

## Prostaglandin F<sub>2α</sub> induced luteolysis is delayed but not prevented by acute administration of luteotropic drugs in lactating nonpregnant Holstein cows

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### Abstract

Inadvertent use of prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) in pregnant cattle could result in luteolysis and pregnancy loss. Using a nonpregnant cow model, we determined if luteotropic agents could counteract luteolytic effects of PGF<sub>2α</sub>. Ovarian status of 20 lactating nonpregnant Holstein cows was synchronized using an Ovsynch protocol and ovulation confirmed by transrectal ultrasonography 48 hours after the second gonadotropin releasing hormone (GnRH). Eight days after ovulation, 25 mg of dinoprost (native PGF<sub>2α</sub>) was administered IM to induce luteolysis. Five minutes after PGF<sub>2α</sub>, cows were treated IM with gonadorelin (GnRH, 100 µg; n = 5), human chorionic gonadotropin (hCG, 1,000 IU; n = 6), porcine pituitary luteinizing hormone (pLH, 25 mg; n = 5), or saline (control, 2 ml; n = 4). Blood samples were collected before PGF<sub>2α</sub> (0 hour) and at 1, 6, 12, 18, 24, 30, 36, 42, 48, 60, 72, and 84 hours after PGF<sub>2α</sub> to monitor subsequent luteal activity by measuring plasma progesterone concentrations. Although none of the treatments counteracted the luteolytic action of PGF<sub>2α</sub>, the rate of decline in plasma progesterone within 1 hour after PGF<sub>2α</sub> was greater (p = 0.04) in control than in GnRH, hCG, and pLH (2.2 versus 0.2, 0.3, and 0.1 ng/ml/hour, respectively). In addition, pLH-treated cows tended (p = 0.07) to have greater overall mean plasma progesterone for up to 84 hours (1.6 ± 0.2 ng/ml) than other treatments combined (1.1 ± 0.1 ng/ml). In the present study, giving GnRH, hCG or pLH 5 minutes after PGF<sub>2α</sub> administration was not effective in countering luteolytic effects of PGF<sub>2α</sub>. Nevertheless, pLH treatment tended to delay PGF<sub>2α</sub> induced luteolysis, which warrants further investigation.

**Keywords:** Bovine pregnancy, abortion, corpus luteum, porcine pituitary LH, progesterone

### Introduction

Corpus luteum (CL), a transient endocrine gland, undergoes dynamic changes to regulate reproductive cyclicity and thereby fertility of mammals. It produces the steroid hormone progesterone (P<sub>4</sub>) that has a key role in establishment and maintenance of pregnancy. In the absence of a conceptus, CL undergoes luteolysis due to prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) released from the uterus.<sup>1</sup> In case of pregnancy, uterine release of PGF<sub>2α</sub> is blocked by bovine interferon-*tau*, the signal of maternal recognition of pregnancy from placental trophoblast cells in ruminants.<sup>2</sup>

As approved products for reproductive management, both native (dinoprost) and synthetic (cloprostenol) preparations of PGF<sub>2α</sub> are used routinely in dairy cattle, especially in estrus and ovulation synchronization protocols, integral to herd breeding programs. As pregnancy maintenance in cattle, at least up to 150 days of gestation is dependent on luteal P<sub>4</sub>,<sup>3</sup> inadvertent use of PGF<sub>2α</sub>, especially during the first 5 months of pregnancy, usually results in abortion. Under such a circumstance, an immediate remedial action that either prevents or delays PGF<sub>2α</sub>-induced luteolysis would be beneficial, at least until exogenous P<sub>4</sub> therapy could be initiated.

That bovine luteinizing hormone (LH) and human chorionic gonadotropin (hCG) have antiluteolytic properties in cattle was first demonstrated in Holstein heifers subjected to oxytocin-induced luteolysis.<sup>4</sup> In early studies in ewes, ovine LH,<sup>5</sup> and gonadotropin releasing hormone (GnRH),<sup>6</sup> extended CL lifespan of, possibly by delaying or countering action of PGF<sub>2α</sub>. In rats, antiluteolytic effect of hCG was evident by pregnancy maintenance in 70% of PGF<sub>2α</sub>-treated pregnant rats.<sup>7</sup> In studies from our laboratory, in cattle treated with exogenous porcine LH (pLH), plasma LH concentrations remained higher than that of controls for up to 20 hours after

treatment.<sup>8-10</sup> Higher than basal concentrations of LH for a prolonged interval in pLH-treated cattle may protect the CL from luteolytic action of PGF<sub>2α</sub> by saturation of luteal membrane LH receptors, as hypothesized.<sup>11</sup> All of the above products (hCG, GnRH, and pLH) are approved for use in dairy cattle in Canada. However, efficacy of these products as luteotropic agents to counteract PGF<sub>2α</sub>-induced luteolysis in cattle has not been adequately investigated.

