# Improving Beef Cattle Reproductive Efficiency: Hormonal and Fertility Responses to Different Doses of Prostaglandin $F_{2\alpha}$ in Postpartum Beef Cows Subjected to a Timed-Artificial Insemination Protocol

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**AUTHORIZATION TO SUBMIT THESIS**

This thesis of McKenzie Reese Corpron, submitted for the degree of Master of Science with a Major in Animal Science and titled "Improving Beef Cattle Reproductive Efficiency: Hormonal and Fertility Responses to Different Doses of Prostaglandin $F_{2\alpha}$ in Postpartum Beef Cows Subjected to a Timed-Artificial Insemination Protocol," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

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# ABSTRACT

Less than 10% of beef cow-calf producers in the United States currently implement reproductive technologies such as estrous synchronization and artificial insemination (AI) as a component of their breeding management program. Synchronization protocols aid in the synchrony of estrus and ovulation to improve cattle fertility, however, many protocols often require multiple hormone injections and animal handlings. Development of estrous synchronization protocols that limit number of injections, cost, and labor inputs, without compromising fertility, may improve the adoption of fixed-time artificial insemination (FTAI) within the beef industry. The main objective of the first study was to compare the effect of a single injection of high-concentration (HICON) prostaglandin $F_{2\alpha}$ (PGF) or two conventional PGF injections (2PGF) administered 8 h apart at controlled internal drug release insert (CIDR) removal, on reducing serum progesterone ($P_4$) before FTAI and on subsequent pregnancy per artificial insemination (P/AI) in postpartum beef cows (n=404) synchronized with a 5-d CO-Synch + CIDR breeding program. Serum $P_4$ concentration at the time of insemination is a determining factor of cattle fertility in FTAI protocols, as lower concentrations have been associated with increased P/AI. A secondary objective of the first study was to assess the relationship between $P_4$ concentration at the time of insemination and probability of pregnancy to AI. Progesterone concentrations at AI were lesser in 2PGF than HICON, however, P/AI was not different between treatments. Probability of pregnancy decreased as $P_4$ concentration at AI increased and the optimal $P_4$ cutoff concentration to optimize fertility was determined at 0.43 ng/mL. Intramuscular (i.m.) route of injection is commonly used to administer products used in estrous synchronization in beef cattle; however, this route causes greater incidence of injection-site lesions and tissue damage

associated with reduced beef carcass quality and tenderness than subcutaneous (s.c.) routes. Therefore, use of s.c. administration is favorable to reduce carcass damage. The objective of the second study was to compare the ability of a single s.c. injection of high-concentration PGF (HICON-SC) with two i.m. injections of conventional PGF 6 h apart (2PGF-IM) to reduce $P_4$ concentration and induce luteolysis by the time of AI in a 5-d CO-Synch + CIDR synchronization program in postpartum beef cows. A similar decline in $P_4$ concentration occurred in both treatments following PGF, indicating s.c. administration of HICON could be an effective alternative to i.m. administration of the conventional double PGF dose to induce luteolysis before AI in the 5-d CO-Synch + CIDR protocol.

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## DEDICATION

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| 3βHSD | 3β-hydroxysteroid-$\Delta^5$-$\Delta^4$-isomerase |
| AI | Artificial Insemination |
| ANGPT | Angiopoietin |
| ANG II | Angiotensin II |
| BCS | Body Condition Score |
| BW | Body Weight |
| CIDR | Controlled Internal Drug Release |
| CL | Corpus Luteum |
| COX | Cyclooxygenase |
| CYP11A1 | Cytochrome P450 Side Chain Cleavage |
| DPP | Days Postpartum |
| $E_2$ | Estradiol |
| EDN1 | Endothelin-1 |
| ET | Embryo Transfer |
| ES | Estrous Synchronization |
| FSH | Follicle Stimulating Hormone |
| FTAI | Fixed-time Artificial Insemination |
| GLM | General Linear Model |
| GLIMMIX | General Linear Mixed Model |
| GnRH | Gonadotropin-Releasing Hormone |
| HDL | High Density Lipoprotein |
| i.m. | Intramuscular |
| i.v. | Intravenous |
| i.v.s.m. | Intravulvosubmucosal |
| LDL | Low Density Lipoprotein |
| LH | Luteinizing Hormone |
| LLC | Large Luteal Cells |
| OT | Oxytocin |
| NO | Nitric Oxide |

| | |
|---|---|
| $P_4$ | Progesterone |
| P/AI | Pregnancy per Artificial Insemination |
| P/ET | Pregnancy per Embryo Transfer |
| PGF | Prostaglandin $F_{2\alpha}$ |
| PKA | Protein Kinase-A |
| PKC | Protein Kinase-C |
| RIA | Radioimmunoassay |
| ROC | Receiver Operating Characteristics |
| SLC | Small Luteal Cells |
| s.c. | Subcutaneous |
| StAR | Steroidogenic Acute Regulatory protein |
| TAI | Timed Artificial Insemination |

# CHAPTER ONE

Review of Literature

## ESTROUS CYCLE

Cattle are considered a polyestrous species, as they have multiple estrous cycles throughout the year. The length of the bovine estrous cycle is 21 ± 3 days and is composed of two distinct phases, the follicular and luteal phases. Each phase is defined by the dominant structure present on the ovary during that time period. The follicular phase is the shortest of the two and spans 3-5 days, comprising approximately 20% of the total cycle, whereas the luteal phase composes the other 80% (16-18 days) of the bovine estrous cycle (Salisbury et al., 1978; Senger, 2012).

