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CAROLINE FERNANDES POSSEBON

INDUÇÃO A PUBERDADE DE NOVILHAS DE CORTE BOS TAURUS COM PROGESTERONA INJETÁVEL E PROSTAGLANDINA

URUGUAIANA-RS, BRASIL

2023

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Dissertação apresentada ao Programa de Pós-Graduação Stricto sensu em Ciência Animal da Universidade Federal do Pampa, como requisito parcial para obtenção do título de Mestre em Ciência Animal.

Orientador: Prof. Dr. Fabio Gallas Leivas
Co-orientador: Profª. Drº. Gilson Antônio Pessoa

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Área de concentração: Reprodução e Produção Animal.

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Banca examinadora:

Prof. Dr. Fabio Gallas Leivas
Universidade Federal do Pampa - UNIPAMPA
Orientador

Profª. Drª. Janislene Mach Trentin
Universidade Federal do Paraná - UFPR

Dr. Manoel Francisco Sá Filho
Alta Genetics Brasil

Assinado eletronicamente por **FABIO GALLAS LEIVAS**, **PROFESSOR MAGISTÉRIO SUPERIOR**, em15/09/2023, às 15:36, conforme horário oficial de Brasília, de acordo com as normativas legaisaplicáveis.

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RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Ciência Animal
Universidade Federal do Pampa

INDUÇÃO A PUBERDADE DE NOVILHAS DE CORTE *BOS TAURUS* COM PROGESTERONA INJETÁVEL E PROSTAGLANDINA

AUTORA: Caroline Fernandes Possebon
ORIENTADOR: Fabio Gallas Leivas
Uruguaiana-RS, 31 de julho de 2023

Novilhas que possuem capacidade de estarem ciclando no início da estação reprodutiva encontram-se em anestro, o que é um fator limitante para a reprodução. Ocasionando atraso na eficiência reprodutiva e rentabilidade do rebanho. Diferentes programas de indução à puberdade vêm sendo utilizados como forma de minimizar essa deficiência, potencializando a produtividade dos rebanhos comerciais e produzindo um ciclo reprodutivo a mais na vida das fêmeas. Com isso, o objetivo do trabalho foi avaliar o efeito da indução a puberdade com progesterona injetável (P4i) e sua associação com prostaglandina (PG) sobre a fertilidade de novilhas taurinas com idade entre 16 e 20 meses submetidas a protocolos de IATF. O experimento foi dividido em duas fases, em três fazendas comerciais da Fronteira Oeste do estado do Rio Grande do Sul. No experimento I foram utilizadas 289 novilhas pré-púberes avaliadas para peso, escore de condição corporal (ECC), escore de trato reprodutivo (ETR), ciclicidade (presença ou não de CL) nos dias D-50, D-26 e D0, taxa de prenhes e perda gestacional no D30 e D60. Foram divididas em: grupo controle negativo (C-) composto por novilhas não tratadas, e quatro grupos tratamento: T-50 receberam uma aplicação de 150mg de P4 (IM) (Sincrogest® injetável, OuroFino) no D-50; T-26 uma aplicação de 150mg de P4 (IM) no D-26, P4P4 duas doses de P4 no D-50 e D-26 (IM) e o grupo P4PG uma dose de P4 (IM) no D-50 e 12,5 mg de PG (IM) (Sincrocio®, OuroFino) no D-26. No D0 as novilhas iniciaram o protocolo de IATF e foram inseminadas no D10. O diagnóstico de gestação foi realizado 30 e 60 dias após a IA e foi calculada a taxa de perda gestacional. No experimento II, 12 novilhas pré-púberes foram distribuídas em quatro grupos tratamento: T-50, T-26, P4P4 e P4PG como descrito no experimento I. O primeiro dia do protocolo de indução à puberdade foi denominado como D-50. As novilhas foram avaliadas a cada dois dias para: peso, ECC, ETR e ciclicidade, as avaliações perduraram 52 dias. Amostras de sangue para avaliação da progesterona sérica foram coletadas por 12 dias consecutivos após cada tratamento (D-50 e D-26). Para a análise estatística utilizou-se GLIMMIX do SAS e as variáveis comparadas pelo teste Tukey ($P<0.05$). O diâmetro uterino dos grupos P4P4 e T26 foram similares entre si e apresentaram melhor diâmetro (16.04±0.34; 16.63±0.32). O grupo C- (15.2±0.3) foi igual aos grupos T-50 (15.8±0.4) e P4PG (15.7±0.4). A taxa de ciclicidade no D0 foi menor no grupo C- (33.9%), e a maior do grupo P4P4 (62.7%). A taxa de estro no dia da IA foi menor no grupo C- (53.6%) e maior nos grupos T-26 (84.0%) e P4P4 (83.0%). A taxa de prenhez aos 30 dias foi menor no grupo C- (50.0%), T-26 (49.0%) e P4PG (48.0%) e maior nos grupos T-50 e P4P4 (55.7%; 67.8%). No DG aos 60 dias o grupo C- e T-26 apresentaram menor média (35.7%; 37.3%) e os grupos T-50 e P4P4 (49.2%; 59.3 %) maior taxa de prenhez. Na perda gestacional não houve diferença entre os grupos tratado e controle ($P=0.43$), assim como não houve diferença entre a tamanho do folículo na IA ($P=1.00$) e taxa de ciclicidade no D30 ($P=0.24$). Entretanto, novilhas que apresentavam diâmetro uterino >20mm tiveram melhores médias de taxa estro na IA (81.2%), prenhez aos 30 dias (68.7%) e 60 dias (68.7%) e menor perda gestacional (0.0%). Com isso, o protocolo de indução com P4i acelerou o desenvolvimento reprodutivo das novilhas pré púberes. Quando utilizada 50 e/ou 25 dias antes do início do protocolo de IATF foi capaz de estimular o desenvolvimento uterino, aumentar a taxa de ciclicidade na IA e maior taxa de prenhez.

Palavras-chave: Novilhas. Ciclicidade. Fertilidade. Puberdade. Prostaglandina.

ABSTRACT

Dissertation of Master's Degree
Programa de Pós-Graduação em Ciência Animal
Universidade Federal do Pampa

INDUCTION TO PUBERTY IN *BOS TAURUS* BEEF HEIFERS WITH INJECTED PROGESTERONE AND PROSTAGLANDIN

AUTHOR: Caroline Fernandes Possebon
ADVISOR: Fabio Gallas Leivas
Uruguaiana-RS, July 31, 2023

Heifers that have the capacity to be cycling at the beginning of the reproductive season are in anestrus, which is a limiting factor for reproduction. Causing delay in the reproductive efficiency and profitability of the herd. Different puberty induction programs have been used to minimize this deficiency, enhancing the productivity of commercial herds and producing an extra reproductive cycle in females' lives. Thus, the objective of this work was to evaluate the effect of inducing puberty with injectable progesterone (iP4) and its association with prostaglandin (PG) on the fertility of taurine heifers aged between 16 and 20 months submitted to FTAI protocols. The experiment was divided into two phases, in three commercial farms in the West Frontier of the state of Rio Grande do Sul. In experiment I, 289 prepubertal heifers were used, evaluated for weight, body condition score (BCS), reproductive tract score (RTS), cyclicity (presence or absence of CL) on days D-50, D-26 and D0, pregnancy rate and pregnancy loss on D30 and D60. They were divided into: negative control group (C-) composed of untreated heifers, and four treatment groups: T-50 received an application of 150mg of P4 (IM) (injectable Sincrogest®, OuroFino) on D-50; T-26 one application of 150mg of P4 (IM) on D-26, P4P4 two doses of P4 on D-50 and D-26 (IM) and the P4PG group one dose of P4 (IM) on D-50 and 12 .5 mg of PG (IM) (Sincrocio®, OuroFino) on D-26. On D0, heifers started the FTAI protocol and were inseminated on D10. Pregnancy diagnosis was performed 30 and 60 days after AI and the pregnancy loss rate was calculated. In experiment II, 12 prepubertal heifers were distributed into four treatment groups: T-50, T-26, P4P4 and P4PG as described in experiment I. The first day of the puberty induction protocol was designated as D-50. The heifers were evaluated every two days for: weight, BCS, RTS and cyclicity, the evaluations lasted 52 days. Blood samples for serum progesterone assessment were collected for 12 consecutive days after each treatment (D-50 and D-26). For the statistical analysis, GLIMMIX from SAS was used and the variables compared by the Tukey test (P<0.05). The uterine diameter of the P4P4 and T26 groups were similar to each other and showed a better diameter (16.04±0.34; 16.63±0.32). The C- group (15.2±0.3) was equal to the T-50 (15.8±0.4) and P4PG (15.7±0.4) groups. The cyclicity rate on D0 was lowest in the C- group (33.9%), and the highest in the P4P4 group (62.7%). The estrus rate on the day of AI was lower in the C- group (53.6%) and higher in the T-26 (84.0%) and P4P4 (83.0%) groups. The pregnancy rate at 30 days was lower in the C- (50.0%), T-26 (49.0%) and P4PG (48.0%) groups and higher in the T-50 and P4P4 groups (55.7%; 67.8%). In the DG at 60 days, the C- and T-26 groups had a lower mean (35.7%; 37.3%) and the T-50 and P4P4 groups (49.2%; 59.3%) had a higher pregnancy rate. There was no difference in pregnancy loss between treated and control groups (P=0.43), as well as there was no difference between follicle size at AI (P=1.00) and cyclicity rate at D30 (P=0.24). However, heifers with uterine diameter >20mm had better means of estrus rate at AI (81.2%), pregnancy at 30 days (68.7%) and 60 days (68.7%) and lower pregnancy loss (0.0%). Thus, the induction protocol with P4i accelerated the reproductive development of prepubertal heifers. When used 50 and/or 25 days before the start of the TAI protocol, it was able to stimulate uterine development, increase the AI cyclicity rate and increase the pregnancy rate.

