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Tese

Efeito da progesterona e $PGF_2\alpha$ na indução da puberdade e fertilidade em fêmeas bovinas de corte

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Resumo

Pfeifer, Luiz Francisco Machado. **Efeito da progesterona e PGF2α na indução da puberdade e fertilidade em fêmeas bovinas de corte.** 2008. 80f. Tese (Doutorado) – Programa de Pós-Graduação em Zootecnia. Universidade Federal de Pelotas, Pelotas.

Os objetivos destes experimentos foram determinar os efeitos do tratamento com progesterona exógena e $PGF_2α$ no desenvolvimento folicular e taxas de ovulação e prenhez em fêmeas de corte. No Experimento 1, vacas de corte lactantes (n=129) e novilhas púberes (n=150) em estágio aleatório do ciclo estral receberam duas injeções de 500 µg de cloprostenol (PGF) em 11 dias de intervalo. Dez dias após a segunda PGF (Dia 0), as fêmeas receberam benzoato de estradiol (1.5 mg para vacas e 1 mg para novilhas) e um dispositivo liberador de progesterona (Cue-Mate; meia-dose). Além disso, o Grupo baixa progesterona (Grupo BP; n=148) recebeu outra injeção de PGF no momento da inserção do Cue-Mate, enquanto que o Grupo alta progesterona (Grupo AP; n=131) não recebeu nenhum tratamento. No Dia 8, os Cue-Mates foram removidos e a PGF foi administrada novamente em todas as fêmeas. Após 54-56 h, todas as fêmeas receberam 12.5 mg de LHp e foram submetidas a IATF. A taxa de prenhez foi comparada entre os grupos por análise de regressão logística. A taxa de prenhez não foi diferente entre os grupos (52.7%, 69/131 e 53.4%, 79/148 para os grupos AP e BP, respectivamente). O Experimento 2 foi realizado em duas etapas, Experimento 1 e Experimento 2. No Experimento 1, novilhas mestiças hereford pré-puberes (n=38) foram separadas aleatoriamente em três grupos e receberam um dispositivo liberador de progesterona, CIDR, (Grupo P, n=13), progesterona e PGF (Grupo PPG, n=11) ou nenhum tratamento (Grupo controle, n=14) em estágios aleatórios do ciclo estral. Logo após a inserção do CIDR os ovários foram monitorados diariamente por ultra-som, sendo que 5 dias após a detecção da emergência folicular, os CIDRs foram removidos e o Grupo PPG recebeu 500 µg de cloprostenol, im. A taxa de ovulação foi comparada entre os grupos pelo teste do qui-quadrado. A taxa de ovulação foi maior nas novilhas do Grupo PPG (72.7%, 8/11), intermediária no Grupo P (30.8%, 4/13), e menor no Grupo controle (7.1%, 1/14; $P<0.001$). No Experimento 2, novilhas pré-puberes mestiças foram separadas aleatoriamente em dois grupos para determinar o efeito da PGF, com ou sem tratamento prévio com progesterona, na indução da puberdade em novilhas de corte. No Dia 0, as novilhas do Grupo Estradiol-Progesterona (Grupo EP; n=8) receberam 1 mg de benzoato de estradiol, im, e um dispositivo de CIDR, sendo que as novilhas do Grupo aspiração folicular (Grupo AF; n=8) foram submetidas à aspiração folicular de todos os folículos ovarianos >5 mm no Dia 3. No Dia 7, todas as novilhas receberam 500 µg de cloprostenol, im, e o CIDR foi removido do Grupo EP. A taxa de ovulação foi a mesma para ambos os grupos (75%, 6/8). A concentração de progesterona durante o crescimento do folículo ovulatório não teve influencia sobre a taxa de prenhez. Já a exposição à progesterona, particularmente em associação com PGF em novilhas peri-púberes foi associado com um incremento na capacidade ovulatória do folículo dominante.

Palavras-chave: Gado de corte; Desenvolvimento Folicular; Competência ovocitária; Progesterona; Puberdade

Abstract

Pfeifer, Luiz Francisco Machado. **Effect of progesterone and $PGF_2\alpha$ on puberty induction and fertility in beef cattle**. 2008. 80p. PhD thesis – Departament of Animal Science. Universidade Federal de Pelotas, Pelotas, Brazil.

The objectives of these experiments were to determine the effects of exogenous progesterone and $PGF_{2\alpha}$ treatments on follicular development and ovulation and pregnancy rates in cattle. In Experiment 1, suckled beef cows (n=129) and pubertal heifers (n=150) at random stages of the estrous cycle were given cloprostenol (PGF) twice, 11-day apart. Ten days after the second PGF (Day 0), cattle received an estradiol benzoate injection (1.5 mg for cows and 1 mg for heifers) and a Cue-Mate device (half dose). In addition, the Grupo baixa progesterona (LP group; n=148) were given PGF at the time of Cue-Mate insertion, whereas the High progesterone group (HP group; n=131) received no further treatment. On Day 8, Cue-Mates were removed and PGF was given to all cattle. Fifty-four to 56 hr later, all cattle received 12.5 mg im of pLH and were concurrently timed-inseminated (TAI). Pregnancy diagnosis was done by ultrasonography 28 d after TAI. The pregnancy rate data was compared by the logistic regression. Pregnancy rates did not differ between groups (52.7%, 69/131 and 53.4%, 79/148 for HP and LP groups respectively). The Experiment 2 was performed in two steps, Experiment 1 and Experiment 2. In Experiment 1, prepubertal Hereford crossbred heifers (n=38), were assigned randomly to three groups and given progesterone (CIDR) insert (P group, n= 13), progesterone insert plus prostaglandin $F_{2\alpha}$ (PPG group, n= 11), or no treatment (Control group, n= 14) at random stages of the follicular wave. The day of follicular wave emergence after CIDR insertion was recorded and CIDR were removed 5 days later. The PPG group received 500 µg cloprostenol ($PGF_{2\alpha}$ analog), im, on the day of CIDR removal. The incidence of ovulation was compared among groups by chi-square analysis. The proportion of heifers that ovulated was highest in the PPG group (72.7%, 8 of 11), intermediate in the P group (30.8%, 4 of 13), and lowest in the control group (7.1%, 1 of 14; $P<0.001$). In Experiment 2, prepubertal crossbred beef heifers were assigned to two groups to determine the effects of progesterone and PGF on the onset of puberty in beef heifers in which follicle wave emergence had been synchronized. On Day 0, heifers in the EP group (n=8) were given 1 mg of estradiol benzoate im and a CIDR insert, while heifers in the FA group (n=8) had all the follicles >5mm ablated on Day 3. On Day 7, all heifers received $PGF_2\alpha$ and CIDR were removed from the EP group. The ovulation rate did not differ for both treatments, 75% (6/8). In summary, low circulating progesterone concentrations during the growing phase of the ovulatory follicle had any apparent effect on pregnancy rate. The progesterone exposure and withdrawal, particularly in combination with post-progesterone prostaglandin treatment, in near-pubertal heifers was associated with an increased capacity of the dominant follicle to ovulate.

Keywords: Beef cattle, Follicle development, Oocyte competence, Progesterone; Puberty

Sumário

1. INTRODUÇÃO

A progesterona é um hormônio esteróide secretado principalmente por glândulas transitórias (CL e placenta) e tem como função a regulação ovariana, o controle do desenvolvimento folicular e a secreção de hormônios que interferem na reprodução, sendo também de fundamental importância no estabelecimento e manutenção da gestação. Tendo em vista que a progesterona está envolvida em várias etapas do processo reprodutivo, o entendimento do mecanismo de ação e dos efeitos deste hormônio são essenciais para o desenvolvimento e maximização de técnicas reprodutivas que utilizam a progesterona visando o incremento da produtividade de um sistema de produção.

As propriedades biológicas da progesterona tem sido amplamente utilizadas em protocolos de sincronização de estro e ovulação visando aumentar a proporção de fêmeas que concebem logo no início da estação reprodutiva (Tauck et al., 2007), além de antecipar a puberdade em novilhas (Anderson et al., 1996; Imwalle et al., 1998; Lucy et al., 2001; Patterson et al., 1990). Embora existam dados que descrevem os eventos foliculares que precedem a puberdade (Adams et al., 1994; Anderson et al., 1996; Evans et al., 1994), a dinâmica folicular ovariana durante o tratamento com progesterona em novilhas pré-púberes, ainda não foi descrita.

Convencionalmente, a progesterona tem sido utilizada para sincronização de cios em associação com outros hormônios (ex. estradiol, GnRH, eCG e $PGF_2\alpha$), sendo que algumas dessas associações devem ser melhor investigadas, principalmente quando aplicado em novilhas pré-puberes. A PGF, por exemplo, é usualmente utilizada como agente luteolítico, porém possui ação direta na hipófise, aumentando a resposta

hipofisária à secreção de GnRH, podendo ser uma alternativa importante na indução da ovulação quando associado à progesterona (Weems et al., 2006). Por sua vez a progesterona atua como responsável pelo feed-back negativo regulador da secreção de LH na fêmea adulta (Roberson et al., 1989), porém em novihas alguns estudos sugerem que a progesterona pode ser ineficiente para inibir a secreção de LH (Kojima et al., 1995; Kojima et al., 1992). A concentração de progesterona determina o nível de inibição do LH, afetando indiretamente o crescimento da onda follicular (Adams et al., 1992) e a competência ovocitária, como pôde ser observado em prévios estudos (Pfeifer et al., 2005).

Vários experimentos têm sido conduzidos com o objetivo de determinar como a variação da concentração e da duração do tratamento de progesterona afeta a fertilidade em fêmeas bovinas (Austin et al., 1999; Roche, 1974; Shaham-Albalancy et al., 2000). Baixas concentrações de progesterona e, consequentemente, alta frequência de LH (Rahe et al., 1980) durante o desenvolvimento do folículo ovulatório foram associadas com redução da fertilidade devido a indução de folículos dominantes persistentes (Shaham-Albalancy et al., 2000). Como o LH é responsável pela maturação ovocitária (retomada da meiose), o ambiente folicular com alta secreção pulsátil de LH, pode ocasionar maturação ovocitária precoce afetando negativamente a competência e qualidade do ovócito (Mihm et al., 1999; Revah and Butler, 1996). Em contraste, baixas concentrações de progesterona promovem um aumento do crescimento do folículo dominante (Adams et al., 1992), melhorando a função do CL subsequente (Perry et al., 2007) devido ao aumento da produção de progesterona no

período pós-ovulação podendo melhorar a fertilidade, desde que a ovulação de um ovócito competente seja atingida.

Estudos que utlizaram a OPU (ovum pick-up) para recuperar ovócitos que se desenvolveram sob diferentes ambientes foliculares indicam que a qualidade ovocitária é afetada pela concentração de progesterona, assim como pela fase do ciclo estral em que são coletados (Hagemann et al., 1999; Hendriksen et al., 2004; Salamone et al., 1999). A progesterona parece exercer um efeito positivo na competência ovocitária (Blondin and Sirard, 1995; Leibfried-Rutledge et al., 1987), pois ovócitos coletados no diestro apresentam maior competência que ovócitos coletados na fase folicular (Machatkova et al., 1996; Machatkova et al., 2004).

Além disso, aparentemente, a concentração de progesterona no início dos tratamentos de superovulação afeta os resultados de programas de transferência de embriões (Bo et al., 2001; Macmillan et al., 1994; Nasser et al., 2002). Um maior número de embriões de boa qualidade foram obtidos quando o tratamento de superovulação em vacas doadoras foi iniciado no diestro (alta concentração de progesterona) do que quando foram iniciados na primeira onda folicular, onde as concentrações de progesterona são baixas. Há uma estreita associação entre a concentração de progesterona na mãe e adequado desenvolvimento embrionário (para revisão Mann e Lamming, 1999). Vacas com adequadas concentrações de progesterona no leite (acima de 3 ng/mL), no dia 5 após a ovulação, tem taxa de prenhez em torno de 50-55%, enquanto que vacas com baixa concentração de progesterona no leite (<1 ng/mL) possuem taxa de prenhez em torno de 10%.

Como pôde ser visto a progesterona é um hormônio não só essencial à vida, mas também tem seu uso vinculado a produtividade de fazendas de leite e corte. Devido ao amplo uso deste hormônio e sua respectiva importância, o estudo de como a progesterona age sobre a onda folicular e na fertillidade, bem como sua associação com outros hormônios, ainda é motivo de vários experimentos realizados por diversos grupos de pesquisa dentro e fora do Brasil. Os objetivos gerais dessa tese foram: 1) avaliar o efeito da concentração de progesterona sobre o desenvolvimento folicular e a fertilidade de vacas e novilhas de corte e 2) avaliar a associação da progesterona e PGF sobre o desenvolvimento folicular e a indução da puberdade em novilhas pré-púberes.

2. ARTIGO 1

Effects of low versus high plasma progesterone concentrations on follicle development and fertility in beef cattle

Effects of low versus high plasma progesterone concentrations on follicle development and fertility in beef cattle

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Abstract

The objective of this study was to determine the effect of low vs high plasma progesterone concentration during the ovulatory wave on fertility in cattle. Suckled beef cows (n=129) and pubertal heifers (n=150) at random stages of the estrous cycle were given a luteolytic dose of prostaglandin F2α (PGF) twice, 11 d apart. Ten days after the second PGF treatment, cattle received estradiol benzoate (1.5 mg for cows and 1 mg for heifers, im) and a progesterone-releasing intravaginal device (Cue-Mate) with a single progesterone-releasing pod (Day 0). The low-progesterone group (n=148) received a luteolytic dose of PGF on Day 0, whereas the high-progesterone group (n=131) cattle were allowed to retain their CL during selection and growth of ovulatory follicle. On Day 8, the Cue-Mate was removed and PGF was given to both groups. Fifty four to 56 hr later, cattle received 12.5 mg pLH im and were concurrently artificially inseminated. The dominant follicle in the low-progesterone group was larger ($P<0.001$) than in the high-progesterone group on the day of insemination (14.9±0.3 vs 12.7±0.3 mm). At 7 d after ovulation, the low-progesterone group had a larger CL (24.5±0.54 vs 21.9±0.64 mm; $P<0.01$), and higher plasma progesterone concentration (4.0±0.3 vs 3.1±0.2) than the high-progesterone group. Pregnancy rates did not differ between groups (52.7%, 69/131 and 53.4%, 79/148 for high- and low-progesterone groups, respectively) and between heifers and cows (52.7% vs 53.5%, respectively). In summary, low circulating progesterone concentrations during the growing phase of the ovulatory follicle resulted in a larger dominant follicle, larger CL that produced more progesterone. We inferred that the mechanisms which regulate oocyte quality and the establishment of pregnancy were not entirely dependent on progesterone concentrations. Finally, in the absence of prolonged follicle dominance, the low progesterone environment did not affect fertility.

Key-words: cattle, corpus luteum, fertility, follicle development, ovary, progesterone, synchronization of ovulation.

1. Introduction

The control and manipulation of the estrous cycle in cattle is an important management tool to improve reproductive performance (17). Several methods have been used to synchronize estrus in cattle (10,18,28,29), including progestin-releasing devices. Progestin treatment for less than 9 days, has been shown to be effective in increasing the proportion of cattle that become pregnant early in the breeding season (48).

Ovarian follicular and oocyte development, and gonadotropin release may vary according to the duration of progesterone treatment (3,21,45). Sanchez et al. (41) reported that one implant containing 3 mg of norgestomet in mature heifers without a CL resulted in an LH pulse frequency typical of the follicular phase (approximately 1 pulse/h). However, two or four norgestomet implants suppressed the LH pulse frequency to that of the luteal phase (2), supporting the contention that the blood progesterone concentrations determine blood LH concentrations and pulsatility, and in turn, growth of the ovulatory follicle (1).

Circulating progesterone concentration is known to affect follicular development (1) and oocyte quality (6,19,32), and several studies have been done to determine how variations in progesterone concentrations and duration of treatment affect fertility after a synchronized breeding (3,38,45). Low progesterone concentrations, and consequently high LH pulse-frequency (34), during the ovulatory wave were associated with decreased fertility attributed to prolonged dominance of the ovulatory follicle (45), possibly through premature oocyte maturation (27,36). However, low progesterone concentrations promoted an overgrowth of the dominant follicle (1), which may improve CL function (31) and fertility, as long as ovulation of a competent oocyte was achieved.

