GILMAR ARANTES ATAIDE JUNIOR

Use of injectable P4 associated with an intravaginal P4 device for early resynchronization of beef cattle submitted to three TAIs in 48 days

Pirassununga

2020

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Dissertation submitted to the Postgraduate Program in Animal Reproduction of the School of Veterinary Medicine and Animal Science of the University of São Paulo to obtain the Master's degree in Sciences.

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Animal Reproduction

Area: Animal Reproduction

Advisor:
Prof. Guilherme Pugliesi, Ph.D.

Approved:______________________________
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CERTIFICADO

Certificamos que a proposta intitulada "Ressincronização superprecoce com progesterona em novilhas e vacas Nelore submetidas à 3 IATFs em 48 dias", protocolada sob o CEUA nº 2769070818 (ID 006701), sob a responsabilidade de **Guilherme Pugliesi** *e equipe; Gilmar Arantes Ataide Júnior* - 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 Medicina Veterinária e Zootecnia da Universidade de São Paulo (CEUA/FMVZ) na reunião de 02/07/2019.

We certify that the proposal "Resynchronization of ovulation with progesterone for 3 TAIs in 48 days in Nelore heifers and cows", utilizing 60 Bovines (60 females), protocol number CEUA 2769070818 (ID 006701), under the responsibility of **Guilherme Pugliesi** *and team; Gilmar Arantes Ataide Júnior* - 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 Veterinary Medicine and Animal Science (University of São Paulo) (CEUA/FMVZ) in the meeting of 07/02/2019.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 09/2018 a 12/2019 Área: Reprodução Animal

Origem:	Prefeitura do Campus da USP de Pirassununga						
Espécie:	Bovinos	sexo:	Fêmeas	idade:	2 a 10 anos	N:	60
Linhagem:	Nelore			Peso:	350 a 600 kg		

Local do experimento: (VRA) Departamento de Reprodução animal da Universidade de São Paulo - Campus Fernando Costa - Pirassununga - SP. Avenida Duque Norte, 225, Jardim Elite.

São Paulo, 27 de julho de 2020

Prof. Dr. Marcelo Bahia Labruna
Coordenador da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

Camilla Mota Mendes
Vice-Coordenador
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

São Paulo, 27 de julho de 2020
CEUA N 2769070818

Ilmo(a). Sr(a).
Responsável: Guilherme Pugliesi
Área: Reprodução Animal

Título da proposta: "Ressincronização superprecoce com progesterona em novilhas e vacas Nelore submetidas à 3 IATFs em 48 dias".

Parecer Consubstanciado da Comissão de Ética no Uso de Animais FMVZ (ID 006690)

A Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, no cumprimento das suas atribuições, analisou e **APROVOU** a Emenda (versão de 11/julho/2020) da proposta acima referenciada.

Resumo apresentado pelo pesquisador: "O motivo da presente solicitação de emenda é a inconsistência do N amostral entre o que foi descrito no projeto e o que preenchido no formulário na submissão e que foram aprovados pelo colegiado desta CEUA. A proposta é para uso de 60 animais no experimento 1 e 1600 no experimento 2. Entretanto, consta no formulário e certificado de aprovação da CEUA apenas o número de animais do experimento 1 (n=60). Desta forma, é necessário que seja incluído o número de animais referente ao experimento 2. Lembrando que tanto os TCLEs e projeto usando estes 1600 animais do Experimento 2 foram já enviados na submissão e foram aprovados, ou seja, ficou errado somente no formulário, mas como preciso do certificado da CEUA para depósito de minha dissertação nesse mês de Julho, vou precisar deste documento corrigido para evitar inconsistência e para refletir o real número de animais usados no projeto. ".

Comentário da CEUA: "".

Prof. Dr. Marcelo Bahia Labruna
Coordenador da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

Camilla Mota Mendes
Vice-Coordenador
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

EVALUATION FORM

Author: ATAIDE JUNIOR, Gilmar Arantes

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Date: _____/_____/_____

Committee Members

Prof. ____
Institution: _____________________________ Decision: ___________________________

Prof. ____
Institution: _____________________________ Decision: ___________________________

Prof. ____
Institution: _____________________________ Decision: ___________________________

DEDICATION

I dedicate this work to myself and everyone who collaborates directly or indirectly to accomplish it.

ACKNOWLEDGEMENTS

I am grateful to my parents (Cleonice and Gilmar) who taught me to dream high and fight tirelessly for my goals, knowing how to respect, enjoy and be thankful for every opportunity. I am very grateful to my sister (Flaviany) and my godmother (Gilmagda), who were always with me, supporting and advising me whenever necessary. They are my foundation and my greatest supporters.

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“Do more than exist, live. Do more than touch, feel it. Do more than look, watch. Do more than read, absorb. Do more than listen, understand.”

“Faça mais do que existir, viva. Faça mais do que tocar, sinta. Faça mais do que olhar, observe. Faça mais do que ler, absorva. Faça mais do que ouvir, compreenda.”

Benjamin Franklin

RESUMO

ATAIDE JUNIOR, G. A. **Uso da progesterona injetável associada a um dispositivo intravaginal de P4 na ressincronização superprecoce de fêmeas de corte submetidas a 3 IATFs em 48 dias**. 2020. 49 f. Dissertação (Mestrado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2020.

Nós objetivamos avaliar a dinâmica folicular e a taxa de prenhez (P/IA) de fêmeas bovinas submetidas à ressincronização, 13 dias após a IATF, utilizando um dispositivo contendo progesterona (P4) associado ou não à progesterona injetável de ação curta (P4i). No Exp. 1, 28 vacas lactantes e 25 novilhas Nelore foram submetidas a um protocolo de sincronização da ovulação (D0 = estro esperado). No Dia 13, os animais foram divididos em dois tratamentos denominados controle: (Apenas o dispositivo; 15 vacas e 13 novilhas) e P4i (Dispositivo + 100 mg P4i; 13 vacas e 12 novilhas), e foram submetidos a ultrassonografia ovariana (US) e colheita de sangue diárias, do Dia 13 até o Dia 22. No Dia 22, os dispositivos foram removidos e os animais com luteólise detectada por US tiveram a ovulação induzida pelo tratamento com cipionato de E2. No Exp. 2, 760 vacas e 498 novilhas foram submetidas à 1ª IATF no Dia 0. No Dia 13 e Dia 22, os animais foram submetidos aos mesmos procedimentos realizados no Exp. 1. As fêmeas não prenhes receberam a 2ª IATF no Dia 24, e no Dia 37 foram ressincronizadas usando o tratamento oposto à 1ª ressincronização. No Dia 37, um diagnóstico de gestação (DG) (visualização de um embrião viável) confirmatório foi realizado em animais que apresentaram CL ativo no Dia 22. Aquelas com potencial perda de gestação entre o Dia 22 e Dia 37 foram ressincronizadas usando um dispositivo P4 e 2 mg de benzoato de estradiol. A terceira IATF foi realizada no Dia 48. O DGs confirmatórios da 2ª e 3ª IATFs foram realizados nos dias 61 e 85, respectivamente. No Exp. 1, o momento e a sincronia da emergência folicular não diferiram entre os tratamentos e paridades (P>0,1). O folículo dominante foi maior em vacas do que em novilhas (P<0,05) no Dia 22 e Dia 24, e no tratamento controle no Dia 24 (P<0,05). Maiores concentrações de P4 foram detectadas no Dia 14 (P<0,05) e Dia 15 (P=0,08) no animais tratados com P4i. As concentrações de P4 reduziram gradualmente entre o Dia 14 e o Dia 22 (P<0,05), e a luteólise ocorreu mais cedo (P=0,07) em vacas do que em novilhas. No Exp. 2, a P/IA geral para a 2ª e 3ª IATF, foi maior (P<0,05) no tratamento P4i do que no controle (43% [178 / 411] vs. 38% [148/387]), independentemente da paridade. Houve uma tendência para maior P/IA (P=0,09) em novilhas tratadas com P4i do que novilhas controle (43,7% [73/167] vs. 38,1% [61/160]), mas não diferiu entre vacas tratadas com P4i (43% [105/244]) e controle (38,3% [87/227]). A P/IA acumulada após 3 IATFs foi de 77,3% (385/498) para novilhas e 83,3% (633/760) para vacas. Em conclusão, o tratamento com 100 mg de P4i de ação curta é eficaz para aumentar a concentração plasmática de P4, entretanto não impactou na sincronia da emergência da onda folicular. Os animais tratados com P4i apresentaram melhor P/IA.

Palavras-chave: Eficiência reprodutiva. Escore de condição corporal. Progesterona de curta ação. Ressincronização. Ultrassonografia Doppler.

ABSTRACT

ATAIDE JUNIOR, G. A. **Use of injectable P4 associated with an intravaginal P4 device for early resynchronization of beef cattle submitted to three TAIs in 48 days.** 2020. 49 f. Dissertação (Mestrado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2020.