Keywords: Heifers. Cyclicity. Fertility. Puberty. Prostaglandin.

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1 INTRODUÇÃO

De acordo com o Instituto Brasileiro de Geografia e Estatística (IBGE, 2021) o Brasil possui o maior rebanho bovino do mundo, estimado em 224,6 milhões de cabeças, representando 14% da produção global de bovinos. Ocupa o 2º lugar na produção de carne (10 milhões de toneladas), utilizando 163,1 milhões de hectares de pasto (1,2 cab/ha) e exportando em torno de 2,48 milhões de toneladas de carcaça (ABIEC, 2022). Em 2023, o Produto Interno Bruto (PIB) brasileiro registrou aumento de 1,9%, sendo o setor da agropecuária brasileira responsável por impulsionar o crescimento em 21,6%. Além disso, é responsável por 10,2% do PIB brasileiro, tornando o Brasil um destaque na produção de carne e leite (Conchon, 2023).

Apesar disso, o sistema de criação de bovinos no país possui baixos índices de precocidade, prenhez, natalidade e desmame. Oriundos do sistema de criação extensiva, baseada em pastagens nativas, com estacionalidade de produção, sem manejo de carga (Lobato et al., 2014) e com uma redução das áreas de campo nativo em decorrência do avanço de culturas agrícolas (IBGE, 2018), que vem ocorrendo ao longo dos anos. Esse cenário, associado às características genéticas e a idade de inserção de novilhas na reprodução são fatores que limitam a eficiência reprodutiva e produtiva dos rebanhos, refletindo diretamente no setor comercial da pecuária (Rodrigues, 2012; Queiroz, 2019).

A pecuária de corte brasileira, ao longo dos anos vem investindo em tecnificação e modernização, a fim de buscar maior produtividade, qualidade e competitividade. Nesse sentido, as estratégias hormonais podem ser utilizadas como alternativa para reduzir a idade da primeira concepção/parto e o intervalo entre partos, melhorando as taxas de ciclicidade e prenhez das matrizes. Consequentemente, gerando menor custo de manutenção desses animais dentro da fazenda, pela maior produtividade e longevidade (Cushman et al., 2013).

Os programas de indução à puberdade são utilizados para melhorar a eficiência reprodutiva dos rebanhos em frente à puberdade tardia. Visto que, novilhas taurinas apresentam baixas taxas de ciclicidade no início da estação de monta, não sendo capazes de expressar seu potencial reprodutivo (Pinto et al., 2020). O uso de progestagenos na indução a puberdade é relacionado a maturação e a regulação hormonal do sistema neuroendócrino, que culmina com a puberdade. O uso da progesterona precisa ser melhor caracterizada, visto que os protocolos de indução já descritos têm variações entre tempo de administração e exposição ao hormônio. Sendo importante levar em consideração a diferença de resposta entre as raças taurinas e zebuínas, visto que novilhas *Bos indicus* possuem menor taxa metabólica quando comparada com *Bos taurus*. Com isso, o objetivo do trabalho foi avaliar o efeito da progesterona injetável e sua associação com prostaglandina na indução da puberdade sobre a fertilidade de novilhas taurinas de corte submetidas a protocolos de IATF.

2 REVISÃO BIBLIOGRÁFICA

2.1 Puberdade

A puberdade é caracterizada pela aquisição da capacidade reprodutiva, marcada pela primeira oportunidade de uma novilha conceber (Atkins et al., 2013). É definida pela ocorrência de sinais de estro, seguido da ovulação e de uma fase lútea funcional (Atkins et al., 2013). No período que antecede a puberdade os níveis circulantes de estradiol inibem a secreção do GnRH, realizando *feedback* negativo sobre o LH, inviabilizando a capacidade ovulatória dos folículos (Moran et al., 1989; Perry, 2016). A redução da sensibilidade do hipotálamo em resposta ao estradiol e o aumento do GnRH em torno de 50 dias antes da puberdade (período peri puberdade), induz ao pico pré-ovulatório de LH. Sendo esse pico suficiente para levar ao desenvolvimento de folículos e marcando o início da puberdade pela ativação do eixo hipotalâmico-hipofisário-gonadal e ovulação (Kinder et al., 1995; Moran et al., 1989; Perry, 2016). Diversos são os fatores envolvidos na caracterização da precocidade sexual das fêmeas bovinas, como genética, nutrição, estação do ano, interações e progestagenos exógenos, sendo esses responsáveis por adiantar ou atrasar o início da puberdade (Kinder et al., 1995).

Os eventos que ocorrem na vida reprodutiva das fêmeas, como as alterações hormonais, estruturais e de manutenção da prenhez exigem uma maior intensidade de energia. Diante disso, a puberdade é estreitamente relacionada à nutrição e a seleção genética para ocorrência de ciclos estrais normais. O estado nutricional é responsável pela regulação de hormônios metabólicos, responsáveis por modular a ação de neuropeptídeos hipotalâmicos na secreção de GnRH e aumento da concentração sérica de progesterona (Amstalden, et al., 2014). Assim como o fator genético também influencia na precocidade. Novilhas *Bos taurus* atingem a puberdade em torno de 09-12 meses com peso entre 200 e 250kg (Forde et al., 2011), já novilhas *Bos indicus* atingem puberdade de forma tardia, em torno dos 24-36 meses (Sartori; Barros, 2011). O hormônio LH é o fator endócrino primário para novilhas tornarem-se púbere, induzindo a primeira ovulação. Para isso, é necessário que o eixo hipotalâmico-hipofisário-gonadal esteja funcional. Sendo necessário, que o E2 reduza o *feedback* negativo sobre o hipotálamo e a adenoipófise, atuando sobre seus receptores para que ocorra o aumento na pulsatilidade de LH (Day, 2010).

A secreção pulsátil de LH inicia por volta de 1 a 2 meses de idade nas terneiras, caracterizada como fase infantil. Na fase de desenvolvimento, que compreende entre o 3º e o

5º mês, a concentração de LH tem um aumento de forma gradativa. Logo após, ocorre a fase estática, onde a concentração de LH reduz e mantém baixos níveis. Na fase peripúbere, que ocorre entre o 6º e 9º mês, há um novo aumento na secreção de LH, culminando com a puberdade (Day, 2010). O hipotálamo é responsável por liberar o hormônio liberador de gonadotrofina (GnRH), o qual age na hipófise estimulando a secreção do FSH e LH que atuam diretamente no ovário determinando a ovulação (Reece, 2017).

Após a ovulação, ocorre a formação do CL o qual é responsável por secretar a progesterona (P4), encarregada de preparar o útero e manter a gestação. Se o oócito for fecundado os níveis de P4 são mantidos, caso não ocorra a fecundação a luteolise inicia pela liberação de ocitocina através do corpo lúteo (CL) que atua no endométrio e desencadeia a secreção de prostaglandina ocasionando a lise do CL e iniciando um novo ciclo estral (Atkins et al., 2013)

2.2 Ciclos Estral e Dinâmica Folicular

O ciclo estral compreende o período entre dois estros, que dura em torno de 20 a 23 dias, sendo variável conforme a categoria e raça dos animais. Em bovinos é composto por quatro fases bem definidas: proestro, estro, metaestro e diestro (Reece, 2017). Os ciclos ocorrem durante todo o ano, caracterizando as fêmeas bovinas como poliéstricas anuais, onde a atividade cíclica só é interrompida em casos de gestação, lactação ou patologias (Hyttel; Sinowatz; Vejlsted, 2012).

O ciclo estral é dividido em duas fases: luteal que compõe o metaestro e diestro, e compreende o período após a ovulação e formação de CL com duração de 16 a 17 dias; fase folicular composta pelo proestro e o estro, compreendendo o período de regressão do CL até a próxima ovulação, com duração de 3 a 6 dias (Forde et al., 2011). O estro tem duração de 8 a 18 horas e é nesse período que a fêmea aceita a monta do macho. Nessa fase ocorre modificações externas e internas no aparelho reprodutor das fêmeas, bem como alterações de comportamento caracterizando o cio (Reece, 2017). Após, ocorre a fase de metaestro que tem duração de 3 a 4 dias, nesse período ocorre inicialmente a ovulação e logo após a formação do corpo lúteo. O diestro é o período mais longo do ciclo estral com 11 a 13 dias de duração, onde o corpo lúteo está ativo. O fim dessa fase é marcado pela luteólise através da secreção da prostaglandina (PG). E por fim, o proestro que é caracterizado pelo crescimento e maturação dos folículos durante 2 a 3 dias, não possuindo CL ativo (Reece, 2017).