The objective of the present experiment was to elucidate the effects of progesterone concentrations on follicular wave dynamics, hormonal profiles and fertility in suckled cows and

pubertal heifers. We tested the hypothesis that low plasma progesterone concentration during selection and growth of the dominant follicle will increase ovulatory follicle size, increase subsequent CL diameter and function, and improve pregnancy rate compared to physiological plasma progesterone concentrations during the luteal phase.

2. Materials and methods

2.1. Animal treatments and ultrasound examinations

Crossbred beef cows (n=129) and heifers (n=150) were used. The cows (Hereford and Hereford x Charolais) were 3 to 14 y of age, 60 to 75 d postpartum (suckled), weighed 450 to 650 kg, and were maintained at the University of Saskatchewan Goodale Research Farm. The heifers were 14 to 16 mo of age, 310 to 430 kg body weight, and were maintained at two locations. A group of Hereford and Hereford x Charolais heifers (n=48) were maintained at the Goodale Research Farm near Saskatoon, SK Canada (Location 1), and a group of Angus heifers (n=102) were maintained at the Agriculture and Agri-Food Canada Research Centre, Brandon, MB Canada, (Location 2). The animals were maintained on pasture, with ad libitum access to water, salt, and a mineral mixture.

Before the start of the experiment, ovarian function was assessed twice (10 d apart) by transrectal ultrasonography (Aloka SSD-900 with 7.5 MHz linear-array transducer; Aloka, Tokyo, Japan); only cows and heifers in which a CL was detected at one or both examinations were included. The experimental design and treatment schedule is shown in Figure 1. Cattle received 500 µg cloprostenol im (PGF; Estrumate, Schering-Plough Animal Health, Pointe-Claire, QC, Canada) twice, 11 d apart. Cattle were randomly allocated by age, location, parity, days postpartum (DPP, cows only), body condition score (BCS, 1 = emaciated and 9 = obese) (51) and body weight into

two groups: High progesterone group (HP, n=131; 70 heifers and 61 cows) and Low progesterone group (LP, n=148; 80 heifers and 68 cows). Ten days after the second PGF, corresponding to approximately 5 to 8 d after ovulation, estradiol benzoate was given im (treatment Day 0) and an intravaginal progesterone-releasing device was inserted (Cue-Mate, Bioniche Animal Health, Belleville, ON, Canada). Estradiol benzoate (Sigma Chemical Co., St. Louis, MO, USA) dissolved in canola oil was used at a dose of 1.5 mg (cows) or 1.0 mg (heifers). Cue-Mate devices were equipped with one progesterone-releasing pod (0.78 g progesterone) and a second blank pod. We expected to achieve a plasma progesterone concentration of less than 1ng/mL after 2 days of insertion of these devices in cattle without a function CL (39). Cattle in the LP group were given 500 μg cloprostenol im on Day 0 to achieve low plasma progesterone concentrations. In the HP group, cattle were allowed to retain their CL (i.e., no PGF treatment on Day 0) to maintain luteal-phase plasma progesterone concentrations. On Day 8, the Cue-Mate was removed and both groups were given 500 μg cloprostenol im. Fixed-time artificial insemination was done 54 to 56 h later, and cattle were concurrently given 12.5 mg pLH im (Lutropin-V, Bioniche Animal Health). Cattle detected in standing estrus between 0 and 36 h after Cue-Mate removal were inseminated 12 h after first detection of estrus and given 12.5 mg pLH concurrently.

The ovaries were examined by transrectal ultrasonography on Day 8 (Cue-Mate removal) and Day 10 (just before insemination) to determine the diameter of the dominant follicle. Transrectal ultrasonography was also done on Day 17 to determine the diameter of the CL, and on Day 38 to determine pregnancy. The diameter of the CL on Day 17 in heifers was measured only at Location 1.

129 2.2. Blood sampling

130 Blood samples were collected by caudal venipuncture into heparinized 10 mL tubes (Becton
131 Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) that were immediately centrifuged
132 (1500 x g for 15 min); plasma was harvested and stored at -20^0 C. Samples collected on Days 0,
133 4, and 8 were used to determine plasma LH concentration, and samples collected on Days 4, 8,
134 and 17 to determine plasma progesterone concentration.

135

136 2.3. Daily ultrasound evaluations and intensive blood sampling

137 On Day 0, a subset of six cows and six heifers per group was selected randomly to monitor
138 ovarian follicular development and to measure plasma concentrations of gonadotropins and
139 steroids. All ultrasonographic examinations were done by one operator, once daily from Day 0 to
140 the day of ovulation, or to Day 12 (in the absence of ovulation). At each examination, a sketch of
141 each ovary was made, and the diameter and location of follicles ≥3 mm in diameter were
142 recorded (11). Ovulation was defined as the disappearance (from one examination to the next) of
143 a previously identified follicle ≥8 mm in diameter (24). The day of wave emergence was defined
144 retrospectively as the day when the dominant follicle was first detected at a diameter of 4 to 5
145 mm (11). Blood samples were collected, as described, once daily from Day 0 to ovulation (or to
146 Day 12) to measure plasma concentrations of LH, progesterone, estradiol, and FSH. In addition,
147 blood samples were collected every 15 min for 8 h on Day 4 (i.e., expected day of wave
148 emergence) to assess the pattern of LH release. Immediately before the frequent sampling period,
149 each animal was fitted with an indwelling catheter (Angiocath, 16 gauge, 8.26 cm; Becton
150 Dickinson Vascular Access, Sandy, UT, USA), held in place with Kamar adhesive glue (Kamar
151 Products, Inc., Steamboat Springs, CO, USA), Vetwrap Bandaging Tape (3M, Animal Care

Products, St. Paul, MN, USA) and 2-inch elastic medical tape. The catheter was filled with heparinized saline (0.9% sodium chloride with 0.1% heparin), and the end of the catheter was sealed with a cap. Before sample collection, the heparinised saline solution was removed and discarded, and following collection, catheters were flushed with fresh heparinized saline to prevent blood clot formation.

2.4. Hormone assays

Plasma LH concentrations were determined by double-antibody radioimmunoassay (12), and expressed in terms of NIDDK-bLH-4. The minimum detection limit was 0.06 ng/mL, with a standard curve ranging from 0.06 to 8.0 ng/mL. Intra-assay coefficients of variation were 8.7% and 7.1% for low- (0.90 ng/mL) and high- (2.03 ng/mL) LH reference samples, whereas inter-assay coefficients of variation were 8.5 and 9.2% for low- and high- LH, respectively.

Plasma progesterone concentrations were evaluated with a solid-phase radioimmunoassay (Coat-a-count, Diagnostic Products Corporation, Los Angeles, CA, USA), with a minimum detection limit of 0.1 ng/mL. Progesterone was extracted with 3 mL hexane from 200 µL aliquots of plasma. The intra-assay coefficients of variation were 12.3% (0.24 ng/mL) and 8.2% (0.93 ng/mL), whereas the inter-assay coefficients of variation were 15.0% and 14.0% for low and high progesterone concentrations, respectively.

Plasma concentrations of estradiol were measured with a modified human double-antibody RIA Kit (Coat-a-count, Diagnostic Products Corporation; (33), using the procedure described by Singh et al.(46). The minimum detection limit was 0.5 pg/mL and the intra-assay coefficients of variation were 10.8% (23.3 pg/mL) and 5.3% (85.4 pg/mL) for low and high estradiol, respectively.

Plasma FSH concentrations were determined with a liquid-phase antibody radioimmunoassay (35). The first antibody used was NIDDK anti-oFSH-1, and FSH concentrations were expressed in terms of USDA-bFSH-1. The range of the standard curve was 0.13 to 16.0 ng/mL, the minimum detection limit was 0.13 mg/mL, and intra-assay coefficients of variation were 4.7% (2.7 ng/mL) and 4.5% (5.9 ng/mL), for low and high FSH, respectively.

2.5. Statistical analyses

All statistical analyses were performed with SAS 9.0 (SAS Institute Inc., Cary, NC, USA) and the statistical model included category (cow vs heifer), treatment (HP vs LP) and their interactions, and location. The location was considered a random effect in the model. Single-point measurements (i.e., maximal diameter of the ovulatory follicle on Day 8 and Day 10, interval to ovulation, growth rate of the ovulatory follicle, diameter of the dominant follicle at selection, plasma concentrations of progesterone on Days 4, 8, and 17, and of LH on Days 0, 4, and 8) were compared among groups by two-way analyses of variance (ANOVA) to detect the effect of category (cow vs heifer) and treatment (LP vs HP), and their interactions. Pearson's correlations were determined for the following pairs of end points: CL diameter and progesterone concentrations on Day 17; dominant follicle diameter on Day 10 and progesterone concentrations on Day 17; and LH and estradiol concentrations on Days 4 to 8. Mean plasma LH concentrations and frequency of LH pulses (pulses/8 h) were calculated from samples collected at 15-min intervals. An LH pulse was defined as an increase in LH concentration that exceeded the previous nadir by two intra-assay standard deviations (44). Logistic regression analyses were performed to examine the effects of treatment, category, and parity on rates of ovulation and pregnancy. Analyses involving repeated measures over time (e.g., follicle diameter and hormonal

198 profiles) were compared between groups (LP vs HP) or category (heifer vs cow), by analysis of
199 variance for repeated measures using the MIXED procedure to evaluate the main effects of
200 group, category and time (day), and their interactions (20). When significant interactions were
201 detected, the least significant difference was used to detect treatment effects at each time.
202 Repeated measures of plasma progesterone, LH, FSH and estradiol concentrations were analyzed
203 over three periods, Days 0 to 4 (from start of progesterone treatment to follicular wave
204 emergence), Days 4 to 8 (from follicular wave emergence to end of progesterone treatment), and
205 Days 8 to 13 (from the end of progesterone treatment to ovulation)..
206
207 **3. Results**
208 3.1. Pregnancy rate and ovarian characteristics
209 3.1.1 All animals
210 Reproductive performance and ovarian characteristics are summarized (Table 1). Overall,
211 pregnancy rate were similar between treatment groups (52.7% (69/131) vs 53.4% (79/148) for
212 HP vs LP, respectively) and between categories (53.5% (69/129) vs 52.7% (70/150) for cows vs
213 heifers, respectively). Ovulation rate was similar between the groups (82.9% vs 82.8%, for HP vs
214 LP group, respectively, combining heifers and cows) and between categories (82.2% vs 80.7%,
215 for cows vs heifers, respectively). The proportion of cattle detected in estrus before scheduled
216 insemination was higher in the LP group than HP group (25%, 37/148 vs 13%, 17/131;
217 P=0.014). When the data were analyzed separately for heifers and cows, the proportion of cows
218 detected in estrus before the insemination tended to be lower in HP group (5/61 (8.2%)) than in
219 LP group (13/68 (19.1%); P=0.08)), whereas the proportion of heifers detected in estrus was
220 24/80 (30%) for LP group and 12/70 (17.1%) for HP group (P=0.08). The diameter of the

dominant follicle at Cue-Mate removal and at insemination was larger (P=0.002) in the LP than HP group. The diameter of the dominant follicle at insemination was larger in cows than heifers (P=0.001). The diameter of the CL on Day 17 was larger (P=0.004) in the LP than HP group. Significant positive correlations were detected between the diameter of the dominant follicle at AI and the diameter of the CL on Day 17 (r=0.52, P<0.001), and between CL diameter and plasma progesterone concentration on Day 17 (r=0.31, P=0.002).

3.1.2 Daily ultrasound evaluations and intensive blood sampling animals

Based on daily ultrasonographic examinations (n=11 per group), the interval from PGF treatment to ovulation was shorter (P<0.001) in the LP (60±0.0 h) than HP group (74.4±3.9 h), and in heifers (64.0±2.6 h) than cows (72.0±4.5 h). A treatment-by-category interaction (P=0.02) on the interval from PGF to ovulation was observed. The heifers in LP group ovulated earlier than heifers in HP group (60±00 and 68±5.05 h, respectively; P=0.05), similarly, cows in LP ovulated earlier than cows in HP group (60±00 and 84±00 h, respectively; P=0.001).

No effect of treatment (P=0.95), category (P=0.57), and interaction treatment-by-category (P=0.17) were detected in the growth rate of the dominant follicle (combined growth rate = 1.81±0.08 mm/day; n=22). Similarly, no effect of treatment (P=0.75), category (P=0.54), and their interaction (P=0.54) was detected on the diameter of dominant follicle at selection (combining all categories, size of dominant follicle at selection = 7.9±0.12 mm; n=22).

The dominant follicle development from emergence to ovulation tended to differ between categories and treatments (P=0.09 and P=0.1; Fig. 2, Panels A and B). The dominant follicle grew from 4.3±0.33 mm on Day 4 (expected day of wave emergence) to 13±0.8mm (just before

the ovulation) in HP heifers, from 4.6±0.6 to 15.6±1.2 mm in LP heifers, from 6±1.3 to 16±2 mm in HP cows and from 6.4±1.3 to16.8±1.73 mm in LP cows.

3.2. Hormone concentrations

3.2.1 All animals

The HP group had higher progesterone concentrations than the LP group on Days 4 and 8 ($P<0.001$), whereas the LP group had a higher progesterone concentration on Day 17 ($P=0.001$; Fig. 3). Heifers had higher concentration of progesterone than cows on Day 17 (4.06±0.16 ng/mL in heifers versus 3.04±0.17 ng/mL in cows).

The LP group had higher LH concentrations than the HP group on Days 0, 4, and 8 ($P<0.05$; Fig. 4). Cows had higher LH concentrations than heifers on Day 4 (0.18±0.02 vs. 0.31±0.01 ng/mL, respectively). Heifers from location 1 had higher LH concentrations (0.29±0.02 ng/mL) than heifer from location 2 (0.17±0.02 ng/mL).

3.2.2 Daily ultrasound evaluations and intensive blood sampling animals

Progesterone hormonal profiles from daily blood samples are presented in Fig. 5 (n=11 per group). A treatment ($P=0.001$) and a treatment-by-day interaction ($P=0.034$) effects were observed on progesterone concentrations from the wave emergence to Cue-Mate withdrawal (Days 4 to 8); mean (±SEM) progesterone concentrations during this period were 1.1±0.41 and 3.8±0.41 ng/mL for LP and HP groups, respectively. In contrast, after Cue-Mate removal, a category effect ($P=0.002$) and a category-by-day interaction was detected (<0.001; Fig. 5, Panel A); the mean progesterone concentrations were 0.61±0.06 and 0.28±0.06 ng/mL for the LP and HP heifers, and 0.33±0.07 and 0.22±0.07 ng/mL for LP and HP cows, respectively.

For LH concentrations, a tendency for treatment effect(P=0.06) and category effect (P=0.009) were detected (Fig. 5, Panel B) from the wave emergence to Cue-Mate withdrawal; averaged over days (Days 4 to 8) LH concentrations were 0.42±0.04 and 0.22±0.04 ng/mL for the LP and HP groups, respectively. After Cue-Mate removal, an effect of day (P=0.005), and a category-by-day interaction (P=0.003) were observed. The LH secretion pattern assessed by 8 hour intensive blood sampling on Day 4, is summarized in Table 2. The LP group had higher basal (P=0.01) and mean LH concentrations (P=0.05) than the HP group. No treatment effect or interaction was detected in LH pulse frequency or amplitude of pulses over the 8-h interval. Examples of LH secretory profiles for individual animals in each treatment group are shown (Fig. 6)

The daily measurements of estradiol and FSH were analysed over three time interval (Fig. 7). An effect of day on estradiol concentrations from Days 0 to 3, a category effect during all periods, and a treatment-by-day interaction from Days 4 to 8 were detected. The category-by-day interaction was found only during Day 0 to 3 (Fig. 7, panel A). From Days 4 to 8, mean estradiol concentrations were 15.7±1.4 pg/mL and 9.6±1.4 pg/mL for the LP and HP groups, respectively. There was a positive correlation between LH and estradiol concentrations from Days 4 to 8 (the period of the dominant follicular growth; $r=0.73$; $P<0.0001$).

During the first period of FSH evaluation (Days 0 to 3), there was only a day effect and category-by-day interaction. During the second period (Days 4 to 8), there were no significant effects or interactions, and in the last period (Days 8 to 12), there was only a significant effect of treatment on FSH secretion.