We aimed to evaluate the follicular dynamic and pregnancy rate (P/AI) of beef cattle submitted to resynchronization 13 days after a TAI using a P4 device associated or not with short-acting injectable progesterone (iP4). In Exp. 1, 28 Nelore suckled cows and 25 heifers were submitted to an ovulation synchronization protocol (Day 0 = expected estrus). On Day 13, animals were split into two treatments, namely control (only P4 device; 15 cows and 13 heifers) and iP4 (P4 device + 100 mg iP4; 13 cows and 12 heifers), and submitted to daily ovarian ultrasonography (US) exams and blood collection, from Day 13 to Day 22. On Day 22, the P4 devices were removed, and animals with detected luteolysis by the US had the ovulation induced by E2 cypionate treatment. In Exp. 2, 760 cows and 498 heifers were submitted to a 1^{st} TAI on Day 0. On Day 13 and Day 22, animals were split into control and iP4 treatments and submitted to the same procedures done in Exp. 1. Also, non-pregnant animals received the 2^{nd} TAI on Day 24, and on Day 37 were resynchronized using the opposite treatments' 1^{st} resynchronization procedure. On Day 37, a pregnancy diagnosis was performed in animals with an active CL on Day 22. Those with potential pregnancy loss between Day 22 and Day 37 were also resynchronized on Day 37. The 3^{rd} TAI was performed on Day 48. The confirmatory PD of the 2^{nd} and 3^{rd} TAIs were performed on Days 61 and D85, respectively. In Exp. 1, the time and synchrony of follicular wave emergence did not differ between treatments and parity. The dominant follicle was larger in cows than in heifers on Day 22 and Day 24, and in the control treatment on Day 24 ($P<0.05$). Increased P4 concentrations were detected on Day 14 ($P<0.05$) and Day 15 ($P=0.08$) in the iP4 treatment. P4 concentrations reduced gradually between Day 14 and Day 22 ($P<0.05$), and luteolysis occurred earlier ($P=0.07$) in cows than in heifers. In Exp. 2, the overall P/AI for 2^{nd} and 3^{rd} TAIs, regardless of parity, was greater ($P<0.05$) in the iP4 than the controls (43% [178/411] vs. 38% [148/387]). The P/AI tended to be greater ($P=0.09$) in iP4-treated heifers than control heifers (44% [73/167] vs. 38% [61/160]), but did not differ between iP4-treated cows (43% [105/244]) and control cows (38% [87/227]). In conclusion, the 100 mg short-acting iP4 treatment associated with the intravaginal P4 device is effective in increasing the plasma P4 concentration, but does not impact the synchrony of the follicular wave emergence. Animals treated with iP4 presented improved P/AI.

Keywords: Body condition score. Doppler ultrasonography. Reproductive efficiency. Resynchronization. Short-acting progesterone.

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1 CHAPTER 1: EARLY RESYNCHRONIZATION IN NELORE CATTLE USING INJECTABLE AND INTRAVAGINAL PROGESTERONE FOR THREE TIMED ARTIFICIAL INSEMINATIONS

1.1 INTRODUCTION

Timed-artificial insemination (TAI) has been the most used assisted reproduction technique, mainly in South America, to maximize the use of frozen semen from superior bulls and its subsequent benefits on genetic enhancement in beef cattle (BARUSELLI et al., 2019). Strategies for increasing TAI use after the first service include protocols for resynchronizing ovulation, which allows for a second TAI earlier in breeding seasons (STEVENSON et al., 2003; BARUSELLI et al., 2017). These strategies are combined with the pregnancy diagnosis (PD) methods able to be performed after the TAI. Consequently, females that failed to become pregnant in the first service are re-exposed as early as possible to another AI after parturition (BARUSELLI et al., 2017). Although starting this resynchronization 30 d after the TAI is most common, this strategy leads to a long interbreeding interval (≈ 40 d) [3].

Most recently, a new strategy – referred to as super-early resynchronization – was developed to identify non-pregnant females that underwent luteolysis via color Doppler ultrasonography (SIQUEIRA et al., 2013; PUGLIESI et al., 2014; PESSOA et al., 2018). In this method, the resynchronization protocol starts between 12 to 14 d after the first TAI, enabling an interbreeding interval of 22 to 24 d (PUGLIESI et al., 2019; MOTTA et al., 2020; PALHÃO et al., 2020). In this regard, studies using this strategy provides satisfactory pregnancy per AI (P/AI), around 40%, in the resynchronization protocol (MOTTA et al., 2020; PALHÃO et al., 2020). In addition, since hormonal treatments start close to the period of maternal pregnancy recognition

(BINELLI et al., 2001), they must not risk pre-existing pregnancies and must be effective in synchronizing the follicular emergence in non-pregnant females for a second service. In this regard, the association between progesterone (P4) and estradiol (E2) benzoate in the super-early protocol may influence pregnancy establishment from first TAI because of E2 role in luteolysis induction (ARAUJO et al., 2009; PUGLIESI et al., 2012). Reduced doses of E2 benzoate might be a safe alternative for not disturbing the pregnancy (MOTTA et al., 2020), but it may also be ineffective to synchronize the follicular wave in suckled beef cows.

Due to the controversial results when using E2 in resynchronization programs (MOTTA et al., 2020; VIEIRA et al., 2014), it is imperative to develop alternatives to E2 esters for synchronization of follicle wave emergence without disturbing the previous pregnancy. Consequently, resynchronizations using intravaginal P4 inserts, alone or associated to injectable P4 (iP4) formulations, recently became significant replacements for super-early resynchronization protocols (PUGLIESI et al., 2019). The use of P4 in TAI protocols has mostly been associated with its inhibitory effect on frequency of luteinizing hormone (LH) pulses, which ultimately causes dominant follicle atresia and suppresses ovulation (ADAMS et al., 1992; HANNAN et al., 2010; WILTBANK et al., 2014). Furthermore, recent studies have indicated that the high P4 concentrations can synchronize the emergence of a new follicular wave when applied at different stages of the follicular development and have a potential role in the growth inhibition of LH- and FSH-dependent follicles in cattle (CAVALIERI, 2018) and mares (GASTAL et al., 1999). Our group, through recent experiments conducted with a limited number of animals (PUGLIESI et al., 2019), reported an increased P/AI after the second TAI of Nelore cows resynchronized with a P4 insert plus 75 mg of long-acting iP4 when compared with those receiving only the P4 insert. Although this evidence indicating a positive effect on P/AI in Bos indicus supplemented with iP4, the possible effects of iP4 on follicular wave synchrony and P/AI in Bos indicus cattle need to be investigated, as the super-early resynchronization protocols start at a moment of high circulating P4 caused by the presence of corpus luteum (CL) and the P4 insert. In addition, the increased liver blood flow observed in dairy lactating cows compared to heifers (SARTORI et al., 2004), may be also increasing the P4 clearance in suckled beef cows and consequently resulting in a different efficacy of supplementary iP4 between suckled cows and heifers.

The use of iP4 allows for a transient increase in P4 (PUGLIESI et al., 2014), and the dose can be easily adjusted for each parity. Nevertheless, use of high doses of long-acting iP4 formulae results in residual circulating P4 that may compromise the follow ovulation and P/AI after second TAI, especially in animals with reduced hepatic clearance as the non-lactating females (VIEIRA et al., 2020). Alternatively, iP4 formulae with a short half-life may allow for better adjustment of the iP4 dose to induce an increase in circulating P4 during resynchronization programs in heifers or suckled cows. Therefore, our goal was to evaluate the circulating P4 concentrations and the follicular wave emergence in non-pregnant cows and heifers submitted to a resynchronization protocol 13 d after TAI using an intravaginal P4 insert associated or not with 100 mg of short-acting iP4 (Exp. 1). We also aimed to compare the effects of these treatments on P/AI performing three consecutive TAIs within 48 d (Exp. 2). Our primary hypothesis was that the additional increase in P4 caused by the iP4 treatment would improve the follicular wave emergence synchrony, leading to increased P/AI in resynchronized beef cows and heifers.

1.2 MATERIAL AND METHODS

This study was carried out in two experiments. Both were performed following principles and procedures approved by the Institutional Animal Care and Use Committee of the School of Veterinary Medicine and Animal Science of the University of São Paulo, São Paulo, Brazil (CEUA-FMVZ/USP 2769070818). Experiment 1 was performed at Fernando Costa campus of the University of São Paulo, located in Pirassununga - SP, Brazil. The cows and heifers were fed on a continuous grazing regimen in Brachiaria brizantha paddocks. They had free access to water and corn silage mixed with concentrate, as established by the Nutrient Requirements of Beef Cattle (NRC, 2016). Experiment 2 was performed using 10 breeding groups of three commercial farms located in São Paulo (Farm 1, n= 333) and Mato Grosso States (Farm 2, n= 455 and Farm 3, n=470), in Brazil. The animals were kept in a rotated grazing regimen on B. *brizantha* or B. *humidicula* paddocks and supplemented with mineralized salt and free access to water.