### Follicular Phase

The follicular phase of the estrous cycle is defined as the period between regression of a corpus luteum (CL) and ovulation of a dominant ovarian follicle. During this phase, the primary ovarian structures are developing follicles that are comprised of two main steroidogenic cell types, theca and granulosa cells. These cells function under the influence of pituitary-derived gonadotropins. Cells of the theca interna synthesize and secrete testosterone under the influence of luteinizing hormone (LH) and the theca externa cell layer provides structural support to the follicle. Testosterone from the theca interna cells diffuses into adjacent granulosa cells where it is converted to 17β-estradiol ($E_2$) under the stimulus of follicle stimulating hormone (FSH; Senger, 2012).

The follicular phase is further subdivided into two stages, proestrus and estrus. Proestrus is defined as the time between functional regression of a CL and the onset of estrus (Chenault et al., 1975). The length of proestrus as well as hormonal patterns may differ by cattle breed, but on average it accounts for approximately 2-3 days of the estrous cycle. Two

critical hormonal changes occur during this stage; a decrease in progesterone ($P_4$) associated with CL regression, and an increase in $E_2$ associated with the growth of one or more dominant follicles (Wiltbank et al., 2014a). Subsequent to diminishing $P_4$ concentrations, the negative feedback on the hypothalamus from $P_4$ is removed allowing for tonic release of gonadotropin-releasing hormone (GnRH) from the paraventricular nucleus in the brain. This sustained release of GnRH signals gonadotroph cells in the anterior pituitary to produce and secrete LH and FSH (Conn and Crowley, 1994; Senger, 2012). It should be noted that during the preceding luteal phase, FSH is highly involved with follicle recruitment. Under the influence of low amplitude, high frequency release of FSH and LH, recruited follicles continue to grow and produce increasing concentrations of $E_2$ and inhibin throughout proestrus (Haughian et al., 2013). Inhibin, derived from the largest follicle, acts together with ovarian $E_2$ upon the anterior pituitary to reduce the release of FSH (without affecting pituitary LH secretion). As a result, the growth of subordinate antral follicles is suppressed. This allows for further growth of selected follicles, which are largely influenced by increasing LH pulse frequency towards the end of the follicular phase (Bleach et al., 2001). Selected follicles will continue on to dominance and ovulation at the end of estrus.

During the estrus stage (late follicular phase), $E_2$ reaches peak concentration and is responsible for inducing sexual behavior as well as profound physiological changes within the reproductive tract before ovulation. Elevated $E_2$ from a growing dominant follicle acts upon the uterus to increase expression of its own receptors as well as receptors for $P_4$ and oxytocin (OT) on the endometrium (Spencer and Bazer, 2004). After reaching a threshold concentration, $E_2$ acts through a positive feedback mechanism to cause the release of GnRH from the hypothalamic surge center, resulting in preovulatory surge of LH and FSH from the

anterior pituitary (Haughian et al., 2004). Ovulation of one or more dominant follicles occurs in response to the large surge in LH (typically one follicle in bovine), and granulosa and theca cells begin to undergo luteinization, marking the end of the follicular phase. Episodic release of LH leading up to its preovulatory surge is important for inducing final maturation of the dominant ovarian follicle, ovulation, and proper development of the CL (Hunter, 1991; Quintal-Franco et al., 1999). In the absence of preovulatory LH pulses, LH receptors on theca and granulosa cells are inadequately expressed, resulting in a smaller ovulatory follicle and CL size (Suter et al., 1980). During the periovulatory period between the LH surge and ovulation, a final event occurs before follicular rupture involving the continued meiotic maturation of the oocyte, which is required for successful fertilization (Liu et al., 2013).

## Luteal Phase

The luteal phase is the longest phase of the estrous cycle that succeeds ovulation and is classified by the presence of one or more corpora lutea on the ovary. This phase is composed of two stages, metestrus and diestrus. In cattle, metestrus starts after ovulation and spans approximately three days. Diestrus begins around three days following ovulation and ends on day 17 to 18 of the estrous cycle when luteolysis occurs. Throughout the luteal phase, four important events take place; CL development and maturation, increasing $P_4$ production, continuation of follicular waves, and luteal regression. In the subsequent sections, $P_4$ synthesis and luteal regression will be discussed in more detail.

