used, not only for animal breeding, but also for infertility therapies faced by modern human society. Changes in habits such as later pregnancy and excessive consumption of high-calorie foods reflects on female reproductive potential. Despite these biotechnologies improvements on the reproductive success rates, in livestock and human scenarios the pregnancy achievement depends on the ideal development of the ovarian follicle and embryo. This is due to oocyte competence and early embryo development, which are susceptible to fluctuations in reproductive environment. In this way, life practices and physiological mechanisms that can possibly impact the female's reproductive life are not fully understood and deserves attention to improve fertility rates. To elucidate one of the mechanisms possibly affecting female reproductive efficiency, the goal of this thesis was to evaluate the consequences of increased body energy reserve on ovarian and oviductal response from bovine females.

Systematically, the ovarian follicle has important functions, such as provide oocyte competency and subsequent embryo survival. A recent study showed that cows with extreme BER had significant differences in serum metabolomic profile (HORN et al., 2022). The authors suggests that these modified metabolites may cross the blood follicular barrier influencing negatively in follicle and oocyte quality (HORN et al., 2022). In a mouse model, the elevated BER is associated with ovarian morphology impairment (HILAL et al., 2020) and reduces oocyte quality by abnormal mitochondrial function and distribution leading to oxidative stress and apoptosis (WU et al., 2015; HOU et al., 2016). In addition, murine diabetes affects the oocyte quality by chromatin defects and mitochondrial abnormalities (WANG et al., 2009). However, even with those negative ovarian responses, high BER females continue to ovulate (BERMEJO-ALVAREZ; ROSENFELD; ROBERTS, 2012). This may have a further effect in other reproductive structures, as the oviduct, where the fertilization and early embryo development takes place. In this way, in **chapter 1**, was aborded a literature review about the oviductal ability based on its dynamic and systemic changes during reproductive events, as well as the extracellular vesicles contribution in this process. Despite the oviductal biological importance, studies involving this reproductive structure are limited due to the difficult to obtain samples. Also, until now, although it is known that high BER influences in ovarian response, much remains to be sough, about its effects on oviduct response.

To compose the reported studies of this thesis we performed different analyses of the ovarian and oviductal responses to high BER (**chapters 2 and 3**). For this purpose, we used the same animal model whose groups were initially well characterized morphologically with different BER. For this, animals were previously subjected to the experimental period which Nellore cows from a same herd were breed in electronic feeder with different nutritional plans. Upon that cows were randomly selected to compose HBER group, which the diet was offered *Ad libitum* to elevate BER, while MBER cows were fed 70% of the daily feed of HBER treatment to maintain BER. Although in our animal model the groups presented BER alteration by different DMI, our aim was that this divergence was not extremely abrupt to the point that it could cause considerable metabolic disorders. In this way, when HBER group showed considerable higher values for BW, ADG and subcutaneous fat thickness compared to MBER group but without metabolic disorders, we assumed that the animals had different BER. Therefore, at the end of the experimental period the animals were submitted to estrous synchronization, FTAI and slaughter approximately 120 hours after ovulation induction, to sample collection. This window time was defined since it contemplates the presence of small antral follicles in the ovary (3-6 mm in diameter) and the period of time that the embryo develops within the oviduct. Thus, it was possible to analyze the effects of BER in ovarian and oviductal response. Additionally, this physiologic animal model allowed that each animal could achieve its own performance by individual analysis throughout the experimental period.

Initially at the ovarian level, the insulin has a positive action such as follicular growth, steroidogenesis, granulosa cells proliferation (KOSTER; OPSOMER, 2013). However, in excessive serum concentration it can lead to hyperinsulinemia or insulin resistance as a consequence of elevated BER, the insulin is harmful for ovarian follicles

(SINCLAIR, 2010). In **chapter 2**, added to the fact that HBER animals had elevated BER than MBER group, these animals showed higher values for insulin serum concentration during experimental period. This may reflect a hyperinsulinemia condition, which is already known in high DMI cows. Also, HBER group presented lower ovulation rate and even lower embryo recovery rate demonstrating reproductive failures in these animals. Changing BER from moderate to high BER modulates the hypothalamic-pituitary-ovarian axis by GnRH, FSH, LH and steroid synthesis and release leading to ovulations problems and fertility impairment (SPICER et al., 1984; BOSSIS et al., 2000; DISKIN et al., 2003). It is important to highlight that in HBER animals, 7 to 12 cows had ovulated and, from those 7 animals, only 3 structures were found. There are two reasonable possibilities to the low number of recovered structures: 1) the method to embryo recovery that was not efficient or 2) the HBER oviductal fimbriae may be under HBER effect and the interaction with the COC was impaired. Unfortunately, we were not able to answer these questions. However, this is an important problem to be solved, once bovine breeders can have problems in producing *in vivo* embryos for ET from high value genetic donors because their high BER.

Despite the great influence of the high BER on ovarian follicle and oocyte, little is kwon about the molecular mechanisms involved in this process and how this occurs. The quality of oocyte from small follicles was not affected in insulin resistant dairy cows (OLIVEIRA et al., 2016) this is because larger follicles apparently are more sensible to high BER effect (ADAMIAK et al., 2005; MOLLO et al., 2017). However, although the great influence of high BER on ovarian follicle and oocyte, little is kwon about the molecular mechanisms involved in this process, how and when this occurs. In this way, also in **chapter 2** our results showed that despite no differences in reproductive hormones (E2 and P4) serum concentration, the communication within ovarian follicles (3-6 mm in diameter) evaluated by miRNA profile of CC and EVs FF, appears to be different between groups. To this end this is first time a work allowed to show molecular alterations within small developing follicles. Also, miRNA profile of the EVs FF from HBER group are more different from CC than HBER group. Once others follicular cells, as granulosa, are influenced by high BER leading to apoptosis and alteration of mitochondrial metabolic function (WU et al., 2015; KORDUS et al., 2020), their secretion of molecules may be different. This suggests that the communication altered by high BER may impact in further follicles stages impairing ovulation and oocyte quality.

The oviduct takes place to important reproductive processes such as final oocyte maturation (ASAADI et al., 2021), fertilization (COY et al., 2012), and early embryo development (KOLLE et al., 2009; AVILÉS; COY; RIZOS, 2015). The knowledge about the anatomy and physiology of this structure, despite being longstanding (EDDY; PAUERSTEIN, 1980; GARCÍA-PASCUAL et al., 1996), is very superficial given the difficulty in obtaining samples. Much of what we know about oviduct was obtained by studies realized *in vitro*, where oviductal samples are cultured in laboratory conditions. Although this model helps to understand oviductal physiology, it is still limited. Moreover, using murine oviduct cultured *in vitro*, Al-Dossary and collages (2013) reported in less than a decade the presence of EVs as a constituent of oviductal fluid. Studies trying to elucidate the role of these biological nanoparticles were performed, but, still in *in vitro* environment (LOPERA-VASQUEZ et al., 2016; ALMIÑANA et al., 2018). However, physiologically studies has been developing aiming to characterize the oviductal response to ovarian hormones (GONELLA-DIAZA et al., 2017, 2018, 2021), embryo presence (RODRÍGUEZ-ALONSO et al., 2020a, 2020b; MAZZARELLA et al., 2021) and now, to high BER in order to better elucidates its impacts in reproduction function. Based on that, in **chapter 3**, several analyses were performed in oviductal EVs, tissue and luminal epithelial cells. To perform analyses, we considered only animals which had an 8-cell embryo to 1) proper comparison between groups, since embryo presence modulates the oviduct and 2) elucidation of BER effects on the environment where embryonic development started and it is propagated. The two oviductal regions were collected independently (ampulla and isthmus) because their morphological/systematical function and to improve our understand of the increased BER effects in each oviductal portion.

Pathological states as a consequence of elevated BER added to hyperinsulinemia increases deposition and remodulation of extracellular matrix in various tissues leading to increase in physical barriers decreasing vascular delivery of metabolites and nutrients as insulin (TESAURO; CARDILLO, 2011; WILLIAMS; KANG; WASSERMAN, 2015). Studies performed in placenta presented that maternal overnutrition, that is, elevated BER, impair placental vascularization and angiogenesis which reduces oxygenation in fetal tissues and contributes to poor neonatal survival (HAYES et al., 2012; GACCIOLI et al., 2013; SON et al., 2019). Data presented in **chapter 3** showed that the ampullary region was widely affected by BER, since the vascularization from

HBER group were different than MBER. Added to that, in ampulla, OF-EVs and OV-Cell were also modulated by high BER and important pathways related to insulin and vascular function were founded by enrichment analysis. This suggests that the elevated BER may be impairing the ideal ampullary vascularization associated with insulin resistance.

In **chapter 3**, although the isthmus was not histomorphologically affected by high BER, molecular alterations were found in IST-Cell. The enrichment analysis performed by miRNA and DEGs demonstrated that in OF-EVs and OV-Cell the insulin and carbohydrate metabolism have an important role in HBER oviduct. The insulin is required in mammalian early embryos and is related to DNA, RNA and protein synthesis (TRAVERS; PRATTEN; BECK, 1989; RAO; WIKARCZUK; HEYNER, 1990). This hormone promotes embryonic growth and differentiation (KAHN; RONALD, 2001) but, in high concentrations or long exposure promotes apoptotic cell death impairing embryo quality (PINTO; SCHLEIN; MOLEY, 2002; WATT et al., 2005; MOLLO et al., 2017). The tissue sensibility by insulin may be specific for a certain metabolic pathway in a certain tissue (KOSTER; OPSOMER, 2013). Thus, once the evaluated cows may have a hyperinsulinemia condition verified in **chapter 2**, it is plausible to suggest that the HBER oviductal environment is insulin resistant. The consequences of this to embryo development were not elucidate in this thesis due to the low number of embryos collected in each cow (single embryo per cow). However, metabolic alterations in IST-Cell may contribute to asynchrony between oviduct-embryo and increased medium toxicity, allowing alterations in embryonic metabolism and quality.

In conclusion, the results showed here indicates that the ovarian and oviductal responses are influenced by elevated BER once the ovulation rate and communication within the follicle is altered, as the embryo recovery rate and environment/composition at the ampulla and isthmus. We believe that future studies will help to elucidate possible solutions to the problems identified in this thesis. Therefore, the results obtained here are interesting and necessary to supply the lack of knowledge in reproductive failures in high BER females.

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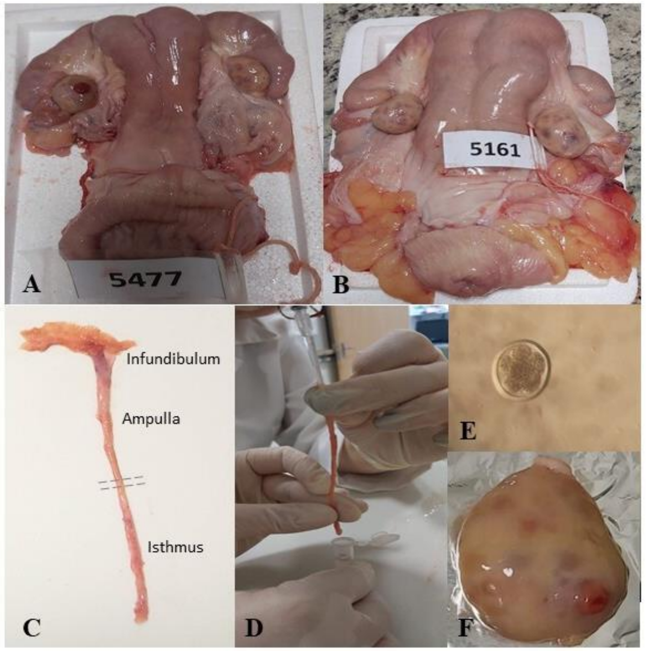
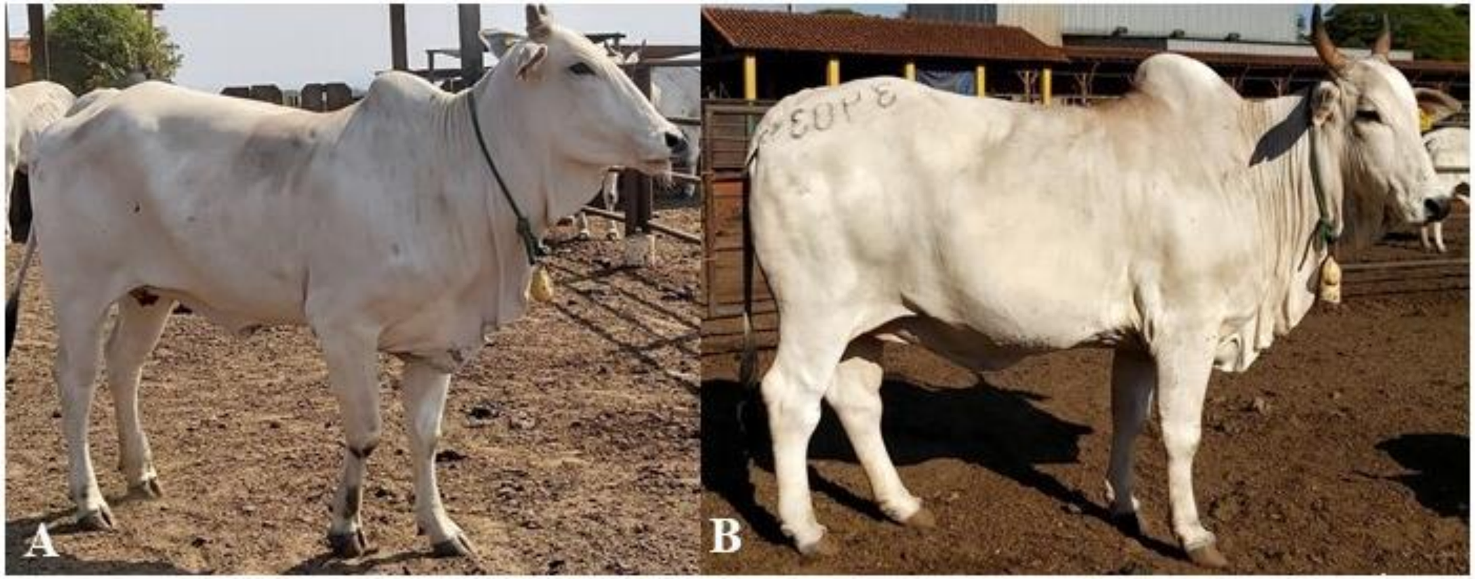
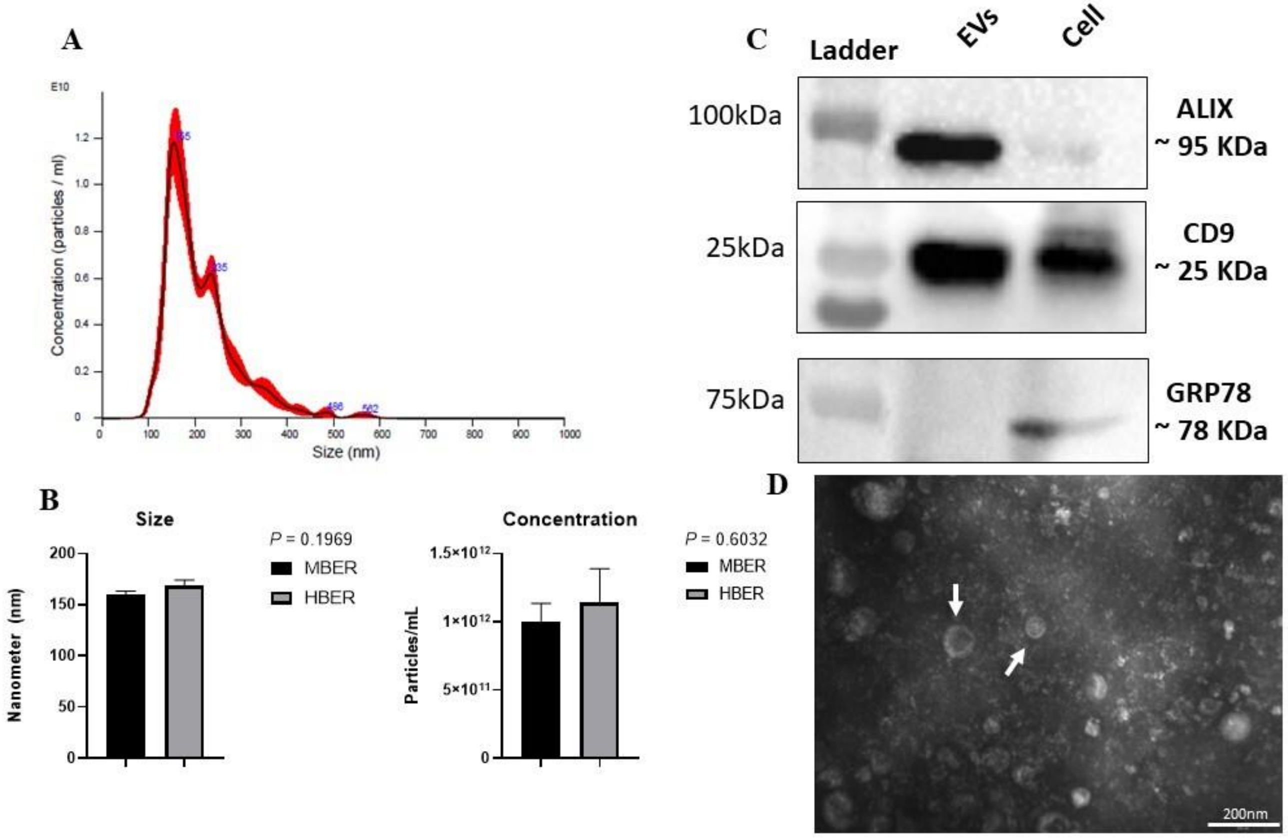
Appendix A – Supplementary material of chapter 2



Supplementary figure 1. Reproductive tract collection for embryo, cumulus cells and follicular fluid recovery. **A.** MBER group reproductive tract **B.** HBER group reproductive tract. **C.** Ipsilateral oviduct was dissected for flushing of its contents. **D.** The isthmus portion was flushed with 1xPBS in the ovary-uterus direction. **E.** A 8-cell embryo representative image obtained by magnifying glass. **F.** Ovarian follicles (3-6 mm) from ipsi and contralateral ovaries were aspirated to obtain cumulus cells and follicular fluid for small extracellular vesicles separation and analysis.



Supplementary figure 2. Representative image of animals with different body energy reserve. A. Moderated body energy reserve group (MBER). **B.** Hight body energy reserve group (HBER).



Supplementary figure 3. Follicular fluid extracellular vesicles characterization. A. Follicular fluid extracellular vesicles from cows with different body energy reserve analyzed by nanoparticle tracking analysis (NTA). **B.** Extracellular vesicles size and concentration were analyzed by NTA. **C.** Western blotting analysis demonstrates the presence of characteristic vesicles proteins (ALIX and CD9) and absence of cell-specific proteins in follicular fluid vesicle samples (GRP78). The western blot images were cropped for the purpose of this figure. **D.** Transmission electron microscopy images shows the presence of extracellular vesicles in follicular fluid.

Supplementary table 1. Ingredients on a dry matter (DM) basis (g/kg) of adaptation diets.

Ingredients [2]	Adaptation diet[1] (g/kg of DM)		
	1	2	3
Corn silage	600	500	550
Finely ground corn	278.4	380	470
Soybean meal	102.4	100	76.3
Mineral supplement	5.6	6.3	6.2
Limestone	5.4	5.6	9.7
Urea	4.5	4.4	6.9
Salt	3.5	4.4	4.4

[1] Adaptation diet: Adaptation diet 1 with 40:60 concentrate and corn silage ratio; Adaptation diet 2 with 50:50 concentrate and corn silage ratio; Adaptation diet 3 with 58:42 concentrate and corn silage ratio. [2] Ingredients: Mineral supplement: Ca (208 g/kg); Co (148 mg/kg), Cu (2.7 mg/kg), S (64 g/kg), F (1.6 mg/kg), P (160 g/kg), I (141 mg/kg), Mn (2.2 mg/kg) Se (37 mg/kg), Zn (79.92 mg/kg) and sodium monensin (4000 mg/kg).

Supplementary table 2. Least squares mean, standard error of mean and probability of performance and carcass characteristics of Nellore cows that changed their body energy reserve after the experimental period.

Item	**Body energy reserve[1]**		**P- value[2]**
	MBER	**HBER**	
Number of animals	9	12	-
Height of withers, cm	144 ± 0.016	145 ± 0.007	0.6204
Dry matter intake, kg	8.20 ± 0.74	12.07 ± 0.76	**<.0001**
Average daily gain, kg	0.41 ± 0.54	1.39 ± 0.60	**<.0001**
Carcass weight, kg	295.82 ± 10.76	363.53 ± 16.39	**0.0020**
Carcass yield, %	58.78 ± 0.47	57.31 ± 0.5	**0.0331**

[1] Body energy reserve: MBER: Cows with moderated body energy reserve; HBER: Cows with high body energy reserve; [2] P-value: P value between animals with different body energy reserve.

Supplementary table 3. Raw cycle threshold levels of the 383 miRNAs profile in cumulus cells (CC) from ipsi and contralateral ovarian follicles (3-6 mm in diameter) from cows with different body energy reserve.

MiRNAs	**Body energy reserve[1]**											
	MBER								**HBER**			
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**1**	**2**	**3**	**4**
bta-let-7a-3p	32.6329	.	.	.	.	.	.	.	.	.	.	.
bta-miR-103	33.4939	.	.	.	.	.	34.1132	36.0767	.	.	.	.
bta-let-7a-5p	27.7830	35.5376	34.8821	.	29.1532	28.4824	.	25.4194	34.8821	34.5822	29.8289	.
bta-miR-105a	.	.	.	.	.	.	33.1376	.	.	.	.	.
bta-let-7b	30.6383	.	35.0286	.	30.6173	30.3857	.	26.7068	35.0286	.	30.3539	.
bta-miR-105b	.	.	.	.	.	.	.	36.5265	.	.	.	.
bta-let-7c	28.3012	33.3092	34.3397	.	29.0178	28.6414	34.4343	25.6382	34.3397	35.0800	29.3478	34.9363
bta-miR-106a	33.2136	.	.	.	34.5924	36.4703	35.3861	32.2902	.	35.4608	.	.
bta-let-7d	29.4991	36.9923	.	.	30.7635	29.9570	.	26.8458	.	.	32.0303	.
bta-miR-106b	35.4937	.	.	.	34.9646	.	.	35.4235	.	.	34.3796	.
bta-let-7e	28.2844	33.9702	32.4129	34.3867	28.4557	27.0000	33.3462	25.0261	32.4129	.	28.4566	.
bta-miR-107	.	.	.	.	.	.	.	.	.	.	.	.
bta-let-7f	28.0775	.	.	.	31.7152	30.6277	.	27.2196	.	34.2189	31.5689	.
bta-miR-10a	31.8335	.	.	.	.	.	.	.	.	.	.	34.6471
bta-let-7g	28.7382	34.3414	.	.	31.7306	34.8084	.	28.8894	.	.	32.5109	.
bta-miR-10b	31.5248	.	.	.	.	.	35.1241	33.1489	.	.	35.4336	.
bta-let-7i	29.6923	.	.	.	.	.	.	30.3218	.	.	.	.
bta-miR-122	.	.	.	.	.	.	.	.	.	.	34.9644	.
bta-miR-1	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-124a	.	.	.	.	.	.	.	.	.	.	.	35.9199
bta-miR-100	34.3975	.	33.9009	.	36.6398	.	34.0635	.	33.9009	.	.	.
bta-miR-124b	.	.	.	.	.	.	.	36.5840	.	.	.	.

bta-miR-101	.	.	.	.	34.4550	.	.	.	.	.	.	.
bta-miR-125a	28.7070	.	35.6764	34.8620	33.8154	31.6039	34.9567	29.2240	35.6764	.	32.9381	.
bta-miR-125b	31.4882	.	36.9339	.	36.4632	33.0967	.	31.9554	36.9339	34.2379	34.9169	.
bta-miR-133b	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-126-3p	.	34.7000	.	33.5459	.	.	31.3812	.	.	.	32.8437	32.6703
bta-miR-133c	34.6735	.	.	.	.	.	31.8928	.	.	.	32.8993	.
bta-miR-126-5p	.	.	.	36.0299	.	.	30.8388	.	.	.	32.5819	32.7644
bta-miR-134	.	.	.	32.9150	.	.	30.1930	.	.	.	34.4423	31.7897
bta-miR-127	35.7024	34.8754	34.2865	33.4015	33.9272	32.8803	32.9846	33.1199	34.2865	.	34.8852	33.4915
bta-miR-135a	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-128	.	.	.	.	.	.	.	33.7580	.	.	34.7147	.
bta-miR-135b	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-129	.	.	.	.	33.9088	.	.	35.1693	.	.	.	.
bta-miR-136	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-129-3p	36.3606	.	.	.	.	.	33.2454	.	.	.	.	35.5468
bta-miR-137	.	.	.	.	.	.	35.2344	.	.	.	.	.
bta-miR-129-5p	.	.	.	.	.	.	.	35.9813	.	.	.	.
bta-miR-138	.	.	.	31.7937	.	.	29.8171	31.7289	.	.	35.2409	30.1953
bta-miR-130a	35.0502	34.4067	31.8284	32.9105	34.7652	32.2672	31.8123	34.8558	31.8284	.	33.5480	32.9560
bta-miR-139	.	.	.	.	.	.	33.4570	.	.	.	.	.
bta-miR-130b	31.7478	33.1327	31.8508	31.7928	33.9760	32.1363	31.3036	33.1302	31.8508	35.9067	32.5855	31.7114
bta-miR-140	35.9445	.	.	.	.	.	33.9359	.	.	.	.	34.1767
bta-miR-132	32.7076	.	.	33.7561	.	.	30.7109	34.1514	.	.	33.9945	33.9350
bta-miR-141	.	.	.	.	.	.	33.0067	.	.	.	34.2814	35.3385
bta-miR-133a	.	.	.	.	.	36.4311	29.8702	.	.	22.8246	32.5369	33.9379
bta-miR-142-3p	.	.	.	.	.	.	.	.	.	.	34.8907	34.8048

bta-miR-142-5p	.	.	.	35.2976	.	.	35.0368	.	.	.	35.0325	.
bta-miR-151-3p	.	.	.	36.7687	.	35.8585	34.7198	33.0143	.	.	.	.
bta-miR-143	33.9600	34.7640	.	34.8022	34.9841	33.8794	31.8105	35.9016	.	.	32.9025	33.2229
bta-miR-151-5p	33.5249	.	.	.	.	36.7780	.	32.9893	.	34.9583	.	.
bta-miR-144	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-152	36.7749	.	36.8008	33.2544	.	.	31.8320	.	36.8008	.	33.1764	.
bta-miR-145	.	.	.	33.8873	34.2177	.	31.8903	33.4795	.	.	36.9522	32.6011
bta-miR-153	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-146a	.	.	.	.	.	.	34.9847	.	.	.	34.5914	.
bta-miR-154a	.	.	.	36.2968	.	35.5757	32.2864	.	.	.	.	34.1084
bta-miR-146b	.	34.2215	.	35.0519	.	.	34.8799	.	.	.	.	34.6747
bta-miR-154b	.	34.7742	32.8739	30.3812	33.9249	33.3504	29.1363	31.7179	32.8739	.	31.9521	30.3117
bta-miR-147	.	.	.	.	.	.	.	35.3285	.	.	.	.
bta-miR-154c	.	36.3512	.	.	36.8795	.	36.6252	.	.	.	.	.
bta-miR-148a	32.8794	.	.	.	34.1772	.	.	.	.	34.2057	.	.
bta-miR-155	32.8174	.	.	.	32.5614	32.8736	33.3902	33.9497	.	.	33.6392	34.2225
bta-miR-148b	31.8361	.	.	.	.	.	.	36.2579	.	34.2469	.	.
bta-miR-15a	34.6343	.	.	.	.	.	35.0208	.	.	.	.	.
bta-miR-149-3p	34.3217	.	.	34.5023	35.2679	33.0436	32.3272	32.7985	.	.	32.9724	32.6484
bta-miR-15b	28.7549	.	33.9609	.	33.8676	30.6392	.	29.7108	33.9609	.	32.8569	.
bta-miR-149-5p	.	.	.	.	.	36.0016	32.7299	34.3492	.	.	33.3838	32.7677
bta-miR-16a	29.6701	.	.	.	.	33.1408	32.8766	31.6752	.	33.5683	.	.
bta-miR-150	.	.	.	.	.	.	32.7471	.	.	.	34.4546	34.9615
bta-miR-16b	29.8265	.	.	.	33.4366	33.1997	32.5006	30.7786	.	33.9599	32.8449	32.9321
bta-miR-17-3p	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-188	.	.	.	.	.	.	32.9035	35.3687	.	.	.	.

bta-miR-17-5p	34.9310	.	.	.	.	.	34.2054	32.9785	.	.	.	36.5868
bta-miR-18a	34.4806	.	.	.	.	.	.	33.3318	.	.	.	.
bta-miR-181a	34.8406	.	.	.	.	.	.	.	.	35.1678	.	.
bta-miR-18b	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-181b	.	.	.	.	.	33.6750	.	34.9148	.	.	.	.
bta-miR-190a	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-181c	.	.	.	.	.	.	.	.	.	.	36.6183	.
bta-miR-190b	36.6755	.	.	.	.	.	34.5340	33.1601	.	.	34.4527	.
bta-miR-181d	33.5128	35.3660	.	34.9862	34.9305	35.0255	32.7695	33.7460	.	.	.	33.7427
bta-miR-191	31.6633	19.6404	27.8390	18.2415	32.7027	33.3956	31.9914	31.4216	27.8390	20.0441	32.8450	21.8092
bta-miR-182	.	.	.	.	.	.	33.2708	.	.	.	.	34.2894
bta-miR-192	34.9210	.	.	36.8534	.	33.8752	.	.	.	.	.	.
bta-miR-183	.	.	.	.	.	.	32.4643	.	.	.	34.8324	36.5417
bta-miR-193a	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-184	.	.	.	.	.	.	33.3915	.	.	.	33.5950	34.8707
bta-miR-193a-3p	.	.	.	.	.	.	.	.	.	.	35.1697	.
bta-miR-185	35.2934	.	.	.	.	.	.	35.3009	.	.	.	.
bta-miR-193a-5p	36.9167	.	36.7786	.	33.9348	31.9433	.	31.6654	36.7786	.	34.0928	.
bta-miR-186	.	.	.	.	.	.	32.0485	32.8926	.	.	33.7941	32.9233
bta-miR-193b	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-187	.	32.0092	.	.	.	.	31.4952	33.8485	.	.	.	32.8885
bta-miR-194	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-195	30.4225	33.9622	35.9576	35.9908	34.4141	32.5607	31.9836	30.3539	35.9576	.	34.3291	33.9686
bta-miR-200c	.	.	.	.	.	.	33.6991	.	.	.	34.2661	.
bta-miR-196a	34.8447	.	.	34.9120	33.8648	.	32.0356	32.8575	.	.	33.6075	33.0888
bta-miR-202	28.1818	.	.	.	.	35.2635	.	30.4398	.	32.5535	30.8038	.

bta-miR-196b	.	.	.	36.2879	.	.	35.7364	33.5800	.	.	34.8288	33.7672
bta-miR-204	.	.	.	36.6348	.	36.6706	31.5981	.	.	.	32.2138	32.9583
bta-miR-197	33.9686	.	.	.	30.3852	28.5821	30.1886	26.9685	.	.	28.8215	31.8557
bta-miR-205	.	.	.	.	.	.	32.9755	35.3986	.	.	31.8619	34.9110
bta-miR-199a-3p	34.7238	.	.	.	.	.	.	.	.	.	.	.
bta-miR-206	.	.	.	35.5308	34.3179	34.6096	31.7284	.	.	.	31.7719	32.1446
bta-miR-199a-5p	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-208a	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-199b	.	.	.	.	.	.	35.7784	.	.	.	.	.
bta-miR-208b	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-199c	.	34.2018	.	.	.	.	31.7519	.	.	.	31.5969	34.1195
bta-miR-20a	31.6567	.	.	34.9325	34.4801	.	33.7804	32.1166	.	35.4886	33.5269	34.7654
bta-miR-19a	.	.	.	.	.	.	.	.	.	35.3081	35.6543	.
bta-miR-20b	34.8625	.	.	.	.	.	36.8952	34.8504	.	.	.	34.9714
bta-miR-19b	34.7653	.	.	.	34.5400	.	.	.	.	35.0393	.	.
bta-miR-21-3p	.	.	.	.	35.1695	35.0111	.	.	.	.	.	.
bta-miR-200a	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-21-5p	31.6939	.	.	.	33.8697	.	.	33.2655	.	34.0121	.	.
bta-miR-200b	34.2378	.	.	.	.	.	32.9298	35.6406	.	.	34.4080	.
bta-miR-210	33.6049	.	.	.	.	34.9643	.	34.1871	.	.	33.8143	34.2843
bta-miR-211	.	.	.	36.3571	.	.	32.0915	.	.	.	32.7494	33.1448
bta-miR-22-5p	34.5064	.	.	.	.	.	.	.	.	.	.	.
bta-miR-212	.	.	.	.	.	.	.	33.6057	.	.	.	.
bta-miR-221	35.1105	.	.	.	.	36.8768	.	.	.	.	.	.
bta-miR-214	35.6785	.	.	.	35.2427	33.9840	36.6934	36.3181	.	.	.	35.3230
bta-miR-222	.	.	.	.	.	.	32.7836	35.1659	.	.	32.8619	.

bta-miR-215	34.8993	34.8933	.	.	.	.	33.3926	.	.	.	35.6990	35.2757
bta-miR-223	.	.	.	36.8756	.	.	34.0398	.	.	.	33.8812	35.9726
bta-miR-216a	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-224	32.8857	.	.	.	.	.	34.9315	34.7126	.	.	.	.
bta-miR-216b	.	.	.	.	.	33.8988	.	35.9551	.	.	.	.
bta-miR-23a	29.8475	.	.	34.3461	35.2246	32.8541	33.9346	32.7422	.	.	33.9055	.
bta-miR-217	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-23b-3p	30.3252	.	.	.	34.8386	32.5033	32.9699	32.1203	.	35.4091	33.7349	.
bta-miR-218	.	.	.	.	.	.	35.4427	35.4001	.	.	.	.
bta-miR-23b-5p	34.9656	.	.	34.3006	.	35.7368	.	.	.	.	.	.
bta-miR-219	36.1105	36.5761	35.9463	.	36.4318	.	31.6548	35.3552	35.9463	.	31.3967	33.6446
bta-miR-24	.	17.8245	.	.	.	.	.	.	.	18.3596	.	.
bta-miR-219-3p	.	.	.	.	36.6639	35.0375	32.8627	35.6858	.	.	33.9014	.
bta-miR-24-3p	33.8440	.	.	.	.	36.6923	.	32.9922	.	.	34.9402	.
bta-miR-219-5p	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-25	29.7013	.	.	36.9239	.	31.9729	.	30.4763	.	35.1271	.	.
bta-miR-22-3p	2.1736	2.3081	2.3994	2.5749	2.4831	2.5526	2.6351	2.3036	2.3994	2.6994	3.0553	2.4952
bta-miR-26a	28.4467	.	.	33.9430	33.2769	32.8148	32.2940	30.8431	.	32.8127	32.6566	32.8577
bta-miR-26b	29.7272	.	.	.	33.7686	35.0710	.	32.9827	.	35.5273	.	.
bta-miR-29d-3p	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-26c	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-29d-5p	.	.	34.9245	.	.	.	.	.	34.9245	.	.	.
bta-miR-27a-3p	34.6542	.	.	.	36.2073	36.1345	31.1250	.	.	.	32.8999	33.6042
bta-miR-29e	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-27a-5p	9.4657	9.2365	8.1142	8.5150	9.1427	35.6654	.	7.3364	8.1142	8.7058	9.5688	10.1206
bta-miR-301a	.	.	.	.	.	.	.	.	.	.	.	34.7270

bta-miR-27b	33.4357	.	.	.	.	.	33.4395	33.9975	.	.	32.3044	34.3072
bta-miR-301b	.	.	.	.	.	.	35.8340	.	.	.	35.5925	.
bta-miR-28	.	.	.	.	.	.	33.9263	.	.	.	.	.
bta-miR-302a	.	.	.	.	35.8240	.	.	.	.	.	34.9293	.
bta-miR-296-3p	32.7752	33.9950	.	35.4993	34.5661	33.3140	35.3540	30.9305	.	.	33.8311	36.4068
bta-miR-302b	.	.	.	.	.	.	.	.	.	.	34.4227	35.8908
bta-miR-296-5p	35.7104	.	36.7894	.	.	35.2243	32.9498	33.9362	36.7894	.	31.8317	32.8323
bta-miR-302c	.	.	.	.	.	.	33.9126	.	.	.	.	35.6125
bta-miR-299	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-302d	.	.	.	34.4043	.	.	.	.	.	.	.	.
bta-miR-29a	32.0420	.	.	.	.	33.8299	.	.	.	33.8721	.	.
bta-miR-3064	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-29b	34.5445	.	.	.	.	.	.	.	.	.	.	.
bta-miR-30a-5p	32.6045	.	.	34.4104	.	36.8179	35.0071	32.0031	.	35.6487	36.4727	.
bta-miR-29c	31.1626	.	.	.	.	34.8972	.	32.7699	.	.	34.7598	.
bta-miR-30b-3p	.	.	.	.	34.6732	.	.	.	.	.	.	.
bta-miR-30b-5p	32.8332	.	.	34.9363	.	35.1110	32.8819	33.2177	.	.	34.1697	34.9144
bta-miR-328	34.1796	.	.	.	.	33.3191	34.0238	.	.	.	35.0194	.
bta-miR-30c	30.2571	.	.	.	.	33.6058	32.4000	31.1427	.	33.5641	32.8968	34.4084
bta-miR-329a	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-30d	33.9221	.	.	.	.	.	34.8932	32.5995	.	35.9774	34.9089	.
bta-miR-329b	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-30e-5p	35.1849	.	.	.	.	.	34.9262	33.0576	.	35.0747	34.0557	.
bta-miR-330	.	.	.	.	.	36.2217	.	33.5945	.	.	35.0027	.
bta-miR-30f	32.0531	.	.	33.8138	.	33.4080	35.1965	33.1698	.	.	34.9343	.
bta-miR-331-3p	.	.	.	.	.	35.3639	.	33.4008	.	.	.	.

bta-miR-31	30.4427	.	.	36.8190	33.4756	31.7775	.	30.0938	.	33.9760	33.8044	.
bta-miR-331-5p	.	.	.	34.5732	.	35.2996	31.8985	.	.	.	31.4883	32.5107
bta-miR-32	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-335	33.9707	.	.	36.0278	34.6767	.	.	.	.	.	36.5548	.
bta-miR-320a	31.8904	36.2960	34.6620	.	31.4937	31.8546	34.4136	28.4305	34.6620	.	31.0635	33.8862
bta-miR-338	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-320b	.	.	.	.	.	34.6263	32.8969	34.7941	.	.	34.1305	33.9190
bta-miR-339a	.	.	.	33.6918	.	.	.	32.5040	.	.	.	34.6318
bta-miR-323	19.3785	17.1192	15.8844	15.7498	19.4385	17.2737	17.6686	20.6150	15.8844	21.6157	18.7191	17.7376
bta-miR-339b	33.0316	.	.	33.5172	31.5418	35.1416	32.0415	31.1874	.	17.1678	31.9507	33.2442
bta-miR-324	.	.	.	.	.	.	32.7139	.	.	.	34.9700	33.9812
bta-miR-33a	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-326	.	.	.	.	35.7449	34.9171	35.1819	.	.	.	.	.
bta-miR-33b	.	36.4775	.	36.0681	34.8983	34.2320	34.8549	35.4230	.	.	.	35.0411
bta-miR-340	.	.	.	.	.	.	33.8994	33.1944	.	.	31.9905	.
bta-miR-365-3p	30.8804	.	.	.	.	.	.	32.3892	.	.	.	.
bta-miR-342	33.9694	.	.	34.9936	.	32.9053	33.6413	31.6317	.	.	33.3334	35.4356
bta-miR-365-5p	.	.	.	.	36.8022	34.9886	.	34.9077	.	.	35.0218	35.4840
bta-miR-345-3p	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-367	.	.	.	.	.	.	36.2053	.	.	.	.	.
bta-miR-345-5p	.	.	.	.	.	.	33.8552	.	.	.	33.9391	33.9637
bta-miR-369-3p	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-346	.	.	.	.	35.3747	36.6267	32.9444	32.8427	.	.	33.2539	.
bta-miR-369-5p	.	.	.	.	.	.	33.3732	35.8516	.	.	33.2680	34.4616
bta-miR-34a	.	.	.	.	35.0421	35.2769	34.5280	32.0705	.	.	.	34.6651
bta-miR-370	.	35.7506	.	34.6013	33.9415	32.1730	34.9481	33.0144	.	35.4226	33.8910	.

bta-miR-34b	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-371	.	35.4800	.	34.9024	.	.	.	.	.	.	36.7333	34.9232
bta-miR-34c	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-374a	34.8218	.	.	.	.	.	.	.	.	.	.	.
bta-miR-361	31.9101	.	.	.	33.9990	32.4140	.	31.4354	.	.	34.1427	.
bta-miR-374b	31.6008	.	.	.	.	.	34.6589	.	.	.	.	.
bta-miR-362-3p	.	.	.	.	.	.	34.9182	.	.	.	.	.
bta-miR-375	.	34.7678	32.9869	30.6016	34.4514	31.7544	30.6241	.	32.9869	.	30.7844	29.7425
bta-miR-362-5p	.	.	.	.	.	36.0219	.	34.8051	.	.	.	.
bta-miR-376a	.	.	.	.	.	.	34.0018	36.9730	.	.	31.9728	.
bta-miR-363	.	.	.	.	.	.	.	.	.	.	33.8912	.
bta-miR-376b	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-376c	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-382	36.5578	34.9303	36.1682	32.7086	33.9920	34.4666	31.7033	33.0485	36.1682	36.3061	33.0123	33.1904
bta-miR-376d	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-383	.	.	.	32.9275	.	.	33.2008	.	.	.	36.8510	.
bta-miR-376e	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-409a	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-377	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-409b	.	.	.	.	.	.	.	.	.	.	.	34.9559
bta-miR-378	.	.	.	.	34.0453	33.4267	31.7683	.	.	.	32.6782	32.6360
bta-miR-410	.	.	.	.	.	.	35.0093	.	.	.	.	.
bta-miR-378b	.	.	.	34.9327	.	.	32.7503	34.6895	.	.	32.1304	32.5901
bta-miR-411a	.	35.9600	36.8022	34.4003	34.6577	35.0102	32.2470	35.6020	36.8022	.	33.1828	32.8486
bta-miR-378c	.	.	.	36.0124	.	35.8411	36.5963	34.7474	.	34.9325	.	.
bta-miR-411b	.	.	34.5095	35.2848	35.5426	33.7818	34.1531	.	34.5095	23.6390	35.1951	34.3176

bta-miR-378d	.	35.9298	.	32.8466	.	34.9704	36.8377	.	.	.	35.0205	33.6833
bta-miR-411c-3p	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-379	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-411c-5p	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-380-3p	.	.	.	.	.	.	33.8743	35.7677	.	.	.	35.7453
bta-miR-412	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-380-5p	.	35.9054	.	34.0579	.	.	31.8243	.	.	34.2154	31.4519	32.3943
bta-miR-421	33.7388	34.4778	32.6496	34.6022	31.9194	.	31.3350	35.1485	32.6496	34.7893	31.8333	33.8691
bta-miR-381	.	.	.	.	.	.	.	.	.	.	35.1595	.
bta-miR-423-3p	35.9268	.	.	.	.	35.7211	.	32.8085	.	.	.	.
bta-miR-423-5p	31.7584	.	.	.	33.2695	32.3116	.	29.7646	.	.	32.7813	.
bta-miR-449c	.	.	.	.	.	.	33.9152	.	.	.	32.8824	35.2932
bta-miR-424-3p	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-449d	.	.	.	.	35.0107	33.1009	33.8596	32.9081	.	.	32.8321	36.0919
bta-miR-424-5p	32.5336	.	.	34.1810	35.5854	.	.	.	.	.	.	.
bta-miR-450a	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-425-3p	32.4723	.	.	.	35.9329	30.7368	33.3083	32.7962	.	.	32.1639	32.7601
bta-miR-450b	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-425-5p	34.8359	.	.	.	.	.	35.0124	.	.	.	.	.
bta-miR-451	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-429	35.9611	32.2459	33.6305	32.6573	32.2034	30.5414	30.3136	33.1151	33.6305	36.3953	32.0754	31.0765
bta-miR-452	.	.	.	.	.	.	34.6085	.	.	.	.	.
bta-miR-431	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-4523	.	.	35.8877	36.4017	.	.	34.8666	36.8509	35.8877	.	35.1428	35.5200
bta-miR-432	34.9424	.	.	35.5499	.	.	31.3461	.	.	.	30.3136	32.9358
bta-miR-453	.	36.4057	36.2101	.	.	.	34.6335	.	.	.	32.9538	34.8814

bta-miR-433	31.8930	30.4069	28.8153	29.3664	32.8493	30.9091	30.2186	33.5136	28.8153	31.9433	30.5722	30.3427
bta-miR-454	.	.	.	.	.	.	32.2671	.	.	.	31.9381	33.1557
bta-miR-448	.	.	.	.	.	.	35.8722	.	.	.	.	.
bta-miR-455-3p	.	.	.	.	.	.	34.6563	.	.	.	.	.
bta-miR-449a	.	.	.	.	.	.	36.0651	33.0950	.	.	.	.
bta-miR-455-5p	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-449b	.	.	.	.	.	.	34.8829	.	.	.	.	.
bta-miR-483	.	.	.	.	.	35.4207	.	.	.	.	35.4481	.
bta-miR-484	34.0364	.	.	.	.	.	.	34.0322	.	35.8419	.	34.9139
bta-miR-496	.	.	.	.	.	.	35.0714	.	.	.	34.4055	33.5788
bta-miR-485	.	.	.	34.8735	.	36.8368	34.9842	.	.	.	34.0263	.
bta-miR-497	.	.	.	.	.	.	.	33.2641	.	.	.	.
bta-miR-486	33.6286	.	.	.	36.3341	32.7828	34.9421	32.2445	.	.	35.4372	.
bta-miR-499	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-487a	.	.	.	.	.	.	.	.	.	.	34.8724	.
bta-miR-500	33.7736	.	.	.	.	.	.	.	.	.	.	.
bta-miR-487b	.	.	.	.	.	.	.	35.6283	.	.	.	.
bta-miR-502a	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-488	.	.	.	.	.	.	34.8244	.	.	.	34.5750	34.4788
bta-miR-502b	.	.	.	.	.	34.4550	.	.	.	.	.	.
bta-miR-489	.	34.9317	.	35.2159	34.9825	34.2332	34.2023	.	.	.	34.4694	34.0032
bta-miR-503-3p	34.4055	.	.	34.8724	34.4553	32.4983	31.5761	30.8984	.	.	31.8525	32.7927
bta-miR-490	.	.	.	.	.	.	33.0715	36.1290	.	.	.	.
bta-miR-503-5p	.	27.9393	.	18.2871	.	.	.	.	.	.	24.4655	34.8125
bta-miR-491	34.0999	.	.	.	.	.	32.8301	34.9047	.	.	33.1930	35.7792
bta-miR-504	35.7637	.	.	.	.	.	.	36.0424	.	.	.	.

bta-miR-493	.	.	.	.	34.5875	34.0990	32.1189	33.0938	.	.	32.3659	34.9122
bta-miR-505	32.5767	.	.	.	.	.	35.0468	32.8204	.	.	35.0382	.
bta-miR-494	28.3453	30.8312	29.8328	30.4536	28.8058	25.8343	29.9510	27.3494	29.8328	30.8125	29.7290	30.7430
bta-miR-532	33.1305	.	.	34.8479	32.9388	.	33.2107	.	.	34.0354	34.5842	33.8858
bta-miR-495	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-539	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-541	.	33.8297	34.6828	33.8748	34.1074	32.3239	31.9207	34.2343	34.6828	.	32.3785	31.9840
bta-miR-582	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-542-5p	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-584	.	.	.	.	.	36.8985	34.8746	.	.	.	.	.
bta-miR-543	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-592	.	.	.	.	.	.	35.6735	.	.	.	35.6957	.
bta-miR-544a	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-599	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-544b	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-615	12.1437	11.4892	11.5965	11.3251	11.7342	11.7814	11.7244	11.3228	11.5965	11.8554	11.5882	11.3326
bta-miR-545-3p	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-628	.	.	34.7308	34.8034	.	.	32.6669	34.0086	34.7308	.	32.8855	32.8347
bta-miR-545-5p	.	.	.	.	.	.	.	.	.	.	34.1350	.
bta-miR-631	22.2985	19.7268	18.9791	18.9787	21.3397	19.5733	19.3643	22.4016	18.9791	23.9478	20.7170	19.9158
bta-miR-551a	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-652	32.7966	.	.	.	.	.	35.1978	.	.	.	.	.
bta-miR-551b	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-653	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-562	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-654	.	.	.	.	.	36.8402	.	32.9645	.	.	.	.

bta-miR-568	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-655	.	.	.	.	.	.	34.9591	.	.	.	34.7929	.
bta-miR-574	31.0758	33.0696	33.1283	32.8303	28.5351	27.5073	31.4290	26.4982	33.1283	36.5842	28.8957	31.4702
bta-miR-656	.	33.8776	32.6654	32.7364	34.2860	33.6520	32.6517	35.6752	32.6654	.	33.4261	32.4987
bta-miR-658	.	.	.	.	.	.	.	.	.	.	36.9044	.
bta-miR-758	.	.	35.3267	.	.	.	.	.	35.3267	.	.	35.0312
bta-miR-660	35.9602	.	.	.	.	.	32.8747	.	.	.	33.8692	35.7858
bta-miR-759	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-664a	.	.	.	.	34.2407	34.4148	33.8070	35.2604	.	.	.	34.2905
bta-miR-760-3p	.	.	.	.	.	.	33.9574	35.8898	.	.	35.3161	35.0605
bta-miR-664b	31.9499	.	.	.	34.3009	33.9447	34.9613	34.1679	.	36.8215	35.1926	.
bta-miR-760-5p	31.8379	.	.	34.1134	33.4687	32.9094	30.7198	31.3532	.	.	32.7478	32.5056
bta-miR-665	34.2483	.	.	.	33.9370	31.4091	.	32.6993	.	.	33.9281	35.1131
bta-miR-761	.	.	.	.	.	.	.	35.4243	.	.	36.9151	.
bta-miR-669	.	.	.	35.5730	31.7902	31.7687	31.5082	30.8516	.	.	31.4566	.
bta-miR-763	.	.	.	34.6551	35.8797	36.7804	.	33.9972	.	.	34.8724	34.9659
bta-miR-670	.	.	.	.	.	.	.	.	.	.	36.1018	.
bta-miR-764	.	.	.	.	.	.	.	35.6364	.	.	.	34.9419
bta-miR-671	.	.	.	.	.	.	.	36.0761	.	.	.	.
bta-miR-767	36.7716	33.9549	32.8315	33.0774	34.9681	32.0659	32.5830	33.9112	32.8315	.	34.9189	32.6276
bta-miR-677	34.3609	.	.	.	.	.	.	36.9806	.	.	35.8614	.
bta-miR-769	.	.	.	.	.	.	35.0842	35.9648	.	.	32.8437	.
bta-miR-7	33.0160	.	.	.	.	.	.	33.3987	.	.	.	.
bta-miR-873	.	.	.	.	.	.	34.0353	.	.	.	.	.
bta-miR-708	.	.	.	.	.	35.9663	35.0038	.	.	.	.	.
bta-miR-874	.	.	.	.	.	.	35.1619	33.4948	.	.	.	.

bta-miR-744	33.9533	33.7916	35.8698	.	30.7157	30.6550	35.0131	30.2562	35.8698	.	33.9336	.
bta-miR-875	.	.	.	.	.	.	33.9062	.	.	.	34.9452	34.9599
bta-miR-876	.	.	.	.	.	.	.	.	.	.	35.5650	.
bta-miR-98	31.4446	.	.	.	34.0537	.	.	33.0543	.	.	.	.
bta-miR-877	.	.	.	.	.	35.8287	32.4225	33.9599	.	.	32.7840	33.7721
bta-miR-99a-3p	.	.	.	.	.	.	32.6334	.	.	.	33.7227	.
bta-miR-885	.	.	.	.	36.5384	.	33.3393	36.9928	.	.	32.4945	33.0170
bta-miR-99a-5p	34.9053	.	.	.	.	.	.	.	.	35.3492	.	.
bta-miR-9-3p	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-99b	24.5998	23.3018	21.1714	22.1699	24.7431	22.8748	23.2209	25.5968	21.1714	24.5977	24.5195	23.7746
bta-miR-9-5p	.	.	.	.	.	.	34.3766	.	.	.	34.3210	33.9900
bta-miR-1179	.	.	.	34.6916	.	.	.	.	.	.	34.7463	.
bta-miR-92a	29.3032	.	.	33.5412	.	32.6235	33.8048	29.5108	.	.	34.8936	.
bta-miR-1185	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-92b	33.1418	32.9753	32.9044	32.8777	32.5948	30.6146	31.0786	31.2819	32.9044	.	29.8071	30.2334
bta-miR-1193	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-93	34.5710	.	.	.	.	.	.	32.1964	.	.	.	36.0023
bta-miR-1197	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-935	.	.	.	.	35.5011	33.4598	32.6111	34.1689	.	.	33.7286	34.6490
bta-miR-122	.	.	.	.	.	.	.	.	.	.	.	34.8658
bta-miR-940	32.4066	.	.	36.8733	34.8249	31.8996	.	31.9588	.	.	32.8201	.
bta-miR-1224	34.2819	.	.	34.4538	33.4669	34.9133	33.5904	31.6506	.	35.4623	32.8038	35.5079
bta-miR-95	.	.	.	.	.	.	.	.	.	.	35.0521	.
bta-miR-1225-3p	33.8604	.	.	.	33.8970	31.9569	35.5129	33.0053	.	.	33.2605	34.1263
bta-miR-96	.	.	.	.	.	.	35.1298	.	.	.	.	.
bta-miR-1246	27.3755	30.0785	28.7901	28.9034	26.5989	25.5448	30.5542	23.7961	28.7901	30.0979	27.6184	32.6107

bta-miR-1247-3p	.	.	33.6832	31.7063	33.9019	34.9955	31.6428	33.6760	33.6832	.	31.9829	33.0339
bta-miR-1296	.	.	.	.	.	33.6681	32.8916	.	.	.	32.7716	34.4487
bta-miR-1247-5p	34.9951	.	34.8871	.	36.9928	.	.	34.7239	34.8871	.	.	.
bta-miR-1298	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-1248	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-1301	36.4894	.	.	.	.	.	.	.	.	.	.	.
bta-miR-1249	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-1306	33.9356	.	.	.	34.4527	34.6863	32.1128	32.5890	.	.	32.7556	33.0154
bta-miR-1260b	27.1906	36.3851	32.0804	35.1181	30.2108	28.2464	32.8457	26.4281	32.0804	29.3194	30.1294	33.7232
bta-miR-1307	34.3105	35.9487	.	34.9091	36.1567	32.9290	35.4056	33.0381	.	36.7601	33.1743	33.7840
bta-miR-1271	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-1343-3p	35.3548	.	.	.	.	34.3209	32.8706	32.7790	.	.	33.4523	.
bta-miR-1277	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-1343-5p	32.8263	.	.	35.5688	34.0025	33.1691	.	32.6796	.	.	36.1962	36.1294
bta-miR-1281	.	.	.	32.9951	.	31.9410	32.3516	32.5159	.	.	31.0455	32.9258
bta-miR-1388-3p	.	36.7863	.	35.3805	.	.	36.5170	35.6263	.	.	35.7396	35.5214
bta-miR-1282	.	.	.	.	.	.	.	.	.	.	.	.
RNT43 snoRNA	30.4981	.	.	.	34.5266	31.8553	.	32.5419	.	34.9616	.	.
bta-miR-1284	.	.	.	.	.	.	.	.	.	.	.	.
Hm/Ms/Rt T1 snRNA	22.6585	29.0975	29.4449	27.4063	27.7294	27.0742	30.2098	25.5978	29.4449	27.7984	26.1580	30.6986
bta-miR-1287	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-99b	24.5360	23.1711	21.3774	21.7380	24.6964	22.8635	23.0394	25.2990	21.3774	24.2534	24.3961	23.6566
bta-miR-1291	.	.	36.8670	.	.	.	.	.	36.8670	.	35.0374	.
Negative control	.	.	.	.	.	.	.	.	.	.	.	.

[1] Body energy reserve: MBER: Cows with moderated body energy reserve; HBER: Cows with high body energy reserve.

Supplementary table 4. Raw cycle threshold levels of the 383 miRNAs profile in follicular fluid extracellular vesicles (FF EVs) from ipsi and contralateral ovarian follicles (3-6 mm in diameter) from cows with different body energy reserve.