O crescimento e desenvolvimento folicular nos bovinos podem ocorrer em duas ou três ondas foliculares durante o ciclo estral. Durante essas ondas existem três fases: o recrutamento, seleção e dominância dos folículos (Oliveira; Sarapião; Quintão, 2005). A fase de recrutamento promove o crescimento de folículos através do hormônio folículo estimulante (FSH) (Moran et al., 1989). A seleção e dominância é o momento que um dos folículos recrutados se destaca e torna-se dominante, provocando a atresia dos demais folículos pela secreção de inibina. Com a alta produção de estrógeno (E2) pelo folículo dominante, a hipófise reduz a secreção dos níveis de FSH e consequentemente ocorre um aumento na secreção de hormônio luteinizante (LH), que será responsável pela ovulação (Vieira, 2020).

2.3 Estratégias Hormonais para Indução da Puberdade

Novilhas reprodutivamente imaturas acarretam menor desempenho produtivo dentro do sistema de criação. Diante disso, diferentes associações hormonais vêm sendo estudadas para a indução da puberdade em novilhas, sendo a progesterona a base dos protocolos. A progesterona pode ser usada de forma isolada ou associada com outros hormônios tais como estradiol, prostaglandinas e GnRH (Araújo et al., 2019). A eficácia dos protocolos de indução de puberdade depende da dose e tempo de exposição das novilhas aos fármacos, variando principalmente pelo padrão racial (Roman, 2018).

O uso de fontes exógenas de P4 aceleram o início da puberdade quando a maturidade do eixo hipotalâmico-hipofisário está completa. Quando há redução dos receptores de E2, há redução do feedback negativo do E2 pelo GnRH e os níveis de LH aumentam, levando a ovulação (Cabral et al., 2013; Rasby et al., 1998**;** Sá Filho et al., 2015). Entretanto, Carvalho et al., (2008) avaliou que a alta exposição de P4 exógena em novilhas pode interferir no crescimento folicular, levando a uma taxa de ovulação e gestação menor do que o proposto. Para novilhas pré-púberes é indicado o uso de dispositivo intravaginal de P4 de terceiro (Sá Filho et al., 2015) ou quarto uso (Magi et al., 2020) ou dose de 150mg de P4i (Lima et al., 2020).

A literatura ainda traz dados similares sobre o uso das diferentes fontes de P4 exógena nos protocolos de indução de puberdade. As principais são os dispositivos intravaginais de progesterona implantes subcutâneos (Baruselli et al., 2004), progesterona injetável (Morotti et al., 2013), adesivos transdérmicos (Kajaysri et al., 2017) e progestagenos no alimento (acetato de melengestrol - MGA) (Pinto et al., 2018). Assim como Sousa et al. (2018)**,** Gottschal et al. (2019) e Nascimento et al. (2021), obtiveram taxas de puberdade abaixo de 40% usando P4i ou dispositivo intravaginal de P4, enquanto outros autores tiveram taxas superiores a 60% de puberdade com ambas as fontes (Rodrigues et al., 2013). Apesar disso, o uso de P4 injetável ou através de dispositivo promove taxas de indução satisfatória (Rasby et al., 1998; Júnior et al., 2010; Morotti et al., 2018), sendo atualmente preconizado o uso da P4i pela maior facilidade de manejo e menor custo.

Além disso, associação com PG também demonstrou eficácia quando usada 12 dias após aplicação de P4. Novilhas que receberam dose de PG tiveram maior taxa de ovulação quando comparada com as que não receberam, onde a PG auxilia no crescimento final do folículo quando aplicada no início ou final do protocolo (Carvalho et al., 2008; Leonardi et al., 2012). A P4 quando utilizada na indução a puberdade tem capacidade de reduzir o número de receptores de estradiol reduzindo o feedback negativo ao GnRH, que sensibiliza a hipófise a secretar LH, promovendo a indução da ovulação. A PG quando usada em novilhas pré-púberes é capaz de aumentar a sensibilidade da hipófise ao GnRH, a secreção de LH induzindo a ovulação sem interferir no desenvolvimento do CL (Leonardi et al., 2012).

Diante disso, a pré-sincronização é uma opção a ser explorada para induzir a puberdade em novilhas pré-púberes. De acordo com a literatura essa técnica demonstra boa eficiência e melhor taxa de ciclicidade e prenhez nos rebanhos comerciais, assim como influencia diretamente a eficiência reprodutiva e produtiva.

3 OBJETIVOS

3.1 Objetivo Geral

Avaliar o efeito da progesterona injetável e sua associação com prostaglandina na indução da puberdade sobre a fertilidade de novilhas taurinas de corte submetidas a protocolos de IATF

3.2 Objetivos Específicos

Avaliar o efeito da indução de ciclicidade utilizando uma ou duas doses de progesterona injetável, ou associação de uma dose de progesterona injetável e prostaglandina sobre:

- Taxa de ciclicidade após 1ª e 2º manejo de indução das novilhas;
- Desenvolvimento uterino;
- Taxa de ciclicidade no D0 e de estro no D10 do protocolo de IATF;
- Tamanho do folículo ovulatório no D10;
- Taxa de concepção aos 30 e 60 dias após IA e de perdas embrionárias.
- Perdas gestacionais

4 ARTIGO CIENTÍFICO

Os resultados que fazem parte desta dissertação estão apresentados sob a forma de artigo científico. As *seções Material e Métodos, Resultados, Discussão e Referências Bibliográficas* encontram-se no próprio manuscrito, que está apresentado da mesma forma que será submetido ao periódico *Theriogenology*.

INJECTABLE PROGESTERONE IMPROVE UTERINE AND OVARIAN ACTIVITY IN PREPUBERTAL *BOS TAURUS* HEIFERS

ABSTRACT

This study evaluates the effect of inducing puberty with injectable progesterone (iP4) and its association with prostaglandin (PG) on the fertility of taurine heifers submitted to FTAI protocols. The work was divided into two experiments using heifers aged between 16 - 20 months. In experiment I 289 heifers were used, distributed into five treatment groups: one group control negative (C-) composed of heifers that did not received any treatment; and four groups treatment: T-50 received an application of 150mg of iP4 on D-50; T-26 received a 150mg of iP4 on D-26, P4P4 received two doses of iP4 on D-50 and D-26, and the P4PG group received one dose of iP4 on D-50 and 12.5 mg of progesterone (PG) on D-26. On D0, heifers started the FTAI protocol and were inseminated on D10. The heifers were evaluated for: weight, body condition score (BCS), reproductive tract score (RTS), cyclicity (presence or absence of CL) on days D-50, D-26 and D0 and pregnancy and pregnancy loss rates on days D30 and D60. In experiment II, 12 prepubertal heifers were used, divided in four treatments: T-50, T-26, P4P4 and P4PG, in the same away as experiment I. The first day of puberty induction was called D-50. Heifers were evaluated every two days for weight, BCS, RTS and cyclicity, the evaluations lasted 52 days. For the statistical analysis, GLIMMIX from SAS was used and the variables compared by the Tukey test (P<0.05). The uterine diameter of the P4P4 and T26 groups were like each other and showed a better diameter (16.04±0.34; 16.63±0.32). The C- group (15.72±0.31) was equal to the T-50 (15.6±0.34) and P4PG (15.49±0.34) groups. The estrus rate on the day of AI was lower in the C- group (53%) and higher in the T-26 (84%) and P4P4 (83%) groups. The 30-day pregnancy rate was lowest in the C- (50%), T-26 (49%) and P4PG (48%) groups and highest in the T-50 and P4P4 groups (55%; 68%). In the DG at 60 days, the C- and T-26 groups had a lower mean (35%; 37%) and the T-50 and P4P4 groups (49%; 59%) had a higher pregnancy rate. There was no difference in pregnancy loss between treated and control groups (P=0.43), in follicle size at AI (P=1.00) and cyclicity rate at D30 (P=0.24). However, heifers with uterine diameter >20mm had better means of estrus rate at AI (84%), pregnancy at 30 days (68.7%) and 60 days (68.7%) and lower pregnancy loss (1%). Thus, the induction protocol with iP4 accelerated the reproductive development of prepubertal heifers. When used 50 and/or 25 days before the start of the FTAI protocol, it was able to stimulate uterine development, increase the cyclicity and pregnancy rate.

Keywords: Heifers. Cyclicity. Fertility. Puberty. Prostaglandin.