We hypothesized that a luteotropic agent given to cows soon after PGF<sub>2α</sub> administration prevents or delays luteolysis, as indicated by greater P<sub>4</sub> concentrations compared to cows not given a luteotropic treatment. Therefore, our objective was to determine if luteolytic effects of PGF<sub>2α</sub> in dairy cattle could be counteracted by GnRH, hCG or pLH given 5 minutes after PGF<sub>2α</sub> administration.

## Materials and methods

### Cattle and housing

The study was conducted at the Dairy Research and Technology Centre of the University of Alberta. Cattle were handled and cared for in accordance with the Canadian Council on Animal Care guidelines (2009)<sup>12</sup> and experimental procedures were approved by the Animal Policy and Welfare Committee, Department of Agricultural, Food and Nutritional Science, University of Alberta. Twenty-four lactating nonpregnant Holstein cows (7 primiparous and 17 multiparous) were enrolled in the study. Cows were individually fed a total mixed ration (primary ingredients were barley silage, alfalfa silage, alfalfa hay, and concentrates) and housed in tie stalls and let out for approximately 2 hours of exercise during weekdays. Diets were formulated according to NRC (2001)<sup>13</sup> to meet requirements of a 650 kg lactating cow producing 30 kg of milk/day and delivered individually by a Data Ranger (American Calan Inc., Northwood, NH), once daily at 0800 hour. Cows had ad libitum access to fresh water. Cows averaged (mean ± standard deviation) 3.0 ± 0.4 lactation and 266 ± 25 day postpartum at the beginning of the experiment. Average 305 days milk yield was 9,540 kg.

### Experimental design and treatments

Ovarian status of cows was synchronized using an Ovsynch protocol.<sup>14</sup> In brief, the protocol consisted of GnRH (100 µg gonadorelin acetate, IM; Fertiline<sup>®</sup>, Vetoquinol N. A. Inc. Lavaltrie QC, Canada), followed 7 days later by PGF<sub>2α</sub> (25 mg dinoprost tromethamine, IM; Lutalyse<sup>®</sup>, Zoetis Canada Inc. Kirkland, QC, Canada), and a second GnRH (100 µg gonadorelin acetate, IM) given 48 hours after PGF<sub>2α</sub>. Transrectal ultrasonography (Aloka 500, Aloka Co Ltd., Tokyo, Japan) using a 7.5 MHz linear-array transducer, was first conducted at the time of the second GnRH of Ovsynch to confirm the presence of putative ovulatory follicle(s) (≥ 10 mm in diameter). Ovulation was confirmed 48 hours later by absence of a follicle previously detected. Eight days after ovulation, PGF<sub>2α</sub> (25 mg dinoprost tromethamine, IM) was administered to induce luteolysis in all cows.

Luteotropic treatments were randomly administered IM (n = 6 per treatment), exactly 5 minutes after PGF<sub>2α</sub> administration. Interval between PGF<sub>2α</sub> and luteotropic treatments was an arbitrary yet realistic time frame under field conditions, to take remedial action, if PGF<sub>2α</sub> was administered inadvertently to a pregnant animal and the mistake quickly discovered. Treatments were: GnRH (100 µg gonadorelin acetate, IM; Fertiline<sup>®</sup>); hCG (1,000 IU human chorionic gonadotropin, IM; Chorulon<sup>®</sup>, Intervet, Kirkland, QC, Canada); pLH (25 mg porcine pituitary LH, IM, Lutropin-V, Bioniche Animal Health, Belleville, ON, Canada); or control (2 ml sterile saline, IM).