MiRNAs	**Body energy reserve[1]**											
	MBER								**HBER**			
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**1**	**2**	**3**	**4**
bta-let-7a-3p	32.4228	31.5078	31.6503	33.6150	30.5825	.	34.8630	29.8423	33.0103	32.6799	32.8767	33.9361
bta-miR-103	31.1236	31.3223	30.2220	31.7174	28.7657	31.8996	32.9519	28.5728	30.8996	31.0321	30.8218	30.6779
bta-let-7a-5p	26.7365	26.2293	25.9156	27.2431	25.5704	27.4169	30.1855	25.7767	27.1868	27.0570	27.5731	26.6384
bta-miR-105a	30.9832	.	33.8898	.	32.8564	33.9507	.	32.9432	.	35.0364	.	32.5419
bta-let-7b	25.5766	24.7403	25.2329	25.7622	24.7464	25.8998	26.9993	24.4538	24.8582	25.7400	25.0831	25.3147
bta-miR-105b	31.0393	34.9981	34.7762	36.7867	.	.	.	32.4225	34.9487	.	.	31.5192
bta-let-7c	25.8030	25.1456	25.1794	26.2232	25.0407	26.1883	27.6499	24.6088	25.4922	26.1605	25.3909	25.5985
bta-miR-106a	25.7693	25.0272	24.3453	24.7342	24.1360	26.5594	29.5632	22.5894	24.3822	26.4648	25.8058	24.5030
bta-let-7d	28.5072	27.7561	27.5883	29.4502	26.9662	29.1954	31.6870	27.0728	29.1154	28.7851	28.7543	28.4652
bta-miR-106b	29.0310	28.7073	28.6133	28.7477	27.0708	30.6888	32.7442	25.7499	28.5190	29.6812	29.2870	28.5875
bta-let-7e	25.8844	25.7143	25.6689	26.8061	25.3125	26.8096	28.5943	25.7777	26.8376	26.6606	26.6757	26.8321
bta-miR-107	33.5579	34.2404	33.8288	.	32.1112	34.7233	.	32.7584	.	.	35.0041	36.5599
bta-let-7f	28.7939	28.0970	27.7909	28.5613	27.4070	28.6922	32.5091	27.3307	28.7672	29.2516	29.2897	28.7572
bta-miR-10a	29.2027	28.7278	29.2311	29.9615	28.4994	30.6999	33.6378	28.5819	30.3422	30.8350	30.4833	29.8286
bta-let-7g	30.2968	28.5931	27.7863	28.9552	26.9986	31.1341	33.9057	26.6684	29.2052	30.3427	30.0564	28.2012
bta-miR-10b	29.6353	28.2817	28.8352	28.9502	27.8972	29.8023	31.8476	27.1947	29.6170	30.0968	29.4343	29.3593
bta-let-7i	30.4678	30.4667	29.7851	31.5483	28.7981	31.8890	32.4128	28.7357	30.8083	32.0591	31.7134	30.7494
bta-miR-122	31.8864	.	35.4849	.	33.9465	36.4848	.	32.4473	.	.	.	.
bta-miR-1	35.6191	33.6520	.	.	.	.	.	36.1135	35.2261	.	.	.
bta-miR-124a	33.6171	.	33.5591	34.9129	33.2353	36.8955	.	33.7149	34.7666	.	.	36.1281
bta-miR-100	27.3328	26.5539	27.2604	28.1472	26.7753	29.1401	31.5578	26.7740	28.2314	28.6143	28.5954	28.1636
bta-miR-124b	.	.	36.3839	33.9487	33.6957	34.4239	.	32.8905	34.0009	35.4418	36.7406	33.8939

bta-miR-101	34.1501	32.3122	30.9559	30.5158	29.9062	.	.	27.6389	30.7502	34.5389	.	29.5032
bta-miR-125a	29.7724	30.3313	29.7410	29.7311	27.7803	30.7677	33.6682	28.1014	29.9611	29.7493	30.4188	29.7426
bta-miR-125b	28.2830	26.6026	26.8217	26.5974	26.4886	27.7183	30.7197	25.7519	25.9847	27.5790	27.7634	26.7529
bta-miR-133b	33.9688	.	35.6960	34.8837	33.9251	.	.	.	.	.	.	.
bta-miR-126-3p	27.6414	29.0583	28.9387	29.7983	29.5036	32.2961	32.9377	27.7751	28.7046	30.9613	30.8280	30.0796
bta-miR-133c	29.7590	.	34.6218	.	33.9014	34.8435	.	.	.	.	.	36.0791
bta-miR-126-5p	26.2022	26.1628	26.4822	27.5442	26.6237	27.8351	28.0916	24.9549	25.7785	27.6425	26.6421	27.2444
bta-miR-134	27.3659	34.1607	33.0286	32.4321	30.2382	33.0082	33.1450	31.1164	31.7912	34.3739	.	30.8096
bta-miR-127	30.7168	31.8859	31.1933	32.0147	30.5582	32.4954	32.3764	30.4066	32.9544	32.9742	31.8468	30.7121
bta-miR-135a	34.9460	34.2594	35.4655	33.5262	34.1691	35.7546	.	32.8487	33.9448	.	35.3489	33.9575
bta-miR-128	28.5798	27.8082	27.8735	29.1496	27.5039	29.7003	32.3674	26.3765	28.7456	29.3745	29.7318	28.8268
bta-miR-135b	36.0047	35.2784	.	.	34.2151	.	.	34.2456	.	36.8814	.	33.9485
bta-miR-129	32.0819	31.9367	33.0180	31.5310	29.4454	32.9453	36.1299	29.9026	31.9060	34.3028	35.0105	29.8934
bta-miR-136	.	35.4264	34.8898	.	34.5331	34.5826	.	33.0342	35.0635	.	35.0949	33.6781
bta-miR-129-3p	32.9595	.	.	35.0417	34.1764	.	.	31.7545	35.0402	.	.	.
bta-miR-137	.	.	33.9069	.	.	.	.	.	33.6531	.	.	.
bta-miR-129-5p	31.0122	32.5931	31.8928	32.5889	29.4655	31.8908	36.3964	30.2408	32.7090	33.0767	34.9641	30.6239
bta-miR-138	26.7509	31.8403	32.2412	34.0342	30.7808	33.3739	33.4450	31.8431	34.1830	33.3263	32.9037	31.1384
bta-miR-130a	29.6399	28.8479	28.5248	29.8705	28.0053	30.7608	29.9978	26.0006	29.2946	30.1403	28.7213	29.0393
bta-miR-139	31.7227	33.9617	33.1589	34.6059	30.8350	.	33.9518	31.5338	33.0909	33.8280	31.7979	32.8215
bta-miR-130b	29.9667	29.8433	28.7499	30.6126	28.1278	30.8411	31.7753	26.7214	29.7086	30.4710	28.8467	29.3173
bta-miR-140	27.9940	27.7804	26.6921	28.8081	26.8220	28.8196	29.7728	25.3496	27.7360	28.4985	26.7216	28.7083
bta-miR-132	26.6552	27.6625	28.3148	27.8400	27.7775	28.2127	33.2859	26.8941	27.6982	29.7819	28.7401	27.7155
bta-miR-141	31.4331	.	.	.	.	.	.	34.0654	.	.	33.6902	.
bta-miR-133a	28.4781	34.0005	31.6101	33.8756	30.5731	31.8095	35.8533	29.7482	31.4540	.	33.5147	30.1999
bta-miR-142-3p	31.4671	.	33.4683	.	32.8181	.	.	.	.	.	.	.

bta-miR-142-5p	32.5181	.	.	.	.	.	.	36.4960	.	.	.	.
bta-miR-151-3p	28.3935	28.3983	27.7243	28.8305	27.7991	29.0531	30.5382	26.7391	28.0278	29.1314	28.7670	28.1975
bta-miR-143	29.5047	30.7542	30.5590	30.7553	29.8380	32.2576	32.5436	29.0104	29.7486	31.4621	33.5028	29.9782
bta-miR-151-5p	30.6188	30.6818	29.7941	30.7249	29.1692	32.1540	32.8775	29.7096	31.4900	31.8498	31.7056	30.7119
bta-miR-144	32.8571	.	.	.	.	.	.	.	.	.	.	.
bta-miR-152	28.6821	31.3618	30.9745	31.3191	30.4051	32.6573	.	29.2707	30.6831	32.0940	32.1316	30.9927
bta-miR-145	27.8867	27.0294	27.3086	27.7874	26.7036	29.6206	29.7461	25.7341	26.9732	29.8425	28.3775	28.1673
bta-miR-153	.	.	35.2468	.	.	35.5119	.	36.8271	35.8931	35.4707	.	35.0823
bta-miR-146a	33.4573	.	34.7184	.	34.6387	.	.	33.3193	33.5267	33.7860	35.0080	34.4454
bta-miR-154a	29.5635	.	.	35.7357	.	36.5649	.	.	.	.	.	.
bta-miR-146b	31.9353	35.4319	.	.	34.1279	.	.	32.2972	.	34.5278	35.0799	36.5694
bta-miR-154b	27.2709	32.8409	32.9530	32.3862	33.9009	32.5109	32.0863	31.8717	33.9719	32.7117	31.7890	32.0118
bta-miR-147	.	.	34.7574	.	33.2167	34.4247	.	32.9357	.	.	.	.
bta-miR-154c	31.7529	33.0618	33.8930	34.3448	32.4900	.	.	31.5802	32.6236	33.7405	35.1018	.
bta-miR-148a	28.4351	26.8332	26.6782	26.6476	25.8886	28.7001	33.1440	24.7356	26.3462	28.7710	28.7396	26.7724
bta-miR-155	29.8867	30.3809	30.7092	30.7577	29.4119	31.4013	32.2543	29.4949	29.5001	30.3795	29.8976	30.8211
bta-miR-148b	28.8819	27.6253	27.7217	27.3010	26.4773	29.4587	.	25.4233	26.7125	29.6019	29.8403	27.1694
bta-miR-15a	26.4079	25.8111	25.5529	25.7798	25.3761	27.2023	33.7772	24.0270	25.3229	27.6821	27.0948	25.4005
bta-miR-149-3p	23.2398	23.4274	22.3210	24.8034	23.3729	24.5964	26.2070	22.4597	23.7692	24.7095	24.2058	24.4370
bta-miR-15b	26.7626	26.4339	25.8663	26.5414	25.2949	27.4094	29.6963	24.4551	26.1153	27.7109	26.7673	26.3455
bta-miR-149-5p	29.0026	31.6956	30.8070	33.0522	29.4158	31.7857	36.2637	29.8734	32.2642	31.7248	31.7554	29.6981
bta-miR-16a	25.9539	25.4842	24.8049	25.5106	24.6162	26.6498	29.7464	23.0574	24.7665	26.4205	26.3375	24.9083
bta-miR-150	30.6527	36.0265	33.9239	36.7149	31.5226	36.0438	35.0545	32.4333	32.8444	.	.	35.3759
bta-miR-16b	24.8358	24.3704	23.7556	24.6230	23.6180	25.6198	28.6124	22.2334	23.8277	25.5424	25.0268	24.1022
bta-miR-17-3p	33.4909	35.5283	34.1641	36.8912	33.0874	34.2547	.	33.1913	34.2541	.	33.7296	32.8376
bta-miR-188	29.0703	28.7557	28.0667	28.4587	27.3090	29.3216	31.1733	26.7711	27.6993	28.4654	28.8869	27.7711

bta-miR-17-5p	29.7769	29.7086	29.0846	30.0129	28.2818	30.7176	33.2897	27.6108	29.7940	30.2762	30.6470	29.5706
bta-miR-18a	30.7425	30.4603	29.7213	30.1411	28.8734	31.7012	.	27.7111	29.3417	32.3479	31.7542	29.3807
bta-miR-181a	30.3294	30.6564	29.8195	31.1566	29.5578	31.7838	32.9816	28.6074	31.2526	31.8053	30.4650	31.0727
bta-miR-18b	31.7586	33.4158	32.7350	31.3404	30.7982	34.1250	.	28.8932	30.5116	33.5739	33.9386	30.6741
bta-miR-181b	29.8830	29.8369	29.7141	29.7779	28.7563	30.7109	33.0760	28.7018	30.0467	30.7994	30.3145	30.6072
bta-miR-190a	32.5633	.	36.3765	.	33.8579	36.5483	.	34.3610	.	.	.	35.4685
bta-miR-181c	32.0773	32.3693	30.2518	32.3770	29.7870	35.5699	33.8783	29.0776	30.7631	31.7118	30.9309	32.4060
bta-miR-190b	32.4437	31.6403	31.2800	32.7257	31.8356	32.8262	34.8259	29.8184	32.2584	33.1410	30.4314	31.2958
bta-miR-181d	28.3788	29.1625	29.6810	29.5540	28.7181	29.7769	31.5329	28.3909	28.7702	29.7495	30.8300	28.8231
bta-miR-191	27.9814	27.9108	28.6909	28.8009	27.7723	29.7825	35.2976	26.4018	28.8762	30.4536	30.5047	28.6149
bta-miR-182	31.6896	.	.	.	35.5619	.	.	35.4662	.	.	.	34.9392
bta-miR-192	30.2134	31.5569	31.7657	32.0465	30.3048	32.8743	36.9990	29.7534	31.8910	33.4457	31.8952	30.7823
bta-miR-183	30.5403	36.9023	.	.	.	.	.	35.9293	.	.	34.1759	35.9822
bta-miR-193a	35.3375	.	.	.	.	.	.	.	.	.	.	.
bta-miR-184	29.7009	.	.	.	.	.	.	.	36.6117	.	.	.
bta-miR-193a-3p	34.1963	33.3968	31.7807	34.3233	30.6144	36.5464	34.0276	29.5353	33.9172	34.5439	32.7295	32.9013
bta-miR-185	30.6256	30.6772	29.9009	30.7008	28.6758	31.7811	31.8797	28.3391	30.7442	31.2711	30.2229	29.6738
bta-miR-193a-5p	27.9134	28.5166	27.7558	28.5190	27.3261	28.2646	28.6544	26.3627	27.8258	29.0177	27.1410	27.8123
bta-miR-186	28.6601	29.7076	28.7322	30.0979	28.1175	30.9023	32.6668	27.1450	28.8653	31.5097	29.7405	29.6772
bta-miR-193b	29.6202	29.7203	29.5541	30.6039	28.4844	30.5189	32.5528	27.4991	29.6967	31.5201	28.9425	29.8528
bta-miR-187	28.4819	30.0615	29.3750	30.6054	28.5156	30.1520	31.9584	28.4913	30.2612	30.0756	30.0847	28.7177
bta-miR-194	32.8445	32.7579	32.7544	32.6847	31.4743	34.8110	.	31.0786	31.9774	34.4434	.	32.1351
bta-miR-195	26.7789	26.1563	25.7267	26.4423	25.7151	27.3380	30.6947	24.7001	25.8388	27.4483	27.0438	25.8064
bta-miR-200c	31.1271	36.6591	35.9860	.	36.3394	.	.	33.3894	36.5223	.	29.7339	35.3729
bta-miR-196a	28.7697	33.9088	32.8617	33.5386	33.8794	32.8099	.	32.0911	34.0478	34.1375	34.7717	32.8648
bta-miR-202	27.0171	26.3465	25.6341	25.0903	24.1578	28.0998	32.2620	22.7310	25.5829	27.0876	27.8403	24.6404

bta-miR-196b	31.4707	33.8507	34.3470	.	34.2962	36.7366	34.9747	32.9237	35.7974	33.9578	.	32.0148
bta-miR-204	28.8547	31.1705	30.5738	31.9238	31.5105	32.4874	33.7327	29.7261	30.7437	33.0044	30.4975	31.9681
bta-miR-197	27.7222	28.8959	28.1172	28.7778	26.7775	28.6520	32.0911	26.6228	28.7198	29.7391	29.3715	27.7523
bta-miR-205	29.4967	.	33.8841	34.1190	30.7973	33.5449	34.5310	32.0085	34.5524	34.1476	31.7630	32.9476
bta-miR-199a-3p	27.2414	26.2073	26.6269	26.8165	25.7540	28.3316	29.6883	25.4449	26.1272	28.0205	28.2462	26.6414
bta-miR-206	27.8370	31.7322	31.1553	30.8235	29.8153	31.7731	33.7511	30.5487	31.3961	31.7619	32.9053	30.2727
bta-miR-199a-5p	32.8806	32.8519	34.1920	33.0442	31.8171	.	34.3415	30.6768	32.9197	.	33.0117	33.9446
bta-miR-208a	.	36.3132	.	.	.	.	.	33.9864	.	.	.	.
bta-miR-199b	33.8675	31.8674	33.2756	32.1033	33.9310	34.9370	.	31.7640	33.2870	34.5386	34.0760	32.9445
bta-miR-208b	.	.	.	.	.	.	36.4235	.	.	.	.	.
bta-miR-199c	25.7261	24.7683	25.4254	25.6791	25.2630	26.8384	28.7195	24.3730	24.8073	26.8186	26.7616	25.6094
bta-miR-20a	25.3155	24.8366	23.9511	24.7968	23.7958	26.1800	27.7894	22.1781	24.1566	25.8426	25.2403	24.4275
bta-miR-19a	35.6973	32.8558	31.3003	30.8129	30.6387	.	34.7350	26.4072	31.2800	34.2570	31.6965	29.7468
bta-miR-20b	26.6881	26.1262	25.4020	25.8479	24.9039	27.6566	29.7357	23.5864	25.3960	27.2815	26.7625	25.7726
bta-miR-19b	31.4194	33.8840	31.6854	32.0065	29.7367	31.4026	32.3749	26.3931	32.2478	32.9410	30.9312	30.1197
bta-miR-21-3p	33.9581	34.5320	31.8231	34.1406	31.2464	35.5155	.	31.6793	32.9643	36.8073	32.8696	32.1199
bta-miR-200a	.	.	34.2440	.	34.4964	.	.	.	.	.	.	34.4191
bta-miR-21-5p	31.2950	30.8349	31.8431	31.1447	30.6039	32.6507	.	31.2218	32.0963	31.7735	32.8760	31.7250
bta-miR-200b	30.3477	31.5795	30.1840	32.4841	30.6253	32.9201	.	29.8784	30.7501	32.8104	27.7001	31.8771
bta-miR-210	29.2249	29.4182	28.2168	29.3312	27.4176	30.5031	30.6963	25.7377	29.1403	29.7547	27.7555	28.7443
bta-miR-211	29.7140	31.0659	30.6635	31.5328	30.8150	33.2661	32.7663	29.8321	32.3835	32.0088	30.5695	31.0697
bta-miR-22-5p	31.9620	31.8519	31.0039	32.6676	29.7973	34.8031	.	29.7672	31.2563	33.8498	31.8522	32.1809
bta-miR-212	.	.	.	.	.	.	35.2012	.	.	.	.	35.1307
bta-miR-221	27.7132	27.3908	27.3457	28.4344	26.9021	29.1730	28.5326	25.8100	27.5146	28.6763	27.4431	27.7396
bta-miR-214	28.6101	28.8466	28.3817	29.9440	27.7837	28.9640	31.1452	27.3499	28.8501	31.6009	30.1580	27.7749
bta-miR-222	28.8342	29.3457	29.5867	30.9557	28.8327	30.9105	30.6162	28.1913	29.5034	30.2645	28.9518	30.4596

bta-miR-215	30.7801	30.4764	31.1897	30.3109	29.7196	31.9536	33.6409	29.4630	30.8296	31.9983	31.6630	30.6811
bta-miR-223	31.5458	33.5204	34.5751	.	33.4819	.	.	32.9085	35.4411	34.4659	.	33.7687
bta-miR-216a	.	.	35.8870	.	32.2047	.	.	33.7839	.	35.3007	.	33.7852
bta-miR-224	31.5254	30.3466	29.3253	30.8745	28.7588	30.8667	32.9469	28.0947	30.0591	30.5149	30.0627	29.6967
bta-miR-216b	.	.	.	.	36.5151	36.5269	.	.	.	.	.	35.0157
bta-miR-23a	24.3164	23.8651	24.4422	24.8625	23.6997	25.2934	26.5342	23.4106	24.4004	25.3886	24.3918	24.8442
bta-miR-217	.	.	.	.	.	.	.	.	.	.	36.5946	.
bta-miR-23b-3p	26.7601	27.1201	26.9342	28.0803	26.5413	27.6612	29.7217	26.6953	28.4917	27.5156	26.9290	27.7170
bta-miR-218	32.1876	33.5794	35.2766	35.6930	35.0120	35.6500	.	33.5224	33.9825	.	.	34.2206
bta-miR-23b-5p	36.3099	35.5891	32.7832	.	33.7719	.	.	32.2090	35.0095	35.7148	32.2199	33.8645
bta-miR-219	28.7147	31.9068	31.8650	32.2806	30.5082	32.3573	33.9981	30.3931	32.2475	34.3453	32.7036	30.8281
bta-miR-24	35.1847	.	.	.	.	.	.	.	.	.	.	.
bta-miR-219-3p	30.7222	.	34.0062	35.5791	31.1091	36.1194	.	31.5257	33.2554	33.7197	.	31.6248
bta-miR-24-3p	26.6153	26.1072	25.7584	25.6605	24.8250	27.4483	30.7487	23.7223	25.2864	27.7219	26.7303	25.6395
bta-miR-219-5p	.	.	.	.	35.7124	.	.	.	.	.	.	.
bta-miR-25	22.8001	22.5804	22.4982	22.7170	22.0300	22.7425	24.8119	20.9735	22.6336	23.5023	22.0723	22.5775
bta-miR-22-3p	.	3.2601	3.0317	2.9693	.	2.8317	2.8798	2.9011	.	3.0328	2.8339	3.0108
bta-miR-26a	26.6917	26.7723	26.5436	28.2898	25.8019	28.3471	31.8221	26.1486	27.7834	28.0645	27.7951	27.3574
bta-miR-26b	30.2085	29.2331	29.2139	30.3954	28.3666	32.3850	.	28.7362	30.3577	30.3925	31.4242	29.7309
bta-miR-29d-3p	29.8317	28.8796	28.7988	29.0397	28.0188	30.2522	32.9151	27.6188	28.8198	30.6049	30.2155	28.5791
bta-miR-26c	.	.	.	.	.	.	.	42.2589	.	.	.	.
bta-miR-29d-5p	31.8798	34.8662	33.3113	.	33.0252	.	34.4433	31.7889	33.6344	34.2056	34.2972	34.2051
bta-miR-27a-3p	27.2697	27.5374	27.1953	27.5753	26.2067	28.6288	29.4542	25.0254	27.0793	28.7165	26.9626	26.7138
bta-miR-29e	.	.	.	.	43.8504	.	.	.	.	.	34.5373	.
bta-miR-27a-5p	.	36.9155	33.9796	8.2319	32.1650	34.4102	8.7284	33.0417	.	28.5175	10.8155	31.8468
bta-miR-301a	34.4754	.	35.3799	36.9715	34.9203	.	.	34.1295	.	.	.	.

bta-miR-27b	25.9579	25.8033	25.0413	24.9141	24.7432	26.8823	26.9167	23.0813	25.2637	26.9333	24.7876	25.0699
bta-miR-301b	34.6598	.	.	.	36.1314	.	.	.	.	.	.	.
bta-miR-28	33.9500	31.7954	31.8460	34.8322	31.4189	33.9546	.	30.4372	31.7813	33.6675	32.3278	31.5523
bta-miR-302a	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-296-3p	25.7393	25.7359	25.5346	26.3561	25.0815	31.8751	27.4460	24.2527	25.8070	33.1374	25.2313	26.4410
bta-miR-302b	32.2820	.	.	.	.	.	.	.	.	.	.	.
bta-miR-296-5p	29.0219	31.3941	30.7528	31.7964	28.8870	31.5767	.	28.4729	30.7918	31.8586	31.9069	29.7891
bta-miR-302c	30.0601	.	35.1261	.	33.8408	.	.	.	.	.	.	32.3891
bta-miR-299	32.1301	.	.	.	34.6484	.	.	.	.	.	.	.
bta-miR-302d	.	.	.	.	.	.	.	34.7441	.	.	.	.
bta-miR-29a	26.1354	25.4836	25.0413	24.7200	24.3337	26.6447	28.5971	23.5024	24.5578	26.6013	25.4807	24.7656
bta-miR-3064	33.9681	.	.	.	.	.	35.7205	.	.	.	.	36.7723
bta-miR-29b	35.4296	.	34.4473	36.8010	32.5539	.	.	32.8340	32.8882	.	.	32.8026
bta-miR-30a-5p	29.4231	28.9331	28.1781	28.9829	26.9188	30.7738	32.9101	25.7407	28.4252	30.3583	29.8262	28.3113
bta-miR-29c	26.2108	25.7291	24.8577	25.2604	24.4422	26.6891	29.4056	23.4650	24.7321	26.7808	25.6061	24.7004
bta-miR-30b-3p	33.0079	.	33.0252	.	33.6104	35.0577	.	32.7210	34.4991	.	.	34.7993
bta-miR-30b-5p	30.3065	31.4023	30.1202	31.5974	29.7465	31.7920	33.8327	28.4130	30.9047	32.6182	30.6738	31.2337
bta-miR-328	28.9470	29.7274	28.7381	29.6897	27.5656	28.6989	31.0280	26.8397	29.0480	31.6243	29.7539	28.4644
bta-miR-30c	29.1946	29.9755	28.7254	30.4782	27.8039	30.3994	32.1287	27.4531	29.8042	30.6989	29.1179	29.8279
bta-miR-329a	.	.	34.5003	.	34.2355	.	.	33.8390	.	.	.	.
bta-miR-30d	28.9748	29.6764	28.1669	28.6981	27.1658	30.3798	31.8552	25.7758	28.7922	29.8655	29.0565	28.8080
bta-miR-329b	.	.	.	.	.	.	.	.	.	.	.	35.5156
bta-miR-30e-5p	29.3592	29.4992	28.2698	28.8159	27.1074	31.0971	31.9210	25.7813	28.7026	29.7130	30.1556	28.6201
bta-miR-330	31.6201	32.2045	32.3054	32.3072	29.8367	30.9183	34.2130	29.8558	32.7595	32.9744	32.8025	29.7451
bta-miR-30f	29.8048	31.0667	29.8962	31.3922	29.1047	31.8221	33.7510	28.4039	31.1925	31.4135	29.9433	31.3408
bta-miR-331-3p	32.6118	32.5433	32.9321	32.5749	31.5949	33.3883	33.9611	30.7397	32.7105	33.9687	32.3635	35.6981

bta-miR-31	28.7125	28.7568	27.8844	29.8044	27.7666	29.8542	31.5704	26.7256	28.9401	29.1951	28.4624	28.6410
bta-miR-331-5p	28.3079	30.4564	28.1238	29.9447	28.0001	30.8055	30.2736	27.7745	29.4745	29.7648	28.4392	28.4614
bta-miR-32	.	.	.	.	.	.	36.0465	.	.	.	.	.
bta-miR-335	31.2325	29.4401	29.7927	29.3810	28.4239	31.8373	34.4914	26.7714	29.9351	31.8268	31.6509	29.1258
bta-miR-320a	21.5181	21.7355	21.8394	22.6954	21.7273	22.7118	22.7781	20.6537	21.9973	22.8727	21.7383	22.1649
bta-miR-338	.	.	33.2737	34.5871	32.5619	35.5626	.	33.3011	.	34.4044	.	34.6877
bta-miR-320b	28.7020	29.0654	28.8042	28.9884	27.8178	29.5763	31.2624	27.6630	28.2209	29.6634	30.4288	27.7636
bta-miR-339a	28.0457	27.9802	27.7445	29.8208	26.8319	29.7223	30.5125	25.8608	28.5863	30.1849	27.7811	28.0003
bta-miR-323	19.5343	19.3921	19.4683	19.1106	20.4556	19.6812	18.8213	20.5126	19.4994	18.2217	19.0694	19.2659
bta-miR-339b	26.5555	26.3640	26.6526	28.8146	25.5567	27.7508	28.6052	24.6548	27.4233	28.3626	26.1593	27.2862
bta-miR-324	28.7976	29.9875	30.1834	30.8388	29.5363	33.5745	32.2625	28.4584	29.6541	32.7399	31.2386	30.0773
bta-miR-33a	.	.	.	.	33.8263	.	.	.	.	.	.	.
bta-miR-326	29.2146	29.7414	28.8136	30.5705	27.1702	29.8182	32.3738	27.3206	28.8338	29.8103	29.8372	27.3586
bta-miR-33b	.	36.8159	.	.	36.0927	34.9609	.	36.0024	36.0410	.	.	34.2862
bta-miR-340	29.8077	.	.	34.9427	36.6068	.	.	.	.	.	.	.
bta-miR-365-3p	32.6354	30.0546	28.5648	28.8642	27.1969	31.8406	31.8183	26.4317	28.7925	31.7168	30.6928	28.2832
bta-miR-342	29.9419	31.1182	29.9635	31.4340	29.4739	31.2461	34.0180	28.2393	30.8258	31.6872	30.6134	30.5925
bta-miR-365-5p	27.6044	25.7018	25.8145	25.7934	23.8229	27.3012	30.7555	24.2640	25.3958	26.7117	27.8238	24.8173
bta-miR-345-3p	29.6828	29.6500	29.2864	29.3639	28.4782	29.9928	30.7074	27.9464	29.2833	29.7541	29.6871	28.7793
bta-miR-367	34.7159	.	.	.	.	.	.	.	.	.	.	.
bta-miR-345-5p	30.7357	33.9442	33.2093	33.9768	30.7399	33.1936	35.2530	30.9650	32.8942	36.0795	35.6768	30.7074
bta-miR-369-3p	33.0428	33.0362	33.0873	32.9728	32.9462	.	.	31.0570	33.0953	.	.	33.8720
bta-miR-346	28.6873	30.2691	28.6778	29.7257	26.9583	29.0786	31.8964	27.5268	29.5271	30.7147	31.2310	26.8104
bta-miR-369-5p	30.7812	.	33.4772	.	33.6968	.	.	33.9530	34.5242	.	.	.
bta-miR-34a	34.0930	32.7157	31.7492	34.4680	31.7485	34.6774	.	31.7766	34.0622	33.9960	33.1614	33.2453
bta-miR-370	32.4314	31.7342	31.1842	31.8268	29.1586	32.4322	32.2833	29.4555	31.2921	33.7397	32.0136	29.7943

bta-miR-34b	33.1425	.	.	33.6535	.	.	.	36.3868	.	.	.	.
bta-miR-371	32.3838	.	.	.	36.1405	.	.	.	35.6168	.	36.9974	.
bta-miR-34c	33.3970	.	.	.	36.7622	.	.	.	36.6227	34.3593	.	.
bta-miR-374a	31.7048	31.3115	30.9747	32.8527	30.0471	32.3217	34.3959	30.6604	31.8787	32.6026	32.4497	31.0056
bta-miR-361	28.0171	27.6947	26.1398	27.2379	26.7693	28.4460	28.7774	25.8052	27.8046	28.6504	27.9647	27.6707
bta-miR-374b	29.2573	29.2956	28.5940	30.3678	27.7700	30.0304	31.6783	27.9883	29.1233	29.4588	28.9218	29.1948
bta-miR-362-3p	28.2825	27.8755	27.8192	28.4072	26.8866	29.6953	32.1331	26.2337	28.2613	29.8339	28.1699	28.8167
bta-miR-375	29.2239	28.7202	30.1555	31.3601	30.0698	31.3811	32.5707	29.6095	.	31.8794	30.7537	29.7311
bta-miR-362-5p	32.9264	33.7169	33.1522	35.0307	31.5381	34.2267	.	31.8588	33.8683	33.3021	34.0050	32.1679
bta-miR-376a	30.2585	.	35.0718	36.0036	36.9752	.	.	33.0261	.	.	.	34.0182
bta-miR-363	31.8270	.	34.9008	.	33.9403	36.5986	.	.	.	.	.	36.4402
bta-miR-376b	34.3792	.	.	.	.	.	.	.	.	.	.	.
bta-miR-376c	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-382	28.7280	29.8014	30.2978	30.7094	29.8281	30.8158	30.8382	28.8351	29.7354	31.8185	30.4791	30.8824
bta-miR-376d	31.6176	.	34.9145	.	.	.	.	32.9241	.	.	.	.
bta-miR-383	29.5382	36.1138	33.9419	33.9873	31.8847	35.1707	.	32.9063	36.9540	36.8475	32.1430	32.8115
bta-miR-376e	.	.	.	.	34.5473	.	.	.	.	.	.	.
bta-miR-409a	34.2874	.	.	.	.	.	.	35.7653	35.6773	36.0067	35.6608	34.7958
bta-miR-377	.	.	.	34.1261	.	.	.	35.8850	.	.	.	.
bta-miR-409b	32.5758	.	.	.	.	.	.	.	.	.	.	.
bta-miR-378	28.4652	30.3029	29.7932	30.3264	29.1232	30.7383	32.6733	27.8393	30.5140	31.0851	30.0792	29.4253
bta-miR-410	32.9122	34.2049	32.6365	35.4684	32.1773	35.0566	.	30.8135	35.2453	.	35.2373	31.7845
bta-miR-378b	29.5039	32.3079	31.3031	31.8249	29.5525	30.6477	32.7431	27.8056	31.7597	30.7648	29.7729	30.3925
bta-miR-411a	28.7740	30.7080	32.4110	31.9038	31.0563	32.1236	32.7028	30.5301	32.1288	32.7645	31.8660	32.8024
bta-miR-378c	33.9019	.	34.1716	36.7926	32.8686	33.3378	35.4795	32.6445	34.5980	36.9652	33.9941	33.6384
bta-miR-411b	31.6881	34.2318	33.4977	35.1399	.	35.3808	34.3713	36.9217	36.0926	32.5750	33.9117	33.9601

bta-miR-378d	29.9578	31.6493	33.0832	32.8155	30.6076	34.4088	33.3600	30.5251	33.8781	33.2931	32.1410	31.0976
bta-miR-411c-3p	34.0439	36.3331	.	36.3452	.	.	.	.	.	.	.	.
bta-miR-379	32.0382	33.8559	32.7478	34.5051	33.5556	.	34.2687	.	.	.	34.6418	.
bta-miR-411c-5p	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-380-3p	30.8003	33.1738	30.7250	32.8114	31.8743	32.7301	31.7244	30.6980	31.8630	34.0011	31.5230	31.8975
bta-miR-412	34.9657	.	36.4852	.	32.8161	34.2180	.	34.8347	.	.	.	33.7187
bta-miR-380-5p	28.3175	33.9779	32.9624	35.0617	31.3854	33.9474	34.1998	31.8654	32.9067	.	35.4233	31.5525
bta-miR-421	27.6532	28.3462	27.8000	29.0799	27.7740	29.7286	29.6419	26.8290	28.8917	28.9164	27.9513	29.3318
bta-miR-381	35.3731	34.9417	32.6047	34.0969	31.6039	34.2196	.	31.4384	33.9008	.	.	31.5532
bta-miR-423-3p	26.6177	26.3132	26.1567	26.8547	25.7165	27.8365	28.9787	24.4354	26.1952	27.7713	26.5401	26.5471
bta-miR-423-5p	23.9366	24.1172	24.1912	23.9972	24.0000	24.3298	25.9930	23.4330	23.6811	24.7609	23.5652	23.4978
bta-miR-449c	30.3339	35.4185	32.8567	35.0980	30.7156	35.9677	.	33.8737	33.8886	34.8873	34.8010	32.7191
bta-miR-424-3p	29.6021	28.2630	28.4275	30.6104	28.3335	30.4195	29.7549	27.4927	29.2678	30.8684	28.3546	29.3579
bta-miR-449d	29.5819	30.6483	30.1490	30.4929	28.0090	30.7917	32.9290	28.5245	30.5292	30.6872	32.3404	28.5593
bta-miR-424-5p	23.8257	22.2529	23.1342	23.6225	22.7487	25.7376	27.9292	21.7414	22.7535	25.2688	23.7377	23.2842
bta-miR-450a	35.9438	34.9584	35.5235	34.9235	33.8663	.	.	33.0735	33.9464	36.4079	.	33.8733
bta-miR-425-3p	26.7430	27.7257	25.4479	28.0999	25.7709	28.4087	27.5735	24.6860	27.3056	27.6856	26.2941	26.6696
bta-miR-450b	32.6831	31.8977	32.6082	31.6919	31.5532	35.9722	.	30.7637	32.5563	34.4145	34.7596	31.8790
bta-miR-425-5p	27.7652	27.6731	27.5562	28.7946	27.2437	29.3416	30.0672	26.3754	28.0130	28.8163	28.4532	28.6448
bta-miR-451	32.8714	32.2473	33.6965	34.4225	32.9252	35.0159	33.6355	31.0317	32.0354	34.6378	34.8470	33.3370
bta-miR-429	29.2020	31.5155	31.5899	30.7711	31.8787	31.5872	30.7956	30.3191	31.2056	31.1995	30.6342	31.2643
bta-miR-452	33.5362	36.3244	35.0514	34.1677	32.1145	.	34.3094	32.7943	36.6242	33.5559	33.5617	34.8693
bta-miR-431	32.0012	36.1599	31.8005	33.2758	30.0162	33.0461	.	30.6408	36.4190	34.6460	33.9526	30.7494
bta-miR-4523	31.7464	33.0613	33.4886	32.2944	30.6594	33.3023	.	31.9736	32.8989	34.6302	35.9951	31.1312
bta-miR-432	27.8987	30.4767	30.2935	30.6013	30.1565	31.5454	30.6923	29.0073	30.3885	31.7811	30.7520	30.0097
bta-miR-453	32.8577	34.3048	34.8210	34.8144	33.7641	33.8836	36.0543	32.9350	33.9052	.	35.3546	35.2606

bta-miR-433	27.8141	31.8505	31.8632	31.8638	29.7696	31.6758	32.7923	30.5532	31.5184	31.4971	32.7715	29.7750
bta-miR-454	28.8730	34.2575	34.2672	35.2446	33.3025	.	.	.	.	.	.	.
bta-miR-448	35.0924	.	.	.	33.8388	.	.	.	.	.	.	34.6332
bta-miR-455-3p	30.8625	35.3241	.	.	34.1433	35.5828	34.5190	34.7922	.	.	33.8564	33.4337
bta-miR-449a	.	33.9624	35.9633	33.0707	36.7609	.	35.0208	.	35.8506	.	36.6686	33.8736
bta-miR-455-5p	.	.	33.8559	33.8862	33.4348	.	.	30.7286	32.6031	.	34.8730	33.2049
bta-miR-449b	.	34.3794	36.9146	36.7370	33.8557	.	.	33.9213	35.0553	.	34.9370	33.5080
bta-miR-483	34.3259	33.2595	31.5598	33.9823	31.8383	32.4629	32.8751	29.8241	30.6547	34.9786	32.8214	32.0629
bta-miR-484	27.3398	26.8588	26.7817	28.3089	25.8353	28.7547	29.5658	24.8226	27.2038	28.2347	26.3709	27.3765
bta-miR-496	31.7996	.	36.0630	.	32.7863	.	36.5241	33.2384	.	.	.	34.9115
bta-miR-485	31.8630	.	34.4322	35.6130	34.2251	.	.	34.7563	.	.	36.7495	.
bta-miR-497	30.8398	30.7341	29.5963	30.4112	29.1796	29.6981	33.6317	27.4979	30.0404	32.0781	30.7469	.
bta-miR-486	28.2447	29.2051	27.7774	29.9264	27.1209	28.9311	30.1478	26.7612	28.8278	30.4912	28.5998	27.6808
bta-miR-499	.	35.4355	34.9341	.	34.0187	.	.	35.3786	33.5062	.	.	.
bta-miR-487a	.	34.1991	35.7615	.	33.3672	.	.	33.9102	34.0744	.	36.2680	.
bta-miR-500	30.8312	30.6882	30.0460	30.7209	29.6303	31.7411	32.6591	28.7066	30.5812	31.7844	30.0304	30.4043
bta-miR-487b	34.7440	32.1305	33.1148	33.9744	31.6759	.	33.6950	31.4314	31.8680	.	32.8707	34.6517
bta-miR-502a	32.9128	32.1267	.	32.9039	32.8861	34.2271	.	33.1397	34.8957	34.1161	35.0320	32.8619
bta-miR-488	32.0157	.	.	.	32.2486	34.9390	.	33.8142	35.5143	36.4275	35.9183	33.5905
bta-miR-502b	30.1885	29.8287	29.9972	29.8445	28.8689	30.7611	32.6378	28.3430	29.4457	30.7860	30.1384	29.8980
bta-miR-489	32.9267	35.2008	35.4119	.	35.5486	35.7445	35.3027	34.5509	36.0319	35.8466	36.0299	.
bta-miR-503-3p	27.3799	28.8159	28.5507	29.5536	27.2864	29.2545	31.7379	27.3678	28.7866	29.9304	28.5408	27.5207
bta-miR-490	31.4561	35.3214	32.2731	32.9987	30.1983	32.2865	36.2800	31.6517	32.9725	35.0128	34.1823	30.7755
bta-miR-503-5p	31.8050	33.8718	34.0736	34.5414	31.9347	35.3721	.	31.4318	33.0897	35.2774	32.9361	31.8386
bta-miR-491	29.7265	32.6368	31.9603	31.8328	30.6389	32.1702	35.4830	30.5065	31.7624	32.8157	32.7558	31.6258
bta-miR-504	32.4787	35.0071	33.9204	32.9877	30.2517	33.6714	32.0805	28.3732	34.8355	33.5791	30.5598	30.5409

bta-miR-493	28.7378	30.8911	29.7207	30.7611	27.6349	29.9247	32.7557	28.4094	29.8051	31.7532	30.8141	27.6975
bta-miR-505	27.3751	27.3217	26.7756	28.3306	26.2777	28.7198	28.8544	25.6005	27.7585	28.5461	26.6983	28.2468
bta-miR-494	23.6960	23.8975	22.7070	25.8406	22.6614	25.1333	25.5433	22.4946	24.5845	24.9326	23.5160	23.5459
bta-miR-532	30.1559	31.6601	30.8111	31.3445	30.6368	32.7750	33.8865	28.3526	30.4087	32.3626	30.9877	30.4407
bta-miR-495	.	.	35.0511	.	33.8317	.	.	31.8101	34.7269	.	.	34.9652
bta-miR-539	33.6338	.	33.8845	36.7008	34.3507	.	.	36.8718	34.8747	.	.	34.8084
bta-miR-541	29.7635	32.7068	31.8001	31.8791	29.5406	31.1867	32.9011	29.9863	31.1629	32.6907	32.7441	29.6118
bta-miR-582	34.9909	.	.	.	.	.	.	.	.	.	.	.
bta-miR-542-5p	31.9530	31.9086	31.8224	32.9912	32.0648	.	34.3456	30.5139	31.7984	35.1698	33.1743	34.7986
bta-miR-584	30.6239	31.3907	30.6969	31.8535	29.4699	31.1608	32.0326	29.7959	30.7539	31.0729	32.4298	29.0787
bta-miR-543	.	.	36.0913	.	.	.	.	35.1840	.	.	.	34.6476
bta-miR-592	29.6881	30.1736	29.3733	32.0904	29.8713	31.9295	35.0220	30.0754	30.7753	30.0886	30.3185	31.2216
bta-miR-544a	.	.	.	34.2023	.	.	.	.	.	.	.	.
bta-miR-599	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-544b	33.9224	.	.	33.8548	32.7872	.	.	36.6403	.	.	.	33.8519
bta-miR-615	12.9510	12.8858	12.7690	12.7656	12.6331	12.7566	12.7762	12.7248	12.7568	12.4785	13.2102	12.7763
bta-miR-545-3p	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-628	28.9576	34.1735	35.7985	.	33.2055	.	36.5633	33.9043	.	.	33.2464	.
bta-miR-545-5p	32.5423	33.6958	.	35.8115	33.1459	.	34.5204	33.3470	33.9047	33.9298	36.3976	32.3153
bta-miR-631	20.5944	20.4416	20.5926	20.4080	21.6651	20.4415	19.9901	21.1955	20.7295	19.8389	20.2980	20.5669
bta-miR-551a	33.8517	.	.	.	35.0002	.	.	.	.	.	.	.
bta-miR-652	29.8711	29.0903	28.7726	30.1914	28.4663	30.6940	30.6852	28.1687	29.3769	30.4416	29.1216	29.9764
bta-miR-551b	34.1637	35.1802	.	35.0919	33.8979	36.7175	.	33.2635	.	36.5289	.	.
bta-miR-653	.	.	.	36.6975	.	.	.	35.1248	.	.	.	.
bta-miR-562	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-654	34.1927	33.6177	.	.	33.3018	35.7362	.	32.5239	36.5564	.	35.3761	32.8881

bta-miR-568	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-655	32.9023	33.8892	35.8603	.	33.4607	.	.	.	36.5255	.	36.5642	.
bta-miR-574	23.0212	23.4137	22.5879	22.8356	20.6028	22.6650	26.4392	21.6005	22.8427	24.1427	24.7051	20.8014
bta-miR-656	31.3137	36.7164	34.2362	35.7623	35.7201	35.4679	35.0241	35.7159	33.9454	36.4669	35.2212	34.2007
bta-miR-658	31.9142	32.3885	31.8807	33.6027	30.9159	32.9277	35.1295	31.0598	31.9031	34.1078	33.2968	31.6936
bta-miR-758	32.2758	.	34.9371	.	32.8780	36.3403	.	.	34.8054	.	34.2496	35.9208
bta-miR-660	26.4971	26.1933	26.1069	26.6604	25.7090	27.2110	28.6780	24.7377	26.5924	27.4752	25.8205	26.5533
bta-miR-759	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-664a	29.1982	28.6178	28.6299	27.8345	26.5134	28.6693	32.5890	26.7243	27.7377	28.7275	30.2303	26.8276
bta-miR-760-3p	27.8034	27.7365	27.7033	27.9579	26.6917	27.8225	28.9686	26.3803	27.1125	28.5277	27.7446	26.7749
bta-miR-664b	31.0692	30.1744	31.2154	32.5852	29.8440	32.2935	33.7166	30.0590	32.5440	31.7844	30.7969	32.2321
bta-miR-760-5p	27.8214	29.7734	29.1172	29.7243	27.4712	29.3893	31.6589	27.9219	29.4528	30.7655	29.9452	27.7499
bta-miR-665	28.4351	28.7950	28.0909	29.3021	26.7651	28.2354	31.4656	27.0304	28.4409	29.7445	29.9112	26.9461
bta-miR-761	.	.	.	.	33.8576	.	36.6585	34.0377	33.3927	.	.	32.8395
bta-miR-669	25.6993	26.5893	25.3764	25.8252	22.9505	25.4692	29.8188	23.8126	25.5616	27.4428	27.8260	23.4048
bta-miR-763	31.8238	32.9253	33.2374	32.6265	29.5895	32.7950	.	31.0324	31.9064	33.2852	34.1051	30.0200
bta-miR-670	34.7366	35.0533	.	36.6449	.	.	34.9367	.	35.4883	34.5060	35.3414	33.9287
bta-miR-764	33.4116	36.3804	35.1791	.	31.7014	34.9224	.	33.2291	34.3646	.	.	33.5640
bta-miR-671	31.7435	30.6125	30.6779	31.6921	30.4000	31.8712	31.7824	29.8858	30.6421	31.5082	30.6326	30.8224
bta-miR-767	32.6307	33.9124	33.7265	33.8807	31.9892	34.1959	33.1502	32.7857	32.9136	34.2967	32.2372	32.5189
bta-miR-677	29.3988	29.0085	28.1391	30.6322	28.5973	31.3111	30.0690	27.0185	31.0643	30.4203	29.3256	31.5912
bta-miR-769	31.8754	.	34.9155	34.6264	30.7490	34.8158	.	31.7737	33.5359	34.3489	34.2070	32.0499
bta-miR-7	.	33.8481	.	.	.	.	.	33.9009	.	35.0304	34.8719	34.5725
bta-miR-873	32.9992	35.2196	35.4196	.	32.6307	.	.	33.1071	33.9157	35.0714	.	33.1870
bta-miR-708	29.9695	31.6810	31.8206	32.6971	31.0047	33.2095	.	30.5298	32.8158	33.7898	31.9847	31.8600
bta-miR-874	29.8215	30.0811	29.4911	30.7779	27.8817	30.3550	31.7845	28.8217	29.7908	30.2308	30.6778	28.4665

bta-miR-744	27.3194	27.7802	27.5578	28.9683	27.2606	28.0824	28.6462	26.5882	27.7785	28.8219	26.9229	28.8926
bta-miR-875	32.9081	.	.	.	35.1776	.	.	.	.	.	.	.
bta-miR-876	36.2115	34.3705	.	35.8240	34.3202	36.7007	.	35.0202	34.5882	35.9453	35.0592	.
bta-miR-98	34.1061	33.8880	33.5127	35.4199	32.8964	34.2395	.	33.7086	33.3808	.	34.8782	33.3749
bta-miR-877	26.7577	26.7596	26.7023	26.8400	25.7602	27.4689	28.0098	25.5300	26.4106	27.8308	26.8512	25.8346
bta-miR-99a-3p	29.7983	29.3708	29.6041	31.4909	29.4581	31.5539	30.4614	27.8282	30.1281	31.7781	28.7707	32.0868
bta-miR-885	31.5688	32.8741	31.9083	32.0423	30.3725	32.1149	.	30.0141	32.8894	33.9263	32.0303	29.7857
bta-miR-99a-5p	26.3265	24.8274	25.5089	25.7044	24.7839	27.6479	29.7567	23.7772	25.3757	26.7446	26.6990	26.7477
bta-miR-9-3p	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-99b	24.0727	23.7476	23.8140	24.1548	24.9161	24.3077	23.6423	24.7382	24.2490	23.0970	24.0140	23.7879
bta-miR-9-5p	31.7910	.	.	.	35.1638	.	.	34.1134	34.5150	.	.	.
bta-miR-1179	31.5101	.	.	.	33.1639	.	.	36.1207	.	.	.	.
bta-miR-92a	22.4779	22.2003	21.9972	22.6397	21.7054	22.8181	24.6015	20.6643	22.3863	23.2544	22.2748	21.8099
bta-miR-1185	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-92b	26.8117	26.7733	26.1626	27.2480	25.3860	27.4747	28.6328	24.8106	26.8070	27.7818	27.2755	25.6847
bta-miR-1193	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-93	24.7906	24.4538	24.0437	25.1375	23.9418	25.7110	26.1923	22.5471	24.6178	25.5644	24.5020	25.1515
bta-miR-1197	33.9846	.	36.9055	.	.	.	.	.	.	.	.	.
bta-miR-935	29.7469	31.4212	31.8857	32.7816	30.1814	31.5656	32.8177	30.6792	31.9088	32.8704	32.6853	30.8057
bta-miR-122	34.0032	.	35.6655	.	34.4213	33.7846	.	33.3930	34.8764	.	34.5701	33.9590
bta-miR-940	25.3142	23.7681	24.0438	24.8187	23.8194	25.7321	26.7162	23.3901	24.7441	25.7801	25.5569	24.1012
bta-miR-1224	19.2112	18.6878	18.5709	20.2122	19.2068	20.4460	20.7146	17.9197	19.4977	20.5220	19.6513	20.2018
bta-miR-95	33.7050	.	.	33.6170	32.8550	34.3331	.	32.9045	34.4899	33.9751	.	.
bta-miR-1225-3p	26.6665	27.5638	26.2550	27.3752	24.8271	26.8512	30.1466	25.3571	26.8466	27.6961	27.9107	25.2518
bta-miR-96	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-1246	15.1996	14.7367	13.8231	14.1552	14.7244	14.1588	16.7983	13.8262	14.0462	14.2366	15.3026	13.7766

bta-miR-1247-3p	28.1163	30.4295	28.7303	30.4517	27.8190	29.1566	31.6961	28.0462	29.7253	30.8576	29.7232	28.6361
bta-miR-1296	28.9338	30.8375	29.5410	30.6602	27.2050	29.9183	32.9625	28.0486	30.4084	32.1671	30.6618	27.9840
bta-miR-1247-5p	27.4084	27.0554	25.7175	27.6566	25.3499	28.3901	28.7333	24.6925	27.1991	28.6008	26.9219	26.6553
bta-miR-1298	.	.	35.9578	.	34.9747	.	.	.	35.1795	35.5576	.	34.6465
bta-miR-1248	31.8950	32.6860	31.8034	32.2809	31.9080	33.9598	32.8221	31.7750	33.1249	32.4276	32.8955	32.9029
bta-miR-1301	35.8258	35.0005	.	.	34.6031	.	34.0729	33.8360	32.8843	.	35.9527	.
bta-miR-1249	36.5169	36.9969	33.3966	32.7972	31.4780	.	33.7038	31.8274	33.2639	36.6343	35.8326	32.8186
bta-miR-1306	28.3227	28.2553	27.9370	28.7809	27.4888	29.1763	30.5620	26.5743	28.3839	30.5165	27.9403	28.0886
bta-miR-1260b	21.7134	21.9263	21.4981	23.3321	20.8133	22.8220	23.9149	21.4490	23.6837	22.5980	20.9101	22.7490
bta-miR-1307	27.4050	27.7342	26.8718	29.1417	26.6454	28.6999	29.0824	26.5657	28.3840	28.8207	27.7509	27.7621
bta-miR-1271	32.0955	32.6496	32.6037	31.8621	30.8371	32.6567	.	31.7353	31.8731	33.1223	32.8880	32.3137
bta-miR-1343-3p	28.4537	28.7484	28.1138	29.1592	26.8150	28.8178	29.9135	26.6676	28.7543	30.8361	28.0120	27.5741
bta-miR-1277	.	.	.	.	.	.	.	.	.	.	.	.
bta-miR-1343-5p	24.4095	24.7643	23.4090	25.3219	22.8058	25.0283	26.8777	23.0432	24.7250	25.4799	24.5666	24.0330
bta-miR-1281	28.2479	29.1126	28.0323	29.1958	25.8625	28.4084	31.8789	26.7124	28.1884	30.0557	29.7884	26.5853
bta-miR-1388-3p	30.1853	29.8920	29.6897	30.3395	28.7682	29.8903	31.8528	28.2345	30.1575	30.7431	30.3377	28.9773
bta-miR-1282	.	.	36.9771	35.7403	32.8224	.	.	35.3923	35.0761	.	34.6061	35.0535
RNT43 snoRNA	29.7641	30.1469	29.2112	30.7354	29.6725	30.6002	30.4378	28.8917	30.7754	31.7674	30.0056	30.7444
bta-miR-1284	33.9575	.	34.5083	.	30.8129	35.9108	.	32.2943	.	.	.	32.2890
Hm/Ms/Rt T1 snRNA	13.8171	13.5662	13.2898	13.7441	13.3647	14.0554	15.7783	12.7134	13.4337	14.3951	14.2455	13.7318
bta-miR-1287	.	.	.	.	32.1608	.	.	34.3599	.	.	.	33.8545
bta-miR-99b	23.8166	23.7110	23.8340	23.7415	24.7679	23.7172	23.5108	24.6369	24.0899	22.9006	23.7781	23.9145
bta-miR-1291	31.6743	.	33.5925	33.2988	30.6159	33.5334	33.9159	31.6542	32.9333	33.4315	33.9577	31.4523
Negative control	.	.	.	.	.	.	.	.	.	.	.	.

[1] Body energy reserve: MBER: Cows with moderated body energy reserve; HBER: Cows with high body energy reserve.

Supplementary table 5. Normalized data of the 6 exclusives miRNAs detected in follicular fluid extracellular vesicles (EV FF) compared to cumulus cells (CC) from ipsi and contralateral ovarian follicles (3-6 mm in diameter) from cows with moderated body energy reserve (MBER)

miRNA	**MBER[1]**															
	CC[2]								**EV FF[3]**							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
bta-miR-181c	.	.	.	.	.	.	.	.	8,132996642	8,640025529	6,427796566	8,429802773	4,945081669	11,55921334	10,30181512	4,39011904
bta-miR-193a-3p	.	.	.	.	.	.	.	.	10,25194653	9,667453203	7,956708157	10,37610328	5,772487599	12,5357644	10,45109949	4,847768271
bta-miR-193b	.	.	.	.	.	.	.	.	5,67589482	5,991000566	5,730170452	6,656687615	3,642534214	6,50828332	8,976314637	2,811650717
bta-miR-29d-3p	.	.	.	.	.	.	.	.	5,887381637	5,150321397	4,974793636	5,092440483	3,176912266	6,241519633	9,338638398	2,93126055
bta-miR-1248	.	.	.	.	.	.	.	.	7,95066365	8,956679735	7,979468548	8,333615181	7,066070515	9,949098292	9,245575043	7,087476218
bta-miR-1249	.	.	.	.	.	.	.	.	12,57262143	13,26765004	9,572621548	8,850003121	6,636095005	13,53172845	10,1273118	7,139872992

[1]MBER: Cows with moderated body energy reserve. [2]CC: Cumulus cells. [3]EV FF: Follicular fluid extracellular vesicles.

Supplementary table 6. Normalized data of the 79 miRNAs commonly detected in cumulus cells (CC) and follicular fluid extracellular vesicles (EV FF) from ipsi and contralateral ovarian follicles (3-6 mm in diameter) from cows with moderated body energy reserve (MBER).

miRNA	**MBER**																***P* - value**
	CC								**EV FF**								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	
bta-let-7a-5p	3.215098	12.30125	13.60788	.	4.433421	5.613194	.	-0.02804	2.792211	2.500034	2.091621	3.295899	0.728554	3.406233	6.609068	1.089246	**0.0338**
bta-let-7b	6.070368	.	13.75444	.	5.897568	7.516566	.	1.259287	1.632321	1.011032	1.408923	1.814925	-0.09545	1.889122	3.42285	-0.2337	**0.024**
bta-let-7c	3.733269	10.07284	13.06554	.	4.298087	5.772217	11.30433	0.190758	1.858687	1.416274	1.355439	2.275935	0.198864	2.177686	4.073399	-0.0787	**0.008933**
bta-miR-106a	8.645741	.	.	.	9.872654	13.6011	12.25613	6.842761	1.825025	1.29792	0.521371	0.786946	-0.70592	2.548731	5.986717	-2.09807	**6.28E-05**
bta-let-7d	4.931174	13.75596	.	.	6.043734	7.087807	.	1.398373	4.562907	4.026845	3.764344	5.502978	2.124357	5.18478	8.110502	2.385335	0.244555
bta-let-7e	3.716477	10.73381	11.13868	12.43376	3.735995	4.130852	10.21619	-0.42136	1.940122	1.984991	1.844918	2.858857	0.47064	2.79898	5.01787	1.09016	**0.0313**
bta-let-7g	4.17034	11.10507	.	.	7.010861	11.93923	.	3.441893	6.352487	4.863756	3.962333	5.007933	2.156702	7.123443	10.32924	1.980941	0.23227
bta-miR-125a	4.139088	.	14.40224	12.90915	9.095719	8.734744	11.82673	3.77657	5.828084	6.60202	5.917001	5.783868	2.938395	6.757005	10.09167	3.413862	0.067123

bta-miR-125b	6.920321	.	15.65969	.	11.74343	10.22754	.	6.507939	4.338666	2.87329	2.997707	2.650128	1.646759	3.707622	7.143185	1.064374	**0.000954**
bta-miR-127	11.13453	11.63901	13.01234	11.44861	9.207448	10.01111	9.854611	7.672476	6.772437	8.156606	7.369352	8.067426	5.716337	8.484728	8.799908	5.719072	**0.000745**
bta-miR-130a	10.48232	11.17032	10.55417	10.9576	10.04546	9.398013	8.682341	9.408354	5.695558	5.118623	4.700797	5.923238	3.163383	6.750129	6.421372	1.313138	**4.04E-06**
bta-miR-130b	7.179871	9.8964	10.57661	9.839933	9.256295	9.267138	8.173609	7.682715	6.022334	6.114021	4.925943	6.665379	3.285904	6.830455	8.19878	2.033929	**0.000876**
bta-miR-143	9.392117	11.52765	.	12.8493	10.26438	11.01025	8.680583	10.4541	5.560336	7.024858	6.73507	6.808034	4.996136	8.246916	8.967114	4.322917	**0.000167**
bta-miR-154b	.	11.53787	11.59966	8.428284	9.205147	10.48119	6.006369	6.270384	3.326585	9.111633	9.129033	8.43895	9.059008	8.500227	8.509853	7.184246	0.308318
bta-miR-155	8.249493	.	.	.	7.841656	10.00446	10.26027	8.502276	5.942416	6.651609	6.885223	6.81046	4.570038	7.390615	8.677853	4.807456	**0.005067**
bta-miR-149-3p	9.753766	.	.	12.54941	10.54814	10.17445	9.197259	7.350993	-0.70452	-0.30188	-1.50298	0.856118	-1.46898	0.585699	2.630514	-2.22775	**0.0024**
bta-miR-15b	4.186984	.	12.6867	.	9.147881	7.770051	.	4.263352	2.818261	2.704601	2.042325	2.594123	0.452984	3.398721	6.119854	-0.23235	**0.005963**
bta-miR-16b	5.258654	.	.	.	8.716912	10.33057	9.370656	5.331124	0.891456	0.641147	-0.0684	0.675811	-1.22391	1.609184	5.035885	-2.45406	**0.00017**
bta-miR-181d	8.944932	12.12964	.	13.03332	10.21073	12.15633	9.639542	8.298541	4.434524	5.433212	5.857043	5.606727	3.876179	5.766222	7.956472	3.703428	**2.17E-05**
bta-miR-191	7.095452	-3.59594	6.564763	-3.71136	7.982953	10.52639	8.861451	5.974104	4.037101	4.181549	4.866939	4.853616	2.930382	5.771816	11.72113	1.714357	0.983223
bta-miR-193a-5p	12.34878	.	15.5044	.	9.215027	9.074168	.	6.21794	3.969068	4.78734	3.931789	4.571732	2.484171	4.253895	5.077896	1.675167	**0.000421**
bta-miR-195	5.854579	10.72583	14.68337	14.03793	9.69439	9.691508	8.853671	4.906474	2.834575	2.426984	1.902732	2.495082	0.873251	3.327295	7.118233	0.012599	**0.000185**
bta-miR-196a	10.27676	.	.	12.95908	9.145028	.	8.905602	7.410037	4.825387	10.17948	9.037694	9.59137	9.037527	8.799249	.	7.403648	0.262127
bta-miR-197	9.400731	.	.	.	5.665461	5.712979	7.058657	1.52106	3.777915	5.166643	4.293205	4.83055	1.935633	4.64139	8.514645	1.935298	0.30031
bta-miR-20a	7.088828	.	.	12.97966	9.760335	.	10.6504	6.669123	1.371223	1.107347	0.12717	0.849587	-1.04604	2.169386	4.212929	-2.50942	**3.3E-05**
bta-miR-214	11.11059	.	.	.	10.523	11.11479	13.56348	10.8706	4.665731	5.117277	4.557758	5.996772	2.941776	4.953314	7.56877	2.662439	**6.5E-06**
bta-miR-23a	5.279618	.	.	12.39326	10.50482	9.984946	10.80466	7.294737	0.372029	0.13577	0.618196	0.915259	-1.14219	1.282781	2.95772	-1.27693	**0.0024**
bta-miR-23b-3p	5.757338	.	.	.	10.11889	9.634185	9.83989	6.672854	2.81582	3.39079	3.110194	4.133073	1.699387	3.650545	6.145268	2.007789	**0.000231**
bta-miR-219	11.54266	13.33979	14.67208	.	11.71203	.	8.524886	9.907771	4.770383	8.177511	8.041068	8.333371	5.666271	8.346689	10.42165	5.705604	**0.002464**
bta-miR-22-3p	-22.3943	-20.9282	-18.8748	-19.378	-22.2366	-20.3166	-20.4949	-23.1439	.	-20.4692	-20.7923	-20.978	.	-21.179	-20.6967	-21.7863	0.984605
bta-miR-26a	3.878838	.	.	11.99011	8.557168	9.945662	9.16404	5.395619	2.747352	3.042995	2.719577	4.342561	0.960012	4.336471	8.24567	1.461111	**0.005972**

bta-miR-27a-5p	-15.1022	-13.9998	-13.16	-13.4379	-15.577	12.79619	.	-18.1111	.	13.1862	10.15561	-15.7153	7.323133	10.3995	-14.8481	8.354232	0.2013
bta-miR-296-3p	8.207261	10.75866	.	13.5464	9.846413	10.44479	12.22404	5.482996	1.795016	2.006643	1.71063	2.408902	0.239622	7.864483	3.869486	-0.43479	**7.46E-05**
bta-miR-296-5p	11.14247	.	15.51521	.	.	12.35511	9.819813	8.488772	5.077575	7.664756	6.928815	7.849133	4.045095	7.566004	.	3.785406	**0.001934**
bta-miR-30a-5p	8.036607	.	.	12.45754	.	13.94876	11.87715	6.555602	5.47878	5.203781	4.354104	5.035621	2.076914	6.763138	9.33358	1.053235	**0.004509**
bta-miR-30b-5p	8.265313	.	.	12.98337	.	12.24184	9.751958	7.770228	6.362189	7.672992	6.29627	7.650136	4.904612	7.781374	10.25624	3.725513	**0.017781**
bta-miR-30f	7.485158	.	.	11.86089	.	10.53886	12.06656	7.722334	5.860526	7.337415	6.07225	7.44496	4.262798	7.81147	10.17448	3.716421	**0.018339**
bta-miR-31	5.874842	.	.	14.86616	8.75589	8.908318	.	4.646374	4.768216	5.027551	4.060386	5.857175	2.924675	5.843519	7.993902	2.03808	**0.036934**
bta-miR-320a	7.322499	13.05963	13.38779	.	6.773951	8.9854	11.28365	2.983008	-2.42618	-1.99377	-1.98459	-1.25187	-3.11454	-1.2989	-0.79841	-4.03383	**0.0015**
bta-miR-323	-5.18944	-6.11715	-5.38978	-6.20306	-5.28123	-5.59549	-5.46139	-4.83251	-4.40998	-4.33718	-4.35565	-4.8366	-4.38625	-4.32944	-4.75513	-4.17489	**4.29E-05**
bta-miR-339b	8.4637	.	.	11.56432	6.8221	12.27246	8.911553	5.739888	2.611194	2.634731	2.828641	4.867333	0.714785	3.740152	5.028696	-0.03264	**0.000186**
bta-miR-342	9.401555	.	.	13.04076	.	10.03609	10.51138	6.184248	5.997533	7.388938	6.139539	7.486733	4.63203	7.235466	10.44154	3.551801	**0.027498**
bta-miR-370	.	12.51423	.	12.64842	9.221791	9.303839	11.81812	7.566905	8.4871	8.004922	7.36024	7.879529	4.316734	8.421587	8.706871	4.768024	**0.007583**
bta-miR-361	7.342177	.	.	.	9.279233	9.544818	.	5.987904	4.072736	3.965442	2.315799	3.290706	1.927412	4.435362	5.200892	1.117671	**0.00039**
bta-miR-375	.	11.53151	11.71266	8.648683	9.731667	8.885255	7.494181	.	5.279582	4.990886	6.331535	7.412812	5.227915	7.370493	8.994206	4.922	**0.001897**
bta-miR-382	11.98994	11.69393	14.89399	10.75571	9.272305	11.59744	8.573325	7.601004	4.783656	6.072122	6.473855	6.76213	4.986256	6.805132	7.261768	4.147624	**9.59E-05**
bta-miR-411a	.	12.7237	15.52795	12.44741	9.937943	12.14102	9.117055	10.15451	4.829652	6.978671	8.587049	7.956605	6.214401	8.112974	9.126362	5.842596	**0.00039**
bta-miR-411b	.	.	13.23535	13.33188	10.82286	10.91266	11.02311	.	7.743793	10.50255	9.673749	11.1927	.	11.37013	10.79482	12.2342	0.12501
bta-miR-421	9.170861	11.24145	11.37545	12.64928	7.199696	.	8.205014	9.701048	3.708911	4.616867	3.976026	5.132626	2.932148	5.717897	6.065451	2.141521	**1.62E-05**
bta-miR-425-3p	7.904435	.	.	.	11.21317	7.867654	10.17831	7.348769	2.798703	3.996406	1.623958	4.152711	0.929003	4.398025	3.996986	-0.00152	**5.07E-05**
bta-miR-429	11.39323	9.009559	12.35634	10.70446	7.483674	7.672282	7.183665	7.667635	5.257691	7.786155	7.765954	6.823909	7.036821	7.576517	7.219162	5.631647	**0.0313**
bta-miR-433	7.325066	7.170512	7.541068	7.413553	8.129597	8.039977	7.088624	8.066107	3.869744	8.121217	8.039245	7.916556	4.92769	7.665172	9.215801	5.865701	0.352673
bta-miR-486	9.060658	.	.	.	11.61433	9.9136	11.81209	6.797068	4.300363	5.475776	3.953422	5.979155	2.278978	4.920465	6.571297	2.073749	**0.00027**
bta-miR-489	.	11.69538	.	13.26299	10.26278	11.364	11.07234	.	8.982335	11.47153	11.58797	.	10.70668	11.73388	11.72618	9.86341	0.321695

bta-miR-503-3p	9.837628	.	.	12.91956	9.735543	9.629145	8.446162	5.450907	3.435557	5.086625	4.726762	5.606315	2.444521	5.24382	8.161388	2.680282	0.001473
bta-miR-494	3.777386	7.594819	8.55856	8.500681	4.0861	2.965115	6.821002	1.901948	-0.2483	0.168199	-1.11696	1.89335	-2.18053	1.122631	1.966812	-2.19289	**0.000172**
bta-miR-541	.	10.59339	13.40863	11.92196	9.387696	9.454722	8.790703	8.786826	5.819163	8.977477	7.976114	7.931903	4.698671	7.176092	9.32465	5.298851	**0.003557**
bta-miR-615	-12.4242	-11.7471	-9.67773	-10.6278	-12.9855	-11.0878	-11.4055	-14.1247	-10.9934	-10.8435	-11.0549	-11.1816	-12.2088	-11.254	-10.8003	-11.9627	0.385984
bta-miR-631	-2.26941	-3.50954	-2.29511	-2.97419	-3.38003	-3.29589	-3.76566	-3.04582	-3.34991	-3.28768	-3.23133	-3.53924	-3.17679	-3.56914	-3.58642	-3.49204	0.115493
bta-miR-574	6.507865	9.833259	11.85414	10.87736	3.815402	4.638098	8.299063	1.05069	-0.92311	-0.31558	-1.23611	-1.1116	-4.23913	-1.34568	2.86277	-3.08695	**8.68E-05**
bta-miR-656	.	10.64125	11.39116	10.7835	9.566304	10.78281	9.521733	10.22771	7.369395	12.98711	10.4122	11.81509	10.87819	11.45726	11.44767	11.02843	0.457325
bta-miR-664b	7.382029	.	.	.	9.581193	11.07551	11.83138	8.720412	7.124858	6.445125	7.391444	8.637925	5.002111	8.282869	10.14012	5.371532	**0.033071**
bta-miR-760-5p	7.270047	.	.	12.1605	8.748971	10.04024	7.589881	5.905694	3.877118	6.044126	5.29326	5.7771	2.629333	5.37866	8.082407	3.234371	**0.005499**
bta-miR-669	.	.	.	13.62011	7.070479	8.899525	8.378209	5.404095	1.754928	2.860008	1.552438	1.878007	-1.89141	1.458511	6.242317	-0.87491	**0.000761**
bta-miR-767	12.20366	10.7186	11.55729	11.12455	10.24837	9.196742	9.453023	8.463755	8.68641	10.18311	9.902524	9.933469	7.147308	10.18529	9.573742	8.098186	**0.074135**
bta-miR-744	9.385419	10.55524	14.59561	.	5.995921	7.785836	11.8831	4.808747	3.375123	4.050882	3.733791	5.021052	2.418705	4.071747	5.069756	1.900695	**0.000734**
bta-miR-92a	4.735291	.	.	11.5883	.	9.754355	10.67486	4.063365	-1.46642	-1.529	-1.82673	-1.30758	-3.13645	-1.19257	1.025005	-4.02321	**0.0043**
bta-miR-92b	8.573891	9.73891	11.63017	10.92478	7.875081	7.745425	7.948674	5.834479	2.867364	3.043954	2.338641	3.300725	0.544138	3.464012	5.056294	0.123144	**5.57E-06**
bta-miR-940	7.838727	.	.	14.92041	10.10513	9.030413	.	6.511315	1.369868	0.038812	0.219827	0.871463	-1.02252	1.721397	3.139744	-1.29735	**2.26E-05**
bta-miR-1224	9.713971	.	.	12.50093	8.747124	12.04413	10.46045	6.203114	-4.73316	-5.04153	-5.25307	-3.73504	-5.63507	-3.5647	-2.86183	-6.76774	**0.0024**
bta-miR-1225-3p	9.292535	.	.	.	9.17725	9.087702	12.38294	7.557841	2.72219	3.834461	2.430978	3.427966	-0.01476	2.840507	6.570076	0.669569	**7.72E-05**
bta-miR-1246	2.807593	6.842181	7.515875	6.950517	1.879206	2.675608	7.424275	-1.65139	-8.74468	-8.99259	-10.0009	-9.79199	-10.1175	-9.85181	-6.77818	-10.8613	**0.0009**
bta-miR-1247-3p	.	.	12.40905	9.753433	9.182152	12.12637	8.512841	8.228522	4.171949	6.700197	4.906282	6.504491	2.977133	5.145967	8.119667	3.35868	**0.000324**
bta-miR-1306	9.367711	.	.	.	9.732987	11.81713	8.982804	7.141501	4.378354	4.526006	4.112977	4.833623	2.646875	5.165622	6.985487	1.886833	**0.000164**
bta-miR-1260b	2.622735	13.1488	10.80618	13.16521	5.491093	5.377198	9.715718	0.980614	-2.23093	-1.80295	-2.32592	-0.61509	-4.02855	-1.18863	0.338402	-3.23851	**7.69E-05**
bta-miR-1307	9.742581	12.71238	.	12.95624	11.43699	10.05981	12.27567	7.590617	3.460687	4.004888	3.047805	5.19447	1.803564	4.689272	5.505934	1.87824	**1.36E-06**
bta-miR-1343-5p	8.258378	.	.	13.61593	9.282803	10.29994	.	7.232092	0.465129	1.035032	-0.41497	1.374661	-2.03604	1.017648	3.301182	-1.64427	**5.72E-06**

Hm/Ms/Rt T1 snRNA	-1.90941	5.861194	8.170708	5.453375	3.009621	4.20506	7.079884	0.150317	-10.1272	-10.1631	-10.5341	-10.2031	-11.4772	-9.95522	-7.79816	-11.974	**2.74E-08**
bta-miR-99b	-0.03187	-0.06523	0.103253	-0.2149	-0.02333	-0.00564	-0.09056	-0.14842	-0.12772	-0.01831	0.010028	-0.20575	-0.07401	-0.29346	-0.06565	-0.05059	0.392802

[1]MBER: Cows with moderated body energy reserve. [2]CC: Cumulus cells. [3]EV FF: Follicular fluid extracellular vesicles.