### *Corpus Luteum Development*

Metestrus begins with the ovulation of a dominant follicle, whereby the granulosa and theca interna cells intermix and undergo luteinization, and ruptured blood vessels form a clot within the follicular cavity, known as a corpus hemorrhagicum (Senger, 2012). This structure

is present on the ovary for 1 to 3 days. During metestrus, follicular cells go through a structural and functional transition, whereby the granulosa and theca cells of the preexisting follicle begin differentiating into large luteal cells (LLCs) and small luteal cells (SLCs), respectively. The CL also contains other non-steroidogenic cell types including vascular endothelial cells, fibroblasts, and immune cells such as leukocytes and macrophages (Lei et al., 1991). Both steroidogenic cell types produce $P_4$; however, the LLCs comprise 30% of the steroidogenic cells of the CL and contribute 70% of the luteal-derived $P_4$. Interestingly, these cells are minimally stimulated by LH to produce $P_4$. The SLCs comprise 70% of the steroidogenic cells and only produce 30% of the $P_4$, but are highly stimulated by LH (Farin et al., 1989; Niswender et al., 1994). Under the influence of pulsatile LH, these cells are maintained and work in synchrony to synthesize and secrete $P_4$, which is required for the establishment and maintenance of pregnancy. As the CL matures and increases in size, blood $P_4$ concentrations increase throughout the luteal phase. The CL continues to produce elevated concentrations of $P_4$ until late in the luteal phase when luteolysis occurs.

***Follicular Waves***

During the luteal phase, follicle growth continues in a wave-like manner under the tonic secretion of LH and low peaks of FSH production; however, because of elevated $P_4$ concentrations, follicles that reach dominance do not ovulate. At the end of each wave during the luteal phase, non-ovulated follicles undergo atresia, $E_2$ concentrations decline temporarily, and a new follicle wave is initiated by another surge in FSH (Bergfelt et al., 1997). Typically, cattle have 2 to 3 waves each estrous cycle. Each follicle wave consists of a group of growing antral follicles from which a dominant follicle is selected. The remaining cohort of follicles become subordinate and undergo atresia (Ginther et al., 1996). Emergence of the first

follicular wave occurs on the day ovulation (Day 0 of estrous cycle), whereas the second wave emerges 9 to 10 days into the estrous cycle in 2-wave cycles, and 8 to 9 days later in 3-wave cycles. In cows with 3-wave cycles, the third wave usually emerges around Day 15 or 16 of the estrous cycle (Colazo and Mapletoft, 2014).

Responsiveness of follicles to FSH and LH determines recruitment of follicular waves and selection of a dominant follicle. Surges in circulating FSH elicit follicle wave emergence, which is quickly followed by suppression of FSH through elevations in inhibin and $E_2$ from the growing follicles (Adams et al., 1992a). In each wave, the follicle to reach dominance is also the first to acquire LH receptors and become FSH independent, while other follicles remain dependent upon FSH to grow and therefore become atretic. Selection of the dominant follicle is determined by a deviation in growth rate between the dominant (~8.5 mm) and largest subordinate follicle, also called diameter deviation (Ginther et al., 1996). Shortly after diameter deviation (~1 day), the dominant follicle acquires ovulatory capacity (increase in LH receptors) through the pulsatile release of LH (Sartori et al., 2001). However, first wave (and second wave, in 3-wave cycles) follicles that reach dominance normally enter a static phase and regress, due to a lack of LH-receptor acquisition on granulosa cells and because the preovulatory surge of LH is suppressed by luteal $P_4$ secretion. Follicle stimulating hormone resurges following the termination of the previous wave, a new wave emerges, and the same pattern is repeated. Luteal regression and reduction of circulating $P_4$ at the end of diestrus allows the dominant follicle present on the ovary at the time (usually second or third wave follicles in natural estrous cycles) to continue developing throughout the proestrus period, and ultimately achieve ovulation (Ginther et al., 1996). As indicated earlier, following ovulation,

follicular cells undergo reconstruction and remodeling to form luteal tissue, which becomes the presumptive CL, and begins synthesizing and secreting $P_4$.

## LUTEAL PROGESTERONE SYNTHESIS

All steroid hormones are derived from cholesterol. Production of $P_4$ is the simplest of the steroidogenic pathway, as it requires few enzymatic conversions. The cholesterol precursor for $P_4$ production within luteal cells can be derived from multiple sources including circulating lipoproteins, cholesterol ester stores, free cholesterol, and de novo synthesis (Knickerbocker, 1988). Lipoproteins in circulation such as high-density lipoprotein (HDL) and low-density lipoprotein (LDL) are the major source of cholesterol for luteal steroidogenesis (Grummer and Carroll, 1988; Wiltbank et al., 1990). Movement of free cholesterol within the cell requires steroidogenic acute regulatory protein (StAR). Steroidogenesis is highly regulated by the activity of the StAR protein, which functions to transport cholesterol from the outer to the inner mitochondrial membrane in luteal cells (Diaz et al., 2002). Once cholesterol reaches the mitochondria, the enzyme cytochrome P450 side chain cleavage (CYP11A1), located on the inner mitochondrial membrane, catalyzes the conversion of cholesterol to pregnenolone. Pregnenolone diffuses out of the mitochondria to the smooth endoplasmic reticulum where it is further converted to $P_4$ by the enzyme 3β-hydroxysteroid-$\Delta^5$-$\Delta^4$-isomerase (3βHSD; Couet et al., 1990). Subsequent progesterone diffuses into the adjacent vasculature and travels to target tissues.