### Blood sampling and progesterone assay

Blood samples were collected into evacuated tubes containing sodium heparin (Vacutainer, Beckton Dickinson and Co., Franklin Lakes, NJ) from an indwelling jugular

catheter. Blood samples were collected immediately before PGF<sub>2α</sub> (0 hour), 1 and 6 hours after PGF<sub>2α</sub>, then every 6 hours until 48 hours, and thereafter every 12 hours for the next 36 hours, up to 86 hours after PGF<sub>2α</sub> treatment. Samples were placed on ice immediately after collection and centrifuged at 1500 x g for 20 minutes at 4°C, plasma harvested and frozen at -20 °C until assayed for P<sub>4</sub>, in duplicate, using a direct enzyme immunoassay (Quanticheck , Faculty of Veterinary Science, Budapest, Hungary). This assay uses an antiP<sub>4</sub> monoclonal antibody and horseradish peroxidase as the enzyme label, with a sensitivity (lowest detection limit) of 0.5 ng/ml and has been compared to established assays.<sup>15</sup> The intra-assay coefficient of variation was 6.5%.

### Statistical analyses

Differences in plasma P<sub>4</sub> concentrations by luteotropic treatment (GnRH, hCG, pLH, or saline), time of sampling (0 hour before PGF<sub>2α</sub> and 1, 6, 12, 18, 24, 30, 36, 42, 48, 60, 72, and 84 hours after PGF<sub>2α</sub>), parity (primiparous and multiparous), and interactions between luteotropic treatment and time were tested by repeated measures analysis using MIXED procedure of SAS. Four cows (GnRH, n = 2; hCG and pLH, n = 1 each) with P<sub>4</sub> concentrations < 1 ng/ml at 0 hour (before PGF<sub>2α</sub>), indicative of either delayed or poor luteal function, were excluded from the analysis. In the remaining 20 cows, P<sub>4</sub> concentration at 0 hour was used as a covariate to account for potential influence of P<sub>4</sub> at time of PGF<sub>2α</sub> administration on subsequent P<sub>4</sub> concentrations. Time of sampling was used as a repeated measurement nested within cow. Covariance structure of the repeated measurement and appropriate final model were chosen based on the lowest Akaike's Information Criteria. Since pLH-treated cows had greater mean plasma P<sub>4</sub> concentration than GnRH-treated cows, a pairwise comparison between pLH and other groups combined (control, GnRH, and hCG) was also performed using an orthogonal contrast statement. In addition, differences among luteotropic treatments in rate of decline in mean plasma P<sub>4</sub> concentrations from 0 to 1 hour after giving PGF<sub>2α</sub>, were tested using MIXED procedure. Differences in least square means were tested using PDIFF option. Significant differences were reported if  $p \leq 0.05$  and considered a tendency if  $p > 0.05$  and  $\leq 0.10$ .

### Results

Changes in mean plasma P<sub>4</sub> concentrations by luteotropic treatment, time and their interactions are presented in Figure 1. None of the luteotropic treatments countered the luteolytic effect of PGF<sub>2α</sub> ( $p = 0.24$ ), as evident from declines in P<sub>4</sub> concentrations. The rate of decline in plasma P<sub>4</sub> concentrations (ng/ml/hour) within the first hour after PGF<sub>2α</sub> administration, however, differed ( $p = 0.04$ ) between luteotropic treatments. In this regard, cows treated with GnRH ( $0.2 \pm 0.2$ ), hCG ( $0.3 \pm 0.2$ ), or pLH ( $0.1 \pm 0.2$ ) had slower rates of decline than cows treated with saline ( $2.2 \pm 0.2$  ng/ml/hour; Figure 2). Moreover, orthogonal contrast analysis revealed that pLH-treated cows tended to have greater overall mean P<sub>4</sub> concentration from both 0 to 84 hours ( $1.6 \pm 0.2$  versus  $1.1 \pm 0.1$  ng/ml;  $p = 0.07$ ) and from 12 to 84 hours ( $1.0 \pm 0.2$  versus  $0.5 \pm 0.1$  ng/ml;  $p = 0.10$ ) after PGF<sub>2α</sub> administration, compared to mean P<sub>4</sub> concentrations for other treatments combined. Regardless of luteotropic treatment, mean ( $\pm$  SEM) plasma P<sub>4</sub> concentrations (ng/ml) differed by time ( $p < 0.01$ ), declining from 0 to 12 hours after PGF<sub>2α</sub> ( $4.1 \pm 0.2$  [0 hour],  $3.6 \pm 0.2$  [1 hour],  $2.3 \pm 0.2$  [6 hour] and  $0.8 \pm 0.2$  [12 hour]) and then remained unchanged from 12 to 84 hours after PGF<sub>2α</sub>, ranging from 0.4 to 0.7 ng/ml.