1 Introduction

In beef cattle production systems, the onset of puberty is an important milestone, this being a signal of production and profitability on farms, which occurs when ovulation is followed by estrus with a normal luteal phase [1]. However, due to genetics, age and nutrition status beef heifers haven’t sexual maturity in the first estrus, occurs early CL regression and low fertility rate [2,1,3]. Heifers that were pubertal by the start of breeding season have a higher conception rate and reproductive life, searching for a better user of females in the production systems [4]. However, to achieve these conditions, adequate nutritional conditions are necessary, which is not always achieved in extensive grazing systems. The use of puberty induction protocols pre-breeding season in heifers is an efficient alternative to anticipate reproductive life and have a better use of females [5].

The injectable progesterone (iP4) has been used to induce sexual precocity in heifers *Bos indicus,* despite the few of studies in *Bos taurus,* it is known that the administration of progesterone in the prepubertal period promotes higher rates of cyclicity and pregnancy [6]. The progesterone when administered within the period leading up to puberty acts on hypothalamus sensitizing the reduction in estradiol secretion, consequently increasing GnRH carry to the preovulatory peak of LH, which stimulates follicular growth and induces ovulation, marking the onset of puberty [7]. The effect of progesterone occurs by changing the amount and location of estrogen receptors in the hypothalamus, being able to increase the sensitivity of estrogen receptors in the LH and GnRH secretion regions [8,9].

Prostaglandin (PG) has great potential for action in the peripubertal period. There are studies that demonstrate that treatment with PG during the late growth phase of the dominant follicle can promote ovulation by an independent luteolysis mechanism, being able to induce ovulation in peripubertal heifers in the absence of CL [10]. Other reports suggest that PG may act in synergism with P4 in inducing puberty, while P4 reduces the negative feedback on estradiol by the hypothalamus by reducing its receptors, PG may increase the pituitary response to GnRH by increasing LH release and ovulation [11]. It is believed that PG is also produced by granulosa cells and acts directly on the preovulatory follicle, making a local role in the ovary [10]. However, more studies are needed to define certain hormonal and structural actions of the reproductive cycle of heifers.

The hypothesis of this study is that beef heifers submitted to the puberty induction protocol with progesterone and prostaglandin and that present more than one estrous cycle before the FTAI protocol obtain better conception rates and less embryonic loss. To answer the response of taurine heifers to the induction of puberty with injectable progesterone, we asked ourselves four questions: [1] The progesterone treatment improved uterine score in *Bos taurus* heifers? [2] Can progesterone induction have the same impact on reproduction as naturally cycling heifers? [3] The moment of progesterone injection before breeding season influences the uterine score and pregnancy of heifers? [4] Combination of two treatments with iP4 or iP4 and PGF can induce more ovulation and have similar effects on reproductive efficiency to a heifer with natural puberty?

Therefore, the objective of this work was to evaluate the effect of puberty induction with iP4 and its association with PG on the reproductive development and fertility of beef heifers submitted to FTAI protocols.

2 MATERIALS AND METHODS

2.1 Animals and location

The experiment was conducted in four commercial beef farms located in the western border and central region of Rio Grande do Sul State, during the period from October of 2022 to January 2023. The study was carried out on 289 taurine beef heifers in experiment I and 12 taurine beef heifers in experiment II, ranging between 16-20 months of age. In both experiments the heifers were evaluated for age, weight, BCS, RTS and presence or absence of CL. The pregnancy and loss pregnancy rates was evaluated in experiment I. Blood samples were collected for measurement of progesterone in experiment II.

All the experimental procedures and protocols described in this study were approved by the Committee for Ethics in Animal Experimentation from the Federal University of Pampa (protocol number 033/2021).

2.2 Experiment I: Induction of puberty of *Bos taurus* heifers pre-FTAI

The objective of experiment I was to evaluate the response rate to cyclicity and pregnancy in taurine heifers induced to puberty using protocols with injectable progesterone associated or not with prostaglandin at different times.

Heifers were divided into prepubertal (non-cyclical) five groups: control group (C-) was composed of heifers did not receive treatment. Group T-50 received an application of 150mg of long-acting progesterone (iP4) (Sincrogest®injetável, OuroFino), intramuscular (IM) on the day D-50; group T-26 received 150mg of iP4 (IM) on day D-26; iP4iP4 received 150mg of iP4 (IM) on D-50 and on D-26 day, the group iP4PG received on application of 150mg iP4 (IM) on D-50 and 12,5mg of prostaglandin (PG) (Sincrocio®, OuroFino) on the day D-26. Assessments as age, weight, body condition (BCS), reproductive tract score (RTS).

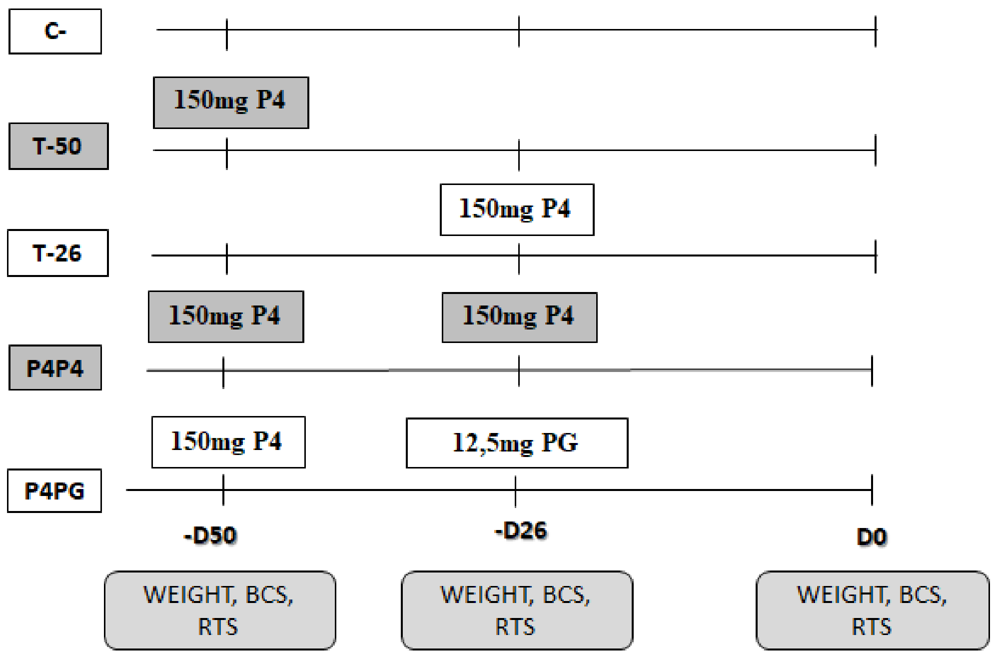

Figure 1. Schematic representation: control -: heifers that had a follicular diameter >10mm and did not receive treatment, T-50: non-cycled heifers that received a dose of iP4 at D-50; T-26: non-cycling heifers that received a dose of iP4 at D-26; P4P4: non-cyclic heifers that received two doses of iP4, at D-50 and D-26; P4PG: non-cycling heifers that received a dose of iP4 at D-50 and a dose of PG at D-26. Assessments were carried out on D-50, D-26 and D0, about weight, BCS, RTS and blood collection for measuring serum P4.

2.3 Experiment II: Evaluation of hormonal behavior, uterine status and follicular dynamics in beef heifers at the puberty induction protocol

The objective of Experiment II was to evaluate the follicular dynamics of heifers during the puberty induction protocol. The heifers were divided into four treatments according to the experiment I: T-50; T-26; P4P4; P4PG. Weight, BCS and ETR were carried out every two days for 52 days and the blood was collected during 12 consecutive days after induction on D-50 and D-26 (Figure 2).

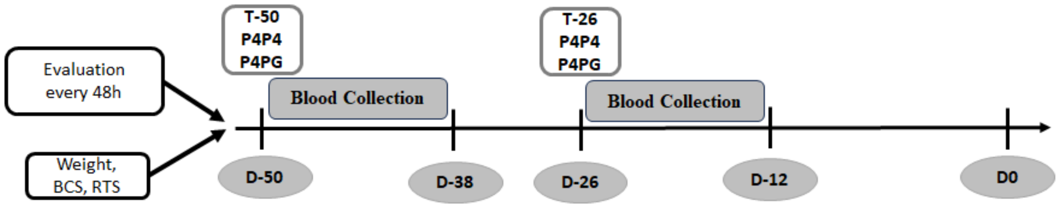

Figure 2: Experimental Design: The design demonstrates the treatment groups (T-50, T-26, P4P4, P4PG), timing of induction puberty for each group (D-50, D-26) and frequency of assessments (every 48h for 52 days) and blood collections (For 12 days after each treatment (D-50, D-26). BCS = Body condition score; RTS = Reproductive Tract Score.

2.4 Weight and Body Condition Score (BCS)

Heifers were weighed using a scale, followed by body condition score (BCS) assessment. BCS was evaluated visually, on a scale from 1 to 5: BCS1 extremely thin animal; BCS2 thin animal; BCS3 animal in ideal condition; BCS4 fat animal and BCS5 very fat/overweight animal. In the Experiment I the BCS was evaluated on days D-50, D-26 and D0, while in Experiment II the BCS occurred every two days.