Supplementary table 7. Normalized data of the 42 exclusives miRNAs detected in follicular fluid extracellular vesicles (EV FF) compared to cumulus cells (CC) from ipsi and contralateral ovarian follicles (3-6 mm in diameter) from cows with high body energy reserve (HBER).

miRNA	**HBER[1]**							
	CC[2]				**EV FF[3]**			
	1	**2**	**3**	**4**	**1**	**2**	**3**	**4**
bta-let-7a-3p	.	.	.	.	8.840998	9.681344	8.980979	10.08494
bta-miR-103	.	.	.	.	6.730314	8.033536	6.926074	6.826751
bta-miR-124b	.	.	.	.	9.831537	12.44323	12.84489	10.04279
bta-miR-135a	.	.	.	.	9.775423	15.06291	11.45315	10.10634
bta-miR-129	.	.	.	.	7.736664	11.30417	11.11477	6.042321
bta-miR-129-5p	.	.	.	.	8.539625	10.07807	11.06834	6.772817
bta-miR-139	.	.	.	.	8.921545	10.82945	7.902148	8.970344
bta-miR-151-3p	.	.	.	.	3.858425	6.132859	4.871276	4.346385
bta-miR-15a	.	.	.	.	1.153528	4.683518	3.199073	1.549346
bta-miR-18a	.	.	.	.	5.172365	9.349324	7.858508	5.529571
bta-miR-192	.	.	.	.	7.721663	10.44714	7.999454	6.931186
bta-miR-185	.	.	.	.	6.574846	8.272486	6.327161	5.822673
bta-miR-199a-3p	.	.	.	.	1.957819	5.021876	4.350421	2.790271
bta-miR-199b	.	.	.	.	9.117686	11.54005	10.1803	9.093337
bta-miR-21-3p	.	.	.	.	8.794981	13.80871	8.973876	8.268748

bta-miR-22-5p	.	.	.	.	7.086943	10.85125	7.956489	8.329776
bta-miR-221	.	.	.	.	3.345238	5.67771	3.547368	3.888532
bta-miR-224	.	.	.	.	5.889757	7.51636	6.166968	5.845554
bta-miR-23b-5p	.	.	.	.	10.84016	12.71617	8.324142	10.01335
bta-miR-29d-3p	.	.	.	.	4.650437	7.606301	6.319762	4.728004
bta-miR-28	.	.	.	.	7.611969	10.6689	8.432037	7.701196
bta-miR-326	.	.	.	.	4.664486	6.811711	5.941442	3.507463
bta-miR-365-3p	.	.	.	.	4.623163	8.718238	6.797076	4.432094
bta-miR-345-3p	.	.	.	.	5.114012	6.7555	5.791398	4.928218
bta-miR-423-3p	.	.	.	.	2.025847	4.772738	2.644385	2.695981
bta-miR-424-3p	.	.	.	.	5.098479	7.869809	4.458875	5.506824
bta-miR-424-5p	.	.	.	.	-1.41587	2.270188	-0.15801	-0.56691
bta-miR-450b	.	.	.	.	8.386999	11.41592	10.8639	8.0279
bta-miR-425-5p	.	.	.	.	3.843693	5.81767	4.557492	4.793695
bta-miR-451	.	.	.	.	7.866064	11.63917	10.95129	9.485839
bta-miR-452	.	.	.	.	12.45491	10.55729	9.665935	11.0182
bta-miR-431	.	.	.	.	12.24971	11.64742	10.05683	6.898252
bta-miR-500	.	.	.	.	6.411852	8.785857	6.134647	6.553189
bta-miR-502b	.	.	.	.	5.276325	7.787417	6.242675	6.046933
bta-miR-490	.	.	.	.	8.803171	12.01421	10.28654	6.924419
bta-miR-504	.	.	.	.	10.66615	10.58048	6.664054	6.689752
bta-miR-542-5p	.	.	.	.	7.629019	12.1712	9.278518	10.94751
bta-miR-584	.	.	.	.	6.584555	8.074359	8.534057	5.227552
bta-miR-652	.	.	.	.	5.207606	7.443011	5.225867	6.125333
bta-miR-671	.	.	.	.	6.472719	8.509576	6.736825	6.971238
bta-miR-708	.	.	.	.	8.646452	10.79121	8.088996	8.008893
bta-miR-874	.	.	.	.	5.621453	7.232259	6.782098	4.615428

[1]HBER: Cows with high body energy reserve. [2]CC: Cumulus cells. [3]EV FF: Follicular fluid extracellular vesicles.

Supplementary table 8. Normalized data of the 46 miRNAs commonly detected in cumulus cells (CC) and follicular fluid extracellular vesicles (EV FF) from ipsi and contralateral ovarian follicles (3-6 mm in diameter) from cows with high body energy reserve (HBER).

miRNA	**HBER[1]**								***P* - value**
	CC[2]				**EV FF[3]**				
	1	**2**	**3**	**4**	**1**	**2**	**3**	**4**	
bta-let-7a-5p	13.60788	10.1573	5.371198	.	3.017429564	4.058363635	3.677334923	2.787291723	0.0518
bta-let-7c	13.06554	10.65505	4.890073	11.22076	1.322870467	3.161875445	1.495160241	1.74738999	0.004460785
bta-miR-125b	15.65969	9.812956	10.45917	.	1.815378401	4.580431659	3.867631096	2.901833731	0.003773581
bta-miR-127	13.01234	.	10.42745	9.776014	8.785116408	9.975571353	7.951077599	6.861024099	0.064900645
bta-miR-130a	10.55417	.	9.090299	9.240465	5.125269149	7.141703326	4.825574059	5.188235479	0.002713422
bta-miR-130b	10.57661	11.48177	8.127817	7.995895	5.539304946	7.472420413	4.95099749	5.466145712	0.011980864
bta-miR-133a	.	-1.60033	8.079141	10.22241	7.284696791	.	9.61900248	6.348824697	0.592955417
bta-miR-154b	11.59966	.	7.494424	6.596182	9.802572463	9.713112552	7.893300194	8.160681921	0.825885231
bta-miR-16b	.	9.53496	8.387182	9.216547	-0.341666963	2.543822009	1.131016869	0.251124578	0.000151921
bta-miR-191	6.564763	-4.38082	8.387235	-1.90635	4.70690367	7.455040652	6.608908791	4.76382752	0.289921261
bta-miR-195	14.68337	.	9.871355	10.25303	1.6695101	4.44974013	3.148009438	1.9552414	0.002010797
bta-miR-20a	.	11.06374	9.069215	11.04984	-0.012710359	2.844031054	1.344552178	0.576401907	0.00016824
bta-miR-219	14.67208	.	6.938932	9.929029	8.078176711	11.34668799	8.807860512	6.976947027	0.468133315
bta-miR-22-3p	-18.8748	-21.7255	-21.4024	-21.2204	.	-19.9657381	-21.06183062	-20.8403335	0.832247831
bta-miR-26a	.	8.387812	8.198869	9.142145	3.614025328	5.06586333	3.899377007	3.506255346	0.000236052

bta-miR-27a-5p	-13.16	-15.7191	-14.8889	-13.5949	13.50524733	5.518896777	-13.08026109	7.995661932	0.0304
bta-miR-296-5p	15.51521	.	7.373966	9.116755	6.622432921	8.860003192	8.011187691	5.937955099	0.194959024
bta-miR-30c	.	9.139177	8.439081	10.6929	5.634832595	7.700289447	5.222193856	5.976788717	0.011850998
bta-miR-320a	13.38779	.	6.6058	10.17063	-2.172078874	-0.125914853	-2.157404762	-1.686190409	0.001137708
bta-miR-323	-5.38978	-2.80919	-5.73866	-5.97797	-4.669976976	-4.776913922	-4.826300483	-4.585221094	0.73155422
bta-miR-339b	.	-7.25706	7.492998	9.5287	3.254015181	5.363964755	2.263535637	3.435103613	0.945235508
bta-miR-375	11.71266	.	6.326641	6.026993	.	8.880825717	6.857951302	5.879986959	0.710679355
bta-miR-382	14.89399	11.88123	8.55459	9.474821	5.566110823	8.819950778	6.583375115	7.031323629	0.03684902
bta-miR-411a	15.52795	.	8.725126	9.133043	7.959509108	9.765904147	7.970257897	8.951308447	0.253754703
bta-miR-411b	13.23535	-0.78591	10.73742	10.60206	11.9232733	9.576386172	10.01599421	10.10902329	0.560427126
bta-miR-380-5p	.	9.790529	6.994192	8.67872	8.737337325	.	11.52760233	7.701348501	0.583888098
bta-miR-421	11.37545	10.3644	7.375608	10.15359	4.7224066	5.917767245	4.055590767	5.480668264	0.00239729
bta-miR-429	12.35634	11.97043	7.617639	7.360996	7.036277983	8.200892169	6.738423485	7.413148123	0.124555599
bta-miR-4523	14.61351	.	10.68509	11.80449	8.729601119	11.63163443	12.09932246	7.280131536	0.207806617
bta-miR-433	7.541068	7.518377	6.114437	6.627138	7.349058364	8.498524386	8.875804085	5.923842626	0.37994595
bta-miR-494	8.55856	6.387634	5.271242	7.027458	0.415170882	1.933998782	-0.379731041	-0.305174631	0.000326941
bta-miR-532	.	9.610541	10.12644	10.17028	6.239338134	9.363980014	7.091916222	6.589635092	0.025888173
bta-miR-541	13.40863	.	7.920755	8.268494	6.993602965	9.692154605	8.84834208	5.760682302	0.312636197
bta-miR-615	-9.67773	-12.5695	-12.8695	-12.3829	-11.41256502	-10.52007608	-10.68553148	-11.07478408	0.260378394
bta-miR-631	-2.29511	-0.4771	-3.74077	-3.79976	-3.439851548	-3.159705442	-3.597781044	-3.284222406	0.35340923
bta-miR-574	11.85414	12.15933	4.437944	7.754669	-1.326600816	1.144066531	0.809407372	-3.049711985	0.003549646

bta-miR-656	11.39116	.	8.968389	8.783125	9.776033614	13.46832724	11.32543101	10.34963532	0.258881244
bta-miR-767	11.55729	.	10.46121	8.912027	8.744245742	11.29809256	8.341426033	8.667781073	0.357199391
bta-miR-92b	11.63017	.	5.349378	6.517881	2.637703154	4.78318854	3.379721779	1.833565908	0.046425672
bta-miR-1224	.	11.03741	8.346044	11.79233	-4.671678331	-2.476556104	-4.244444709	-3.649300379	0.0518
bta-miR-1246	7.515875	5.672995	3.160711	8.895163	-10.12315443	-8.762016576	-8.593123298	-10.07455043	0.0304
bta-miR-1247-3p	12.40905	.	7.525156	9.318415	5.555956099	7.85905524	5.827456612	4.784997183	0.046512931
bta-miR-1260b	10.80618	4.89451	5.671657	10.00764	-0.485650804	-0.400582593	-2.985649925	-1.102085871	0.001337021
bta-miR-1307	.	12.33522	8.716535	10.06846	4.214714263	5.822113711	3.855122704	3.910992819	0.002328034
Hm/Ms/Rt T1 snRNA	8.170708	3.373505	1.700271	6.983095	-10.7356489	-8.603463452	-9.650225502	-10.11936168	8.27384E-05
bta-miR-99b	0.103253	-0.17155	-0.06163	-0.05894	-0.079404619	-0.097962659	-0.117640358	0.063430029	0.88376036

[1]HBER: Cows with high body energy reserve. [2]CC: Cumulus cells. [3]EV FF: Follicular fluid extracellular vesicles.

Supplementary table 9. Biological patwhays predicted as modulated by exclusives miRNAs detected in follicular fluid extracellular vesicles (EV FF) compared to cumulus cells (CC) from ipsi and contralateral ovarian follicles (3-6 mm in diameter) from cows with moderated body energy reserve (MBER).

Pathway	%[1]	BH[2]
bta04144 Endocytosis	28.57143	0.0
bta05200 Pathways in cancer	23.24723	0.0092
bta05205 Proteoglycans in cancer	28.78049	0.0092
bta04010 MAPK signaling pathway	25.68493	0.0138
bta04014 Ras signaling pathway	26.44628	0.0138
bta04022 cGMP-PKG signaling pathway	28.40237	0.0138

bta04530 Tight junction	28.24859	0.0138
bta05202 Transcriptional misregulation in cancer	27.74869	0.0138
bta04150 mTOR signaling pathway	28.66242	0.0153
bta01522 Endocrine resistance	32.97872	0.0165
bta04310 Wnt signaling pathway	27.16049	0.0275
bta04360 Axon guidance	26.96629	0.0275
bta04520 Adherens junction	34.28571	0.0275
bta04921 Oxytocin signaling pathway	27.63158	0.0275
bta05224 Breast cancer	28	0.0275
bta05223 Non-small cell lung cancer	34.32836	0.0309
bta04670 Leukocyte transendothelial migration	29.20354	0.034
bta04066 HIF-1 signaling pathway	29.09091	0.0382
bta04371 Apelin signaling pathway	27.14286	0.04
bta04660 T cell receptor signaling pathway	28.97196	0.04
bta05215 Prostate cancer	29.59184	0.04
bta05225 Hepatocellular carcinoma	25.86207	0.04
bta04390 Hippo signaling pathway	26.28205	0.0401
bta05412 Arrhythmogenic right ventricular cardiomyopathy (ARVC)	31.57895	0.0401
bta05226 Gastric cancer	26.14379	0.0451
bta04710 Circadian rhythm	41.93548	0.0476
bta04068 FoxO signaling pathway	26.71756	0.0511
bta04810 Regulation of actin cytoskeleton	24.17062	0.0511
bta04510 Focal adhesion	24.24242	0.0588
bta04380 Osteoclast differentiation	26.1194	0.0632
bta05220 Chronic myeloid leukemia	29.87013	0.0639
bta04012 ErbB signaling pathway	28.57143	0.0778
bta04514 Cell adhesion molecules (CAMs)	24.68354	0.0778
bta04550 Signaling pathways regulating pluripotency of stem cells	25.35211	0.0778

bta04919 Thyroid hormone signaling pathway	26.27119	0.0778
bta00564 Glycerophospholipid metabolism	26.92308	0.078
bta01521 EGFR tyrosine kinase inhibitor resistance	28.75	0.078
bta04211 Longevity regulating pathway	27.77778	0.0796
bta04370 VEGF signaling pathway	31.03448	0.0839
bta04934 Cushing syndrome	24.35897	0.0866
bta04072 Phospholipase D signaling pathway	24.34211	0.0875
bta04960 Aldosterone-regulated sodium reabsorption	35.13514	0.0875
bta05213 Endometrial cancer	30.50847	0.0875
bta05218 Melanoma	28.76712	0.0875
bta04152 AMPK signaling pathway	25.20325	0.0947
bta05210 Colorectal cancer	26.96629	0.0986
bta00310 Lysine degradation	28.78788	0.1023
bta04910 Insulin signaling pathway	24.28571	0.1023
bta04931 Insulin resistance	25.45455	0.1023
bta05230 Central carbon metabolism in cancer	28.78788	0.1023
bta04213 Longevity regulating pathway	29.03226	0.1073
bta04925 Aldosterone synthesis and secretion	26.04167	0.1074
bta04015 Rap1 signaling pathway	22.22222	0.11
bta05214 Glioma	27.27273	0.11
bta05217 Basal cell carcinoma	28.57143	0.11
bta05410 Hypertrophic cardiomyopathy (HCM)	26.08696	0.11
bta05221 Acute myeloid leukemia	27.94118	0.1114
bta04666 Fc gamma R-mediated phagocytosis	25.80645	0.1157
bta04978 Mineral absorption	29.09091	0.1208
bta05231 Choline metabolism in cancer	25.25253	0.1208
bta05414 Dilated cardiomyopathy (DCM)	25.25253	0.1208
bta04340 Hedgehog signaling pathway	29.41176	0.1283

bta04911 Insulin secretion	25.88235	0.1283
bta04130 SNARE interactions in vesicular transport	33.33333	0.1289
bta01100 Metabolic pathways	17.94368	0.1338
bta05212 Pancreatic cancer	26.31579	0.1338
bta04151 PI3K-Akt signaling pathway	20.10724	0.1383
bta00410 beta-Alanine metabolism	32.35294	0.1411
bta04662 B cell receptor signaling pathway	25.28736	0.1411
bta04261 Adrenergic signaling in cardiomyocytes	22.66667	0.1477
bta05235 PD-L1 expression and PD-1 checkpoint pathway in cancer	24.73118	0.1491
bta04070 Phosphatidylinositol signaling system	24.24242	0.1585
bta00512 Mucin type O-glycan biosynthesis	32.25806	0.1632
bta00514 Other types of O-glycan biosynthesis	28.88889	0.1632
bta00533 Glycosaminoglycan biosynthesis	42.85714	0.1632
bta04611 Platelet activation	23.1405	0.1646
bta04664 Fc epsilon RI signaling pathway	25.71429	0.1693
bta03430 Mismatch repair	34.78261	0.1751
bta04930 Type II diabetes mellitus	28.26087	0.1751
bta00770 Pantothenate and CoA biosynthesis	36.84211	0.1759
bta04218 Cellular senescence	21.68675	0.1759
bta04916 Melanogenesis	23.52941	0.1759
bta05211 Renal cell carcinoma	25.35211	0.1759
bta04120 Ubiquitin mediated proteolysis	22.14286	0.1837
bta00561 Glycerolipid metabolism	25.37313	0.186
bta04024 cAMP signaling pathway	20.52402	0.1897
bta04914 Progesterone-mediated oocyte maturation	23.86364	0.1897
bta00562 Inositol phosphate metabolism	24.65753	0.191
bta04020 Calcium signaling pathway	20.79208	0.191
bta05100 Bacterial invasion of epithelial cells	24.65753	0.191

bta04140 Autophagy	21.83099	0.1922
bta01040 Biosynthesis of unsaturated fatty acids	30	0.2191
bta05166 Human T-cell leukemia virus 1 infection	20.08547	0.2253
bta04668 TNF signaling pathway	22.0339	0.2261
bta04961 Endocrine and other factor-regulated calcium reabsorption	26	0.2261
bta04114 Oocyte meiosis	21.84874	0.2276
bta04137 Mitophagy	24.24242	0.2276
bta04750 Inflammatory mediator regulation of TRP channels	22.3301	0.2276
bta04926 Relaxin signaling pathway	21.53846	0.2276
bta04971 Gastric acid secretion	23.68421	0.2276
bta05135 Yersinia infection	21.53846	0.2276
bta00790 Folate biosynthesis	27.77778	0.2279
bta03030 DNA replication	27.77778	0.2279
bta04350 TGF-beta signaling pathway	22.58065	0.2279
bta04924 Renin secretion	23.61111	0.2279
bta04928 Parathyroid hormone synthesis. secretion and action	22.11538	0.2279
bta04964 Proximal tubule bicarbonate reclamation	31.81818	0.2279
bta05160 Hepatitis C	20.73171	0.2279
bta04976 Bile secretion	22.89157	0.2331
bta04062 Chemokine signaling pathway	20.21277	0.2335
bta04142 Lysosome	21.21212	0.2335
bta05020 Prion diseases	28.125	0.2335
bta05219 Bladder cancer	26.19048	0.2344
bta04141 Protein processing in endoplasmic reticulum	20.48193	0.2347
bta04270 Vascular smooth muscle contraction	21.05263	0.2356
bta04722 Neurotrophin signaling pathway	21.31148	0.2356
bta00360 Phenylalanine metabolism	30.43478	0.2373
bta00515 Mannose type O-glycan biosynthesis	30.43478	0.2373

bta04923 Regulation of lipolysis in adipocytes	24.13793	0.2373
bta05165 Human papillomavirus infection	18.84058	0.2379
bta04935 Growth hormone synthesis. secretion and action	21.18644	0.2473
bta04659 Th17 cell differentiation	21.23894	0.2509
bta04972 Pancreatic secretion	21.56863	0.2509
bta04933 AGE-RAGE signaling pathway in diabetic complications	21.35922	0.2627
bta05032 Morphine addiction	21.73913	0.2627
bta04071 Sphingolipid signaling pathway	20.83333	0.2669
bta00532 Glycosaminoglycan biosynthesis	30	0.2761
bta03420 Nucleotide excision repair	24.44444	0.2761
bta04216 Ferroptosis	24.44444	0.2761
bta05167 Kaposi sarcoma-associated herpesvirus infection	19.41748	0.2761
bta00230 Purine metabolism	20.14925	0.2983
bta00510 N-Glycan biosynthesis	23.07692	0.3146
bta00250 Alanine. aspartate and glutamate metabolism	24.32432	0.3347
bta04725 Cholinergic synapse	20.17544	0.3347
bta04658 Th1 and Th2 cell differentiation	20.40816	0.3502
bta00620 Pyruvate metabolism	23.68421	0.3537
bta04912 GnRH signaling pathway	20.43011	0.3537
bta04927 Cortisol synthesis and secretion	21.53846	0.3537
bta04929 GnRH secretion	21.53846	0.3537
bta00600 Sphingolipid metabolism	22.44898	0.3581
bta03410 Base excision repair	24.24242	0.3581
bta00340 Histidine metabolism	26.08696	0.3703
bta05162 Measles	19.07895	0.3713
bta01524 Platinum drug resistance	20.51282	0.3808
bta04146 Peroxisome	20.2381	0.3858
bta01230 Biosynthesis of amino acids	20.54795	0.3905

bta00220 Arginine biosynthesis	26.31579	0.4044
bta04727 GABAergic synapse	19.78022	0.4044
bta04913 Ovarian steroidogenesis	21.05263	0.4044
bta04720 Long-term potentiation	20.28986	0.4202
bta05418 Fluid shear stress and atherosclerosis	18.62069	0.4278
bta00010 Glycolysis Gluconeogenesis	20.3125	0.4324
bta04110 Cell cycle	18.69919	0.4504
bta04975 Fat digestion and absorption	20.83333	0.4542
bta05161 Hepatitis B	18.12865	0.4542
bta04210 Apoptosis	18.30986	0.4612
bta04512 ECM-receptor interaction	19.10112	0.4612
bta04917 Prolactin signaling pathway	19.27711	0.4612
bta00513 Various types of N-glycan biosynthesis	20.93023	0.4618
bta05163 Human cytomegalovirus infection	17.55102	0.4666
bta04721 Synaptic vesicle cycle	19.23077	0.4685
bta04540 Gap junction	18.88889	0.47
bta05017 Spinocerebellar ataxia	18.75	0.47
bta05170 Human immunodeficiency virus 1 infection	17.52137	0.47
bta04915 Estrogen signaling pathway	18.11594	0.4735
bta04973 Carbohydrate digestion and absorption	20.45455	0.474
bta03018 RNA degradation	18.98734	0.4751
bta00240 Pyrimidine metabolism	19.64286	0.4822
bta00350 Tyrosine metabolism	20.51282	0.4891
bta04215 Apoptosis	20.58824	0.5123
bta00062 Fatty acid elongation	20.68966	0.5144
bta00565 Ether lipid metabolism	19.23077	0.5144
bta00592 alpha-Linolenic acid metabolism	20.68966	0.5144
bta00601 Glycosphingolipid biosynthesis	20.68966	0.5144

bta01212 Fatty acid metabolism	18.96552	0.5144
bta04625 C-type lectin receptor signaling pathway	17.92453	0.5144
bta05169 Epstein-Barr virus infection	17.10526	0.5144
bta05216 Thyroid cancer	20	0.5144
bta05222 Small cell lung cancer	18.08511	0.5144
bta01200 Carbon metabolism	17.69912	0.5211
bta03015 mRNA surveillance pathway	17.89474	0.5211
bta04260 Cardiac muscle contraction	17.97753	0.5211
bta04330 Notch signaling pathway	18.86792	0.5211
bta05206 MicroRNAs in cancer	16.78082	0.5265
bta00630 Glyoxylate and dicarboxylate metabolism	20	0.5354
bta04064 NF-kappa B signaling pathway	17.43119	0.546
bta05142 Chagas disease (American trypanosomiasis)	17.3913	0.546
bta04970 Salivary secretion	17.2043	0.5933
bta00563 Glycosylphosphatidylinositol (GPI)-anchor biosynthesis	19.23077	0.5999
bta04650 Natural killer cell mediated cytotoxicity	16.79389	0.6024
bta00983 Drug metabolism	17.10526	0.6195
bta04061 Viral protein interaction with cytokine and cytokine receptor	16.84211	0.6221
bta04136 Autophagy	18.18182	0.6221
bta04744 Phototransduction	18.51852	0.6244
bta04115 p53 signaling pathway	16.88312	0.6302
bta04728 Dopaminergic synapse	16.41791	0.6367
bta00051 Fructose and mannose metabolism	17.64706	0.6414
bta04922 Glucagon signaling pathway	16.50485	0.6414
bta00380 Tryptophan metabolism	17.02128	0.643
bta04640 Hematopoietic cell lineage	16.36364	0.643
bta04920 Adipocytokine signaling pathway	16.66667	0.643
bta04966 Collecting duct acid secretion	17.85714	0.643

bta00071 Fatty acid degradation	16.66667	0.6665
bta03020 RNA polymerase	17.24138	0.6665
bta04060 Cytokine-cytokine receptor interaction	15.78947	0.6665
bta04145 Phagosome	15.88235	0.6665
bta04392 Hippo signaling pathway	17.24138	0.6665
bta04918 Thyroid hormone synthesis	16.21622	0.6665
bta04713 Circadian entrainment	16	0.6696
bta00270 Cysteine and methionine metabolism	16.32653	0.6736
bta00260 Glycine. serine and threonine metabolism	16.27907	0.6829
bta05132 Salmonella infection	15.625	0.6898
bta03022 Basal transcription factors	15.90909	0.7015
bta05321 Inflammatory bowel disease (IBD)	15.71429	0.7015
bta05146 Amoebiasis	15.38462	0.7232
bta00760 Nicotinate and nicotinamide metabolism	15.38462	0.7378
bta04657 IL-17 signaling pathway	15.21739	0.7378
bta00640 Propanoate metabolism	15.15152	0.7552
bta00140 Steroid hormone biosynthesis	14.92537	0.7611
bta04217 Necroptosis	14.94253	0.7726
bta00330 Arginine and proline metabolism	14.58333	0.7826
bta04630 JAK-STAT signaling pathway	14.85149	0.7852
bta05031 Amphetamine addiction	14.49275	0.7885
bta04962 Vasopressin-regulated water reabsorption	14.28571	0.7958
bta03013 RNA transport	14.60674	0.8021
bta04932 Non-alcoholic fatty liver disease (NAFLD)	14.55696	0.8021
bta00280 Valine. leucine and isoleucine degradation	13.72549	0.8262
bta04724 Glutamatergic synapse	14.15929	0.8262
bta00980 Metabolism of xenobiotics by cytochrome P450	13.43284	0.85
bta03320 PPAR signaling pathway	13.58025	0.85

bta04730 Long-term depression	13.33333	0.85
bta05014 Amyotrophic lateral sclerosis (ALS)	13.33333	0.85
bta04620 Toll-like receptor signaling pathway	13.63636	0.8606
bta00860 Porphyrin and chlorophyll metabolism	12.5	0.8772
bta03040 Spliceosome	13.60544	0.8812
bta00982 Drug metabolism	12.69841	0.8839
bta05145 Toxoplasmosis	13.27434	0.8839
bta03440 Homologous recombination	11.90476	0.9016
bta05010 Alzheimer disease	13.33333	0.9123
bta05134 Legionellosis	12.06897	0.9123
bta00970 Aminoacyl-tRNA biosynthesis	12.12121	0.9131
bta00480 Glutathione metabolism	11.66667	0.9263
bta04714 Thermogenesis	13.38912	0.9263
bta04974 Protein digestion and absorption	12.39669	0.9412
bta00830 Retinol metabolism	10.9375	0.967
bta05168 Herpes simplex virus 1 infection	13.3995	0.9687
bta05203 Viral carcinogenesis	12.86307	0.9687
bta05323 Rheumatoid arthritis	11.53846	0.9698
bta04623 Cytosolic DNA-sensing pathway	10.44776	0.9807
bta04726 Serotonergic synapse	11.2069	0.9851
bta05152 Tuberculosis	12.12121	0.9851
bta05204 Chemical carcinogenesis	10.38961	0.9851
bta05140 Leishmaniasis	10.25641	0.9884
bta03008 Ribosome biogenesis in eukaryotes	9.638554	0.9942
bta04610 Complement and coagulation cascades	9.782609	0.9942
bta04621 NOD-like receptor signaling pathway	11.41304	0.9942
bta04622 RIG-I-like receptor signaling pathway	9.803922	0.9942
bta04672 Intestinal immune network for IgA production	8.928571	0.9942

bta04940 Type I diabetes mellitus	8.474576	0.9942
bta05012 Parkinson disease	11.33333	0.9942
bta05144 Malaria	8.474576	0.9942
bta05164 Influenza A	11.60221	0.9942
bta04723 Retrograde endocannabinoid signaling	10.52632	0.995
bta04742 Taste transduction	8.860759	0.995
bta00190 Oxidative phosphorylation	7.857143	1.0
bta00590 Arachidonic acid metabolism	7.317073	1.0
bta03010 Ribosome	4.375	1.0
bta04080 Neuroactive ligand-receptor interaction	11.29477	1.0
bta04612 Antigen processing and presentation	5.882353	1.0
bta04740 Olfactory transduction	2.09607	1.0
bta05016 Huntington disease	10.94891	1.0
bta05034 Alcoholism	10.04367	1.0
bta05133 Pertussis	6.493506	1.0
bta05150 Staphylococcus aureus infection	6.666667	1.0
bta05322 Systemic lupus erythematosus	3.296703	1.0

[1]%: Percent of genes predicted to be modulated. [2]BH: Benjamini – Hochberg

Supplementary table 10. Biological patwhays predicted as modulated by miRNAs up regulated in cumulus cells (CC) compared to follicular fluid extracellular vesicles (EV FF) from ipsi and contralateral ovarian follicles (3-6 mm in diameter) from cows with moderated body energy reserve (MBER).

Pathway	**%[1]**	**BH[2]**
bta04360 Axon guidance	21.91011	0.0
bta04520 Adherens junction	30	0.0
bta05200 Pathways in cancer	16.23616	0.0

bta04722 Neurotrophin signaling pathway	21.31148	0.028
bta04014 Ras signaling pathway	16.52893	0.0576
bta04022 cGMP-PKG signaling pathway	18.3432	0.0576
bta04924 Renin secretion	23.61111	0.0576
bta04921 Oxytocin signaling pathway	18.42105	0.0582
bta04931 Insulin resistance	20	0.0582
bta05031 Amphetamine addiction	23.18841	0.0582
bta04150 mTOR signaling pathway	17.83439	0.0631
bta04010 MAPK signaling pathway	14.72603	0.0784
bta04020 Calcium signaling pathway	15.84158	0.0784
bta04024 cAMP signaling pathway	15.72052	0.0784
bta04130 SNARE interactions in vesicular transport	27.27273	0.0784
bta04216 Ferroptosis	24.44444	0.0784
bta04261 Adrenergic signaling in cardiomyocytes	17.33333	0.0784
bta04270 Vascular smooth muscle contraction	18.04511	0.0784
bta04713 Circadian entrainment	19	0.0784
bta04728 Dopaminergic synapse	17.91045	0.0784
bta04744 Phototransduction	29.62963	0.0784
bta04910 Insulin signaling pathway	17.14286	0.0784
bta04916 Melanogenesis	18.62745	0.0784
bta04925 Aldosterone synthesis and secretion	19.79167	0.0784
bta04934 Cushing syndrome	16.66667	0.0784
bta04961 Endocrine and other factor-regulated calcium reabsorption	24	0.0784
bta04510 Focal adhesion	15.65657	0.0838
bta00270 Cysteine and methionine metabolism	22.44898	0.099
bta04310 Wnt signaling pathway	16.04938	0.099
bta05032 Morphine addiction	18.47826	0.099
bta05214 Glioma	19.48052	0.099

bta04142 Lysosome	16.66667	0.1018
bta05211 Renal cell carcinoma	19.71831	0.1018
bta05152 Tuberculosis	15.15152	0.1061
bta05224 Breast cancer	16	0.1101
bta04928 Parathyroid hormone synthesis. secretion and action	17.30769	0.1188
bta04810 Regulation of actin cytoskeleton	14.69194	0.1189
bta05163 Human cytomegalovirus infection	14.28571	0.1189
bta05226 Gastric cancer	15.68627	0.1189
bta05215 Prostate cancer	17.34694	0.1226
bta04012 ErbB signaling pathway	17.85714	0.1263
bta04720 Long-term potentiation	18.84058	0.1263
bta04971 Gastric acid secretion	18.42105	0.1263
bta05212 Pancreatic cancer	18.42105	0.1263
bta04151 PI3K-Akt signaling pathway	13.13673	0.1292
bta04970 Salivary secretion	17.2043	0.1292
bta04978 Mineral absorption	20	0.1292
bta01522 Endocrine resistance	17.02128	0.1363
bta05100 Bacterial invasion of epithelial cells	17.80822	0.1605
bta04340 Hedgehog signaling pathway	19.60784	0.1634
bta05167 Kaposi sarcoma-associated herpesvirus infection	14.07767	0.1634
bta04625 C-type lectin receptor signaling pathway	16.03774	0.1667
bta04015 Rap1 signaling pathway	13.88889	0.1668
bta04371 Apelin signaling pathway	15	0.1668
bta05231 Choline metabolism in cancer	16.16162	0.1668
bta05414 Dilated cardiomyopathy (DCM)	16.16162	0.1668
bta05412 Arrhythmogenic right ventricular cardiomyopathy (ARVC)	17.10526	0.1749
bta04911 Insulin secretion	16.47059	0.183
bta04912 GnRH signaling pathway	16.12903	0.183

bta04072 Phospholipase D signaling pathway	14.47368	0.1836
bta04919 Thyroid hormone signaling pathway	15.25424	0.1836
bta04114 Oocyte meiosis	15.12605	0.1874
bta05205 Proteoglycans in cancer	13.65854	0.1874
bta05217 Basal cell carcinoma	17.46032	0.1925
bta00532 Glycosaminoglycan biosynthesis	25	0.2006
bta04915 Estrogen signaling pathway	14.49275	0.2006
bta04920 Adipocytokine signaling pathway	16.66667	0.2006
bta04926 Relaxin signaling pathway	14.61538	0.2006
bta05210 Colorectal cancer	15.73034	0.2055
bta04923 Regulation of lipolysis in adipocytes	17.24138	0.2214
bta00230 Purine metabolism	14.1791	0.2327
bta05223 Non-small cell lung cancer	16.41791	0.2327
bta00514 Other types of O-glycan biosynthesis	17.77778	0.2513
bta04972 Pancreatic secretion	14.70588	0.2513
bta05220 Chronic myeloid leukemia	15.58442	0.2513
bta05222 Small cell lung cancer	14.89362	0.2513
bta05418 Fluid shear stress and atherosclerosis	13.7931	0.2513
bta04071 Sphingolipid signaling pathway	14.16667	0.2519
bta04721 Synaptic vesicle cycle	15.38462	0.2566
bta05135 Yersinia infection	13.84615	0.2618
bta04218 Cellular senescence	13.25301	0.2628
bta04659 Th17 cell differentiation	14.15929	0.2628
bta04710 Circadian rhythm	19.35484	0.2642
bta01230 Biosynthesis of amino acids	15.06849	0.2802
bta04062 Chemokine signaling pathway	12.76596	0.2802
bta04140 Autophagy	13.38028	0.2802
bta04658 Th1 and Th2 cell differentiation	14.28571	0.2802

bta04660 T cell receptor signaling pathway	14.01869	0.2802
bta05216 Thyroid cancer	17.5	0.2802
bta05218 Melanoma	15.06849	0.2802
bta04913 Ovarian steroidogenesis	15.78947	0.289
bta04144 Endocytosis	12.2449	0.2922
bta04657 IL-17 signaling pathway	14.13043	0.2964
bta05410 Hypertrophic cardiomyopathy (HCM)	14.13043	0.2964
bta04977 Vitamin digestion and absorption	19.23077	0.298
bta05160 Hepatitis C	12.80488	0.298
bta05225 Hepatocellular carcinoma	12.64368	0.3048
bta05213 Endometrial cancer	15.25424	0.3072
bta04750 Inflammatory mediator regulation of TRP channels	13.59223	0.3116
bta04922 Glucagon signaling pathway	13.59223	0.3116
bta05014 Amyotrophic lateral sclerosis (ALS)	15	0.3178
bta04110 Cell cycle	13.00813	0.3325
bta04725 Cholinergic synapse	13.15789	0.3325
bta05321 Inflammatory bowel disease (IBD)	14.28571	0.3354
bta01521 EGFR tyrosine kinase inhibitor resistance	13.75	0.3514
bta05161 Hepatitis B	12.2807	0.3514
bta05202 Transcriptional misregulation in cancer	12.04188	0.3611
bta04727 GABAergic synapse	13.18681	0.3812
bta04390 Hippo signaling pathway	12.17949	0.3851
bta00350 Tyrosine metabolism	15.38462	0.3868
bta04927 Cortisol synthesis and secretion	13.84615	0.3868
bta04976 Bile secretion	13.25301	0.3868
bta05030 Cocaine addiction	14.58333	0.39
bta05166 Human T-cell leukemia virus 1 infection	11.53846	0.39
bta05170 Human immunodeficiency virus 1 infection	11.53846	0.39

bta04611 Platelet activation	12.39669	0.3937
bta04962 Vasopressin-regulated water reabsorption	14.28571	0.4043
bta04080 Neuroactive ligand-receptor interaction	11.01928	0.4081
bta05165 Human papillomavirus infection	11.01449	0.4183
bta00480 Glutathione metabolism	13.33333	0.4422
bta03018 RNA degradation	12.65823	0.4521
bta04211 Longevity regulating pathway	12.22222	0.4803
bta04330 Notch signaling pathway	13.20755	0.481
bta04514 Cell adhesion molecules (CAMs)	11.39241	0.4885
bta03013 RNA transport	11.23596	0.4923
bta04120 Ubiquitin mediated proteolysis	11.42857	0.5008
bta04917 Prolactin signaling pathway	12.04819	0.5085
bta04350 TGF-beta signaling pathway	11.82796	0.5093
bta05235 PD-L1 expression and PD-1 checkpoint pathway in cancer	11.82796	0.5093
bta01100 Metabolic pathways	10.01965	0.51
bta00250 Alanine. aspartate and glutamate metabolism	13.51351	0.5103
bta04724 Glutamatergic synapse	11.50442	0.5103
bta04152 AMPK signaling pathway	11.38211	0.511
bta03015 mRNA surveillance pathway	11.57895	0.5264
bta00140 Steroid hormone biosynthesis	11.9403	0.5394
bta04975 Fat digestion and absorption	12.5	0.5394
bta05133 Pertussis	11.68831	0.5409
bta00760 Nicotinate and nicotinamide metabolism	12.82051	0.5434
bta00600 Sphingolipid metabolism	12.2449	0.5467
bta04530 Tight junction	10.73446	0.5467
bta05221 Acute myeloid leukemia	11.76471	0.5467
bta04668 TNF signaling pathway	11.01695	0.5493
bta05169 Epstein-Barr virus infection	10.52632	0.5493

bta04070 Phosphatidylinositol signaling system	11.11111	0.5588
bta04664 Fc epsilon RI signaling pathway	11.42857	0.566
bta04066 HIF-1 signaling pathway	10.90909	0.5689
bta00510 N-Glycan biosynthesis	11.53846	0.5761
bta04550 Signaling pathways regulating pluripotency of stem cells	10.56338	0.5761
bta04650 Natural killer cell mediated cytotoxicity	10.68702	0.5761
bta04723 Retrograde endocannabinoid signaling	10.52632	0.5761
bta05162 Measles	10.52632	0.5761
bta05219 Bladder cancer	11.90476	0.5761
bta04621 NOD-like receptor signaling pathway	10.32609	0.5888
bta04380 Osteoclast differentiation	10.44776	0.5971
bta00564 Glycerophospholipid metabolism	10.57692	0.5997
bta04146 Peroxisome	10.71429	0.5997
bta04918 Thyroid hormone synthesis	10.81081	0.5997
bta04726 Serotonergic synapse	10.34483	0.6134
bta05034 Alcoholism	10.04367	0.6134
bta00310 Lysine degradation	10.60606	0.6307
bta00561 Glycerolipid metabolism	10.44776	0.635
bta00980 Metabolism of xenobiotics by cytochrome P450	10.44776	0.635
bta04115 p53 signaling pathway	10.38961	0.635
bta05204 Chemical carcinogenesis	10.38961	0.635
bta04914 Progesterone-mediated oocyte maturation	10.22727	0.6367
bta05132 Salmonella infection	9.821429	0.6414
bta04068 FoxO signaling pathway	9.923664	0.6416
bta04260 Cardiac muscle contraction	10.11236	0.6416
bta04512 ECM-receptor interaction	10.11236	0.6416
bta04217 Necroptosis	9.770115	0.6478
bta04540 Gap junction	10	0.6478

bta03320 PPAR signaling pathway	9.876543	0.6607
bta04670 Leukocyte transendothelial migration	9.734513	0.6607
bta05145 Toxoplasmosis	9.734513	0.6607
bta05206 MicroRNAs in cancer	9.589041	0.6607
bta04213 Longevity regulating pathway	9.677419	0.6941
bta04061 Viral protein interaction with cytokine and cytokine receptor	9.473684	0.6975
bta04630 JAK-STAT signaling pathway	9.405941	0.6975
bta00982 Drug metabolism	9.52381	0.7005
bta04612 Antigen processing and presentation	9.411765	0.7005
bta05017 Spinocerebellar ataxia	9.375	0.7005
bta04935 Growth hormone synthesis. secretion and action	9.322034	0.7014
bta00830 Retinol metabolism	9.375	0.703
bta04064 NF-kappa B signaling pathway	9.174312	0.7103
bta04662 B cell receptor signaling pathway	9.195402	0.7103
bta04929 GnRH secretion	9.230769	0.7103
bta04137 Mitophagy	9.090909	0.7215
bta01524 Platinum drug resistance	8.974359	0.7312
bta00240 Pyrimidine metabolism	8.928571	0.734
bta04742 Taste transduction	8.860759	0.7381
bta04622 RIG-I-like receptor signaling pathway	8.823529	0.7427
bta04933 AGE-RAGE signaling pathway in diabetic complications	8.737864	0.7481
bta05134 Legionellosis	8.62069	0.7481
bta05164 Influenza A	8.839779	0.7481
bta05142 Chagas disease (American trypanosomiasis)	8.695652	0.749
bta04666 Fc gamma R-mediated phagocytosis	8.602151	0.7517
bta03008 Ribosome biogenesis in eukaryotes	8.433735	0.7639
bta04730 Long-term depression	8.333333	0.7639
bta04210 Apoptosis	8.450704	0.7795

bta04141 Protein processing in endoplasmic reticulum	8.433735	0.7875
bta00983 Drug metabolism	7.894737	0.8006
bta05010 Alzheimer disease	8.333333	0.8006
bta05416 Viral myocarditis	7.894737	0.8006
bta05230 Central carbon metabolism in cancer	7.575758	0.8214
bta04620 Toll-like receptor signaling pathway	7.272727	0.8745
bta04640 Hematopoietic cell lineage	7.272727	0.8745
bta04932 Non-alcoholic fatty liver disease (NAFLD)	7.594937	0.8745
bta05203 Viral carcinogenesis	7.883817	0.8745
bta00562 Inositol phosphate metabolism	6.849315	0.8755
bta04060 Cytokine-cytokine receptor interaction	7.430341	0.9563
bta01200 Carbon metabolism	6.19469	0.959
bta04145 Phagosome	6.470588	0.9654
bta05146 Amoebiasis	5.982906	0.9654
bta05323 Rheumatoid arthritis	5.769231	0.9654
bta04610 Complement and coagulation cascades	5.434783	0.9691
bta05012 Parkinson disease	6	0.9736
bta05016 Huntington disease	6.569343	0.983
bta03040 Spliceosome	5.442177	0.9879
bta04974 Protein digestion and absorption	4.958678	0.9879
bta05168 Herpes simplex virus 1 infection	6.699752	0.9879
bta00190 Oxidative phosphorylation	4.285714	1.0
bta04714 Thermogenesis	5.020921	1.0
bta04740 Olfactory transduction	1.834061	1.0
bta05322 Systemic lupus erythematosus	2.747253	1.0

[1] %: Percent of genes predicted to be modulated. [2] BH: Benjamini – Hochberg

Supplementary table 11. Biological patwhays predicted as modulated by exclusives miRNAs detected in follicular fluid extracellular vesicles (EV FF) compared to cumulus cells (CC) from ipsi and contralateral ovarian follicles (3-6 mm in diameter) from cows with high body energy reserve (HBER).

Pathway	%[1]	BH[2]
bta01100 Metabolic pathways	62.93386	0.0
bta04010 MAPK signaling pathway	72.26027	0.0128
bta04014 Ras signaling pathway	73.96694	0.0128
bta04144 Endocytosis	75.91837	0.0128
bta05200 Pathways in cancer	66.42066	0.0128
bta04360 Axon guidance	75.2809	0.0367
bta05205 Proteoglycans in cancer	73.17073	0.0367
bta04810 Regulation of actin cytoskeleton	72.03791	0.0482
bta04071 Sphingolipid signaling pathway	78.33333	0.0606
bta04910 Insulin signaling pathway	75.71429	0.0642
bta04015 Rap1 signaling pathway	69.90741	0.0688
bta04072 Phospholipase D signaling pathway	73.68421	0.0688
bta04150 mTOR signaling pathway	73.24841	0.0688
bta04310 Wnt signaling pathway	72.83951	0.0688
bta04142 Lysosome	75	0.0728
bta00564 Glycerophospholipid metabolism	75.96154	0.1009
bta04070 Phosphatidylinositol signaling system	76.76768	0.1009
bta04270 Vascular smooth muscle contraction	72.93233	0.1009
bta04722 Neurotrophin signaling pathway	74.59016	0.1009
bta04921 Oxytocin signaling pathway	71.71053	0.1009
bta05165 Human papillomavirus infection	64.34783	0.1009
bta04934 Cushing syndrome	70.51282	0.1117
bta05231 Choline metabolism in cancer	75.75758	0.1117

bta04012 ErbB signaling pathway	77.38095	0.1161
bta04611 Platelet activation	72.72727	0.1161
bta05212 Pancreatic cancer	78.94737	0.1161
bta04928 Parathyroid hormone synthesis. secretion and action	74.03846	0.126
bta05220 Chronic myeloid leukemia	77.92208	0.1273
bta04750 Inflammatory mediator regulation of TRP channels	73.78641	0.1295
bta00230 Purine metabolism	69.40299	0.1302
bta00562 Inositol phosphate metabolism	75.34247	0.1302
bta01522 Endocrine resistance	72.34043	0.1302
bta04022 cGMP-PKG signaling pathway	66.86391	0.1302
bta04062 Chemokine signaling pathway	67.02128	0.1302
bta04068 FoxO signaling pathway	69.46565	0.1302
bta04120 Ubiquitin mediated proteolysis	70	0.1302
bta04140 Autophagy	68.30986	0.1302
bta04261 Adrenergic signaling in cardiomyocytes	68	0.1302
bta04390 Hippo signaling pathway	67.30769	0.1302
bta04510 Focal adhesion	65.65657	0.1302
bta04514 Cell adhesion molecules (CAMs)	68.35443	0.1302
bta04659 Th17 cell differentiation	71.68142	0.1302
bta04666 Fc gamma R-mediated phagocytosis	73.11828	0.1302
bta04668 TNF signaling pathway	70.33898	0.1302
bta04728 Dopaminergic synapse	68.65672	0.1302
bta04730 Long-term depression	78.33333	0.1302
bta04912 GnRH signaling pathway	73.11828	0.1302
bta04916 Melanogenesis	73.52941	0.1302
bta04919 Thyroid hormone signaling pathway	72.0339	0.1302
bta05132 Salmonella infection	64.73214	0.1302
bta05163 Human cytomegalovirus infection	64.4898	0.1302

bta05211 Renal cell carcinoma	77.46479	0.1302
bta05214 Glioma	75.32468	0.1302
bta05225 Hepatocellular carcinoma	67.24138	0.1302
bta04520 Adherens junction	75.71429	0.1325
bta04371 Apelin signaling pathway	67.85714	0.1352
bta05418 Fluid shear stress and atherosclerosis	67.58621	0.1352
bta04550 Signaling pathways regulating pluripotency of stem cells	67.60563	0.1355
bta04931 Insulin resistance	70	0.1355
bta04660 T cell receptor signaling pathway	70.09346	0.1386
bta04141 Protein processing in endoplasmic reticulum	65.66265	0.1469
bta04658 Th1 and Th2 cell differentiation	70.40816	0.1469
bta04933 AGE-RAGE signaling pathway in diabetic complications	69.90291	0.1469
bta05100 Bacterial invasion of epithelial cells	73.9726	0.1469
bta05135 Yersinia infection	67.69231	0.1469
bta05215 Prostate cancer	70.40816	0.1469
bta04370 VEGF signaling pathway	75.86207	0.161
bta05210 Colorectal cancer	70.78652	0.161
bta05224 Breast cancer	66	0.161
bta01200 Carbon metabolism	68.14159	0.1637
bta04926 Relaxin signaling pathway	66.92308	0.1637
bta04721 Synaptic vesicle cycle	71.79487	0.1645
bta04066 HIF-1 signaling pathway	68.18182	0.1667
bta00330 Arginine and proline metabolism	77.08333	0.1732
bta04151 PI3K-Akt signaling pathway	60.05362	0.1732
bta04218 Cellular senescence	64.45783	0.1732
bta04625 C-type lectin receptor signaling pathway	67.92453	0.1732
bta04720 Long-term potentiation	72.46377	0.1732
bta04935 Growth hormone synthesis. secretion and action	66.94915	0.1732

bta05161 Hepatitis B	64.32749	0.1732
bta05166 Human T-cell leukemia virus 1 infection	62.39316	0.1732
bta04530 Tight junction	63.84181	0.1733
bta04670 Leukocyte transendothelial migration	67.25664	0.1733
bta04020 Calcium signaling pathway	62.87129	0.1743
bta04914 Progesterone-mediated oocyte maturation	69.31818	0.1743
bta05235 PD-L1 expression and PD-1 checkpoint pathway in cancer	68.8172	0.1743
bta05170 Human immunodeficiency virus 1 infection	61.96581	0.176
bta05223 Non-small cell lung cancer	71.64179	0.1864
bta04925 Aldosterone synthesis and secretion	67.70833	0.1958
bta05226 Gastric cancer	64.05229	0.1958
bta04137 Mitophagy	71.21212	0.1964
bta05230 Central carbon metabolism in cancer	71.21212	0.1964
bta00514 Other types of O-glycan biosynthesis	75.55556	0.199
bta04114 Oocyte meiosis	65.54622	0.199
bta05217 Basal cell carcinoma	71.42857	0.199
bta05221 Acute myeloid leukemia	70.58824	0.199
bta01521 EGFR tyrosine kinase inhibitor resistance	68.75	0.2022
bta04152 AMPK signaling pathway	65.04065	0.2023
bta04726 Serotonergic synapse	65.51724	0.2023
bta04024 cAMP signaling pathway	61.13537	0.2063
bta05167 Kaposi sarcoma-associated herpesvirus infection	61.65049	0.2063
bta04146 Peroxisome	67.85714	0.2077
bta00600 Sphingolipid metabolism	73.46939	0.208
bta04922 Glucagon signaling pathway	66.01942	0.208
bta04130 SNARE interactions in vesicular transport	78.78788	0.2106
bta04350 TGF-beta signaling pathway	66.66667	0.2106
bta04971 Gastric acid secretion	68.42105	0.2106

bta00240 Pyrimidine metabolism	71.42857	0.2128
bta04064 NF-kappa B signaling pathway	65.13761	0.217
bta04911 Insulin secretion	67.05882	0.2177
bta05032 Morphine addiction	66.30435	0.2192
bta04216 Ferroptosis	73.33333	0.2241
bta04961 Endocrine and other factor-regulated calcium reabsorption	72	0.2241
bta04924 Renin secretion	68.05556	0.2272
bta00410 beta-Alanine metabolism	76.47059	0.2347
bta04710 Circadian rhythm	77.41935	0.2427
bta00533 Glycosaminoglycan biosynthesis	92.85714	0.2455
bta00591 Linoleic acid metabolism	75	0.2455
bta05160 Hepatitis C	61.58537	0.2455
bta01212 Fatty acid metabolism	68.96552	0.2549
bta04330 Notch signaling pathway	69.81132	0.2549
bta04927 Cortisol synthesis and secretion	67.69231	0.2579
bta04110 Cell cycle	62.60163	0.2714
bta01524 Platinum drug resistance	65.38462	0.2845
bta00310 Lysine degradation	66.66667	0.285
bta01230 Biosynthesis of amino acids	65.75342	0.2864
bta00670 One carbon pool by folate	83.33333	0.2914
bta04664 Fc epsilon RI signaling pathway	65.71429	0.297
bta04727 GABAergic synapse	63.73626	0.297
bta05202 Transcriptional misregulation in cancer	59.68586	0.297
bta00340 Histidine metabolism	78.26087	0.2972
bta04920 Adipocytokine signaling pathway	65.27778	0.2974
bta00561 Glycerolipid metabolism	65.67164	0.3011
bta04061 Viral protein interaction with cytokine and cytokine receptor	63.15789	0.3011
bta04540 Gap junction	63.33333	0.3059

bta05412 Arrhythmogenic right ventricular cardiomyopathy (ARVC)	64.47368	0.3059
bta00062 Fatty acid elongation	72.41379	0.3087
bta00220 Arginine biosynthesis	78.94737	0.3087
bta00270 Cysteine and methionine metabolism	67.34694	0.3087
bta00510 N-Glycan biosynthesis	67.30769	0.3087
bta00565 Ether lipid metabolism	67.30769	0.3087
bta00592 alpha-Linolenic acid metabolism	72.41379	0.3087
bta00760 Nicotinate and nicotinamide metabolism	69.23077	0.3087
bta00770 Pantothenate and CoA biosynthesis	78.94737	0.3087
bta03015 mRNA surveillance pathway	62.10526	0.3087
bta04115 p53 signaling pathway	63.63636	0.3087
bta04215 Apoptosis	70.58824	0.3087
bta04724 Glutamatergic synapse	61.06195	0.3087
bta04744 Phototransduction	74.07407	0.3087
bta04915 Estrogen signaling pathway	60.14493	0.3087
bta04930 Type II diabetes mellitus	67.3913	0.3087
bta04970 Salivary secretion	62.36559	0.3087
bta05017 Spinocerebellar ataxia	62.5	0.3087
bta05020 Prion diseases	71.875	0.3087
bta05133 Pertussis	63.63636	0.3087
bta05145 Toxoplasmosis	61.06195	0.3087
bta05321 Inflammatory bowel disease (IBD)	64.28571	0.3087
bta04210 Apoptosis	59.85915	0.3103
bta05142 Chagas disease (American trypanosomiasis)	60.86957	0.3103
bta04917 Prolactin signaling pathway	62.6506	0.311
bta04657 IL-17 signaling pathway	61.95652	0.3126
bta00515 Mannose type O-glycan biosynthesis	73.91304	0.3197
bta03420 Nucleotide excision repair	66.66667	0.3197