Expression of 3βHSD, CYP11A1, and StAR mRNA are all increased under the influence of LH, with peak expression occurring 8 to 11 d after ovulation in cattle (Couet et al., 1990; Niswender et al., 2000). Luteinizing hormone stimulates the cAMP/protein kinase-A (PKA) pathway in luteal cells, which further activates other intracellular mechanisms

involved in steroid synthesis. Although LH does not acutely regulate luteal steroidogenesis, as this process is predominately mediated by cholesterol availability, it has been shown to increase $P_4$ synthesis, especially in SLCs (Wiltbank et al., 1993). In addition, 3βHSD and CYP11A1 have been shown to decrease within 24 h after a luteolytic dose of PGF is administered; however, given that luteal $P_4$ production decreased more rapidly (within 12 h following PGF), it appears unlikely that suppression of 3βHSD and CYP11A1 expression is a critical point of steroidogenic regulation in the CL (Hawkins et al., 1993; Niswender et al., 2000, Diaz et al., 2002). It does appear, however, that the key rate-limiting factor in luteal $P_4$ production is the movement of cholesterol from the outer to the inner mitochondrial membrane by StAR (Diaz et al., 2002). For instance, in cattle under environmental heat stress, reduced expression of mRNA for StAR has been observed, which was associated with impaired luteal steroidogenesis (Lian et al., 2016). Negative energy balance also influences StAR transcription. Specifically, the primary regulators of StAR-transcription (i.e. LH) decrease during states of negative energy balance, leading to decreased StAR expression and luteal $P_4$ production in cattle (Staples et al., 1990). Hence, luteal steroid production is a dynamic process highly mediated by precursor and transport protein availability, and in part by the influence of LH.

## LUTEOLYSIS

If maternal recognition of the conceptus is not achieved by the end of diestrus the CL must be regressed, resulting in reduced circulating $P_4$ and luteal apoptosis; a process collectively known as luteolysis. Spontaneous regression of the bovine CL normally occurs between day 16 and 19 of the estrous cycle in cattle (Ginther, 1974). It has been established that luteolysis in ruminant species is stimulated by the episodic release of PGF from the

uterus, which is delivered to the CL by a countercurrent exchange system between the uterine vein and the ovarian artery (McCracken et al., 1970; Ginther, 1974; McCracken et al., 2012; Meidan et al., 2017). In cattle, multiple distinct pulses of PGF are necessary for luteolysis to occur. Typically, four to eight discrete pulses occurring 6 to 14 hours apart are required to initiate and maintain luteal regression (Mann and Lamming, 2006). The utero-ovarian vasculature provides a convoluted network of vessels allowing for luteolysis to be initiated in a local manner, rather than systemically as seen in the horse (Knickerbocker et al., 1988). Intraluteal production of PGF and OT are also involved in the series of events associated with luteolysis (Wiltbank and Ottbre, 2003). Oxytocin from the posterior pituitary and luteal cells acts to initiate and enhance the secretion of PGF by the endometrium and CL (Silva et al., 1991). Because of its luteolytic capacity, PGF and its analogues may also be given exogenously, and has been widely used to induce luteal regression for purposes of synchronizing ovulation in cattle.

Although luteolysis is a continuous process, CL regression can be characterized in two stages, which are defined as a loss in luteal steroidogenesis capacity (functional luteolysis) and apoptosis of the luteal cells (structural luteolysis; McCracken et al., 1999). During functional luteolysis, $P_4$ production decreases rapidly within several hours (Schams and Berisha, 2004; Miyamoto et al., 2010). Structural regression of the CL is the second, more prolonged phase, whereby luteal size decreases, cellular integrity is lost, and luteal cells undergo apoptosis. Normally, acute decrease in $P_4$ production precedes any form of morphological regression during the luteolytic process (Ginther, 1974; O'Shea and McCoy, 1988). The main effects of the PGF-induced luteolytic cascade include a decline in steroidogenesis, decreased luteal cell size and viability, vascular disruption, and immune cell

activation (O'Shea and McCoy, 1988; Niswender et al., 1994; Kobayashi et al., 2001; Meidan et al., 2017). Prostaglandin $F_{2\alpha}$ acts directly upon steroidogenic luteal and endothelial cells, which express PGF receptors. In addition, PGF indirectly affects immune cells, which do not express PGF receptors, and are activated locally by other intra-luteal factors (Meidan et al., 2017). There are multiple proposed mechanisms associated with the luteolytic process initiated by uterine or exogenous PGF, which are all highly mediated by locally produced factors.