2.5 Uterus Diameter and Reproductive Tract Score (RTS)

Uterus diameter and RTS were classified through gynecological examination with an ultrasonic scanner Mindray DP10 equipped with a 5.0 MHz linear transducer on days D-50, D-26 and D0 in experiment I and every two days in experiment II. The uterus diameter was evaluated as the average of the height and width of a cross section of the uterine horn just cranial to the external bifurcation of the uterus (Figure 3). The classification of RTS was measured considering the morphometry of the ovaries and uterus using scores on a scale of 1 to 5 (MIHURA; CASALO, 1999).

- RTS 1-2: indicates sexual immaturity, uterus is small (<10mm), without tone and with the presence of small follicles in the ovary (<8mm);
- RTS 3-4: indicates pre-puberty, uterus is larger (10 - 15mm) and have slight uterine tone and slightly larger follicles (8-10mm);

-RTS 5: indicates cyclicity, large uterus (>20mm) and evident uterine tone, presence of corpus luteum in one or both ovaries.

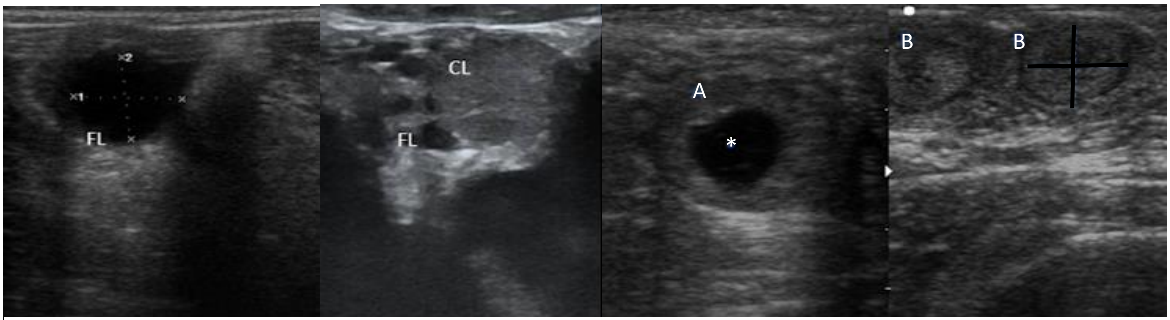

Figure 3. Uterine image of heifers: FL indicates mean follicle. CL indicates corpus luteum. Letter A indicates CL cavity and an asterisk indicates the cavity of CL. Letter B means transversion uterine section and the dotted line indicates the mean uterine diameter.

2.6 Cyclicity

The cyclicity of heifers were evaluated on days D-26 and D0 in experiment I and every two days in experiment II. The presence or absence of CL in the ovaries was identified, cyclic animals with CL (pubescent heifers) and non-cyclic animals without CL (heifers in anestrus).

2.7 Follicular dynamics

The follicular dynamics was performed in experiment II, using an ultrasonic scanner Mindray DP10 equipped with 5.0 MHz linear transducer every two days, for 52 days. Was evaluated: uterine diameter through the average of the height and width of a cross section of the uterine horn just cranial to the external bifurcation of the uterus; the follicular diameter was also measured through the average of the height and width of the follicle and the total number of follicles present in each ovary was counted.

2.8 Serum P4 measurement

To measure serum P4, blood samples were collected by venipuncture using a vacutainer needle and a coupler in EDTA tubes. Samples were collected in experiment II from all animals, during 12 consecutive days after each treatment (D-50 and D-26). The plasma was separated by centrifugation at 4ºC, 1500 xg for 30 minutes and stored at – 20ºC right after collection. After, the blood samples from days D-50 to D-17 were sent to a specialized clinical analysis using the electrochemiluminescence kit (ADVIA Centaur, Siemens Healthcare, Munich, Germany), with a sensitivity of 0,05ng/ml of progesterone.

2.9 FTAI

The FTAI protocol (Figure 4) was performed only in experiment I and started on D0. The synchronization protocol consisted of the insertion of an intravaginal P4 device on D0 (Sincrogest® 1g; Ouro Fino) and 2 mg of estradiol benzoate (IM) (Sincrodiol®, Ouro Fino). On the eighth day of protocol (D8) the P4 device was removed, and all heifers received 12,5mg of prostaglandina (IM) (Sincrocio®, Ouro fino), 0,5mg estradiol cypionate (IM) (SincroCP®, Ouro Fino), 300IU of eCG (IM) (Sincro eCG®, Ouro Fino). The heifers were inseminated two days after (D10) and received 0,01mg Buserelin Acetate (IM) (GnRH analogue, Sincroforte®, Ouro Fino). Pregnancy diagnosis was performed at 30 days and 60 days after AI.

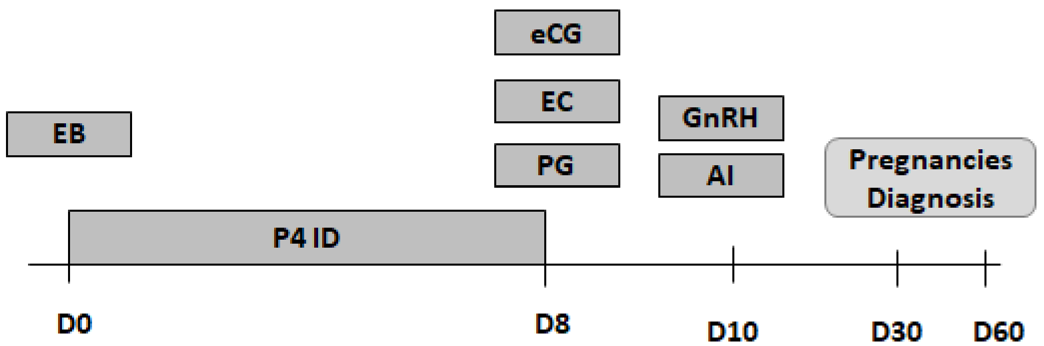

Figure 4. Schematic representation of the Fixed-Time Artificial Insemination (FTAI) protocol performed in heifers after the puberty induction protocol, followed by the management diagnosis at 30 and 60 days after AI. EB: 2 mg estradiol benzoate, P4 ID: intravaginal P4 device 1g, PG: 12.5 mg de prostaglandin; EC: 0,5 mg estradiol cypionate, eCG: 300 IU equine chorionic gonadotropin; GnRH: 0.01mg Buserelin Acetate; AI: artificial insemination.

3 RESULTS

3.1 Effects of treatments on weight, BCS and RTS

The body score (BCS) of the heifers was not different among the treatment groups, but it was different between the evaluations (P= 0.001; Figure 5). Likewise, the average weight of heifers did not differ between treatments, but there was a difference between assessments (P=0.001; Figure 6). The RTS of heifers showed an increase during the evaluations in the control and treatment groups. The RTS, on the other hand, showed difference between the treated groups (P=0.005) and the evaluations (P=0.001). The P4PG group had a lower mean RTS at the end of the evaluations (3.79±0.14) and P4P4 showed better evolution in the ETR (4.35±0.12), when compared to the other groups (Figure 7).

3.2 Effect of P4 on uterine development

The effect of P4 on the average uterine diameter was observed between treated groups (T-50, T-26, P4P4P, P4PG) with positive impact on the uterine development of heifers that received iP4 ($P<0.001$; Table 1). Regarding the evolution in the uterine diameter, both the treated and the control groups had increased uterine development. The P4PG, T-50 and C- groups did not differ between themselves at the end of the evaluations, while the P4P4 and T-26 groups were similar to each other and different from the other groups, showing the best averages of uterine development.

3.3 Effect of treatment on ovarian activity, estrus rate and follicular diameter

Regarding the cyclicity in the D0, the C- group had the lowest average (33.9%), followed by P4PG group (38.7%) and T-26 (41.2%). The P4P4 and T-50 groups had the best CL rates in the D0 (62.7%; 57.4%). The estrus rate in AI, heifers from T-26 and P4P4 groups showed similar means (84.0% vs 83.0%). The T-50 group was like the P4PG group (72.1% vs 64.0%), while the C- group presented a lower average (53.6%) in relation to the treatment groups (Table 2). In relation to the size of the follicle in AI, there was no significant difference between control and treatment groups ($P=1.00$; Table 2)

3.4 Effect of treatments on pregnancy at 30 and 60 days and pregnancy loss

About the pregnancy rate at 30 days, the T-50 group (55%) had a mean equal to the P4P4 group (68%). The T-26 (49%) and P4PG (48%) groups were like each other and equal to the C- group (50%). In the pregnancy rate at 60 days, the similarity between the groups was equal to the pregnancy at 30 days, exception the P4PG group, which, besides to being like the T-26 and C- groups, was like the T-50 and P4P4 groups. Pregnancy loss was the lowest in the T-50 (11.7%) and P4P4 (12.5%) groups compared to the T-26 (24.0%), P4PG (20.0%) and C- (28.6%) groups, which presented the highest means of pregnancy loss (Table 3).