bta04380 Osteoclast differentiation	59.70149	0.3197
bta04966 Collecting duct acid secretion	71.42857	0.3197
bta05213 Endometrial cancer	64.40678	0.3197
bta05218 Melanoma	63.0137	0.3197
bta04713 Circadian entrainment	61	0.3235
bta00020 Citrate cycle (TCA cycle)	70	0.3301
bta05219 Bladder cancer	66.66667	0.3301
bta00500 Starch and sucrose metabolism	68.75	0.3361
bta04340 Hedgehog signaling pathway	64.70588	0.3361
bta04923 Regulation of lipolysis in adipocytes	63.7931	0.3361
bta05164 Influenza A	58.01105	0.3361
bta05410 Hypertrophic cardiomyopathy (HCM)	60.86957	0.3361
bta05414 Dilated cardiomyopathy (DCM)	60.60606	0.3361
bta00480 Glutathione metabolism	63.33333	0.3371
bta05031 Amphetamine addiction	62.31884	0.3382
bta05222 Small cell lung cancer	60.6383	0.3382
bta04978 Mineral absorption	63.63636	0.3424
bta00010 Glycolysis Gluconeogenesis	62.5	0.3441
bta05169 Epstein-Barr virus infection	57.01754	0.3441
bta00513 Various types of N-glycan biosynthesis	65.11628	0.3451
bta03030 DNA replication	66.66667	0.3451
bta04913 Ovarian steroidogenesis	63.15789	0.3451
bta00512 Mucin type O-glycan biosynthesis	67.74194	0.3505
bta04136 Autophagy	66.66667	0.3616
bta05216 Thyroid cancer	65	0.3616
bta00071 Fatty acid degradation	64.28571	0.3669
bta04725 Cholinergic synapse	58.77193	0.3669
bta04962 Vasopressin-regulated water reabsorption	63.26531	0.3669

bta01040 Biosynthesis of unsaturated fatty acids	66.66667	0.37
bta03430 Mismatch repair	69.56522	0.37
bta04662 B cell receptor signaling pathway	59.77011	0.37
bta04960 Aldosterone-regulated sodium reabsorption	64.86486	0.37
bta05014 Amyotrophic lateral sclerosis (ALS)	61.66667	0.37
bta05152 Tuberculosis	56.56566	0.3788
bta00100 Steroid biosynthesis	70	0.3822
bta00532 Glycosaminoglycan biosynthesis	70	0.3822
bta05162 Measles	57.23684	0.3822
bta05340 Primary immunodeficiency	63.41463	0.3822
bta04640 Hematopoietic cell lineage	58.18182	0.3855
bta00260 Glycine. serine and threonine metabolism	62.7907	0.3863
bta04211 Longevity regulating pathway	58.88889	0.3863
bta03460 Fanconi anemia pathway	61.53846	0.3868
bta03020 RNA polymerase	65.51724	0.39
bta04918 Thyroid hormone synthesis	59.45946	0.3936
bta00620 Pyruvate metabolism	63.15789	0.3943
bta00380 Tryptophan metabolism	61.70213	0.395
bta00052 Galactose metabolism	64.51613	0.3951
bta00280 Valine. leucine and isoleucine degradation	60.78431	0.4038
bta00563 Glycosylphosphatidylinositol (GPI)-anchor biosynthesis	65.38462	0.4038
bta00640 Propanoate metabolism	63.63636	0.4038
bta04621 NOD-like receptor signaling pathway	55.97826	0.4038
bta00900 Terpenoid backbone biosynthesis	66.66667	0.4174
bta05030 Cocaine addiction	60.41667	0.4238
bta04976 Bile secretion	57.83133	0.4291
bta04512 ECM-receptor interaction	57.30337	0.4399
bta04972 Pancreatic secretion	56.86275	0.4399

bta05146 Amoebiasis	56.41026	0.44
bta05323 Rheumatoid arthritis	56.73077	0.44
bta00590 Arachidonic acid metabolism	57.31707	0.4468
bta00601 Glycosphingolipid biosynthesis	62.06897	0.4501
bta04217 Necroptosis	55.17241	0.4501
bta04392 Hippo signaling pathway	62.06897	0.4501
bta00531 Glycosaminoglycan degradation	63.63636	0.455
bta04964 Proximal tubule bicarbonate reclamation	63.63636	0.455
bta05204 Chemical carcinogenesis	57.14286	0.455
bta00250 Alanine. aspartate and glutamate metabolism	59.45946	0.471
bta00910 Nitrogen metabolism	64.70588	0.471
bta04145 Phagosome	54.70588	0.471
bta04975 Fat digestion and absorption	58.33333	0.471
bta04977 Vitamin digestion and absorption	61.53846	0.471
bta00030 Pentose phosphate pathway	60.71429	0.4712
bta00650 Butanoate metabolism	60.71429	0.4712
bta04122 Sulfur relay system	70	0.4712
bta04929 GnRH secretion	56.92308	0.4712
bta00630 Glyoxylate and dicarboxylate metabolism	60	0.4775
bta04723 Retrograde endocannabinoid signaling	54.60526	0.48
bta00051 Fructose and mannose metabolism	58.82353	0.4897
bta00061 Fatty acid biosynthesis	61.11111	0.5172
bta00730 Thiamine metabolism	61.11111	0.5172
bta01210 2-Oxocarboxylic acid metabolism	61.11111	0.5172
bta04650 Natural killer cell mediated cytotoxicity	54.19847	0.5172
bta00520 Amino sugar and nucleotide sugar metabolism	56	0.5268
bta00511 Other glycan degradation	59.09091	0.532
bta00534 Glycosaminoglycan biosynthesis	58.33333	0.538

bta00430 Taurine and hypotaurine metabolism	61.53846	0.5385
bta03018 RNA degradation	54.43038	0.5385
bta03320 PPAR signaling pathway	54.32099	0.5385
bta03450 Non-homologous end-joining	61.53846	0.5385
bta04213 Longevity regulating pathway	54.83871	0.5385
bta04614 Renin-angiotensin system	57.69231	0.5385
bta00983 Drug metabolism	53.94737	0.5529
bta05416 Viral myocarditis	53.94737	0.5529
bta05140 Leishmaniasis	53.84615	0.5535
bta00360 Phenylalanine metabolism	56.52174	0.5602
bta00980 Metabolism of xenobiotics by cytochrome P450	53.73134	0.5602
bta03022 Basal transcription factors	54.54545	0.5602
bta03060 Protein export	56.52174	0.5602
bta03440 Homologous recombination	54.7619	0.5602
bta04672 Intestinal immune network for IgA production	53.57143	0.5766
bta00920 Sulfur metabolism	60	0.578
bta03013 RNA transport	52.24719	0.578
bta03410 Base excision repair	54.54545	0.578
bta00350 Tyrosine metabolism	53.84615	0.5823
bta01523 Antifolate resistance	53.48837	0.5859
bta00604 Glycosphingolipid biosynthesis	56.25	0.5873
bta00140 Steroid hormone biosynthesis	52.23881	0.6033
bta00982 Drug metabolism	52.38095	0.6033
bta04623 Cytosolic DNA-sensing pathway	52.23881	0.6033
bta05033 Nicotine addiction	52.5	0.6126
bta04973 Carbohydrate digestion and absorption	52.27273	0.6141
bta04620 Toll-like receptor signaling pathway	50.90909	0.6514
bta04940 Type I diabetes mellitus	50.84746	0.6555

bta03008 Ribosome biogenesis in eukaryotes	50.60241	0.6622
bta04060 Cytokine-cytokine receptor interaction	50.77399	0.6641
bta03040 Spliceosome	50.34014	0.6748
bta04979 Cholesterol metabolism	50	0.6748
bta05134 Legionellosis	50	0.6748
bta05332 Graft-versus-host disease	50	0.6748
bta04950 Maturity onset diabetes of the young	50	0.6758
bta04612 Antigen processing and presentation	49.41176	0.7012
bta00040 Pentose and glucuronate interconversions	48.3871	0.7142
bta05203 Viral carcinogenesis	49.79253	0.715
bta00053 Ascorbate and aldarate metabolism	48	0.7171
bta00830 Retinol metabolism	48.4375	0.723
bta00603 Glycosphingolipid biosynthesis	47.05882	0.7305
bta00860 Porphyrin and chlorophyll metabolism	47.5	0.7327
bta04714 Thermogenesis	49.37238	0.7327
bta00790 Folate biosynthesis	47.22222	0.7329
bta00072 Synthesis and degradation of ketone bodies	45.45455	0.7484
bta03050 Proteasome	45.65217	0.7857
bta05143 African trypanosomiasis	45.45455	0.7861
bta05144 Malaria	45.76271	0.7938
bta04080 Neuroactive ligand-receptor interaction	48.76033	0.7952
bta04630 JAK-STAT signaling pathway	47.52475	0.8171
bta05330 Allograft rejection	44.64286	0.8171
bta05150 Staphylococcus aureus infection	45.71429	0.8334
bta00450 Selenocompound metabolism	38.88889	0.8505
bta02010 ABC transporters	43.33333	0.8505
bta04932 Non-alcoholic fatty liver disease (NAFLD)	46.20253	0.8505
bta04610 Complement and coagulation cascades	44.56522	0.8511

bta05010 Alzheimer disease	45.55556	0.8818
bta04742 Taste transduction	41.77215	0.9116
bta04974 Protein digestion and absorption	42.97521	0.9216
bta04260 Cardiac muscle contraction	41.57303	0.923
bta04622 RIG-I-like receptor signaling pathway	41.17647	0.94
bta05016 Huntington disease	44.89051	0.94
bta00120 Primary bile acid biosynthesis	29.41176	0.9464
bta05310 Asthma	34.21053	0.9558
bta05168 Herpes simplex virus 1 infection	45.16129	0.9583
bta05320 Autoimmune thyroid disease	36.61972	0.9712
bta05012 Parkinson disease	40	0.9799
bta00970 Aminoacyl-tRNA biosynthesis	33.33333	0.9883
bta05206 MicroRNAs in cancer	42.12329	0.9883
bta00190 Oxidative phosphorylation	37.14286	0.9951
bta05034 Alcoholism	39.30131	0.9967
bta03010 Ribosome	25	1.0
bta04740 Olfactory transduction	6.550218	1.0
bta05322 Systemic lupus erythematosus	24.72527	1.0

[1]%: Percent of genes predicted to be modulated. [2]BH: Benjamini – Hochberg

Supplementary table 12. Biological patwhays predicted as modulated by miRNAs up regulated in follicular fluid extracellular vesicles (EV FF) compared to cumulus cells (CC) from ipsi and contralateral ovarian follicles (3-6 mm in diameter) from cows with high body energy reserve (HBER).

Pathway	**%[1]**	**BH[2]**
bta04010 MAPK signaling pathway	57.87671	0.0
bta04144 Endocytosis	57.95918	0.0

bta04360 Axon guidance	62.92135	0.0
bta05200 Pathways in cancer	51.10701	0.0
bta01100 Metabolic pathways	44.53176	0.0053
bta04014 Ras signaling pathway	56.19835	0.0053
bta04072 Phospholipase D signaling pathway	60.52632	0.0071
bta04910 Insulin signaling pathway	62.14286	0.0071
bta05205 Proteoglycans in cancer	57.07317	0.0071
bta04015 Rap1 signaling pathway	56.01852	0.0087
bta04310 Wnt signaling pathway	59.25926	0.0087
bta04390 Hippo signaling pathway	58.97436	0.0098
bta04810 Regulation of actin cytoskeleton	55.45024	0.0098
bta04012 ErbB signaling pathway	66.66667	0.0137
bta04660 T cell receptor signaling pathway	62.61682	0.0149
bta04068 FoxO signaling pathway	59.54198	0.016
bta04062 Chemokine signaling pathway	54.78723	0.0192
bta05211 Renal cell carcinoma	67.60563	0.0192
bta05220 Chronic myeloid leukemia	66.23377	0.0192
bta05225 Hepatocellular carcinoma	55.74713	0.0192
bta05212 Pancreatic cancer	65.78947	0.0213
bta01522 Endocrine resistance	61.70213	0.0248
bta04071 Sphingolipid signaling pathway	58.33333	0.0248
bta04120 Ubiquitin mediated proteolysis	56.42857	0.0248
bta04150 mTOR signaling pathway	55.41401	0.0248
bta04510 Focal adhesion	53.0303	0.0248
bta04550 Signaling pathways regulating pluripotency of stem cells	56.33803	0.0248
bta04611 Platelet activation	57.85124	0.0248
bta04659 Th17 cell differentiation	59.29204	0.0248

bta04722 Neurotrophin signaling pathway	58.19672	0.0248
bta05210 Colorectal cancer	61.79775	0.0248
bta00562 Inositol phosphate metabolism	64.38356	0.0252
bta05214 Glioma	63.63636	0.0252
bta04530 Tight junction	53.67232	0.0254
bta01212 Fatty acid metabolism	67.24138	0.0272
bta01521 EGFR tyrosine kinase inhibitor resistance	62.5	0.0272
bta04070 Phosphatidylinositol signaling system	59.59596	0.0272
bta04921 Oxytocin signaling pathway	54.60526	0.0272
bta04931 Insulin resistance	58.18182	0.0272
bta05132 Salmonella infection	51.33929	0.0272
bta05224 Breast cancer	54.66667	0.0273
bta04514 Cell adhesion molecules (CAMs)	53.79747	0.0312
bta04934 Cushing syndrome	53.84615	0.0313
bta05231 Choline metabolism in cancer	58.58586	0.0313
bta04666 Fc gamma R-mediated phagocytosis	59.13978	0.032
bta05235 PD-L1 expression and PD-1 checkpoint pathway in cancer	59.13978	0.032
bta04916 Melanogenesis	57.84314	0.0334
bta04020 Calcium signaling pathway	50.9901	0.0378
bta04933 AGE-RAGE signaling pathway in diabetic complications	57.28155	0.0378
bta05223 Non-small cell lung cancer	62.68657	0.0378
bta05135 Yersinia infection	54.61538	0.0389
bta04625 C-type lectin receptor signaling pathway	56.60377	0.0394
bta05163 Human cytomegalovirus infection	49.38776	0.0397
bta05226 Gastric cancer	52.94118	0.0397
bta04520 Adherens junction	61.42857	0.0407

bta05230 Central carbon metabolism in cancer	62.12121	0.0411
bta05165 Human papillomavirus infection	46.95652	0.0488
bta04928 Parathyroid hormone synthesis. secretion and action	55.76923	0.0502
bta04658 Th1 and Th2 cell differentiation	56.12245	0.0519
bta04919 Thyroid hormone signaling pathway	54.23729	0.0519
bta05215 Prostate cancer	56.12245	0.0519
bta04261 Adrenergic signaling in cardiomyocytes	52	0.052
bta04664 Fc epsilon RI signaling pathway	60	0.052
bta04728 Dopaminergic synapse	52.98507	0.052
bta04024 cAMP signaling pathway	48.9083	0.0527
bta05166 Human T-cell leukemia virus 1 infection	48.71795	0.0533
bta04370 VEGF signaling pathway	62.06897	0.0544
bta04140 Autophagy	52.11268	0.056
bta04371 Apelin signaling pathway	52.14286	0.057
bta05219 Bladder cancer	66.66667	0.0576
bta04022 cGMP-PKG signaling pathway	50.29586	0.0603
bta04151 PI3K-Akt signaling pathway	45.8445	0.0603
bta04152 AMPK signaling pathway	52.84553	0.0603
bta04750 Inflammatory mediator regulation of TRP channels	54.36893	0.0603
bta04920 Adipocytokine signaling pathway	58.33333	0.0603
bta04922 Glucagon signaling pathway	54.36893	0.0603
bta05160 Hepatitis C	50.60976	0.0603
bta04540 Gap junction	55.55556	0.0619
bta00533 Glycosaminoglycan biosynthesis	92.85714	0.062
bta04670 Leukocyte transendothelial migration	53.09735	0.064
bta04730 Long-term depression	60	0.064
bta05100 Bacterial invasion of epithelial cells	57.53425	0.064
bta05418 Fluid shear stress and atherosclerosis	51.03448	0.064

bta04912 GnRH signaling pathway	54.83871	0.0644
bta04935 Growth hormone synthesis. secretion and action	52.54237	0.0651
bta05161 Hepatitis B	49.7076	0.0651
bta04662 B cell receptor signaling pathway	55.17241	0.068
bta04926 Relaxin signaling pathway	51.53846	0.068
bta00410 beta-Alanine metabolism	67.64706	0.0703
bta04066 HIF-1 signaling pathway	52.72727	0.0703
bta05213 Endometrial cancer	59.32203	0.0703
bta05218 Melanoma	56.16438	0.0797
bta04721 Synaptic vesicle cycle	55.12821	0.0853
bta04392 Hippo signaling pathway	68.96552	0.0861
bta05167 Kaposi sarcoma-associated herpesvirus infection	47.57282	0.0879
bta04218 Cellular senescence	48.79518	0.089
bta00071 Fatty acid degradation	61.90476	0.0934
bta00230 Purine metabolism	50	0.0934
bta04380 Osteoclast differentiation	50	0.0934
bta04668 TNF signaling pathway	50.84746	0.0944
bta05170 Human immunodeficiency virus 1 infection	46.5812	0.0944
bta05020 Prion diseases	65.625	0.0982
bta04720 Long-term potentiation	55.07246	0.1041
bta04911 Insulin secretion	52.94118	0.1061
bta05412 Arrhythmogenic right ventricular cardiomyopathy (ARVC)	53.94737	0.1061
bta04917 Prolactin signaling pathway	53.01205	0.1077
bta05414 Dilated cardiomyopathy (DCM)	51.51515	0.1077
bta01200 Carbon metabolism	50.44248	0.1084
bta04130 SNARE interactions in vesicular transport	63.63636	0.113
bta04710 Circadian rhythm	64.51613	0.1137

bta05221 Acute myeloid leukemia	54.41176	0.1147
bta05216 Thyroid cancer	60	0.1243
bta05410 Hypertrophic cardiomyopathy (HCM)	51.08696	0.1317
bta04142 Lysosome	48.48485	0.1336
bta00514 Other types of O-glycan biosynthesis	57.77778	0.1346
bta01040 Biosynthesis of unsaturated fatty acids	63.33333	0.1346
bta00650 Butanoate metabolism	64.28571	0.1359
bta04061 Viral protein interaction with cytokine and cytokine receptor	50.52632	0.1361
bta05217 Basal cell carcinoma	53.96825	0.1361
bta04146 Peroxisome	51.19048	0.1429
bta00310 Lysine degradation	53.0303	0.1471
bta05017 Spinocerebellar ataxia	50	0.1471
bta05142 Chagas disease (American trypanosomiasis)	48.69565	0.1491
bta05145 Toxoplasmosis	48.67257	0.1533
bta00062 Fatty acid elongation	62.06897	0.1556
bta04141 Protein processing in endoplasmic reticulum	46.38554	0.1557
bta04211 Longevity regulating pathway	50	0.1562
bta04971 Gastric acid secretion	51.31579	0.1562
bta00515 Mannose type O-glycan biosynthesis	65.21739	0.1571
bta00600 Sphingolipid metabolism	55.10204	0.1571
bta04064 NF-kappa B signaling pathway	48.62385	0.1571
bta05202 Transcriptional misregulation in cancer	45.54974	0.1571
bta04929 GnRH secretion	52.30769	0.1586
bta04215 Apoptosis	58.82353	0.1655
bta04724 Glutamatergic synapse	47.78761	0.1766
bta00564 Glycerophospholipid metabolism	48.07692	0.1818
bta01524 Platinum drug resistance	50	0.1818

bta04657 IL-17 signaling pathway	48.91304	0.1818
bta04930 Type II diabetes mellitus	54.34783	0.1846
bta04725 Cholinergic synapse	47.36842	0.1854
bta05031 Amphetamine addiction	50.72464	0.1854
bta01210 2-Oxocarboxylic acid metabolism	66.66667	0.1909
bta00280 Valine. leucine and isoleucine degradation	52.94118	0.1916
bta04923 Regulation of lipolysis in adipocytes	51.72414	0.1931
bta04927 Cortisol synthesis and secretion	50.76923	0.1931
bta00270 Cysteine and methionine metabolism	53.06122	0.1948
bta05164 Influenza A	44.75138	0.1963
bta05169 Epstein-Barr virus infection	43.85965	0.1963
bta04270 Vascular smooth muscle contraction	45.86466	0.2089
bta05032 Morphine addiction	47.82609	0.2089
bta04961 Endocrine and other factor-regulated calcium reabsorption	52	0.2153
bta00900 Terpenoid backbone biosynthesis	61.90476	0.216
bta00770 Pantothenate and CoA biosynthesis	63.15789	0.218
bta04713 Circadian entrainment	47	0.218
bta04914 Progesterone-mediated oocyte maturation	47.72727	0.218
bta03320 PPAR signaling pathway	48.14815	0.2195
bta04350 TGF-beta signaling pathway	47.31183	0.2195
bta04330 Notch signaling pathway	50.9434	0.2244
bta05162 Measles	44.73684	0.226
bta05321 Inflammatory bowel disease (IBD)	48.57143	0.2354
bta04915 Estrogen signaling pathway	44.92754	0.2361
bta01230 Biosynthesis of amino acids	47.94521	0.2462
bta04114 Oocyte meiosis	45.37815	0.2462
bta04137 Mitophagy	48.48485	0.2488

bta04210 Apoptosis	44.3662	0.2576
bta04727 GABAergic synapse	46.15385	0.2681
bta04925 Aldosterone synthesis and secretion	45.83333	0.2694
bta05014 Amyotrophic lateral sclerosis (ALS)	48.33333	0.2745
bta05222 Small cell lung cancer	45.74468	0.2764
bta00440 Phosphonate and phosphinate metabolism	83.33333	0.281
bta04340 Hedgehog signaling pathway	49.01961	0.2856
bta00250 Alanine. aspartate and glutamate metabolism	51.35135	0.2882
bta04672 Intestinal immune network for IgA production	48.21429	0.2897
bta00030 Pentose phosphate pathway	53.57143	0.292
bta00220 Arginine biosynthesis	57.89474	0.292
bta04640 Hematopoietic cell lineage	44.54545	0.292
bta04966 Collecting duct acid secretion	53.57143	0.292
bta00910 Nitrogen metabolism	58.82353	0.3024
bta00640 Propanoate metabolism	51.51515	0.3027
bta00052 Galactose metabolism	51.6129	0.3133
bta00512 Mucin type O-glycan biosynthesis	51.6129	0.3133
bta04924 Renin secretion	45.83333	0.3181
bta03450 Non-homologous end-joining	61.53846	0.3186
bta04964 Proximal tubule bicarbonate reclamation	54.54545	0.3193
bta04972 Pancreatic secretion	44.11765	0.3221
bta05152 Tuberculosis	41.91919	0.3241
bta04110 Cell cycle	43.08943	0.3408
bta04970 Salivary secretion	44.08602	0.3408
bta04726 Serotonergic synapse	43.10345	0.3481
bta05416 Viral myocarditis	44.73684	0.3481
bta04960 Aldosterone-regulated sodium reabsorption	48.64865	0.3493
bta00020 Citrate cycle (TCA cycle)	50	0.3542

bta00380 Tryptophan metabolism	46.80851	0.3586
bta04213 Longevity regulating pathway	45.16129	0.3664
bta05133 Pertussis	44.15584	0.3676
bta03420 Nucleotide excision repair	46.66667	0.3678
bta04216 Ferroptosis	46.66667	0.3678
bta04620 Toll-like receptor signaling pathway	42.72727	0.3706
bta00513 Various types of N-glycan biosynthesis	46.51163	0.3811
bta05030 Cocaine addiction	45.83333	0.3827
bta05134 Legionellosis	44.82759	0.3835
bta05140 Leishmaniasis	43.58974	0.3835
bta00534 Glycosaminoglycan biosynthesis	50	0.3896
bta05340 Primary immunodeficiency	46.34146	0.3896
bta00240 Pyrimidine metabolism	44.64286	0.3938
bta00592 alpha-Linolenic acid metabolism	48.27586	0.3939
bta00051 Fructose and mannose metabolism	47.05882	0.3968
bta00350 Tyrosine metabolism	46.15385	0.3968
bta00760 Nicotinate and nicotinamide metabolism	46.15385	0.3968
bta05146 Amoebiasis	41.88034	0.3968
bta00010 Glycolysis Gluconeogenesis	43.75	0.3973
bta04962 Vasopressin-regulated water reabsorption	44.89796	0.3973
bta00510 N-Glycan biosynthesis	44.23077	0.406
bta00565 Ether lipid metabolism	44.23077	0.406
bta03015 mRNA surveillance pathway	42.10526	0.406
bta03440 Homologous recombination	45.2381	0.406
bta04115 p53 signaling pathway	42.85714	0.406
bta04976 Bile secretion	42.16867	0.4242
bta00061 Fatty acid biosynthesis	50	0.4267
bta00072 Synthesis and degradation of ketone bodies	54.54545	0.4348

bta03013 RNA transport	39.88764	0.4572
bta03030 DNA replication	44.44444	0.4572
bta04145 Phagosome	40	0.4572
bta00561 Glycerolipid metabolism	41.79104	0.4689
bta04623 Cytosolic DNA-sensing pathway	41.79104	0.4689
bta03020 RNA polymerase	44.82759	0.4739
bta04913 Ovarian steroidogenesis	42.10526	0.4739
bta05323 Rheumatoid arthritis	40.38462	0.4845
bta00500 Starch and sucrose metabolism	43.75	0.4896
bta04978 Mineral absorption	41.81818	0.4896
bta04744 Phototransduction	44.44444	0.4909
bta00531 Glycosaminoglycan degradation	45.45455	0.4912
bta00330 Arginine and proline metabolism	41.66667	0.5074
bta00630 Glyoxylate and dicarboxylate metabolism	43.33333	0.5074
bta00532 Glycosaminoglycan biosynthesis	45	0.5154
bta04060 Cytokine-cytokine receptor interaction	38.39009	0.5166
bta03410 Base excision repair	42.42424	0.5215
bta00340 Histidine metabolism	43.47826	0.5346
bta00591 Linoleic acid metabolism	41.66667	0.5358
bta00670 One carbon pool by folate	44.44444	0.5366
bta04621 NOD-like receptor signaling pathway	38.58696	0.5366
bta04650 Natural killer cell mediated cytotoxicity	38.9313	0.5366
bta04512 ECM-receptor interaction	39.32584	0.5433
bta04950 Maturity onset diabetes of the young	42.30769	0.5435
bta03018 RNA degradation	39.24051	0.5559
bta00601 Glycosphingolipid biosynthesis	41.37931	0.5587
bta04975 Fat digestion and absorption	39.58333	0.5787
bta01523 Antifolate resistance	39.53488	0.5846

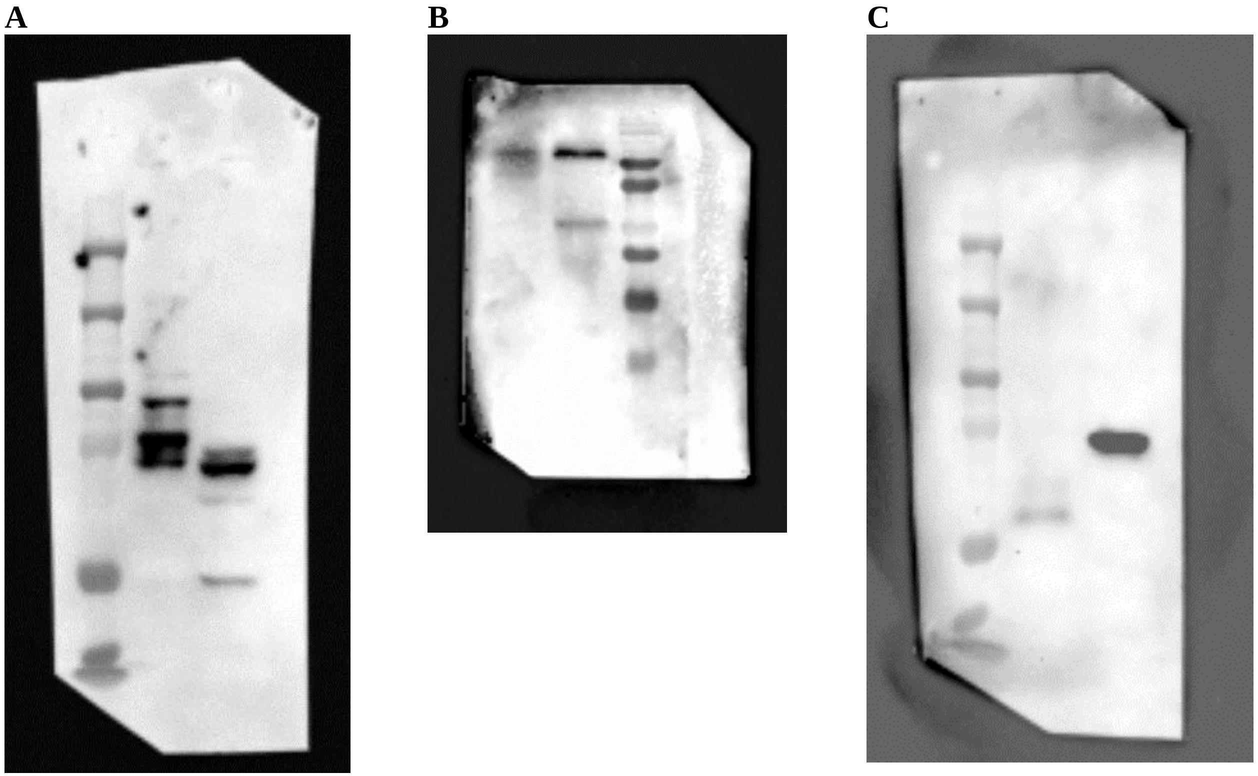
bta04932 Non-alcoholic fatty liver disease (NAFLD)	37.97468	0.5846
bta05144 Malaria	38.98305	0.5846
bta00480 Glutathione metabolism	38.33333	0.6162
bta03460 Fanconi anemia pathway	38.46154	0.6163
bta04973 Carbohydrate digestion and absorption	38.63636	0.6165
bta04723 Retrograde endocannabinoid signaling	37.5	0.618
bta00100 Steroid biosynthesis	40	0.6209
bta04918 Thyroid hormone synthesis	37.83784	0.6209
bta00520 Amino sugar and nucleotide sugar metabolism	38	0.6293
bta00360 Phenylalanine metabolism	39.13043	0.6321
bta00563 Glycosylphosphatidylinositol (GPI)-anchor biosynthesis	38.46154	0.6412
bta04614 Renin-angiotensin system	38.46154	0.6412
bta05033 Nicotine addiction	37.5	0.6531
bta00260 Glycine. serine and threonine metabolism	37.2093	0.6606
bta04630 JAK-STAT signaling pathway	36.63366	0.6691
bta00430 Taurine and hypotaurine metabolism	38.46154	0.6778
bta00604 Glycosphingolipid biosynthesis	37.5	0.6851
bta03022 Basal transcription factors	36.36364	0.6851
bta05143 African trypanosomiasis	36.36364	0.6851
bta00511 Other glycan degradation	36.36364	0.6986
bta00983 Drug metabolism	35.52632	0.72
bta04714 Thermogenesis	35.98326	0.72
bta00603 Glycosphingolipid biosynthesis	35.29412	0.7289
bta03430 Mismatch repair	34.78261	0.7368
bta04977 Vitamin digestion and absorption	34.61538	0.7393
bta00620 Pyruvate metabolism	34.21053	0.7531
bta05310 Asthma	34.21053	0.7531

bta00140 Steroid hormone biosynthesis	34.32836	0.7641
bta00730 Thiamine metabolism	33.33333	0.7652
bta04136 Autophagy	33.33333	0.7709
bta04940 Type I diabetes mellitus	33.89831	0.7709
bta00790 Folate biosynthesis	33.33333	0.771
bta05332 Graft-versus-host disease	33.33333	0.7803
bta05204 Chemical carcinogenesis	33.76623	0.7833
bta03008 Ribosome biogenesis in eukaryotes	33.73494	0.7869
bta00040 Pentose and glucuronate interconversions	32.25806	0.7903
bta04610 Complement and coagulation cascades	33.69565	0.7903
bta04612 Antigen processing and presentation	32.94118	0.8209
bta00120 Primary bile acid biosynthesis	29.41176	0.8342
bta00982 Drug metabolism	31.74603	0.836
bta03040 Spliceosome	33.33333	0.836
bta04260 Cardiac muscle contraction	32.58427	0.836
bta05206 MicroRNAs in cancer	34.24658	0.836
bta04217 Necroptosis	33.33333	0.8465
bta00450 Selenocompound metabolism	27.77778	0.8481
bta00590 Arachidonic acid metabolism	31.70732	0.8481
bta00860 Porphyrin and chlorophyll metabolism	30	0.8481
bta00980 Metabolism of xenobiotics by cytochrome P450	31.34328	0.8481
bta00053 Ascorbate and aldarate metabolism	28	0.8567
bta04979 Cholesterol metabolism	30	0.8567
bta05330 Allograft rejection	30.35714	0.8567
bta05168 Herpes simplex virus 1 infection	33.99504	0.8643
bta04080 Neuroactive ligand-receptor interaction	33.60882	0.8801
bta05203 Viral carcinogenesis	32.78008	0.8865
bta04742 Taste transduction	29.11392	0.9183

bta05010 Alzheimer disease	31.11111	0.9287
bta05150 Staphylococcus aureus infection	29.52381	0.9287
bta05016 Huntington disease	31.75182	0.9422
bta00830 Retinol metabolism	26.5625	0.9453
bta03060 Protein export	21.73913	0.9453
bta04622 RIG-I-like receptor signaling pathway	28.43137	0.9453
bta05320 Autoimmune thyroid disease	26.76056	0.9475
bta03050 Proteasome	21.73913	0.9871
bta00190 Oxidative phosphorylation	20.71429	1.0
bta00970 Aminoacyl-tRNA biosynthesis	18.18182	1.0
bta02010 ABC transporters	20	1.0
bta03010 Ribosome	13.125	1.0
bta04740 Olfactory transduction	4.89083	1.0
bta04974 Protein digestion and absorption	20.66116	1.0
bta05012 Parkinson disease	22.66667	1.0
bta05034 Alcoholism	24.89083	1.0
bta05322 Systemic lupus erythematosus	15.93407	1.0

[1] %: Percent of genes predicted to be modulated. [2] BH: Benjamini – Hochberg

Appendix B – Supplementary material of chapter 3



Supplementary Figure 1. Original images of Western blot technique for oviductal flushing extracellular vesicles characterization from cows with different body energy reserve. **A.** ALIX: ~95KDa. **B.** CD9: ~25KDa. **C.** GRP78: ~75KDa.

Supplementary Table 1. Raw cycle threshold levels of the 383 miRNAs profile in ampullary extracellular vesicles (AMP-EVs) of cows with different body energy reserve.

miRNA	Body energy reserve[1]					
	MBER			HBER		
	1	2	3	1	2	3
bta-let-7a-3p	.	.	.	.	.	.
bta-miR-103	.	.	34.518	36.761	33.676	.
bta-let-7a-5p	.	.	34.751	35.297	.	35.264
bta-miR-105a	.	.	.	35.080	32.600	.
bta-let-7b	34.632	34.202	32.937	33.431	.	32.930
bta-miR-105b	.	35.565	.	.	30.540	32.957
bta-let-7c	34.790	36.019	32.699	32.809	34.623	35.017
bta-miR-106a	.	34.985	35.851	35.357	32.993	35.940
bta-let-7d	.	.	.	33.883	.	.
bta-miR-106b	.	34.129	.	.	33.942	.
bta-let-7e	35.329	33.640	34.926	33.393	30.727	33.186
bta-miR-107	.	.	.	.	34.961	.
bta-let-7f	.	34.687	36.295	.	.	.
bta-miR-10a	.	34.762	.	33.858	30.475	33.736
bta-let-7g	.	.	.	.	.	34.903
bta-miR-10b	.	.	.	.	32.743	32.906
bta-let-7i	.	.	.	.	34.529	.
bta-miR-122	.	.	.	.	32.729	.
bta-miR-1	.	.	.	33.554	.	.
bta-miR-124a	33.884	.	.	.	33.742	.
bta-miR-100	.	33.772	34.756	.	33.779	.
bta-miR-124b	32.678	36.117	.	35.023	33.607	.

bta-miR-101	.	34.564	.	.	.	.
bta-miR-125a	.	32.708	.	.	30.781	33.541
bta-miR-125b	.	33.864	34.422	33.907	.	.
bta-miR-133b	.	.	.	.	34.662	.
bta-miR-126-3p	32.809	31.971	35.146	32.834	28.605	31.443
bta-miR-133c	33.906	33.865	35.011	.	29.726	33.203
bta-miR-126-5p	36.018	30.753	33.542	36.788	28.562	30.509
bta-miR-134	.	31.277	38.317	32.777	28.111	30.157
bta-miR-127	34.320	33.201	33.627	34.885	30.837	33.986
bta-miR-135a	.	36.908	38.325	.	33.855	.
bta-miR-128	.	.	.	.	.	.
bta-miR-135b	.	.	.	.	28.941	.
bta-miR-129	.	.	36.358	.	35.028	35.715
bta-miR-136	.	.	.	.	36.421	.
bta-miR-129-3p	34.812	32.888	33.547	.	33.598	35.347
bta-miR-137	.	.	.	.	35.272	.
bta-miR-129-5p	34.596	34.041	34.093	33.895	32.798	35.052
bta-miR-138	.	27.830	.	30.972	26.587	28.720
bta-miR-130a	33.872	33.970	33.623	35.025	32.912	33.231
bta-miR-139	.	31.929	34.402	32.300	30.733	33.900
bta-miR-130b	32.559	33.756	33.328	33.750	31.402	32.405
bta-miR-140	.	33.941	.	.	31.731	33.088
bta-miR-132	29.716	29.560	29.695	28.014	27.314	28.714
bta-miR-141	.	39.979	34.637	33.199	31.548	32.648
bta-miR-133a	.	31.881	32.788	39.090	27.951	30.797
bta-miR-142-3p	.	35.518	.	.	32.677	34.256

bta-miR-142-5p	.	.	.	.	32.733	37.078
bta-miR-151-3p	.	34.106	34.480	.	30.729	34.091
bta-miR-143	31.694	33.880	30.784	32.027	29.712	30.962
bta-miR-151-5p	.	34.498	35.275	.	34.965	.
bta-miR-144	.	.	.	.	31.463	34.269
bta-miR-152	34.451	32.912	.	.	28.908	31.946
bta-miR-145	.	32.509	32.644	36.472	29.280	31.860
bta-miR-153	.	.	.	.	.	.
bta-miR-146a	.	.	.	.	32.533	.
bta-miR-154a	.	32.610	31.097	34.090	28.698	31.404
bta-miR-146b	35.090	.	.	.	32.316	34.639
bta-miR-154b	33.962	28.671	29.749	32.834	26.066	28.890
bta-miR-147	.	.	.	.	.	.
bta-miR-154c	.	34.182	.	.	31.365	33.571
bta-miR-148a	.	34.769	34.239	.	.	.
bta-miR-155	.	33.973	34.289	37.831	32.094	34.951
bta-miR-148b	.	36.953	33.938	.	.	.
bta-miR-15a	.	.	34.719	.	34.810	.
bta-miR-149-3p	30.392	30.528	30.585	32.399	28.617	31.402
bta-miR-15b	.	.	.	.	.	.
bta-miR-149-5p	.	32.538	32.571	33.898	28.556	30.995
bta-miR-16a	.	32.928	33.807	.	31.158	34.747
bta-miR-150	.	33.188	33.401	36.229	29.754	32.898
bta-miR-16b	.	32.324	32.825	34.973	29.709	32.185
bta-miR-17-3p	.	.	.	.	.	.
bta-miR-188	35.693	35.103	34.003	33.905	32.407	33.910

bta-miR-17-5p	.	.	34.757	35.774	31.878	.
bta-miR-18a	.	.	.	.	35.337	.
bta-miR-181a	.	.	.	.	.	.
bta-miR-18b	.	.	.	.	.	.
bta-miR-181b	.	34.102	34.090	33.974	31.618	.
bta-miR-190a	.	36.050	.	.	33.984	.
bta-miR-181c	.	.	.	.	.	.
bta-miR-190b	.	33.857	35.140	33.929	33.893	.
bta-miR-181d	.	31.800	32.887	33.840	29.492	32.177
bta-miR-191	35.361	31.593	31.861	31.185	29.095	30.597
bta-miR-182	.	36.843	36.750	.	31.094	33.962
bta-miR-192	35.904	34.097	34.809	38.528	32.949	33.507
bta-miR-183	.	33.580	35.110	.	30.244	32.651
bta-miR-193a	.	.	.	.	35.995	.
bta-miR-184	.	33.531	34.727	.	30.222	33.770
bta-miR-193a-3p	.	.	38.056	.	.	.
bta-miR-185	.	.	.	.	33.972	.
bta-miR-193a-5p	.	34.095	33.888	31.866	32.194	34.729
bta-miR-186	.	33.348	34.705	.	29.625	33.876
bta-miR-193b	.	.	35.586	38.128	.	.
bta-miR-187	34.646	32.549	31.584	33.449	29.652	32.872
bta-miR-194	.	.	.	.	34.933	.
bta-miR-195	.	34.738	33.253	.	31.690	.
bta-miR-200c	.	32.946	32.412	33.148	31.071	31.490
bta-miR-196a	35.362	34.114	32.431	33.436	29.029	30.860
bta-miR-202	.	.	.	.	34.386	.

bta-miR-196b	35.467	33.675	33.923	34.912	31.739	34.601
bta-miR-204	.	34.996	33.093	34.941	29.013	32.000
bta-miR-197	32.567	30.683	31.110	33.945	27.747	30.743
bta-miR-205	.	33.749	32.887	36.855	29.355	31.845
bta-miR-199a-3p	.	.	33.591	34.772	.	.
bta-miR-206	.	31.123	33.343	.	28.055	30.710
bta-miR-199a-5p	.	.	.	.	.	.
bta-miR-208a	.	.	.	.	35.597	.
bta-miR-199b	.	.	.	.	33.931	34.307
bta-miR-208b	.	33.219	.	.	.	.
bta-miR-199c	.	34.152	32.568	33.815	29.823	31.933
bta-miR-20a	.	32.590	33.731	34.376	30.673	33.131
bta-miR-19a	.	.	.	36.619	32.003	34.667
bta-miR-20b	.	.	35.438	.	32.885	35.963
bta-miR-19b	.	36.717	.	.	33.277	35.839
bta-miR-21-3p	.	.	.	.	34.875	.
bta-miR-200a	.	36.919	34.778	.	.	.
bta-miR-21-5p	.	.	.	.	33.986	.
bta-miR-200b	32.223	31.572	29.893	29.276	31.022	30.984
bta-miR-210	35.530	34.741	34.564	32.655	31.713	.
bta-miR-211	.	32.115	31.463	33.708	29.750	31.878
bta-miR-22-5p	36.890	.	34.284	34.022	.	35.219
bta-miR-212	34.781	34.989	.	.	31.651	33.783
bta-miR-221	.	.	35.060	35.213	29.682	.
bta-miR-214	32.729	36.796	34.230	35.600	32.539	.
bta-miR-222	34.237	32.668	33.787	32.882	30.300	32.856

bta-miR-215	.	33.415	33.115	.	32.965	.
bta-miR-223	.	34.381	32.058	.	30.180	34.721
bta-miR-216a	.	.	.	35.238	38.024	.
bta-miR-224	.	.	.	.	.	35.260
bta-miR-216b	.	.	34.539	.	34.164	.
bta-miR-23a	35.373	32.240	30.806	31.137	32.236	32.729
bta-miR-217	.	.	.	36.704	.	.
bta-miR-23b-3p	.	34.087	32.462	34.386	30.677	34.619
bta-miR-218	.	34.053	.	.	32.294	36.501
bta-miR-23b-5p	.	34.976	.	34.466	33.068	34.320
bta-miR-219	35.825	31.870	31.530	32.779	28.649	30.540
bta-miR-24	.	.	.	.	34.979	.
bta-miR-219-3p	.	34.834	34.864	35.272	31.839	33.848
bta-miR-24-3p	.	34.437	.	29.709	.	33.971
bta-miR-219-5p	.	.	.	.	.	.
bta-miR-25	.	33.335	34.567	35.430	33.846	.
bta-miR-22-3p	2.823	2.682	2.766	2.810	2.679	2.393
bta-miR-26a	.	32.832	30.825	31.558	29.076	30.261
bta-miR-26b	.	35.845	.	33.859	32.779	.
bta-miR-29d-3p	.	.	.	35.871	.	.
bta-miR-26c	.	.	.	.	.	.
bta-miR-29d-5p	.	35.334	35.060	.	.	.
bta-miR-27a-3p	.	31.744	30.704	32.107	28.431	30.156
bta-miR-29e	.	.	.	.	.	.
bta-miR-27a-5p	8.599	8.632	8.796	8.373	8.489	8.489
bta-miR-301a	.	33.694	38.287	.	34.381	.

bta-miR-27b	.	.	33.220	.	32.815	34.369
bta-miR-301b	.	39.946	.	.	.	.
bta-miR-28	.	.	34.334	.	.	.
bta-miR-302a	.	.	.	.	33.745	.
bta-miR-296-3p	33.682	31.755	30.402	38.177	30.638	35.254
bta-miR-302b	.	34.651	.	.	33.799	.
bta-miR-296-5p	.	32.384	31.797	33.059	28.886	32.743
bta-miR-302c	.	33.970	33.125	.	30.383	33.602
bta-miR-299	.	35.043	.	.	31.876	33.650
bta-miR-302d	.	.	.	.	.	.
bta-miR-29a	34.439	34.195	34.838	30.683	34.928	33.792
bta-miR-3064	.	34.333	.	.	32.645	44.188
bta-miR-29b	.	.	.	.	33.723	.
bta-miR-30a-5p	.	32.717	33.732	34.674	31.440	33.496
bta-miR-29c	34.590	36.829	35.026	32.695	35.435	.
bta-miR-30b-3p	35.722	.	.	.	.	.
bta-miR-30b-5p	.	31.214	35.043	43.045	30.817	34.700
bta-miR-328	30.869	29.200	30.608	35.022	29.259	31.623
bta-miR-30c	.	33.031	32.816	.	29.721	32.161
bta-miR-329a	.	.	.	.	.	.
bta-miR-30d	.	32.829	34.145	36.060	31.156	.
bta-miR-329b	.	.	.	.	.	.
bta-miR-30e-5p	34.184	36.391	33.854	38.028	31.908	33.158
bta-miR-330	.	36.537	33.625	36.068	32.705	.
bta-miR-30f	.	32.793	33.576	.	29.885	32.112
bta-miR-331-3p	.	.	.	.	35.560	.

bta-miR-31	35.062	32.330	32.574	35.623	32.746	.
bta-miR-331-5p	34.364	31.107	33.186	35.655	29.065	32.858
bta-miR-32	.	.	.	.	36.281	42.902
bta-miR-335	.	33.797	35.109	.	32.865	36.335
bta-miR-320a	30.649	30.113	30.799	29.176	30.712	32.180
bta-miR-338	.	.	.	.	.	.
bta-miR-320b	33.929	32.810	33.063	33.680	30.256	31.930
bta-miR-339a	.	36.786	.	.	30.383	33.624
bta-miR-323	19.181	18.600	19.330	18.984	18.811	19.004
bta-miR-339b	.	31.239	31.743	32.867	28.721	32.854
bta-miR-324	.	32.585	32.024	35.473	29.676	33.667
bta-miR-33a	.	34.938	34.186	36.925	.	.
bta-miR-326	.	36.577	34.473	.	32.167	36.784
bta-miR-33b	35.327	.	36.042	.	36.035	36.724
bta-miR-340	.	32.152	31.527	.	28.964	34.189
bta-miR-365-3p	.	.	.	.	35.726	.
bta-miR-342	.	31.364	32.643	35.056	29.617	34.491
bta-miR-365-5p	.	33.532	36.340	33.306	30.597	34.215
bta-miR-345-3p	.	.	34.240	34.100	33.930	.
bta-miR-367	.	.	.	36.864	.	.
bta-miR-345-5p	34.100	33.457	32.945	34.193	30.762	32.618
bta-miR-369-3p	.	.	.	.	.	.
bta-miR-346	.	31.858	32.800	31.833	30.373	33.459
bta-miR-369-5p	.	34.343	33.523	.	29.791	32.440
bta-miR-34a	.	33.341	.	.	32.882	.
bta-miR-370	35.330	32.018	34.650	34.816	32.127	35.014

bta-miR-34b	.	34.776	.	.	33.481	.
bta-miR-371	.	34.451	.	.	31.637	.
bta-miR-34c	.	.	34.809	.	34.938	.
bta-miR-374a	.	.	.	35.620	35.830	.
bta-miR-361	.	.	34.877	36.962	35.669	.
bta-miR-374b	.	34.941	35.533	34.851	33.299	34.408
bta-miR-362-3p	.	.	.	.	.	.
bta-miR-375	34.110	33.401	31.936	32.485	27.805	34.220
bta-miR-362-5p	.	.	34.980	.	32.940	.
bta-miR-376a	.	33.790	.	.	30.122	33.050
bta-miR-363	.	.	34.801	.	32.561	33.597
bta-miR-376b	.	.	.	.	.	.
bta-miR-376c	.	.	.	.	.	.
bta-miR-382	33.637	30.578	30.409	33.798	28.176	31.447
bta-miR-376d	.	33.771	34.470	.	31.843	.
bta-miR-383	.	33.847	31.886	38.380	29.533	32.895
bta-miR-376e	.	.	.	.	34.870	.
bta-miR-409a	37.538	35.430	35.388	.	33.540	.
bta-miR-377	.	.	.	.	.	36.175
bta-miR-409b	.	33.078	.	.	30.729	35.215
bta-miR-378	.	32.781	31.821	33.560	28.483	33.669
bta-miR-410	.	34.352	34.072	.	32.887	.
bta-miR-378b	34.250	31.997	31.924	.	28.652	31.842
bta-miR-411a	33.323	31.613	30.634	33.638	28.168	30.897
bta-miR-378c	36.738	36.238	33.426	.	34.139	.
bta-miR-411b	34.200	32.872	31.547	33.710	30.386	34.346

bta-miR-378d	33.996	32.417	32.316	34.915	29.740	31.571
bta-miR-411c-3p	.	.	.	.	36.054	.
bta-miR-379	.	.	.	.	35.395	.
bta-miR-411c-5p	.	.	.	.	36.877	.
bta-miR-380-3p	.	.	.	.	31.058	.
bta-miR-412	.	34.581	.	.	32.210	.
bta-miR-380-5p	31.810	31.800	29.693	33.849	27.925	30.749
bta-miR-421	36.488	31.866	32.158	32.658	28.586	31.757
bta-miR-381	.	.	.	.	.	.
bta-miR-423-3p	.	36.066	33.736	34.106	34.183	.
bta-miR-423-5p	.	.	.	34.977	35.532	.
bta-miR-449c	.	33.190	34.393	35.607	31.739	33.803
bta-miR-424-3p	.	.	.	.	.	.
bta-miR-449d	.	33.107	32.767	33.384	31.749	33.686
bta-miR-424-5p	.	.	.	33.532	.	.
bta-miR-450a	.	.	.	.	.	.
bta-miR-425-3p	32.793	31.796	30.714	30.752	28.890	31.219
bta-miR-450b	.	.	36.498	.	.	.
bta-miR-425-5p	.	.	34.875	34.549	35.365	.
bta-miR-451	.	33.261	.	32.664	.	.
bta-miR-429	30.733	31.330	29.953	31.620	28.882	30.780
bta-miR-452	.	.	33.070	.	31.613	34.539
bta-miR-431	.	.	36.117	34.627	33.817	.
bta-miR-4523	.	32.482	29.862	34.823	31.866	36.992
bta-miR-432	33.855	30.780	30.588	36.427	28.130	30.258
bta-miR-453	35.348	34.947	35.474	34.669	32.819	36.060

bta-miR-433	33.232	29.087	28.215	31.149	26.494	29.849
bta-miR-454	.	31.582	31.204	.	23.887	32.807
bta-miR-448	.	.	.	.	33.740	.
bta-miR-455-3p	.	32.872	32.841	.	29.684	33.954
bta-miR-449a	34.088	35.318	.	.	33.919	.
bta-miR-455-5p	.	.	.	.	34.240	.
bta-miR-449b	.	35.995	35.009	.	35.641	.
bta-miR-483	.	.	.	.	32.517	.
bta-miR-484	.	.	.	.	34.851	.
bta-miR-496	35.496	.	34.547	.	30.935	.
bta-miR-485	.	.	34.549	34.562	32.739	36.730
bta-miR-497	.	.	.	.	36.494	34.507
bta-miR-486	35.856	33.890	35.702	31.922	31.354	34.315
bta-miR-499	.	.	.	.	.	.
bta-miR-487a	.	.	34.945	.	33.235	.
bta-miR-500	33.934	36.710	34.000	32.257	35.486	35.591
bta-miR-487b	.	35.579	35.879	.	33.246	34.732
bta-miR-502a	.	.	.	.	33.900	.
bta-miR-488	.	32.888	33.530	.	30.717	34.964
bta-miR-502b	33.980	32.034	32.262	.	31.891	33.590
bta-miR-489	33.877	33.146	33.276	33.896	32.981	33.381
bta-miR-503-3p	34.723	31.661	31.091	33.310	28.727	31.622
bta-miR-490	.	34.365	33.511	.	30.758	.
bta-miR-503-5p	8.329	34.852	34.368	33.872	33.386	.
bta-miR-491	.	34.146	33.789	34.889	30.201	32.919
bta-miR-504	.	.	34.851	.	34.400	.

bta-miR-493	.	31.725	31.840	33.591	30.514	31.754
bta-miR-505	34.398	32.924	32.553	36.508	30.624	.
bta-miR-494	28.972	27.123	27.790	29.456	28.938	30.083
bta-miR-532	36.666	32.197	31.438	.	29.507	32.040
bta-miR-495	.	.	.	.	.	.
bta-miR-539	.	33.263	34.583	.	30.611	.
bta-miR-541	33.191	31.179	31.098	31.128	28.791	31.756
bta-miR-582	.	.	.	.	.	.
bta-miR-542-5p	.	34.399	.	.	31.742	.
bta-miR-584	28.706	28.825	27.752	25.796	29.385	28.652
bta-miR-543	.	.	.	.	.	.
bta-miR-592	.	36.846	.	.	32.947	.
bta-miR-544a	.	.	.	.	.	.
bta-miR-599	.	.	.	.	.	.
bta-miR-544b	.	.	.	.	.	.
bta-miR-615	12.365	12.258	12.393	12.197	12.459	12.531
bta-miR-545-3p	.	.	.	.	.	.
bta-miR-628	35.184	31.803	31.220	.	29.181	33.615
bta-miR-545-5p	.	34.393	.	.	35.390	.
bta-miR-631	20.314	19.721	20.427	20.540	19.749	20.220
bta-miR-551a	.	35.301	.	.	33.608	34.681
bta-miR-652	.	.	36.886	35.497	31.730	39.695
bta-miR-551b	.	.	.	35.046	33.164	.
bta-miR-653	.	.	.	.	33.046	.
bta-miR-562	.	.	.	.	.	.
bta-miR-654	.	.	.	34.714	31.859	37.725

bta-miR-568	.	.	.	.	.	.
bta-miR-655	.	34.890	32.877	.	30.810	34.911
bta-miR-574	32.918	30.661	29.318	27.965	30.375	32.000
bta-miR-656	35.891	31.834	30.824	34.303	29.280	32.817
bta-miR-658	.	.	36.969	33.987	31.670	35.942
bta-miR-758	.	33.444	33.478	.	32.245	35.743
bta-miR-660	.	32.795	31.778	.	29.214	32.834
bta-miR-759	.	.	.	.	.	.
bta-miR-664a	33.899	34.488	34.366	32.957	32.393	33.635
bta-miR-760-3p	34.233	34.958	33.355	31.791	32.448	33.917
bta-miR-664b	34.797	34.890	34.017	32.798	32.422	.
bta-miR-760-5p	33.865	30.813	29.752	33.512	28.016	30.881
bta-miR-665	.	34.901	33.942	32.358	31.380	33.469
bta-miR-761	.	.	34.115	36.049	35.886	.
bta-miR-669	.	32.149	31.530	34.211	30.799	.
bta-miR-763	.	31.824	32.124	34.913	30.597	33.951
bta-miR-670	.	32.922	34.274	.	33.633	34.925
bta-miR-764	.	32.948	35.039	.	31.727	.
bta-miR-671	.	.	36.110	.	.	.
bta-miR-767	33.929	33.170	32.071	34.002	32.519	34.099
bta-miR-677	.	36.714	35.609	43.593	.	36.388
bta-miR-769	36.128	33.614	33.226	.	30.811	.
bta-miR-7	.	.	.	.	.	.
bta-miR-873	.	32.369	32.899	.	30.957	33.813
bta-miR-708	34.596	31.987	31.805	36.149	30.657	32.899
bta-miR-874	33.127	33.544	32.950	31.781	34.826	.

bta-miR-744	38.312	.	33.107	39.681	32.855	36.974
bta-miR-875	.	35.406	35.484	.	32.326	.
bta-miR-876	.	.	33.551	.	33.807	.
bta-miR-98	.	.	34.604	.	.	.
bta-miR-877	31.907	31.066	30.680	30.845	30.515	31.880
bta-miR-99a-3p	.	31.817	30.612	33.633	30.175	33.553
bta-miR-885	.	31.090	30.709	31.599	29.550	32.504
bta-miR-99a-5p	.	34.616	32.201	33.811	33.782	36.117
bta-miR-9-3p	.	.	36.824	.	.	.
bta-miR-99b	23.718	23.178	23.734	24.117	23.310	23.718
bta-miR-9-5p	34.481	33.938	32.573	35.125	31.872	34.317
bta-miR-1179	.	36.699	32.979	.	31.780	34.051
bta-miR-92a	34.996	31.881	30.770	33.697	31.175	.
bta-miR-1185	.	.	.	.	.	.
bta-miR-92b	31.773	29.291	29.156	30.845	28.447	30.085
bta-miR-1193	.	.	.	.	.	.
bta-miR-93	34.769	33.808	32.861	32.673	34.493	34.805
bta-miR-1197	.	34.306	32.950	.	33.452	34.881
bta-miR-935	.	32.886	31.508	34.026	29.793	32.328
bta-miR-122	.	34.587	.	.	.	.
bta-miR-940	32.009	30.705	31.479	29.604	31.829	32.893
bta-miR-1224	24.900	25.713	27.910	29.959	29.310	30.647
bta-miR-95	.	.	33.438	.	32.925	.
bta-miR-1225-3p	33.011	32.420	31.850	31.898	31.047	32.988
bta-miR-96	.	.	.	.	.	.
bta-miR-1246	23.722	24.838	26.603	25.687	27.098	28.811

bta-miR-1247-3p	32.742	31.118	28.761	29.821	28.145	30.858
bta-miR-1296	.	33.039	30.471	36.794	29.795	33.482
bta-miR-1247-5p	32.937	32.258	31.801	31.993	32.896	35.039
bta-miR-1298	.	34.731	.	.	.	.
bta-miR-1248	.	.	.	.	35.369	.
bta-miR-1301	.	34.466	35.030	34.912	37.031	.
bta-miR-1249	.	33.911	35.021	.	32.989	.
bta-miR-1306	.	31.871	30.615	35.299	29.533	32.304
bta-miR-1260b	27.401	25.752	23.817	26.847	27.211	27.710
bta-miR-1307	34.822	32.335	31.669	32.587	31.158	33.927
bta-miR-1271	.	.	.	.	32.903	.
bta-miR-1343-3p	32.661	31.478	29.529	32.661	30.365	32.447
bta-miR-1277	.	.	.	.	.	.
bta-miR-1343-5p	31.741	31.325	31.284	30.624	30.797	33.935
bta-miR-1281	30.822	31.613	29.405	30.435	28.264	31.439
bta-miR-1388-3p	32.396	33.790	31.570	34.049	31.379	33.982
bta-miR-1282	.	.	36.964	35.906	.	.
RNT43 snoRNA	36.013	.	32.870	30.826	32.828	.
bta-miR-1284	.	.	.	.	33.310	.
Hm/Ms/Rt T1 snRNA	22.121	23.108	24.833	24.953	24.086	27.795
bta-miR-1287	35.394	33.569	33.108	33.396	32.945	36.599
bta-miR-99b	23.738	22.997	23.288	23.916	23.135	23.751
bta-miR-1291	.	35.553	32.033	35.101	.	.
Negative control	.	.	.	.	.	.

[1] Body energy reserve: MBER: Cows with moderated body energy reserve; HBER: Cows with high body energy reserve.