### Discussion

Bovine LH and hCG in heifers,<sup>4</sup> ovine LH and GnRH in ewes,<sup>5,6</sup> and hCG in rats,<sup>7</sup> were reported to have antiluteolytic properties against exogenous oxytocin, endogenous PGF<sub>2α</sub>, exogenous estradiol and PGF<sub>2α</sub>, respectively. On the contrary, in the present study, none of the agents, at the administered dose, prevented luteolysis in the nonpregnant cow model, as evident from declines in P<sub>4</sub> concentrations from 0 to 84 hours after PGF<sub>2α</sub> administration.

Few studies have evaluated efficacy of luteotropic agents in preventing or reversing luteolysis in ruminants. In a pioneering study<sup>4</sup> in cyclic Holstein heifers, antiluteolytic effects of bovine LH (30 mg daily), hCG (2,000 IU), urea-treated hCG (2,000 IU) and bovine prolactin (60 mg daily) on oxytocin (0.33 USP units/kg BW) induced luteolysis were evaluated. Both bovine LH and hCG when given concurrently with oxytocin treatment not only overcame luteolytic effects of oxytocin, but also increased CL weight and luteal progesterone concentration, indicative of luteotropic effects. When the LH component of hCG was destroyed by urea treatment before administration, the preparation was no longer luteotropic, indicating that LH is the luteotropin in cattle. Likewise, prolactin also had no luteotropic effect. In a contrasting study,<sup>16</sup> continuous IV infusions of purified bovine LH (10 µg/minute) for 10 hours, starting 4 hours prior to giving 25 mg PGF<sub>2α</sub>, at days 10 - 12 of the estrous cycle in beef cattle, failed to prevent PGF<sub>2α</sub>-induced luteolysis. Intravenous infusions of ovine LH (4 µg/minute), ovine prolactin (42 µg/minute), or LH + prolactin for 12 hours, failed to prevent luteolysis in ewes following IM injections of 6.66 mg PGF<sub>2α</sub> given 2 and 6 hours after infusions began.<sup>17</sup> On the contrary, prolactin had an antiluteolytic effect in pregnant rats when given on day 4 of gestation,<sup>18</sup> and maintained pregnancy in 90% of rats when given on day 10 of gestation.<sup>7</sup> Prolactin and prolactin receptor mRNA are expressed in bovine CL throughout the estrous cycle, suggesting a role for prolactin in luteal function in cattle.<sup>19</sup> However, as pioneering studies in cattle<sup>4</sup> and sheep<sup>17</sup> reported prolactin not to prevent luteolysis, it may not be a suitable candidate for further consideration as an antiluteolytic agent.

Conversely, pLH is a potential candidate for further research as an antiluteolytic agent for two reasons. First, the rate of P<sub>4</sub> decline in the first hour after PGF<sub>2α</sub> administration was the smallest and second, the overall mean plasma P<sub>4</sub> concentration up to 84 hours was highest in pLH-treated cows in the present study. Although the overall mean P<sub>4</sub> concentration of 1.6 ng/ml in pLH-treated cows was greater than that of all other treatments, such a low P<sub>4</sub> concentration during diestrus is likely insufficient to sustain pregnancy, particularly in lactating dairy cows that have lower plasma P<sub>4</sub> concentrations, likely due to increased P<sub>4</sub> metabolism.<sup>20</sup> Small and repeated doses of hCG prevented estradiol-induced luteolysis in macaques (*Macaca fascicularis*).<sup>21</sup> Therefore, using a conceptually similar approach, it would be worth investigating whether repeated doses of pLH treatment would effectively counteract luteolysis in cattle.