3.5 Effects of uterine diameter on ciclicity, pregnancy and loss rates

Heifers that had a uterine diameter greater than 16mm, the estrus rate was above 75% compared to those that had a diameter smaller than 16mm (65%). Despite the pregnancy rate at 30 days showing no difference between uterine diameter (P=0.12), the pregnancy rate at 60 days P=0.09) and pregnancy loss (P=0.04) showed better averages. As well as heifers that had a uterine diameter greater than 20mm, had better cyclicity, pregnancy and loss rates, when compared to heifers that had a uterus smaller than 20mm (Table 4).

3.6 Progesterone concentration and ovulations

The average concentration of P4 measured between days D-50 and D-17 in heifers from experiment II are described in figure 4. The T-26 group maintained the P4 concentration below 1.5ng/ml until the first iP4 treatment (D-26), which coincided with the development of a CL, influencing the P4 concentration. T-50 and P4PG groups showed P4 concentrations greater than 1.5ng/ml during the evaluations, justifying the curves due to the presence of follicles followed by ovulation and the presence of CL. The discrepant measurements presented between days D-23 and D-17 occurred due to double ovulation in a heifer in the T-50 group and in the P4PG group, influencing the average of P4. The P4P4 group made the same curve as the T-50 group, but in a regular way. After the increase in the concentration of P4, there is a reduction influenced by the presence of a follicle, keeping the concentration above 1.5ng/ml. Then, a new regression occurs around six days after the second application, coinciding again with the presence of a follicle, which ovulates, under the influence of the CL there is a gradual increase in the concentration of P4.

4 Statistical analysis

All statistical analyses were carried out using SAS (Sas on Demand for Academics, 2023 SAS Institute Inc., Cary, NC, USA) considering heifer as the experimental unit. In the experiment 1 arrangement of 6 treatments in a completely randomized design. All variables were submitted to the normality test. Model included fixed effects of treatments, pubertal status and interaction. The variable farm and bull was also included as a random effect in the model. The binomial dependent variables were analyzed by logistic regression using the GLIMMIX procedure with the models fitted to binomial distributions. For estrus, P/ AI, P d60 and pregnancy loss, secondary analyzes were generated including the fixed effect of each of the following variables individually: (a) estrus expression at FTAI, (b) uterine horn diameter (D-50, D-26 and D0), (c) Follicle diameter at FTAI. Analyzes were also run including the variable total antral follicle count and RTS on D0 as a covariate and interactions. For the induction response analysis using GLIMMIX procedure, an additional model was used considering the uterine horn diameter and RTS evolution from D-50 to D0. Results containing continuous and discrete variables were reported as LSMEANS ± S.E.M and binomial variables were reported as arithmetic means. For the comparison of means, the DIFF command incorporating the Tukey test correction was used. A probability of P 0.05 indicated a significant difference.

5 Discussion

This study demonstrated for the first time in *Bos taurus* heifers the use of different protocols for puberty induction using a protocol based on an iP4, on uterine evolution, pregnancy rate and pregnancy loss in beef heifers. Double dose of iP4, 25 days apart increases uterine diameter and pregnancy in prepubertal heifers like heifers that were pubescent fifty days before the breeding season.

Puberty usually occurs when heifers reach goof BSC and weight. The recommendation is heifers reach 50 to 57% of their expected adult weight at the beginning of the breeding season [1]. Despite racial differences that directly interfere with body development, nutrition is an important key point during the growth and development phase for the heifer to reach early puberty and demonstrate its performance [2]. In this work, the BCS and weight did not make a significant difference between the groups but showed a positive evolution during the evaluation (Figure 5, 6). The T-26 group showed the greatest BCS development and P4P4 showed the best weight gain when compared to the other groups. It can be linked to the literature, which brings us data that the higher the BCS in heifers, the better pregnancy rate of these animals [3].

In this work, when we evaluated the uterine development (Table 1) and RTS (Figure 7) of heifers that were treated with iP4, all groups had an increase showing that iP4 promoted a positive influence on the uterine development, probably due to the increase in the concentration of estrogen, responsible for causing changes in the uterine tissues [4,5,6,7]. Associated with this, we identify that *Bos taurus* heifers that had uterine diameter >20mm had higher estrus rate, conception rate at 60 days and less pregnancy loss when compared to heifers that had a diameter <16mm (Table 4). The greater uterine diameter, the greater cyclicity rate ate the beginning of the FTAI protocol, resulting in a higher pregnancy rate and lower pregnancy loss (Figure 5). Thus, it is important that pubescent heifers have stiff and mature uteruses, well developed to provide a favorable environment. We see that the evaluation of the reproductive system in prepubertal heifers before the breeding season allows identify the infantilism, predict pregnancy rates, and select the most fertile heifers [8]. In this work, we identified that the selection of heifers pre breeding season that have uterine diameter with >20mm is a key point for the good performance of the reproductive tract, the larger uterine diameter, the greater the positive response about puberty.

When we evaluate the P4 concentration curve in Figure 4, we identified that all groups reacted after treatment, maintain P4 above 1.5ng/ml for six days. These data corroborate those obtained by Simões et al., 2018 [9], [10], [11] where is identified that heifers *Bos indicus* maintain a plasma concentration of P4 above 1.5ng/ml for 5-7 days after the treatment with iP4. This reduction on the sixth day culminates in the presence of largest follicle (LF), which had its development accelerated and reached ovulation early. With this, identified that the first CL formed after treatment suffers premature regression due to the influence of LF. Like [12] identified CL in prepubertal heifers with less pronounced vascular characteristics and smaller diameter than active CL. The action of P4 in heifers before puberty has a positive influence on preovulatory follicles and CL function [13,14]. Influencing greater secretion of FSH and LH leading to greater development of the dominant follicle. As well as it can prolong the persistence of the CL, preventing premature PG secretion, consequently promoting a longer shelf life and hormone production, obtaining good pregnancy rates [11, 13, 15].

When we evaluated the cyclicity rate at the beginning of the FTAI protocol, we verified that the control group (C-) had a lower rate when compared to the treatment groups. The groups that received iP4 had the highest cyclicity rates on D0. These data can explain the capacity of iP4 to promote adequate performance like the physiological cycle. Likewise, P4 was able to promote more than one ovulation between treatments and the beginning of the FTAI protocol (P=0.001). Except for the T-26 group, which presented only one ovulation, justified by the shorter time of exposure to P4 and the beginning of FTAI. As well, P4 was able to reduce the anestrus return rate after treatment. The group that received two doses of P4 had a lower rate of return to anestrus, showing that the good reproductive performance of prepubertal heifers.

The diameter follicular did not show difference between the treated and control groups, even as it did not show positive relation with the estrus rate. The high concentrations of P4 during follicular growth can promote a shorter life of the LF and an early peak of FSH, hastening the next wave, thus not allowing the follicle to reach the maximum diameter, as well as low concentrations of P4 promote the opposite effect**.** Despite this, follicular capacity remained normal, because P4 has no direct influence on FSH action. Where P4 suppresses LF only in the growth phase, not influencing the static or regressive phase. Some reports demonstrate [16, 17,18] that the short cycle CL were functionally like the normal cycle, but with less P4 secretion due to its smaller size, it is justifying the indifference in the follicular diameter in the present work.

Like progesterone, prostaglandin (PG) also influences the induction of cyclicity in TAI programs and puberty induction in heifers. Studies report that PG increases the pituitary response to GnRH, which leads to an increase in LH culminating in ovulation and seems to play a local role in the ovary, being produced by granulosa cells and acting directly on the preovulatory follicle, while iP4 being responsible for the reduction of estradiol receptors in the hypothalamus [13,18,19]. In puberty induction protocols, PG acts only in the prepubertal period, that is, without the presence of CL [20]. In this work, the P4PG group had a lower RTS, pregnancy and estrus rates were lower than the other groups. The high embryonic loss and CL index at D30 in the P4PG group, cannot be fully explained in this study, so a new assay must be developed to verify the administration of PGF after an induction with iP4. Our hypothesis was partially refuted in this group to the point that the administration of PG at D-26 would induce new ovulations but not increase pregnancy rates.

Despite the use of iP4 having influenced the performance of the reproductive tract, the pregnancy rates showed significant difference between C- and the treatment groups. The highest index in pregnant at 30 days and 60 days was in P4P4 group. Combined with this, P4 alone in the cyclicity protocol was insufficient to obtain a fertility rate like pubescent heifers, requiring pre-puberty stimulation, being able to keep the pregnancy [2]. These data confirm that the use of two doses of iP4, 50 days before the FTAI protocol improves the cyclicity rate and influences the pregnancy rate in prepubertal *Bos taurus* heifers in weight gain during fifty days pre-breeding season, allowing AI to occur from the second estrus.

Therefore, starting the protocol of cyclicity fifty days before FTAI is a more accurate moment for a good return on the reproductive performance of heifer, being a practical and effective strategy to accelerate puberty. Suggesting that, the treatment with iP4 influenced the maturation of the reproductive tract, improving the uterine score, quality of cyclicity, estrus and preovulatory follicle development and CL function. The occurrence of natural cyclicity fifty days before artificial insemination still provides better pregnancy rate and low pregnancy loss. The best conception rate and the lowest embryonic loss is related to the uterine diameter, and those heifers with greater responsiveness to the puberty induction protocol were those with uterine diameter >16.0mm.