Supplementary Table 2. Raw cycle threshold levels of the 383 miRNAs profile in isthmic extracellular vesicles (IST-EVs) of cows with different body energy reserve.

miRNA	**Body energy reserve**[1]					
	MBER			**HBER**		
	1	**2**	**3**	**1**	**2**	**3**
bta-let-7a-3p	30.912	.	.	.	.	.
bta-miR-103	34.695	34.376	32.239	36.857	32.349	34.130
bta-let-7a-5p	29.729	30.657	29.096	30.277	30.139	31.654
bta-miR-105a	.	34.168	.	.	38.184	.
bta-let-7b	30.701	31.168	29.565	29.822	29.711	32.994
bta-miR-105b	35.809	36.879	33.952	.	33.951	.
bta-let-7c	29.545	29.777	29.345	29.695	.	31.467
bta-miR-106a	.	34.753	32.686	34.743	32.941	35.383
bta-let-7d	30.784	32.269	31.683	33.311	.	34.876
bta-miR-106b	32.899	31.484	33.108	31.789	32.746	33.322
bta-let-7e	29.193	30.203	29.262	30.011	31.875	31.728
bta-miR-107	.	.	.	.	.	.
bta-let-7f	30.925	32.137	30.481	33.456	31.259	34.192
bta-miR-10a	34.560	34.911	31.090	32.899	31.893	33.817
bta-let-7g	35.953	34.366	32.417	.	32.868	.
bta-miR-10b	33.910	36.377	32.862	.	31.194	34.382
bta-let-7i	.	32.861	32.842	36.339	31.982	.
bta-miR-122	.	34.927	.	35.472	.	.
bta-miR-1	31.381	30.412	25.670	33.345	28.407	30.427
bta-miR-124a	33.134	32.259	32.713	32.818	33.430	32.244
bta-miR-100	30.732	33.559	30.750	31.874	31.286	34.945
bta-miR-124b	31.935	32.089	32.832	33.840	32.927	32.530

bta-miR-101	.	34.438	31.036	.	32.397	34.782
bta-miR-125a	33.828	34.730	32.908	33.883	32.209	.
bta-miR-125b	30.839	30.711	29.306	31.847	28.407	32.690
bta-miR-133b	35.110	34.110	31.440	.	32.356	34.896
bta-miR-126-3p	32.327	30.676	28.736	31.532	28.063	32.289
bta-miR-133c	.	35.506	.	34.290	35.054	.
bta-miR-126-5p	33.241	32.046	29.562	32.205	29.383	32.929
bta-miR-134	34.045	33.503	36.551	32.766	35.060	33.456
bta-miR-127	30.698	29.990	30.832	31.253	30.471	30.777
bta-miR-135a	33.087	36.448	35.600	33.547	33.241	.
bta-miR-128	.	34.320	.	.	.	34.672
bta-miR-135b	36.649	.	.	35.694	35.661	.
bta-miR-129	32.882	31.593	33.799	31.468	33.966	31.858
bta-miR-136	.	.	.	.	.	.
bta-miR-129-3p	.	.	.	35.504	.	.
bta-miR-137	.	.	.	.	.	36.088
bta-miR-129-5p	32.862	31.351	35.156	30.795	32.851	32.876
bta-miR-138	33.688	33.884	35.629	32.914	34.689	33.930
bta-miR-130a	30.892	31.074	31.505	30.870	30.800	30.712
bta-miR-139	30.724	28.718	31.915	29.816	31.903	31.036
bta-miR-130b	30.586	30.537	30.445	30.574	30.292	30.683
bta-miR-140	.	35.341	.	33.952	36.051	.
bta-miR-132	31.482	29.091	31.824	29.167	30.841	31.505
bta-miR-141	32.845	33.491	.	33.882	34.175	36.953
bta-miR-133a	31.838	31.478	26.602	34.220	28.585	30.747
bta-miR-142-3p	34.903	.	.	.	38.475	.

bta-miR-142-5p	.	34.890	.	.	.	.
bta-miR-151-3p	35.104	.	33.895	34.125	33.851	.
bta-miR-143	32.295	30.747	30.695	31.619	30.655	32.480
bta-miR-151-5p	33.870	33.854	31.844	33.852	30.750	35.784
bta-miR-144	.	.	.	35.693	.	.
bta-miR-152	36.266	36.291	35.855	36.369	35.379	.
bta-miR-145	33.028	.	32.440	31.826	30.868	.
bta-miR-153	.	.	.	.	.	.
bta-miR-146a	.	.	.	.	34.831	.
bta-miR-154a	33.507	34.855	35.202	35.191	34.086	36.299
bta-miR-146b	.	.	.	.	36.720	.
bta-miR-154b	29.499	30.229	30.132	29.731	29.597	30.387
bta-miR-147	.	.	35.549	.	35.038	23.662
bta-miR-154c	34.420	35.268	34.925	35.043	33.391	33.390
bta-miR-148a	32.932	32.297	31.767	33.738	30.474	34.967
bta-miR-155	.	33.826	.	33.955	.	36.801
bta-miR-148b	33.964	33.892	32.963	32.844	31.014	34.349
bta-miR-15a	33.627	.	31.548	34.752	31.745	34.654
bta-miR-149-3p	29.739	30.380	30.240	29.824	30.227	29.725
bta-miR-15b	.	35.026	32.828	35.054	36.544	34.925
bta-miR-149-5p	.	34.133	.	.	.	35.396
bta-miR-16a	33.278	33.737	29.811	33.619	30.024	33.948
bta-miR-150	.	.	.	.	32.450	.
bta-miR-16b	31.955	32.821	29.831	32.267	30.241	34.607
bta-miR-17-3p	34.842	34.971	39.653	.	34.447	.
bta-miR-188	32.890	32.089	33.239	31.509	32.182	32.819

bta-miR-17-5p	34.471	32.681	34.297	33.044	32.943	33.882
bta-miR-18a	.	33.908	35.887	.	34.866	34.913
bta-miR-181a	.	34.270	32.836	34.912	32.866	.
bta-miR-18b	.	.	.	.	.	.
bta-miR-181b	33.868	31.974	32.039	32.412	32.300	34.420
bta-miR-190a	.	.	35.973	.	.	.
bta-miR-181c	.	.	32.891	.	34.001	.
bta-miR-190b	35.525	.	.	.	34.870	.
bta-miR-181d	33.034	30.958	32.761	31.900	31.901	33.402
bta-miR-191	31.756	29.103	30.907	28.777	30.663	31.365
bta-miR-182	.	35.822	.	.	.	.
bta-miR-192	36.807	33.480	33.951	33.491	35.025	33.973
bta-miR-183	.	.	.	.	.	.
bta-miR-193a	.	35.050	.	.	.	35.837
bta-miR-184	35.467	32.229	.	32.637	33.984	.
bta-miR-193a-3p	.	.	.	35.861	35.579	34.773
bta-miR-185	.	.	.	.	33.555	.
bta-miR-193a-5p	30.793	28.699	32.234	30.763	29.738	31.168
bta-miR-186	.	39.685	33.886	.	33.531	.
bta-miR-193b	32.847	31.836	31.817	32.469	32.767	32.981
bta-miR-187	32.455	30.668	30.326	30.753	30.913	31.409
bta-miR-194	35.993	.	34.476	.	33.941	.
bta-miR-195	33.123	31.588	31.564	32.513	30.224	35.083
bta-miR-200c	31.798	32.190	33.795	31.797	31.090	32.932
bta-miR-196a	.	.	33.999	36.654	.	.
bta-miR-202	.	.	.	34.948	.	.

bta-miR-196b	.	.	33.868	.	.	.
bta-miR-204	35.948	33.545	35.715	.	33.573	.
bta-miR-197	.	30.807	34.912	32.402	32.745	.
bta-miR-205	34.048	34.214	33.622	35.054	34.759	33.599
bta-miR-199a-3p	.	32.774	31.906	.	30.737	.
bta-miR-206	32.934	31.780	29.310	32.836	30.689	35.163
bta-miR-199a-5p	34.744	33.317	33.167	.	32.607	.
bta-miR-208a	35.103	36.603	.	.	.	.
bta-miR-199b	.	34.549	32.910	.	33.546	34.760
bta-miR-208b	.	.	33.523	.	36.124	.
bta-miR-199c	32.684	31.939	31.059	32.580	29.677	33.972
bta-miR-20a	33.647	35.902	33.073	34.957	31.746	33.129
bta-miR-19a	.	35.907	.	.	34.548	.
bta-miR-20b	33.975	32.938	33.846	.	33.429	.
bta-miR-19b	.	35.012	.	.	33.928	34.542
bta-miR-21-3p	34.540	32.707	.	34.988	35.560	35.608
bta-miR-200a	33.916	36.139	33.798	.	32.863	33.984
bta-miR-21-5p	.	.	.	.	.	36.532
bta-miR-200b	29.869	30.353	31.851	31.379	29.835	31.333
bta-miR-210	33.799	33.098	33.984	33.232	33.099	32.856
bta-miR-211	35.820	.	.	34.449	33.955	.
bta-miR-22-5p	33.187	34.224	30.160	33.620	32.772	32.163
bta-miR-212	28.074	27.476	31.761	31.302	30.859	29.730
bta-miR-221	32.747	32.702	32.481	32.521	.	32.916
bta-miR-214	33.856	33.604	32.934	32.272	33.040	33.056
bta-miR-222	34.934	33.328	32.951	34.762	32.980	34.936

bta-miR-215	38.749	.	.	.	35.740	.
bta-miR-223	.	.	35.559	34.302	34.717	.
bta-miR-216a	.	.	.	35.944	.	34.393
bta-miR-224	34.158	.	.	.	.	.
bta-miR-216b	.	.	.	.	34.597	.
bta-miR-23a	30.830	31.515	29.366	31.155	28.649	31.622
bta-miR-217	.	41.657	.	.	.	.
bta-miR-23b-3p	33.888	34.274	32.213	34.968	31.775	33.882
bta-miR-218	34.751	.	.	.	.	.
bta-miR-23b-5p	.	.	.	.	.	.
bta-miR-219	31.040	29.592	31.841	30.692	30.658	29.326
bta-miR-24	.	.	.	.	.	.
bta-miR-219-3p	36.101	36.430	.	35.075	35.103	36.966
bta-miR-24-3p	31.610	30.754	29.416	32.029	29.371	32.237
bta-miR-219-5p	.	.	.	.	.	.
bta-miR-25	34.914	32.812	32.759	32.860	32.154	34.838
bta-miR-22-3p	2.953	2.836	.	3.157	3.057	2.795
bta-miR-26a	30.824	31.496	28.752	32.782	29.285	32.639
bta-miR-26b	35.908	32.536	30.760	34.317	31.277	.
bta-miR-29d-3p	38.858	33.223	30.653	34.496	31.874	34.745
bta-miR-26c	.	.	.	.	.	.
bta-miR-29d-5p	35.140	35.073	33.848	34.272	35.984	35.408
bta-miR-27a-3p	35.418	31.886	29.930	34.288	30.725	34.635
bta-miR-29e	.	36.627	34.884	.	35.210	.
bta-miR-27a-5p	.	36.884	.	36.307	29.751	18.623
bta-miR-301a	.	34.780	35.986	.	36.863	.

bta-miR-27b	32.827	34.875	30.230	35.042	29.823	32.999
bta-miR-301b	36.974	.	.	.	33.977	.
bta-miR-28	.	.	42.951	.	33.314	.
bta-miR-302a	.	.	.	.	.	.
bta-miR-296-3p	32.824	34.090	35.343	29.873	36.886	33.805
bta-miR-302b	.	.	.	.	.	.
bta-miR-296-5p	33.004	33.887	30.810	35.788	33.895	29.245
bta-miR-302c	.	34.568	.	.	.	.
bta-miR-299	.	34.876	.	.	.	.
bta-miR-302d	.	.	35.565	34.342	.	.
bta-miR-29a	31.895	31.901	29.748	.	29.802	.
bta-miR-3064	.	.	.	.	.	.
bta-miR-29b	33.867	32.199	31.935	.	32.866	32.871
bta-miR-30a-5p	33.910	34.280	31.352	.	30.711	32.726
bta-miR-29c	31.180	32.611	29.835	32.525	29.549	32.944
bta-miR-30b-3p	.	.	33.730	.	.	.
bta-miR-30b-5p	32.908	32.023	33.376	34.550	33.032	35.838
bta-miR-328	33.822	31.444	33.769	31.793	33.510	33.300
bta-miR-30c	32.713	31.817	31.660	32.292	30.693	33.882
bta-miR-329a	.	.	.	.	.	.
bta-miR-30d	34.718	32.972	32.245	.	31.745	33.841
bta-miR-329b	.	.	.	.	.	.
bta-miR-30e-5p	33.819	.	31.471	.	32.057	34.938
bta-miR-330	36.480	33.307	33.875	34.821	33.270	.
bta-miR-30f	35.281	33.919	32.428	35.033	33.539	38.461
bta-miR-331-3p	.	.	35.431	35.944	.	.

bta-miR-31	31.934	32.390	34.792	33.815	32.491	32.892
bta-miR-331-5p	31.856	30.429	32.749	29.923	31.866	31.833
bta-miR-32	.	.	.	.	.	.
bta-miR-335	36.598	34.518	.	.	34.897	36.140
bta-miR-320a	28.197	27.892	28.711	28.451	28.046	27.969
bta-miR-338	.	.	34.898	.	.	.
bta-miR-320b	31.919	31.488	32.930	31.873	31.835	32.008
bta-miR-339a	34.793	31.654	33.064	32.935	32.712	32.923
bta-miR-323	16.848	16.762	17.303	16.799	16.512	16.828
bta-miR-339b	34.947	31.369	32.897	31.847	34.136	33.063
bta-miR-324	34.905	35.101	33.950	34.326	34.292	34.283
bta-miR-33a	.	.	.	.	.	36.438
bta-miR-326	.	.	35.343	.	35.019	35.620
bta-miR-33b	33.988	32.893	33.818	34.372	33.277	33.896
bta-miR-340	.	.	.	.	35.177	.
bta-miR-365-3p	33.521	.	30.054	.	30.629	34.709
bta-miR-342	.	34.932	.	34.788	33.561	.
bta-miR-365-5p	.	33.948	33.518	33.770	35.516	33.418
bta-miR-345-3p	34.350	32.798	35.027	34.764	36.352	34.753
bta-miR-367	.	.	.	.	.	38.216
bta-miR-345-5p	32.841	32.257	33.302	32.350	32.524	32.805
bta-miR-369-3p	.	36.111	.	.	35.568	.
bta-miR-346	32.666	31.406	34.940	31.856	32.827	32.927
bta-miR-369-5p	35.281	.	36.386	.	.	.
bta-miR-34a	34.859	33.883	32.868	33.408	.	34.995
bta-miR-370	34.869	33.718	36.698	33.924	33.923	34.900

bta-miR-34b	.	34.483	.	.	34.464	35.727
bta-miR-371	.	33.512	.	32.978	34.934	36.988
bta-miR-34c	34.160	34.281	.	.	.	35.638
bta-miR-374a	.	.	36.131	.	36.524	.
bta-miR-361	.	34.442	.	35.361	35.055	.
bta-miR-374b	.	.	.	.	33.887	.
bta-miR-362-3p	.	.	34.604	.	.	.
bta-miR-375	31.811	31.920	32.692	30.759	32.262	31.858
bta-miR-362-5p	.	35.168	35.068	.	34.048	36.933
bta-miR-376a	.	35.566	.	36.689	.	.
bta-miR-363	36.871	36.137	36.206	35.316	.	.
bta-miR-376b	.	.	.	.	.	.
bta-miR-376c	.	.	.	.	.	.
bta-miR-382	29.562	29.022	29.850	28.833	29.821	29.711
bta-miR-376d	.	.	.	.	.	.
bta-miR-383	34.266	32.393	36.044	31.800	33.273	32.876
bta-miR-376e	.	.	.	.	.	.
bta-miR-409a	.	35.121	36.454	36.521	34.420	.
bta-miR-377	.	.	.	.	.	.
bta-miR-409b	.	.	.	.	.	.
bta-miR-378	33.442	31.811	30.296	35.416	31.856	33.004
bta-miR-410	34.435	32.662	35.648	32.919	35.599	.
bta-miR-378b	34.541	33.334	30.283	35.692	31.819	33.806
bta-miR-411a	31.906	31.829	33.098	31.534	32.568	32.664
bta-miR-378c	.	34.506	32.954	32.767	33.962	34.505
bta-miR-411b	34.464	33.528	36.051	34.764	33.462	34.129

bta-miR-378d	33.267	35.121	34.123	32.852	34.272	36.093
bta-miR-411c-3p	.	36.699	37.809	34.761	.	.
bta-miR-379	.	.	35.306	.	.	.
bta-miR-411c-5p	.	.	34.925	.	.	.
bta-miR-380-3p	.	33.105	.	36.746	.	.
bta-miR-412	.	.	.	.	.	.
bta-miR-380-5p	35.793	34.577	34.394	35.104	35.268	.
bta-miR-421	30.127	26.887	29.802	29.099	29.894	30.235
bta-miR-381	.	.	.	35.095	34.408	.
bta-miR-423-3p	34.969	32.258	33.384	32.315	34.893	34.702
bta-miR-423-5p	33.155	32.921	34.000	32.200	32.792	35.814
bta-miR-449c	35.472	34.984	36.190	36.139	36.098	34.476
bta-miR-424-3p	.	33.916	33.786	35.445	.	.
bta-miR-449d	33.340	32.066	33.649	32.562	34.877	32.918
bta-miR-424-5p	36.874	.	32.253	34.977	31.011	.
bta-miR-450a	.	.	.	.	.	.
bta-miR-425-3p	28.567	27.743	28.899	28.282	28.885	28.789
bta-miR-450b	.	.	.	.	.	.
bta-miR-425-5p	.	.	36.004	33.576	33.298	35.426
bta-miR-451	.	34.968	32.164	.	31.193	34.683
bta-miR-429	29.161	28.776	29.585	29.673	29.390	29.284
bta-miR-452	.	36.963	.	35.044	.	.
bta-miR-431	.	33.583	.	34.471	.	36.346
bta-miR-4523	33.322	32.292	35.671	27.756	32.799	33.300
bta-miR-432	36.329	34.144	35.529	34.465	35.368	.
bta-miR-453	32.945	32.125	33.515	32.282	32.873	33.532

bta-miR-433	30.055	30.088	29.724	29.246	29.897	29.961
bta-miR-454	.	.	.	.	.	.
bta-miR-448	.	.	.	.	.	.
bta-miR-455-3p	.	.	.	.	.	.
bta-miR-449a	34.028	35.205	.	34.985	35.106	33.783
bta-miR-455-5p	.	.	.	.	.	.
bta-miR-449b	.	33.885	35.032	.	35.625	34.301
bta-miR-483	.	32.882	33.895	34.532	32.754	.
bta-miR-484	35.226	33.164	33.333	33.892	36.448	.
bta-miR-496	36.012	35.934	.	36.090	.	.
bta-miR-485	36.627	35.341	.	34.629	33.421	35.085
bta-miR-497	.	36.027	34.555	.	34.052	.
bta-miR-486	29.766	29.778	29.652	30.954	29.541	29.374
bta-miR-499	.	.	33.184	.	34.015	.
bta-miR-487a	.	.	.	.	.	.
bta-miR-500	26.271	23.834	26.762	24.358	25.554	25.895
bta-miR-487b	.	35.280	.	.	.	.
bta-miR-502a	.	.	.	.	.	.
bta-miR-488	.	.	.	.	.	.
bta-miR-502b	.	35.511	.	34.478	.	.
bta-miR-489	31.722	31.787	31.635	30.677	31.845	31.794
bta-miR-503-3p	31.973	31.630	31.237	31.932	32.040	31.584
bta-miR-490	.	33.587	40.638	35.298	34.970	.
bta-miR-503-5p	.	35.633	.	35.169	6.299	36.056
bta-miR-491	35.752	35.671	35.166	.	33.903	35.036
bta-miR-504	.	36.823	.	.	.	.

bta-miR-493	31.888	30.456	32.766	31.142	30.647	31.088
bta-miR-505	31.819	29.806	31.414	31.269	30.460	31.152
bta-miR-494	21.884	22.199	22.810	22.776	22.804	21.759
bta-miR-532	35.667	32.596	.	32.942	33.862	34.546
bta-miR-495	.	36.432	.	35.743	.	.
bta-miR-539	.	34.921	34.082	.	.	.
bta-miR-541	29.918	29.711	30.472	29.272	29.821	30.010
bta-miR-582	.	.	.	.	.	.
bta-miR-542-5p	.	36.564	.	.	36.552	.
bta-miR-584	29.708	28.592	30.800	30.158	29.727	29.706
bta-miR-543	.	36.330	.	33.399	.	.
bta-miR-592	36.790	33.441	34.741	33.409	34.989	35.037
bta-miR-544a	.	.	.	.	35.267	.
bta-miR-599	.	.	.	.	.	.
bta-miR-544b	.	.	.	.	.	.
bta-miR-615	9.446	9.728	9.552	9.426	9.499	9.341
bta-miR-545-3p	.	.	.	.	.	.
bta-miR-628	34.785	.	.	.	.	.
bta-miR-545-5p	.	.	.	.	.	.
bta-miR-631	19.250	19.267	19.343	19.132	18.990	19.307
bta-miR-551a	.	.	.	33.878	.	.
bta-miR-652	34.443	32.294	32.904	33.099	33.475	34.983
bta-miR-551b	.	35.745	.	35.642	36.023	.
bta-miR-653	.	.	.	.	.	.
bta-miR-562	.	.	.	.	.	.
bta-miR-654	.	.	33.918	.	.	.

bta-miR-568	.	.	.	.	.	.
bta-miR-655	.	.	.	36.124	.	36.607
bta-miR-574	26.798	27.533	26.760	28.002	27.831	26.869
bta-miR-656	31.849	30.794	31.229	30.739	30.985	31.303
bta-miR-658	34.876	35.812	35.741	35.997	36.413	35.045
bta-miR-758	.	43.649	.	.	.	.
bta-miR-660	.	34.133	35.626	34.223	33.329	36.997
bta-miR-759	36.797	.	.	36.789	.	.
bta-miR-664a	32.847	32.915	34.084	31.817	31.011	33.120
bta-miR-760-3p	35.011	34.021	35.274	33.499	34.068	33.955
bta-miR-664b	.	.	33.772	33.874	32.248	36.437
bta-miR-760-5p	32.821	32.528	30.697	32.043	31.224	32.865
bta-miR-665	32.643	29.298	31.813	29.714	30.658	30.612
bta-miR-761	.	33.070	34.860	32.945	.	.
bta-miR-669	31.842	31.358	32.745	32.465	31.840	32.053
bta-miR-763	33.214	33.217	33.503	31.125	34.180	33.891
bta-miR-670	.	.	.	35.026	.	.
bta-miR-764	35.596	35.453	.	.	.	36.771
bta-miR-671	.	.	.	35.570	.	36.555
bta-miR-767	30.807	30.930	30.775	30.889	30.876	30.987
bta-miR-677	36.989	33.108	31.884	35.316	32.351	33.777
bta-miR-769	.	.	.	34.560	.	.
bta-miR-7	.	34.924	33.820	.	36.050	.
bta-miR-873	.	34.989	36.058	.	.	33.963
bta-miR-708	35.873	33.826	32.958	.	33.890	.
bta-miR-874	31.882	29.699	30.890	30.752	31.588	30.688

bta-miR-744	36.682	36.467	.	34.828	.	.
bta-miR-875	.	36.951	.	36.638	.	.
bta-miR-876	.	34.375	36.874	.	35.304	.
bta-miR-98	.	.	34.574	.	.	.
bta-miR-877	31.219	29.820	31.445	30.105	30.539	30.365
bta-miR-99a-3p	.	.	36.088	.	34.573	33.827
bta-miR-885	34.301	28.281	34.549	29.973	31.931	32.899
bta-miR-99a-5p	32.367	33.877	30.954	33.754	30.249	.
bta-miR-9-3p	.	.	.	.	.	.
bta-miR-99b	23.789	23.279	23.829	23.520	23.671	23.838
bta-miR-9-5p	.	34.142	34.957	36.065	35.966	.
bta-miR-1179	.	35.305	.	.	.	.
bta-miR-92a	30.715	29.097	30.769	29.679	30.506	31.774
bta-miR-1185	.	.	.	.	.	.
bta-miR-92b	28.976	28.137	27.970	28.434	28.714	28.738
bta-miR-1193	.	.	.	.	.	.
bta-miR-93	.	.	.	32.583	32.271	33.908
bta-miR-1197	.	36.989	.	.	.	.
bta-miR-935	33.970	31.947	32.998	32.728	32.963	34.294
bta-miR-122	35.518	35.547	.	34.556	.	.
bta-miR-940	27.816	27.350	27.119	27.962	27.811	25.940
bta-miR-1224	27.264	27.739	28.098	27.191	26.566	27.746
bta-miR-95	.	.	.	.	.	.
bta-miR-1225-3p	29.641	29.131	28.887	28.842	29.791	28.127
bta-miR-96	.	.	.	.	.	.
bta-miR-1246	22.725	24.781	23.757	24.731	24.603	23.892

bta-miR-1247-3p	31.768	30.837	32.030	31.129	.	31.663
bta-miR-1296	34.580	33.897	33.524	32.333	.	34.034
bta-miR-1247-5p	29.776	29.676	30.572	28.070	30.475	29.924
bta-miR-1298	.	.	33.697	.	.	.
bta-miR-1248	34.766	33.957	30.762	33.825	34.528	36.635
bta-miR-1301	35.652	33.773	.	33.949	36.676	34.968
bta-miR-1249	.	34.615	34.871	.	33.913	33.373
bta-miR-1306	34.969	33.665	34.740	35.254	33.991	36.515
bta-miR-1260b	26.184	25.703	22.348	26.404	23.837	26.254
bta-miR-1307	27.374	27.434	28.195	27.826	28.665	27.632
bta-miR-1271	.	.	.	.	.	.
bta-miR-1343-3p	31.899	30.196	32.034	30.578	30.744	31.995
bta-miR-1277	.	.	.	.	.	.
bta-miR-1343-5p	28.548	28.941	28.706	28.789	28.480	28.654
bta-miR-1281	30.284	30.658	29.368	31.017	30.493	28.494
bta-miR-1388-3p	32.077	30.502	31.580	30.699	32.184	33.441
bta-miR-1282	33.850	.	32.655	35.668	36.681	32.918
RNT43 snoRNA	31.708	29.973	30.693	32.919	30.410	31.315
bta-miR-1284	.	.	.	.	.	.
Hm/Ms/Rt T1 snRNA	20.780	22.578	20.722	20.857	21.073	22.287
bta-miR-1287	33.305	30.752	31.736	31.880	32.466	32.866
bta-miR-99b	23.250	23.681	23.591	23.228	23.400	23.637
bta-miR-1291	35.168	32.169	.	33.554	34.192	35.562
Negative control	.	.	.	.	.	.

[1] Body energy reserve: MBER: Cows with moderated body energy reserve; HBER: Cows with high body energy reserve.

Supplementary Table 3. Normalized data of the 82 miRNAs commonly detected in ampullary extracellular vesicles (AMP-EVs) of cows with different body energy reserve.

miRNA	**Body energy reserve[1]**						**P – Value[2]**
	MBER			**HBER**			
	1	**2**	**3**	**1**	**2**	**3**	
bta-let-7c	11.0621	12.932	9.18838	8.79235	11.4008	11.2821	0.7002
bta-let-7e	11.6012	10.5522	11.415	9.37628	7.50493	9.45158	**0.0278**
bta-miR-126-3p	9.08123	8.88399	11.6352	8.81788	5.38234	7.70872	0.1294
bta-miR-126-5p	12.2904	7.6655	10.0312	12.7715	5.33977	6.77431	0.5544
bta-miR-127	10.5917	10.1137	10.1161	10.8689	7.61446	10.2515	0.5291
bta-miR-129-5p	10.8675	10.954	10.5817	9.87842	9.57595	11.3171	0.3777
bta-miR-130a	10.1439	10.8825	10.1123	11.0089	9.68973	9.49678	0.5901
bta-miR-130b	8.83089	10.6688	9.81708	9.73361	8.17945	8.67004	0.2639
bta-miR-132	5.9878	6.47301	6.18405	3.99811	4.09169	4.97969	**0.0056**
bta-miR-143	7.96597	10.7928	7.27338	8.011	6.48937	7.2275	0.2847
bta-miR-154b	10.2338	5.58387	6.23785	8.81732	2.84346	5.15574	0.4840
bta-miR-149-3p	6.66376	7.44094	7.0742	8.38257	5.39473	7.66739	0.9286
bta-miR-188	11.9648	12.0152	10.4921	9.88893	9.18494	10.1757	**0.0398**
bta-miR-191	11.6329	8.50585	8.35005	7.16905	5.87247	6.86252	0.0658
bta-miR-192	12.1762	11.0094	11.2977	14.512	9.72696	9.77262	0.9276
bta-miR-187	10.9184	9.46205	8.07356	9.43272	6.42917	9.13785	0.4126
bta-miR-196a	11.6335	11.0261	8.91992	9.41997	5.806	7.12542	0.0831
bta-miR-196b	11.7392	10.5872	10.4118	10.896	8.51673	10.8669	0.4100
bta-miR-197	8.83907	7.59514	7.59913	9.92843	4.5243	7.00817	0.6237
bta-miR-200b	8.49529	8.48439	6.38252	5.25968	7.79942	7.2493	0.3845
bta-miR-222	10.5093	9.58057	10.2765	8.86577	7.07774	9.12184	0.0653
bta-miR-23a	11.6449	9.15274	7.29485	7.12093	9.01353	8.99435	0.5215

bta-miR-219	12.0973	8.78258	8.01917	8.76235	5.4264	6.80554	0.1712
bta-miR-22-3p	-20.905	-20.406	-20.745	-21.206	-20.544	-21.341	0.2953
bta-miR-27a-5p	-15.129	-14.456	-14.715	-15.643	-14.733	-15.246	0.2505
bta-miR-296-3p	9.95352	8.6679	6.89117	14.1605	7.41522	11.519	0.3057
bta-miR-29a	10.7105	11.1072	11.3269	6.66653	11.7053	10.057	0.3521
bta-miR-328	7.14054	6.11301	7.0974	11.0058	6.03677	7.88802	0.3630
bta-miR-30e-5p	10.4556	13.3034	10.3432	14.0121	8.68574	9.42369	0.7492
bta-miR-331-5p	10.6358	8.01999	9.67472	11.6381	5.84218	9.12341	0.7705
bta-miR-320a	6.92081	7.02597	7.28813	5.15997	7.48946	8.44566	0.9644
bta-miR-320b	10.2005	9.72285	9.55215	9.66412	7.03392	8.19534	0.1237
bta-miR-323	-4.5472	-4.4872	-4.1813	-5.0323	-4.4111	-4.7305	0.2067
bta-miR-345-5p	10.3723	10.3695	9.43375	10.1764	7.53952	8.88375	0.2210
bta-miR-370	11.6017	8.93063	11.1389	10.7995	8.90479	11.2795	0.8448
bta-miR-375	10.3824	10.314	8.42493	8.46894	4.58295	10.4853	0.3706
bta-miR-382	9.90872	7.49054	6.89775	9.78149	4.95354	7.71239	0.7314
bta-miR-411a	9.59504	8.52523	7.12279	9.62146	4.94539	7.16202	0.4862
bta-miR-411b	10.4723	9.78499	8.03618	9.69325	7.16376	10.6116	0.8380
bta-miR-378d	10.2675	9.32972	8.80507	10.8991	6.51737	7.83601	0.4852
bta-miR-380-5p	8.08176	8.71244	6.18232	9.8324	4.70248	7.0141	0.7894
bta-miR-421	12.7602	8.77852	8.64749	8.64186	5.36335	8.02264	0.1814
bta-miR-425-3p	9.06518	8.70843	7.2035	6.73594	5.66731	7.48394	0.0943
bta-miR-429	7.00543	8.24254	6.44189	7.60376	5.65993	7.04501	0.5892
bta-miR-432	10.1271	7.69254	7.07727	12.4105	4.90779	6.52308	0.8933
bta-miR-453	11.6197	11.8591	11.9636	10.6526	9.59657	12.325	0.2985
bta-miR-433	9.5037	5.99969	4.70409	7.13223	3.27165	6.11463	0.5408
bta-miR-486	12.128	10.8021	12.1915	7.90527	8.13105	10.5804	**0.0430**

bta-miR-500	10.2064	13.6221	10.4891	8.24091	12.2639	11.8563	0.7181
bta-miR-489	10.1489	10.0588	9.76537	9.87999	9.7588	9.64645	0.1622
bta-miR-503-3p	10.9951	8.57308	7.58061	9.29317	5.50423	7.88695	0.3774
bta-miR-494	5.24436	4.0354	4.27906	5.43921	5.71526	6.34817	**0.0451**
bta-miR-541	9.46329	8.09198	7.58742	7.1116	5.56874	8.02098	0.1787
bta-miR-584	4.97765	5.7377	4.24138	1.77982	6.16257	4.91763	0.6377
bta-miR-615	-11.363	-10.83	-11.118	-11.82	-10.763	-11.203	0.6676
bta-miR-631	-3.4143	-3.3663	-3.0837	-3.4765	-3.474	-3.5148	0.1263
bta-miR-574	9.19042	7.5736	5.80692	3.94818	7.15248	8.26524	0.5460
bta-miR-656	12.1634	8.74656	7.31314	10.2865	6.05749	9.0827	0.6513
bta-miR-664a	10.1708	11.4007	10.855	8.94062	9.17005	9.90061	**0.0326**
bta-miR-760-3p	10.5051	11.8708	9.84443	7.77466	9.2257	10.1824	0.1419
bta-miR-760-5p	10.1365	7.72575	6.24114	9.49587	4.79339	7.14682	0.6417
bta-miR-767	10.2007	10.0826	8.55982	9.98548	9.29688	10.3648	0.6850
bta-miR-708	10.8677	8.89958	8.29393	12.1322	7.43417	9.16491	0.8942
bta-miR-877	8.17861	7.9789	7.169	6.82851	7.29244	8.14586	0.5142
bta-miR-9-5p	10.7526	10.851	9.06167	11.109	8.64983	10.5827	0.9147
bta-miR-92b	8.04497	6.2034	5.6455	6.82875	5.22439	6.35031	0.5973
bta-miR-93	11.0407	10.7207	9.35035	8.65664	11.2708	11.0708	0.9713
bta-miR-940	8.28086	7.61757	7.96802	5.58729	8.606	9.15872	0.8864
bta-miR-1224	1.17228	2.62523	4.39874	5.94262	6.08771	6.91198	**0.0217**
bta-miR-1225-3p	9.28329	9.33284	8.33943	7.88148	7.82493	9.2532	0.3064
bta-miR-1246	-0.0058	1.75044	3.09178	1.67041	3.8758	5.07595	0.2238
bta-miR-1247-3p	9.01418	8.0301	5.24986	5.80438	4.92255	7.12389	0.3169
bta-miR-1247-5p	9.20904	9.17074	8.29065	7.97692	9.67395	11.3045	0.4913
bta-miR-1260b	3.67273	2.66492	0.30634	2.83056	3.98884	3.97571	0.2651

bta-miR-1307	11.0942	9.24795	8.15813	8.57067	7.93526	10.1929	0.6107
bta-miR-1343-3p	8.93341	8.39081	6.01836	8.64445	7.14289	8.71285	0.7273
bta-miR-1343-5p	8.01281	8.23736	7.77357	6.60803	7.57402	10.2005	0.9173
bta-miR-1281	7.09441	8.52587	5.89419	6.41905	5.0412	7.70427	0.5090
bta-miR-1388-3p	8.66794	10.7026	8.05964	10.0327	8.15634	10.2473	0.7630
Hm/Ms/Rt T1 snRNA	-1.6069	0.02083	1.32196	0.9367	0.86333	4.06024	0.2056
bta-miR-1287	11.666	10.4819	9.59731	9.37915	9.72279	12.8647	0.9561
bta-miR-99b	0.01028	-0.0906	-0.2231	-0.1005	-0.0878	0.01659	0.5998

[1] Body energy reserve: MBER: Cows with moderated body energy reserve; HBER: Cows with high body energy reserve; [2] P-value: P value between animals with different body energy reserve.

Supplementary Table 4. Normalized data of the 150 miRNAs commonly detected in isthmic extracellular vesicles (IST-EVs) of cows with different body energy reserve.

miRNA	**Body energy reserve[1]**						**P-value[2]**
	MBER			**HBER**			
	1	**2**	**3**	**1**	**2**	**3**	
bta-miR-103	11.17591	10.89641	8.865318	13.4834	8.81394	10.39238	0.7259
bta-let-7a-5p	6.209712	7.177778	5.721577	6.90333	6.603207	7.916258	0.2570
bta-let-7b	7.182044	7.688715	6.190813	6.448496	6.175463	9.256462	0.8127
bta-miR-106b	9.37997	8.004498	9.73423	8.414736	9.210135	9.584236	0.9642
bta-let-7e	5.67361	6.723055	5.887817	6.636835	8.33988	7.990408	0.0627
bta-let-7f	7.406226	8.657598	7.107239	10.0822	7.72377	10.45418	0.1578
bta-miR-10a	11.04091	11.4319	7.715961	9.525482	8.357744	10.07971	0.5942
bta-miR-1	7.861703	6.932756	2.296492	9.971348	4.871855	6.689949	0.5511
bta-miR-124a	9.614612	8.779001	9.338577	9.444054	9.89451	8.506268	0.9411
bta-miR-100	7.213111	10.07969	7.375648	8.500069	7.750546	11.20747	0.5435
bta-miR-124b	8.415971	8.609091	9.458015	10.46604	9.392008	8.792458	0.2843
bta-miR-125b	7.319982	7.231377	5.931747	8.473083	4.871719	8.952972	0.6803

bta-miR-126-3p	8.808276	7.196796	5.361818	8.157825	4.527639	8.55196	0.9801
bta-miR-126-5p	9.722304	8.566599	6.188192	8.831066	5.847662	9.191471	0.8982
bta-miR-134	10.52569	10.02379	13.17724	9.39164	11.52422	9.718689	0.4324
bta-miR-127	7.178924	6.510365	7.458509	7.879025	6.935253	7.039467	0.5970
bta-miR-129	9.362922	8.113626	10.42503	8.093742	10.43033	8.120363	0.7030
bta-miR-129-5p	9.342723	7.871872	11.78217	7.420998	9.315248	9.138828	0.4652
bta-miR-138	10.16926	10.40484	12.25554	9.539974	11.15303	10.19297	0.4681
bta-miR-130a	7.372909	7.594852	8.1308	7.496222	7.264242	6.974224	0.1687
bta-miR-139	7.205222	5.238153	8.540672	6.442274	8.367054	7.298517	0.7525
bta-miR-130b	7.067014	7.057287	7.071411	7.199686	6.756367	6.945711	0.4882
bta-miR-132	7.96256	5.611607	8.449974	5.792992	7.305504	7.767563	0.7341
bta-miR-133a	8.318871	7.998035	3.227861	10.84563	5.049467	7.009317	0.6609
bta-miR-143	8.77576	7.267372	7.321341	8.245388	7.119818	8.74276	0.7372
bta-miR-151-5p	10.35062	10.37486	8.470007	10.4777	7.214531	12.04701	0.9129
bta-miR-154a	9.988315	11.37512	11.82846	11.8175	10.55095	12.56194	0.5123
bta-miR-154b	5.980039	6.74918	6.758263	6.357443	6.061993	6.649214	0.6745
bta-miR-154c	10.90113	11.78828	11.55069	11.66859	9.855604	9.652596	0.2149
bta-miR-148a	9.413035	8.817762	8.392921	10.3641	6.938593	11.23001	0.6604
bta-miR-148b	10.44492	10.41232	9.588634	9.470139	7.478998	10.61174	0.3719
bta-miR-149-3p	6.219905	6.900366	6.86586	6.449944	6.691326	5.987778	0.3986
bta-miR-16a	9.758548	10.25755	6.437122	10.24496	6.488596	10.21028	0.9292
bta-miR-16b	8.435746	9.341108	6.456987	8.893066	6.70562	10.86926	0.6398
bta-miR-188	9.371188	8.609522	9.865204	8.135046	8.646627	9.08176	0.2211
bta-miR-17-5p	10.95165	9.201868	10.92313	9.670048	9.40737	10.14426	0.3733
bta-miR-181b	10.34857	8.494308	8.665272	9.037815	8.764345	10.68224	0.7188
bta-miR-181d	9.514861	7.478064	9.386669	8.526175	8.365341	9.664276	0.9432
bta-miR-191	8.236646	5.623587	7.533197	5.403269	7.127773	7.627281	0.7101
bta-miR-192	13.28819	10.00078	10.57664	10.11746	11.48944	10.23597	0.5745

bta-miR-193a-5p	7.273412	5.219772	8.860382	7.389532	6.202488	7.43029	0.9267
bta-miR-193b	9.327529	8.356787	8.443254	9.095001	9.231794	9.243614	0.2002
bta-miR-187	8.936263	7.18799	6.952196	7.379562	7.377857	7.671806	0.7505
bta-miR-195	9.603553	8.107912	8.190243	9.139324	6.689001	11.3458	0.7815
bta-miR-200c	8.278815	8.710485	10.4213	8.423227	7.554483	9.194416	0.4079
bta-miR-205	10.5288	10.73453	10.24799	11.67985	11.22398	9.861271	0.5000
bta-miR-206	9.414786	8.300842	5.936382	9.461899	7.153978	11.42537	0.4135
bta-miR-199c	9.164618	8.45962	7.685457	9.205608	6.141253	10.23488	0.9478
bta-miR-20a	10.12747	12.42266	9.699039	11.58327	8.210178	9.391457	0.4762
bta-miR-200b	6.349769	6.873535	8.477457	8.004877	6.299713	7.595516	0.9394
bta-miR-210	10.27993	9.618109	10.60997	9.857819	9.563421	9.118778	0.1443
bta-miR-22-5p	9.667888	10.74456	6.786339	10.24586	9.236731	8.425941	0.8638
bta-miR-212	4.554889	3.996458	8.387453	7.927616	7.323542	5.992317	0.3912
bta-miR-214	10.33705	10.12446	9.560478	8.898308	9.504087	9.318687	0.0589
bta-miR-222	11.41473	9.848307	9.576859	11.38837	9.444368	11.19868	0.6622
bta-miR-23a	7.311198	8.035294	5.992023	7.781424	5.113171	7.884476	0.8721
bta-miR-23b-3p	10.369	10.79425	8.838745	11.59378	8.239296	10.14441	0.9946
bta-miR-219	7.520541	6.112766	8.466751	7.318523	7.122595	5.588818	0.4747
bta-miR-24-3p	8.090997	7.274107	6.042261	8.655083	5.835579	8.499947	0.6541
bta-miR-25	11.39487	9.332655	9.385155	9.486008	8.61886	11.10056	0.7763
bta-miR-26a	7.305032	8.016808	5.378508	9.40815	5.749537	8.901428	0.4656
bta-miR-29d-3p	15.3386	9.74296	7.279274	11.12197	8.338264	11.00791	0.8169
bta-miR-29d-5p	11.62032	11.5936	10.4739	10.89832	12.44872	11.67089	0.4912
bta-miR-27a-3p	11.89868	8.406197	6.556074	10.91428	7.189367	10.89806	0.7389
bta-miR-27b	9.308311	11.3959	6.856426	11.66791	6.287188	9.26141	0.9578
bta-miR-296-3p	9.304938	10.61022	11.96944	6.49894	13.35006	10.06803	0.7728
bta-miR-296-5p	9.485057	10.40759	7.436438	12.4138	10.35925	5.507814	0.8936
bta-miR-29c	7.660571	9.130949	6.460747	9.151276	6.013288	9.206993	0.7896

bta-miR-30b-5p	9.38911	8.542952	10.00223	11.17567	9.496073	12.10077	0.1380
bta-miR-328	10.30239	7.964647	10.39543	8.41931	9.974607	9.563034	0.8111
bta-miR-30c	9.194003	8.337814	8.286472	8.917786	7.15764	10.14469	0.8908
bta-miR-30f	11.76166	10.43936	9.05447	11.65874	10.00362	14.72308	0.3422
bta-miR-31	8.414468	8.910322	11.41829	10.44111	8.955149	9.154339	0.9538
bta-miR-331-5p	8.336928	6.949476	9.375286	6.549242	8.330029	8.095434	0.5650
bta-miR-320a	4.677434	4.412218	5.337252	5.0774	4.510035	4.231899	0.6140
bta-miR-320b	8.399459	8.008684	9.556301	8.498648	8.29949	8.270191	0.5597
bta-miR-339a	11.27357	8.174273	9.689762	9.561447	9.176149	9.185978	0.6775
bta-miR-323	-6.67114	-6.71724	-6.07045	-6.57503	-7.02365	-6.90895	0.2315
bta-miR-339b	11.42752	7.889271	9.523026	8.472776	10.60036	9.325298	0.9079
bta-miR-324	11.38555	11.62117	10.5756	10.95225	10.75658	10.54525	0.2601
bta-miR-33b	10.46886	9.413832	10.44423	10.99823	9.74122	10.15875	0.7265
bta-miR-345-3p	10.83063	9.318048	11.65296	11.39053	12.81662	11.01578	0.2632
bta-miR-345-5p	9.322089	8.777731	9.927963	8.975727	8.988501	9.068054	0.3760
bta-miR-346	9.147067	7.926579	11.56571	8.482477	9.291876	9.189407	0.6381
bta-miR-370	11.35029	10.2386	13.32426	10.55026	10.38768	11.16211	0.3715
bta-miR-375	8.292018	8.440569	9.318443	7.385071	8.726767	8.120335	0.2945
bta-miR-382	6.042964	5.542351	6.476446	5.45953	6.28528	5.973738	0.7676
bta-miR-383	10.74668	8.913145	12.66998	8.425671	9.737921	9.138218	0.2184
bta-miR-378	9.923236	8.331881	6.922289	12.04178	8.320988	9.266216	0.3531
bta-miR-378b	11.02169	9.854086	6.908686	12.3183	8.283663	10.0681	0.5999
bta-miR-411a	8.387261	8.349604	9.72422	8.159906	9.032549	8.926533	0.8399
bta-miR-411b	10.94478	10.04873	12.67674	11.39021	9.926291	10.39131	0.5004
bta-miR-378d	9.748084	11.64137	10.74906	9.477818	10.73676	12.35579	0.8921
bta-miR-421	6.608041	3.407516	6.427677	5.725368	6.358215	6.497095	0.5401
bta-miR-423-3p	11.44991	8.778803	10.01	8.941216	11.3575	10.9641	0.7668
bta-miR-423-5p	9.635451	9.441198	10.62646	8.82563	9.256472	12.07618	0.8954

bta-miR-449c	11.95242	11.50408	12.81578	12.76463	12.56254	10.73838	0.9313
bta-miR-449d	9.82054	8.586172	10.27478	9.188492	11.34187	9.180714	0.7159
bta-miR-425-3p	5.047448	4.263044	5.525396	4.908121	5.349876	5.052002	0.7063
bta-miR-429	5.642312	5.296574	6.211414	6.298655	5.854845	5.5462	0.6234
bta-miR-4523	9.802554	8.812407	12.29737	4.382282	9.263468	9.56263	0.2631
bta-miR-453	9.426059	8.645651	10.14067	8.908218	9.33708	9.794998	0.9145
bta-miR-433	6.535562	6.608486	6.350296	5.872278	6.361917	6.223902	0.1041
bta-miR-486	6.246661	6.298117	6.278273	7.579851	6.005039	5.63677	0.8345
bta-miR-500	2.751954	0.354319	3.38809	0.983736	2.018721	2.157902	0.6781
bta-miR-489	8.202521	8.306927	8.261436	7.302591	8.309669	8.056166	0.2932
bta-miR-503-3p	8.45361	8.150631	7.863565	8.55769	8.504259	7.847012	0.6331
bta-miR-493	8.368438	6.976126	9.392044	7.768055	7.111837	7.35008	0.3138
bta-miR-505	8.300122	6.326246	8.039667	7.894651	6.924662	7.414911	0.8426
bta-miR-494	-1.63496	-1.28054	-0.56408	-0.59792	-0.73111	-1.97832	0.9206
bta-miR-541	6.39844	6.231714	7.097651	5.897614	6.285688	6.27231	0.2229
bta-miR-584	6.188684	5.11237	7.426083	6.784405	6.191706	5.968166	0.9239
bta-miR-592	13.27041	9.961813	11.36683	10.03513	11.45333	11.29977	0.5991
bta-miR-615	-14.0734	-13.7512	-13.8219	-13.948	-14.0369	-14.3961	0.2195
bta-miR-631	-4.26953	-4.2129	-4.03086	-4.24188	-4.54529	-4.43034	0.1086
bta-miR-652	10.92366	8.814116	9.530449	9.725567	9.939753	11.24534	0.5217
bta-miR-574	3.279292	4.053377	3.385927	4.628204	4.29557	3.131907	0.4350
bta-miR-656	8.329532	7.31398	7.855247	7.364714	7.449041	7.565872	0.2804
bta-miR-658	11.35638	12.33285	12.36686	12.62336	12.87776	11.30797	0.6917
bta-miR-664a	9.328307	9.435633	10.71014	8.443344	7.475161	9.382137	0.1205
bta-miR-760-3p	11.49194	10.5415	11.89981	10.12498	10.53274	10.2173	0.0726
bta-miR-760-5p	9.301696	9.048285	7.322767	8.669281	7.688808	9.12743	0.9379
bta-miR-665	9.124274	5.818836	8.439383	6.340084	7.122225	6.874781	0.3815
bta-miR-669	8.32293	7.877956	9.371353	9.091324	8.304583	8.315643	0.9323

bta-miR-763	9.695251	9.737562	10.12927	7.75111	10.64444	10.15372	0.7279
bta-miR-767	7.288161	7.450369	7.400957	7.51528	7.340191	7.24999	0.9073
bta-miR-677	13.46955	9.627939	8.509909	11.94178	8.815424	10.03988	0.8852
bta-miR-874	8.3628	6.219395	7.516171	7.378412	8.052797	6.950726	0.8992
bta-miR-877	7.699403	6.340729	8.071043	6.731041	7.003788	6.628012	0.3394
bta-miR-885	10.78184	4.801031	11.17554	6.598738	8.39591	9.161667	0.7132
bta-miR-92a	7.19538	5.61788	7.395399	6.304861	6.970467	8.036798	0.6517
bta-miR-92b	5.457097	4.656933	4.59609	5.059885	5.178087	5.000406	0.5666
bta-miR-935	10.45087	8.46699	9.623718	9.354088	9.427416	10.55638	0.7218
bta-miR-940	4.296811	3.870539	3.744774	4.588444	4.275368	2.202326	0.7318
bta-miR-1224	3.74479	4.25971	4.724144	3.816919	3.030884	4.008503	0.2041
bta-miR-1225-3p	6.121905	5.651754	5.513281	5.468018	6.255581	4.38986	0.5311
bta-miR-1246	-0.79415	1.301109	0.382721	1.356659	1.067633	0.15473	0.4700
bta-miR-1247-5p	6.256555	6.196478	7.19758	4.696552	6.939753	6.186398	0.4534
bta-miR-1248	11.24695	10.47711	7.387741	10.45074	10.99259	12.8976	0.2791
bta-miR-1306	11.44998	10.18557	11.36619	11.87962	10.45579	12.77763	0.4232
bta-miR-1260b	2.665286	2.223625	-1.02544	3.029955	0.301739	2.516743	0.6684
bta-miR-1307	3.854907	3.954506	4.820864	4.451782	5.129292	3.894249	0.5818
bta-miR-1343-3p	8.379815	6.716177	8.659832	7.203905	7.20858	8.25737	0.6326
bta-miR-1343-5p	5.028543	5.461735	5.332357	5.41522	4.944231	4.916775	0.4276
bta-miR-1281	6.764709	7.178242	5.994081	7.64258	6.95764	4.756683	0.8466
bta-miR-1388-3p	8.557364	7.022686	8.206028	7.325203	8.648565	9.703217	0.4899
RNT43 snoRNA	8.188893	6.493582	7.319403	9.545467	6.874199	7.577818	0.5171
Hm/Ms/Rt T1 snRNA	-2.73956	-0.90206	-2.65185	-2.51664	-2.46216	-1.4505	0.9509
bta-miR-1287	9.786246	7.2726	8.362364	8.50585	8.930414	9.128262	0.6383
bta-miR-99b	-0.26955	0.20104	0.216698	-0.14635	-0.13543	-0.1005	0.3315

[1] Body energy reserve: MBER: Cows with moderated body energy reserve; HBER: Cows with high body energy reserve; [2] P-value: P value between animals with different body energy reserve.

Supplementary table 5. Biological patwhays predicted as modulated by miRNAs up regulated in ampullary extracellular vesicles (AMP-EVs) in moderated body energy reserve (MBER) group.

Pathway	**%^{1}**	**BH^2**
bta04360 Axon guidance	27.53	0.0094
bta04664 Fc epsilon RI signaling pathway	37.14	0.0094
bta04728 Dopaminergic synapse	29.85	0.0094
bta04928 Parathyroid hormone synthesis. secretion and action	32.69	0.0094
bta05200 Pathways in cancer	21.77	0.0094
bta05214 Glioma	36.36	0.0094
bta04014 Ras signaling pathway	24.79	0.0121
bta04015 Rap1 signaling pathway	25.00	0.0121
bta04024 cAMP signaling pathway	24.89	0.0121
bta04062 Chemokine signaling pathway	26.60	0.0121
bta04071 Sphingolipid signaling pathway	29.17	0.0121
bta04261 Adrenergic signaling in cardiomyocytes	27.33	0.0121
bta04371 Apelin signaling pathway	27.86	0.0121
bta05225 Hepatocellular carcinoma	26.44	0.0121
bta04012 ErbB signaling pathway	32.14	0.0124
bta04072 Phospholipase D signaling pathway	26.97	0.0124
bta05212 Pancreatic cancer	32.89	0.0126
bta05223 Non-small cell lung cancer	34.33	0.0126
bta04810 Regulation of actin cytoskeleton	24.64	0.0149
bta04660 T cell receptor signaling pathway	28.97	0.017
bta04933 AGE-RAGE signaling pathway in diabetic complications	29.13	0.0175
bta00564 Glycerophospholipid metabolism	28.85	0.0185

bta05160 Hepatitis C	25.61	0.0185
bta04144 Endocytosis	23.27	0.0212
bta04010 MAPK signaling pathway	22.26	0.0232
bta04730 Long-term depression	33.33	0.0232
bta04912 GnRH signaling pathway	29.03	0.0232
bta05205 Proteoglycans in cancer	23.90	0.0232
bta04919 Thyroid hormone signaling pathway	27.12	0.0234
bta04530 Tight junction	24.29	0.0274
bta05211 Renal cell carcinoma	30.99	0.0274
bta04150 mTOR signaling pathway	24.84	0.0283
bta04625 C-type lectin receptor signaling pathway	27.36	0.0283
bta05220 Chronic myeloid leukemia	29.87	0.0283
bta05218 Melanoma	30.14	0.0307
bta04350 TGF-beta signaling pathway	27.96	0.0322
bta01522 Endocrine resistance	27.66	0.0343
bta05210 Colorectal cancer	28.09	0.0343
bta05230 Central carbon metabolism in cancer	30.30	0.0385
bta04925 Aldosterone synthesis and secretion	27.08	0.04
bta05017 Spinocerebellar ataxia	27.08	0.04
bta01100 Metabolic pathways	17.55	0.0412
bta04370 VEGF signaling pathway	31.03	0.0412
bta04916 Melanogenesis	26.47	0.0412
bta04152 AMPK signaling pathway	25.20	0.0421
bta04666 Fc gamma R-mediated phagocytosis	26.88	0.0434
bta05215 Prostate cancer	26.53	0.0434
bta04725 Cholinergic synapse	25.44	0.0454

bta05231 Choline metabolism in cancer	26.26	0.0462
bta05163 Human cytomegalovirus infection	21.63	0.047
bta04921 Oxytocin signaling pathway	23.68	0.0472
bta05219 Bladder cancer	33.33	0.0506
bta04971 Gastric acid secretion	27.63	0.0539
bta04390 Hippo signaling pathway	23.08	0.0561
bta04670 Leukocyte transendothelial migration	24.78	0.0561
bta04724 Glutamatergic synapse	24.78	0.0561
bta04910 Insulin signaling pathway	23.57	0.0561
bta04922 Glucagon signaling pathway	25.24	0.0561
bta04934 Cushing syndrome	23.08	0.0561
bta04935 Growth hormone synthesis. secretion and action	24.58	0.0561
bta04926 Relaxin signaling pathway	23.85	0.0585
bta04962 Vasopressin-regulated water reabsorption	30.61	0.0612
bta04550 Signaling pathways regulating pluripotency of stem cells	23.24	0.0623
bta04720 Long-term potentiation	27.54	0.0623
bta05226 Gastric cancer	22.88	0.0623
bta01521 EGFR tyrosine kinase inhibitor resistance	26.25	0.0672
bta04722 Neurotrophin signaling pathway	23.77	0.0672
bta04972 Pancreatic secretion	24.51	0.0741
bta05032 Morphine addiction	25.00	0.0767
bta04920 Adipocytokine signaling pathway	26.39	0.0786
bta05133 Pertussis	25.97	0.0786
bta05202 Transcriptional misregulation in cancer	21.47	0.0786
bta04022 cGMP-PKG signaling pathway	21.89	0.0787
bta04068 FoxO signaling pathway	22.90	0.0815

bta04070 Phosphatidylinositol signaling system	24.24	0.083
bta04931 Insulin resistance	23.64	0.0838
bta05161 Hepatitis B	21.64	0.0853
bta04713 Circadian entrainment	24.00	0.086
bta04915 Estrogen signaling pathway	22.46	0.086
bta05031 Amphetamine addiction	26.09	0.0891
bta05224 Breast cancer	22.00	0.0891
bta04020 Calcium signaling pathway	20.79	0.0946
bta04750 Inflammatory mediator regulation of TRP channels	23.30	0.106
bta04662 B cell receptor signaling pathway	24.14	0.1061
bta00561 Glycerolipid metabolism	25.37	0.1086
bta04066 HIF-1 signaling pathway	22.73	0.1086
bta04218 Cellular senescence	21.08	0.1086
bta04611 Platelet activation	22.31	0.1086
bta05020 Prion diseases	31.25	0.1086
bta05142 Chagas disease (American trypanosomiasis)	22.61	0.1086
bta05167 Kaposi sarcoma-associated herpesvirus infection	20.39	0.1086
bta05414 Dilated cardiomyopathy (DCM)	23.23	0.1086
bta04726 Serotonergic synapse	22.41	0.1111
bta04270 Vascular smooth muscle contraction	21.80	0.1123
bta04310 Wnt signaling pathway	20.99	0.115
bta05221 Acute myeloid leukemia	25.00	0.115
bta05164 Influenza A	20.44	0.1263
bta00010 Glycolysis Gluconeogenesis	25.00	0.1271
bta04727 GABAergic synapse	23.08	0.1275
bta04140 Autophagy	21.13	0.1307

bta04215 Apoptosis	29.41	0.1307
bta04520 Adherens junction	24.29	0.1307
bta03320 PPAR signaling pathway	23.46	0.131
bta04929 GnRH secretion	24.62	0.131
bta05410 Hypertrophic cardiomyopathy (HCM)	22.83	0.131
bta01524 Platinum drug resistance	23.08	0.1568
bta04151 PI3K-Akt signaling pathway	18.23	0.1568
bta00562 Inositol phosphate metabolism	23.29	0.1587
bta01230 Biosynthesis of amino acids	23.29	0.1587
bta03030 DNA replication	27.78	0.1587
bta04211 Longevity regulating pathway	22.22	0.1615
bta04540 Gap junction	22.22	0.1615
bta01200 Carbon metabolism	21.24	0.1653
bta04911 Insulin secretion	22.35	0.1661
bta00071 Fatty acid degradation	26.19	0.1676
bta05213 Endometrial cancer	23.73	0.1852
bta00340 Histidine metabolism	30.43	0.1887
bta03430 Mismatch repair	30.43	0.1887
bta05418 Fluid shear stress and atherosclerosis	20.00	0.1895
bta04380 Osteoclast differentiation	20.15	0.1944
bta05014 Amyotrophic lateral sclerosis (ALS)	23.33	0.1944
bta00310 Lysine degradation	22.73	0.1979
bta04137 Mitophagy	22.73	0.1979
bta05132 Salmonella infection	18.75	0.1979
bta00410 beta-Alanine metabolism	26.47	0.1996
bta00592 alpha-Linolenic acid metabolism	27.59	0.1996

bta04668 TNF signaling pathway	20.34	0.1996
bta04924 Renin secretion	22.22	0.1996
bta04961 Endocrine and other factor-regulated calcium reabsorption	24.00	0.1996
bta03420 Nucleotide excision repair	24.44	0.204
bta04141 Protein processing in endoplasmic reticulum	19.28	0.2046
bta04510 Focal adhesion	18.69	0.2219
bta00510 N-Glycan biosynthesis	23.08	0.2291
bta00565 Ether lipid metabolism	23.08	0.2291
bta04923 Regulation of lipolysis in adipocytes	22.41	0.2363
bta00380 Tryptophan metabolism	23.40	0.2364
bta05162 Measles	19.08	0.239
bta04950 Maturity onset diabetes of the young	26.92	0.243
bta05412 Arrhythmogenic right ventricular cardiomyopathy (ARVC)	21.05	0.2468
bta00330 Arginine and proline metabolism	22.92	0.2505
bta04927 Cortisol synthesis and secretion	21.54	0.2505
bta05222 Small cell lung cancer	20.21	0.2505
bta05135 Yersinia infection	19.23	0.2539
bta04917 Prolactin signaling pathway	20.48	0.2584
bta00270 Cysteine and methionine metabolism	22.45	0.2628
bta04650 Natural killer cell mediated cytotoxicity	19.08	0.2628
bta04721 Synaptic vesicle cycle	20.51	0.2676
bta03410 Base excision repair	24.24	0.2724
bta04130 SNARE interactions in vesicular transport	24.24	0.2724
bta00230 Purine metabolism	18.66	0.2921
bta00514 Other types of O-glycan biosynthesis	22.22	0.2921
bta00515 Mannose type O-glycan biosynthesis	26.09	0.2921

bta00051 Fructose and mannose metabolism	23.53	0.2932
bta00280 Valine. leucine and isoleucine degradation	21.57	0.2938
bta04621 NOD-like receptor signaling pathway	17.93	0.2953
bta05170 Human immunodeficiency virus 1 infection	17.52	0.2953
bta05235 PD-L1 expression and PD-1 checkpoint pathway in cancer	19.35	0.3039
bta04914 Progesterone-mediated oocyte maturation	19.32	0.3199
bta00220 Arginine biosynthesis	26.32	0.323
bta00770 Pantothenate and CoA biosynthesis	26.32	0.323
bta00020 Citrate cycle (TCA cycle)	23.33	0.3243
bta03008 Ribosome biogenesis in eukaryotes	19.28	0.3287
bta04260 Cardiac muscle contraction	19.10	0.3287
bta00053 Ascorbate and aldarate metabolism	24.00	0.3367
bta00052 Galactose metabolism	22.58	0.3428
bta00100 Steroid biosynthesis	25.00	0.3428
bta00532 Glycosaminoglycan biosynthesis	25.00	0.3428
bta04064 NF-kappa B signaling pathway	18.35	0.3428
bta05166 Human T-cell leukemia virus 1 infection	17.09	0.3428
bta05169 Epstein-Barr virus infection	17.11	0.3428
bta03018 RNA degradation	18.99	0.3514
bta05100 Bacterial invasion of epithelial cells	19.18	0.3514
bta04620 Toll-like receptor signaling pathway	18.18	0.3532
bta04210 Apoptosis	17.61	0.3635
bta04213 Longevity regulating pathway	19.35	0.3669
bta04973 Carbohydrate digestion and absorption	20.45	0.3669
bta00900 Terpenoid backbone biosynthesis	23.81	0.3679
bta04136 Autophagy	21.21	0.3824

bta04216 Ferroptosis	20.00	0.3824
bta04340 Hedgehog signaling pathway	19.61	0.3824
bta04913 Ovarian steroidogenesis	19.30	0.3824
bta03015 mRNA surveillance pathway	17.89	0.3939
bta04061 Viral protein interaction with cytokine and cytokine receptor	17.89	0.3939
bta04514 Cell adhesion molecules (CAMs)	17.09	0.3939
bta04723 Retrograde endocannabinoid signaling	17.11	0.3939
bta04930 Type II diabetes mellitus	19.57	0.3986
bta05152 Tuberculosis	16.67	0.4047
bta04146 Peroxisome	17.86	0.413
bta05144 Malaria	18.64	0.413
bta04217 Necroptosis	16.67	0.4215
bta04640 Hematopoietic cell lineage	17.27	0.4215
bta05146 Amoebiasis	17.09	0.4298
bta04975 Fat digestion and absorption	18.75	0.4344
bta05030 Cocaine addiction	18.75	0.4344
bta00591 Linoleic acid metabolism	19.44	0.441
bta01040 Biosynthesis of unsaturated fatty acids	20.00	0.4418
bta00600 Sphingolipid metabolism	18.37	0.4452
bta04142 Lysosome	16.67	0.4452
bta04918 Thyroid hormone synthesis	17.57	0.4452
bta04932 Non-alcoholic fatty liver disease (NAFLD)	16.46	0.4452
bta04970 Salivary secretion	17.20	0.4452
bta00260 Glycine. serine and threonine metabolism	18.60	0.4471
bta04659 Th17 cell differentiation	16.81	0.4471
bta00512 Mucin type O-glycan biosynthesis	19.35	0.4571

bta04120 Ubiquitin mediated proteolysis	16.43	0.4587
bta04979 Cholesterol metabolism	18.00	0.4597
bta00982 Drug metabolism	17.46	0.46
bta04714 Thermogenesis	15.90	0.46
bta05165 Human papillomavirus infection	15.65	0.46
bta00620 Pyruvate metabolism	18.42	0.4675
bta04976 Bile secretion	16.87	0.4682
bta05321 Inflammatory bowel disease (IBD)	17.14	0.4682
bta05016 Huntington disease	15.69	0.4695
bta05204 Chemical carcinogenesis	16.88	0.4732
bta01212 Fatty acid metabolism	17.24	0.477
bta05134 Legionellosis	17.24	0.477
bta04744 Phototransduction	18.52	0.5035
bta05216 Thyroid cancer	17.50	0.5068
bta00480 Glutathione metabolism	16.67	0.5165
bta05145 Toxoplasmosis	15.93	0.5178
bta00030 Pentose phosphate pathway	17.86	0.5284
bta04080 Neuroactive ligand-receptor interaction	15.15	0.5337
bta04060 Cytokine-cytokine receptor interaction	15.17	0.5361
bta00062 Fatty acid elongation	17.24	0.5434
bta00590 Arachidonic acid metabolism	15.85	0.5434
bta00601 Glycosphingolipid biosynthesis	17.24	0.5434
bta03020 RNA polymerase	17.24	0.5434
bta04392 Hippo signaling pathway	17.24	0.5434
bta04512 ECM-receptor interaction	15.73	0.5434
bta00983 Drug metabolism	15.79	0.5489

bta05217 Basal cell carcinoma	15.87	0.5553
bta00513 Various types of N-glycan biosynthesis	16.28	0.5559
bta00520 Amino sugar and nucleotide sugar metabolism	16.00	0.5608
bta04960 Aldosterone-regulated sodium reabsorption	16.22	0.5706
bta05010 Alzheimer disease	15.00	0.5741
bta04610 Complement and coagulation cascades	15.22	0.5806
bta04710 Circadian rhythm	16.13	0.5858
bta00500 Starch and sucrose metabolism	15.63	0.6133
bta00980 Metabolism of xenobiotics by cytochrome P450	14.93	0.6199
bta00860 Porphyrin and chlorophyll metabolism	15.00	0.6341
bta05033 Nicotine addiction	15.00	0.6341
bta05340 Primary immunodeficiency	14.63	0.6571
bta00240 Pyrimidine metabolism	14.29	0.6617
bta04114 Oocyte meiosis	14.29	0.6617
bta04115 p53 signaling pathway	14.29	0.6617
bta04658 Th1 and Th2 cell differentiation	14.29	0.6617
bta04672 Intestinal immune network for IgA production	14.29	0.6617
bta03440 Homologous recombination	14.29	0.6656
bta05140 Leishmaniasis	14.10	0.6689
bta00790 Folate biosynthesis	13.89	0.6911
bta04110 Cell cycle	13.82	0.6979
bta05143 African trypanosomiasis	13.64	0.7012
bta04630 JAK-STAT signaling pathway	13.86	0.7015
bta00140 Steroid hormone biosynthesis	13.43	0.7127
bta04623 Cytosolic DNA-sensing pathway	13.43	0.7127
bta05323 Rheumatoid arthritis	13.46	0.7193

bta04330 Notch signaling pathway	13.21	0.7208
bta05206 MicroRNAs in cancer	13.70	0.7292
bta05416 Viral myocarditis	13.16	0.7292
bta00350 Tyrosine metabolism	12.82	0.7308
bta00760 Nicotinate and nicotinamide metabolism	12.82	0.7308
bta04657 IL-17 signaling pathway	13.04	0.7391
bta04978 Mineral absorption	12.73	0.7411
bta03040 Spliceosome	12.93	0.7736
bta03013 RNA transport	12.92	0.7856
bta03460 Fanconi anemia pathway	11.54	0.8118
bta05203 Viral carcinogenesis	12.86	0.8129
bta03050 Proteasome	10.87	0.8379
bta04742 Taste transduction	11.39	0.8441
bta00830 Retinol metabolism	10.94	0.8532
bta00970 Aminoacyl-tRNA biosynthesis	10.61	0.8727
bta04612 Antigen processing and presentation	10.59	0.8937
bta05012 Parkinson disease	11.33	0.8967
bta05034 Alcoholism	11.79	0.9003
bta04622 RIG-I-like receptor signaling pathway	9.80	0.9424
bta04974 Protein digestion and absorption	9.92	0.949
bta04145 Phagosome	10.00	0.97
bta05150 Staphylococcus aureus infection	8.57	0.9762
bta05168 Herpes simplex virus 1 infection	11.17	0.9762
bta00190 Oxidative phosphorylation	7.14	1.0
bta03010 Ribosome	3.13	1.0
bta04740 Olfactory transduction	1.92	1.0

bta05322 Systemic lupus erythematosus	3.85	1.0

[1]%: Percent of genes predicted to be modulated. [2]BH: Benjamini – Hochberg

Supplementary table 6. Biological patwhays predicted as modulated by miRNAs up regulated in ampullary extracellular vesicles (AMP-EVs) in high body energy reserve (HBER) group.