1.2.1 Experiment 1

1.2.1.1 Herd and animal management

For this experiment, we enrolled 30 Nelore (*Bos taurus indicus*) suckled multiparous cows 5 to 15 years of age, ranging from 32 to 62 d postpartum, and 31 Nelore heifers 20 to 24 months of age. Animals had body condition scores (BCS) between 3 and 4 (scale from 1 to 5; AYRES et al., 2009). The animals were selected through a reproductive tract development and cyclicity criterions (CLARO JUNIOR et al., 2010). The heifers were examined twice in a 10 d interval. Those heifers with absence of reproductive abnormalities, presence of CL in one of the exams and a well-developed reproductive tract were considered cycling, mature and were used. Lactating multiparous cows were selected based on the reproductive tract criterions, good uterine involution, and absence of abnormalities.

1.2.1.2 Experimental design and treatments

All cows and heifers had their estrus and ovulation synchronized with a P4/ estradiol benzoate (EB) based protocol considering the day of the expected estrus as Day 0 (D0) (Fig. 1). For this, on Day –11, the animals received an intravaginal P4 insert (1.9 g, CIDR®, Zoetis, São Paulo, SP, Brazil) and 2 mg EB intramuscularly (Gonadiol®, Zoetis, Brazil). On Day –4, all animals received 12.5 mg of dinoprost tromethamine (PGF2α; Lutalyse®, Zoetis, Brazil). On Day –2, the P4 inserts were removed and animals received a second dose of PGF2α (12.5 mg; Lutalyse®, Zoetis, Brazil), 0.6 mg of estradiol cypionate (ECP; ECP®, Zoetis, Brazil), and equine chorionic gonadotropin (eCG; Novormon®, Zoetis, Brazil; 200 IU for heifers and 300 IU for cows) to induce ovulation.

The animals were not bred, as we aimed to evaluate follicle and CL dynamics in non-pregnant animals in a subsequent TAI. Therefore, 13 d after the expected estrus (D13), all animals were evaluated via transrectal ultrasonography, and the individuals with well-developed CL that had ovulated (CL area and blood perfusion > 2cm^2 and 25%, respectively) were randomly assigned into two treatments for an ovulation resynchronization protocol. The ovulation was resynchronized in controls (13 heifers and 15 cows) with a new intravaginal insert containing 1.9 g P4 (CIDR®, Zoetis, Brazil), and in iP4 treatment (12 heifers and 13 cows) with a similar P4 insert plus 100 mg of short-acting iP4 intramuscularly (Afisterone®, Ceva, Paulínia, SP, Brazil). The intravaginal insert was removed after 9 d (D22).

1.2.1.3 Ultrasound scanning and ovarian end-points

During the resynchronization protocol (Day 13 to Day 22), the animals' ovaries were scanned daily with a B-mode and color Doppler ultrasound instrument (MyLab Delta, Esaote, Italy) equipped with a multifrequency linear-array transductor (settings: B-mode: RES-A, gain 70%, P 59 mm, X/M, PRS 1; Color Doppler mode: frequency 6.3 MHz, gain 70%, PRF 730Hz, WF 4, PRS 3, and PRC M/2). The diameter of all visible follicles was calculated as the mean of the largest follicular diameter in two perpendicular directions using the electronic calipers. Each follicle was represented in a schematic drawing for the retrospective identification of the follicular atresia or growth. These data were used to determine the turnover of dominant follicle and to evaluate the synchrony of the onset of the follicular wave emergence in the respective treatments and parities. The day of follicular wave emergence was determined retrospectively based on the first appearance of a dominant follicle with 4 to 5 mm, as proposed by Ginther *et al*. (1997). A synchronized follicle wave emergence was considered when the dominant follicle first appeared from 3 to 5 days after beginning of treatments for resynchronization (Days 16 to 18). The luteolysis was determined as the day the CL presented reduction of 50% in the blood perfusion and 25% in the area. The CL blood perfusion and area were evaluated with color Doppler and B-mode ultrasonography respectively to predict luteal function (PUGLIESI et al., 2014; ROCHA et al., 2019). The ovulations were verified every 12 h from 36 to 84 h after the P4 inserts' removal. The time of ovulation was defined as the average between the time that the dominant follicle disappearance was verified and the time of the previous evaluation (Fig. 1).

1.2.1.4 Blood samples and hormone assays

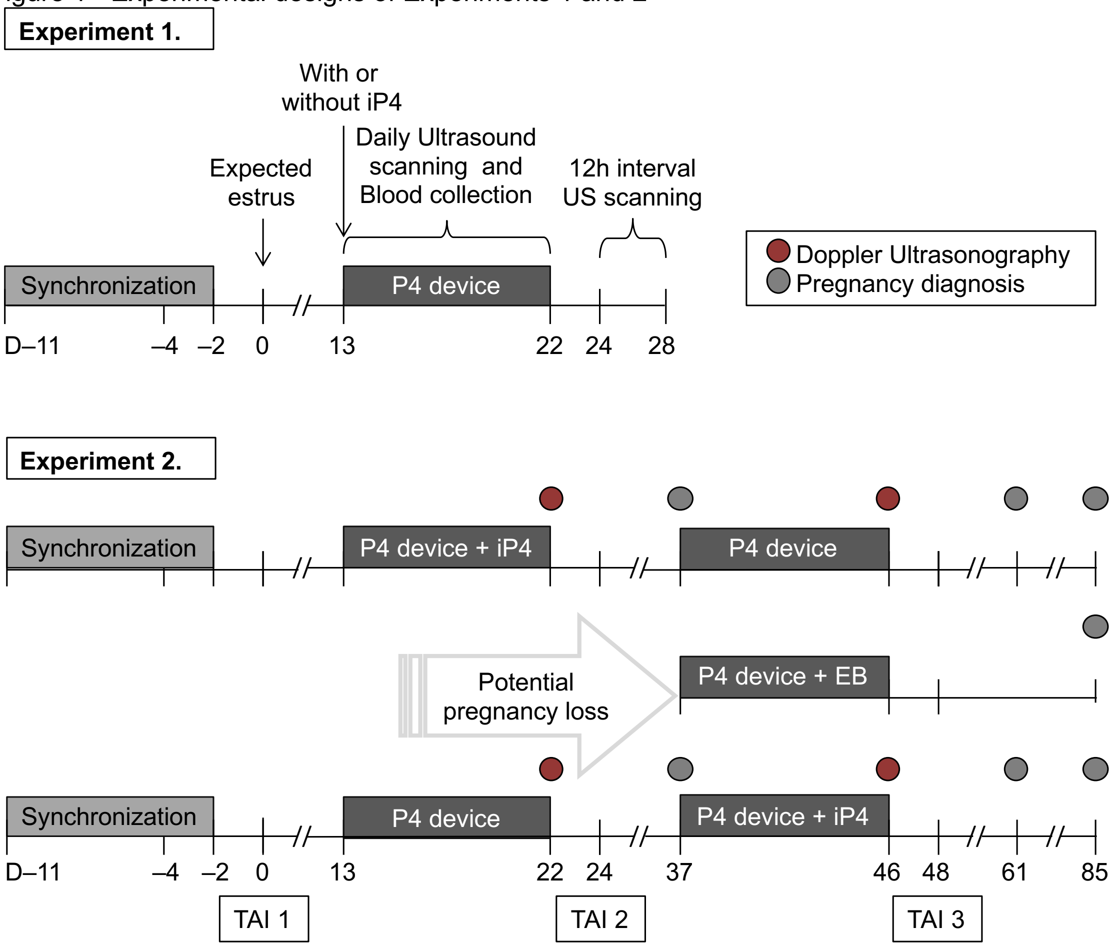
Blood samples were daily collected into 10-mL evacuated and heparinized tubes (BD Vacutainer®, São Paulo, Brazil) from the animals' jugular veins from Day 13 to Day 22 to quantify plasma P4 concentrations (Fig. 1). The samples were inverted several times by hand and stored in "thermos" containing iced water before centrifugation for 30 min to 4 h. They were then centrifuged at 3600 x g for 15 min at 4ºC, and the isolated plasma was stored at –20ºC until subsequent measurements. Plasma P4 concentrations were measured in the Laboratory of Endocrinology from São Paulo State University in Araçatuba, SP, Brazil. The measurements were performed directly in 50 µL of plasma using a validated commercial kit (Immuchem™ Double Antibody Progesterone Kit; Cat. 07e170105, MP Biomedicals, NY, USA; ECKTERNKAMP et al., 2006). The standard curve ranged from 0.1 to 80 ng/mL, and the intra- and inter-assays' coefficients of variation were 6.9% and 6.6%, respectively.