**Functional Luteolysis**

It has been determined that PGF causes rapid fluctuations in blood flow to the CL, activates protein kinase/second messenger systems, and stimulates luteal and endothelial cells to produce various factors affecting $P_4$ production. Some studies have suggested the involvement of PGF in LH receptor actions (Garverick et al., 1985), $Ca^{2+}$/protein kinase C (PKC) second messenger systems (Niswender et al., 2000), and lipoprotein-directed $P_4$ synthesis (Wiltbank et al., 1990). Nonetheless, no specific model for the anti-steroidogenic actions of PGF has been agreed upon. Some *in vivo* studies have demonstrated PGF-induced increases in $P_4$ synthesis rather than decreases in steroidogenesis (Davis et al., 1989; Wiltbank et al., 1991). However, Girsch et al. (1996a) observed PGF-induced inhibition of $P_4$ production in luteal cells only when co-cultured with endothelial cells, implying that the presence of endothelial cells, which also express receptors for PGF (Mamluk et al., 1999), is imperative for PGF-dependent inhibition of steroid production.

In livestock species, more than 80% of ovarian blood flow is directed to luteal tissues and the endothelial cells, which line the capillaries and comprise half of the cells within the CL (Lei et al., 1991). As a result, luteal steroidogenic capacity is highly regulated by its

vasculature and resulting blood supply (Meidan et al., 2017). One of the first effects of PGF during early luteolysis is a drastic decrease in luteal blood flow (Niswender et al., 1967). Nevertheless, before this marked decrease in blood supply, there is an initial acute vasodilation following the first PGF pulse in spontaneous luteolysis. During the early stages of the luteolytic cascade, PGF causes an increase in nitric oxide (NO) production as well as the expression of its receptors on luteal cells (Kobayashi et al., 2001; Miyamoto et al., 2009). This elevation in NO in response to PGF has been associated with increased peripheral blood flow to the CL via acute vasodilation of ovarian blood vessels and is considered the earliest physiological sign of the luteolytic cascade. Subsequent to the increase in luteal blood flow, PGF significantly reduces expression of the angiogenic factor VEGF, promoting severe vasoconstriction and angiolysis within the CL (Miyamoto et al., 2009). This process is thought to indirectly accelerate the luteolytic cascade and decrease steroidogenic capacity of the CL.

At the cellular level, PGF acts by way of second messenger systems involved in steroid synthesis and cellular degeneration. Specifically, PGF binds transmembrane G-coupled protein receptors primarily located on LLCs (Niswender et al., 1994). Binding of PGF significantly increases intracellular levels of $Ca^{2+}$, which activates PKC in the LLCs. Activation of PKC in LLCs is thought to inhibit LH-induced $P_4$ synthesis in both luteal cell types through cell-to-cell communication mechanisms (Wiltbank et al., 1991; McGuire et al., 1992). Specifically, PKC activation by PGF results in inhibition of lipoprotein uptake to limit intracellular cholesterol and steroid precursor availability, resulting in decreased $P_4$ production (Niswender et al., 2000). One of the key rate-limiting steps in $P_4$ production is the movement of cholesterol from the outer to the inner mitochondrial membrane by the StAR

transport protein (Diaz et al., 2002). Protein kinase C activation has been associated with decreases in cholesterol transport to the inner mitochondrial membrane (Wiltbank et al., 1993), as well as downregulation of 3βHSD, the enzyme responsible for converting pregnenolone to $P_4$ (Hawkins et al., 1993). However, it has been proposed that downregulation of 3βHSD by PGF is most likely not the initial factor involved with reductions in luteal $P_4$ synthesis during luteolysis (Hawkins et al., 1993; Niswender et al., 2000). Therefore, one mode in which PGF acts to mediate the luteolytic cascade may be via second messenger systems involving PKC and downregulation of other rate-limiting factors such as steroid precursor transporters involved in the synthesis of $P_4$.

Despite the previously proposed modes of action of PGF in inhibiting luteal steroidogenesis, there are inconsistencies among research that make defining a concrete anti-steroidogenic mechanism difficult. While PGF has long been recognized as the main luteolysin in ruminants, Meidan et al. (2017) proposed a new model for the actions of PGF on $P_4$ production and its co-dependency on other intraluteal factors. The model hypothesizes that, upon reaching the CL via the vasculature, uterine or exogenously derived PGF upregulates the expression, synthesis, and release of vasoactive factors such as endothelin-1 (EDN1), angiopoietin (ANGPT) and nitric oxide synthase. Specifically, it is thought that EDN1 binds receptors on both SLCs and LLCs and acts to reduce basal and LH-induced $P_4$ synthesis. Supporting data from Girsch et al. (1996b) indicates that PGF facilitates the action of EDN-1 by several mechanisms: 1) by acting directly on endothelial cells in the CL; 2) by causing hypoxia of the CL (by EDN-1-induced vasoconstriction); and 3) indirectly, by increasing OT release from LLCs (which has also been shown to increase EDN-1 expression in endothelial

cells; Shirasuna et al., 2007). Without the mediatory role of EDN-1, it appears that PGF-induced luteal regression may be compromised in the bovine CL.