Pathway	%[1]	BH[2]
bta04144 Endocytosis	48.98	0.0
bta04360 Axon guidance	49.44	0.0
bta04722 Neurotrophin signaling pathway	53.28	0.0
bta05200 Pathways in cancer	40.41	0.0
bta04010 MAPK signaling pathway	43.15	0.0045
bta04014 Ras signaling pathway	45.04	0.0045
bta05212 Pancreatic cancer	59.21	0.0045
bta04062 Chemokine signaling pathway	45.74	0.0078
bta04390 Hippo signaling pathway	46.15	0.0171
bta04514 Cell adhesion molecules (CAMs)	45.57	0.0171
bta05220 Chronic myeloid leukemia	54.55	0.0171
bta01522 Endocrine resistance	48.94	0.025
bta04072 Phospholipase D signaling pathway	44.74	0.025
bta04150 mTOR signaling pathway	43.95	0.025
bta04310 Wnt signaling pathway	44.44	0.025
bta04550 Signaling pathways regulating pluripotency of stem cells	45.07	0.025
bta04611 Platelet activation	46.28	0.025
bta04810 Regulation of actin cytoskeleton	42.18	0.025
bta04910 Insulin signaling pathway	45.00	0.025
bta04934 Cushing syndrome	44.23	0.025

bta05100 Bacterial invasion of epithelial cells	52.05	0.025
bta05132 Salmonella infection	41.07	0.025
bta05211 Renal cell carcinoma	53.52	0.025
bta05214 Glioma	51.95	0.025
bta05224 Breast cancer	44.00	0.025
bta05205 Proteoglycans in cancer	41.46	0.0253
bta04931 Insulin resistance	46.36	0.0302
bta05223 Non-small cell lung cancer	52.24	0.0302
bta05231 Choline metabolism in cancer	47.47	0.0302
bta04261 Adrenergic signaling in cardiomyocytes	43.33	0.0303
bta04721 Synaptic vesicle cycle	50.00	0.0303
bta05163 Human cytomegalovirus infection	39.59	0.0304
bta05225 Hepatocellular carcinoma	41.95	0.0304
bta05215 Prostate cancer	46.94	0.0322
bta01100 Metabolic pathways	32.61	0.033
bta04710 Circadian rhythm	64.52	0.033
bta04928 Parathyroid hormone synthesis. secretion and action	46.15	0.033
bta04520 Adherens junction	50.00	0.0377
bta05210 Colorectal cancer	47.19	0.0377
bta04012 ErbB signaling pathway	47.62	0.0388
bta04921 Oxytocin signaling pathway	42.11	0.0388
bta05219 Bladder cancer	57.14	0.0388
bta05160 Hepatitis C	40.85	0.0524
bta05226 Gastric cancer	41.18	0.0555
bta05217 Basal cell carcinoma	49.21	0.0612
bta04071 Sphingolipid signaling pathway	42.50	0.0667

bta04750 Inflammatory mediator regulation of TRP channels	43.69	0.0673
bta00562 Inositol phosphate metabolism	46.58	0.0676
bta04015 Rap1 signaling pathway	38.43	0.0676
bta04140 Autophagy	40.85	0.0676
bta04350 TGF-beta signaling pathway	44.09	0.0676
bta04659 Th17 cell differentiation	42.48	0.0676
bta04666 Fc gamma R-mediated phagocytosis	44.09	0.0676
bta05032 Morphine addiction	44.57	0.0676
bta05213 Endometrial cancer	49.15	0.0676
bta05218 Melanoma	46.58	0.0676
bta00600 Sphingolipid metabolism	51.02	0.0679
bta04070 Phosphatidylinositol signaling system	43.43	0.0679
bta04916 Melanogenesis	43.14	0.0679
bta04530 Tight junction	38.98	0.0741
bta05167 Kaposi sarcoma-associated herpesvirus infection	37.86	0.0836
bta05221 Acute myeloid leukemia	45.59	0.0959
bta04727 GABAergic synapse	42.86	0.0964
bta04926 Relaxin signaling pathway	40.00	0.1001
bta05135 Yersinia infection	40.00	0.1001
bta05170 Human immunodeficiency virus 1 infection	36.75	0.1001
bta00564 Glycerophospholipid metabolism	41.35	0.1027
bta04068 FoxO signaling pathway	39.69	0.1027
bta04371 Apelin signaling pathway	39.29	0.1027
bta04660 T cell receptor signaling pathway	41.12	0.1027
bta04670 Leukocyte transendothelial migration	40.71	0.1027
bta04110 Cell cycle	39.84	0.1073

bta04151 PI3K-Akt signaling pathway	34.58	0.1073
bta04152 AMPK signaling pathway	39.84	0.1073
bta04912 GnRH signaling pathway	41.94	0.1073
bta04625 C-type lectin receptor signaling pathway	40.57	0.1125
bta04668 TNF signaling pathway	39.83	0.1125
bta04919 Thyroid hormone signaling pathway	39.83	0.1125
bta05216 Thyroid cancer	50.00	0.1125
bta04024 cAMP signaling pathway	36.24	0.1135
bta01521 EGFR tyrosine kinase inhibitor resistance	42.50	0.1137
bta05133 Pertussis	42.86	0.1137
bta04728 Dopaminergic synapse	38.81	0.1169
bta04370 VEGF signaling pathway	44.83	0.1271
bta04966 Collecting duct acid secretion	53.57	0.1293
bta04664 Fc epsilon RI signaling pathway	42.86	0.1313
bta04961 Endocrine and other factor-regulated calcium reabsorption	46.00	0.1313
bta05412 Arrhythmogenic right ventricular cardiomyopathy (ARVC)	42.11	0.1327
bta05165 Human papillomavirus infection	34.20	0.1354
bta00604 Glycosphingolipid biosynthesis	62.50	0.1436
bta05169 Epstein-Barr virus infection	35.53	0.1438
bta04022 cGMP-PKG signaling pathway	36.69	0.1504
bta04730 Long-term depression	43.33	0.1504
bta04725 Cholinergic synapse	38.60	0.1548
bta05414 Dilated cardiomyopathy (DCM)	39.39	0.1558
bta05166 Human T-cell leukemia virus 1 infection	35.04	0.1637
bta01230 Biosynthesis of amino acids	41.10	0.1666
bta04914 Progesterone-mediated oocyte maturation	39.77	0.1666

bta05222 Small cell lung cancer	39.36	0.1666
bta04130 SNARE interactions in vesicular transport	48.48	0.1693
bta01200 Carbon metabolism	38.05	0.1717
bta04066 HIF-1 signaling pathway	38.18	0.1717
bta04726 Serotonergic synapse	37.93	0.1717
bta04510 Focal adhesion	35.35	0.1735
bta05410 Hypertrophic cardiomyopathy (HCM)	39.13	0.1735
bta00052 Galactose metabolism	48.39	0.1819
bta04137 Mitophagy	40.91	0.1828
bta04215 Apoptosis	47.06	0.1828
bta04720 Long-term potentiation	40.58	0.1828
bta04920 Adipocytokine signaling pathway	40.28	0.1828
bta05017 Spinocerebellar ataxia	38.54	0.1828
bta05230 Central carbon metabolism in cancer	40.91	0.1828
bta01210 2-Oxocarboxylic acid metabolism	55.56	0.1856
bta04933 AGE-RAGE signaling pathway in diabetic complications	37.86	0.1883
bta05202 Transcriptional misregulation in cancer	35.08	0.1883
bta04392 Hippo signaling pathway	48.28	0.1894
bta04911 Insulin secretion	38.82	0.1923
bta03015 mRNA surveillance pathway	37.89	0.2038
bta04658 Th1 and Th2 cell differentiation	37.76	0.2038
bta04217 Necroptosis	35.06	0.2071
bta05161 Hepatitis B	35.09	0.208
bta04211 Longevity regulating pathway	37.78	0.2214
bta01524 Platinum drug resistance	38.46	0.2265
bta04020 Calcium signaling pathway	34.16	0.2274

bta04114 Oocyte meiosis	36.13	0.2274
bta04145 Phagosome	34.71	0.2274
bta04915 Estrogen signaling pathway	35.51	0.2274
bta05014 Amyotrophic lateral sclerosis (ALS)	40.00	0.2274
bta00650 Butanoate metabolism	46.43	0.2277
bta04142 Lysosome	35.61	0.2277
bta04724 Glutamatergic synapse	36.28	0.2277
bta04210 Apoptosis	35.21	0.2333
bta00532 Glycosaminoglycan biosynthesis	50.00	0.2338
bta04270 Vascular smooth muscle contraction	35.34	0.2338
bta04657 IL-17 signaling pathway	36.96	0.2338
bta04923 Regulation of lipolysis in adipocytes	39.66	0.2338
bta04962 Vasopressin-regulated water reabsorption	40.82	0.2338
bta05134 Legionellosis	39.66	0.2338
bta04115 p53 signaling pathway	37.66	0.2443
bta04935 Growth hormone synthesis. secretion and action	35.59	0.245
bta04925 Aldosterone synthesis and secretion	36.46	0.2462
bta00500 Starch and sucrose metabolism	43.75	0.2463
bta04064 NF-kappa B signaling pathway	35.78	0.2463
bta04540 Gap junction	36.67	0.2463
bta05020 Prion diseases	43.75	0.2463
bta04146 Peroxisome	36.90	0.2493
bta05418 Fluid shear stress and atherosclerosis	34.48	0.2564
bta05145 Toxoplasmosis	35.40	0.2567
bta05164 Influenza A	33.70	0.2575
bta05323 Rheumatoid arthritis	35.58	0.2642

bta00280 Valine. leucine and isoleucine degradation	39.22	0.2654
bta04340 Hedgehog signaling pathway	39.22	0.2654
bta00514 Other types of O-glycan biosynthesis	40.00	0.2662
bta04971 Gastric acid secretion	36.84	0.2673
bta04218 Cellular senescence	33.73	0.2674
bta00770 Pantothenate and CoA biosynthesis	47.37	0.2801
bta04978 Mineral absorption	38.18	0.2833
bta05235 PD-L1 expression and PD-1 checkpoint pathway in cancer	35.48	0.2869
bta00030 Pentose phosphate pathway	42.86	0.2892
bta00051 Fructose and mannose metabolism	41.18	0.2893
bta04713 Circadian entrainment	35.00	0.2945
bta04213 Longevity regulating pathway	37.10	0.296
bta00533 Glycosaminoglycan biosynthesis	50.00	0.3003
bta04141 Protein processing in endoplasmic reticulum	33.13	0.3048
bta00310 Lysine degradation	36.36	0.3138
bta00515 Mannose type O-glycan biosynthesis	43.48	0.3152
bta04061 Viral protein interaction with cytokine and cytokine receptor	34.74	0.3152
bta05321 Inflammatory bowel disease (IBD)	35.71	0.3305
bta00330 Arginine and proline metabolism	37.50	0.332
bta00561 Glycerolipid metabolism	35.82	0.332
bta04970 Salivary secretion	34.41	0.3364
bta04922 Glucagon signaling pathway	33.98	0.3399
bta03030 DNA replication	38.89	0.3405
bta00640 Propanoate metabolism	39.39	0.3418
bta00630 Glyoxylate and dicarboxylate metabolism	40.00	0.3423
bta04640 Hematopoietic cell lineage	33.64	0.3423

bta04927 Cortisol synthesis and secretion	35.38	0.3523
bta04924 Renin secretion	34.72	0.3659
bta00240 Pyrimidine metabolism	35.71	0.367
bta05031 Amphetamine addiction	34.78	0.3686
bta05142 Chagas disease (American trypanosomiasis)	33.04	0.3699
bta00130 Ubiquinone and other terpenoid-quinone biosynthesis	50.00	0.3704
bta04723 Retrograde endocannabinoid signaling	32.24	0.3704
bta04972 Pancreatic secretion	33.33	0.3704
bta05162 Measles	32.24	0.3704
bta00220 Arginine biosynthesis	42.11	0.3753
bta04973 Carbohydrate digestion and absorption	36.36	0.3769
bta04913 Ovarian steroidogenesis	35.09	0.3784
bta04976 Bile secretion	33.73	0.3784
bta00010 Glycolysis Gluconeogenesis	34.38	0.3866
bta04120 Ubiquitin mediated proteolysis	32.14	0.3866
bta04621 NOD-like receptor signaling pathway	31.52	0.3866
bta04662 B cell receptor signaling pathway	33.33	0.3866
bta00062 Fatty acid elongation	37.93	0.3898
bta00230 Purine metabolism	32.09	0.3898
bta00592 alpha-Linolenic acid metabolism	37.93	0.3898
bta00601 Glycosphingolipid biosynthesis	37.93	0.3898
bta01212 Fatty acid metabolism	34.48	0.3898
bta04216 Ferroptosis	35.56	0.3898
bta03430 Mismatch repair	39.13	0.3959
bta00565 Ether lipid metabolism	34.62	0.3997
bta00603 Glycosphingolipid biosynthesis	41.18	0.3997

bta00910 Nitrogen metabolism	41.18	0.3997
bta00591 Linoleic acid metabolism	36.11	0.4028
bta00270 Cysteine and methionine metabolism	34.69	0.4029
bta01040 Biosynthesis of unsaturated fatty acids	36.67	0.4157
bta04330 Notch signaling pathway	33.96	0.4231
bta05033 Nicotine addiction	35.00	0.4244
bta00520 Amino sugar and nucleotide sugar metabolism	34.00	0.4281
bta04380 Osteoclast differentiation	31.34	0.4281
bta04960 Aldosterone-regulated sodium reabsorption	35.14	0.4281
bta00900 Terpenoid backbone biosynthesis	38.10	0.4298
bta00380 Tryptophan metabolism	34.04	0.4306
bta00410 beta-Alanine metabolism	35.29	0.4322
bta00053 Ascorbate and aldarate metabolism	36.00	0.4614
bta04612 Antigen processing and presentation	31.76	0.4614
bta03420 Nucleotide excision repair	33.33	0.4689
bta04940 Type I diabetes mellitus	32.20	0.4859
bta05416 Viral myocarditis	31.58	0.4859
bta04917 Prolactin signaling pathway	31.33	0.4888
bta04930 Type II diabetes mellitus	32.61	0.4964
bta04614 Renin-angiotensin system	34.62	0.4967
bta00260 Glycine. serine and threonine metabolism	32.56	0.5057
bta00360 Phenylalanine metabolism	34.78	0.5076
bta00100 Steroid biosynthesis	35.00	0.5224
bta04975 Fat digestion and absorption	31.25	0.5499
bta05030 Cocaine addiction	31.25	0.5499
bta05332 Graft-versus-host disease	31.25	0.5499

bta05340 Primary immunodeficiency	31.71	0.5499
bta00040 Pentose and glucuronate interconversions	32.26	0.5512
bta00512 Mucin type O-glycan biosynthesis	32.26	0.5512
bta00071 Fatty acid degradation	30.95	0.5761
bta04672 Intestinal immune network for IgA production	30.36	0.5768
bta00730 Thiamine metabolism	33.33	0.5782
bta04932 Non-alcoholic fatty liver disease (NAFLD)	29.11	0.5783
bta00511 Other glycan degradation	31.82	0.5804
bta00513 Various types of N-glycan biosynthesis	30.23	0.5804
bta00531 Glycosaminoglycan degradation	31.82	0.5804
bta00760 Nicotinate and nicotinamide metabolism	30.77	0.5804
bta00790 Folate biosynthesis	30.56	0.5804
bta03020 RNA polymerase	31.03	0.5804
bta04650 Natural killer cell mediated cytotoxicity	29.01	0.5804
bta04918 Thyroid hormone synthesis	29.73	0.5804
bta05146 Amoebiasis	29.06	0.5804
bta05152 Tuberculosis	28.79	0.5804
bta05203 Viral carcinogenesis	28.63	0.5804
bta00590 Arachidonic acid metabolism	29.27	0.5844
bta00860 Porphyrin and chlorophyll metabolism	30.00	0.5929
bta04136 Autophagy	30.30	0.5929
bta04714 Thermogenesis	28.45	0.5937
bta04929 GnRH secretion	29.23	0.5937
bta03022 Basal transcription factors	29.55	0.6002
bta00250 Alanine. aspartate and glutamate metabolism	29.73	0.6014
bta00510 N-Glycan biosynthesis	28.85	0.6216

bta03460 Fanconi anemia pathway	28.85	0.6216
bta03320 PPAR signaling pathway	28.40	0.6281
bta05140 Leishmaniasis	28.21	0.6406
bta04260 Cardiac muscle contraction	28.09	0.6427
bta04742 Taste transduction	27.85	0.6608
bta04620 Toll-like receptor signaling pathway	27.27	0.7008
bta05016 Huntington disease	27.37	0.7037
bta04964 Proximal tubule bicarbonate reclamation	27.27	0.7117
bta00980 Metabolism of xenobiotics by cytochrome P450	26.87	0.7158
bta04950 Maturity onset diabetes of the young	26.92	0.7158
bta00020 Citrate cycle (TCA cycle)	26.67	0.7218
bta00830 Retinol metabolism	26.56	0.7275
bta00340 Histidine metabolism	26.09	0.7361
bta03060 Protein export	26.09	0.7361
bta03440 Homologous recombination	26.19	0.7361
bta04744 Phototransduction	25.93	0.739
bta00534 Glycosaminoglycan biosynthesis	25.00	0.7707
bta04060 Cytokine-cytokine receptor interaction	26.63	0.7764
bta00140 Steroid hormone biosynthesis	25.37	0.7812
bta03008 Ribosome biogenesis in eukaryotes	25.30	0.7891
bta03040 Spliceosome	25.85	0.7891
bta05330 Allograft rejection	25.00	0.7891
bta04512 ECM-receptor interaction	24.72	0.8215
bta04977 Vitamin digestion and absorption	23.08	0.8215
bta05310 Asthma	23.68	0.8215
bta05150 Staphylococcus aureus infection	24.76	0.8293

bta04623 Cytosolic DNA-sensing pathway	23.88	0.8342
bta05144 Malaria	23.73	0.8342
bta05168 Herpes simplex virus 1 infection	26.05	0.8342
bta03013 RNA transport	24.72	0.8704
bta03410 Base excision repair	21.21	0.8775
bta04630 JAK-STAT signaling pathway	24.75	0.8775
bta03050 Proteasome	21.74	0.8806
bta04979 Cholesterol metabolism	22.00	0.8806
bta00620 Pyruvate metabolism	21.05	0.8813
bta00982 Drug metabolism	22.22	0.8813
bta03018 RNA degradation	22.78	0.8813
bta00350 Tyrosine metabolism	20.51	0.8869
bta00480 Glutathione metabolism	21.67	0.8869
bta00983 Drug metabolism	22.37	0.8869
bta01523 Antifolate resistance	20.93	0.8869
bta00563 Glycosylphosphatidylinositol (GPI)-anchor biosynthesis	19.23	0.887
bta05204 Chemical carcinogenesis	22.08	0.8888
bta05206 MicroRNAs in cancer	24.32	0.9106
bta04080 Neuroactive ligand-receptor interaction	24.52	0.9144
bta04610 Complement and coagulation cascades	21.74	0.9144
bta05012 Parkinson disease	22.67	0.9171
bta05034 Alcoholism	23.58	0.9171
bta05010 Alzheimer disease	22.22	0.9507
bta04622 RIG-I-like receptor signaling pathway	19.61	0.9699
bta05320 Autoimmune thyroid disease	18.31	0.9699
bta05143 African trypanosomiasis	15.91	0.9707

bta00970 Aminoacyl-tRNA biosynthesis	16.67	0.9837
bta04974 Protein digestion and absorption	19.01	0.9837
bta00190 Oxidative phosphorylation	19.29	0.9848
bta02010 ABC transporters	15.00	0.9873
bta03010 Ribosome	10.63	1.0
bta04740 Olfactory transduction	3.49	1.0
bta05322 Systemic lupus erythematosus	13.74	1.0

[1]%: Percent of genes predicted to be modulated. [2]BH: Benjamini – Hochberg

Supplementary Table 7. Raw cycle threshold levels of the 383 miRNAs profile in ampullary luminal epithelial cells (AMP-Cell) of cows with different body energy reserve.

miRNA	**Body energy reserve[1]**					
	MBER			**HBER**		
	1	**2**	**3**	**1**	**2**	**3**
bta-let-7a-3p	28.835	27.905	28.312	25.948	26.802	27.546
bta-miR-103	27.812	26.750	29.643	25.125	26.348	26.224
bta-let-7a-5p	22.837	21.861	22.618	19.972	21.518	21.505
bta-miR-105a	.	.	.	33.127	.	34.920
bta-let-7b	24.590	24.268	23.595	20.940	23.208	22.363
bta-miR-105b	33.512	33.868	36.658	31.140	31.606	32.872
bta-let-7c	23.417	22.683	22.704	20.415	21.867	21.436
bta-miR-106a	28.883	28.003	29.743	25.633	27.330	26.732
bta-let-7d	24.394	23.719	23.721	21.575	22.964	22.700
bta-miR-106b	30.825	30.006	.	27.614	29.287	28.813
bta-let-7e	22.094	22.168	22.372	19.524	20.673	20.859
bta-miR-107	31.754	31.482	32.886	29.026	30.573	30.988

bta-let-7f	23.724	22.874	24.335	21.619	22.299	22.780
bta-miR-10a	26.805	25.602	26.620	24.553	25.531	26.361
bta-let-7g	25.214	24.478	25.771	22.793	23.713	23.792
bta-miR-10b	27.011	25.852	27.361	24.765	25.771	26.661
bta-let-7i	27.645	26.284	28.800	24.358	25.833	25.575
bta-miR-122	.	.	.	33.595	.	.
bta-miR-1	29.753	28.446	33.147	27.745	27.849	29.217
bta-miR-124a	.	.	.	34.805	35.930	34.748
bta-miR-100	28.730	27.774	28.272	25.067	26.785	25.748
bta-miR-124b	.	.	.	33.665	35.529	.
bta-miR-101	32.592	31.466	30.767	27.666	29.578	29.209
bta-miR-125a	24.784	23.869	24.832	22.493	23.143	23.747
bta-miR-125b	24.080	23.013	24.680	20.651	21.742	21.709
bta-miR-133b	.	.	.	34.926	.	.
bta-miR-126-3p	33.414	32.750	33.554	29.441	32.930	.
bta-miR-133c	34.550	.	.	32.839	36.070	.
bta-miR-126-5p	.	30.810	.	28.720	30.636	.
bta-miR-134	35.056	.	.	28.700	35.250	34.010
bta-miR-127	33.307	33.842	33.644	32.041	32.896	33.192
bta-miR-135a	26.402	24.728	25.220	22.100	24.096	23.752
bta-miR-128	28.859	28.779	30.507	26.734	28.423	28.488
bta-miR-135b	27.902	26.433	26.624	23.815	25.702	25.244
bta-miR-129	31.674	32.034	.	31.742	31.864	.
bta-miR-136	.	.	.	.	.	.
bta-miR-129-3p	.	35.744	33.760	30.791	35.106	32.848
bta-miR-137	.	.	.	35.818	.	.
bta-miR-129-5p	33.022	32.341	34.813	31.803	31.792	31.725

bta-miR-138	35.016	32.743	.	27.814	33.678	31.880
bta-miR-130a	32.313	32.528	32.820	29.783	30.976	30.824
bta-miR-139	31.501	29.952	32.037	28.014	30.638	29.797
bta-miR-130b	25.594	24.895	26.192	23.511	24.646	24.840
bta-miR-140	29.823	28.927	31.548	27.449	27.975	29.328
bta-miR-132	31.642	31.600	33.805	28.651	30.746	31.117
bta-miR-141	29.031	27.926	29.172	25.790	27.271	26.804
bta-miR-133a	36.909	33.939	35.422	29.166	33.570	31.756
bta-miR-142-3p	.	.	.	32.758	.	.
bta-miR-142-5p	34.961	35.636	34.315	31.234	36.592	.
bta-miR-151-3p	27.805	26.687	28.651	25.495	26.320	27.273
bta-miR-143	31.686	32.371	30.728	29.197	31.419	30.724
bta-miR-151-5p	26.272	24.914	27.127	23.602	24.557	25.122
bta-miR-144	.	.	.	.	.	.
bta-miR-152	33.159	32.584	.	29.687	31.876	31.904
bta-miR-145	31.762	31.846	31.320	27.749	30.596	29.834
bta-miR-153	34.399	33.401	31.806	29.802	34.479	30.867
bta-miR-146a	34.960	33.890	35.063	33.883	35.807	33.491
bta-miR-154a	35.936	36.820	34.401	29.947	34.946	35.040
bta-miR-146b	34.632	34.690	.	31.843	33.390	36.375
bta-miR-154b	30.700	32.932	31.903	27.781	31.925	31.812
bta-miR-147	.	33.405	.	33.918	33.248	.
bta-miR-154c	35.103	.	.	32.848	.	36.964
bta-miR-148a	24.389	22.910	23.444	20.194	21.834	21.838
bta-miR-155	34.052	31.806	33.705	29.827	32.312	32.198
bta-miR-148b	24.995	23.991	23.896	20.644	22.812	22.580

bta-miR-15a	30.751	29.750	32.645	27.554	29.380	28.807
bta-miR-149-3p	31.909	31.799	.	28.584	30.734	29.409
bta-miR-15b	27.065	26.754	27.581	24.709	26.432	26.269
bta-miR-149-5p	32.558	34.231	36.096	28.856	32.426	31.866
bta-miR-16a	26.764	25.925	27.543	23.811	25.523	24.872
bta-miR-150	29.708	29.241	31.672	28.154	29.265	29.766
bta-miR-16b	25.933	24.921	26.809	23.068	24.606	24.132
bta-miR-17-3p	34.500	33.246	.	31.534	33.478	32.799
bta-miR-188	34.972	33.322	33.988	31.070	32.326	32.885
bta-miR-17-5p	33.416	31.832	33.064	29.806	30.832	30.786
bta-miR-18a	34.906	32.654	.	31.415	32.135	31.831
bta-miR-181a	33.587	33.170	.	.	32.883	32.293
bta-miR-18b	.	35.070	.	32.947	36.846	33.759
bta-miR-181b	31.764	30.696	31.904	29.556	30.700	31.731
bta-miR-190a	34.394	32.705	32.833	29.726	31.777	30.289
bta-miR-181c	.	.	.	32.692	.	33.543
bta-miR-190b	25.796	25.115	28.669	25.165	24.937	26.408
bta-miR-181d	30.807	29.738	31.808	28.025	30.100	29.310
bta-miR-191	25.370	24.613	26.775	23.111	23.834	24.759
bta-miR-182	29.791	30.084	31.363	27.572	29.714	29.672
bta-miR-192	32.004	31.491	33.972	29.769	30.531	32.207
bta-miR-183	30.827	30.566	31.693	28.764	30.167	30.438
bta-miR-193a	.	.	.	35.188	.	.
bta-miR-184	.	.	.	31.793	.	36.414
bta-miR-193a-3p	.	35.014	.	.	36.340	.
bta-miR-185	29.644	28.796	30.671	27.251	28.716	28.708

bta-miR-193a-5p	30.655	30.149	30.544	27.558	30.134	29.755
bta-miR-186	29.080	27.631	29.724	25.128	27.208	27.000
bta-miR-193b	.	.	.	32.825	34.998	.
bta-miR-187	.	32.502	33.116	29.305	.	30.796
bta-miR-194	30.761	29.967	30.603	27.455	28.925	28.776
bta-miR-195	26.657	25.709	28.131	24.318	24.966	25.005
bta-miR-200c	21.721	20.956	22.677	19.736	20.662	21.409
bta-miR-196a	.	.	35.109	30.781	35.183	36.841
bta-miR-202	33.491	.	.	31.283	31.828	32.778
bta-miR-196b	.	.	.	33.530	35.010	34.292
bta-miR-204	26.846	25.974	26.055	23.693	26.473	28.142
bta-miR-197	27.332	26.531	27.947	25.844	26.708	27.691
bta-miR-205	33.866	31.613	30.762	28.270	28.695	29.835
bta-miR-199a-3p	32.917	35.011	34.318	30.784	32.895	33.034
bta-miR-206	34.792	33.830	34.962	29.437	34.110	34.425
bta-miR-199a-5p	.	.	.	.	.	.
bta-miR-208a	34.497	34.456	.	.	34.525	.
bta-miR-199b	.	.	.	32.949	.	.
bta-miR-208b	.	.	.	36.671	.	.
bta-miR-199c	31.962	30.932	32.293	29.676	30.751	32.958
bta-miR-20a	29.204	27.933	29.435	25.667	27.265	26.721
bta-miR-19a	32.147	30.673	32.467	27.365	29.809	28.222
bta-miR-20b	30.457	29.449	31.599	26.901	28.642	28.599
bta-miR-19b	32.102	31.636	30.183	27.324	29.703	27.771
bta-miR-21-3p	.	.	.	32.905	33.884	33.987
bta-miR-200a	30.186	28.698	30.670	26.354	27.854	27.702

bta-miR-21-5p	32.131	29.940	29.834	27.958	28.761	28.822
bta-miR-200b	20.223	19.532	21.196	18.334	18.728	19.820
bta-miR-210	31.818	29.791	31.889	28.218	28.792	29.306
bta-miR-211	27.641	26.691	26.805	24.637	26.876	28.372
bta-miR-22-5p	31.126	29.986	32.133	28.048	30.635	30.055
bta-miR-212	.	.	.	34.160	.	.
bta-miR-221	28.811	27.818	30.797	26.706	27.690	27.702
bta-miR-214	33.435	34.511	34.053	31.701	35.289	32.421
bta-miR-222	27.784	27.437	.	25.758	26.832	26.933
bta-miR-215	31.831	30.796	31.723	29.240	29.756	31.665
bta-miR-223	33.788	33.279	35.684	28.814	31.614	30.789
bta-miR-216a	36.766	34.494	34.498	31.970	33.476	33.176
bta-miR-224	29.616	29.013	31.536	27.568	29.527	29.674
bta-miR-216b	34.293	32.585	.	32.167	32.638	33.970
bta-miR-23a	22.011	21.564	23.064	20.748	21.025	22.047
bta-miR-217	.	.	.	.	.	.
bta-miR-23b-3p	24.927	24.817	26.077	23.656	23.708	24.815
bta-miR-218	30.723	29.691	32.643	29.594	30.321	31.038
bta-miR-23b-5p	34.004	33.238	.	32.678	34.004	36.965
bta-miR-219	34.481	34.798	.	29.768	32.951	32.606
bta-miR-24	.	.	.	36.538	.	.
bta-miR-219-3p	32.403	32.930	33.141	30.234	31.693	31.581
bta-miR-24-3p	25.695	24.822	26.477	22.951	24.224	24.419
bta-miR-219-5p	.	.	.	.	.	.
bta-miR-25	25.477	24.793	26.566	23.831	24.310	25.220
bta-miR-22-3p	2.426	2.748	2.817	2.779	2.272	2.824

bta-miR-26a	22.296	21.383	22.637	19.573	20.403	21.331
bta-miR-26b	23.787	22.722	24.187	21.106	22.425	23.050
bta-miR-29d-3p	26.838	25.756	26.265	23.757	24.800	24.739
bta-miR-26c	.	.	.	.	.	.
bta-miR-29d-5p	31.457	29.689	31.184	28.423	29.279	30.028
bta-miR-27a-3p	27.001	25.765	26.328	23.690	25.366	24.769
bta-miR-29e	.	34.765	.	33.198	.	34.809
bta-miR-27a-5p	33.258	32.788	.	31.417	32.716	32.869
bta-miR-301a	.	.	.	33.652	.	.
bta-miR-27b	26.736	25.919	26.629	23.715	24.787	25.205
bta-miR-301b	.	.	.	.	.	36.137
bta-miR-28	30.780	30.021	31.635	27.934	29.013	29.857
bta-miR-302a	.	.	.	35.965	.	.
bta-miR-296-3p	.	.	31.529	29.507	30.258	30.581
bta-miR-302b	.	.	.	32.691	34.086	.
bta-miR-296-5p	33.955	32.162	32.812	28.963	31.262	.
bta-miR-302c	36.755	.	.	31.919	.	33.931
bta-miR-299	35.290	35.000	.	32.070	.	.
bta-miR-302d	.	35.783	.	.	.	34.683
bta-miR-29a	24.390	23.484	24.061	21.406	22.752	22.878
bta-miR-3064	34.924	33.333	.	31.804	36.939	34.220
bta-miR-29b	36.727	34.860	33.639	29.961	34.999	31.695
bta-miR-30a-5p	29.462	28.402	29.398	25.178	27.236	26.524
bta-miR-29c	24.531	23.498	23.849	21.560	22.749	22.781
bta-miR-30b-3p	32.126	31.335	33.585	30.268	30.855	31.989
bta-miR-30b-5p	27.879	26.553	26.713	23.186	25.649	24.640

bta-miR-328	33.452	30.231	30.745	28.935	30.591	29.813
bta-miR-30c	25.861	24.721	25.570	22.164	23.815	23.703
bta-miR-329a	.	.	.	.	.	.
bta-miR-30d	29.191	28.184	29.073	25.392	27.689	26.625
bta-miR-329b	.	36.599	.	33.880	.	35.698
bta-miR-30e-5p	29.510	28.806	29.265	25.336	27.518	26.691
bta-miR-330	35.360	32.912	33.658	32.604	36.909	33.687
bta-miR-30f	27.767	26.589	27.257	23.781	25.788	25.486
bta-miR-331-3p	31.495	30.017	35.825	28.384	29.707	30.159
bta-miR-31	25.694	24.773	26.884	23.463	24.245	24.853
bta-miR-331-5p	31.676	30.093	31.759	28.548	30.732	30.750
bta-miR-32	.	35.721	.	.	.	.
bta-miR-335	32.824	33.905	32.646	29.548	30.733	30.568
bta-miR-320a	27.035	26.753	28.570	24.800	26.661	26.722
bta-miR-338	.	34.444	34.839	31.796	33.689	31.697
bta-miR-320b	36.668	35.745	.	30.833	33.919	33.817
bta-miR-339a	30.081	29.411	30.795	26.760	28.460	28.690
bta-miR-323	17.002	17.610	17.163	17.530	17.301	17.567
bta-miR-339b	29.516	28.775	29.980	26.255	27.905	27.812
bta-miR-324	33.974	32.930	34.575	29.170	.	32.384
bta-miR-33a	.	.	36.053	33.196	.	35.250
bta-miR-326	33.450	33.690	34.390	30.716	33.025	32.868
bta-miR-33b	35.322	34.995	35.637	33.830	34.906	36.357
bta-miR-340	33.591	31.617	31.917	29.389	31.467	31.684
bta-miR-365-3p	29.511	28.546	29.166	25.737	27.111	27.454
bta-miR-342	30.818	29.725	30.784	28.004	29.429	29.838

bta-miR-365-5p	34.477	36.270	36.208	31.658	32.394	30.757
bta-miR-345-3p	32.174	30.720	32.769	29.837	31.870	.
bta-miR-367	.	.	.	.	.	.
bta-miR-345-5p	.	32.643	33.992	.	33.970	32.129
bta-miR-369-3p	.	.	.	.	.	.
bta-miR-346	33.183	33.227	34.958	30.469	32.055	30.545
bta-miR-369-5p	.	.	35.106	31.816	.	34.953
bta-miR-34a	29.152	28.654	30.835	26.658	28.177	28.560
bta-miR-370	32.842	34.995	33.266	.	32.875	33.714
bta-miR-34b	27.224	25.684	28.835	24.836	25.094	25.802
bta-miR-371	35.613	.	.	.	35.956	.
bta-miR-34c	26.951	25.733	28.755	24.820	24.810	25.786
bta-miR-374a	28.828	27.692	28.416	26.132	27.980	26.844
bta-miR-361	26.762	26.083	28.811	25.779	25.475	28.334
bta-miR-374b	27.182	26.167	27.199	24.608	25.373	25.841
bta-miR-362-3p	.	35.325	.	31.076	34.849	33.103
bta-miR-375	26.079	25.066	27.323	23.827	24.228	25.806
bta-miR-362-5p	34.126	32.550	36.970	30.984	32.807	32.890
bta-miR-376a	.	.	.	31.729	.	.
bta-miR-363	.	34.602	.	32.925	.	.
bta-miR-376b	.	.	.	.	.	34.535
bta-miR-376c	.	.	.	.	.	.
bta-miR-382	31.764	31.328	32.227	30.037	.	31.861
bta-miR-376d	.	35.981	.	32.290	.	33.333
bta-miR-383	35.141	33.559	34.125	30.614	36.551	33.645
bta-miR-376e	.	.	.	35.506	.	.

bta-miR-409a	.	.	35.602	.	34.721	36.084
bta-miR-377	.	.	.	.	.	.
bta-miR-409b	.	.	.	.	.	.
bta-miR-378	29.716	29.678	32.337	27.724	29.970	29.140
bta-miR-410	.	.	.	36.175	36.913	.
bta-miR-378b	31.109	30.521	31.674	27.725	30.321	29.319
bta-miR-411a	32.670	.	33.814	29.806	32.729	34.270
bta-miR-378c	33.248	32.861	34.888	31.891	32.884	32.719
bta-miR-411b	33.491	33.963	34.665	.	33.719	.
bta-miR-378d	35.327	34.162	36.272	30.841	33.998	33.688
bta-miR-411c-3p	.	.	.	33.922	36.201	36.208
bta-miR-379	.	.	.	33.855	.	.
bta-miR-411c-5p	.	.	35.487	.	.	.
bta-miR-380-3p	.	35.276	35.918	32.942	.	35.238
bta-miR-412	.	.	36.377	.	.	.
bta-miR-380-5p	.	33.087	34.060	28.800	36.685	35.113
bta-miR-421	30.452	29.271	30.517	27.551	28.945	29.641
bta-miR-381	.	35.702	.	34.884	.	36.628
bta-miR-423-3p	29.915	28.741	30.677	26.757	28.148	28.612
bta-miR-423-5p	28.294	27.919	28.555	25.766	27.315	27.534
bta-miR-449c	30.713	31.839	34.718	28.273	30.803	.
bta-miR-424-3p	33.331	34.016	34.597	32.846	32.637	33.877
bta-miR-449d	31.872	31.960	32.926	31.387	31.899	31.799
bta-miR-424-5p	29.527	27.918	29.336	25.522	27.321	26.397
bta-miR-450a	32.786	31.792	35.011	30.053	31.811	31.108
bta-miR-425-3p	27.744	27.306	.	.	26.527	.

bta-miR-450b	32.926	30.926	33.000	29.509	30.840	30.708
bta-miR-425-5p	31.107	29.744	30.491	26.684	28.892	27.918
bta-miR-451	.	34.841	33.943	27.833	33.327	35.041
bta-miR-429	27.329	26.222	27.231	24.016	25.701	25.428
bta-miR-452	36.197	33.959	.	32.952	34.206	34.657
bta-miR-431	.	.	.	35.435	34.715	35.705
bta-miR-4523	34.641	34.109	34.321	32.581	.	34.950
bta-miR-432	.	.	.	29.622	35.152	33.214
bta-miR-453	34.943	34.959	35.731	36.961	.	.
bta-miR-433	30.100	30.733	29.874	28.333	29.818	30.138
bta-miR-454	35.169	32.433	34.513	29.421	31.613	31.902
bta-miR-448	.	.	.	33.636	.	.
bta-miR-455-3p	32.869	32.845	34.081	29.746	30.594	31.688
bta-miR-449a	26.179	26.265	29.951	23.642	26.312	25.892
bta-miR-455-5p	34.807	34.390	34.633	32.295	32.891	32.928
bta-miR-449b	27.884	28.618	31.719	25.820	28.764	27.938
bta-miR-483	34.663	.	.	33.309	.	35.610
bta-miR-484	31.893	31.384	31.942	28.437	29.867	30.123
bta-miR-496	.	.	.	.	.	.
bta-miR-485	34.167	.	36.107	33.699	.	35.361
bta-miR-497	32.337	30.921	36.436	30.479	30.687	30.800
bta-miR-486	.	.	.	.	.	.
bta-miR-499	34.910	33.579	33.964	32.086	33.025	31.844
bta-miR-487a	.	.	.	32.918	.	.
bta-miR-500	32.120	30.968	34.942	29.218	30.692	31.051
bta-miR-487b	.	.	.	34.417	.	.

bta-miR-502a	34.631	33.885	.	34.385	.	33.387
bta-miR-488	36.865	.	.	31.938	.	.
bta-miR-502b	31.518	31.795	33.148	29.735	30.910	30.837
bta-miR-489	34.079	33.899	34.796	34.504	33.862	36.445
bta-miR-503-3p	.	.	33.512	29.566	.	30.768
bta-miR-490	36.296	.	.	33.243	33.052	34.042
bta-miR-503-5p	34.326	34.962	36.058	31.136	.	33.898
bta-miR-491	29.872	29.780	30.421	26.703	28.529	27.953
bta-miR-504	35.430	33.494	.	32.628	33.326	33.358
bta-miR-493	32.599	31.695	36.937	30.625	32.213	30.678
bta-miR-505	29.542	27.865	30.567	26.688	27.841	28.099
bta-miR-494	27.511	28.261	.	.	28.747	.
bta-miR-532	34.468	33.097	32.882	30.119	32.966	33.977
bta-miR-495	.	.	.	.	.	.
bta-miR-539	.	.	.	32.287	.	.
bta-miR-541	31.961	31.662	32.882	30.619	31.648	32.453
bta-miR-582	.	.	33.985	33.214	.	.
bta-miR-542-5p	35.302	32.747	35.118	31.868	32.857	31.734
bta-miR-584	33.443	33.544	35.383	31.448	33.975	31.723
bta-miR-543	.	34.369	.	33.886	35.859	.
bta-miR-592	34.365	35.598	.	31.668	.	35.295
bta-miR-544a	.	.	.	33.700	.	.
bta-miR-599	.	.	.	.	.	.
bta-miR-544b	34.909	.	.	33.965	34.675	.
bta-miR-615	.	.	.	.	.	.
bta-miR-545-3p	.	35.430	.	33.971	.	34.513

bta-miR-628	31.721	31.835	33.753	30.212	30.804	33.936
bta-miR-545-5p	.	.	.	33.510	.	.
bta-miR-631	19.223	19.256	19.071	19.237	19.265	19.075
bta-miR-551a	.	.	.	.	.	.
bta-miR-652	28.512	28.080	29.746	26.711	27.682	28.167
bta-miR-551b	.	.	.	34.900	.	33.889
bta-miR-653	.	34.723	.	.	.	.
bta-miR-562	.	.	.	.	.	.
bta-miR-654	33.910	32.220	33.876	32.006	33.584	31.904
bta-miR-568	.	.	.	.	.	.
bta-miR-655	.	.	.	33.406	.	.
bta-miR-574	28.024	27.497	28.995	26.279	26.988	26.593
bta-miR-656	33.756	32.817	34.048	31.464	33.886	33.249
bta-miR-658	.	.	.	34.583	34.326	34.895
bta-miR-758	.	34.534	.	31.638	.	.
bta-miR-660	31.819	29.208	30.743	27.314	28.815	29.168
bta-miR-759	.	.	.	.	.	.
bta-miR-664a	33.912	33.231	32.683	30.592	33.548	31.842
bta-miR-760-3p	34.218	34.880	.	.	33.409	32.840
bta-miR-664b	26.094	25.398	27.231	23.974	24.735	25.743
bta-miR-760-5p	26.565	25.881	27.841	24.685	25.621	25.624
bta-miR-665	32.160	30.975	32.706	29.245	29.722	29.718
bta-miR-761	36.911	.	.	33.737	36.423	35.403
bta-miR-669	29.697	29.769	31.281	28.695	29.053	29.454
bta-miR-763	34.394	35.194	35.377	31.805	34.820	34.199
bta-miR-670	.	36.846	.	33.905	.	34.161

bta-miR-764	34.513	.	34.129	33.938	34.444	35.484
bta-miR-671	34.371	34.105	.	33.604	32.921	34.537
bta-miR-767	32.941	33.419	32.860	32.480	32.898	33.614
bta-miR-677	36.510	31.822	32.867	30.685	33.067	31.787
bta-miR-769	.	33.714	.	32.303	32.945	33.869
bta-miR-7	29.961	28.793	30.072	27.396	28.290	29.301
bta-miR-873	35.734	36.633	.	32.270	35.653	.
bta-miR-708	30.485	30.214	31.399	28.216	29.779	29.709
bta-miR-874	.	30.838	33.778	28.838	30.137	30.163
bta-miR-744	27.715	27.171	29.264	26.635	26.975	28.533
bta-miR-875	.	.	.	35.117	.	.
bta-miR-876	.	36.465	.	34.560	.	.
bta-miR-98	28.116	26.973	28.706	25.939	26.757	27.274
bta-miR-877	.	.	33.890	.	31.278	.
bta-miR-99a-3p	32.901	33.773	.	29.722	31.884	33.014
bta-miR-885	26.808	25.971	26.752	24.202	25.384	25.746
bta-miR-99a-5p	28.859	27.684	28.020	23.985	26.406	24.944
bta-miR-9-3p	35.036	32.956	35.582	30.523	33.878	35.116
bta-miR-99b	22.763	22.870	22.371	22.761	22.832	22.684
bta-miR-9-5p	25.929	24.742	27.609	24.751	24.480	27.300
bta-miR-1179	.	36.693	.	33.350	.	.
bta-miR-92a	26.815	25.977	26.838	24.499	25.677	25.697
bta-miR-1185	.	.	.	.	.	.
bta-miR-92b	25.326	24.827	25.579	22.878	24.381	24.509
bta-miR-1193	.	.	.	34.182	36.734	.
bta-miR-93	28.927	28.114	30.276	26.477	27.741	28.221

bta-miR-1197	.	35.079	.	.	.	.
bta-miR-935	.	34.270	.	30.976	33.736	32.567
bta-miR-122	.	.	.	36.863	.	.
bta-miR-940	27.742	26.434	28.823	25.770	26.670	26.424
bta-miR-1224	29.751	30.847	.	.	.	.
bta-miR-95	30.819	31.715	30.379	29.644	31.645	31.885
bta-miR-1225-3p	28.949	27.791	30.173	26.998	27.833	27.817
bta-miR-96	.	34.891	.	31.115	35.032	33.443
bta-miR-1246	.	.	.	.	.	.
bta-miR-1247-3p	31.869	31.843	34.907	.	31.622	31.877
bta-miR-1296	33.883	32.446	34.593	30.244	31.743	.
bta-miR-1247-5p	31.906	30.836	33.943	29.752	30.272	30.031
bta-miR-1298	.	.	.	.	.	35.924
bta-miR-1248	32.811	32.326	32.897	30.226	32.785	30.511
bta-miR-1301	34.920	35.727	.	.	34.066	.
bta-miR-1249	29.913	28.696	29.621	27.829	28.740	29.144
bta-miR-1306	29.660	29.396	30.292	27.678	28.830	28.776
bta-miR-1260b	24.173	23.208	24.032	22.220	23.695	23.673
bta-miR-1307	29.177	28.654	.	.	27.997	28.237
bta-miR-1271	33.649	34.548	.	31.712	35.825	32.918
bta-miR-1343-3p	31.949	31.131	30.335	28.578	30.118	29.798
bta-miR-1277	.	.	.	.	.	.
bta-miR-1343-5p	30.119	29.557	30.922	28.653	29.330	28.967
bta-miR-1281	31.735	30.904	.	29.626	30.909	30.356
bta-miR-1388-3p	32.924	30.932	32.521	28.377	32.227	31.557
bta-miR-1282	.	.	.	.	.	.

RNT43 snoRNA	28.618	27.985	26.365	24.704	27.720	24.754
bta-miR-1284	.	34.943	.	.	33.711	34.147
Hm/Ms/Rt T1 snRNA	20.971	19.574	19.682	16.686	19.004	17.789
bta-miR-1287	.	34.347	.	33.926	.	34.939
bta-miR-99b	22.604	22.688	22.760	22.483	22.798	22.774
bta-miR-1291	34.557	32.943	.	.	35.021	32.946
Negative control	.	.	.	.	.	.

[1] Body energy reserve: MBER: Cows with moderated body energy reserve; HBER: Cows with high body energy reserve.