1.2.2 Experiment 2

From a total of 1,258 Nelore females (Bos taurus indicus), we used 760 suckled multiparous cows (35 to 75 d postpartum) and 498 heifers (22 to 24 months of age), with BCS ranging from 2 to 4 (AYRES et al., 2009). Ovulation was synchronized using a P4/EB based protocol described in Experiment 1. In contrast, all animals received a TAI (TAI-1) 48 h after the P4 removal, and this day was set as Day 0 (Fig. 1). Thirteen days after the TAI-1 (Day 13), all females were randomly assigned to the same two treatments described in Experiment 1 in the respective treatments. The control consisted of 377 cows and 248 heifers and the iP4 treatment included 383 cows and 250 heifers. So, the P4 intravaginal insert was removed after 9 d (Day 22), and all animals were submitted to a CL blood perfusion evaluation via color Doppler ultrasonography. This technique was employed to identify luteolysis occurrence in non-pregnant animals, as described by Pugliesi *et al*. (2014). The ultrasound evaluation was performed with a portable Duplex (B and Color Doppler modes) ultrasound equipment (Z5VET, Mindray, São Paulo, SP, Brazil) with a linear transducer (Settings: B-mode: Frequency 7.5 MHz, Gain: 71; Color Doppler mode: Frequency 5.7 MHz, gain 72, PRF 0.7, WF 260). When identified, the non-pregnant animals received 12.5 mg PGF2α, 0.5 mg ECP, and 200 (heifers) or 300 (cows) IU eCG immediately after the scanning. All non-pregnant animals received a second TAI (TAI-2) 48 h after P4 insert removal (D24).

On Day 37, all animals that received the TAI-2 were resynchronized using the opposite Day 13 treatment (Control: 153 cows and 111 heifers and iP4: 149 cows and 103 heifers). The animals detected with an functional CL on Day 22 were submitted, on Day 37, to a confirmative PD by visualization of the embryonic heartbeat. The animals with potential pregnancy loss (false-positive results of doppler ultrasound or pregnancy loss) between Day 22 and Day 37 were resynchronized through a P4/EB based protocol (Fig. 1). Then, following the same protocol used at the first resynchronization, all animals submitted to the second resynchronization had their P4 inserts removed and were submitted to a second CL blood perfusion analysis on Day 46 (9 d after P4 insert introduction). After that, all animals identified as non-pregnant received the same treatment for induction of ovulation as performed for TAI-1 and TAI-2. On Day 48, animals with potential pregnancy loss detected on Day 37, as well as non-pregnant animals detected by the second CL blood perfusion evaluation on Day 46, were submitted, respectively, to their second and third TAIs (2^{nd} and 3^{rd} TAIs). Two additional PDs were performed on Day 61 and Day 85 to confirm the pregnancies from the 2^{nd} and 3^{rd} TAIs, respectively (Fig. 1).

Figure 1 - Experimental designs of Experiments 1 and 2



Source: (ATAIDE JUNIOR, G. A., 2020)

Notes: Experimental design of Experiment 1 (n= 28 cows and 25 heifers) and Experiment 2 (n=498 heifers and 760 cows). All females were submitted to an ovulation synchronization protocol started on Day –11, but only animals of the Experiment 2 were inseminated. On Day 13, the animals were split on two resynchronization treatments. The Control received one P4 insert containing 1.9 g of progesterone. The iP4 group received a P4 insert and 100 mg of short acting iP4 im. On Day 22 the inserts were removed, and animals were evaluated by Doppler Ultrasonography. Animals with detected luteolysis received 1 mg estradiol cypionate, 12.5 mg dinoprost tromethamine and 200 or 300 IU of equine chorionic gonadotropin (heifers and cows, respectively). The second TAI was performed on Day 24. On Day 37 another resynchronization protocol was performed in a similar way as performed on Day 13 and a confirmatory pregnancy diagnosis was performed in animals with a functional corpus luteum on Day 22. The animals with potential pregnancy loss (presence of a functional corpus luteum on Day 22 but not pregnant on Day 37) were resynchronized using a conventional TAI protocol. On Day 46 the resynchronized animals were submitted to a similar handling as performed on Day 22. The third TAI was performed on Day 48 and the confirmatory pregnancy of the second and third TAI was performed on Days 61 and 85, respectively.

1.2.3 Statistical analysis

Experiment 1 was a completely randomized design, considering each animal as an experimental unit. All continuous data (plasma P4 concentrations, day of follicular wave emergence, CL area and blood perfusion, day of luteolysis, dominant follicle diameter at Days 22 and 24, follicular growth rate and time of ovulation) were analyzed for normality of the residuals and applying to the Shapiro Wilk test. Variables that did not follow a normal distribution were transformed to logarithmic scale (day of follicular emergence) or ranked (follicular growth rate between Day 22 and Day 24, diameter of the dominant follicle on Day 22 and Day 24 and ovulation moment). The data were analyzed with analysis of variance (ANOVA) using the SAS software (Version 9.2 SAS Institute Inc., Cary, NC, USA) with the PROC MIXED procedure, considering effects of parity (heifer vs. cow), treatment (control vs. iP4), day (for repeated measures of CL area, CL blood perfusion, and plasma P4 concentrations), and possible interactions. The synchrony of the follicular wave emergence was analyzed by Bartletts' test and the frequency of the follicular wave emergence between Day 3 and 5 after the resynchronization treatment was analyzed by Fisher's exact test using the PROC FREQ of SAS. For Experiment 2, the data were analyzed with the PROC GLIMMIX of the SAS software, considering the fixed effects of parity (heifer vs. cow), treatment (control vs. iP4), farms (1, 2 or 3), and possible interactions. Considering that in each farm were used different sires (n= Farm 1: 14, Farm 2: 16 and farm 3: 21) and insemination technicians (Farm 2: 2 and Farm 3: 7), these effects were considered as part of farm variation. included in the model as a random effect nested within farm. Variables were removed using backward elimination based on the Wald statistics criterion when $P > 0.20$. Data were presented with mean ± standard error of the mean

(SEM). Differences were considered significant at $P < 0.05$ and tendencies $0.05 \leq P \leq 0.1$.

1.3 RESULTS

1.3.1 Experiment 1

Eight animals (six heifers and two cows) were excluded from Experiment 1 because of the following reasons: one animal from each parity presented a follicular cyst during the synchronization protocol; three heifers had double ovulations; two heifers had delayed ovulation or presented a regressed CL on Day 13; and one cow presented an underdeveloped CL, respectively.

1.3.1.1 Follicle characteristics during the resynchronization protocol

During the resynchronization protocol (Day 13 to Day 22), the day, the dispersion and the frequency of the follicular wave emergence did not differ ($P > 0.1$) between heifers and cows and between treatments (Table 1, Fig. 2). The proportion of synchronized follicular wave emergence (between Day 15 to 17) was 73.3 and 46.1% for cows, and 69.2 and 58.3% for heifers of the control and iP4 treatments, respectively. The diameter of the dominant follicle was larger ($P < 0.05$) in cows than in heifers on Day 22 and Day 24. In addition, the dominant follicle tended to be larger ($P = 0.1$) and was larger ($P < 0.05$) in the control than the iP4, respectively, on Day 22 and Day 24 (Table 1). The follicular growth rate and time of ovulation were not affected ($P > 0.1$) by the treatment or parity (Table 1). The ovulation risk after the resynchronization protocol was 96% (24 out of 25) for heifers and 79.3% (23 out of 29) for cows ($P > 0.1$).

Table 1. Ovarian characteristics in heifers and cows submitted to early resynchronization using an intravaginal P4 device (CIDR) with (iP4 group) or without (control group) 100 mg injectable P4 13 days after the expected estrus (Day 0).

	Heifers		**Cows**		**P value**		
	Control (n=13)	**iP4 (n=12)**	**Control (n=15)**	**iP4 (n=13)**	**G**	**C**	**G*C**
Follicular emergence (days)	17.2 ± 0.5	17.6 ± 0.5	16.8 ± 0.6	18.1 ± 0.7	0.15	0.81	0.56
Time of Luteolysis (days)[c]	19.7 ± 0.5	19 ± 0.4	18.6 ± 0.3	18.6 ± 0.5	0.39	**0.07**	0.34
Dominant follicle diameter (mm)							
Day 22	10.2 ± 0.7	9.5 ± 0.7	12.2 ± 0.8	10.4 ± 1.0	**0.10**	**0.02**	0.46
Day 24	12.7 ± 0.8	11.3 ± 0.7	14.7 ± 0.8	13.1 ± 1.0	**0.04**	**0.002**	0.92
Follicular growth rate (mm/day)[a]	1.3 ± 0.2	1.3 ± 0.2	1.2 ± 0.1	1.1 ± 0.5	0.64	0.62	0.90
Time of ovulation (hours)[b]	64.1 ± 3.3	58.2 ± 4.5	72.0 ± 2.7	74.6 ± 1.7	0.13	0.95	0.80

Source: (ATAIDE JUNIOR, G. A., 2020)
Notes: The effects of treatment group (T), parity order (P) and the interaction between treatment and parity T*P were tested.

[a] The day of follicular wave emergence was determined retrospectively based on the first appearance of the ovulatory follicle with 4 to 5 mm, in relation to Day 0.