4. Discussion

The results of the present study support the hypothesis that low plasma progesterone concentration during selection and growth of the dominant follicle will increase ovulatory follicle size, increasing subsequent CL diameter and function, however, the second part of this hypothesis that such treatment will improve pregnancy rate compared to physiological plasma progesterone concentrations during the luteal phase was not supported. Ovulation and pregnancy rates following insemination did not differ between treatment groups, agreeing with other studies in which progestin treatments were used to synchronize ovulation of postpartum cows (pregnancy rates ranged from 45 to 70%; (5,8,14,21,23,43)). Conversely, our observations on more than 275 cattle clearly indicate that low progesterone milieu during the growing and preovulatory phases of the dominant follicle does not have deleterious effect on oocyte competence under two extreme different physiological conditions, i.e., in lactating postpartum beef cows and heifers. It is important to mention that the life span of the ovulatory follicle under both low and high progesterone was tightly controlled in the present study and was less than 8 days from wave emergence at 4mm to ovulation. One caveat of the present study is that a high proportion of cattle showing estrus signs before the scheduled insemination (~20 %), more so in the low progesterone heifers. By design, the study included early estrus detection followed by insemination that allowed us to optimize the fertility in the cattle used in this experiment, thereby minimizing any confounding that might have resulted if we would have followed strict fixed-time artificial schedule.

The current study provided further evidence that synchronization protocols using progestins, in low or high concentrations, can yield high pregnancy rates. In another study involving previously used CIDRs in TAI protocols in suckled beef cows, the numbers of times the CIDR was used

affected pregnancy rate (8). Cows synchronized with a once-used CIDR had a higher pregnancy rate than cows synchronized with twice-used CIDR (62.4 and 48.4%, respectively). However, in the same study (8), authors did not find any difference in the pregnancy rate of heifers receiving one new, one once-used, one twice-used or two twice-used CIDR. The authors concluded that some cows may have ovulated early following the use of a twice-used CIDR, and thus would not become pregnant following TAI.

As the dominant follicle grew in different progesterone environments, LH secretion also differed between groups (Table 2). As expected, use of one progestone containing pod with a blank pod on the second arm of Cue-Mate device achieved a markedly lower plasma progesterone levels in LP group (1.1±0. 41ng/mL during selection and growth of dominant follicle; Figure 5) than the normal luteal phase levels or those in the HP group (3.8±0.41 ng/mL).The high LH secretory activity in the LP group would appear to be responsible for the greater growth of the dominant follicle. Since subluteal concentrations of progesterone have been associated with an increase in LH pulse frequency (34,37), the higher frequency of LH pulses apparently stimulated dominant follicle growth and estradiol secretion by the follicle (47). Indeed, the LP group had higher LH secretion (Table 2; Fig. 6) during the period of progesterone treatment, and there was a positive correlation between LH and estradiol secretion from Days 4 to 8. As LH stimulates estradiol secretion by increasing steroidogenesis (26,34) in granulosa cells, the high estrogenic activity of the dominant follicle enhances the expression of the genes for aromatase, 3β-hydroxy-steroid dehydrogenase and FSH receptors, and the acquisition of LH receptors in granulosa cells (4,9,13,52). In turn, the LP group had a larger dominant follicle at the time of Cue-Mate removal, and AI, and consequently a larger CL formed when compared to HP group. There was a positive correlation between the diameter of the ovulatory follicle, CL and progesterone concentrations, 7

d after AI. Therefore, the anatomic and steroidal function of the CL was clearly associated with the characteristics of the dominant follicle. A larger CL and higher progesterone concentration may increase fertility, since the high progesterone concentration in the early luteal phase augments embryo development and pregnancy establishment (22). Treatment with eCG at the end of a norgestomet-based protocol in Nelore heifers has also been shown to result in a larger dominant follicle and consequently, a larger CL and higher progesterone concentrations (40), supporting our findings.

The development of a normal CL capable of maintaining a pregnancy depends on an adequate number of granulosa cells, adequate numbers of LH receptors on granulosa and theca cells, and granulosa cells capable of synthesizing adequate amounts of progesterone after luteinization (25). Therefore, it would seem important to optimize ovulatory follicle size at the time of ovulation; small follicles have fewer granulosa cells prior to ovulation, resulting in fewer number of large luteal cells in the newly formed CL (31). It is likely that the dominant follicle growth in the high progesterone heifers and cows in the present study was sufficient enough to mask any additional oocyte competence/luteal function advantage that we were expecting to obtain under low progesterone milieu.

The difference in the diameter of the dominant follicle in LP and HP groups at the time of Cue-Mate removal was 2 mm. An apparently similar difference, (1.6 mm) between heifers in low and high progesterone groups was reported by Carvalho et al. (7). In a 7-d progestin synchronization protocol where estradiol cypionate was used to induce the new follicular wave and either estradiol or GnRH was used to induce the ovulation, authors (30) detected ovulatory follicles of 13.0±0.5 mm and 14.1±0.4 mm in diameter, respectively. Apparently, it is not only the concentration of progesterone that affects the development of the dominant follicle, but also, the

358 interval from follicular wave emergence to progestin withdrawal (50), the progestin
359 administration period (50), the follicle age (15), and the treatment used to induce the new
360 follicular wave (i.e. esters of estradiol or GnRH) (15,30).
361 The LP group ovulated earlier than HP group in the present study. Since the LP group had higher
362 LH concentrations during dominant follicle growth, and presumably earlier induction of the LH
363 receptors and earlier induction of ovulatory competence (52). Nonetheless, Colazo et al. (8) did
364 not detect any difference in the interval from CIDR removal to ovulation of heifers synchronized
365 under various concentrations of progesterone. The ovulation time in the LP group (60±00 hours)
366 seemed earlier than that reported in other studies that evaluated the interval to ovulation after
367 progestin treatment (5,30,49).
368 The diameter of the dominant follicle at selection was similar for both treatment groups, and did
369 not differ from that reported for dairy heifers (8.3±0.2 mm), but did differ from that reported for
370 dairy cows (9.8±0.3 mm) (42). The physiological growth rate of antral follicles during the rapid
371 growth phase may be up to 2 mm/day (26), similar to that in the present study; however, others
372 have reported growth rates of 1.2±0.1 and 0.9±0.1 mm/day, for low and high progesterone
373 groups in cross-breed heifers, respectively (7), and 1.2±0.08 mm/day for dairy cows (42).
374 Although progesterone has been reported to be the primary feed-back regulator of LH secretion
375 in the adult cattle (37), there is at least one study that reported the failure of progestin treatment
376 to suppress LH secretion (16), and another reporting that progesterone enhanced the pulsatile
377 release of LH in prepubertal heifers (2). However, as detected in the present study and others
378 (34,37,41), LH secretion depends on progesterone concentrations; higher LH secretion and
379 pulsatility occurred in the LP than in the HP group during progesterone treatments. The HP
380 group had higher concentrations of progesterone during the treatment period (Days 4 to 8, Fig.

3), reflecting the presence of progesterone from the CL and the Cue-Mate, whereas the progesterone in the LP group came from the Cue-Mate per se. The difference in LH secretion on Days 4 and 8 was likely due the effect of PGF on CL of the LP group cows; this was confirmed by circulating progesterone concentrations.

In summary, although low circulating progesterone concentrations during the periods of wave emergence, selection and growing phase of the dominant follicle resulted in an increased diameter of the ovulatory follicle and consequently the diameter of the resulting CL and plasma progesterone; no effect on fertility was detected. We inferred that the mechanisms which regulate oocyte quality and the establishment of pregnancy were not entirely dependent on progesterone concentrations. However, we were able to demonstrate that, in the absence of prolonged follicle dominance, the low progesterone environment did not decrease fertility.

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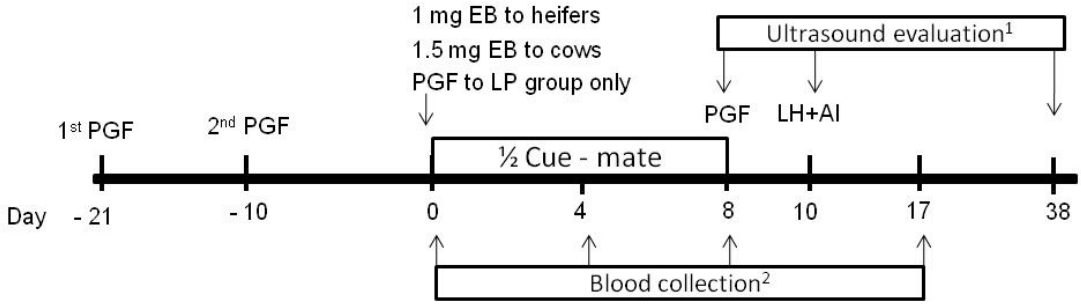

Fig. 1: Time-line for treatment of the high progesterone (HP) and low progesterone (LP) groups. Cattle were treated with 500 µg cloprostenol (PGF) 11 d apart, and assigned randomly into two groups 10 d after the second PGF (Day 0). On Day 0, cattle received a Cue-Mate with a single progesterone-releasing pod and a blank pod, and either 1.5 mg (cows; n=129)) or 1.0 mg (heifers; n=150) of estradiol benzoate. In addition, cattle in the LP group was given PGF. On Day 8, the Cue-Mate was removed and PGF was given to all animals. At 54-56 hours after Cue-Mate removal, cattle were concurrently treated with 12.5 mg porcine LH (Lutropin-V) and artificially inseminated.

[1]The dominant follicle diameter was measured ultrasonographically on Days 8 and 10, and pregnancy diagnosis was performed on Day 38.

[2] Progesterone concentrations were measured on Days 4, 8, and 17, and LH concentrations were measured on Days 0, 4, and 8.

592 Table 1. Pregnancy and ovulation rates (percentage), diameter of the dominant follicle (DF) at
593 Cue-Mate removal and artificial insemination (AI), growth rate of DF, and diameter of the
594 corpus luteum (CL) 7 days after AI (Mean ±SEM) in cows and heifers assigned to treatment with
595 low- (LP) or high- (HP) progesterone. Pregnancy rate and ovulation data were analyzed by
596 logestic regression; rest of the data were analyzed by ANOVA using 2x2 factorial design. P-
597 values from statistical analyses for treatment (Trt; LP vs HP), category (Cat; cows vs heifers) and
598 treatment x Category interaction (Trt*Cat) are provided in last 3 columns.

	Heifer		Cow		P-Val		
	LP	HP	LP	HP	Trt	Cat	Trt*Cat
Pregnancy rate	55% (44/80)	50% (35/70)	51.5% (35/68)	57.4% (35/61)	0.9	0.81	0.37
Ovulation rate	83.75% (67/80)	77.14% (54/70)	81.4% (55/68)	84.0% (51/61)	0.64	0.76	0.33
Diameter of DF at Cue-Mate removal (mm)	12.20±0.34	10.19±0.55	14.3±0.51	12.3±0.34	0.002	0.07	0.93
Diameter of DF at AI (mm)	13.57±0.35	11.49±0.48	16.5±0.52	14.2±0.34	0.001	0.001	0.76
CL diameter 7 d after AI (mm)	24.17±0.83	20.79±1.06	24.7±0.69	22.2±0.76	0.004	0.3	0.64

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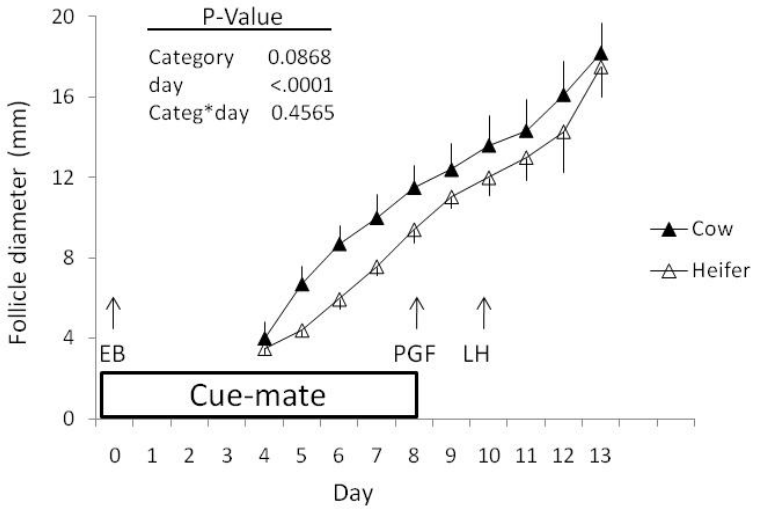
A)



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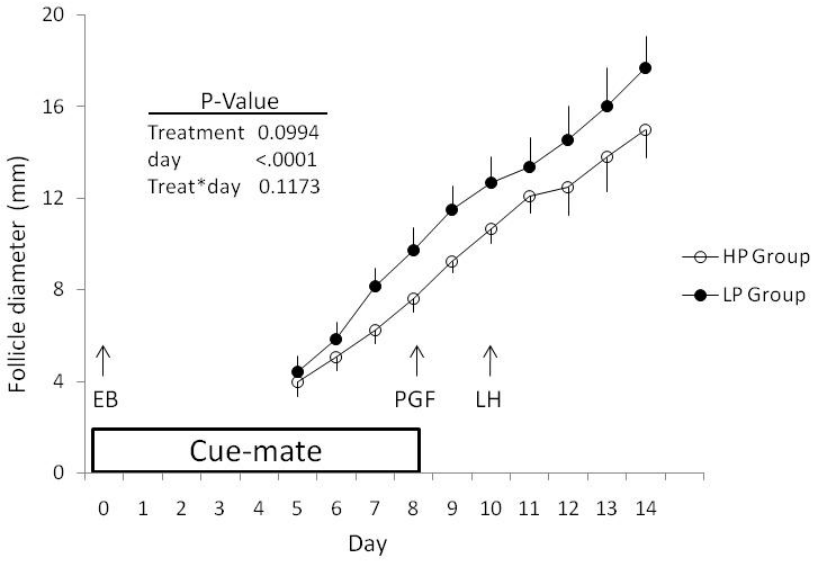


Fig. 2: Dominant follicle profiles (Cows vs Heifers in Panel A and High progesterone (HP) vs Low progesterone (LP) groups in Panel B) from the subset of animals that were examined daily by ultrasonography (n=22). Data were centralized to the day of wave emergence for each group. Timing of estradiol benzoate (EB), progesterone implants (Cue-Mate), cloprostenol (PGF) and luteinizing hormone (LH) treatments are indicated by arrows. P-values from repeated measured mixed model analyses for treatment (Cow vs heifer in Fig. A and HP vs LP in Fig B), Day (repeated factor) and the interaction term are indicated on the figures.