Supplementary Table 8. Raw cycle threshold levels of the 383 miRNAs profile in isthmic luminal epithelial cells (IST-Cell) of cows with different body energy reserve.

miRNA	**Body energy reserve**[1]					
	MBER			**HBER**		
	1	**2**	**3**	**1**	**2**	**3**
bta-let-7a-3p	25.990	25.861	26.589	26.341	25.703	25.818
bta-miR-103	26.724	26.018	26.196	27.613	25.873	26.133
bta-let-7a-5p	20.054	20.193	20.655	19.635	19.990	19.510
bta-miR-105a	.	34.467	.	34.991	36.061	.
bta-let-7b	21.352	21.414	21.781	20.803	21.046	20.781
bta-miR-105b	32.580	.	33.433	34.999	33.052	32.271
bta-let-7c	20.133	20.319	20.795	19.782	20.108	19.663
bta-miR-106a	26.833	26.105	26.339	27.831	25.936	25.912
bta-let-7d	21.181	21.247	21.813	20.775	21.361	20.730
bta-miR-106b	28.682	28.306	27.710	30.484	27.783	27.955
bta-let-7e	19.902	19.786	20.549	19.455	19.799	19.273
bta-miR-107	30.803	29.818	29.737	31.490	30.721	29.872

bta-let-7f	21.254	21.633	.	21.123	21.488	20.720
bta-miR-10a	23.713	24.278	25.267	24.722	23.856	24.276
bta-let-7g	22.611	22.397	23.187	23.183	22.447	22.181
bta-miR-10b	24.178	24.511	25.279	25.391	24.581	24.701
bta-let-7i	25.512	24.768	24.967	25.712	24.973	24.744
bta-miR-122	.	35.070	.	35.871	.	35.229
bta-miR-1	29.811	29.135	27.477	30.174	29.256	29.464
bta-miR-124a	35.880	.	.	.	35.866	.
bta-miR-100	25.112	24.993	25.732	26.396	24.593	24.665
bta-miR-124b	.	34.278	34.685	.	35.929	.
bta-miR-101	28.967	28.035	28.578	29.799	27.937	28.311
bta-miR-125a	22.391	22.390	22.741	22.934	22.147	22.090
bta-miR-125b	22.522	21.990	21.808	23.142	21.649	21.570
bta-miR-133b	.	34.838	36.832	.	.	.
bta-miR-126-3p	.	.	30.562	30.625	28.322	29.888
bta-miR-133c	.	35.158	.	.	35.355	.
bta-miR-126-5p	27.834	27.630	.	28.636	27.074	28.683
bta-miR-134	.	34.625	.	.	.	36.640
bta-miR-127	30.601	30.003	30.395	31.418	30.730	31.724
bta-miR-135a	22.708	21.910	22.071	22.831	21.816	21.765
bta-miR-128	26.316	25.826	26.839	27.352	26.426	25.833
bta-miR-135b	23.775	23.217	23.354	24.275	23.511	22.952
bta-miR-129	.	31.492	32.714	.	31.769	31.887
bta-miR-136	.	35.791	34.875	.	.	34.935
bta-miR-129-3p	33.941	31.524	32.862	32.715	31.312	31.527
bta-miR-137	.	.	.	.	.	.
bta-miR-129-5p	32.118	31.390	32.647	.	31.643	32.839

bta-miR-138	.	.	.	33.559	32.482	32.917
bta-miR-130a	29.769	30.011	30.061	31.151	30.816	31.582
bta-miR-139	29.032	28.480	29.112	28.833	28.541	27.646
bta-miR-130b	23.399	22.786	23.269	23.960	23.283	23.389
bta-miR-140	28.801	28.318	28.471	29.566	27.936	28.587
bta-miR-132	29.747	29.887	31.131	30.790	30.798	29.491
bta-miR-141	27.789	26.559	26.807	28.779	25.961	26.413
bta-miR-133a	32.859	33.287	31.095	35.314	32.928	33.836
bta-miR-142-3p	.	32.537	.	33.500	34.854	34.756
bta-miR-142-5p	33.958	33.910	32.668	35.567	33.270	33.641
bta-miR-151-3p	26.746	26.129	26.231	26.746	25.988	25.942
bta-miR-143	26.369	25.403	25.827	26.520	25.808	25.470
bta-miR-151-5p	24.455	23.801	24.064	24.525	23.700	23.801
bta-miR-144	.	.	.	.	.	.
bta-miR-152	31.422	31.677	31.078	32.252	31.910	30.263
bta-miR-145	28.787	28.514	30.487	29.813	27.976	28.047
bta-miR-153	29.636	28.890	29.667	30.742	29.742	28.635
bta-miR-146a	31.897	33.328	31.348	32.190	31.530	36.107
bta-miR-154a	32.897	32.670	35.049	34.938	32.962	32.449
bta-miR-146b	31.151	32.254	33.893	.	31.903	31.961
bta-miR-154b	29.662	29.420	28.640	30.762	30.136	29.723
bta-miR-147	32.934	33.209	31.827	.	31.114	32.276
bta-miR-154c	32.997	34.613	34.129	36.239	34.285	35.574
bta-miR-148a	20.977	20.355	20.486	21.057	19.956	19.812
bta-miR-155	29.424	29.864	31.256	29.816	31.147	30.219
bta-miR-148b	21.781	20.857	20.812	21.716	20.712	20.461

bta-miR-15a	28.822	28.348	27.878	30.287	28.190	27.929
bta-miR-149-3p	29.403	30.068	30.456	31.537	30.320	30.570
bta-miR-15b	24.793	25.190	25.308	25.231	25.120	24.534
bta-miR-149-5p	31.732	31.630	32.784	32.930	31.090	30.860
bta-miR-16a	24.768	24.352	24.053	25.689	24.059	24.015
bta-miR-150	26.697	27.785	28.357	28.519	27.803	27.922
bta-miR-16b	24.591	24.090	23.896	25.461	23.991	23.827
bta-miR-17-3p	33.997	32.837	32.127	33.178	31.567	33.419
bta-miR-188	32.812	30.537	.	32.338	.	31.012
bta-miR-17-5p	29.763	29.463	29.562	30.436	29.432	29.293
bta-miR-18a	31.830	31.882	32.154	.	32.829	31.751
bta-miR-181a	31.571	32.116	31.819	31.856	31.437	31.591
bta-miR-18b	33.829	33.146	33.759	35.029	33.072	33.348
bta-miR-181b	28.682	28.680	29.311	29.347	28.646	28.478
bta-miR-190a	30.571	30.163	29.826	32.503	30.665	30.617
bta-miR-181c	32.841	33.750	31.649	34.091	33.340	32.834
bta-miR-190b	25.972	25.555	25.297	25.957	26.399	25.736
bta-miR-181d	27.827	27.797	28.438	28.071	27.699	27.637
bta-miR-191	23.644	22.729	23.441	23.829	23.605	23.308
bta-miR-182	27.768	28.130	28.567	28.387	28.577	27.671
bta-miR-192	30.493	30.428	30.275	31.066	29.465	29.829
bta-miR-183	28.737	29.404	29.197	28.785	29.228	29.029
bta-miR-193a	.	.	38.254	.	.	.
bta-miR-184	.	35.337	35.518	36.988	36.418	33.993
bta-miR-193a-3p	35.711	.	34.337	.	35.308	.
bta-miR-185	28.775	28.284	27.876	29.727	27.829	28.250

bta-miR-193a-5p	29.145	28.684	29.447	28.735	29.503	29.489
bta-miR-186	26.642	25.967	26.040	27.744	25.662	25.686
bta-miR-193b	34.819	35.330	33.387	.	32.593	33.618
bta-miR-187	31.205	30.495	.	.	30.706	30.785
bta-miR-194	28.759	27.815	28.081	29.731	27.319	28.075
bta-miR-195	25.594	25.279	24.869	25.722	24.817	24.692
bta-miR-200c	20.501	20.443	20.744	20.705	20.473	20.067
bta-miR-196a	31.422	35.741	.	36.917	30.887	35.398
bta-miR-202	30.811	29.627	29.758	30.603	29.359	30.332
bta-miR-196b	30.727	32.847	32.778	32.768	31.535	31.924
bta-miR-204	24.579	23.980	24.562	24.727	24.033	24.696
bta-miR-197	26.459	25.760	26.813	26.548	26.008	25.894
bta-miR-205	28.544	28.705	29.806	30.090	26.039	27.644
bta-miR-199a-3p	27.767	27.807	30.827	28.757	26.779	28.680
bta-miR-206	34.021	33.390	35.000	35.028	34.011	34.760
bta-miR-199a-5p	34.673	33.222	35.728	35.048	31.313	34.105
bta-miR-208a	35.883	36.087	36.782	34.993	.	36.831
bta-miR-199b	32.751	32.885	34.959	.	32.105	.
bta-miR-208b	35.211	35.733	36.768	34.946	.	35.517
bta-miR-199c	27.312	27.054	30.146	27.964	26.123	27.692
bta-miR-20a	26.685	26.402	26.455	27.740	26.230	25.995
bta-miR-19a	27.609	27.174	27.461	29.736	27.330	27.044
bta-miR-20b	28.566	27.758	28.079	29.751	27.807	27.784
bta-miR-19b	27.692	27.028	27.309	29.480	27.007	26.889
bta-miR-21-3p	35.028	32.738	32.819	34.177	33.885	35.258
bta-miR-200a	27.039	26.876	26.993	28.304	26.768	26.839

bta-miR-21-5p	28.081	28.447	28.006	28.767	27.564	27.780
bta-miR-200b	18.582	18.632	18.853	18.991	18.490	18.153
bta-miR-210	29.771	28.971	29.124	30.730	28.814	28.735
bta-miR-211	25.427	24.672	25.429	24.798	23.983	25.469
bta-miR-22-5p	28.588	28.538	28.721	28.969	28.369	27.691
bta-miR-212	.	.	37.477	.	34.772	.
bta-miR-221	27.328	27.425	25.667	26.851	27.022	26.780
bta-miR-214	.	.	.	33.951	.	31.902
bta-miR-222	27.728	27.388	25.189	26.812	26.826	26.706
bta-miR-215	29.815	29.406	29.610	30.534	29.409	29.594
bta-miR-223	30.830	31.064	30.393	30.809	29.853	30.616
bta-miR-216a	32.851	31.677	32.106	35.342	.	.
bta-miR-224	27.679	27.355	28.305	27.789	28.429	27.537
bta-miR-216b	.	.	32.942	35.052	33.115	32.904
bta-miR-23a	20.746	20.722	21.084	21.153	20.823	20.559
bta-miR-217	.	.	.	.	.	.
bta-miR-23b-3p	23.332	23.424	23.832	23.699	23.531	23.360
bta-miR-218	30.233	29.785	29.589	30.759	30.649	29.908
bta-miR-23b-5p	32.865	33.608	32.900	33.499	32.751	33.202
bta-miR-219	33.090	.	32.608	33.931	31.870	.
bta-miR-24	.	.	.	.	.	.
bta-miR-219-3p	29.201	29.365	29.720	30.655	29.760	30.449
bta-miR-24-3p	23.799	23.313	23.607	24.690	23.216	23.386
bta-miR-219-5p	.	.	.	.	.	.
bta-miR-25	23.740	23.708	24.152	24.413	23.837	23.631
bta-miR-22-3p	2.767	2.434	2.516	2.776	2.694	2.685

bta-miR-26a	20.554	19.983	20.324	20.860	19.919	19.960
bta-miR-26b	21.233	20.888	21.445	21.231	20.886	20.798
bta-miR-29d-3p	22.933	22.134	21.997	23.191	22.020	22.137
bta-miR-26c	.	.	.	.	.	.
bta-miR-29d-5p	30.284	28.793	28.593	29.774	28.666	28.969
bta-miR-27a-3p	24.725	23.654	24.074	25.961	24.097	24.358
bta-miR-29e	33.631	32.911	32.107	35.478	32.102	31.861
bta-miR-27a-5p	36.255	32.803	31.895	33.268	32.904	33.261
bta-miR-301a	.	33.974	.	.	.	.
bta-miR-27b	24.701	24.343	25.161	25.377	24.082	24.236
bta-miR-301b	.	.	.	.	.	.
bta-miR-28	29.601	28.725	29.577	29.891	28.470	28.744
bta-miR-302a	.	.	34.830	.	.	34.350
bta-miR-296-3p	29.230	29.246	30.806	29.750	29.823	29.545
bta-miR-302b	.	.	.	34.768	.	.
bta-miR-296-5p	31.645	30.714	31.100	31.797	30.479	30.354
bta-miR-302c	33.869	33.978	37.498	.	34.691	36.281
bta-miR-299	.	.	.	.	35.637	.
bta-miR-302d	.	33.975	.	.	.	.
bta-miR-29a	20.487	20.229	20.468	20.997	19.787	20.067
bta-miR-3064	35.491	32.164	31.837	32.936	.	33.013
bta-miR-29b	31.246	29.701	30.208	31.044	29.756	29.846
bta-miR-30a-5p	27.166	26.360	26.508	27.772	26.039	26.256
bta-miR-29c	20.322	19.984	20.390	20.794	19.831	20.218
bta-miR-30b-3p	31.221	30.995	30.820	29.827	31.121	30.486
bta-miR-30b-5p	24.992	24.383	24.128	25.810	23.809	24.098

bta-miR-328	30.149	27.465	28.974	29.392	27.824	27.075
bta-miR-30c	23.710	23.360	23.275	24.381	22.941	23.220
bta-miR-329a	.	.	.	.	.	.
bta-miR-30d	27.272	26.517	26.135	27.784	26.072	26.298
bta-miR-329b	.	36.088	.	35.783	.	.
bta-miR-30e-5p	27.110	26.557	26.214	28.085	26.291	26.141
bta-miR-330	34.091	32.715	32.878	35.654	32.661	33.965
bta-miR-30f	25.586	25.121	24.813	26.377	24.686	24.939
bta-miR-331-3p	29.742	29.677	29.418	28.571	28.797	29.374
bta-miR-31	23.978	23.742	23.974	25.184	23.659	23.819
bta-miR-331-5p	29.499	29.412	29.667	30.337	29.745	29.762
bta-miR-32	.	.	.	.	34.551	.
bta-miR-335	29.794	30.371	28.748	33.346	28.599	29.665
bta-miR-320a	25.495	25.618	25.841	25.421	25.828	25.546
bta-miR-338	34.167	31.586	30.734	33.193	30.916	31.468
bta-miR-320b	33.848	33.127	.	34.830	33.870	35.308
bta-miR-339a	27.925	27.575	27.663	28.699	27.478	27.712
bta-miR-323	15.702	15.593	15.838	16.003	16.430	16.207
bta-miR-339b	27.712	27.064	26.782	28.481	26.723	27.177
bta-miR-324	30.804	30.081	31.366	31.581	29.989	30.513
bta-miR-33a	35.915	.	33.465	.	34.320	34.877
bta-miR-326	33.114	31.860	35.137	32.894	32.086	32.793
bta-miR-33b	33.260	32.866	34.022	34.431	35.989	35.362
bta-miR-340	31.070	31.267	30.927	30.985	31.744	30.278
bta-miR-365-3p	25.993	25.262	26.690	26.004	25.213	25.110
bta-miR-342	28.199	27.831	28.649	29.246	28.100	28.115

bta-miR-365-5p	35.622	34.275	34.611	33.113	32.696	32.748
bta-miR-345-3p	30.347	30.020	30.737	30.265	30.184	29.917
bta-miR-367	.	.	.	.	.	.
bta-miR-345-5p	31.454	31.156	31.149	32.700	31.030	.
bta-miR-369-3p	32.339	33.222	34.349	33.224	33.562	33.956
bta-miR-346	31.775	30.769	.	31.432	30.926	30.835
bta-miR-369-5p	.	.	.	36.024	33.577	.
bta-miR-34a	26.333	25.960	26.827	27.825	26.278	26.440
bta-miR-370	35.370	34.517	33.181	.	.	32.552
bta-miR-34b	25.998	25.238	24.859	27.363	24.793	25.299
bta-miR-371	35.132	34.350	34.222	.	34.897	36.526
bta-miR-34c	25.931	25.240	25.056	27.066	24.709	25.078
bta-miR-374a	26.394	26.357	26.343	27.480	25.949	25.767
bta-miR-361	26.310	26.489	26.723	24.800	26.384	26.250
bta-miR-374b	24.766	24.822	24.815	25.379	24.616	24.493
bta-miR-362-3p	32.906	31.757	31.975	.	31.573	31.954
bta-miR-375	24.371	23.595	24.394	23.761	23.494	23.824
bta-miR-362-5p	32.287	32.834	31.645	32.821	32.085	31.777
bta-miR-376a	.	.	.	.	.	.
bta-miR-363	36.142	34.933	.	.	.	33.916
bta-miR-376b	.	.	.	.	.	.
bta-miR-376c	.	32.851	.	.	33.998	32.935
bta-miR-382	.	28.784	28.767	30.624	.	29.300
bta-miR-376d	34.788	.	.	.	36.072	.
bta-miR-383	.	35.938	33.396	34.511	33.922	33.280
bta-miR-376e	.	.	.	34.834	.	.

bta-miR-409a	35.315	.	34.646	36.539	34.408	34.664
bta-miR-377	.	.	.	.	.	.
bta-miR-409b	.	35.525	.	.	.	.
bta-miR-378	28.750	28.532	29.109	29.337	28.780	28.606
bta-miR-410	35.344	.	.	.	.	.
bta-miR-378b	28.803	28.560	28.791	29.819	29.485	28.609
bta-miR-411a	.	30.751	31.801	33.815	.	32.860
bta-miR-378c	31.706	30.793	31.594	31.950	32.539	31.022
bta-miR-411b	.	32.556	32.881	.	.	.
bta-miR-378d	32.564	31.948	32.356	34.361	33.860	33.029
bta-miR-411c-3p	.	.	34.993	.	.	33.966
bta-miR-379	.	.	32.839	.	.	.
bta-miR-411c-5p	.	.	.	34.374	35.145	.
bta-miR-380-3p	34.276	33.931	.	35.458	.	.
bta-miR-412	.	34.923	33.826	.	34.509	.
bta-miR-380-5p	36.275	.	.	.	32.738	32.895
bta-miR-421	26.607	25.858	27.215	27.343	28.022	27.435
bta-miR-381	36.259	32.912	35.137	.	35.840	34.135
bta-miR-423-3p	27.148	26.889	27.532	27.930	26.973	26.773
bta-miR-423-5p	25.827	25.799	26.914	26.556	26.413	25.780
bta-miR-449c	30.707	29.816	32.139	31.688	32.102	30.985
bta-miR-424-3p	32.791	31.797	31.620	33.981	33.121	31.907
bta-miR-449d	32.533	31.884	32.728	32.930	31.204	32.755
bta-miR-424-5p	27.536	27.461	25.749	28.142	27.487	26.202
bta-miR-450a	30.779	31.632	30.078	31.893	31.625	30.699
bta-miR-425-3p	.	.	.	.	.	.

bta-miR-450b	31.279	32.286	29.888	30.616	31.869	30.706
bta-miR-425-5p	28.296	27.667	27.565	28.988	27.546	27.470
bta-miR-451	30.730	30.405	34.523	30.845	29.676	29.643
bta-miR-429	24.534	24.113	24.150	25.970	24.059	23.773
bta-miR-452	33.969	32.186	33.023	.	33.198	32.888
bta-miR-431	.	33.046	35.232	35.116	34.142	35.546
bta-miR-4523	.	.	.	.	33.793	34.745
bta-miR-432	35.416	33.921	.	.	.	.
bta-miR-453	33.014	32.799	33.392	33.917	33.769	33.506
bta-miR-433	28.532	28.101	28.243	29.782	29.504	29.627
bta-miR-454	32.997	30.670	31.180	30.661	31.695	30.763
bta-miR-448	.	36.350	.	.	.	.
bta-miR-455-3p	29.714	29.702	30.304	30.887	29.260	30.243
bta-miR-449a	24.799	24.320	25.823	25.713	25.717	25.083
bta-miR-455-5p	31.760	31.852	31.803	34.153	31.154	33.793
bta-miR-449b	26.825	26.582	28.253	27.694	28.216	27.572
bta-miR-483	.	32.801	.	.	.	.
bta-miR-484	29.356	28.738	29.210	29.820	28.778	29.280
bta-miR-496	.	36.478	34.596	.	.	.
bta-miR-485	33.852	32.851	32.795	33.981	35.433	33.893
bta-miR-497	29.938	29.683	29.752	30.581	30.639	30.088
bta-miR-486	28.980	28.710	29.396	30.133	29.236	29.703
bta-miR-499	31.895	29.853	30.470	31.010	29.812	30.434
bta-miR-487a	34.422	35.550	.	.	.	.
bta-miR-500	30.692	29.784	30.081	29.782	29.473	29.692
bta-miR-487b	.	.	.	.	.	.

bta-miR-502a	33.676	34.963	33.010	32.913	34.999	34.381
bta-miR-488	.	35.573	.	35.732	.	33.887
bta-miR-502b	29.846	30.272	30.735	30.376	30.154	29.662
bta-miR-489	30.605	30.611	29.806	32.207	32.296	32.508
bta-miR-503-3p	31.243	30.365	31.709	31.997	31.608	31.643
bta-miR-490	34.570	31.783	.	35.204	33.783	.
bta-miR-503-5p	.	.	33.469	.	33.541	33.919
bta-miR-491	28.541	28.356	28.775	27.700	28.446	27.747
bta-miR-504	33.003	32.431	.	33.933	32.048	31.855
bta-miR-493	32.533	31.251	32.838	34.317	30.830	32.128
bta-miR-505	27.670	26.743	26.768	.	.	27.254
bta-miR-494	27.714	26.755	27.798	28.638	27.719	27.812
bta-miR-532	32.071	31.212	31.882	33.137	30.681	29.974
bta-miR-495	32.878	33.162	33.613	34.825	32.907	34.190
bta-miR-539	35.325	.	.	.	.	.
bta-miR-541	30.019	29.753	.	30.828	30.784	30.261
bta-miR-582	.	.	.	.	36.438	.
bta-miR-542-5p	33.831	34.821	32.893	35.011	.	33.812
bta-miR-584	32.322	31.530	32.861	33.369	33.309	34.111
bta-miR-543	34.776	33.831	.	.	34.583	35.466
bta-miR-592	31.862	30.764	.	32.311	30.472	31.697
bta-miR-544a	36.307	.	34.958	.	34.244	.
bta-miR-599	.	.	.	.	.	.
bta-miR-544b	.	.	.	.	.	.
bta-miR-615	.	.	.	.	.	.
bta-miR-545-3p	36.064	33.731	35.041	33.935	32.219	.

bta-miR-628	31.828	31.648	31.900	32.458	31.765	31.885
bta-miR-545-5p	.	.	.	34.175	.	34.181
bta-miR-631	18.456	18.015	18.360	18.707	18.774	18.645
bta-miR-551a	37.694	38.295	36.610	40.579	38.198	40.428
bta-miR-652	27.675	26.913	26.784	27.399	26.963	27.096
bta-miR-551b	34.789	33.056	.	.	33.278	34.586
bta-miR-653	.	.	44.541	.	40.601	42.456
bta-miR-562	.	.	.	.	.	.
bta-miR-654	31.833	31.967	32.269	31.795	33.098	33.863
bta-miR-568	.	.	.	.	.	.
bta-miR-655	36.729	34.929	.	.	33.566	.
bta-miR-574	27.783	26.821	27.978	27.813	26.362	27.220
bta-miR-656	30.265	29.720	30.339	31.103	31.577	30.780
bta-miR-658	36.388	.	36.057	.	.	36.561
bta-miR-758	.	36.062	.	.	.	.
bta-miR-660	28.947	28.168	27.889	28.617	27.995	27.755
bta-miR-759	.	.	.	.	.	.
bta-miR-664a	31.805	30.747	31.230	32.339	31.765	31.606
bta-miR-760-3p	33.315	31.911	32.936	32.717	31.516	32.667
bta-miR-664b	24.642	24.111	24.351	24.756	23.840	23.780
bta-miR-760-5p	25.769	.	25.601	26.775	25.271	24.698
bta-miR-665	29.856	29.352	30.145	31.488	29.631	29.868
bta-miR-761	33.776	34.561	33.735	38.253	33.829	38.139
bta-miR-669	30.720	29.811	29.716	30.068	28.750	30.833
bta-miR-763	34.231	33.822	33.544	35.917	35.355	33.860
bta-miR-670	35.027	34.811	.	.	34.712	33.198

bta-miR-764	32.838	32.228	33.327	34.064	34.124	35.476
bta-miR-671	33.439	33.782	35.034	33.438	32.826	33.250
bta-miR-767	30.388	30.620	31.027	31.467	31.716	31.871
bta-miR-677	30.625	29.894	31.012	31.485	30.532	30.678
bta-miR-769	31.386	32.803	32.090	32.477	31.830	31.708
bta-miR-7	26.392	26.672	26.805	26.469	26.108	26.072
bta-miR-873	.	33.540	34.912	35.914	34.900	.
bta-miR-708	29.234	28.390	29.585	29.213	29.151	28.525
bta-miR-874	31.062	30.638	30.764	31.320	30.532	30.319
bta-miR-744	26.907	27.255	27.331	27.642	27.420	27.495
bta-miR-875	36.641	.	.	.	.	.
bta-miR-876	.	35.514	.	.	.	.
bta-miR-98	25.433	25.547	25.754	25.658	25.701	24.716
bta-miR-877	30.640	.	30.865	.	.	.
bta-miR-99a-3p	31.752	32.599	32.732	33.492	32.025	31.880
bta-miR-885	24.967	24.004	24.628	24.829	23.934	24.083
bta-miR-99a-5p	24.991	24.485	24.695	26.464	23.826	24.153
bta-miR-9-3p	32.808	31.300	32.924	35.259	32.123	33.800
bta-miR-99b	22.765	22.204	22.607	22.714	22.656	22.724
bta-miR-9-5p	25.524	24.575	25.512	25.522	24.135	26.018
bta-miR-1179	33.744	.	.	.	.	.
bta-miR-92a	23.772	23.875	24.284	24.723	23.944	23.821
bta-miR-1185	.	.	.	.	.	.
bta-miR-92b	23.582	23.088	23.422	23.723	23.261	23.256
bta-miR-1193	.	.	38.136	.	.	35.056
bta-miR-93	26.793	26.929	26.795	27.749	26.771	26.603

bta-miR-1197	.	.	.	.	.	.
bta-miR-935	32.880	31.783	32.893	32.514	.	32.517
bta-miR-122	.	36.345	35.831	36.491	.	.
bta-miR-940	26.266	25.639	26.228	26.917	25.808	25.900
bta-miR-1224	29.840	29.350	31.121	30.194	29.655	29.737
bta-miR-95	28.255	28.758	29.833	30.319	28.836	29.583
bta-miR-1225-3p	28.148	27.486	27.831	28.810	27.543	28.058
bta-miR-96	32.527	33.976	31.624	.	32.795	31.940
bta-miR-1246	.	.	23.826	.	.	.
bta-miR-1247-3p	31.781	31.060	.	.	.	.
bta-miR-1296	31.822	32.795	33.861	32.526	31.160	32.635
bta-miR-1247-5p	30.126	29.654	30.062	31.259	29.297	29.793
bta-miR-1298	.	.	.	.	.	.
bta-miR-1248	.	29.024	30.086	32.160	29.708	30.373
bta-miR-1301	.	33.529	35.400	.	32.861	32.254
bta-miR-1249	27.154	26.896	27.767	27.099	27.009	26.534
bta-miR-1306	28.205	27.732	28.093	28.537	27.830	28.189
bta-miR-1260b	22.015	21.570	22.252	22.756	21.682	21.752
bta-miR-1307	27.320	.	27.532	28.207	27.236	26.978
bta-miR-1271	34.748	34.026	34.777	34.396	32.280	31.857
bta-miR-1343-3p	.	28.830	29.786	.	28.709	28.830
bta-miR-1277	.	.	.	.	.	.
bta-miR-1343-5p	28.756	28.829	29.594	29.745	28.116	28.951
bta-miR-1281	30.625	29.949	30.561	31.610	29.726	29.971
bta-miR-1388-3p	30.691	29.790	30.730	30.400	31.356	30.642
bta-miR-1282	.	.	34.911	.	34.270	.

RNT43 snoRNA	23.832	22.872	23.785	26.580	23.579	23.671
bta-miR-1284	34.252	33.714	.	34.702	36.830	34.160
Hm/Ms/Rt T1 snRNA	17.126	16.434	16.607	18.796	16.172	16.331
bta-miR-1287	.	33.872	34.786	36.843	34.914	34.008
bta-miR-99b	22.463	22.062	22.425	22.757	22.648	22.487
bta-miR-1291	35.330	34.765	32.843	34.785	31.319	35.626
Negative control	.	.	.	.	.	.

[1] Body energy reserve: MBER: Cows with moderated body energy reserve; HBER: Cows with high body energy reserve.

Supplementary Table 9. Normalized data of the 210 miRNAs commonly detected in ampullary luminal epithelial cells (AMP-Cell) of cows with different body energy reserve.

miRNA	**Body energy reserve[1]**						**P – value[2]**
	MBER			**HBER**			
	1	**2**	**3**	**1**	**2**	**3**	
bta-let-7a-3p	4.957328	4.710137	5.603642	4.900023	3.895859	5.995809	0.8212
bta-miR-103	3.934743	3.555394	6.934411	4.077137	3.441912	4.673582	0.5450
bta-let-7a-5p	-1.0407	-1.33307	-0.08998	-1.07646	-1.3883	-0.04521	0.9791
bta-let-7b	0.713087	1.074027	0.886325	-0.10842	0.301775	0.812448	0.1239
bta-miR-105b	9.634544	10.67383	13.94961	10.09149	8.699806	11.32177	0.4105
bta-let-7c	-0.4601	-0.51107	-0.00443	-0.6334	-1.03963	-0.11397	0.4357
bta-miR-106a	5.005369	4.808623	7.03427	4.585173	4.423685	5.18202	0.3018
bta-let-7d	0.517088	0.524291	1.012826	0.527407	0.057995	1.149061	0.7796
bta-let-7e	-1.78345	-1.02627	-0.33653	-1.5242	-2.23294	-0.69096	0.5167
bta-miR-107	7.876983	8.288043	10.17733	7.977879	7.667219	9.437534	0.6631
bta-let-7f	-0.15379	-0.32066	1.626803	0.571257	-0.6073	1.229589	0.9875
bta-miR-10a	2.927812	2.407809	3.911635	3.504654	2.62499	4.810312	0.5059
bta-let-7g	1.336505	1.283955	3.062423	1.745238	0.807112	2.24152	0.7017

bta-miR-10b	3.133373	2.658036	4.652266	3.716582	2.864817	5.110916	0.6640
bta-let-7i	3.767807	3.089242	6.092017	3.310448	2.92713	4.02478	0.4056
bta-miR-1	5.875831	5.251748	10.43913	6.697046	4.943197	7.666963	0.7001
bta-miR-100	4.852184	4.579281	5.564168	4.019203	3.878679	4.197662	**0.0348**
bta-miR-101	8.715038	8.27199	8.058301	6.617766	6.671478	7.658285	**0.0248**
bta-miR-125a	0.906248	0.674683	2.123738	1.444843	0.236759	2.196766	0.9403
bta-miR-125b	0.202713	-0.18106	1.971929	-0.39658	-1.16439	0.158065	0.2134
bta-miR-127	9.42955	10.64718	10.93601	10.99283	9.989996	11.64132	0.4653
bta-miR-135a	2.525102	1.533659	2.511962	1.052307	1.189773	2.201788	0.2207
bta-miR-128	4.982053	5.584651	7.7984	5.685682	5.516405	6.937538	0.9417
bta-miR-135b	4.024982	3.23815	3.915713	2.7673	2.795528	3.693962	0.1769
bta-miR-129-5p	9.144226	9.146725	12.1047	10.75544	8.885351	10.17447	0.8724
bta-miR-130a	8.436031	9.333249	10.11151	8.734917	8.07004	9.273494	0.3706
bta-miR-139	7.624116	6.757847	9.328551	6.966444	7.732016	8.246996	0.7770
bta-miR-130b	1.716774	1.700852	3.483785	2.462773	1.739601	3.289281	0.8040
bta-miR-140	5.945303	5.733063	8.839791	6.400744	5.068563	7.77746	0.7556
bta-miR-132	7.764364	8.405299	11.09631	7.60248	7.840177	9.567003	0.5629
bta-miR-141	5.154054	4.731154	6.463277	4.742146	4.364565	5.253402	0.3180
bta-miR-133a	13.03179	10.74463	12.71399	8.118136	10.66413	10.20535	0.0779
bta-miR-151-3p	3.927322	3.492854	5.942998	4.447006	3.413942	5.722093	0.9455
bta-miR-143	7.808214	9.176606	8.019809	8.148466	8.512464	9.173742	0.6232
bta-miR-151-5p	2.394638	1.719218	4.418401	2.554201	1.650949	3.571273	0.8103
bta-miR-145	7.884601	8.65127	8.611838	6.700665	7.689562	8.283195	0.1910
bta-miR-153	10.52148	10.20687	9.097773	8.75362	11.57278	9.316486	0.9525
bta-miR-146a	11.08276	10.69557	12.35482	12.83467	12.90081	11.94099	0.1154
bta-miR-154a	12.0584	13.62574	11.69236	8.898516	12.04021	13.48956	0.5428
bta-miR-154b	6.822372	9.738045	9.194911	6.733311	9.018598	10.26114	0.9529
bta-miR-148a	0.511392	-0.28448	0.735795	-0.85391	-1.07194	0.287237	0.1726

bta-miR-155	10.17506	8.611487	10.99639	8.778744	9.405512	10.6473	0.7394
bta-miR-148b	1.117612	0.797009	1.187858	-0.40383	-0.0945	1.029152	0.1306
bta-miR-15a	6.873283	6.555979	9.937133	6.505936	6.474205	7.256885	0.3998
bta-miR-15b	3.18791	3.559334	4.872838	3.660479	3.525618	4.718773	0.8884
bta-miR-149-5p	8.680679	11.03685	13.38745	7.807711	9.51959	10.31572	0.3045
bta-miR-16a	2.886583	2.730166	4.834689	2.763123	2.616796	3.32142	0.4575
bta-miR-150	5.830991	6.046217	8.963254	7.106392	6.35895	8.215044	0.8189
bta-miR-16b	2.055996	1.72654	4.100799	2.019779	1.699417	2.581775	0.5390
bta-miR-188	11.09493	10.12727	11.27974	10.0219	9.420259	11.3343	0.4382
bta-miR-17-5p	9.538467	8.637652	10.35567	8.758341	7.925947	9.236011	0.2371
bta-miR-181b	7.88706	7.501322	9.195849	8.507481	7.793415	10.18061	0.5092
bta-miR-190a	10.51693	9.510144	10.12479	8.677942	8.870993	8.738355	**0.0125**
bta-miR-190b	1.918253	1.920249	5.960677	4.116883	2.030336	4.857496	0.8130
bta-miR-181d	6.929351	6.54324	9.099317	6.977419	7.193397	7.759197	0.8091
bta-miR-191	1.492349	1.418519	4.067147	2.063374	0.92824	3.208051	0.8238
bta-miR-182	5.913456	6.889178	8.654361	6.524439	6.80759	8.121711	0.9991
bta-miR-192	8.126216	8.296093	11.26366	8.720597	7.624346	10.65624	0.8740
bta-miR-183	6.949541	7.371787	8.985028	7.715531	7.26104	8.887725	0.8248
bta-miR-185	5.766346	5.601605	7.962577	6.203346	5.809851	7.157741	0.9536
bta-miR-193a-5p	6.778129	6.954251	7.835959	6.50992	7.22813	8.204071	0.8431
bta-miR-186	5.20302	4.436335	7.015626	4.07954	4.301729	5.449216	0.3423
bta-miR-194	6.883619	6.772456	7.894576	6.40671	6.019218	7.22571	0.2774
bta-miR-195	2.779762	2.514582	5.422271	3.270064	2.059559	3.455003	0.5643
bta-miR-200c	-2.15609	-2.2389	-0.03099	-1.31245	-2.24428	-0.14123	0.8099
bta-miR-204	2.968595	2.779143	3.346501	2.644581	3.567303	6.591396	0.3624
bta-miR-197	3.455089	3.336913	5.238709	4.795603	3.802135	6.14084	0.3799
bta-miR-205	9.989063	8.418688	8.053657	7.222338	5.788675	8.284412	0.1395
bta-miR-199a-3p	9.039775	11.81688	11.61018	9.735724	9.989141	11.48393	0.7091

bta-miR-206	10.91479	10.63577	12.25337	8.388529	11.20376	12.87485	0.7664
bta-miR-199c	8.084816	7.737692	9.584502	8.628023	7.844433	11.40716	0.5365
bta-miR-20a	5.327055	4.73905	6.726535	4.618719	4.359238	5.170383	0.2381
bta-miR-19a	8.269191	7.478623	9.759181	6.316694	6.902447	6.671137	0.0533
bta-miR-20b	6.57929	6.254099	8.891171	5.853029	5.735495	7.04841	0.3305
bta-miR-19b	8.224975	8.441882	7.474994	6.276274	6.797153	6.2201	**0.0095**
bta-miR-200a	6.308195	5.503607	7.96161	5.305886	4.947872	6.151333	0.2364
bta-miR-21-5p	8.253513	6.745862	7.125411	6.910181	5.854633	7.271232	0.3250
bta-miR-200b	-3.65475	-3.66191	-1.51197	-2.71407	-4.1783	-1.72997	0.9489
bta-miR-210	7.940921	6.596594	9.181121	7.17041	5.886116	7.755981	0.3557
bta-miR-211	3.763914	3.496691	4.096919	3.588801	3.969715	6.821828	0.3853
bta-miR-22-5p	7.248733	6.791432	9.424888	6.999909	7.728886	8.504377	0.9372
bta-miR-221	4.933702	4.623969	8.088994	5.657812	4.783888	6.151453	0.7803
bta-miR-214	9.557331	11.31614	11.34486	10.65302	12.38289	10.87007	0.5224
bta-miR-215	7.954111	7.6013	9.014977	8.191481	6.849884	10.11438	0.8601
bta-miR-223	9.911121	10.08429	12.97595	7.765795	8.707937	9.238763	0.0893
bta-miR-216a	12.88902	11.29924	11.79018	10.92185	10.57015	11.62514	0.1656
bta-miR-224	5.738582	5.818274	8.82767	6.519949	6.620836	8.123159	0.8100
bta-miR-23a	-1.86661	-1.63058	0.355559	-0.30047	-1.88067	0.49636	0.6502
bta-miR-23b-3p	1.049789	1.622374	3.368932	2.608238	0.801729	3.264572	0.8453
bta-miR-218	6.84526	6.496445	9.934554	8.545811	7.414841	9.48776	0.5924
bta-miR-219-3p	8.526126	9.735291	10.43225	9.18644	8.787033	10.03008	0.7474
bta-miR-24-3p	1.817974	1.627251	3.768222	1.903317	1.318138	2.868271	0.6715
bta-miR-25	1.599582	1.598759	3.858111	2.783463	1.403734	3.66918	0.8031
bta-miR-22-3p	-21.4511	-20.446	-19.8915	-18.2688	-20.6345	-18.7262	0.1807
bta-miR-26a	-1.58122	-1.81146	-0.07098	-1.47496	-2.50348	-0.21981	0.7892
bta-miR-26b	-0.0908	-0.47214	1.479104	0.057619	-0.48149	1.499914	0.9525
bta-miR-29d-3p	2.960552	2.561276	3.557193	2.708985	1.894112	3.188808	0.4183

bta-miR-29d-5p	7.57998	6.494301	8.475402	7.375174	6.372974	8.477535	0.9033
bta-miR-27a-3p	3.123663	2.570502	3.619794	2.641772	2.459412	3.218078	0.4317
bta-miR-27b	2.858566	2.725079	3.920865	2.666798	1.881233	3.654609	0.5334
bta-miR-28	6.90302	6.826672	8.92665	6.88584	6.106817	8.306438	0.6561
bta-miR-29a	0.513004	0.289727	1.352984	0.358208	-0.15441	1.327531	0.7204
bta-miR-29b	12.84971	11.66599	10.93024	8.912522	12.09274	10.14478	0.2561
bta-miR-30a-5p	5.584798	5.207531	6.689511	4.130207	4.329611	4.974004	0.0580
bta-miR-29c	0.65352	0.303526	1.140702	0.511557	-0.15671	1.230138	0.7336
bta-miR-30b-3p	8.248336	8.140564	10.8772	9.220459	7.948723	10.43823	0.9258
bta-miR-30b-5p	4.001749	3.358878	4.005103	2.137993	2.743048	3.09003	**0.0323**
bta-miR-328	9.574749	7.036929	8.036223	7.887081	7.685032	8.262836	0.7385
bta-miR-30c	1.983312	1.526789	2.861464	1.116154	0.909161	2.152183	0.2535
bta-miR-30d	5.313299	4.989874	6.364535	4.343756	4.782478	5.074335	0.1524
bta-miR-30e-5p	5.632255	5.611674	6.556521	4.287729	4.611883	5.140152	**0.0347**
bta-miR-330	11.4823	9.717606	10.94924	11.55547	14.00247	12.13604	0.1105
bta-miR-30f	3.889562	3.394658	4.548922	2.733134	2.881733	3.935281	0.2063
bta-miR-331-3p	7.617387	6.822918	13.11705	7.336321	6.801004	8.608095	0.4777
bta-miR-31	1.817062	1.578612	4.176082	2.414753	1.33897	3.302107	0.8724
bta-miR-331-5p	7.798816	6.898755	9.051114	7.499821	7.825375	9.199062	0.7663
bta-miR-335	8.946167	10.71075	9.938197	8.499825	7.827169	9.017814	0.0830
bta-miR-320a	3.157922	3.558272	5.8616	3.752091	3.754589	5.171535	0.9740
bta-miR-339a	6.203893	6.216353	8.086615	5.712172	5.554058	7.139061	0.4324
bta-miR-323	-6.87547	-5.58457	-5.5455	-3.51853	-5.60501	-3.98383	0.1009
bta-miR-339b	5.638906	5.580695	7.272023	5.207388	4.999304	6.261123	0.3761
bta-miR-326	9.572479	10.49514	11.68122	9.667879	10.11928	11.31741	0.7977
bta-miR-33b	11.44429	11.80042	12.92859	12.78211	12.00009	14.80659	0.2962
bta-miR-340	9.713878	8.42305	9.208716	8.340616	8.561211	10.13351	0.8861
bta-miR-365-3p	5.63322	5.3513	6.457684	4.689039	4.204908	5.90361	0.2185

bta-miR-342	6.940176	6.530652	8.07594	6.956169	6.522608	8.287063	0.9224
bta-miR-365-5p	10.59943	13.07515	13.49961	10.60972	9.487909	9.207008	0.0587
bta-miR-346	9.305862	10.03293	12.24984	9.42115	9.148424	8.994718	0.2079
bta-miR-34a	5.275007	5.459939	8.126225	5.6096	5.270756	7.00909	0.7759
bta-miR-34b	3.346759	2.489499	6.126975	3.788027	2.187342	4.251066	0.6706
bta-miR-34c	3.073589	2.538954	6.046487	3.772135	1.904272	4.235586	0.6781
bta-miR-374a	4.951097	4.497391	5.707644	5.084009	5.073393	5.29326	0.7987
bta-miR-361	2.884279	2.8886	6.10241	4.730881	2.569126	6.784	0.6734
bta-miR-374b	3.304306	2.972098	4.490649	3.559471	2.466431	4.29009	0.8409
bta-miR-375	2.202114	1.871428	4.614825	2.779177	1.322126	4.255132	0.9315
bta-miR-362-5p	10.24869	9.355896	14.26163	9.936245	9.90101	11.33936	0.6010
bta-miR-383	11.26408	10.36483	11.41637	9.565891	13.64528	12.09446	0.5743
bta-miR-378	5.838514	6.483336	9.628344	6.675989	7.063993	7.589452	0.8715
bta-miR-378b	7.231424	7.326777	8.965785	6.676981	7.41473	7.768686	0.4405
bta-miR-378c	9.37022	9.666203	12.17972	10.84306	9.977945	11.16837	0.8015
bta-miR-378d	11.44958	10.96785	13.56378	9.792742	11.09139	12.1375	0.3993
bta-miR-421	6.574243	6.076994	7.808314	6.502897	6.038385	8.09057	0.9467
bta-miR-423-3p	6.037948	5.546311	7.969039	5.708933	5.241442	7.061456	0.6059
bta-miR-423-5p	4.416965	4.724724	5.846557	4.718205	4.40864	5.983078	0.9531
bta-miR-424-3p	9.453476	10.82149	11.88903	11.79803	9.730635	12.32632	0.6231
bta-miR-449d	7.994285	8.765257	10.2173	10.33865	8.993185	10.24875	0.3299
bta-miR-424-5p	5.649225	4.723309	6.627332	4.4744	4.414358	4.846441	0.1269
bta-miR-450a	8.908485	8.598023	12.30236	9.004648	8.904821	9.557563	0.5520
bta-miR-450b	9.048915	7.731995	10.29144	8.460501	7.933653	9.15736	0.5697
bta-miR-425-5p	7.230026	6.54917	7.782964	5.635527	5.985878	6.367429	**0.0454**
bta-miR-429	3.451511	3.027434	4.522788	2.96813	2.795107	3.877306	0.4613
bta-miR-433	6.222456	7.538983	7.165563	7.28479	6.911556	8.58745	0.3893
bta-miR-454	11.29195	9.238556	11.80501	8.372565	8.70669	10.35154	0.1755

bta-miR-455-3p	8.991249	9.650998	11.37304	8.697527	7.687487	10.1378	0.3111
bta-miR-449a	2.301609	3.070122	7.242812	2.593554	3.40563	4.341501	0.6635
bta-miR-455-5p	10.92947	11.19606	11.92483	11.24675	9.984358	11.37715	0.4194
bta-miR-449b	4.006616	5.423253	9.010396	4.771773	5.857822	6.38756	0.7767
bta-miR-484	8.015934	8.189942	9.233613	7.389044	6.961212	8.572319	0.2434
bta-miR-497	8.459186	7.726315	13.72761	9.431275	7.781032	9.249073	0.5889
bta-miR-499	11.0326	10.38487	11.25529	11.03837	10.11902	10.29397	0.3490
bta-miR-500	8.242279	7.773655	12.23378	8.169827	7.785417	9.500553	0.5701
bta-miR-502b	7.640432	8.60034	10.43956	8.686476	8.003576	9.286436	0.8074
bta-miR-489	10.20182	10.7048	12.08776	13.45629	10.95536	14.89445	0.1760
bta-miR-491	5.994773	6.585576	7.712851	5.654963	5.622787	6.402773	0.1979
bta-miR-493	8.721435	8.500248	14.22863	9.576653	9.306368	9.127399	0.5745
bta-miR-505	5.664777	4.670451	7.858706	5.639826	4.934887	6.548732	0.7514
bta-miR-532	10.59041	9.903054	10.17387	9.071078	10.0598	12.42695	0.7846
bta-miR-541	8.084024	8.467709	10.17366	9.570917	8.742039	10.90252	0.4082
bta-miR-542-5p	11.42513	9.552121	12.40988	10.81999	9.950926	10.18377	0.4078
bta-miR-584	9.565757	10.3498	12.67492	10.40006	11.0692	10.17248	0.7611
bta-miR-628	7.843614	8.640168	11.04425	9.164461	7.897455	12.38596	0.7173
bta-miR-631	-4.65467	-3.93848	-3.63716	-1.81055	-3.64133	-2.47585	0.0798
bta-miR-652	4.634822	4.885613	7.037564	5.663447	4.776258	6.61639	0.8669
bta-miR-654	10.03233	9.025322	11.16792	10.95773	10.67788	10.35334	0.4124
bta-miR-574	4.147062	4.30226	6.286393	5.230932	4.081882	5.042827	0.8781
bta-miR-656	9.878585	9.622822	11.33995	10.41637	10.97996	11.6982	0.3128
bta-miR-660	7.941553	6.01377	8.034806	6.265495	5.908876	7.617126	0.4319
bta-miR-664a	10.03448	10.03658	9.974559	9.544131	10.642	10.29162	0.6800
bta-miR-664b	2.216659	2.203379	4.522262	2.926233	1.829159	4.192325	0.9987
bta-miR-760-5p	2.687721	2.686995	5.132523	3.637402	2.71516	4.073179	0.9776
bta-miR-665	8.282791	7.780324	9.998149	8.196509	6.815786	8.16773	0.3021

bta-miR-669	5.820089	6.574873	8.57245	7.647051	6.1469	7.903379	0.8174
bta-miR-763	10.51684	11.99927	12.66828	10.75734	11.91386	12.64857	0.9598
bta-miR-767	9.063957	10.22474	10.15205	11.43194	9.991815	12.06306	0.1338
bta-miR-677	12.63295	8.627518	10.1587	9.636494	10.16105	10.23672	0.7160
bta-miR-7	6.08333	5.598288	7.3642	6.348009	5.383436	7.750675	0.8748
bta-miR-708	6.607313	7.019991	8.690469	7.16761	6.872496	8.158764	0.9602
bta-miR-744	3.837909	3.976883	6.555474	5.587012	4.068935	6.982872	0.5690
bta-miR-98	4.239114	3.778428	5.997329	4.890709	3.850599	5.723762	0.8709
bta-miR-885	2.930585	2.776177	4.043368	3.154339	2.477434	4.195877	0.9697
bta-miR-99a-5p	4.982006	4.489634	5.311686	2.936751	3.50029	3.393957	**0.0050**
bta-miR-9-3p	11.15855	9.761532	12.87353	9.474606	10.97231	13.56591	0.9634
bta-miR-9-5p	2.051412	1.547396	4.900798	3.703411	1.574169	5.749338	0.6252
bta-miR-92a	2.937822	2.782414	4.129571	3.45143	2.770748	4.146324	0.7812
bta-miR-92b	1.448965	1.63283	2.870503	1.829718	1.474815	2.958782	0.8777
bta-miR-93	5.049568	4.919138	7.567954	5.42902	4.835024	6.670244	0.8532
bta-miR-940	3.864715	3.23975	6.114692	4.721945	3.764242	4.873165	0.9627
bta-miR-95	6.941335	8.520415	7.670815	8.596195	8.738373	10.33501	0.1037
bta-miR-1225-3p	5.072008	4.596341	7.464915	5.95036	4.927195	6.266348	0.9973
bta-miR-1247-5p	8.028785	7.641915	11.23444	8.70414	7.365669	8.48081	0.5524
bta-miR-1248	8.933826	9.131554	10.18869	9.178377	9.878683	8.960094	0.8768
bta-miR-1249	6.035329	5.501704	6.912585	6.781233	5.834093	7.593181	0.4206
bta-miR-1306	5.782592	6.201235	7.5836	6.629732	5.923698	7.225586	0.9202
bta-miR-1260b	0.295866	0.014042	1.323609	1.17152	0.788597	2.122493	0.2198
bta-miR-1343-3p	8.071203	7.936371	7.626569	7.530056	7.211784	8.247701	0.5544
bta-miR-1343-5p	6.241722	6.36274	8.213984	7.604767	6.424332	7.417011	0.7903
bta-miR-1388-3p	9.046562	7.737687	9.812991	7.328572	9.320352	10.00703	0.9854
RNT43 snoRNA	4.740491	4.790825	3.656894	3.655661	4.813814	3.203348	0.4512
Hm/Ms/Rt T1 snRNA	-2.90639	-3.62009	-3.0259	-4.36248	-3.90197	-3.76165	**0.0447**

bta-miR-99b	-1.27366	-0.50674	0.051812	1.434611	-0.10847	1.22393	0.0820

[1] Body energy reserve: MBER: Cows with moderated body energy reserve; HBER: Cows with high body energy reserve; [2] P-value: P value between animals with different body energy reserve.

Supplementary Table 10. Normalized data of the 242 miRNAs commonly detected in isthmic luminal epithelial cells (IST-Cell) of cows with different body energy reserve.

miRNA	**Body energy reserve**						**P - value**
	MBER			**HBER**			
	1	**2**	**3**	**1**	**2**	**3**	
bta-let-7a-3p	5.014058	5.598746	5.870683	3.862506	5.185162	5.219811	0.2235
bta-miR-103	5.7476	5.75528	5.477537	5.133969	5.354506	5.534808	0.0967
bta-let-7a-5p	-0.92236	-0.06985	-0.06351	-2.84431	-0.52793	-1.08804	0.2067
bta-let-7b	0.375876	1.151021	1.062555	-1.67577	0.527716	0.183511	0.1785
bta-let-7c	-0.84358	0.056178	0.07607	-2.69746	-0.4098	-0.93443	0.2156
bta-miR-106a	5.857308	5.842846	5.620696	5.35158	5.418284	5.314286	**0.0075**
bta-let-7d	0.205119	0.984046	1.093778	-1.70406	0.843077	0.132378	0.2823
bta-miR-106b	7.706094	8.043633	6.991632	8.005068	7.26532	7.357137	0.9268
bta-let-7e	-1.07442	-0.47689	-0.16977	-3.02424	-0.7187	-1.32474	0.2059
bta-miR-107	9.826512	9.555918	9.018369	9.011109	10.20278	9.274215	0.9496
bta-miR-10a	2.736472	4.015843	4.548503	2.242858	3.337664	3.678697	0.3801
bta-let-7g	1.635184	2.134858	2.468021	0.703808	1.929216	1.583384	0.1985
bta-miR-10b	3.202386	4.248843	4.560674	2.912436	4.063286	4.103265	0.6124
bta-let-7i	4.536073	4.505307	4.247873	3.233325	4.454567	4.14589	0.2685
bta-miR-1	8.834472	8.872365	6.758165	7.694642	8.737869	8.865985	0.7431
bta-miR-100	4.135989	4.729983	5.013723	3.916819	4.074991	4.067153	0.0828
bta-miR-101	7.990672	7.772967	7.859718	7.320513	7.419317	7.71271	**0.0433**
bta-miR-125a	1.414516	2.127108	2.022575	0.454739	1.62866	1.49235	0.1999
bta-miR-125b	1.545478	1.7272	1.089355	0.662634	1.13051	0.972049	0.0855

bta-miR-127	9.625056	9.740813	9.676689	8.93908	10.21166	11.12668	0.5523
bta-miR-135a	1.731618	1.647423	1.351795	0.352223	1.297976	1.166995	0.1149
bta-miR-128	5.33953	5.563132	6.120567	4.872746	5.908394	5.234996	0.4292
bta-miR-135b	2.798474	2.954587	2.634874	1.796271	2.992836	2.354432	0.3108
bta-miR-129-3p	12.96511	11.26148	12.14277	10.23562	10.79368	10.92876	0.0516
bta-miR-130a	8.793391	9.748334	9.341924	8.671897	10.29843	10.9843	0.4034
bta-miR-139	8.055615	8.217722	8.392846	6.3539	8.023321	7.048568	0.0940
bta-miR-130b	2.422862	2.523005	2.549922	1.480776	2.764788	2.791217	0.7423
bta-miR-140	7.825021	8.054993	7.752752	7.086857	7.418432	7.988973	0.2450
bta-miR-132	8.770837	9.624299	10.41184	8.310572	10.27967	8.892789	0.5889
bta-miR-141	6.812951	6.296645	6.087903	6.299546	5.443306	5.81483	0.1714
bta-miR-133a	11.88279	13.02466	10.37612	12.83485	12.40954	13.2381	0.2551
bta-miR-142-5p	12.98166	13.64743	11.94916	13.08802	12.75187	13.04353	0.8502
bta-miR-151-3p	5.769769	5.866543	5.511983	4.267396	5.470459	5.343924	0.1570
bta-miR-143	5.393212	5.140639	5.108753	4.041085	5.289835	4.87206	0.2730
bta-miR-151-5p	3.478557	3.538138	3.34563	2.045605	3.18154	3.203078	0.1710
bta-miR-152	10.4461	11.41471	10.35937	9.773167	11.39149	9.665105	0.5169
bta-miR-145	7.810885	8.250996	9.767846	7.333567	7.457817	7.449572	0.1143
bta-miR-153	8.660161	8.627259	8.94827	8.263373	9.223749	8.037099	0.5642
bta-miR-146a	10.92113	13.06501	10.62962	9.71069	11.01191	15.50878	0.7927
bta-miR-154a	11.92101	12.40761	14.3301	12.45866	12.44417	11.85169	0.4518
bta-miR-154b	8.686307	9.157603	7.92126	8.283393	9.617508	9.124993	0.4726
bta-miR-154c	12.02114	14.35039	13.40978	13.76032	13.76711	14.97584	0.3137
bta-miR-148a	0.000928	0.092606	-0.23298	-1.42246	-0.562	-0.78548	**0.0334**
bta-miR-155	8.448035	9.601348	10.5373	7.337124	10.6291	9.620753	0.7857
bta-miR-148b	0.805135	0.594369	0.093545	-0.76306	0.193863	-0.13647	0.1051
bta-miR-15a	7.845773	8.085436	7.159084	7.808217	7.671515	7.331668	0.7803
bta-miR-149-3p	8.426673	9.8053	9.737117	9.05818	9.801554	9.971974	0.6157

bta-miR-15b	3.817199	4.927165	4.589036	2.752047	4.601879	3.935864	0.3423
bta-miR-149-5p	10.75598	11.3678	12.06535	10.45097	10.57228	10.26221	0.0676
bta-miR-16a	3.791547	4.08923	3.334339	3.20999	3.541458	3.417693	0.2198
bta-miR-150	5.720658	7.522349	7.638507	6.040198	7.284548	7.32463	0.9228
bta-miR-16b	3.615251	3.827019	3.177383	2.981677	3.472607	3.228825	0.2599
bta-miR-17-3p	13.02121	12.5747	11.40833	10.69931	11.04911	12.82093	0.3751
bta-miR-17-5p	8.787074	9.200785	8.843517	7.957453	8.913721	8.695012	0.2541
bta-miR-181a	10.59502	11.85379	11.10004	9.377148	10.91944	10.99276	0.3053
bta-miR-18b	12.85321	12.88397	13.03988	12.55037	12.55393	12.74977	**0.0247**
bta-miR-181b	7.705634	8.417124	8.5927	6.868292	8.12777	7.880553	0.2632
bta-miR-190a	9.595342	9.900099	9.107391	10.02386	10.14735	10.01969	0.0871
bta-miR-181c	11.86494	13.48751	10.93024	11.61166	12.82214	12.23603	0.8833
bta-miR-190b	4.996127	5.292887	4.577983	3.478425	5.880549	5.137879	0.8756
bta-miR-181d	6.850434	7.534533	7.718877	5.591824	7.181168	7.038709	0.2528
bta-miR-191	2.667972	2.465989	2.722373	1.349711	3.086868	2.710254	0.6804
bta-miR-182	6.791671	7.866995	7.84872	5.907662	8.059439	7.073035	0.5322
bta-miR-192	9.516469	10.16513	9.556138	8.587098	8.946852	9.230847	**0.0425**
bta-miR-183	7.7609	9.141015	8.477947	6.305752	8.710102	8.431201	0.4942
bta-miR-185	7.798493	8.021853	7.157155	7.248461	7.310776	7.652276	0.4253
bta-miR-193a-5p	8.16892	8.421969	8.72787	6.256016	8.984762	8.891351	0.6859
bta-miR-186	5.666333	5.704051	5.321752	5.265299	5.144419	5.088349	**0.0397**
bta-miR-194	7.782418	7.552685	7.362674	7.252328	6.801315	7.477075	0.1701
bta-miR-195	4.617475	5.016005	4.150314	3.242531	4.299162	4.094369	0.1549
bta-miR-200c	-0.47534	0.180125	0.02494	-1.77444	-0.04471	-0.53054	0.2774
bta-miR-202	9.835101	9.364703	9.039592	8.123553	8.841034	9.734458	0.3794
bta-miR-196b	9.75085	12.58412	12.0594	10.2894	11.01683	11.32587	0.5590
bta-miR-204	3.602443	3.717791	3.843686	2.248456	3.514757	4.098376	0.4744
bta-miR-197	5.483346	5.49701	6.093912	4.068701	5.489664	5.296071	0.2043

bta-miR-205	7.568263	8.442936	9.087009	7.610861	5.521452	7.04608	0.0982
bta-miR-199a-3p	6.791068	7.5448	10.10825	6.277922	6.261404	8.0822	0.3380
bta-miR-206	13.04464	13.12702	14.28164	12.54882	13.49288	14.16206	0.8990
bta-miR-199a-5p	13.69724	12.95925	15.00907	12.56866	10.79499	13.50682	0.1837
bta-miR-199c	6.336116	6.791163	9.427719	5.485499	5.604649	7.094384	0.2538
bta-miR-20a	5.709094	6.139279	5.736732	5.260811	5.712469	5.396968	0.1038
bta-miR-19a	6.633136	6.910985	6.742391	7.257266	6.812296	6.446612	0.7729
bta-miR-20b	7.590354	7.495923	7.360698	7.272442	7.288988	7.186023	**0.0343**
bta-miR-19b	6.715774	6.765019	6.590709	7.000804	6.488607	6.290944	0.6789
bta-miR-21-3p	14.05192	12.475	12.10068	11.69803	13.36714	14.66014	0.7439
bta-miR-200a	6.063376	6.613152	6.273824	5.824778	6.249947	6.241435	0.3770
bta-miR-21-5p	7.105353	8.184441	7.287528	6.287842	7.046015	7.1822	0.1888
bta-miR-200b	-2.39428	-1.63084	-1.86586	-3.48785	-2.02786	-2.44529	0.2313
bta-miR-210	8.794599	8.70843	8.405443	8.250804	8.295727	8.136901	**0.0325**
bta-miR-211	4.451064	4.409524	4.709934	2.319464	3.465157	4.871602	0.2617
bta-miR-22-5p	7.611852	8.275225	8.00225	6.489533	7.851028	7.093697	0.1353
bta-miR-221	6.351449	7.162781	4.948736	4.372502	6.503683	6.18191	0.6398
bta-miR-222	6.75174	7.125237	4.469825	4.332827	6.308071	6.108096	0.6357
bta-miR-215	8.83846	9.14322	8.891467	8.054566	8.890598	8.996377	0.3764
bta-miR-223	9.853538	10.80155	9.674573	8.329907	9.334555	10.01868	0.2169
bta-miR-224	6.702703	7.092955	7.586704	5.309844	7.91057	6.939475	0.6376
bta-miR-23a	-0.23056	0.459809	0.365154	-1.32644	0.305039	-0.03862	0.3661
bta-miR-23b-3p	2.356233	3.161372	3.113101	1.220069	3.013449	2.762584	0.4280
bta-miR-218	9.257287	9.522938	8.87046	8.279776	10.13095	9.309901	0.9692
bta-miR-23b-5p	11.88884	13.34525	12.18149	11.01982	12.23339	12.60382	0.4709
bta-miR-219-3p	8.224544	9.102631	9.00077	8.176404	9.24214	9.85123	0.6065
bta-miR-24-3p	2.822959	3.05058	2.888036	2.211421	2.697897	2.787946	0.1375
bta-miR-25	2.763907	3.445628	3.432813	1.933774	3.319048	3.03291	0.3981

bta-miR-22-3p	-18.2088	-17.8284	-18.2024	-19.7035	-17.8238	-17.9125	0.5570
bta-miR-26a	-0.42233	-0.27994	-0.39492	-1.61929	-0.59868	-0.63789	0.1566
bta-miR-26b	0.257333	0.625229	0.72619	-1.24749	0.367643	0.200356	0.2251
bta-miR-29d-3p	1.957015	1.871808	1.278142	0.712258	1.501602	1.539622	0.2599
bta-miR-29d-5p	9.308381	8.530068	7.87449	7.295355	8.147854	8.371676	0.2973
bta-miR-27a-3p	3.748609	3.391341	3.355571	3.482306	3.57897	3.760354	0.5078
bta-miR-29e	12.65453	12.64819	11.38868	12.99933	11.58405	11.26307	0.6998
bta-miR-27a-5p	15.27923	12.54086	11.17645	10.789	12.38586	12.66342	0.4761
bta-miR-27b	3.725251	4.080929	4.442474	2.898156	3.56428	3.638701	0.0845
bta-miR-28	8.625113	8.462901	8.858301	7.411669	7.951702	8.146514	**0.0306**
bta-miR-296-3p	8.253835	8.983847	10.08742	7.270764	9.305233	8.94756	0.5060
bta-miR-296-5p	10.66868	10.45125	10.38125	9.317898	9.960854	9.756024	**0.0169**
bta-miR-29a	-0.48915	-0.03382	-0.25121	-1.48166	-0.7311	-0.53071	0.1078
bta-miR-29b	10.26993	9.438336	9.489019	8.564574	9.23785	9.247874	0.1115
bta-miR-30a-5p	6.189916	6.097872	5.789558	5.292966	5.520737	5.658364	**0.0294**
bta-miR-29c	-0.65381	-0.27892	-0.32911	-1.68458	-0.68671	-0.37945	0.2938
bta-miR-30b-3p	10.2445	10.73278	10.10119	7.34784	10.60309	9.888661	0.3437
bta-miR-30b-5p	4.016	4.120818	3.40973	3.330708	3.290714	3.500241	0.1087
bta-miR-328	9.173272	7.202376	8.255618	6.912718	7.305767	6.476725	0.1009
bta-miR-30c	2.734138	3.097594	2.556579	1.901742	2.422821	2.621713	0.1465
bta-miR-30d	6.296201	6.254281	5.416284	5.305119	5.553543	5.700114	0.2033
bta-miR-30e-5p	6.133832	6.294758	5.494893	5.605797	5.77277	5.542972	0.2584
bta-miR-330	13.11448	12.45263	12.15894	13.17513	12.14251	13.36757	0.5368
bta-miR-30f	4.610293	4.858435	4.09418	3.897696	4.168215	4.340947	0.2116
bta-miR-331-3p	8.766389	9.414858	8.699653	6.091933	8.279287	8.775842	0.2194
bta-miR-31	3.002374	3.479563	3.255655	2.705102	3.141035	3.221026	0.3501
bta-miR-331-5p	8.522555	9.149098	8.948512	7.857825	9.227329	9.164395	0.8108
bta-miR-335	8.817826	10.10871	8.029214	10.86719	8.080793	9.06729	0.7457

bta-miR-320a	4.518819	5.355746	5.12185	2.941613	5.309673	4.94847	0.4842
bta-miR-338	13.19093	11.32336	10.01563	10.71396	10.39749	10.86987	0.4135
bta-miR-339a	6.948821	7.312199	6.944728	6.219826	6.959591	7.114495	0.3708
bta-miR-323	-5.2744	-4.6691	-4.881	-6.47588	-4.08838	-4.39126	0.9575
bta-miR-339b	6.736081	6.801528	6.063487	6.002049	6.204981	6.578816	0.4019
bta-miR-324	9.828362	9.818715	10.64694	9.10217	9.471275	9.915244	0.1712
bta-miR-326	12.13818	11.59761	14.41823	10.41459	11.56798	12.19566	0.2595
bta-miR-33b	12.28352	12.60393	13.30357	11.9516	15.47146	14.76386	0.2987
bta-miR-340	10.09411	11.00488	10.20856	8.506339	11.22563	9.680531	0.4929
bta-miR-365-3p	5.017211	4.999428	5.971593	3.525033	4.695229	4.512545	0.0889
bta-miR-342	7.223161	7.568062	7.930648	6.766715	7.581707	7.51769	0.4386
bta-miR-365-5p	14.64637	14.01295	13.89201	10.63417	12.17808	12.15036	**0.0108**
bta-miR-345-3p	9.371299	9.757783	10.01872	7.785796	9.66641	9.319592	0.2624
bta-miR-369-3p	11.36245	12.95933	13.63027	10.74481	13.04416	13.3579	0.8132
bta-miR-34a	5.357274	5.697754	6.107812	5.346141	5.760262	5.84213	0.8014
bta-miR-34b	5.021716	4.975718	4.140537	4.884041	4.275307	4.701659	0.7985
bta-miR-34c	4.955005	4.977719	4.337448	4.586949	4.190998	4.480565	0.2340
bta-miR-374a	5.418345	6.094426	5.623819	5.000966	5.430847	5.169423	0.0959
bta-miR-361	5.334367	6.226658	6.003921	2.321213	5.865725	5.652604	0.3515
bta-miR-374b	3.789855	4.559187	4.096612	2.900077	4.098002	3.895469	0.2977
bta-miR-375	3.394494	3.3322	3.675582	1.281767	2.976419	3.226195	0.1917
bta-miR-362-5p	11.31131	12.57104	10.92657	10.34203	11.56742	11.17929	0.4034
bta-miR-378	7.773622	8.269103	8.389985	6.857569	8.262306	8.008439	0.4087
bta-miR-378b	7.82738	8.297679	8.072358	7.339795	8.967104	8.011575	0.9385
bta-miR-378c	10.72954	10.53061	10.87492	9.471051	12.02069	10.42391	0.9270
bta-miR-378d	11.58809	11.68516	11.63767	11.88221	13.34205	12.43081	0.0987
bta-miR-421	5.631049	5.595049	6.496208	4.86444	7.503883	6.837678	0.5900
bta-miR-423-3p	6.171523	6.626698	6.813514	5.451205	6.454752	6.175279	0.2237

bta-miR-423-5p	4.850791	5.536217	6.195455	4.077311	5.895271	5.182692	0.5085
bta-miR-449c	9.731004	9.55341	11.42038	9.209319	11.58352	10.38735	0.8699
bta-miR-424-3p	11.81463	11.53438	10.90143	11.50197	12.60313	11.30946	0.4686
bta-miR-449d	11.5567	11.62142	12.0094	10.45136	10.6855	12.15702	0.3168
bta-miR-424-5p	6.55956	7.198752	5.030576	5.663058	6.969181	5.604167	0.8255
bta-miR-450a	9.802566	11.36946	9.359458	9.413947	11.10705	10.10073	0.9712
bta-miR-450b	10.30254	12.02315	9.169435	8.137387	11.3507	10.1086	0.6394
bta-miR-425-5p	7.320157	7.404872	6.846038	6.508801	7.027506	6.872512	0.1704
bta-miR-451	9.754111	10.14227	13.80425	8.365565	9.157499	9.045533	0.1446
bta-miR-429	3.558394	3.850819	3.431362	3.491009	3.540848	3.175057	0.2793
bta-miR-453	12.03793	12.53639	12.67309	11.43785	13.25052	12.90821	0.8529
bta-miR-433	7.556252	7.837981	7.524665	7.302979	8.986443	9.0292	0.2380
bta-miR-454	12.02074	10.40735	10.46161	8.181786	11.17678	10.16509	0.3358
bta-miR-455-3p	8.738175	9.439183	9.585046	8.40815	8.741982	9.645663	0.5160
bta-miR-449a	3.822822	4.057034	5.104501	3.234034	5.199415	4.484771	0.9763
bta-miR-455-5p	10.78429	11.5894	11.08457	11.67449	10.63552	13.19529	0.4308
bta-miR-449b	5.848422	6.319052	7.534467	5.214974	7.697748	6.973973	0.9483
bta-miR-484	8.379521	8.475962	8.490985	7.341483	8.260044	8.682364	0.4231
bta-miR-485	12.87615	12.58848	12.07602	11.50171	14.9145	13.29554	0.5145
bta-miR-497	8.962152	9.420386	9.033563	8.10172	10.12062	9.4903	0.8797
bta-miR-486	8.004127	8.447638	8.677207	7.654208	8.718218	9.10533	0.8193
bta-miR-499	10.91901	9.590022	9.751634	8.531211	9.294228	9.836017	0.1995
bta-miR-500	9.715467	9.521489	9.36214	7.302811	8.954811	9.093882	0.1375
bta-miR-502a	12.70024	14.70059	12.29136	10.43428	14.48105	13.78278	0.8309
bta-miR-502b	8.869939	10.00977	10.01663	7.897471	9.635557	9.063834	0.2957
bta-miR-489	9.628812	10.34882	9.087676	9.727723	11.7776	11.90982	0.1423
bta-miR-503-3p	10.26737	10.10245	10.98999	9.518477	11.08982	11.04549	0.8749
bta-miR-491	7.564424	8.093924	8.056672	5.221011	7.928236	7.14943	0.2384

bta-miR-493	11.55699	10.9886	12.1195	11.83807	10.31236	11.53042	0.5950
bta-miR-494	6.738244	6.492917	7.079395	6.159207	7.201214	7.214686	0.8317
bta-miR-532	11.09519	10.94959	11.16328	10.65834	10.16268	9.376082	0.0569
bta-miR-495	11.90181	12.89914	12.89399	12.34646	12.38926	13.59249	0.7087
bta-miR-584	11.34574	11.26745	12.14259	10.88976	12.79128	13.5135	0.3833
bta-miR-628	10.85224	11.38554	11.18168	9.979291	11.24648	11.28718	0.5443
bta-miR-631	-2.52009	-2.24706	-2.35921	-3.77232	-1.74366	-1.95297	0.8689
bta-miR-551a	16.718	18.03275	15.89081	18.10016	17.67995	19.83067	0.1417
bta-miR-652	6.699313	6.650698	6.065015	4.920191	6.445282	6.498006	0.4050
bta-miR-654	10.85642	11.70439	11.55074	9.316033	12.58001	13.26485	0.7928
bta-miR-574	6.807334	6.558814	7.259492	5.333838	5.843565	6.621928	0.0920
bta-miR-656	9.2888	9.457944	9.620752	8.62435	11.05886	10.18243	0.5252
bta-miR-660	7.971335	7.90569	7.169874	6.138308	7.477373	7.157269	0.1884
bta-miR-664a	10.82934	10.48487	10.51131	9.859707	11.24731	11.00771	0.8381
bta-miR-760-3p	12.339	11.64805	12.21755	10.23851	10.99815	12.06922	0.1665
bta-miR-664b	3.665677	3.848155	3.63243	2.277445	3.322199	3.182126	0.0778
bta-miR-665	8.879533	9.089121	9.425968	9.008702	9.112637	9.270551	0.9961
bta-miR-761	12.80004	14.29878	13.01652	15.77387	13.31119	17.54101	0.1736
bta-miR-669	9.744294	9.548213	8.997495	7.589114	8.231897	10.23474	0.4190
bta-miR-763	13.25446	13.55987	12.82568	13.43759	14.83698	13.26247	0.3080
bta-miR-764	11.86199	11.96511	12.60858	11.58466	13.60625	14.8785	0.2871
bta-miR-671	12.46265	13.5199	14.31529	10.9595	12.30797	12.65171	0.1216
bta-miR-767	9.41221	10.35706	10.30791	8.987918	11.19835	11.27352	0.5998
bta-miR-677	9.648664	9.631325	10.29298	9.006171	10.01351	10.0807	0.7204
bta-miR-769	10.40995	12.54076	11.37106	9.998288	11.31208	11.1102	0.4396
bta-miR-7	5.41571	6.409682	6.08649	3.989897	5.590059	5.473842	0.1831
bta-miR-708	8.257844	8.127245	8.865819	6.734326	8.633329	7.927642	0.3377
bta-miR-874	10.08578	10.37512	10.04527	8.84089	10.01436	9.721263	0.1550

bta-miR-744	5.930629	6.992056	6.611841	5.162875	6.902435	6.897639	0.7862
bta-miR-98	4.456887	5.284974	5.034805	3.179478	5.183373	4.118491	0.2905
bta-miR-99a-3p	10.77608	12.33601	12.01367	11.0135	11.5072	11.28267	0.4247
bta-miR-885	3.99133	3.741908	3.909133	2.350375	3.41563	3.485291	0.1004
bta-miR-99a-5p	4.014935	4.222853	3.976694	3.985146	3.307646	3.555118	0.0983
bta-miR-9-3p	11.83178	11.03754	12.20527	12.77955	11.60473	13.20247	0.2282
bta-miR-9-5p	4.548188	4.312962	4.793363	3.0427	3.617445	5.420522	0.5119
bta-miR-92a	2.796199	3.612198	3.565219	2.244271	3.426226	3.223222	0.4692
bta-miR-92b	2.606015	2.825274	2.703485	1.244496	2.743364	2.658521	0.3688
bta-miR-93	5.81737	6.666306	6.07596	5.269581	6.252794	6.005376	0.4251
bta-miR-940	5.289645	5.376177	5.508874	4.438143	5.28981	5.302211	0.2628
bta-miR-1224	8.863509	9.087712	10.40271	7.714696	9.13662	9.139422	0.3080
bta-miR-95	7.27937	8.49578	9.114004	7.839528	8.317492	8.9849	0.9005
bta-miR-1225-3p	7.171399	7.223744	7.112594	6.330517	7.02466	7.460498	0.5237
bta-miR-1296	10.84594	12.53263	13.14261	10.04705	10.64211	12.03753	0.2349
bta-miR-1247-5p	9.149631	9.391488	9.343318	8.779523	8.77894	9.195201	0.0744
bta-miR-1249	6.178208	6.633202	7.048063	4.62023	6.491089	5.936585	0.1987
bta-miR-1306	7.228688	7.46913	7.374322	6.058217	7.311533	7.590869	0.4802
bta-miR-1260b	1.039008	1.307196	1.533065	0.276944	1.164446	1.15422	0.2608
bta-miR-1271	13.77235	13.7635	14.05845	11.91673	11.76174	11.25932	**0.0006**
bta-miR-1343-5p	7.779841	8.566683	8.875038	7.266026	7.598217	8.35278	0.2183
bta-miR-1281	9.648555	9.686188	9.842166	9.131419	9.207657	9.373164	**0.0062**
bta-miR-1388-3p	9.715383	9.527745	10.01165	7.921414	10.8377	10.044	0.8727
RNT43 snoRNA	2.855496	2.609049	3.066083	4.101084	3.060717	3.07343	0.1986
Hm/Ms/Rt T1 snRNA	-3.85053	-3.8285	-4.11174	-3.68292	-4.34564	-4.26635	0.5018
bta-miR-99b	1.487073	1.799285	1.706603	0.278105	2.130096	1.889638	0.7140
bta-miR-1291	14.35359	14.50239	12.12416	12.3064	10.80065	15.02783	0.5505