Inducing formation of a new CL could be another strategy to increase endogenous P<sub>4</sub> to maintain pregnancy. However, this process takes time and cannot be implemented quickly when PGF<sub>2α</sub> is inadvertently administered to pregnant cattle under field conditions. In this regard, when an induced CL formed on the ovary ipsilateral to the pregnant horn after forced lysis of the original CL (while the pregnancy was temporarily sustained by exogenous P<sub>4</sub>), was capable of maintaining the pregnancy even after exogenous P<sub>4</sub> supplementation ended.<sup>22,23</sup> More recently, it was reported<sup>24</sup> that 72% (18 of 25) of pregnant beef cows and heifers maintained their pregnancies for at least 7 days after induced luteolysis, when exogenous P<sub>4</sub> treatments (100 mg SC daily for 7 days) were given, with the first P<sub>4</sub> injection starting 2, 6, 10, 12, or 18 hours after PGF<sub>2α</sub> was given during days 30 - 90 of pregnancy. When an accessory CL was successfully induced using hCG in a subset of those cattle (mean ± SD, 38 ± 5.5 days after PGF<sub>2α</sub>), 80% carried the pregnancy to term.<sup>24</sup> Authors concluded that pregnancies could be maintained following an accidental administration of PGF<sub>2α</sub> without the need for extended P<sub>4</sub> supplementation, when an accessory CL was successfully induced.

An accessory CL could be induced with GnRH or pLH, perhaps as effectively as hCG. However, to prevent P<sub>4</sub> concentrations from plummeting to basal concentrations after accidental PGF<sub>2α</sub> administration, a quick remedial measure may be to concurrently insert two intravaginal P<sub>4</sub> devices to maintain higher circulating P<sub>4</sub> concentration (2.7 ng/ml)<sup>25</sup> than attainable with a single P<sub>4</sub> device (1.0 ng/ml),<sup>26</sup> thereby protecting the embryo from exposure to very low P<sub>4</sub> concentrations due to PGF<sub>2α</sub>-induced decline in luteal P<sub>4</sub>. As many dairy farms and bovine practitioners are likely to have intravaginal progesterone devices readily available, this approach

would be more practical than progesterone injections. Thus, future studies should investigate a combined treatment approach of repeated injections of pLH to maximize LH support to the CL exposed to PGF<sub>2α</sub> and insertion of two intravaginal progesterone devices to maintain P<sub>4</sub> concentrations that could sustain a pregnancy. If a pregnancy could be sustained temporarily with this combined treatment strategy, inducing an accessory CL at the earliest opportunity should be actively considered as a permanent solution to sustain the pregnancy to term, as demonstrated in cattle.<sup>24</sup> Although pLH is available in the Canadian market, to the authors' knowledge there is no FDA approved pLH that is currently available in the United States. The treatment strategies proposed in this paper would be extra label use in many jurisdictions; therefore, any such treatment must occur under the order and discretion of a veterinarian.

### **Conclusion**

In the present study, giving GnRH, hCG or pLH 5 minutes after PGF<sub>2α</sub> administration were not effective in countering luteolytic effects of PGF<sub>2α</sub> in a nonpregnant lactating cow model. Nevertheless, pLH treatment tended to delay PGF<sub>2α</sub>-induced luteolysis, which warrants further investigation.

### **Annotation**

Using any of the luteotropic drugs or exogenous progesterone for the purpose described in the manuscript may constitute extra label use and should only be used at the discretion of a practitioner who has an established veterinarian-patient-client-relationship.

### **Acknowledgement**

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### **Authors' contribution**

DA: Study concept, experimental design, data collection, manuscript writing; MC: experimental design, data collection, progesterone assays, manuscript review; MG: data analysis, manuscript writing.