[b] Mean follicular growth between P4 insert withdrawal (Day 22) and Day 24.

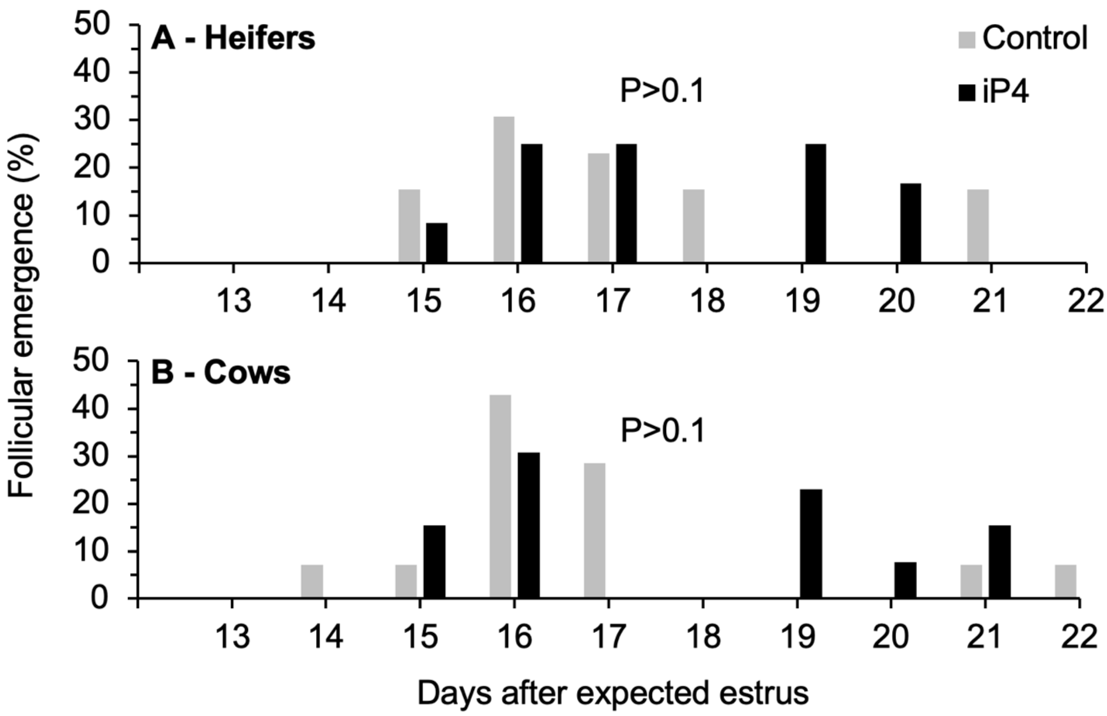
[c] Mean time from P4 insert withdrawal (D22) to time when dominant follicle disappeared (midpoint between measures made 12 h apart starting on Day 24).

[d] Luteolysis was determined as the day the CL presented reduction of 50% in the blood perfusion and 25% in the area.

1.3.1.2 Plasma progesterone concentrations

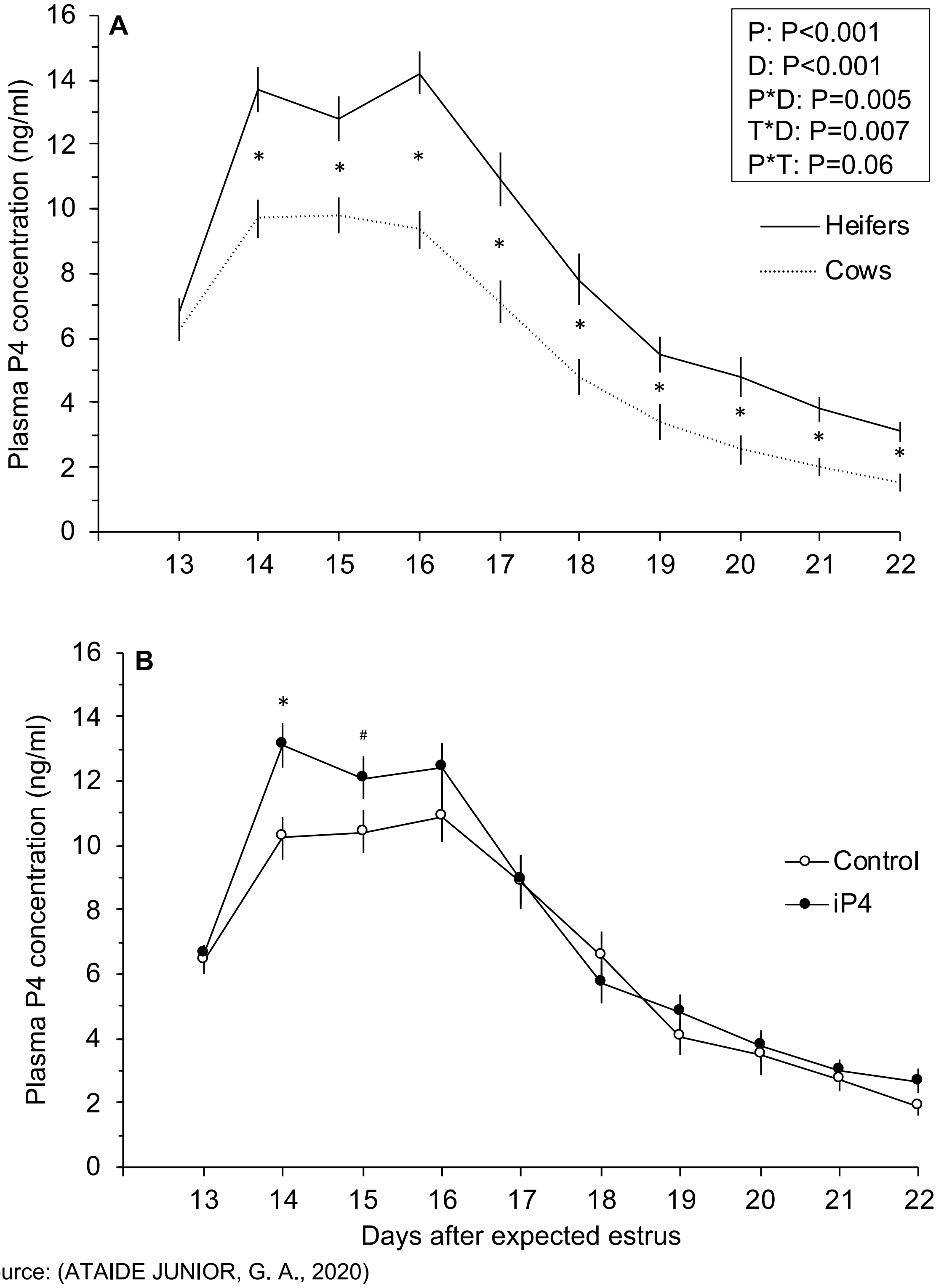
For plasma P4 concentrations from Day 13 to Day 22, a triple interaction between parity, day and treatment effects was not detected (P>0.1); however, significant effects of day and parity (P < 0.05), and interactions of parity by day (P < 0.05), treatment by day (P < 0.05) and treatment by parity (P = 0.06) were observed (Fig. 3). The day effect reflected in an increase in P4 concentrations between Day 13 and Day 14, followed by a progressive decrease from Day 16 until Day 22 (Fig. 3). The parity effect indicated a greater (P < 0.05) P4 concentration in heifers than in cows from Day 14 to Day 22 (Fig. 3A). On Day 13 (before the beginning of resynchronization; Fig. 3) the P4 concentration did not differ (P > 0.1) between treatments or parities. The parity by day interaction reflected greater P4 concentrations in heifers than in cows from Day 14 to Day 22 (Fig. 3A). Also, P4 concentrations in heifers increased ($P < 0.05$) from Day 14 to Day 16, with a reduction to pre-Day 14 concentrations on Day 18, and a progressive decrease ($P < 0.05$) until Day 22. On the other hand, in cows, the reduction to pre-Day 14 P4 concentrations occurred earlier, on Day 17, but also followed a progressive decrease ($P < 0.05$) until Day 22 (Fig. 3A). The interaction between treatment and day ($P = 0.06$; Fig. 3) reflected in greater P4 concentrations on Day 14 ($P < 0.05$) and Day 15 ($P = 0.07$) in the iP4 treatment (Fig. 3B). In addition, a tendency for treatment by parity interaction reflected in a greater increase of P4 concentration in the iP4-treated heifers than cows (Fig. 3).

Figure 2. Dispersion of follicular wave onset in heifers and cows



Source: (Ataide Junior, G. A., 2020)
Notes: Proportion of heifers **(Panel A)** and cows **(Panel B)** in the control (P4 insert) or iP4 (P4 insert plus 100 mg iP4) treatments detected with onset of the follicular wave emergence in each day after expected estrus.