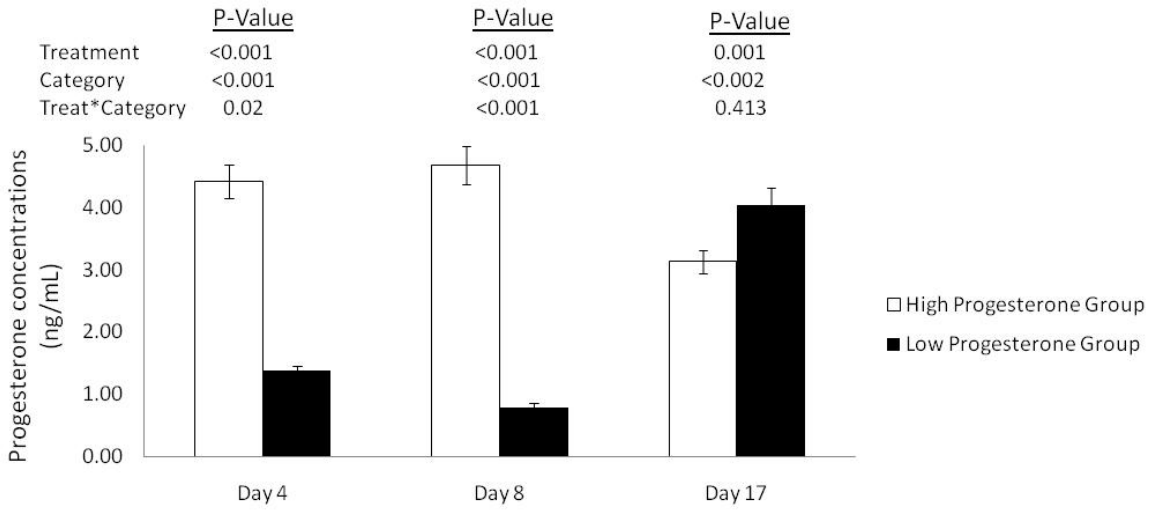
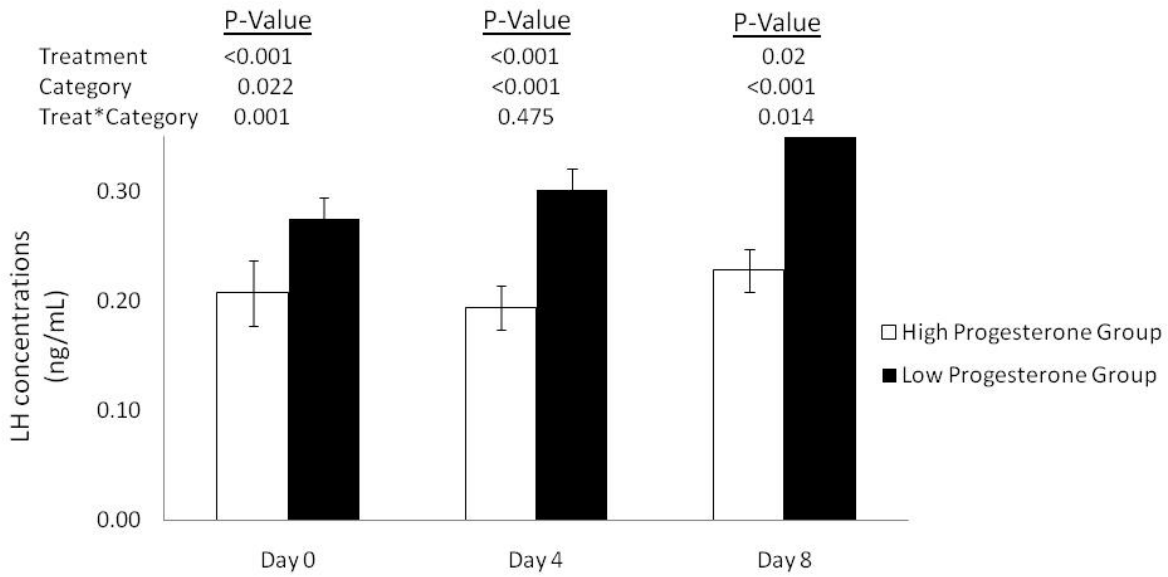


Fig. 3: Mean plasma progesterone concentrations in beef cattle in high- and low-progesterone treatment groups on Day 4 (expected day of wave emergence; Day 0 = Day of estradiol benzoate injection + Cue-Mate insertion), Day 8 (Cue-Mate removal) and Day 17 (7 days after artificial insemination). Data were analyzed by ANOVA for treatment (low versus high progesterone), category (cows vs heifers) and their interaction on Days 4, 8, and 17.



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624 Fig. 4: Mean plasma LH concentrations in beef cattle under high and low progesterone treatment
625 on Day 0 (Cue-Mate insertion), Day 4 (expected day of follicular wave emergence), and Day 8
626 (Cue-Mate removal). Data were analyzed by ANOVA for treatment (low versus high
627 progesterone), category (cows vs heifers) and their interaction on Days 0, 4, and 8.

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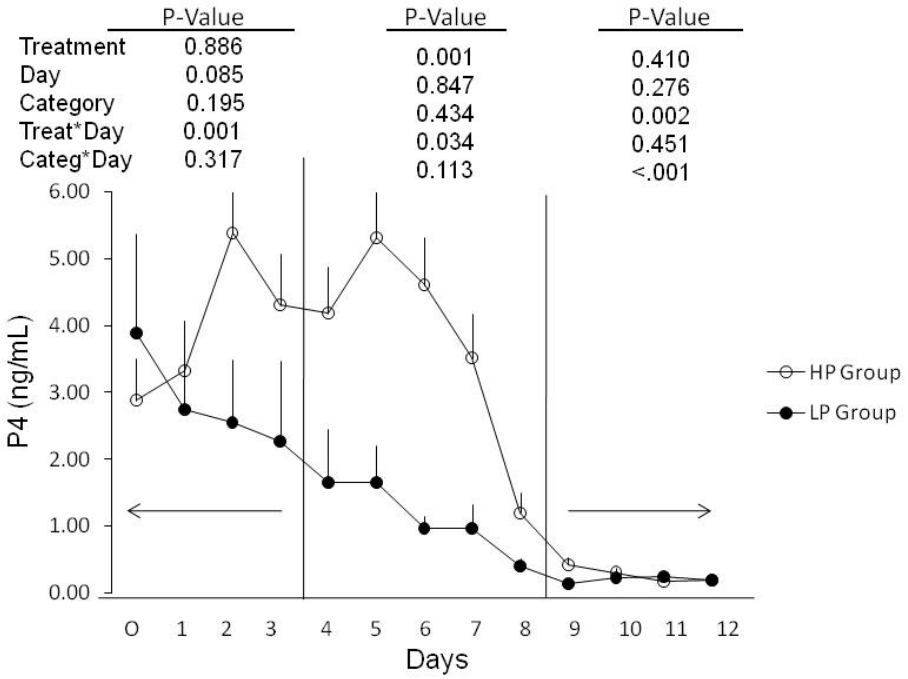

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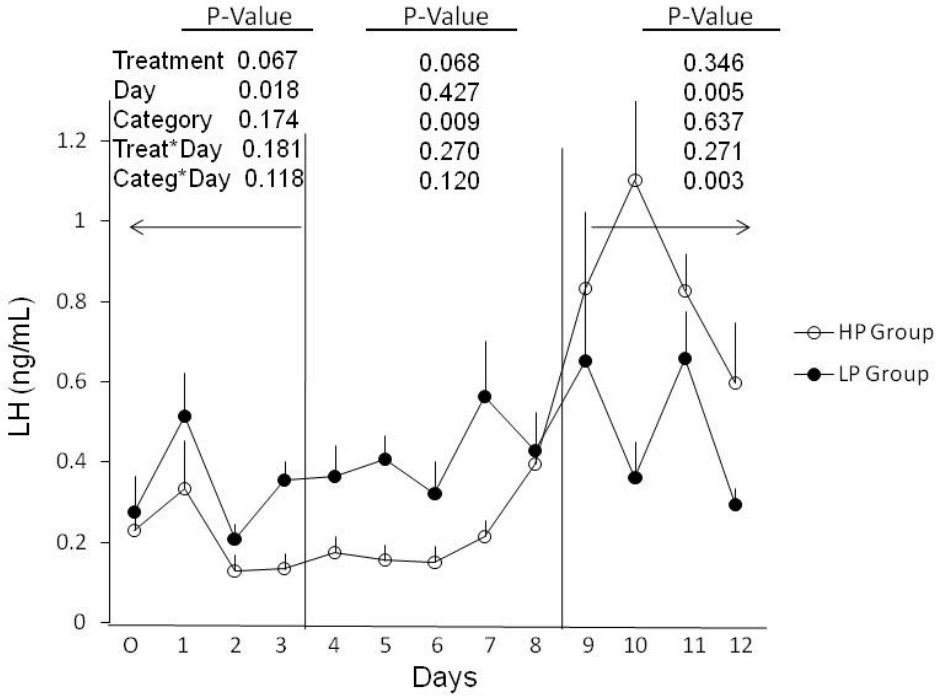

Fig. 5: Mean (±SEM) plasma concentrations of progesterone (P4; Panel A) and LH (Panel B) in cattle in the High progesterone (HP group; ○) and Low progesterone (LP group; ●). The data were analysed by repeated measures ANOVA for three time periods: before expected follicular wave emergence (Day 0 to Day 4), from the expected follicular wave emergence to Cue-Mate removal (Days 4 to 8) and from Cue-Mate removal to ovulation (Day 8 to 12).

639 Table 2: Characteristics of plasma LH concentration (mean±SEM) in cows and heifers given
640 high- (HP) or low- (LP) progesterone treatments on Day 4 (expected time of wave emergence,
641 Day 0=Day of estradiol injecton+Cue-Mate insertion). Blood samples were collected every 15
642 min for 8 hours.

	Heifer		Cow		P-Value		
	LP	HP	LP	HP	Trt	Cat	Trt*Cat
Number of animals (n)	6	6	5	5			
Basal secretion (ng/mL)	0.21±0.02	0.13±0.01	0.26±0.04	0.21±0.02	0.01	0.02	0.63
Mean (ng/mL)	0.31±0.07	0.18±0.03	0.32±0.03	0.23±0.03	0.05	0.65	0.77
Pulse frequency	3.66±0.8	2.5±0.34	3.5±0.86	2±0.71	0.21	0.62	0.81
Pulse amplitude (ng/mL)	1.28±1.06	0.58±0.36	0.23±0.02	0.20±0.08	0.64	0.43	0.61

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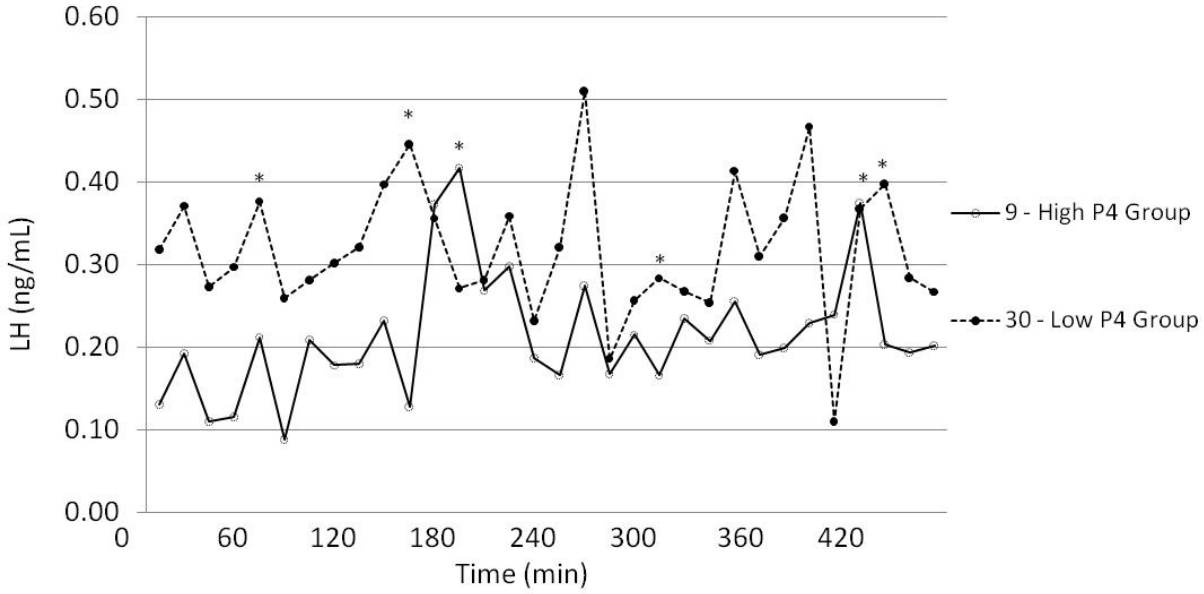

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646 Fig. 6: Plasma concentrations of LH in two representative individuals from the High
647 progesterone (Heifer # 9) and Low progesterone (Heifer # 30) groups on Day 4 after Cue-Mate
648 insertion. Blood was collected every 15 min for 8 h. Pulses of LH are indicated by asterisks.
649 These representative animals for each treatment group were chosen based on the LH mean and
650 LH pulse frequency that best represented the average of the group.

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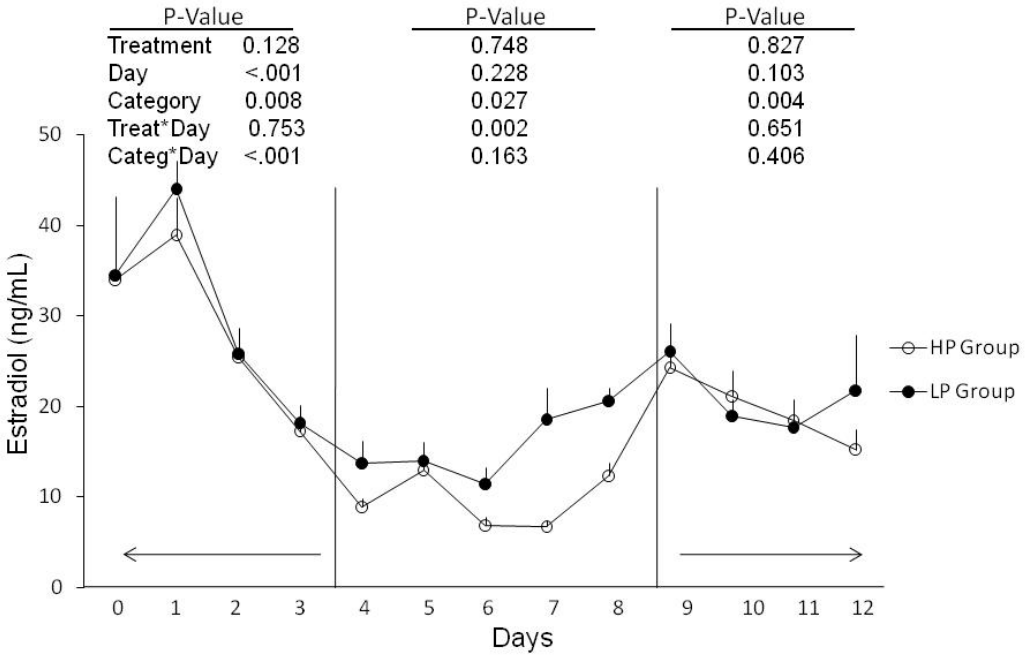
A)



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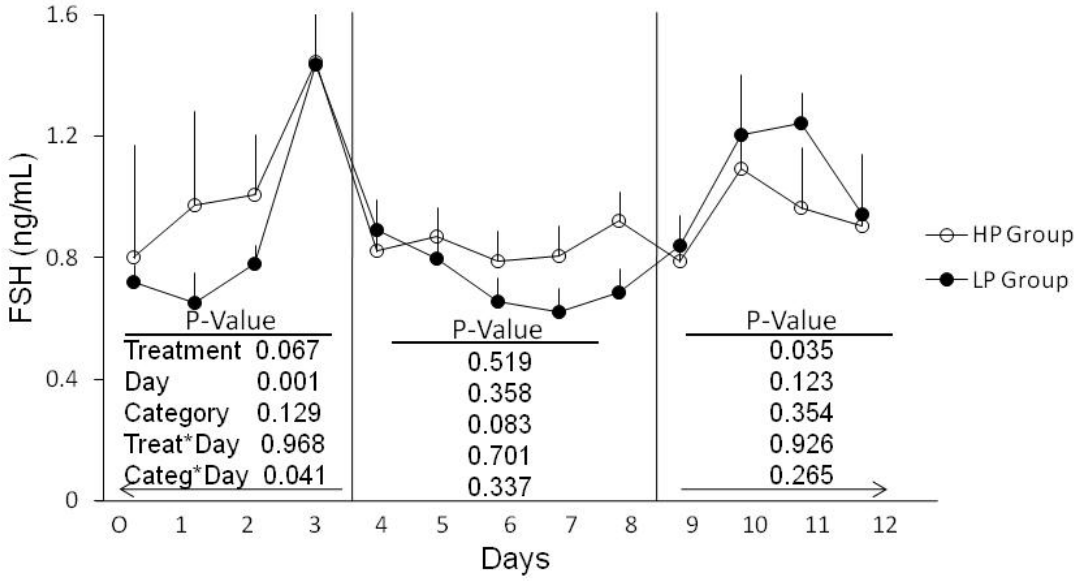

Fig. 7: Mean (±SEM) plasma concentrations of estradiol (E2; Panel A) and FSH (Panel B) in cattle in the High progesterone (HP group; -o-) and Low progesterone (LP group; ●). The data were analysed by repeated measures ANOVA for three time periods: before expected follicular wave emergence (Day 0 to Day 4), from the expected follicular wave emergence to Cue-Mate removal (Days 4 to 8) and from Cue-Mate removal to ovulation (Day 8 to 12).