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[1] Body energy reserve: MBER: Cows with moderated body energy reserve; HBER: Cows with high body energy reserve; [2] P-value: P value between animals with different body energy reserve.

Supplementary table 11. Biological patwhays predicted as modulated by exclusive miRNAs in ampullary luminal epithelial cells (AMP-Cell) in high body energy reserve (HBER) group.

Pathway	**%[1]**	**BH[2]**
bta04144 Endocytosis	52.65	0.0
bta05200 Pathways in cancer	44.28	0.0
bta04010 MAPK signaling pathway	47.26	0.0052
bta04014 Ras signaling pathway	50.00	0.0052
bta04514 Cell adhesion molecules (CAMs)	53.16	0.0052
bta05205 Proteoglycans in cancer	50.24	0.0052
bta04810 Regulation of actin cytoskeleton	48.34	0.018
bta01100 Metabolic pathways	37.46	0.0315
bta04360 Axon guidance	48.88	0.0315
bta04310 Wnt signaling pathway	48.15	0.0492
bta04390 Hippo signaling pathway	48.08	0.0492
bta04659 Th17 cell differentiation	52.21	0.0492
bta04910 Insulin signaling pathway	49.29	0.0492
bta04921 Oxytocin signaling pathway	48.68	0.0492
bta04922 Glucagon signaling pathway	52.43	0.0492
bta05212 Pancreatic cancer	56.58	0.0492
bta04015 Rap1 signaling pathway	44.91	0.0501
bta04120 Ubiquitin mediated proteolysis	48.57	0.0501
bta04530 Tight junction	46.33	0.0501
bta04658 Th1 and Th2 cell differentiation	52.04	0.0501
bta04670 Leukocyte transendothelial migration	50.44	0.0501
bta05163 Human cytomegalovirus infection	44.08	0.0501

bta00564 Glycerophospholipid metabolism	50.96	0.0525
bta01212 Fatty acid metabolism	58.62	0.0525
bta04062 Chemokine signaling pathway	45.21	0.0572
bta04270 Vascular smooth muscle contraction	48.12	0.0572
bta04750 Inflammatory mediator regulation of TRP channels	50.49	0.0572
bta04022 cGMP-PKG signaling pathway	45.56	0.0663
bta04371 Apelin signaling pathway	47.14	0.0663
bta04024 cAMP signaling pathway	43.23	0.0699
bta04668 TNF signaling pathway	48.31	0.0699
bta04912 GnRH signaling pathway	50.54	0.0699
bta04925 Aldosterone synthesis and secretion	50.00	0.0725
bta04068 FoxO signaling pathway	46.56	0.0767
bta04072 Phospholipase D signaling pathway	45.39	0.0767
bta05132 Salmonella infection	42.86	0.0767
bta05160 Hepatitis C	45.12	0.0767
bta05214 Glioma	51.95	0.0767
bta05220 Chronic myeloid leukemia	51.95	0.0767
bta01522 Endocrine resistance	48.94	0.0784
bta04070 Phosphatidylinositol signaling system	48.48	0.0784
bta04261 Adrenergic signaling in cardiomyocytes	45.33	0.0784
bta04625 C-type lectin receptor signaling pathway	48.11	0.0784
bta04722 Neurotrophin signaling pathway	46.72	0.0784
bta05211 Renal cell carcinoma	52.11	0.0784
bta05235 PD-L1 expression and PD-1 checkpoint pathway in cancer	49.46	0.0784
bta05414 Dilated cardiomyopathy (DCM)	48.48	0.0784
bta04660 T cell receptor signaling pathway	47.66	0.0788
bta04931 Insulin resistance	47.27	0.081
bta05167 Kaposi sarcoma-associated herpesvirus infection	42.72	0.0813

bta00562 Inositol phosphate metabolism	50.68	0.0886
bta04934 Cushing syndrome	44.23	0.0886
bta05100 Bacterial invasion of epithelial cells	50.68	0.0886
bta00071 Fatty acid degradation	57.14	0.0901
bta01200 Carbon metabolism	46.02	0.0901
bta04012 ErbB signaling pathway	48.81	0.0901
bta04020 Calcium signaling pathway	42.08	0.0901
bta04066 HIF-1 signaling pathway	46.36	0.0901
bta04130 SNARE interactions in vesicular transport	60.61	0.0901
bta04728 Dopaminergic synapse	44.78	0.0901
bta04916 Melanogenesis	47.06	0.0901
bta04924 Renin secretion	50.00	0.0901
bta05170 Human immunodeficiency virus 1 infection	41.45	0.0901
bta05410 Hypertrophic cardiomyopathy (HCM)	47.83	0.0901
bta05418 Fluid shear stress and atherosclerosis	44.14	0.0901
bta04142 Lysosome	44.70	0.0903
bta04710 Circadian rhythm	61.29	0.0903
bta04911 Insulin secretion	48.24	0.0903
bta04137 Mitophagy	50.00	0.0996
bta04218 Cellular senescence	42.77	0.0996
bta04550 Signaling pathways regulating pluripotency of stem cells	43.66	0.0996
bta04971 Gastric acid secretion	48.68	0.0996
bta05202 Transcriptional misregulation in cancer	41.88	0.0996
bta05412 Arrhythmogenic right ventricular cardiomyopathy (ARVC)	48.68	0.0996
bta04611 Platelet activation	44.63	0.1025
bta00010 Glycolysis Gluconeogenesis	50.00	0.1035
bta00600 Sphingolipid metabolism	53.06	0.1035
bta05219 Bladder cancer	54.76	0.1074

bta04064 NF-kappa B signaling pathway	44.95	0.1114
bta05225 Hepatocellular carcinoma	41.95	0.1114
bta05321 Inflammatory bowel disease (IBD)	48.57	0.112
bta05224 Breast cancer	42.67	0.1149
bta01230 Biosynthesis of amino acids	47.95	0.1158
bta04510 Focal adhesion	40.91	0.1241
bta05230 Central carbon metabolism in cancer	48.48	0.1249
bta04926 Relaxin signaling pathway	43.08	0.1274
bta05161 Hepatitis B	41.52	0.1274
bta04928 Parathyroid hormone synthesis. secretion and action	44.23	0.1392
bta05218 Melanoma	46.58	0.1511
bta04150 mTOR signaling pathway	41.40	0.1522
bta04961 Endocrine and other factor-regulated calcium reabsorption	50.00	0.1523
bta04713 Circadian entrainment	44.00	0.1537
bta00310 Lysine degradation	46.97	0.1585
bta00640 Propanoate metabolism	54.55	0.1585
bta05166 Human T-cell leukemia virus 1 infection	39.32	0.1585
bta05215 Prostate cancer	43.88	0.1585
bta04115 p53 signaling pathway	45.45	0.162
bta04152 AMPK signaling pathway	42.28	0.162
bta04720 Long-term potentiation	46.38	0.162
bta01521 EGFR tyrosine kinase inhibitor resistance	45.00	0.1634
bta04920 Adipocytokine signaling pathway	45.83	0.1634
bta04215 Apoptosis	52.94	0.1711
bta04392 Hippo signaling pathway	55.17	0.1711
bta04520 Adherens junction	45.71	0.1711
bta05135 Yersinia infection	41.54	0.1746
bta05221 Acute myeloid leukemia	45.59	0.181

bta04071 Sphingolipid signaling pathway	41.67	0.1878
bta04330 Notch signaling pathway	47.17	0.1963
bta04919 Thyroid hormone signaling pathway	41.53	0.1964
bta05031 Amphetamine addiction	44.93	0.1964
bta05231 Choline metabolism in cancer	42.42	0.1989
bta00280 Valine. leucine and isoleucine degradation	47.06	0.2047
bta00561 Glycerolipid metabolism	44.78	0.2047
bta05223 Non-small cell lung cancer	44.78	0.2047
bta05226 Gastric cancer	39.87	0.2104
bta00512 Mucin type O-glycan biosynthesis	51.61	0.2138
bta05032 Morphine addiction	42.39	0.2138
bta00230 Purine metabolism	40.30	0.2147
bta04061 Viral protein interaction with cytokine and cytokine receptor	42.11	0.2147
bta04621 NOD-like receptor signaling pathway	38.59	0.2371
bta04726 Serotonergic synapse	40.52	0.2371
bta05133 Pertussis	42.86	0.2371
bta05145 Toxoplasmosis	40.71	0.2371
bta04140 Autophagy	39.44	0.2467
bta04340 Hedgehog signaling pathway	45.10	0.25
bta04380 Osteoclast differentiation	39.55	0.25
bta04657 IL-17 signaling pathway	41.30	0.25
bta04933 AGE-RAGE signaling pathway in diabetic complications	40.78	0.25
bta05210 Colorectal cancer	41.57	0.25
bta04151 PI3K-Akt signaling pathway	36.19	0.2518
bta00270 Cysteine and methionine metabolism	44.90	0.2589
bta00650 Butanoate metabolism	50.00	0.2589
bta04350 TGF-beta signaling pathway	40.86	0.2589
bta04540 Gap junction	41.11	0.2589

bta04666 Fc gamma R-mediated phagocytosis	40.86	0.2589
bta04927 Cortisol synthesis and secretion	43.08	0.2589
bta04640 Hematopoietic cell lineage	40.00	0.2598
bta05217 Basal cell carcinoma	42.86	0.2668
bta04727 GABAergic synapse	40.66	0.2693
bta04978 Mineral absorption	43.64	0.2693
bta04614 Renin-angiotensin system	50.00	0.2737
bta05165 Human papillomavirus infection	35.94	0.2806
bta00062 Fatty acid elongation	48.28	0.2859
bta05164 Influenza A	37.57	0.2859
bta04940 Type I diabetes mellitus	42.37	0.297
bta04662 B cell receptor signaling pathway	40.23	0.2971
bta04724 Glutamatergic synapse	38.94	0.3006
bta04970 Salivary secretion	39.78	0.3006
bta05416 Viral myocarditis	40.79	0.3006
bta04114 Oocyte meiosis	38.66	0.3045
bta00620 Pyruvate metabolism	44.74	0.3075
bta01040 Biosynthesis of unsaturated fatty acids	46.67	0.3075
bta04141 Protein processing in endoplasmic reticulum	37.35	0.3075
bta04730 Long-term depression	41.67	0.3075
bta04930 Type II diabetes mellitus	43.48	0.3075
bta03013 RNA transport	37.08	0.311
bta04973 Carbohydrate digestion and absorption	43.18	0.3228
bta00100 Steroid biosynthesis	50.00	0.3229
bta04370 VEGF signaling pathway	41.38	0.3229
bta05142 Chagas disease (American trypanosomiasis)	38.26	0.3229
bta05140 Leishmaniasis	39.74	0.3277
bta00052 Galactose metabolism	45.16	0.3372

bta00514 Other types of O-glycan biosynthesis	42.22	0.3382
bta01210 2-Oxocarboxylic acid metabolism	50.00	0.3382
bta03420 Nucleotide excision repair	42.22	0.3382
bta04211 Longevity regulating pathway	38.89	0.3382
bta05169 Epstein-Barr virus infection	35.96	0.3382
bta05213 Endometrial cancer	40.68	0.3382
bta04950 Maturity onset diabetes of the young	46.15	0.3403
bta04725 Cholinergic synapse	37.72	0.3458
bta00601 Glycosphingolipid biosynthesis	44.83	0.3476
bta03020 RNA polymerase	44.83	0.3476
bta05216 Thyroid cancer	42.50	0.3476
bta01523 Antifolate resistance	41.86	0.3539
bta00500 Starch and sucrose metabolism	43.75	0.3548
bta00534 Glycosaminoglycan biosynthesis	45.83	0.3601
bta04962 Vasopressin-regulated water reabsorption	40.82	0.3601
bta05152 Tuberculosis	35.86	0.3601
bta04744 Phototransduction	44.44	0.3683
bta01524 Platinum drug resistance	38.46	0.3689
bta00220 Arginine biosynthesis	47.37	0.3696
bta00380 Tryptophan metabolism	40.43	0.3782
bta04623 Cytosolic DNA-sensing pathway	38.81	0.3782
bta04664 Fc epsilon RI signaling pathway	38.57	0.3782
bta04972 Pancreatic secretion	37.25	0.3782
bta00760 Nicotinate and nicotinamide metabolism	41.03	0.3905
bta04914 Progesterone-mediated oocyte maturation	37.50	0.3905
bta05222 Small cell lung cancer	37.23	0.3912
bta00603 Glycosphingolipid biosynthesis	47.06	0.3914
bta00910 Nitrogen metabolism	47.06	0.3914

bta00030 Pentose phosphate pathway	42.86	0.3917
bta04975 Fat digestion and absorption	39.58	0.3939
bta05030 Cocaine addiction	39.58	0.3939
bta05332 Graft-versus-host disease	39.58	0.3939
bta04976 Bile secretion	37.35	0.3966
bta00051 Fructose and mannose metabolism	41.18	0.3986
bta03015 mRNA surveillance pathway	36.84	0.3986
bta05162 Measles	35.53	0.4071
bta00592 alpha-Linolenic acid metabolism	41.38	0.4228
bta00061 Fatty acid biosynthesis	44.44	0.4252
bta00730 Thiamine metabolism	44.44	0.4252
bta05020 Prion diseases	40.63	0.4252
bta05134 Legionellosis	37.93	0.4252
bta00900 Terpenoid backbone biosynthesis	42.86	0.4332
bta04210 Apoptosis	35.21	0.4332
bta04935 Growth hormone synthesis. secretion and action	35.59	0.4332
bta00630 Glyoxylate and dicarboxylate metabolism	40.00	0.453
bta04620 Toll-like receptor signaling pathway	35.45	0.453
bta03410 Base excision repair	39.39	0.454
bta00591 Linoleic acid metabolism	38.89	0.4561
bta04216 Ferroptosis	37.78	0.4631
bta00511 Other glycan degradation	40.91	0.4696
bta00531 Glycosaminoglycan degradation	40.91	0.4696
bta04721 Synaptic vesicle cycle	35.90	0.4696
bta04060 Cytokine-cytokine receptor interaction	33.44	0.4806
bta00410 beta-Alanine metabolism	38.24	0.4834
bta00250 Alanine. aspartate and glutamate metabolism	37.84	0.4837
bta00072 Synthesis and degradation of ketone bodies	45.45	0.484

bta00565 Ether lipid metabolism	36.54	0.484
bta04923 Regulation of lipolysis in adipocytes	36.21	0.484
bta00533 Glycosaminoglycan biosynthesis	42.86	0.49
bta00532 Glycosaminoglycan biosynthesis	40.00	0.5022
bta03430 Mismatch repair	39.13	0.5041
bta00563 Glycosylphosphatidylinositol (GPI)-anchor biosynthesis	38.46	0.5048
bta00520 Amino sugar and nucleotide sugar metabolism	36.00	0.5061
bta04110 Cell cycle	34.15	0.5061
bta04146 Peroxisome	34.52	0.5231
bta00330 Arginine and proline metabolism	35.42	0.5371
bta00670 One carbon pool by folate	38.89	0.5371
bta03030 DNA replication	36.11	0.5371
bta04650 Natural killer cell mediated cytotoxicity	33.59	0.5371
bta03018 RNA degradation	34.18	0.5436
bta04915 Estrogen signaling pathway	33.33	0.55
bta00510 N-Glycan biosynthesis	34.62	0.5592
bta00770 Pantothenate and CoA biosynthesis	36.84	0.5766
bta04217 Necroptosis	32.76	0.5766
bta04672 Intestinal immune network for IgA production	33.93	0.5766
bta04964 Proximal tubule bicarbonate reclamation	36.36	0.5766
bta05017 Spinocerebellar ataxia	33.33	0.5766
bta05330 Allograft rejection	33.93	0.5766
bta05014 Amyotrophic lateral sclerosis (ALS)	33.33	0.6037
bta04913 Ovarian steroidogenesis	33.33	0.6053
bta00340 Histidine metabolism	34.78	0.6102
bta00360 Phenylalanine metabolism	34.78	0.6102
bta00515 Mannose type O-glycan biosynthesis	34.78	0.6102
bta03060 Protein export	34.78	0.6102

bta00350 Tyrosine metabolism	33.33	0.6195
bta04917 Prolactin signaling pathway	32.53	0.6195
bta05203 Viral carcinogenesis	31.95	0.6201
bta04918 Thyroid hormone synthesis	32.43	0.6248
bta00020 Citrate cycle (TCA cycle)	33.33	0.6315
bta00260 Glycine. serine and threonine metabolism	32.56	0.6414
bta00240 Pyrimidine metabolism	32.14	0.6482
bta04960 Aldosterone-regulated sodium reabsorption	32.43	0.6505
bta04612 Antigen processing and presentation	31.76	0.6524
bta05143 African trypanosomiasis	31.82	0.6674
bta04966 Collecting duct acid secretion	32.14	0.6693
bta03440 Homologous recombination	30.95	0.7052
bta04723 Retrograde endocannabinoid signaling	30.92	0.7052
bta03460 Fanconi anemia pathway	30.77	0.7074
bta05323 Rheumatoid arthritis	30.77	0.7074
bta04213 Longevity regulating pathway	30.65	0.7091
bta00604 Glycosphingolipid biosynthesis	31.25	0.7099
bta00513 Various types of N-glycan biosynthesis	30.23	0.7218
bta04136 Autophagy	30.30	0.7218
bta00860 Porphyrin and chlorophyll metabolism	30.00	0.7296
bta05168 Herpes simplex virus 1 infection	30.77	0.7299
bta05204 Chemical carcinogenesis	29.87	0.7428
bta03022 Basal transcription factors	29.55	0.7436
bta03320 PPAR signaling pathway	29.63	0.7512
bta05340 Primary immunodeficiency	29.27	0.7512
bta00040 Pentose and glucuronate interconversions	29.03	0.7513
bta04512 ECM-receptor interaction	29.21	0.7761
bta00790 Folate biosynthesis	27.78	0.7975

bta05146 Amoebiasis	29.06	0.7975
bta00140 Steroid hormone biosynthesis	28.36	0.7977
bta04979 Cholesterol metabolism	28.00	0.7977
bta00830 Retinol metabolism	28.13	0.8025
bta04977 Vitamin digestion and absorption	26.92	0.8025
bta04630 JAK-STAT signaling pathway	29.21	0.8143
bta04929 GnRH secretion	27.69	0.8175
bta04145 Phagosome	28.82	0.8241
bta05310 Asthma	26.32	0.8327
bta04622 RIG-I-like receptor signaling pathway	27.45	0.8593
bta00053 Ascorbate and aldarate metabolism	24.00	0.8655
bta03040 Spliceosome	27.89	0.8655
bta04260 Cardiac muscle contraction	26.97	0.8655
bta04742 Taste transduction	26.58	0.8655
bta05033 Nicotine addiction	25.00	0.8655
bta05144 Malaria	25.42	0.8818
bta00982 Drug metabolism	25.40	0.886
bta04080 Neuroactive ligand-receptor interaction	28.65	0.8865
bta05320 Autoimmune thyroid disease	25.35	0.8935
bta00590 Arachidonic acid metabolism	25.61	0.8948
bta00983 Drug metabolism	25.00	0.9046
bta04714 Thermogenesis	27.62	0.9046
bta00980 Metabolism of xenobiotics by cytochrome P450	23.88	0.9269
bta00480 Glutathione metabolism	23.33	0.9294
bta04932 Non-alcoholic fatty liver disease (NAFLD)	25.95	0.9344
bta00970 Aminoacyl-tRNA biosynthesis	22.73	0.9427
bta04610 Complement and coagulation cascades	23.91	0.9427
bta05206 MicroRNAs in cancer	27.05	0.9427

bta05010 Alzheimer disease	25.56	0.9477
bta05034 Alcoholism	26.20	0.9477
bta03008 Ribosome biogenesis in eukaryotes	22.89	0.9507
bta05150 Staphylococcus aureus infection	22.86	0.9689
bta03050 Proteasome	17.39	0.9904
bta02010 ABC transporters	18.33	0.9926
bta00190 Oxidative phosphorylation	12.14	1.0
bta03010 Ribosome	13.13	1.0
bta04740 Olfactory transduction	4.63	1.0
bta04974 Protein digestion and absorption	17.36	1.0
bta05012 Parkinson disease	17.33	1.0
bta05016 Huntington disease	21.53	1.0
bta05322 Systemic lupus erythematosus	14.29	1.0

[1]%: Percent of genes predicted to be modulated. [2]BH: Benjamini – Hochberg

Supplementary table 12. Biological patwhays predicted as modulated by miRNAs up regulated in ampullary luminal epithelial cells (AMP-Cell) in high body energy reserve (HBER) group.

Pathway	**%[1]**	**BH[2]**
bta05205 Proteoglycans in cancer	18.05	0.0
bta04810 Regulation of actin cytoskeleton	16.59	0.0066
bta05211 Renal cell carcinoma	23.94	0.0066
bta04014 Ras signaling pathway	14.46	0.0239
bta04360 Axon guidance	15.73	0.0239
bta04012 ErbB signaling pathway	19.05	0.0318
bta04144 Endocytosis	13.88	0.0318
bta04660 T cell receptor signaling pathway	17.76	0.0318
bta04722 Neurotrophin signaling pathway	17.21	0.0318

bta05212 Pancreatic cancer	19.74	0.0318
bta05214 Glioma	19.48	0.0326
bta04550 Signaling pathways regulating pluripotency of stem cells	15.49	0.0398
bta04625 C-type lectin receptor signaling pathway	16.98	0.0398
bta04370 VEGF signaling pathway	20.69	0.0455
bta05223 Non-small cell lung cancer	19.40	0.0478
bta01522 Endocrine resistance	17.02	0.0492
bta04022 cGMP-PKG signaling pathway	14.20	0.0492
bta05200 Pathways in cancer	11.07	0.0492
bta05220 Chronic myeloid leukemia	18.18	0.0492
bta05225 Hepatocellular carcinoma	13.79	0.0637
bta04919 Thyroid hormone signaling pathway	15.25	0.0654
bta00564 Glycerophospholipid metabolism	15.38	0.0812
bta04010 MAPK signaling pathway	11.99	0.0812
bta04020 Calcium signaling pathway	12.87	0.0812
bta04921 Oxytocin signaling pathway	13.82	0.0812
bta04664 Fc epsilon RI signaling pathway	17.14	0.088
bta05210 Colorectal cancer	15.73	0.0929
bta05414 Dilated cardiomyopathy (DCM)	15.15	0.0952
bta04510 Focal adhesion	12.63	0.0961
bta05100 Bacterial invasion of epithelial cells	16.44	0.0995
bta05410 Hypertrophic cardiomyopathy (HCM)	15.22	0.1021
bta04666 Fc gamma R-mediated phagocytosis	15.05	0.107
bta05226 Gastric cancer	13.07	0.1135
bta05412 Arrhythmogenic right ventricular cardiomyopathy (ARVC)	15.79	0.1135
bta04150 mTOR signaling pathway	12.74	0.1342
bta01521 EGFR tyrosine kinase inhibitor resistance	15.00	0.135
bta04071 Sphingolipid signaling pathway	13.33	0.135

bta04151 PI3K-Akt signaling pathway	10.72	0.135
bta04261 Adrenergic signaling in cardiomyocytes	12.67	0.135
bta05135 Yersinia infection	13.08	0.135
bta05163 Human cytomegalovirus infection	11.43	0.135
bta05202 Transcriptional misregulation in cancer	12.04	0.135
bta04611 Platelet activation	13.22	0.1402
bta04140 Autophagy	12.68	0.1406
bta05218 Melanoma	15.07	0.1406
bta04270 Vascular smooth muscle contraction	12.78	0.1427
bta05235 PD-L1 expression and PD-1 checkpoint pathway in cancer	13.98	0.1427
bta04925 Aldosterone synthesis and secretion	13.54	0.1696
bta04915 Estrogen signaling pathway	12.32	0.1779
bta04961 Endocrine and other factor-regulated calcium reabsorption	16.00	0.1814
bta05213 Endometrial cancer	15.25	0.1814
bta05224 Breast cancer	12.00	0.1814
bta05231 Choline metabolism in cancer	13.13	0.184
bta04068 FoxO signaling pathway	12.21	0.1885
bta04072 Phospholipase D signaling pathway	11.84	0.1885
bta04380 Osteoclast differentiation	11.94	0.2072
bta04724 Glutamatergic synapse	12.39	0.2072
bta04924 Renin secretion	13.89	0.2072
bta04390 Hippo signaling pathway	11.54	0.2076
bta04912 GnRH signaling pathway	12.90	0.2076
bta04630 JAK-STAT signaling pathway	10.89	0.2222
bta04015 Rap1 signaling pathway	10.65	0.2388
bta04960 Aldosterone-regulated sodium reabsorption	16.22	0.2388
bta05230 Central carbon metabolism in cancer	13.64	0.2388
bta04926 Relaxin signaling pathway	11.54	0.2484

bta05215 Prostate cancer	12.24	0.2484
bta01524 Platinum drug resistance	12.82	0.256
bta04066 HIF-1 signaling pathway	11.82	0.2587
bta04062 Chemokine signaling pathway	10.64	0.2658
bta04141 Protein processing in endoplasmic reticulum	10.84	0.2658
bta05206 MicroRNAs in cancer	9.93	0.2679
bta04728 Dopaminergic synapse	11.19	0.2711
bta04916 Melanogenesis	11.76	0.2748
bta00512 Mucin type O-glycan biosynthesis	16.13	0.2759
bta04917 Prolactin signaling pathway	12.05	0.2936
bta04976 Bile secretion	12.05	0.2936
bta05165 Human papillomavirus infection	9.57	0.2936
bta05219 Bladder cancer	14.29	0.2939
bta03015 mRNA surveillance pathway	11.58	0.3045
bta04910 Insulin signaling pathway	10.71	0.3075
bta04911 Insulin secretion	11.76	0.3075
bta05017 Spinocerebellar ataxia	11.46	0.3075
bta00514 Other types of O-glycan biosynthesis	13.33	0.308
bta04114 Oocyte meiosis	10.92	0.308
bta04142 Lysosome	10.61	0.308
bta04210 Apoptosis	10.56	0.308
bta04218 Cellular senescence	10.24	0.308
bta04650 Natural killer cell mediated cytotoxicity	10.69	0.308
bta04662 B cell receptor signaling pathway	11.49	0.308
bta04978 Mineral absorption	12.73	0.308
bta05160 Hepatitis C	10.37	0.308
bta05166 Human T-cell leukemia virus 1 infection	9.83	0.308
bta04934 Cushing syndrome	10.26	0.316

bta05418 Fluid shear stress and atherosclerosis	10.34	0.3178
bta00790 Folate biosynthesis	13.89	0.3236
bta04913 Ovarian steroidogenesis	12.28	0.3248
bta04670 Leukocyte transendothelial migration	10.62	0.3334
bta05167 Kaposi sarcoma-associated herpesvirus infection	9.71	0.3334
bta04720 Long-term potentiation	11.59	0.3361
bta04750 Inflammatory mediator regulation of TRP channels	10.68	0.3415
bta04520 Adherens junction	11.43	0.3462
bta04310 Wnt signaling pathway	9.88	0.347
bta04928 Parathyroid hormone synthesis. secretion and action	10.58	0.347
bta00600 Sphingolipid metabolism	12.24	0.3496
bta04371 Apelin signaling pathway	10.00	0.3541
bta05222 Small cell lung cancer	10.64	0.3541
bta04530 Tight junction	9.60	0.3641
bta04935 Growth hormone synthesis. secretion and action	10.17	0.3641
bta04340 Hedgehog signaling pathway	11.76	0.3732
bta04918 Thyroid hormone synthesis	10.81	0.3824
bta04064 NF-kappa B signaling pathway	10.09	0.3825
bta05340 Primary immunodeficiency	12.20	0.3825
bta04024 cAMP signaling pathway	9.17	0.3853
bta00230 Purine metabolism	9.70	0.3943
bta04110 Cell cycle	9.76	0.3958
bta04152 AMPK signaling pathway	9.76	0.3958
bta04927 Cortisol synthesis and secretion	10.77	0.3958
bta04929 GnRH secretion	10.77	0.3958
bta04137 Mitophagy	10.61	0.4076
bta04659 Th17 cell differentiation	9.73	0.4076
bta04721 Synaptic vesicle cycle	10.26	0.4076

bta05152 Tuberculosis	9.09	0.4076
bta04540 Gap junction	10.00	0.4082
bta00561 Glycerolipid metabolism	10.45	0.4108
bta00240 Pyrimidine metabolism	10.71	0.4173
bta04216 Ferroptosis	11.11	0.4225
bta04350 TGF-beta signaling pathway	9.68	0.4392
bta04668 TNF signaling pathway	9.32	0.4443
bta04923 Regulation of lipolysis in adipocytes	10.34	0.4443
bta04061 Viral protein interaction with cytokine and cytokine receptor	9.47	0.4583
bta05161 Hepatitis B	8.77	0.4809
bta04060 Cytokine-cytokine receptor interaction	8.36	0.4825
bta04962 Vasopressin-regulated water reabsorption	10.20	0.4825
bta04213 Longevity regulating pathway	9.68	0.4987
bta05217 Basal cell carcinoma	9.52	0.5134
bta04914 Progesterone-mediated oocyte maturation	9.09	0.5159
bta04971 Gastric acid secretion	9.21	0.5183
bta00510 N-Glycan biosynthesis	9.62	0.522
bta04725 Cholinergic synapse	8.77	0.522
bta04115 p53 signaling pathway	9.09	0.5235
bta00310 Lysine degradation	9.09	0.5449
bta04970 Salivary secretion	8.60	0.5688
bta04672 Intestinal immune network for IgA production	8.93	0.5854
bta04640 Hematopoietic cell lineage	8.18	0.6192
bta04730 Long-term depression	8.33	0.6485
bta05014 Amyotrophic lateral sclerosis (ALS)	8.33	0.6485
bta05170 Human immunodeficiency virus 1 infection	7.69	0.6621
bta04211 Longevity regulating pathway	7.78	0.6759
bta04260 Cardiac muscle contraction	7.87	0.6759

bta04621 NOD-like receptor signaling pathway	7.61	0.6759
bta04922 Glucagon signaling pathway	7.77	0.6759
bta04933 AGE-RAGE signaling pathway in diabetic complications	7.77	0.6759
bta05133 Pertussis	7.79	0.6791
bta05169 Epstein-Barr virus infection	7.46	0.6975
bta04620 Toll-like receptor signaling pathway	7.27	0.7417
bta04931 Insulin resistance	7.27	0.7417
bta03008 Ribosome biogenesis in eukaryotes	7.23	0.7418
bta04120 Ubiquitin mediated proteolysis	7.14	0.7418
bta04658 Th1 and Th2 cell differentiation	7.14	0.7418
bta05031 Amphetamine addiction	7.25	0.7418
bta05168 Herpes simplex virus 1 infection	7.20	0.7418
bta05321 Inflammatory bowel disease (IBD)	7.14	0.7418
bta04713 Circadian entrainment	7.00	0.7579
bta04920 Adipocytokine signaling pathway	6.94	0.7612
bta04932 Non-alcoholic fatty liver disease (NAFLD)	6.96	0.7632
bta04217 Necroptosis	6.90	0.7753
bta04512 ECM-receptor interaction	6.74	0.7796
bta00983 Drug metabolism	6.58	0.7817
bta03013 RNA transport	6.74	0.7817
bta04080 Neuroactive ligand-receptor interaction	6.89	0.7817
bta04657 IL-17 signaling pathway	6.52	0.7817
bta04727 GABAergic synapse	6.59	0.7817
bta05032 Morphine addiction	6.52	0.7817
bta05416 Viral myocarditis	6.58	0.7817
bta05140 Leishmaniasis	6.41	0.7862
bta05164 Influenza A	6.63	0.7862
bta03018 RNA degradation	6.33	0.7895

bta05203 Viral carcinogenesis	6.64	0.7935
bta04145 Phagosome	6.47	0.7991
bta04514 Cell adhesion molecules (CAMs)	6.33	0.8101
bta05145 Toxoplasmosis	6.19	0.8101
bta04070 Phosphatidylinositol signaling system	6.06	0.8143
bta04146 Peroxisome	5.95	0.8143
bta01100 Metabolic pathways	6.94	0.8181
bta03040 Spliceosome	6.12	0.8181
bta04612 Antigen processing and presentation	5.88	0.8181
bta04972 Pancreatic secretion	5.88	0.8181
bta05132 Salmonella infection	6.25	0.8206
bta04723 Retrograde endocannabinoid signaling	5.92	0.8221
bta04974 Protein digestion and absorption	5.79	0.8221
bta05162 Measles	5.92	0.8221
bta05323 Rheumatoid arthritis	5.77	0.8221
bta05142 Chagas disease (American trypanosomiasis)	5.22	0.8788
bta04726 Serotonergic synapse	5.17	0.8796
bta04714 Thermogenesis	5.44	0.9138
bta05010 Alzheimer disease	5.00	0.9221
bta05034 Alcoholism	5.24	0.9221
bta05016 Huntington disease	5.11	0.9437
bta04740 Olfactory transduction	0.96	1.0

[1]%: Percent of genes predicted to be modulated. [2]BH: Benjamini – Hochberg

Supplementary table 13. Biological patwhays predicted as modulated by miRNAs up regulated in isthmic luminal epithelial cells (IST-Cell) in high body energy reserve (HBER) group.

Pathway	**%[1]**	**BH[2]**

bta04010 MAPK signaling pathway	50.34	0.0
bta04014 Ras signaling pathway	53.31	0.0
bta04015 Rap1 signaling pathway	53.24	0.0
bta04360 Axon guidance	55.62	0.0
bta05200 Pathways in cancer	47.79	0.0
bta04144 Endocytosis	50.61	0.0039
bta04810 Regulation of actin cytoskeleton	52.13	0.0039
bta04934 Cushing syndrome	55.13	0.0039
bta04072 Phospholipase D signaling pathway	53.95	0.0105
bta04062 Chemokine signaling pathway	50.53	0.0126
bta04140 Autophagy	54.23	0.0126
bta04150 mTOR signaling pathway	52.87	0.0126
bta04390 Hippo signaling pathway	52.56	0.0126
bta04910 Insulin signaling pathway	54.29	0.0126
bta05205 Proteoglycans in cancer	49.76	0.0126
bta01522 Endocrine resistance	57.45	0.0192
bta05212 Pancreatic cancer	60.53	0.0192
bta05215 Prostate cancer	57.14	0.0192
bta05224 Breast cancer	51.33	0.0231
bta04550 Signaling pathways regulating pluripotency of stem cells	51.41	0.0267
bta04611 Platelet activation	52.89	0.0284
bta04520 Adherens junction	60.00	0.0285
bta04012 ErbB signaling pathway	57.14	0.0287
bta04931 Insulin resistance	53.64	0.0288
bta01100 Metabolic pathways	38.18	0.0289
bta04020 Calcium signaling pathway	47.03	0.0289
bta04310 Wnt signaling pathway	48.77	0.0289
bta04510 Focal adhesion	47.47	0.0289

bta04659 Th17 cell differentiation	52.21	0.0289
bta04660 T cell receptor signaling pathway	53.27	0.0289
bta04668 TNF signaling pathway	51.69	0.0289
bta04722 Neurotrophin signaling pathway	51.64	0.0289
bta04922 Glucagon signaling pathway	53.40	0.0289
bta05100 Bacterial invasion of epithelial cells	57.53	0.0289
bta05135 Yersinia infection	50.77	0.0289
bta05210 Colorectal cancer	55.06	0.0289
bta05211 Renal cell carcinoma	59.15	0.0289
bta05230 Central carbon metabolism in cancer	59.09	0.0289
bta05226 Gastric cancer	49.02	0.0306
bta00514 Other types of O-glycan biosynthesis	64.44	0.0345
bta05214 Glioma	55.84	0.036
bta04928 Parathyroid hormone synthesis. secretion and action	51.92	0.0396
bta04916 Melanogenesis	51.96	0.0416
bta04530 Tight junction	46.89	0.0428
bta04071 Sphingolipid signaling pathway	50.00	0.0447
bta04514 Cell adhesion molecules (CAMs)	47.47	0.0451
bta04919 Thyroid hormone signaling pathway	50.00	0.0451
bta05220 Chronic myeloid leukemia	54.55	0.0451
bta04024 cAMP signaling pathway	44.54	0.0521
bta04142 Lysosome	48.48	0.0521
bta04625 C-type lectin receptor signaling pathway	50.00	0.0598
bta05225 Hepatocellular carcinoma	45.98	0.0598
bta04664 Fc epsilon RI signaling pathway	54.29	0.0599
bta05163 Human cytomegalovirus infection	43.67	0.0599
bta05231 Choline metabolism in cancer	50.51	0.0599
bta01521 EGFR tyrosine kinase inhibitor resistance	52.50	0.0611

bta04921 Oxytocin signaling pathway	46.71	0.0611
bta04270 Vascular smooth muscle contraction	47.37	0.0664
bta04720 Long-term potentiation	53.62	0.0664
bta04935 Growth hormone synthesis. secretion and action	48.31	0.0664
bta04961 Endocrine and other factor-regulated calcium reabsorption	58.00	0.0664
bta04925 Aldosterone synthesis and secretion	50.00	0.0679
bta04728 Dopaminergic synapse	47.01	0.0703
bta04371 Apelin signaling pathway	46.43	0.0754
bta05217 Basal cell carcinoma	53.97	0.0754
bta04926 Relaxin signaling pathway	46.92	0.0756
bta04658 Th1 and Th2 cell differentiation	48.98	0.0803
bta04912 GnRH signaling pathway	49.46	0.0803
bta05017 Spinocerebellar ataxia	48.96	0.0846
bta00564 Glycerophospholipid metabolism	48.08	0.0884
bta04920 Adipocytokine signaling pathway	51.39	0.0902
bta04915 Estrogen signaling pathway	45.65	0.0946
bta01212 Fatty acid metabolism	53.45	0.0951
bta04152 AMPK signaling pathway	46.34	0.0963
bta05221 Acute myeloid leukemia	51.47	0.0971
bta05235 PD-L1 expression and PD-1 checkpoint pathway in cancer	48.39	0.0987
bta05132 Salmonella infection	42.41	0.1036
bta00512 Mucin type O-glycan biosynthesis	61.29	0.1053
bta04120 Ubiquitin mediated proteolysis	45.00	0.1053
bta00533 Glycosaminoglycan biosynthesis	78.57	0.1082
bta04392 Hippo signaling pathway	62.07	0.1082
bta04924 Renin secretion	50.00	0.1107
bta00562 Inositol phosphate metabolism	49.32	0.1139
bta04215 Apoptosis	58.82	0.1139

bta04261 Adrenergic signaling in cardiomyocytes	44.00	0.1139
bta04666 Fc gamma R-mediated phagocytosis	47.31	0.1139
bta04730 Long-term depression	51.67	0.1139
bta04750 Inflammatory mediator regulation of TRP channels	46.60	0.1139
bta04911 Insulin secretion	48.24	0.1139
bta04914 Progesterone-mediated oocyte maturation	47.73	0.1139
bta04917 Prolactin signaling pathway	48.19	0.1139
bta00310 Lysine degradation	50.00	0.1143
bta04022 cGMP-PKG signaling pathway	43.20	0.1143
bta04064 NF-kappa B signaling pathway	45.87	0.1143
bta04137 Mitophagy	50.00	0.1143
bta04340 Hedgehog signaling pathway	52.94	0.1143
bta05412 Arrhythmogenic right ventricular cardiomyopathy (ARVC)	48.68	0.1143
bta04070 Phosphatidylinositol signaling system	46.46	0.1153
bta05213 Endometrial cancer	50.85	0.1168
bta05219 Bladder cancer	54.76	0.1168
bta00510 N-Glycan biosynthesis	51.92	0.1219
bta04670 Leukocyte transendothelial migration	45.13	0.122
bta05165 Human papillomavirus infection	39.71	0.122
bta05223 Non-small cell lung cancer	49.25	0.122
bta04927 Cortisol synthesis and secretion	49.23	0.1274
bta05014 Amyotrophic lateral sclerosis (ALS)	50.00	0.1274
bta04350 TGF-beta signaling pathway	46.24	0.1277
bta05202 Transcriptional misregulation in cancer	41.88	0.1294
bta04330 Notch signaling pathway	50.94	0.131
bta04370 VEGF signaling pathway	50.00	0.131
bta04066 HIF-1 signaling pathway	44.55	0.1403
bta04068 FoxO signaling pathway	43.51	0.1403

bta05161 Hepatitis B	42.11	0.1403
bta00230 Purine metabolism	43.28	0.1427
bta05166 Human T-cell leukemia virus 1 infection	40.60	0.1431
bta01040 Biosynthesis of unsaturated fatty acids	56.67	0.1442
bta04151 PI3K-Akt signaling pathway	38.87	0.1442
bta04211 Longevity regulating pathway	45.56	0.1442
bta04726 Serotonergic synapse	43.97	0.1442
bta05160 Hepatitis C	42.07	0.1442
bta00770 Pantothenate and CoA biosynthesis	63.16	0.156
bta01524 Platinum drug resistance	46.15	0.156
bta04978 Mineral absorption	49.09	0.156
bta05170 Human immunodeficiency virus 1 infection	40.17	0.1588
bta00513 Various types of N-glycan biosynthesis	51.16	0.1615
bta04971 Gastric acid secretion	46.05	0.1625
bta04713 Circadian entrainment	44.00	0.1699
bta04930 Type II diabetes mellitus	50.00	0.1702
bta04540 Gap junction	44.44	0.1749
bta05167 Kaposi sarcoma-associated herpesvirus infection	40.29	0.1749
bta04725 Cholinergic synapse	42.98	0.1764
bta00100 Steroid biosynthesis	60.00	0.1808
bta00071 Fatty acid degradation	50.00	0.1891
bta04721 Synaptic vesicle cycle	44.87	0.1903
bta05218 Melanoma	45.21	0.1935
bta04923 Regulation of lipolysis in adipocytes	46.55	0.2048
bta04114 Oocyte meiosis	42.02	0.206
bta04061 Viral protein interaction with cytokine and cytokine receptor	43.16	0.2071
bta00240 Pyrimidine metabolism	46.43	0.2148
bta04210 Apoptosis	40.85	0.2227

bta05414 Dilated cardiomyopathy (DCM)	42.42	0.2285
bta00592 alpha-Linolenic acid metabolism	51.72	0.235
bta04724 Glutamatergic synapse	41.59	0.235
bta00565 Ether lipid metabolism	46.15	0.2388
bta04146 Peroxisome	42.86	0.2439
bta05032 Morphine addiction	42.39	0.2439
bta04662 B cell receptor signaling pathway	42.53	0.2493
bta05216 Thyroid cancer	47.50	0.2599
bta00020 Citrate cycle (TCA cycle)	50.00	0.2638
bta00010 Glycolysis Gluconeogenesis	43.75	0.2707
bta01200 Carbon metabolism	40.71	0.2759
bta05340 Primary immunodeficiency	46.34	0.2882
bta04657 IL-17 signaling pathway	41.30	0.2899
bta04933 AGE-RAGE signaling pathway in diabetic complications	40.78	0.2899
bta05410 Hypertrophic cardiomyopathy (HCM)	41.30	0.2899
bta04710 Circadian rhythm	48.39	0.2923
bta05418 Fluid shear stress and atherosclerosis	39.31	0.2962
bta04380 Osteoclast differentiation	39.55	0.2969
bta00760 Nicotinate and nicotinamide metabolism	46.15	0.297
bta05142 Chagas disease (American trypanosomiasis)	40.00	0.302
bta00534 Glycosaminoglycan biosynthesis	50.00	0.3086
bta05169 Epstein-Barr virus infection	37.72	0.3086
bta04141 Protein processing in endoplasmic reticulum	38.55	0.31
bta05031 Amphetamine addiction	42.03	0.31
bta05222 Small cell lung cancer	40.43	0.318
bta04964 Proximal tubule bicarbonate reclamation	50.00	0.3272
bta04650 Natural killer cell mediated cytotoxicity	38.93	0.3312
bta05321 Inflammatory bowel disease (IBD)	41.43	0.3312

bta00620 Pyruvate metabolism	44.74	0.3408
bta00640 Propanoate metabolism	45.45	0.3465
bta04130 SNARE interactions in vesicular transport	45.45	0.3465
bta04962 Vasopressin-regulated water reabsorption	42.86	0.3465
bta05162 Measles	38.16	0.3472
bta05145 Toxoplasmosis	38.94	0.3555
bta00515 Mannose type O-glycan biosynthesis	47.83	0.3556
bta04115 p53 signaling pathway	40.26	0.3556
bta04621 NOD-like receptor signaling pathway	37.50	0.3556
bta04972 Pancreatic secretion	39.22	0.3556
bta00052 Galactose metabolism	45.16	0.3592
bta00061 Fatty acid biosynthesis	50.00	0.3592
bta04976 Bile secretion	39.76	0.3608
bta00410 beta-Alanine metabolism	44.12	0.3697
bta04216 Ferroptosis	42.22	0.3759
bta00062 Fatty acid elongation	44.83	0.3787
bta04960 Aldosterone-regulated sodium reabsorption	43.24	0.3787
bta01230 Biosynthesis of amino acids	39.73	0.3836
bta05020 Prion diseases	43.75	0.3896
bta00260 Glycine. serine and threonine metabolism	41.86	0.3937
bta00600 Sphingolipid metabolism	40.82	0.4066
bta04727 GABAergic synapse	38.46	0.4066
bta04918 Thyroid hormone synthesis	39.19	0.4066
bta05133 Pertussis	38.96	0.4066
bta00531 Glycosaminoglycan degradation	45.45	0.4123
bta04136 Autophagy	42.42	0.4183
bta04973 Carbohydrate digestion and absorption	40.91	0.4183
bta05134 Legionellosis	39.66	0.4189

bta00340 Histidine metabolism	43.48	0.4539
bta04213 Longevity regulating pathway	38.71	0.4539
bta04218 Cellular senescence	36.14	0.4539
bta04929 GnRH secretion	38.46	0.4539
bta05030 Cocaine addiction	39.58	0.4539
bta05152 Tuberculosis	35.86	0.4539
bta00280 Valine. leucine and isoleucine degradation	39.22	0.4549
bta05164 Influenza A	35.91	0.4579
bta04913 Ovarian steroidogenesis	38.60	0.4626
bta00601 Glycosphingolipid biosynthesis	41.38	0.4634
bta00730 Thiamine metabolism	44.44	0.4634
bta00500 Starch and sucrose metabolism	40.63	0.4715
bta03320 PPAR signaling pathway	37.04	0.4878
bta00561 Glycerolipid metabolism	37.31	0.4969
bta04970 Salivary secretion	36.56	0.4969
bta00380 Tryptophan metabolism	38.30	0.4985
bta05416 Viral myocarditis	36.84	0.5024
bta00591 Linoleic acid metabolism	38.89	0.5094
bta03030 DNA replication	38.89	0.5094
bta03440 Homologous recombination	38.10	0.5188
bta03420 Nucleotide excision repair	37.78	0.5214
bta04966 Collecting duct acid secretion	39.29	0.5289
bta00051 Fructose and mannose metabolism	38.24	0.5392
bta04080 Neuroactive ligand-receptor interaction	34.16	0.5425
bta00270 Cysteine and methionine metabolism	36.73	0.5548
bta03460 Fanconi anemia pathway	36.54	0.5553
bta03430 Mismatch repair	39.13	0.5556
bta04672 Intestinal immune network for IgA production	35.71	0.5892

bta00670 One carbon pool by folate	38.89	0.5906
bta01210 2-Oxocarboxylic acid metabolism	38.89	0.5906
bta04060 Cytokine-cytokine receptor interaction	33.75	0.5906
bta04620 Toll-like receptor signaling pathway	34.55	0.5906
bta04640 Hematopoietic cell lineage	34.55	0.5906
bta04744 Phototransduction	37.04	0.5961
bta04975 Fat digestion and absorption	35.42	0.6045
bta05146 Amoebiasis	34.19	0.6074
bta00604 Glycosphingolipid biosynthesis	37.50	0.6351
bta01523 Antifolate resistance	34.88	0.6351
bta03015 mRNA surveillance pathway	33.68	0.6537
bta03008 Ribosome biogenesis in eukaryotes	33.73	0.6542
bta00520 Amino sugar and nucleotide sugar metabolism	34.00	0.6626
bta04110 Cell cycle	33.33	0.6626
bta00563 Glycosylphosphatidylinositol (GPI)-anchor biosynthesis	34.62	0.6654
bta03020 RNA polymerase	34.48	0.6654
bta04950 Maturity onset diabetes of the young	34.62	0.6654
bta04977 Vitamin digestion and absorption	34.62	0.6654
bta00532 Glycosaminoglycan biosynthesis	35.00	0.6691
bta00910 Nitrogen metabolism	35.29	0.6705
bta00480 Glutathione metabolism	33.33	0.6708
bta00330 Arginine and proline metabolism	33.33	0.6771
bta00590 Arachidonic acid metabolism	32.93	0.6772
bta00983 Drug metabolism	32.89	0.6772
bta04612 Antigen processing and presentation	32.94	0.6772
bta04742 Taste transduction	32.91	0.6772
bta00900 Terpenoid backbone biosynthesis	33.33	0.7014
bta00250 Alanine. aspartate and glutamate metabolism	32.43	0.7131

bta04723 Retrograde endocannabinoid signaling	32.24	0.716
bta05140 Leishmaniasis	32.05	0.7212
bta00053 Ascorbate and aldarate metabolism	32.00	0.7286
bta03022 Basal transcription factors	31.82	0.7286
bta05323 Rheumatoid arthritis	31.73	0.7388
bta00140 Steroid hormone biosynthesis	31.34	0.7517
bta00350 Tyrosine metabolism	30.77	0.768
bta00360 Phenylalanine metabolism	30.43	0.7709
bta03013 RNA transport	31.46	0.7709
bta03410 Base excision repair	30.30	0.7763
bta00630 Glyoxylate and dicarboxylate metabolism	30.00	0.7834
bta00603 Glycosphingolipid biosynthesis	29.41	0.7894
bta00982 Drug metabolism	30.16	0.7894
bta04217 Necroptosis	31.03	0.7894
bta04979 Cholesterol metabolism	30.00	0.7894
bta04512 ECM-receptor interaction	30.34	0.7946
bta00980 Metabolism of xenobiotics by cytochrome P450	29.85	0.7993
bta03040 Spliceosome	30.61	0.7993
bta04623 Cytosolic DNA-sensing pathway	29.85	0.7993
bta00030 Pentose phosphate pathway	28.57	0.8039
bta00650 Butanoate metabolism	28.57	0.8039
bta04630 JAK-STAT signaling pathway	30.69	0.8079
bta00511 Other glycan degradation	27.27	0.8296
bta00790 Folate biosynthesis	27.78	0.8339
bta04260 Cardiac muscle contraction	29.21	0.8339
bta00220 Arginine biosynthesis	26.32	0.8382
bta04614 Renin-angiotensin system	26.92	0.8382
bta04932 Non-alcoholic fatty liver disease (NAFLD)	29.75	0.8392

bta05033 Nicotine addiction	27.50	0.8392
bta00040 Pentose and glucuronate interconversions	25.81	0.8704
bta05310 Asthma	26.32	0.8704
bta03018 RNA degradation	27.85	0.8712
bta04714 Thermogenesis	29.71	0.8719
bta05206 MicroRNAs in cancer	29.79	0.8839
bta05204 Chemical carcinogenesis	27.27	0.8852
bta00830 Retinol metabolism	26.56	0.8925
bta05143 African trypanosomiasis	25.00	0.9067
bta04145 Phagosome	28.24	0.9135
bta04940 Type I diabetes mellitus	25.42	0.9155
bta03060 Protein export	21.74	0.9209
bta05016 Huntington disease	28.83	0.9209
bta05150 Staphylococcus aureus infection	26.67	0.9209
bta04610 Complement and coagulation cascades	26.09	0.9257
bta00860 Porphyrin and chlorophyll metabolism	22.50	0.9421
bta05144 Malaria	23.73	0.9432
bta05332 Graft-versus-host disease	22.92	0.9432
bta04622 RIG-I-like receptor signaling pathway	25.49	0.944
bta05010 Alzheimer disease	26.67	0.9582
bta05168 Herpes simplex virus 1 infection	28.29	0.9681
bta05330 Allograft rejection	21.43	0.9746
bta05203 Viral carcinogenesis	26.56	0.979
bta00190 Oxidative phosphorylation	12.86	1.0
bta00970 Aminoacyl-tRNA biosynthesis	16.67	1.0
bta02010 ABC transporters	16.67	1.0
bta03010 Ribosome	11.88	1.0
bta03050 Proteasome	15.22	1.0

bta04740 Olfactory transduction	3.84	1.0
bta04974 Protein digestion and absorption	22.31	1.0
bta05012 Parkinson disease	20.67	1.0
bta05034 Alcoholism	24.89	1.0
bta05320 Autoimmune thyroid disease	16.90	1.0
bta05322 Systemic lupus erythematosus	11.54	1.0

[1] %: Percent of genes predicted to be modulated. [2] BH: Benjamini – Hochberg