**Structural Luteolysis**

Structural regression entails the activation of various cytotoxic and immune factors that induce programmed cell death (apoptosis) of the CL. Intra-luteal mediators such as cytokines, EDN1, and OT are suspected to play pro-apoptotic roles in the bovine CL. Prostaglandin-induced release of luteal OT is thought to activate T-lymphocytes within the CL (Davis and Rueda, 2002). Activated T-lymphocytes secrete cytokines such as tumor necrosis factor-α (TNFα) and interferon-γ (IFN-γ) within 30 min to 12 h after PGF (Davis and Rueda, 2002). Together, TNFα and IFN-γ activate macrophages within the CL as well as inhibit luteal $P_4$ production and stimulate luteal PGF synthesis (Pate and Townson, 1994). Additionally, it has been proposed that increased EDN1 expression in response to PGF plays a role in this process by promoting leukocyte migration and stimulating macrophages to release TNFα and IFN-γ (Meidan et al., 1999). These cytokines also increase the activity of phospholipase $A_2$, causing intra-luteal elevations in arachidonic acid and free $Ca^{2+}$ ions, resulting in loss of normal morphological integrity in luteal cells (Pate and Townson, 1994). Increases in intra-luteal $Ca^{2+}$ also occurs in direct response to PGF binding, most likely through PGF-gated $Ca^{2+}$ channels or through ion channels coupled with the G-protein coupled PGF receptors (Brown and Birnbaumer, 1988). Effects of intracellular rises in $Ca^{2+}$ in luteal cells include decreased metabolic enzyme activity (Rao et al., 1984) and elevations in autolytic and cytotoxic activities (Braden et al., 1988). Throughout the apoptotic process, luteal cell DNA is fragmented by endonucleases and packaged into oligonucleosomes. This process, however, appears not to be initiated by PKC (Niswender et al., 1994).

**Luteal Refractoriness to $PGF_{2\alpha}$**

Previous studies have indicated that treatment with exogenous PGF earlier than 6 days after estrus was not effective in causing luteolysis (Lauderdale et al., 1974). It is known that the early CL (< 5 days old) is refractory to the luteolytic effect of PGF when compared with the later, mature CL (Tsai and Wiltbank, 1998; Miyamoto et al., 2009). The refractory period exists despite acquisition of PGF receptors in luteal cells two days after ovulation (Wiltbank et al., 1995). In fact, PGF-induced chemical and cellular responses are still elicited during this period including changes in hormone secretion (OT and fibroblast growth factors) and gene expression patterns; however, initiation of intracellular signal transduction pathways associated with complete luteolysis do not occur in the early CL (Pate and Townson, 1994; Wiltbank et al., 1995; Skarzynski and Okuda, 1999; Levy et al., 2000; Zalman et al., 2012).

It has been discovered that certain factors that are luteolytic in the late-CL are, in fact, luteotrophic in the early bovine CL. For example, NO production during the early luteal phase has been shown to play a role in luteal development and angiogenesis; however, in the mid to late phase CL, NO functions to inhibit basal and LH-induced $P_4$ synthesis and induces luteal apoptosis (Weems, 2004; Skarzynski et al., 2008). Likewise, in the early CL, PGF acutely stimulates VEGF expression, which activates luteal angiotensin II (ANG II). Increased ANG II production supports local angiogenesis and increases blood flow to the CL. Miyamoto et al. (2009) proposed that this increase in blood flow may help maintain luteal $P_4$ production and thereby elicit PGF-resistance. It is also suggested that luteal OT directly stimulates $P_4$ secretion within the CL during the early luteal and midluteal phases of the estrous cycle but has luteolytic actions (which regulate the magnitude of intra-luteal PGF, EDN1 and ANG II secretion) during late diestrus in the cow (Shirasuna et al., 2007). This implies that luteal OT

may function as a luteotrophic paracrine/autocrine factor in the CL during the early luteal phase. Other luteolytic mechanisms are speculated to simply not be in place in the early bovine CL. Levy et al. (2000) observed that CL exposed to PGF before day 5 of the estrous cycle fail to initiate the production EDN1, as seen normally in day 7 to 14 CL. It has been proposed that luteal refractoriness is less likely attributable to the resistance of steroidogenic luteal cells to PGF, but rather related to inability of endothelial cells to respond to PGF and produce EDN1 to prevent $P_4$ synthesis in luteal cells (Meidan et al., 2017). Nonetheless, the exact mechanism by which early CL become resistant to spontaneous or induced luteolysis is still not completely understood.