Figure 3. Progesterone concentration during the resynchronization protocol



Source: (ATAIDE JUNIOR, G. A., 2020)

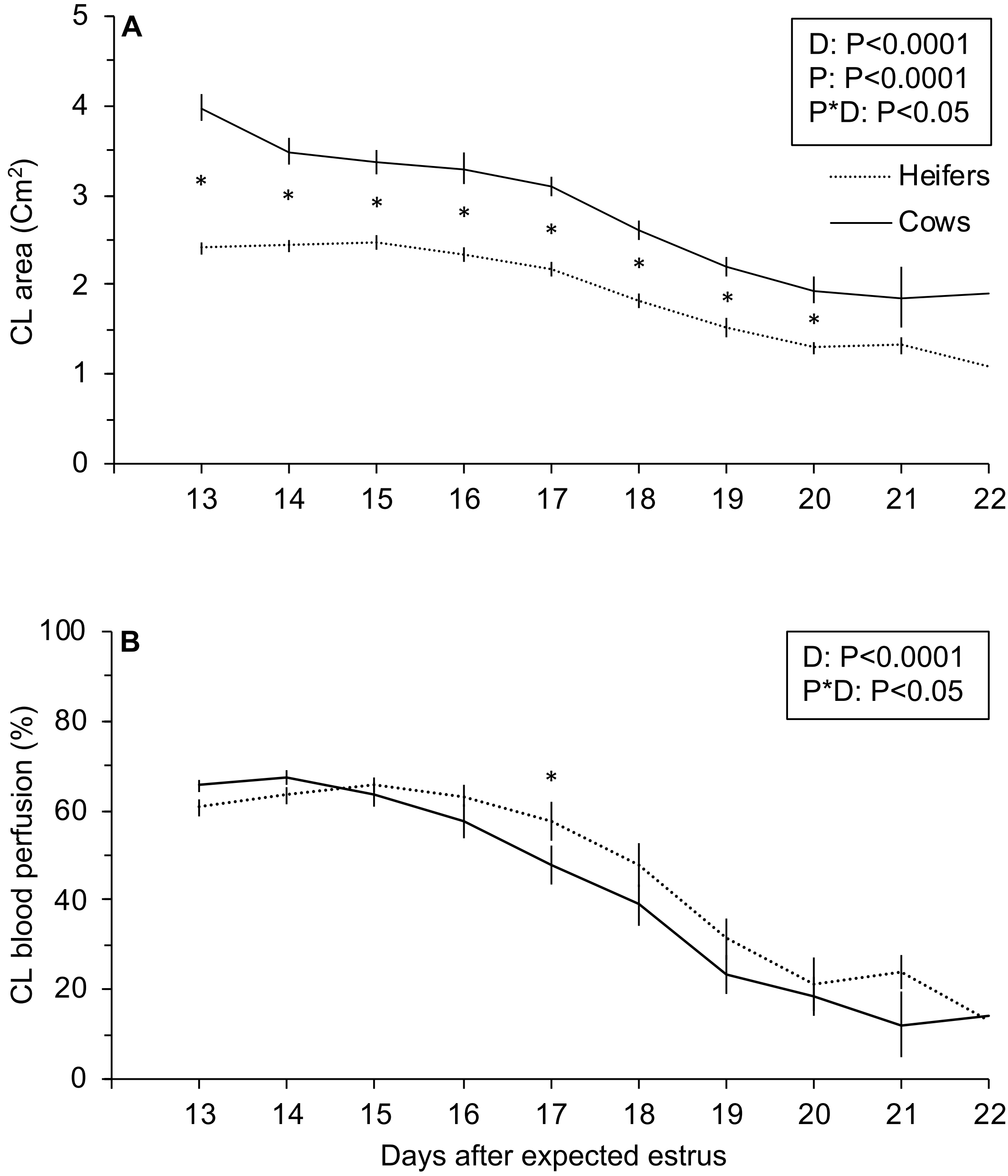
Notes: **Panel A**: Mean ± SEM for plasma P4 concentrations during the resynchronization protocol in heifers (n=25) and cows (n=28), regardless of the treatment group (T). **Panel B**: Mean ± SEM for plasma P4 concentrations during the resynchronization protocol in all animals resynchronized with a P4 insert (control treatment; n= 28) or the P4 insert plus iP4 (iP4 treatment; n=25) on Day 13 after expected estrus, regardless of the parity order (P). It was considered effects of T, P, day (D) and their possible interactions. Data were tested by the LSD test. Days of significant (P<0.05) or an approached difference (P<0.1) in P4 between treatment or parity groups are indicated by an asterisk (*) and hatch mark (#), respectively.

1.3.1.3 Ultrasonography-accessed luteal characteristics

The day for structural luteolysis (Table 1) tended to be shorter (P = 0.07) in cows (18.6 ± 0.3 d) than in heifers (19.4 ± 0.5 d). A triple interaction was not detected for CL area or blood perfusion, however, significant effects of parity and day as well as interaction between them were detected for CL area (P < 0.05; Fig. 4A). The parity effect reflected in smaller CL in heifers than cows from Day 13 to Day 22. The day effect indicated a progressive reduction in CL area over the days. The parity by day interaction reflected, in cows, in an initial reduction in CL area from Day 13 to Day 14, followed by a subsequent progressive reduction from Day 16 to Day 21. In heifers, the initial reduction in CL area started on Day 15, followed by a progressive reduction until Day 21 (Fig. 4A).

For CL blood perfusion, only a day effect (P < 0.05) and a parity by day interaction (P < 0.05) were detected. The day effect reflected in a progressive reduction in CL blood perfusion over the days (Fig. 4B), and the interaction reflected in an earlier reduction in CL blood perfusion in cows than in heifers (Fig. 4B).

Figure 4. Corpus luteum area and blood perfusion in heifers and cows



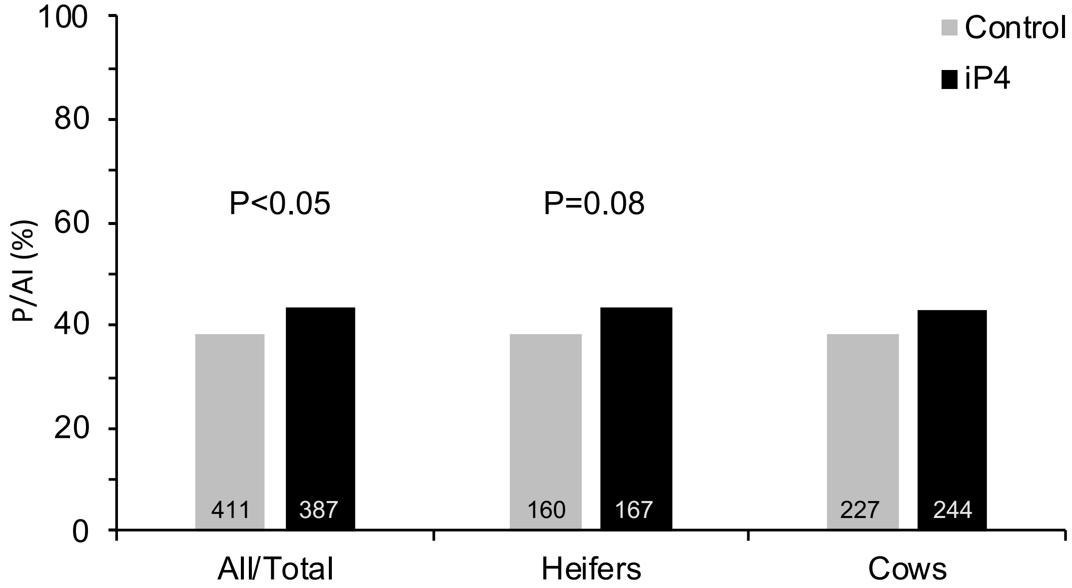
Source: (Ataide Junior, G. A., 2020)
Notes: Mean ± SEM of CL area **(Panel A)** and blood perfusion **(Panel B)** of heifers and cows during the resynchronization protocol regardless of treatment group (T), considering effects of day (D) and parity order (P). Data were tested by LSD test. Days of significant ($P<0.05$) difference ($P<0.1$) in CL area or blood perfusion between treatment groups are indicated by an asterisk (*).

1.3.2 Experiment 2

As expected, the P/AI in heifers and cows after the 1st TAI was not different (P > 0.1) between control and iP4 treatments (45.2% [112/248] vs. 45.2% [113/250] for heifers, and 56.0% [211/377] vs. 53.8% [206/383] for cows respectively). When the total data of the 2nd and 3rd TAIs from heifers and cows were combined, a significant effect of the treatment in the overall P/AI was detected (Fig. 5). In contrast, combining the 2nd and 3rd TAIs data and evaluating it in each parity, only a tendency (P = 0.08) for higher P/AI was observed for heifers in the iP4 treatment compared with the control treatment (Fig. 5). The P/AI in resynchronized heifers and cows did not differ (P > 0.1) between the control and iP4 treatments was not different, when we analyzed separately the 2nd (38.8 % and 45%; 37.6% and 41% for heifers and cows of the control and iP4 treatment, respectively) and 3rd TAIs (36.8 and 41,1%; 39.7 and 45.1% for heifers and cows of the control and iP4 treatment, respectively).