With this, we conclude that the strategy using the long-acting injectable P4 in prepubertal heifers, brought good rates when used in two doses with an interval of 50 days before breeding season when heifers are not in puberal condition. As well as the selection of heifers with uterine diameter >20mm have better rates of cyclicity, pregnancy and less embryonic loss. Being an alternative to improve the fertility of pre-breeding season heifers, with low cost and efficient management and promoting similar results to heifers that cycle naturally.

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Credit authorship contribution statement

Caroline Fernandes Possebon, Fabio Gallas Leivas, Gilson Antônio Pessoa: Conceptualization, Methodology, Resources, Writing e original draft, Writing e review & editing, Review & editing, Supervision.

Caroline Fernandes Possebon, Gilson Antônio Pessoa, Ana Paula Martini, Camila Rohde Brondani: Conceptualization, Methodology, Resources, Formal analysis, Visualization.

Caroline Fernandes Possebon, Fabio Gallas Leivas, Daniela Brum: Formal analysis, Investigation, Methodology, Validation.

Declaration of competing interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

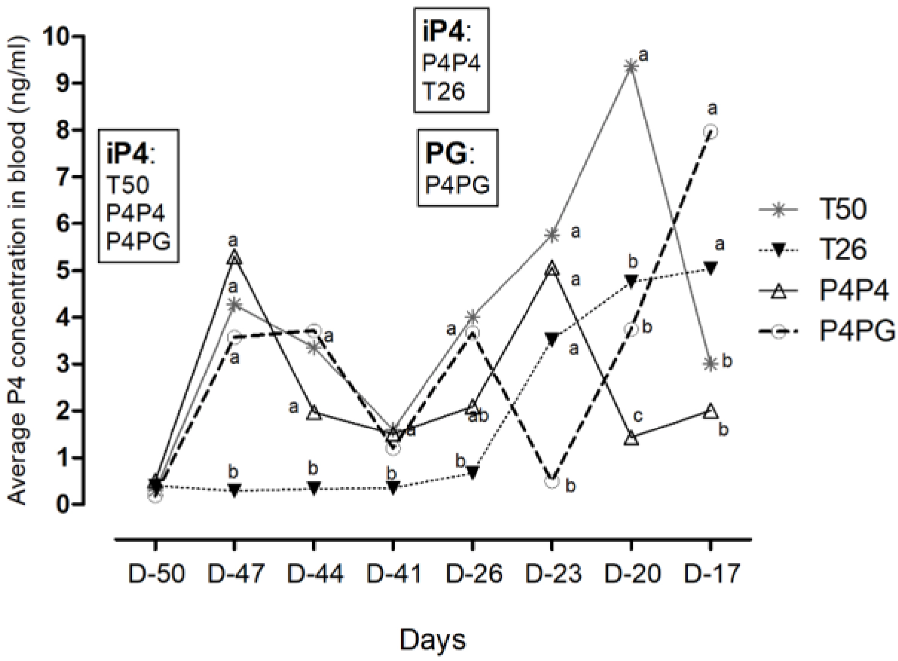

Figure 5. Average P4 concentration in blood (ng/ml) after treatment. Different letters indicate statistically differences (P<0.05) at same day of collection levels after treatment.

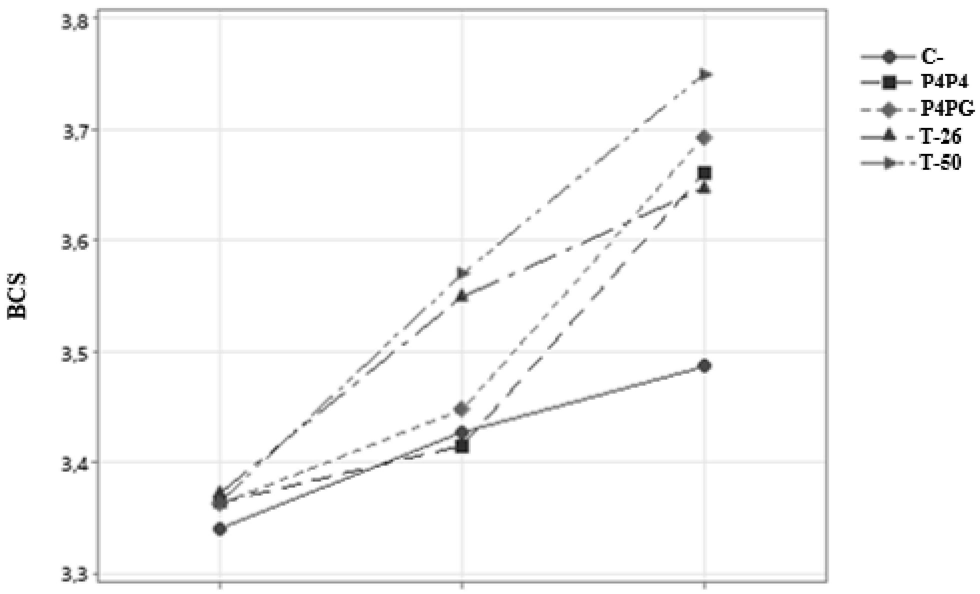

Figure 6. Demonstration of the average evolution of BCS of heifers in the control (C-) and treatments (T-50, T-26, P4P4P, P4PG) groups (P=0.0001) during the evaluations (D-50, D-26 and D0)

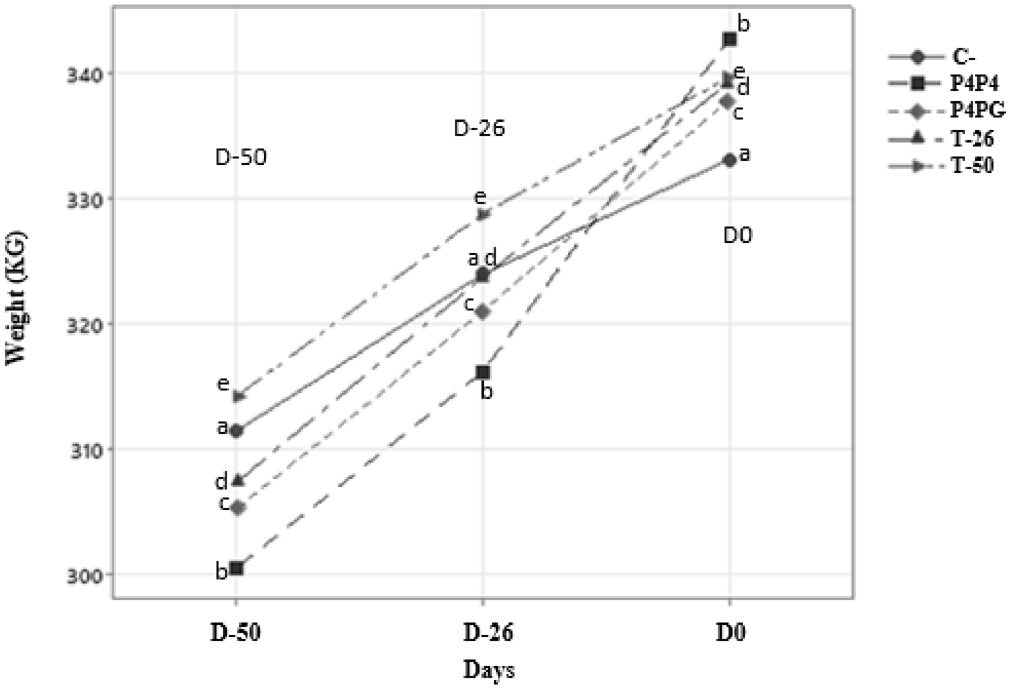

Figure 7. Demonstration of the average of weight of heifers in the control (C-) and treatments (T-50, T-26, P4P4P, P4PG) groups (P=0.0001) during the evaluations (D-50, D-26 and D0).