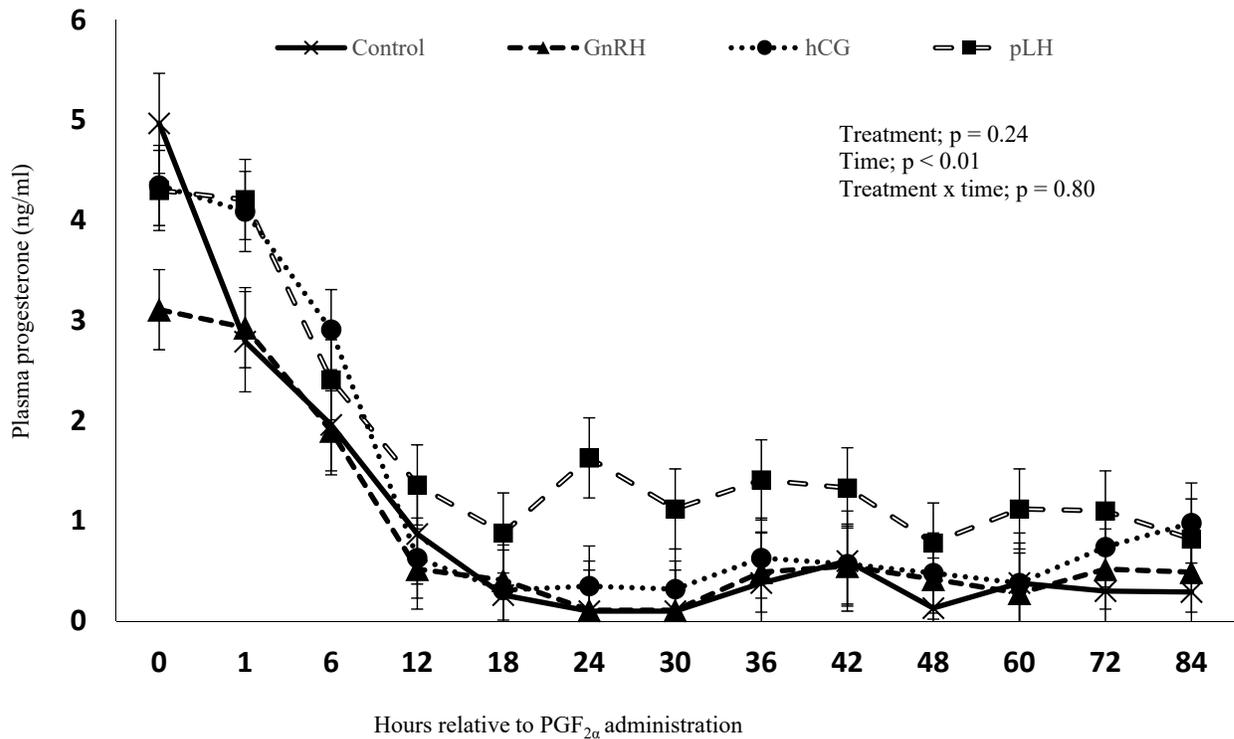
### **Conflict of interest**

Authors declare no conflicts of interest.