3. ARTIGO 2

Effect of exogenous progesterone and $PGF_2\alpha$ analog on ovarian follicular development and first ovulation in prepubertal heifers

Effect of exogenous progesterone and $PGF_2\alpha$ analog on ovarian follicular development and first ovulation in prepubertal heifers

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Abstract

Although $PGF_2\alpha$ increases the pituitary response to GnRH, the influence of the $PGF_2\alpha$ associated with progesterone treatment on the ovulation in non-cycling cattle remains unclear. The objective of these studies was to determine the effects of progesterone and $PGF_2\alpha$ on ovulation rate in prepubertal heifers. In Experiment 1, prepubertal heifers (10 to 12 month, 255 to 320 kg), were assigned randomly to three groups and given 1) an intravaginal progesterone-releasing device (CIDR; P group, n=13), 2) a CIDR plus 500 μg of cloprostenol ($PGF_2\alpha$ analog) im at CIDR removal (PPG group, n= 11), or no treatment (Control group, n=14). Ovarian follicular dynamics and ovulation were monitored by daily transrectal ultrasonography. The CIDR were removed 5 d after the wave emergence. Progesterone treated heifers (P and PPG groups) had larger ovulatory follicle than Control group (P=0.01). The ovulation rate was highest in the PPG group (8 of 11, 73%), intermediate in the P group (31%, 4 of 13), and lowest in the control group (1 of 14, 7%; P<0.001). In Experiment 2, emergence of follicular wave was synchronized; prepubertal heifers (14 to 16 month, 300 to 330 kg) were given 1) a CIDR and 1 mg of estradiol benzoate im on Day 0 (EP group, n=8), or 2) underwent transvaginal ultrasound-guided ablation of all follicles ≥5 mm on Day 3 (FA group, n=8). On Day 7, CIDR were removed and all heifers received 500 μg of cloprostenol im. Ovulation was detected in 6 of 8 heifers (75%) in both groups. In summary, progesterone treatment of prepubertal heifers, particularly in combination with $PGF_2\alpha$ promoted dominant follicle growth and ovulation.

Key-words: Cattle, follicular development, prepubertal heifers, progesterone, prostaglandin, puberty, ovulation.

1. Introduction

In cattle, large antral follicles are observed immediately after birth and continue to grow throughout prepubertal period. A wave-like pattern of follicular development has been observed in heifer calves as early as 2 week of age (12). The maximum diameter of dominant follicles continues to increase through the peripubertal period up to puberty (6,12). The pattern of follicular wave (1,39) and the mechanism controlling follicle growth appears to be similar between the late prepubertal period and during normal cycles (11). Higher secretion of 17β-oestradiol by the dominant follicle near puberty, i.e., first ovulation of life, is responsible for the increase of its diameter and the decrease of negative feed-back of 17β-estradiol on LH secretion (11). In the short period preceding the puberty, there is an increase in LH concentrations and LH pulse frequency (10,19) and this progressive increase in pulsatile LH secretion is considered a critical signal involved in the onset of puberty (11,23).

As one of the main demands of the beef cattle industry is to accelerate age at puberty in order to increase productivity, treatments (nutritional or hormonal) that attempt to hasten the onset of puberty are considered very important for beef heifers. To achieve optimal lifetime productivity, the early conception in the initial breeding season is important in order to avoid the proportion of heifers that do not become pregnant during their first breeding season (20,45). Puberty can be hastened by the use of steroid hormones, such as progesterone. The use of progestins implants for 8 days exhibited an increase in the pulsatile secretion of LH after progesterone device withdrawal (3). The increase in LH pulse frequency after progesterone treatment (3,18) is believed to occur as a result of decrease in the number of hypothalamic estradiol receptors (3). Ovulation induction ability of progesterone is well documented in literature (3,9,15,18,37),

however, the underlying effects of progesterone on follicular wave emergence, selection and growth of dominant follicle are not well characterized.

Although prostaglandin $F_2\alpha$ (PGF) acts mainly on CL by inducing luteal regression, however, indirectly it also affects ovulation, implantation and maintenance of pregnancy, parturition, and postpartum physiology. Therefore, PGF is widely used in clinical practice for synchronization of estrus alone or in combination with progestins, oestrogens, and gonadotropin releasing hormone (GnRH) (42). One of the possible direct effects of PGF during the ovulation process include the increase in pituitary responsiveness to GnRH resulting in release of LH in postpartum cows (32). Furthermore, PGF may enhance the progesterone effect on the hypothalamus and increase pituitary responsiveness to GnRH (32), thereby inducing the ovulation in ewes and cattle (26,42). Presently, it is not know if PGF will have any direct effect on ovulation in prepubertal heifers in the absence of exogenous progesterone treatment.

With these points in mind, the present study was designed with the objectives to investigate the 1) effect of exogenous progesterone on dominant follicle growth and ovulation in prepubertal heifers and 2) effect of prostaglandin $F_2\alpha$ on ovulation with or without previous treatment with exogenous progesterone. In this study, 2 hypotheses were tested: a) Exogenous progesterone treatment beginning at or before wave emergence will increase growth rate of dominant follicle resulting in ovulation following progesterone withdrawal; b) Exogenous $PGF_2\alpha$ treatment at the end of growth phase of dominant follicle, with or without pre-treatment with exogenous progesterone during dominant follicle growth, will enhance ovulation rate.

2. Materials and methods

2.1. Experiment 1

The experiment was designed to study the effect of progesterone on wave emergence and subsequent ovulation rate with or without prostaglandin $F_2\alpha$ in prepubertal heifers. This study was conducted with 38 Hereford crossbred prepubertal heifers (255 to 320 kg of body weight , 10 to 12 mo in age) maintained in outdoor corrals at the University of Saskatchewan Goodale Research Farm, SK (52° 03' N, 106° 30' W). The heifers were fed barley silage and alfalfa hay, *ad libitum* access to water, salt, and a mineral mixture. Heifers were examined by transrectal ultrasonography (Aloka SSD-900, B-mode scanner with a 7.5 MHz linear transducer, Tokyo, Japan), on two occasions 11 d apart, to confirm the absence of a CL (i.e., all heifers were prepubertal). The experimental design is summarized in Fig. 1A. The heifers were assigned randomly into three groups and given: 1) an intravaginal progesterone-releasing device (CIDR; P group, n=13); 2) a CIDR followed by 500 μg of prostaglandin $F_2\alpha$ analog, cloprostenol (Estrumate, Schering-Plough Animal Health, Pointe-Claire, QC, Canada) im at CIDR removal (PPG group, n= 11); or no treatment (Control group, n= 14). The CIDR (1.9 g of progesterone, Pfizer Animal Health, Montreal, QC, Canada) were given to heifers in the progesterone-treated groups at random stages of follicular wave. Heifers were examined daily by transrectal ultrasonography to monitor ovarian follicular dynamics and to detect ovulation. At each examination, a sketch of each ovary was made, and the diameter and location of all follicles ≥4 mm in diameter were recorded. The day of wave emergence was determined retrospectively, and was defined as the day when the dominant follicle of a wave was first detected at a diameter between 4 and 5 mm. The day of follicular wave emergence (=Day 0) after CIDR insertion was recorded for each heifer in the progesterone-treated groups, and CIDR were removed 5 d later

(Day 5). Ultrasonographic examinations were conducted daily from beginning of the experiment to ovulation (all groups) or, in the absence of ovulation, up to 5 d after CIDR removal (P and PPG groups), or until the detection of a dominant follicle of the subsequent follicular wave at ≥8 mm in diameter (Control group). Ovulation was defined as the disappearance of a previously identified follicle ≥ 8 mm in diameter (25). In addition to ovarian ultrasound examinations, the uterus of each heifer was carefully examined by rectal palpation and ultrasonography to identify signs of proestrus, i.e., increased uterine tonus and the presence of fluid in the uterine lumen, along with the presence of a large dominant follicle (29,36). Once the ovulation was detected, two ultrasound exams were performed 7 and 10 days after the ovulation in order to check the CL diameter and if premature luteolyses have occurred, respectively.

Blood samples were collected by caudal venipuncture into heparinized 10 mL tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) just prior to CIDR insertion, and then daily until 5 d after CIDR removal (P and PPG groups), or until detection of of an 8 mm dominant follicle of the subsequent follicular wave (Control group). Blood samples were centrifuged (1500 X g for 15 min) and the plasma was harvested and stored at -20 ^{0}C.

2.2. Experiment 2

Experiment 2 was designed to study the effect of $PGF_2\alpha$ on ovulation rate after synchronized wave emergence and development of dominant follicle in the presence or absence of exogenous progesterone treatment. Twenty-four Hereford crossbred heifers (14 to 16 mo old, body weight from 300 to 330 kg) were examined ultrasonographically on two occasions, 7 d apart, to confirm the absence of CL. Heifers in which a CL was not detected at both ultrasound examinations were used in this study (n=16). Heifers were housed, fed, and handled as in Experiment 1. The

experimental design is summarized in Fig. 1B. Peripubertal heifers were randomly assigned to one of two treatment groups (n=8/group) to induce synchronous wave emergence with either progesterone-estradiol treatment (EP group) or by follicle aspiration (FA group). Heifers in the EP group received a CIDR insert and 1 mg estradiol benzoate (Sigma Chemical Company, St. Louis, MO, USA) in 2 mL canola oil (2 mL; No Name®, Montreal, QC, Canada) irrespective of the stage of follicular wave (Day 0). The CIDR inserts were removed on Day 7. Heifers in the FA group were subjected to ablation of all follicles >5 mm in diameter (24), on Day 3 (Day 0 = start of experiment). Emergence of a new follicular wave was expected on Day 4 in both groups (24). All heifers received 500 μg of cloprostenol im on Day 7. Transrectal ultrasonography was done as in Experiment 1, once daily from Day 3 to ovulation or to Day 12 (in the absence of ovulation).

Blood samples were collected and handled as in Experiment 1. The samples used to measure plasma LH concentrations were collected once daily from Days 3 to 7, and then every 12 h to ovulation or to Day 12. The samples used to measure plasma concentrations of progesterone and estradiol were collected on Days 3 (12 h after ablation in the FA group), 5, and 7.

2.3. Radioimmunoassays

Plasma progesterone concentrations were assayed in a solid-phase radioimmunoassay, with a minimum detection limit of 0.1 ng/mL. Progesterone was analyzed after extraction with 3 mL hexane from 200 μL aliquots of plasma. In Experiment 1, the intra-assay coefficients of variation (CV) were 5.7% (1.6 ng/mL) and 5.7% (16.5 ng/mL) for low and high progesterone reference samples, respectively. In Experiment 2, the intra-assay CVs were 10% (0.36 ng/mL) and 8.6% (1.11 ng/mL) for low and high progesterone reference samples, respectively.

Concentrations of estradiol were measured by a modified human double-antibody RIA Kit (DPC coat-a-count, Diagnostic Products Corporation, Los Angeles, CA, USA; (30)) using a procedure described by Singh et al. (38). The sensitivity of the assay was 0.5 pg/mL and the intra-assay CVs were 4.2% (21.1 pg/mL) and 2.8% (89.1 pg/mL) for low and high estradiol concentrations. Plasma LH concentrations were determined by double-antibody radioimmunoassay (17) and are expressed in terms of NIDDK-bLH-4. The minimum detection limit was 0.06 ng/mL, with a standard curve ranging from 0.06 to 8 ng/mL. In Experiment 1, intra-assay CVs were 7.6% (0.65 ng/mL) and 8.3% (2.05 ng/mL), for low and high LH concentrations, respectively. In Experiment 2, the intra-assay coefficients of variation were 3.9% (0.95 ng/mL) and 6.0% (2.4 ng/mL) for low and high LH concentrations, respectively.

2.4. Statistical analyses

Single-point measures (e.g., maximal diameter of the ovulatory follicle, growth rate of the ovulatory follicle, CL diameter 7 days after ovulation, day of ovulation, and progesterone concentrations at the beginning of treatment), were compared among groups and between heifers that ovulated and did not ovulate (when applicable) by one-way ANOVA. Ovulation rate and the proportion of heifers showing signs of proestrus were compared by chi-square analysis. The pregnancy rate was also analysed combining groups P and PPG versus Control group by chi-square analysis. Analyses involving repeated measures over time (e.g., follicle diameter, plasma LH concentrations, and plasma concentrations of progesterone and estradiol on Days 3, 5 and 7) were compared among groups and also between heifers that ovulated or did not ovulate by the MIXED procedure, using SAS 9.0 (SAS Institute Inc., Cary, NC, USA; (35) for repeated measures to evaluate the main effects of treatment and time (days), and their interactions (21).

When interactions were significant ($P<0.05$), a least significant difference test was used to detect treatment effects at each time. As two treatment groups received progesterone treatment, the dominant follicle growth data of the P and PPG groups were combined and compared to Control groups using MIXED procedure. In Experiment 2, data for LH concentration were centralized for individual heifers to the LH peak.

3. Results

3.1. Experiment 1

The characteristics of the follicular wave pattern, number of heifers that exhibited signs of proestrus, and ovulation rate for each group are summarized in Table 1. Ovulation rate tended to be higher in the PPG group than in the P group ($P=0.09$). Overall, progesterone treatment (P and PPG groups combined) resulted in a higher ($P<0.01$) incidence of ovulation (50%, 12 of 24) compared to the control group (7.1%, 1 of 14). Ovarian follicular growth profiles are summarized in Fig. 2.

Growth rate of the ovulatory follicle was higher (1.1±0.05 mm/d) and the maximal diameter was larger (12.9±0.27 mm) in heifers that ovulated than in those that did not ovulate (0.96±0.02 mm/d and 11.8±0.20 mm, $P<0.002$), regardless of treatment.

The CL diameter 7 d after ovulation did not differ between the P and PPG groups (19.5±2.5 and 22.9±1.3 mm, respectively) and seemed to be larger compared to the single Control heifer that ovulated (11.0 mm). Ten days after ovulation, the CL was nondetectable in the control heifer indicating short estrous cycle. None of the heifers in the P and PPG groups had a short estrous cycle following ovulation.

There was no difference in progesterone concentrations on Day 0 among groups. As expected, heifers in the P and PPG groups had higher plasma progesterone concentrations than those in the Control group 5 d after follicular wave emergence (due to CIDR treatment) and 7 d after ovulation, due to the presence of a CL (Fig. 3).

Plasma LH concentrations from 5 d after follicle wave emergence to Day 9 are shown in Fig. 4. There was no treatment effect, but there tended to be a day effect. There was no difference between heifers that ovulated versus those that did not ovulate.

3.2. Experiment 2

Follicular wave emergence occurred earlier in the EP group (two heifers each on Days 2 and 3, and 4 on Day 4) than in the FA group (all heifers had follicular wave emergence on Day 4, 1 d after follicle ablation; $P<0.03$).

There was no significant difference between the two treatment groups for: the proportion of heifers that ovulated after PGF treatment (6 of 8; 75% for both groups); the interval from PGF treatment to ovulation (Fig 5A); the diameter of the dominant follicle at selection and its growth rate; the duration of the ovulatory wave (from wave emergence detected to its ovulation); and the diameter of the largest follicle prior to ovulation. Overall mean ± SEM for both groups were 8.1±0.56 mm for diameter of the dominant follicle at selection, 1.71±0.09 mm/day for growth rate of the dominant follicle, 7.6±0.36 d for duration of the ovulatory wave and 13.4±0.55 mm for largest diameter of the ovulatory follicle.

There was a day effect in the dominant follicle development between treatments (Fig. 5A). When the dominant follicle growth was compared between heifers that ovulated and did not ovulate, there was a tendency for treatment and a day effect (Fig. 5C).

Plasma LH concentrations before the LH peak were affected by treatment and day; heifers in the FA group had higher mean LH concentrations than those in the EP group (0.61±0.09 and 0.32±0.08 ng/mL, Fig. 5B). Irrespective of the treatment, the ovulated heifers had higher mean LH concentration than non-ovulated heifers after the LH peak (P=0.0334; Fig. 5D).

There was an effect of treatment and day on plasma estradiol concentrations (Fig. 6). Although heifers in EP group had higher estradiol concentrations on Days 3 and 5 than the FA group, there was no difference on Day 7 (the time of CIDR removal in the EP group).

There were treatment and day effects, and a treatment-by-day interaction on plasma progesterone concentrations on Days 3, 5, and 7 (Fig. 7).