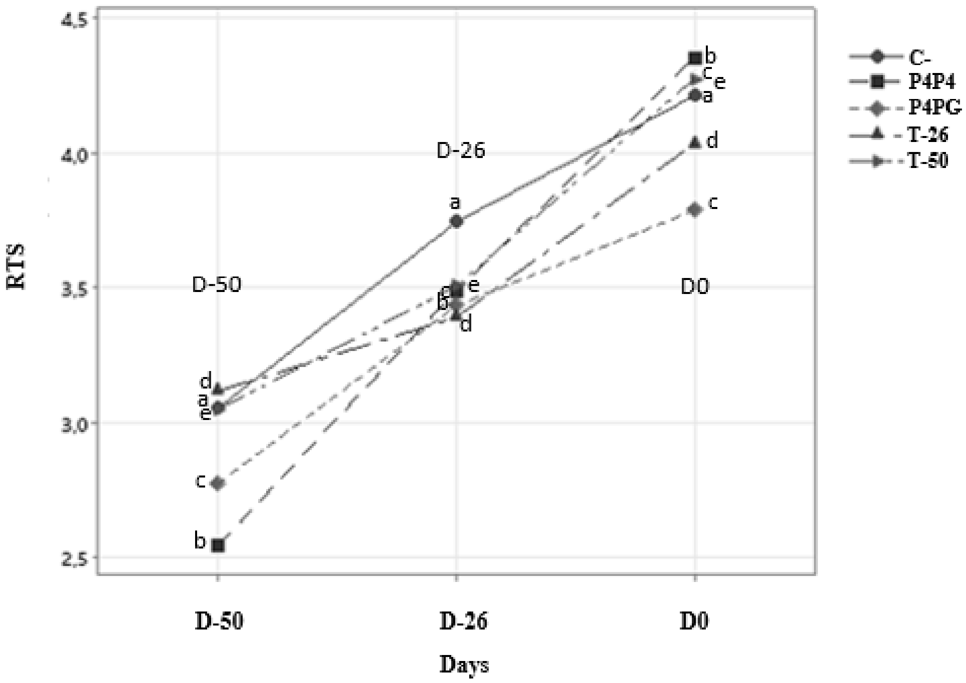

Figure 8. Demonstration of the average evolution of RTS of heifers in the control (C-) and treatments (T-50, T-26, P4P4P, P4PG) groups (P=0.05) during the evaluations (D-50, D-26 and D0)

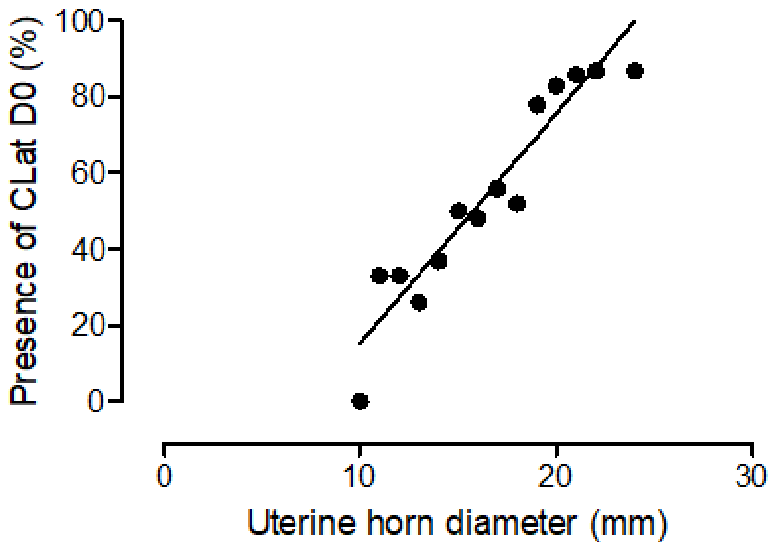

Figure 9. Relationship between the uterine diameter of prepubertal heifers and the presence of CL (cyclic heifers) after puberty induction treatments, at the beginning of the FTAI protocol (D0)

Table 1. Ultrasonography evaluation in three times of uterine diameter (mm) of Bos taurus heifers in six groups during fifty days.

Item	*N*	*D-50*	*D-26*	*D0*	*p-value*
C-	56	13,3 ±0,33 C^{b}	14,21±0,28 B^{bc}	15,72±0,31 A^{c}	0.001
T-50	61	11,997±0,254 C^{bc}	14,774±0,255 B^{b}	15,6± 0,344 A^{c}	0,001
T-26	51	12,69±0,48 C^{b}	15,09±0,37 B^{b}	16,63±0,32 A^{b}	0,003
P4PG	62	12,113±0,267 C^{b}	14,889±0,347 B^{b}	15,494±0,347 A^{c}	0,02
P4P4	59	12,051±0,343 C^{b}	13,927±0,357 B^{c}	16,429±0,349 A^{b}	0,02
Overall	289	12,83±0,15	14,96±0,15	16,2±0,16	0.0001

D-50 induction with P4 of the T-50, P4P4 and P4PG groups. D-26 induction with P4 of the T-26 and P4P4 groups; induction with PG of the P4PG group and D0 start of FTAI protocol. Means that do not share a letter are significantly different: letters A, B, C in the same row refer to the difference in rates among evaluations D-50, D-26 and D0; letters a,b,c in the same column refer to the difference in rates among treatments C+,C-, T-50, T-26, P4PG and P4P4

Table 2. Effect of treatments on uterus diameter and ovarian activity.

Item	*Treatments*					
	C-	T-50	T-26	P4P4	P4PG	*P-values*
CL D0 (%)	33.9%[c] (19/56)	57.4%[ab] (35/61)	41.2[b] (21/51)	62.7 [a] (37/59)	38.7 [bc] (24/62)	*0.005*
Uterine diameter (mm)	15.2±0.3[b]	15.8±0.4[ab]	16.7±0.3[a]	16.6±0.4[a]	15.7±0.4[ab]	*0.05*
Estrus/AI (%)	53.6[c] (30/56)	72.1[ab] (44/61)	84.0[a] (43/51)	83.0[a] (49/59)	64.0[b] (40/62)	*0.004*
LF/AI (mm)	12.8±2.7	11.7±2.6	12.7±2.4	12.69±2.1	12.5±2.4	*1.00*
CL 30d non-pregnant (%)	57.1[b] (16/28)	69.2[a] (18/27)	42.3[c] (11/26)	52.6[b] (10/19)	65.6[a] (21/32)	*0.03*

Abbreviation: AI – artificial insemination; LF/AI – diameter of the largest follicle on AI; CL 30d - corpus luteum in the non-pregnant heifers in diagnosis of pregnancy at 30 days. Different letters represent significant difference between treatment and control groups (a,b,c) on the line.

Table 3. Effect of treatments on pregnancy at 30, 60 days and pregnancy loss

Item	***Treatments***					
	C-	**T-50**	**T-26**	**P4P4**	**P4PG**	***P-values***
	Pregnancy responses					
P 30d (%)	50.0%[b] (28/56)	55.7%[a] (34/61)	49.0%[b] (25/51)	67.8%[a] (40/59)	48.0%[b] (30/62)	*0.02*
P 60d (%)	35.7%[b] (20/56)	49.2%[ab] (30/61)	37.3%[b] (19/51)	59.3%[a] (35/59)	38.7%[b] (24/62)	*0.04*
PL (%)	28.6% (8/28)	11.7% (4/34)	24% (6/25)	12.% (5/40)	20% (6/30)	*0.43*

Abbreviation: P 30d - pregnant at 30 days; P 60d – pregnant at 60 days; PL – pregnancy loss. Different letters represent significant difference between treatment and control groups (a,b,c) on the line.

Table 4. Effect of evaluated variables in influence of the uterine diameter

Item	<16mm	16-20mm	*>20mm*	*P-values*
Average UD	13.7 ± 0.10[c]	17.6 ± 0.11 [b]	22.0 ± 0.22 [a]	*0.0001*
Estrus/AI rates (%)	65.6%[c] (103/157)	77.0%[b] (77/100)	81.2%[a] (26/32)	*0.0001*
Pregnancy 30d rates (%)	49.7 % (78/157)	57.0 % (57/100)	68.7% (22/32)	*0.12*
Pregnancy 60d rates (%)	38.2%[b] (60/157)	46.0%[b] (46/100)	68.7%[a] (22/32)	*0.006*
PL (%)	23.0% [a] (18/78)	19.3%[b] (11/57)	0.0%[c] (0/00)	*0.04*

Average UD: average uterine diameter; CL rates D0: presence of corpus luteum on D0; Estrus/AI rates: presence estrus in artificial insemination; Pregnancy 30d rates: pregnancy rate at 30 days after AI; Pregnancy 60d rates: pregnancy rate at 60 days after AI; PL: Pregnancy Loss rates. Different letters represent significant difference between treatment and control groups (A.B) on the line.

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5 CONCLUSÃO

- A P4i quando utilizada 50 e/ou 25 dias antes de iniciar protocolo de IATF em novilhas pré-púberes, é capaz de melhorar o desenvolvimento uterino de novilhas taurinas, incrementando as taxas de ciclicidade e prenhez e estimulando o desenvolvimento uterino;

- A seleção de novilhas com diâmetro uterino >20mm resulta em melhores índices reprodutivos, aumentando a taxa de ciclicidade no início dos protocolos de IATF, taxa de prenhez aos 30 e 60 dias e reduzindo a perda gestacional.

6 PERSPECTIVAS

Com base nos resultados obtidos nesse trabalho, a perspectiva é buscar identificar o perfil genético para precocidade e diâmetro uterino, definindo quais são as características importantes na seleção de novilhas pré púberes que influenciam na maturidade reprodutiva. Mensurar os níveis de produção de LH em novilhas precoces e a expressão de genes de progesterona sobre o útero das novilhas precoces e tardias, identificando a atuação na maturidade uterina para alcançar resultados satisfatórios de concepção e manutenção da prenhez.

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