**Regulation of Prostaglandin $F_{2\alpha}$ Production**

In cattle, multiple endocrine factors regulate the synthesis and secretion of PGF by the uterus, including ovarian $E_2$, $P_4$, and OT. Predominately, pulsatile secretion of PGF is generated by a positive feedback loop between luteal (and possibly hypophyseal) OT, and uterine and intra-luteal PGF (Okuda et al., 2002). This loop is initiated by the production of PGF from the uterus, which stimulates OT release from granules stored in the LLCs (Lamsa et al., 1989). In endometrial cells (and luteal cells), OT stimulates intracellular PKC and $Ca^{2+}$ to increase phospholipase $A_2$ and cyclooxygenase (COX) gene expression (Thatcher et al., 2001). Cyclooxygenase occurs as both a constitutive (COX-1) and an inducible (COX-2) enzyme. Arachidonic acid released from the endometrial cells, under the influence of $E_2$, is oxidized by COX enzymes to $PGG_2$ and then to $PGH_2$, which is reduced by the PGF-synthase enzyme to PGF (Smith and DeWitt, 1996).

Pulsatile secretion of PGF is thought to be determined by uterine responsiveness to OT, which entails a combination of acute stimulatory effects and uterine refractoriness to OT

(Silva et al., 1991). However, the uterus requires 10 to 14 days of $P_4$ exposure before PGF production can be facilitated, as uterine responsiveness to OT must be acquired for endogenous PGF secretion to occur during the estrous cycle. In the presence of $P_4$, uterine secretory responsiveness to OT develops gradually (Silva et al., 1991). Progesterone exerts two specific effects that regulate PGF synthesis and secretion. First, under the influence of $P_4$, concentrations of lipid and eicosanoid precursors for prostaglandins, such as arachidonic acid, are increased in the endometrial cells (Brinsfield and Hawk, 1973). As the predominant ovarian steroid in circulation shifts from $P_4$ to $E_2$ at the end of the diestrus these precursor stores are released and converted to PGF under the action of OT (Silva et al., 1991). Second, $P_4$ has a suppressive effect on PGF synthesis from the endometrium, which diminishes after prolonged exposure. It has been proposed that $P_4$ exerts this action by inhibiting the synthesis of receptors for $E_2$ and that the expression of OT receptors is dependent upon $E_2$-receptor binding in the endometrium (McCracken, 1980). It is also suggested by McCracken (1980) that, after prolonged exposure, the uterus becomes refractory to $P_4$, thus allowing $E_2$ and OT receptor expression and PGF synthesis.

**Importance of Reduced Progesterone before AI and Subsequent Fertility**

Incomplete luteal regression in response to exogenous PGF may result in elevation of circulating $P_4$ around the time of artificial insemination (AI), and reduced fertility in dairy and beef cattle (Souza et al., 2007; Brusveen et al., 2009; Santos et al., 2010; Martins et al., 2011; Giordano et al., 2012; Stevenson et al., 2015). Results from studies in lactating dairy cows support the concept that greater circulating $P_4$ near the time of AI decreases fertility; however, this decrease was only seen in cows with high $P_4$ that ovulated before or around the time of insemination, suggesting a negative effect on oocyte quality (Souza et al., 2007; Brusveen et

al., 2009). Data from the same two studies suggest that there is dramatic decrease in pregnancy per AI (P/AI) when blood $P_4$ is above 0.4 to 0.5 ng/mL at the time of insemination. In another study, Santos et al. (2010) concluded that P/AI was optimized when $P_4$ was as low as 0.24 ng/mL at AI. Nonetheless, a review by Stevenson and Lamb (2016) suggested that $P_4$ bears a greater influence on fertility in lactating dairy cattle compared with beef cows. Specifically, it was proposed that complete luteolysis is not as necessary to achieve pregnancy in beef cows, as P/AI did not differ in postpartum beef cows with $P_4$ less than or greater than 0.8 ng/mL at the time of AI. In contrast, P/AI was greatly reduced at $P_4$ concentrations > 0.80 ng/mL in lactating dairy cows (Stevenson and Lamb, 2016). Collectively, the consensus among several studies in beef and dairy cows (Stevenson et al., 2015; Santos et al., 2010; Wiltbank et al., 2014; Stevenson, 2016) suggest that circulating $P_4$ concentrations should be at least 0.5 ng/mL at the time of insemination for optimal fertility to AI.