4. Discussion

The higher incidence of ovulation in heifers given progesterone compared to control is similar to that reported previously (3,22,27). Surprisingly, the ovulation rate tended to be higher in the prepubetal heifers that were treated with prostaglandin at end of exogenous treatment in Experiment 1 and equal number of animals ovulated after prostaglandin treatment with or without exogenous progesterone in Experiment 2, providing evidence that prostaglandin $F_2\alpha$ may promote ovulation by mechanisms other than luteolysis. Weens et al. (42) suggested a direct effect of the prostaglandin $F_{2\alpha}$ on the pituitary gland. The combination of progesterone and prostaglandin $F_2\alpha$ appeared to act in synergy, but PGF along with follicle ablation seemed just as efficacious in Experiment 2. Progesterone is expected to reduce the negative feedback of estradiol on the hypothalamus by reducing the number of estradiol receptors (10), whereas prostaglandin $F_2\alpha$ is likely to increase the responsiveness of the pituitary to GnRH (32).

Although heifers in Experiment 2 were acyclic, they were older and heavier than heifers in

248 Experiment 1. The high ovulation observed in the FA group could be an effect of the
249 prostaglandin $F_2\alpha$ in association with follicular ablation; the latter could have affected the
250 feedback mechanisms of estradiol that control the onset of puberty.
251 Heifers that received progesterone plus PGF (PPG group in Experiment 1 and EP group in
252 Experiment 2) had similar ovulation rates indicating that it is possible to induce successful
253 ovulation (and hypothalamo-pituiary axis is ready to respond the treatment) at least 2 months
254 before expected natural ovulations (11). This effect is evidenced in the PPG group, since these
255 heifers were around 4 months younger than heifer in the EP group.
256 Estradiol has been reported to decrease the LH pulse amplitude in sheep and cattle (33), and
257 estradiol alone (7,43) or in combination with progesterone (4) suppressed FSH. Thus, the sudden
258 removal of the negative estradiol feedback in the FA group through the removal of the follicular
259 fluid, could have allowed the secretion of LH, in addition to the FSH surge that was expected to
260 precede the new follicular wave (2,8). Furthermore, reduced estradiol concentrations after
261 follicle ablation (Fig. 6) in association with the $PGF_2\alpha$ treatment, may have increased pituitary
262 responsiveness to endogenous GnRH secretion (32,42). Conversely, follicular ablation may have
263 resulted in partial luteinisation of remaining follicular wall to produce small amounts of
264 endogenous progesterone, although in Experiment 2, plasma concentration were not different
265 (above, slightly increase numerically but around 0.6ng/mL) 4 days after ablation compared with
266 2 days after ablation (Fig 7).
267 Within progesterone-treated heifers in the Experiment 1, five of 12 heifers that did not ovulate
268 had proestrus signs, suggesting that, following progesterone treatment, they had increased
269 estradiol concentrations (11,41) which induced signs of proestrus (36).

The higher rate of growth of the dominant follicle in progesterone-treated heifers as compared to Controls in the Experiment 1 was probably a consequence of the suppressive effect of progesterone on the estradiol negative feedback on the hypothalamus. Once the number of estradiol receptors in the hypothalamus are reduced, follicular steroidogenesis increases as a result of an increase in gonadotropin (FSH and LH) release (2,11,31), resulting in an increased rate of growth and an increased size of the dominant follicle. The effect of progesterone on the hypothalamus was presumably exerted during the period the CIDR was in place. Similar effects were not observed in the heifers of the Experiment 2; progesterone-treated and non-treated heifers had the same rate of dominant follicular growth. Furthermore, these heifers were also nearing puberty; therefore hypothalamus may be sufficiently mature to mask the effects of progesterone on follicular growth. The positive effect of progesterone on the diameter of the dominant follicle in prepubertal heifers has been previously reported (3,40).

Although the effect of progesterone on follicle dynamics in prepubertal heifers has been reported (3,40), this is the first report of a positive effect of progesterone on follicular growth in prepubertal heifers during a spontaneous (non-induced) follicular wave. It has been reported that the pattern of follicular growth during the late prepubertal period and first cycles following the onset of puberty was similar (11,14,39). In the current study, the dominant follicles of the ovulatory waves were larger (similar to cycling heifers) than of non-ovulatory waves (similar to prepubertal period). However, dominant follicle growth profiles in progesterone treated heifers that did not ovulate were larger than in heifers not treated with progesterone (Experiment 1), illustrating the positive effect progesterone on the follicular development in prepubertal heifers. In contrast, there was no difference among groups when the development of the ovulatory wave was compared (Experiment 1); however, only one animal ovulated in the control group in

Experiment 1, making it difficult to make inferences about this group. Notwithstanding it was clearly possible to distinguish the difference in follicular growth between ovulatory and non-ovulatory waves. Progesterone enhanced follicular growth in prepubertal heifers, and status of the follicular wave (ovulatory or not) also affected the growth rate of the dominant follicle. Progestin treatment increased follicular development in prepubertal heifers, even when LH secretion was not influenced (40). Anderson et al. (3) suggested that the use of a progestin could lead to the development of a single large follicle. A similar effect was observed in cows that received progestin treatment during postpartum anestrus (13). Conversely, low progesterone concentrations in cycling cattle resulted in development of a large follicle, compared to those under physiologic progesterone concentrations (28).

As expected, all heifers in the present experiment were considered prepubertal because they had progesterone concentrations <1 ng/mL at the beginning of the study (3). Also as expected, the groups that received the progesterone (CIDR) treatment had higher progesterone concentrations than the Control group at the time of CIDR removal (5 d after follicle wave emergence) (25). However, it was noteworthy that progesterone concentrations 7 d after ovulation in the Experiment 1 were higher in progesterone-treated groups than in the Control group (only one ovulation) which had progesterone concentration lower than expected for a 7-d old CL (34). Moreover, the CL in this heifer had apparently regressed by 10 d after ovulation, suggesting that a short cycle occurred. An increased incidence of short estrous cycles following the first pubertal ovulation has been reported (5,16). Evans et al. (11) reported that all prepubertal heifers had a CL with a short lifespan after the first ovulation. In contrast, in the present study, the only heifer with a CL with a short lifespan was from the Control group. Consistent with previous reports, CIDR treatment overcame the problem of short cycles after the first ovulation in heifers from the

316 P and PPG groups (11,27). Therefore, the CL (7 d after ovulation) had a larger diameter and
317 higher progesterone secretion in heifers progesterone-treated groups, compared to Controls,
318 resulting in normal length cycles, followed first ovulation. Unfortunately, CL development in
319 Experiment 2 was not followed after ovulation, so it was not possible to determine if the high
320 ovulation rate observed in the FA group resulted in corpora lutea with normal lifespan.
321 As mentioned elsewhere, as one of the main demands of the beef cattle industry is to accelerate
322 age at puberty, the practical ovulation synchronization purpose in yearling heifers, EP treatment
323 and progesterone plus PGF treatment can be given irrespective of the cyclic status and will be
324 equally effective to induce ovulation for fixed-time insemination in both pubertal and prepubertal
325 heifers. We can expect similar pregnancy rates because none of the resulting CLs were short
326 lived in prepubertal heifers (Experiment 1).
327 As expected, LH secretion was reduced during the CIDR treatment in EP group in Experiment 2
328 (Fig. 5B). In contrast, after CIDR removal, there was no effect of group in either experiment
329 (Figs. 4 and 5B). The higher LH secretion in the FA group during dominant follicle selection and
330 growth can be associated with the high ovulation rate observed in this group, as long as this
331 heifers were near puberty and the LH secretion could enhanced the number of LH receptors in
332 the dominant follicle and consequently increase its ovulatory competence (44). Although it was
333 not possible to detect an effect of progesterone on LH secretion in the Experiment 1 (due to
334 limited statistical power), it was possible to detect an earlier increase in LH secretion in P and
335 PPG groups, compared to Controls (Fig. 4), which corroborates previous studies that suggested a
336 progesterone treatment effect on the pattern of LH secretion in prepubertal heifers (3,18).
337 In summary, progesterone treatment had a positive effect on dominant follicle development after
338 spontaneous wave emergence in prepubertal heifers, and the use of $PGF_2\alpha$ in association with

exogenous progesterone treatment hastened first ovulation. $PGF_2\alpha$ treatment without exogenous progesterone also induced ovulation after wave synchronization in heifers nearing puberty providing a possible alternative method to hasten the onset of puberty. The question that remains unanswered is whether the use of $PGF_2\alpha$ alone will overcome the occurrence of short cycles after the first ovulation. Furthermore, ovulatory dominant follicles compared to those that failed to ovulate and the exogenous progesterone treatment lead to enhanced growth of dominant follicle in prepubertal heifers possibly through physiologic feedback effects on hypothalamus and/or pituitary.

Acknowledgements

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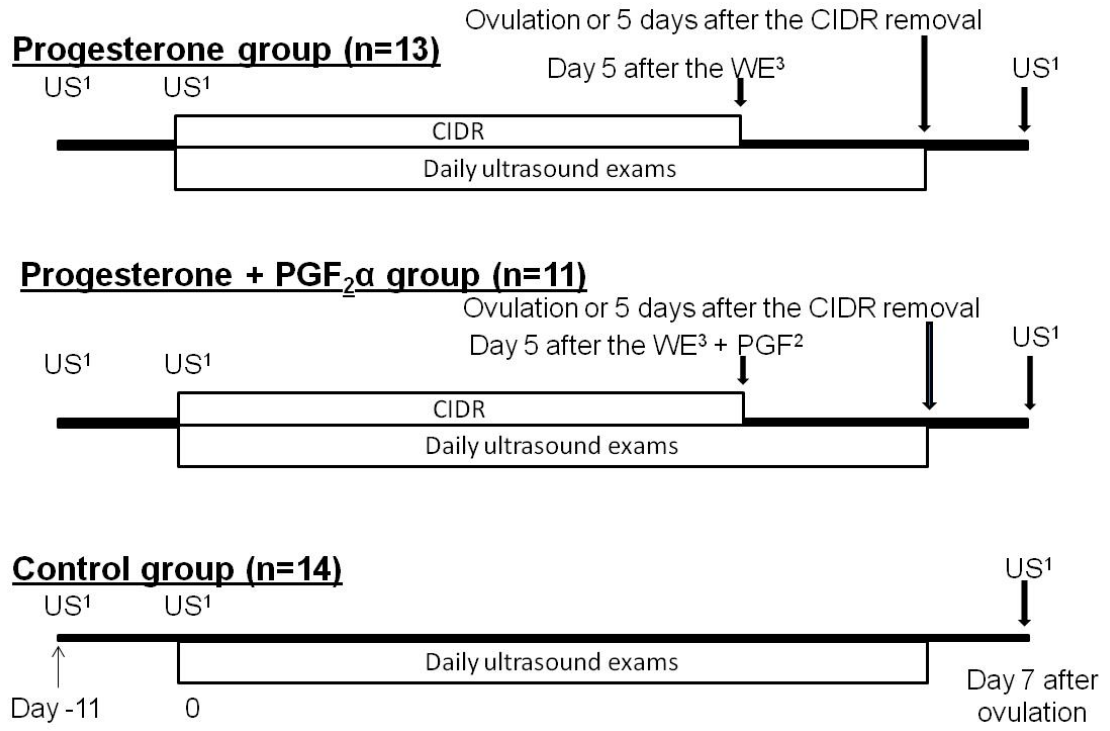
A)



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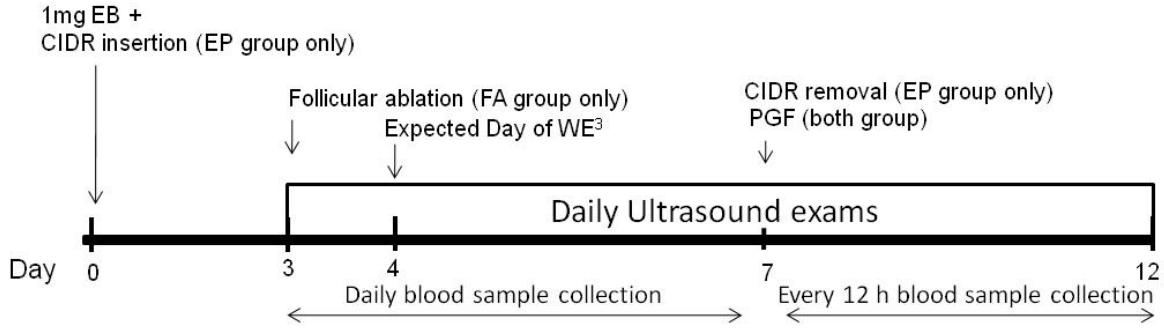

Fig. 1. A) Timeline of treatments in Experiment 1. Prepubertal heifers were given 1) an intravaginal progesterone-releasing device (CIDR; P group, n=13), 2) a CIDR plus 500 µg of cloprostenol (PGF) im at CIDR removal (PPG group, n= 11), or no treatment (Control group, n=14). The CIDR were removed 5 d after the wave emergence. B) Timeline of treatments in Experiment 2. Prepubertal beef heifers received a CIDR insert containing 1.9 g of progesterone and 1 mg of estradiol benzoate (EB; EP group, n=8) or were subjected to follicular ablation (FA group, n=8). All heifers received PGF on Day 7 and CIDRs were removed in EP group.

[1] Ultrasound exams

[2] 500 µg of cloprostenol (PGF2α analog)

[3] Wave emergence

Table 1: Ovulation rate and mean (±SEM) characteristics of the follicular wave of prepubertal heifers treated with CIDR (P group), CIDR plus PGF (PPG group) or no treatment (Control group) for Experiment 1.

	P group	PPG group	Control Group	P-value
Ovulation rate	4/13 (30.8%)[ab]	8/11 (72.7%)[a]	1/14 (7.1%)[b]	<0.001
Proestrus signs	8/13 (61.5%)[a]	9/11 (81.8%)[a]	2/14 (14.3%)[b]	<0.002
Interval from wave emergence to ovulation (days)	8.0±0.0[a]	8.5±0.25[ab]	10.0[b]	0.03
Maximum diameter of the ovulatory follicle (mm)	12.5±0.25[a]	12.7±0.34[a]	11.5±0.25[b]	<0.01
Maximum diameter of anovulatory follicle (mm)	12.5±0.26[a]	11.3±0.16[b]	11.3±0.29[b]	0.017
Growth rate of the dominant follicle (mm/d)	1.1±0.04[a]	1.1±0.06[a]	0.9±0.02[b]	<0.03

Different subscripts in a row indicate difference.