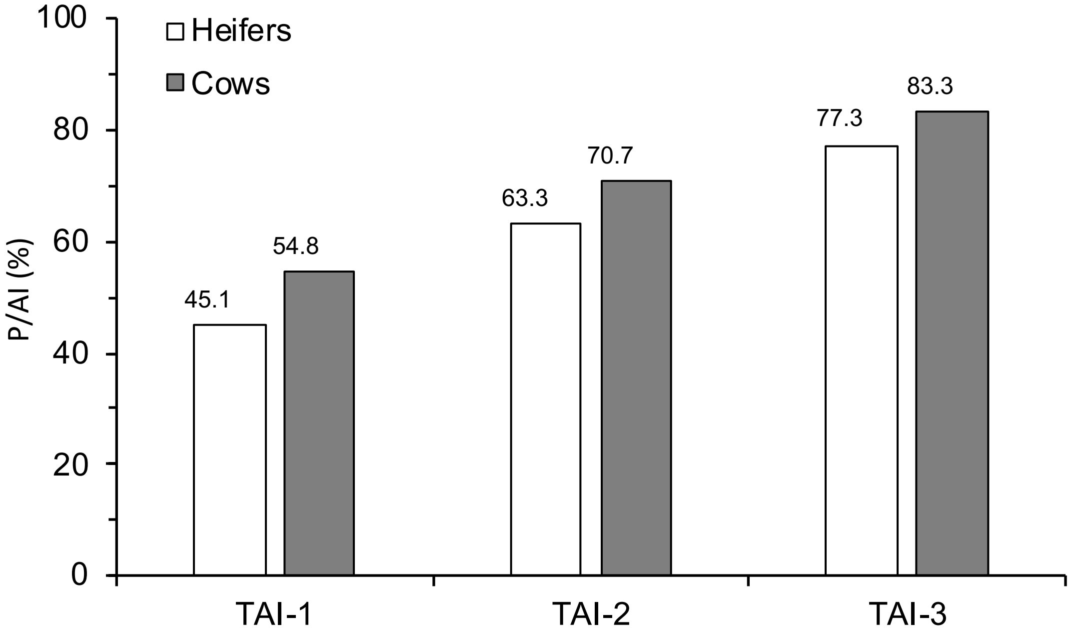
For the treatment effect, there was an 11.8% increase in overall P/AI for the 2nd and 3rd TAIs in the iP4 treatment compared with the control treatment, regardless of the parity. The potential pregnancy loss between the Doppler PD (22 d after TAIs 1 and 2) and the confirmative PD (37 d after TAIs 1 and 2) was greater for heifers than cows (18.2% [70/385] vs. 9.1% [54/591]; P < 0.05). Also, the P/AI of the animals resynchronized with P4/EB on Day 37 was 50.0% (50/100). When we analyzed the full data for the three TAIs within 48 d, the cumulative P/AI increased progressively with the TAIs and reached 83.3% (633/760) for cows and 77.3% (385/498) for heifers (Fig. 6).

Figure 5. Overall pregnancy rate



Source: (Ataide Junior, G. A., 2020)
Notes: Overall pregnancy rate (P/AI; %) of heifers and cows resynchronized with a P4 insert (control treatment) or the P4 insert plus 100 mg iP4 (iP4 treatment) on Day 13 after the previous TAI.

Figure 6. Cumulative pregnancy rate after TAIs 1, 2 and 3



Source: (Ataide Junior, G. A., 2020)
Notes: Cumulative P/AI after TAIs 1, 2 (TAI-1+TAI-2), and 3 (TAI-1+TAI-2+TAI-3) of heifers (n=498) and cows (n=760) resynchronized on day 13 after the first TAI and submitted to three TAIs within 48 d.

1.4 DISCUSSION

The recent development in resynchronization protocols starting between 12 to 14 d after the 1st TAI allows a reduced interbreeding interval (BARUSELLI et al., 2017; PUGLIESI et al., 2019; MOTTA et al., 2020). However, there is a need for definition of the hormonal treatments that effectively synchronize follicle wave emergence and optimize P/AI in these protocols. Therefore, we aimed to evaluate if an additional treatment with 100 mg short-acting iP4 in beef cows and heifers receiving an intravaginal P4 insert 13 d after the 1st TAI, could improve the synchrony of the follicular wave emergence and P/AI of subsequent TAIs. Our results indicated no significant effects on follicle wave emergence and a reduced dominant follicle size in cows and heifers treated with iP4. On the other hand, we observed a consistent increase in P/AI in females resynchronized with the P4 insert associated with iP4. The cumulative P/AI after three consecutive TAIs in 48 d indicated it is an effective hormonal protocol for super-early resynchronization in beef cows and heifers.

The employed dose of short-acting iP4 (100 mg) efficiently increased circulating P4 concentrations in cows and heifers, but this effect was limited to the first 2 d after the treatment (Day 14 and Day 15). In a recent study, Cavalieri (2018) reported that supraphysiologic P4 concentrations (up to 50 ng/mL) induced by insertion of 2 intravaginal inserts containing a total of 6.24 g P4 for 3 d, was adequate in promoting follicle atresia and inducing a new follicular wave emergence. Differently, in the present study, the maximum averaged P4 concentration detected 24 h after the iP4 treatment was 13.1 ng/mL (range: 11.3 to 18.0 ng/mL for heifers and 7.4 to 14.8 ng/mL for cows). Therefore, despite the increased P4 concentrations for 2 d in iP4-treated females, doses greater than 100 mg iP4 may be required for more direct influence on circulating P4 concentrations and subsequent effects on gonadotropin release.

Our central hypothesis was partially supported: The additional increase in P4 concentrations caused by the exogenous iP4 did not improve the follicular wave emergence synchrony; however, the P/AI in resynchronized Nelore cattle increased on about 11.8%, independently of parity order. Therefore, the pregnancy outcomes suggest that iP4 enables the super-early resynchronization protocol to be more effective unless of parity. Although we were unable to precisely determine the mechanisms involved in this improvement through the follicular dynamics, two factors need to be considered for discussion of why supplementary iP4 increased P/AI. Firstly, although a significant difference in follicle wave emergence was not observed, a slight delay (0.4 to 1.3 d) in the day of follicular wave emergence was observed in both classes of parity treated with iP4. This one-day delay in follicle wave emergence is also in agreement with the reduction of 1.3 mm on the dominant follicle diameter at the TAI and the increased circulating P4 for 2 d in iP4-treated animals.

A second factor that may have affected the lack of significant iP4 effects on synchrony of follicular wave emergence was the distinctive diet regimen between the animals used for Experiments 1 and 2. Cows and heifers of both experiments were maintained in pastures; however, animals from Experiment 1 were supplemented with corn silage, as they were maintained in small paddocks. Increased food intake induces distinct liver activity and blood flow in dairy cows (SANGSRITAVONG et al., 2002), leading to an increase in P4 clearance by the liver enzymatic apparatus– also applicable in beef cattle (BATISTA et al., 2020). Thus, one can speculate that the diet regimen and dry matter intake may have influenced the hepatic steroid metabolism, reducing the effects of iP4 treatment on follicle dynamics in Experiment 1. In this regard, Rezende *et al*. (2016) also reported no difference in follicular wave emergence after inserting a P4 insert containing 1 g P4 alone or combined with 100 or 200 mg of short-acting iP4. The mechanism and concentration threshold by which the P4 concentration impacts the follicular growth from emergence to selection phases remains unknown. However, evidence indicates that it happens through a negative feedback on gonadotropins pulsatility in cattle (CAVALIERI et al., 2018) and mares (GASTAL et al., 1999). The follicular atresia in response to high circulating P4 is well known for its adverse effects on LH pulses, causing LH-dependent follicles regression after P4 treatments in cattle (ADAMS et al., 1992; CAVALIERI et al., 2003). Yet, the influence in FSH pulsatility has not been reported. Adams *et al*. (1992) reported that daily treatments with 150 and 300 mg iP4 during early diestrus inhibited dominant follicle growth in Holstein heifers in a dose-dependent manner, but did not suppress FSH. Interestingly, Cavalieri (2018) suggested that high P4 concentrations can induce atresia of FSH-dependent follicles through a potential inhibition of FSH release from the hypophysis. If confirmed, this observation may result in a new follicular wave emergence after the reduction of circulating P4 concentrations.