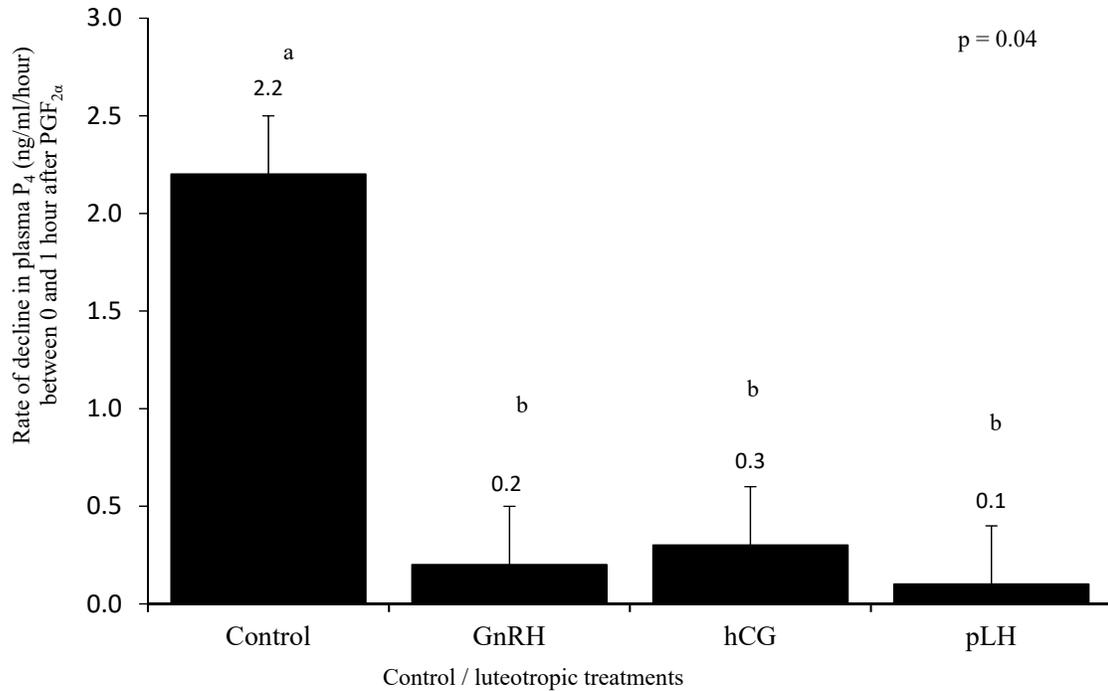
### **References**

1. Thatcher WW, Wolfenson D, Curl JS, et al: Prostaglandin dynamics associated with development of the bovine conceptus. *Anim Reprod Sci* 1984;7:149-176.
2. Bazer FW, Song G, Thatcher WW: Roles of Conceptus secretory proteins in establishment and maintenance of pregnancy in ruminants. *Asian Australas J Anim Sci* 2012;25:1-16.
3. Gross TS, Williams WF: In vitro steroid synthesis by the placenta of cows in late gestation and parturition. *J Reprod Fertil* 1988;83:565-573.
4. Donaldson LE, Hansel W, Van Vleck LD: Luteotropic properties of luteinizing hormone and nature of oxytocin induced luteal inhibition in cattle. *J Dairy Sci* 1965;48:331-337.
5. Karsch FJ, Roche JF, Noveroske JW, et al: Prolonged maintenance of the corpus luteum of the ewe by continuous infusion of luteinizing hormone. *Boil Reprod* 1971;4:129-136.
6. Adams TE, Kinder JE, Chakraborty PK, et al: Ewe luteal function influenced by pulsatile administration of synthetic LHRH/FSHRH. *Endocrinol* 1976;97:1460-1467.
7. Chatterjee A: The possible mode of action of prostaglandins: X. Antagonism between prostaglandin F<sub>2a</sub> and prolactin or human chorionic gonadotropin: a comparative study. *Prostaglandins* 1976;12:525-534.
8. Ambrose JD, Kastelic JP, Rajamahendran R, et al: Progesterone (CIDR)-based timed AI protocols using GnRH, porcine LH or estradiol cypionate for dairy heifers: ovarian and endocrine responses and pregnancy rates. *Theriogenology* 2005;64:1457-1474.

9. Ree TO, Colazo MG, Lamont AGA, et al: The effect of porcine luteinizing hormone in the synchronization of ovulation and corpus luteum development in nonlactating cows. *Theriogenology* 2009;72:120-128.
10. Behrouzi A, Colazo MG, Ambrose DJ: Alterations in bone morphogenetic protein 15, growth differentiation factor 9, and gene expression in granulosa cells in preovulatory follicles of dairy cows given porcine LH. *Theriogenology* 2016;85:1249-1257.
11. Henderson KM, McNatty KP: A biochemical hypothesis to explain the mechanisms of luteal regression. *Prostaglandins* 1975;9:779-797.
12. Canadian Council on Animal Care Guidelines on the Care and Use of Farm Animals in Research, Teaching and Testing. 2009. [http://ccac.ca/Documents/Standards/Guidelines/Farm\\_Animals.pdf](http://ccac.ca/Documents/Standards/Guidelines/Farm_Animals.pdf)
13. National Research Council. Nutrient Requirements of Dairy Cattle. 7<sup>th</sup> edition, National Academic Press, Washington, DC. 2009.
14. Pursley JR, Mee MO, Wiltbank, MC: Synchronization of ovulation in dairy cows using PGF<sub>2α</sub> and GnRH. *Theriogenology* 1995;44:915-923.
15. Colazo MG, Ambrose DJ, Kastelic JP, et al: Comparison of 2 enzyme immunoassays and a radioimmunoassay for measurement of progesterone concentrations in bovine plasma, skim milk, and whole milk. *Can J Vet Res* 2008;72:32-36.
16. González-menció F, Murphy BD, Manns J: Failure of exogenous LH to prevent PGF<sub>2α</sub>-induced luteolysis in beef cows. *Prostaglandins* 1977;14:535-542.
17. Sasser RG, Niswender GD, Nett TM: Failure of LH and/or prolactin to prevent PGF<sub>2α</sub>-induced luteolysis of ovine corpora lutea. *Prostaglandins* 1977;13:1201-1208.
18. Fuchs A, Mok E, Sundaram K: Luteolytic effects of prostaglandins in rat pregnancy, and reversal by luteinizing hormone. *Eur J Endocrinol* 1974;76:583-596.
19. Shibaya M, Murakami S, Tatsukawa Y, et al: Bovine corpus luteum is an extrapituitary site of prolactin production. *Mol Reprod Dev* 2006;73:512-519.
20. Sangsritavong S, Combs DK, Sartori RF, et al: High feed intake increases liver blood flow and metabolism of progesterone and estradiol 17b in dairy cattle. *J Dairy Sci* 2002;85:2831-2842.
21. Westfahl PK, Horton LE, Stadelman HL, et al: Small doses of human chorionic gonadotropin prevent estradiol-induced luteolysis. *Endocrinol. Metab* 1984;10:E84-E87.
22. Lulai C, Dobrinski I, Kastelic JP, et al: Induction of luteal regression, ovulation and development of new luteal tissue during early pregnancy in heifers. *Anim Reprod Sci* 1994;35:163-172.
23. Bridges PJ, Wright DJ, Buford WI, et al: Ability of induced corpora lutea to maintain pregnancy in beef cows. *J Anim Sci* 2000;78:2942-2949.
24. Ferguson CE, Kesler DJ, Godke RA: Maintenance of pregnancy in beef cattle after a luteolytic dose of prostaglandin F<sub>2α</sub>. *Vet Med Anim Sci* 2014;2:1.
25. Bisinotto RS, Ribeiro ES, Lima F, et al: Targeted progesterone supplementation improves fertility in lactating dairy cows without a corpus luteum at the initiation of the timed artificial insemination protocol. *J Dairy Sci* 2013;96:2214-2225.
26. Cerri RL, Rutigliano HM, Chebel RC, et al: Progesterone concentration, follicular development and induction of cyclicity in dairy cows receiving intravaginal progesterone inserts. *Anim Reprod Sci* 2009;110:56-70.