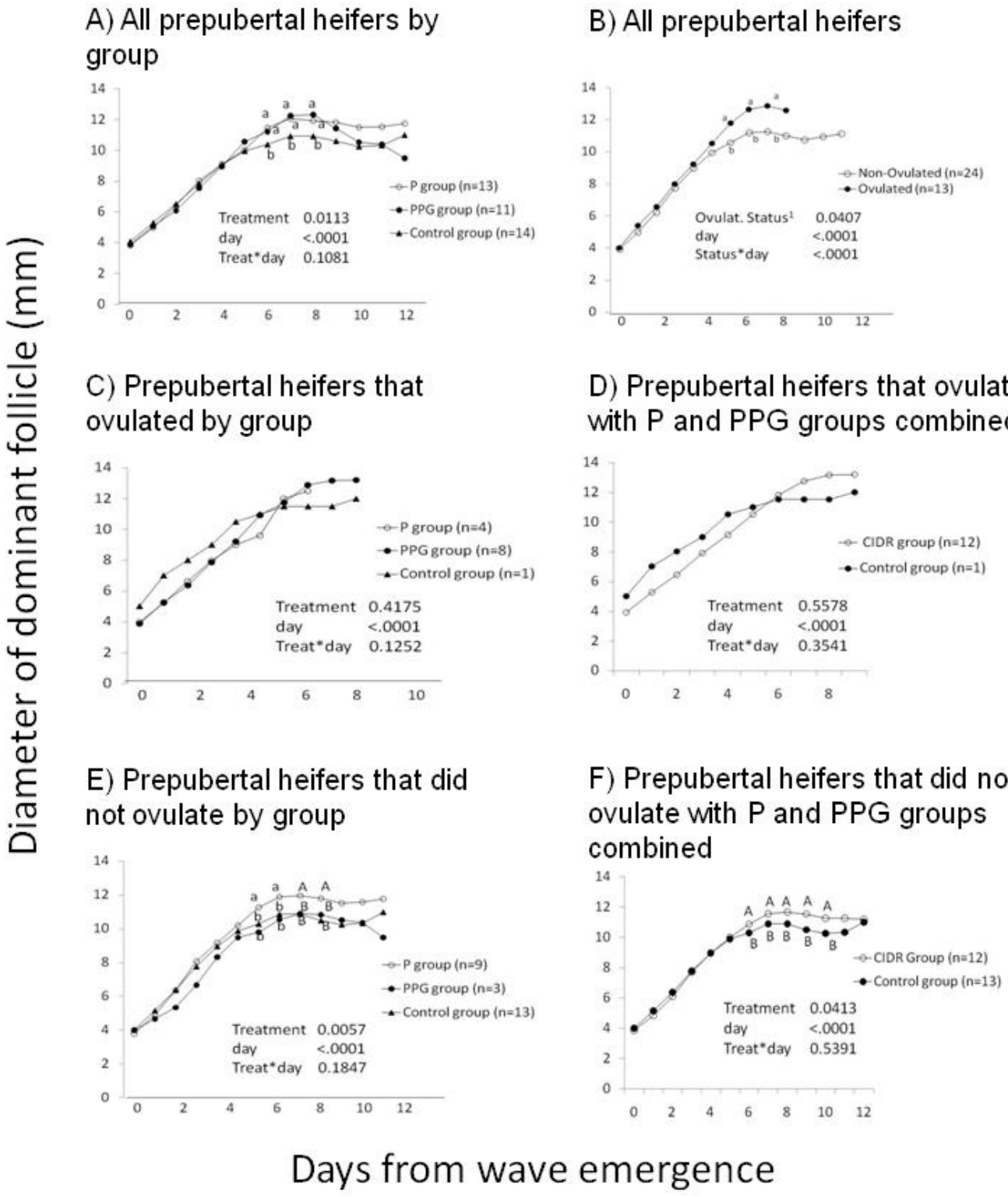

Fig. 2: Pattern of dominant ovarian follicle growth in all pre-pubertal heifers used in Experiment 1. Panel A),C) and E) Heifers in the P (-○-) and PPG (-●-) groups received a CIDR and heifers in the PPG group received 500 µg cloprostenol on the day of CIDR removal (5 d after follicle

wave emergence), whereas the Control group (-▲-) received no treatment. Panel B) Pattern of dominant ovarian follicle growth in prepubertal heifers comparing heifers that ovulated (-●-) with those that did not ovulate (-○-) in all heifers regardless of treatment group. Panel D) and F) the P and PPG groups were combined (-○-), and compared with the Controls (-●-). Bars showing the standard error of the means have been omitted for clarity.

[a,b] Lower case letters within the same day show significant difference ($P<0.05$).

[A,B] Upper case letters within the same day show tendency ($P<0.1$).

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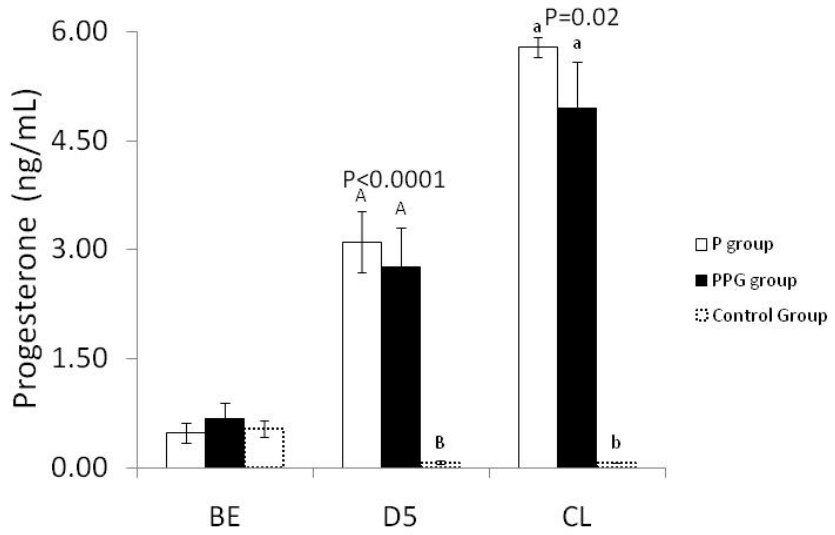

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600 Fig. 3: Mean plasma progesterone concentrations in prepubertal heifers that received CIDR (P
601 group), CIDR plus 500 µg cloprostenol (PPG group) and no treatment (Control group). BE =
602 beginning of the experiment; D5= 5 d after follicular wave emergence; CL = 7 d after ovulation.

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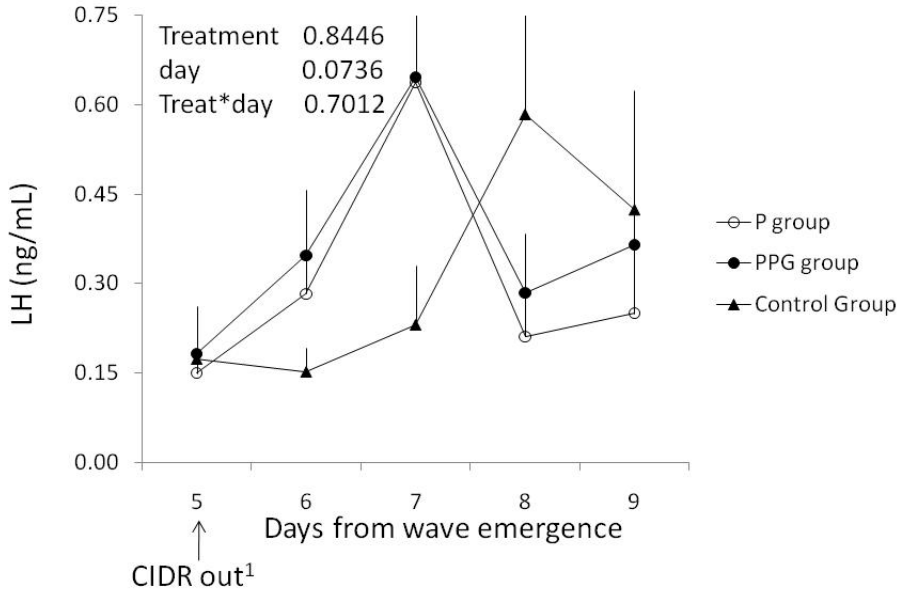

Fig. 4: Profiles of LH concentration in prepubertal that received CIDR (P group; -○-), CIDR plus 500 µg cloprostenol (PPG group; -●-), or no treatment (Control group; -▲-). [1]CIDR out means the Day of the CIDR removal in the P and PPG group.

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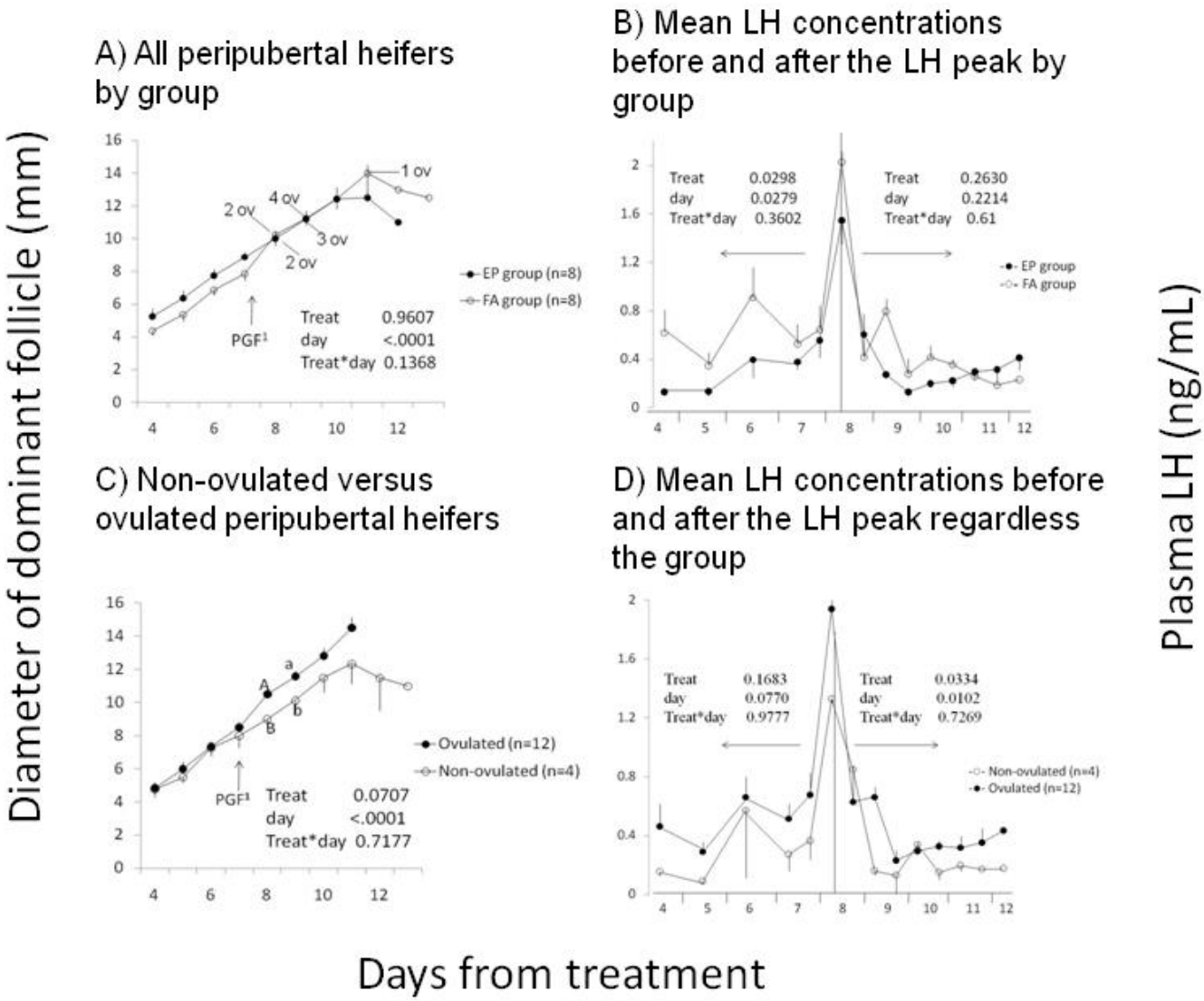

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640 Fig. 5. Panel A) - Pattern of the dominant ovarian follicle growth in peripubertal heifers treated
641 with CIDR and estradiol benzoate (EP group, -●-) and no progesterone (FA group, -○-). Panel B)
642 Mean LH concentrations before and after the LH peak in CIDR treated (EP group, - ○-) and non-
643 treated heifers (FA group,- ●-). Day 4 = 1 day after follicle ablation in FA group. Panel C) -
644 Pattern of the dominant ovarian follicle growth in the peripubertal heifers that ovulated and that
645 did not ovulate. Day 3 = day of follicle ablation in the FA group. The day of follicular wave
646 emergency was normalized to mean for each treatment group. Panel D) Mean LH concentration
647 in ovulated and non-ovulated peripubertal heifers. The black bar meaning the repeated measures
648 results during and after CIDR treatment period. LH data was normalized for each heifer
649 according to its peak. Experiment 2.
650 [a,b] Lower case letters within the same day show significant difference (P<0.05).
651 [A,B] Upper case letters within the same day show tendency (P<0.1). Experiment 2.
652 [1]500 µg cloprostenol i.m.

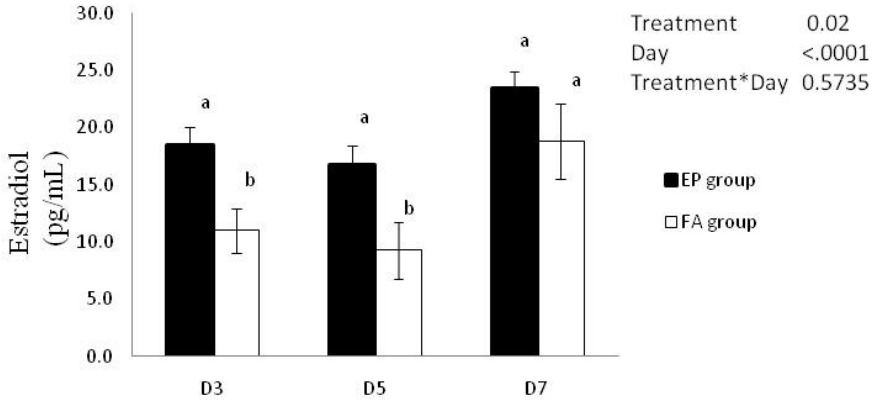

Fig. 6: Mean plasma estradiol concentrations in CIDR treated heifers (EP group) and non-treated heifers (FA group). Estradiol concentrations differed between groups on D3 and on D5, but not at the end of the treatment (D7).

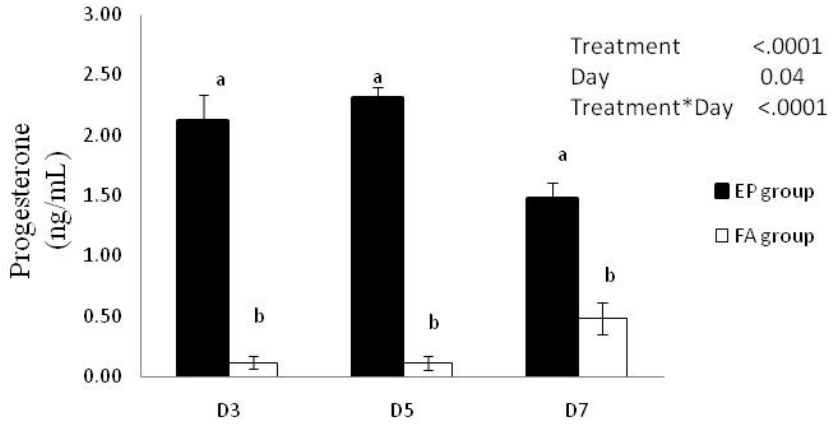

Fig. 7: Mean plasma progesterone concentrations in CIDR treated heifers (EP group) and non-treated heifers (FA group). Progesterone concentrations were higher in EP group ($P<0.0001$) on D 3, 5 and 7.

CONCLUSÕES GERAIS

Como pôde ser visto nesta tese, a progesterona influencia o crescimento folicular, o diâmetro do CL formado e consequente produção de progesterona. Assim, mesmo que de forma indireta, a progesterona está envolvida nos mais importantes eventos de regulação e funcionamento da reprodução dos mamíferos.

Durante o trabalho de doutorado foi possível verificar a importância da progesterona desde as etapas mais fundamentais do início da vida reprodutiva, a puberdade, bem como a sua influência no ciclo estral associado à ação sinérgica com outros hormônios como a prostaglandina, o LH e o estradiol.

De acordo com os dados registrados foi possível verificar que o desenvolvimento folicular sob baixas concentrações de progesterona afeta positivamente o diâmetro do folículo ovulatório e do CL, porém sem afetar a taxa de prenhez. Em novilhas pré-púberes, a progesterona demonstrou um aumento do crescimento folicular, e seu uso associado à $PGF_2\alpha$ apresentou uma efetiva alternativa para a indução da primeira ovulação em novilhas de corte.

Implicações:

Embora as diferentes concentrações de progesterona afetaram tanto o crescimento folicular em fêmeas sincronizadas, quanto a consequentemente a produção de progesterona no período pós-concepção, o protocolo experimental utilizado não permitiu a inferência desta condição sobre a competência ovocitária. Pois, sabe-se que entre a ovulação de um ovócito competente e o estabelecimento da prenhez há uma série de fatores (ex. alimentação, ambiente, stress, etc...) que influenciam as interações

hormonais, a regulação gênica e o ambiente uterino para o desenvolvimento embrionário. Para esclarecer efetivamente o efeito das concentrações de progesterona sobre a competência ovocitária, um novo modelo de estudo deve ser proposto, como por ex., a utillização de punção folicular para recuperar ovócitos submetidos as mesmas condições de crescimento folicular utilizadas neste experimento. Para tanto, é necessário a obtenção de um grande número de animais, para que seja coletado um ovócito de cada folículo dominante por animal. Assim, poderia-se melhor inferir sobre o efeito de altos e baixos níveis de progesterona sobre a competência ovocitária.