Despite the potential effects on follicle dynamics and gonadotropins, a consistent difference in P/AI between control and iP4-treated heifers and cows (about 38% vs. 43%) for the 2^{nd} and 3^{rd} TAIs was observed. In line with these findings, a recent study from our group using 75 mg of long-acting iP4 associated with a P4 insert (0.96 g) on Day 12 after the TAI resulted in 26.7% increase on P/AI in suckled Nelore cows (PUGLIESI et al., 2019). In contrast, the use of an insert containing 0.75 to 1 g P4 associated or not with 50 mg iP4 in beef heifers did not impact P/AI of resynchronized animals (SIMÕES et al., 2018). The lack of differences in these studies may be due to a suboptimal P4 dose and an increased metabolism according to the diet regimen or body growth phase. Also, the increased P/AI observed in our study may be a consequence of the increased P4 supply (100 mg iP4 + insert containing 1.9

g P4) compared with previous studies (SANGSRITAVONG et al., 2002; BATISTA et al., 2020). Therefore, a minimal dose of 100 mg iP4 may be needed to optimize the P/AI in resynchronized animals.

In addition to the differences in circulating P4 concentrations between heifers and cows, a small dominant follicle and a late luteolysis were observed in heifers. Increased plasma P4 concentrations allows for an reduced LH pulsatility, leading to less significant follicle growth and size in heifers (BERGFELD et al., 1995; KOJIMA et al., 2003). Thus, the reduced follicle growth and late E2 secretion in heifers (ADAMS et al,. 1992) may delay the endometrial PGF2α synthesis, conferring the late luteolysis in relation to cows. The greater frequency of 3 to 4 follicular waves in heifers (MOLLO et al., 2017) may be a consequence of the delayed luteolysis, and the increased follicle diameter in cows (TARSO et al., 2016) a result of a dominant follicle from the second wave growing under low P4 concentrations caused by an earlier luteolysis and P4 clearance.

The CL was about 60% larger in cows than heifers in the present study. But, despite the fact that the amount of P4 secreted by luteal cells increases proportionally with the amount of large luteal cells, the plasma P4 concentrations at the beginning of the resynchronization protocol were not different between cows and heifers. It may be driven by the lactation and increased body size in cows, resulting in greater daily food intake and hepatic clearance (SANGSRITAVONG et al., 2002; WILTBANK et al., 2006) and altering circulating P4. An increased total feed intake leads to increased liver blood flow (SANGSRITAVONG et al., 2002) and hepatic clearance of P4 (VASCONCELOS et al., 2003; WILTBANK et al., 2006; SARTORI et al., 2016). The higher plasma P4 concentrations during the P4 insert maintenance in heifers supported the theory that the hepatic metabolism of steroid hormones is faster in suckled Nelore cows than non-lactating animals. In this regard, the precise difference between the metabolism of Bos indicus beef cows and heifers is unknown; however, in each parity, differences in P4 clearance according to the dry matter intake have been reported (BATISTA et al., 2020). Still, further studies are warranted to investigate the effectiveness of iP4 treatment on FSH pulsatility and follicle dynamics considering differences in parities and feed intake.

In conclusion, the addition of 100 mg iP4 for ovulation resynchronization 13 d after the TAI when using an intravaginal insert containing 1.9 g P4 efficiently increases the P4 concentrations for the first 2 d after treatment in Nelore suckled cows and heifers. The iP4 treatment does not improve the synchrony of follicular wave emergence, but reduces the dominant follicle size at the 1st TAI and improves the P/AI in the subsequent TAIs. In addition, non-pregnant Nelore heifers have greater P4 concentrations, smaller CL, later luteolysis during the resynchronization protocol, and a reduced dominant follicle diameter at TAI than suckled cows. Also, the increased rate of potential pregnancy loss (false-positive) between 22 and 37 d of pregnancy in heifers may be caused by the delayed luteolysis in this parity category.

2 CHAPTER 2: FINAL CONSIDERATIONS

As mentioned on chapter 1, the mechanism by which iP4 could favor the synchrony of follicular wave emergence and the specific dose, for different animal categories and breed, needs to be elucidated. In the present study, only one supplementary dose (P4 device + 100 mg iP4) has been tested. Eventually, increased doses and the respective P4 concentrations in circulation need to be investigated, as well as its effects on gonadotropins release modulation. In *Bos taurus* cattle, the metabolic difference that impacts on P4 concentration are well documented and results of the present work in *Bos indicus* indicates similar findings. Thus, P4 concentration needs to be explored with suitable experimental designs considering animal parity, feed intake, P4 doses and BCS.

Although the iP4 treatment increased the P/AI, the resulted economic gains of the proposed resynchronization protocol needs to be analyzed. The current cost of the short-acting iP4 treatment can achieve 40% (R$11.25/28.12) of the total cost of the protocol in Brazil. Also, if further studies indicate that greater doses are necessary to achieve the maximal P/AI, the total costs could become unfeasible. Therefore, a further economic analysis of this strategy of resynchronization associated or not with the usage of iP4 is highly indicated. This is the most relevant subject that influences the choice of the resynchronization method and the reproductive strategy adopted in the beef cattle farms. In this regard, a recent simulation performed in our research group based on field data revealed that the most rentable resynchronization model is two TAIs in 24 days followed by natural service, and the model with three TAIs in 48 days has increased expenses but similar rentability. Moreover, when performing the second resynchronization in the present proposed protocol, the false-positive animals detected between Doppler ultrasonography and confirmatory diagnosis of pregnancy have a second chance to became pregnant by AI compared to when only one super-early resynchronization is performed.

In addition, another factor that needs to be highlighted is the total volume of the treatment applied via intramuscular using the commercial product available (100 mg diluted in 10 mL of oily vehicle). This factor results in extra time-consuming for refilling the syringes and the injection delays the procedures once the animals may react during the injection. Lastly, although the realization of 3 TAIs in 48 days have drastically reduced the interval between TAIs (24 days), this process has also resulted in a more intensive animal management. For instance, when applying the hormonal protocol proposed for resynchronization in several breeding groups or farms simultaneously, as performed during part of the Experiment 2, several procedures had to be done at weekends. Despite the considerations listed above, the super-early resynchronization programs allow good P/AI in a reduced period and the use of short-acting iP4 is a safe alternative for super-early resynchronization programs to avoid the risks provided by the use of estradiol and long-acting iP4.

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Title: Early resynchronization in beef cattle using injectable and intravaginal P4 for three TAIs in 48 days

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Corresponding Author: Mr. Guilherme Pugliesi, Ph.D

Corresponding Author's Institution: Federal University of Minas Gerais

First Author: Gilmar A Ataide Jr., DVM

Order of Authors: Gilmar A Ataide Jr., DVM; Anderson Kloster, DVM; Émerson G Moraes; Igor G Motta, DVM, MS; Izaias Claro Jr., DVM, MS; José Luiz M Vasconcelos, PhD; Priscila A Ferraz, PhD; Guilherme P Nogueira, PhD; Guilherme Pugliesi, Ph.D

Abstract: We aimed to evaluate the follicular dynamic and pregnancy rate (P/AI) of beef cattle submitted to resynchronization 13 days after a TAI using a P4 device associated or not with short-acting injectable progesterone (iP4). In Exp. 1, Nelore suckled cows (C) and heifers (H) were submitted to an ovulation synchronization protocol (D0 = expected estrus). On D13, animals were split into two groups, namely control (only P4 device; 15 C and 13 H) and iP4 (P4 device + 100 mg iP4; 13 C and 12 H), and submitted to daily ovarian ultrasonography (US) exams and blood collection, from D13 to D22. On D22, the P4 devices were removed, and animals with detected luteolysis by the US had the ovulation induced by E2 cypionate treatment. In Exp. 2, 760 C and 498 H were submitted to a 1st TAI on D0. On D13 and D22, animals were split into control and iP4 groups and submitted to the same procedures done in Exp. 1. Also, non-pregnant animals received the 2nd TAI on D24, and on D37 were resynchronized using the opposite groups' 1st resynchronization procedure. On D37, a pregnancy diagnosis was performed in animals with an active CL on D22. Those with potential pregnancy loss between D22 and D37 were also resynchronized on D37. The 3rd TAI was performed on D48. In Exp. 1, the time of follicular wave emergence did not differ between groups and parity categories. The dominant follicle was larger in C than in H on D22 and D24, and in the control group on D24 (P<0.05). Increased P4 concentrations were detected on D14 (P<0.05) and D15 (P=0.08) in the iP4 group. P4 concentrations reduced gradually between D14 and D22 (P<0.05), and luteolysis occurred earlier (P=0.07) in C than in H. In Exp. 2, the P/AI tended to be greater (P=0.09) in iP4-treated H than control H (44% [73/167] vs. 38% [61/160]), but did not differ between iP4-treated C (43% [105/244]) and control C (38% [87/227]). The overall P/AI for 2nd and 3rd TAIs, regardless of category, was greater (P<0.05) in the iP4 group than the controls (43% [178/411] vs. 38% [148/387]). An interaction of group by BCS (P=0.06) indicated increased P/AI due to the iP4 treatment only in females with BCS javascript:insert_text('≤')2 (54% [37/69] vs. 28% [15/53]). In conclusion, the 100 mg short-acting iP4

treatment associated with the intravaginal P4 device is effective in increasing the plasma P4 concentration, but does not impact the synchrony of the follicular wave emergence. Animals treated with iP4 presented improved P/AI, especially those with low BCS.