**Figure 1.** Plasma progesterone (P<sub>4</sub>) concentrations at administration of PGF<sub>2α</sub> (25 mg dinoprost; 0 hour) and at 1, 6, 12, 18, 24, 30, 36, 42, 48, 60, 72 and 84 hours after either a luteotropic or saline (control) treatment given 5 minutes after PGF<sub>2α</sub> in dairy cows. Luteotropic treatments were gonadotropin releasing hormone (GnRH; 100 μg IM; n = 5), human chorionic gonadotropin (hCG; 1,000 IU; n = 6), porcine pituitary luteinizing hormone (pLH; 25 mg IM; n = 5) or control (sterile saline; 2 ml IM; n = 4). There was no overall counteracting effect of luteotropic treatment on PGF<sub>2α</sub>-induced luteolysis (p = 0.24); however, orthogonal contrast analysis revealed that mean P<sub>4</sub> concentration tended to be greater in pLH treated cows up to 84 hours after PGF<sub>2α</sub> administration compared to the overall mean for other treatments (1.6 ± 0.2 versus 1.1 ± 0.1 ng/ml; p = 0.07). Irrespective of the luteotropic treatment given, plasma P<sub>4</sub> concentrations differed (p < 0.01) by time, with the time effect being most evident between 0 and 12 hours. Concentrations of P<sub>4</sub> remained unchanged between 12 and 84 hours after PGF<sub>2α</sub> treatment. However, there was no interaction between categories of luteotropic treatment and time (p = 0.80).



**Figure 2.** Mean ( $\pm$  SEM) rate of decline in plasma progesterone ( $P_4$ ) concentrations between 0 and 1 hour after  $PGF_{2\alpha}$  (25 mg dinoprost) administration in lactating dairy cows. A luteotropic agent or control treatment was given 5 minutes after  $PGF_{2\alpha}$  (0 hour). Luteotropic treatments were gonadotropin releasing hormone (GnRH; 100  $\mu$ g IM;  $n = 5$ ), human chorionic gonadotropin (hCG; 1,000 IU;  $n = 6$ ), porcine pituitary luteinizing hormone (pLH; 25 mg IM;  $n = 5$ ) or control (sterile saline; 2 ml IM;  $n = 4$ ). Cows treated with GnRH ( $0.2 \pm 0.2$ ), hCG ( $0.3 \pm 0.2$ ) or pLH ( $0.1 \pm 0.2$ ) had a smaller rate of decline than those treated with saline ( $2.2 \pm 0.2$  ng/ml/hour).  
<sup>a,b</sup>Columns without a common superscript differed ( $p = 0.04$ ).