Em relação ao experimento 2, um projeto já está em andamento em parceria da UFPel com a USASK, no intuito de aumentar o número de animais e trabalhar com animais mais jovens do que os utilizados no experimento 2, pois se a $PGF_2\alpha$ sem a prévia exposição à progesterona irá induzir a ovulação de um ovócito competente sem induzir a formação de um CL de vida curta ainda é uma incógnita.

4. Anexo

Figuras do segundo artigo que estão compactadas como Figura 2 e Figura 5

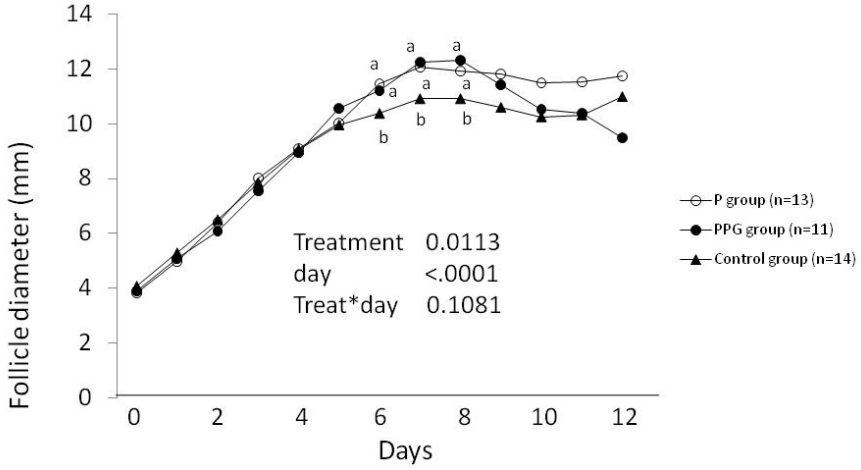

Fig 2 A) All prepubertal heifers by group

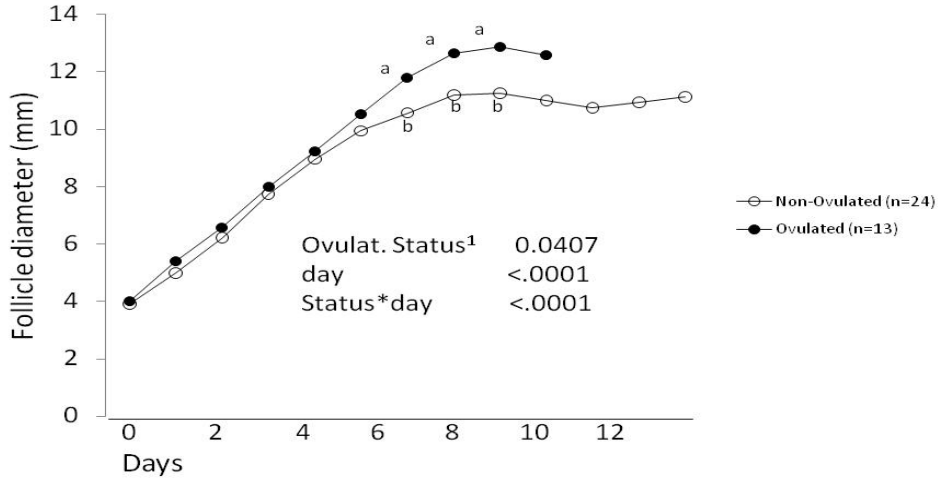

Fig 2 B) All prepubertal heifers

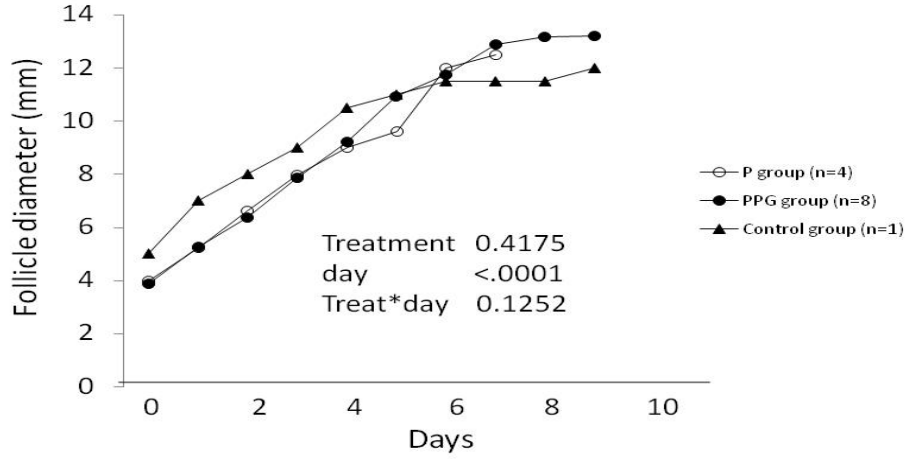

Fig 2 C) Prepubertal heifers that ovulated by group

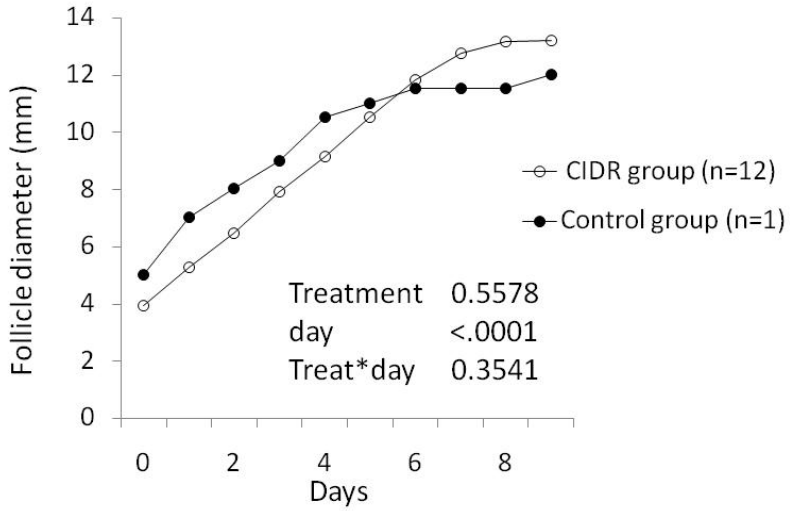

Fig 2 D) Prepubertal heifers that ovulated with P and PPG groups combined

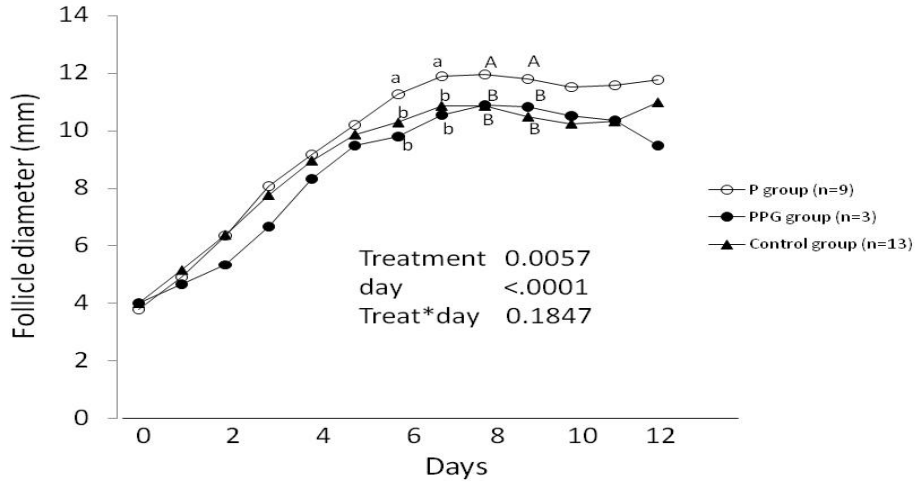

Fig 2 E) Prepubertal heifers that did not ovulate by group

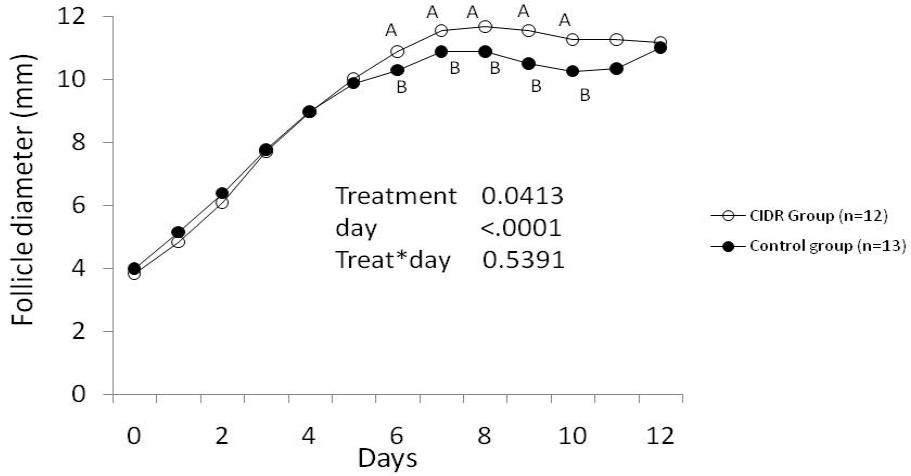

Fig 2 F) Prepubertal heifers that did not ovulate with P and PPG groups combined

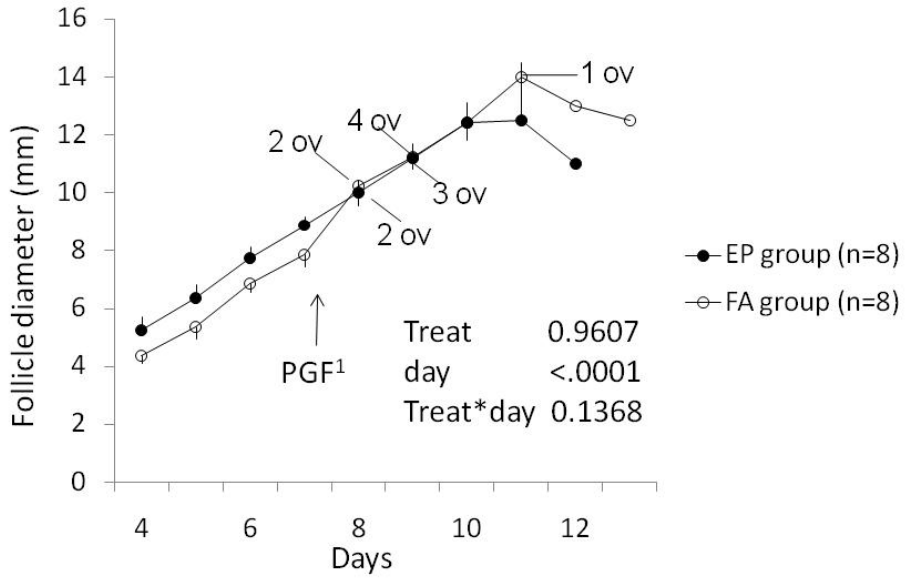

Fig 5 A) All peripubertal heifers

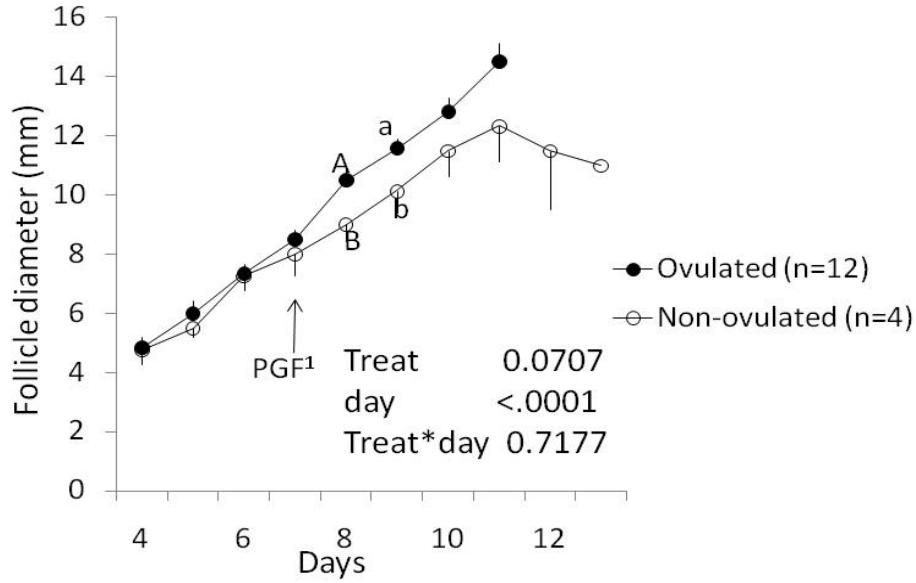

Fig 5 C) Non-ovulated versus ovulated peripubertal heifers

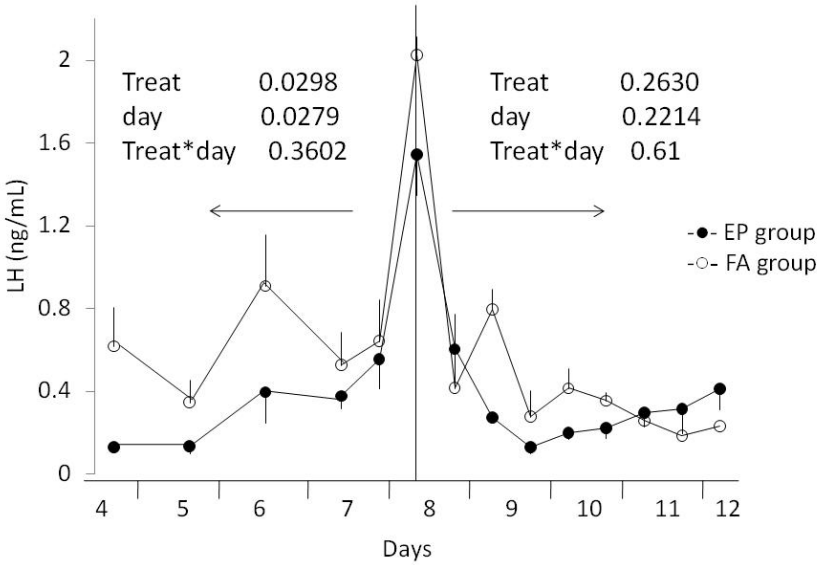

Fig 5 B) Means LH concentrations before and after the LH peak by group

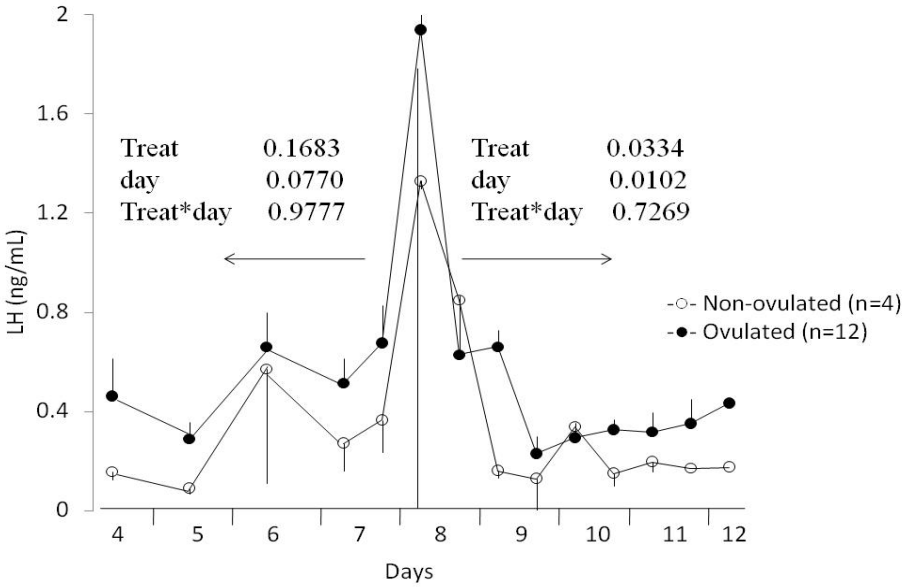

Fig 5 D) Means LH concentrations before and after the LH peak regardless the group

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