Elevated concentrations of $P_4$ at breeding may interfere with multiple physiological mechanisms resulting in reduced fertility. First, $P_4$ may inhibit GnRH-induced preovulatory LH release, thus hindering ovulatory response (Colazo et al., 2008; Giordano et al., 2012). Second, $P_4$ reduces uterine and oviductal contractility which may impair spermatozoa or oocyte transport and fertilization (Hawk, 1983; Hunter, 2005). Third, decreased endometrial thickness seen with elevations in blood $P_4$ may indicate other effects on the uterus that could result in reduced embryo development and survival (Souza et al., 2011). It has also been observed that addition of $P_4$ to *in vitro* fertilization media resulted in reduced rate of embryo development to blastocyst stage (Silva and Knight, 2000) as well as elevations in inhibin A production by the cumulus-oocyte complex (Silva et al., 1999). Large amounts of inhibin A and its α-subunit free forms are normally found within follicular fluid; however, greater

concentrations have been associated with reduced embryo cleavage (Silva et al., 1999). Together, this evidence suggests that there may be direct and indirect effects of $P_4$ on early embryo development. Nonetheless, the underlying physiological mechanisms by which elevated $P_4$ at AI reduces fertility are not well understood. Provided that $P_4$ concentrations at the time of AI play important roles in fertility in cattle, estrous synchronization protocols should be developed such that circulating $P_4$ at the time of AI is optimized so that maximum P/AI can be achieved. The following sections briefly discuss a few timed-AI protocols and the roles of various hormones in estrous synchronization.

## ESTROUS SYNCHRONIZATION

Estrous synchronization protocols have become a useful management tool for improving reproductive performance, decreasing calving intervals and producing more uniform calf crops, and help facilitate the use of AI. The use of AI in estrous synchronization enables producers to rapidly introduce superior genetics into cattle herds and improve profitability. Synchronization protocols are designed to mimic the normal physiological events that occur in the bovine estrous cycle through use of exogenous hormones, including but not limited to GnRH, PGF, and progestins. Some examples of common synchronization programs are the Ovsynch (Pursley et al., 1997; Figure 1.1) and CO-Synch protocols (Geary and Whittier, 1998; Figure 1.2), which utilize a combination of GnRH and PGF injections to synchronize ovulation. Ovsynch protocols were originally developed for use in dairy cattle, whereas CO-Synch, (a modification of Ovsynch) is commonly used in beef cattle because it requires only 3 animal handlings for AI (Geary and Whittier, 1998; Colazo and Mapletoft, 2014). Progesterone in the form of controlled internal drug release insert (CIDR) may also be added to the CO-Synch protocol (Figure 1.3) to improve synchrony of estrus or ovulation, as

well as pregnancy rates by preventing premature ovulation and estrus (Xu and Burton, 2000; Larson et al., 2006). Various modified versions of these protocols have been developed for use in dairy and beef cattle to provide producers with the opportunity to improve fertility in their cows.

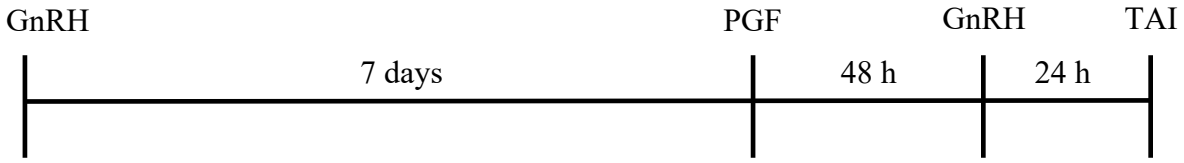


**Figure 1.1** The original Ovsynch protocol for use in timed artificial insemination (TAI) breeding systems. Gonadotropin-releasing hormone (GnRH) is initially administered to induce ovulation in the presence of a dominant follicle. Prostaglandin $F_{2\alpha}$ (PGF) is administered 7 days later to regress any corpus luteum (CL) that may have developed in response to the first GnRH or that was already present on the ovary at initiation. A second GnRH injection is given 48 hours later to induce ovulation. Cows are subjected to TAI 24 hours after the second GnRH.

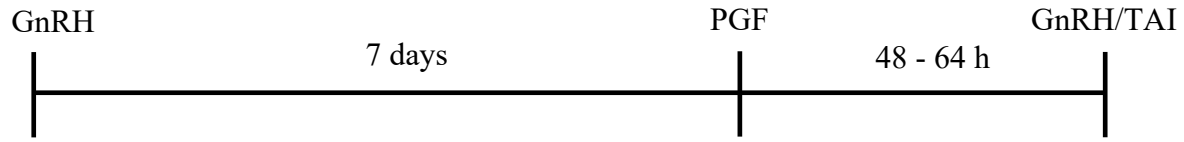


**Figure 1.2** The original CO-Synch protocol for use in timed artificial insemination (TAI) breeding systems. Gonadotropin-releasing hormone (GnRH) is initially administered to induce ovulation in the presence of a dominant follicle. Prostaglandin $F_{2\alpha}$ (PGF) is administered 7 days later to regress any corpus luteum (CL) that may have developed in response to the first GnRH or that was already present on the ovary at initiation. A second GnRH injection is given 48 – 64 hours later to induce ovulation and cows are concurrently subjected to TAI.

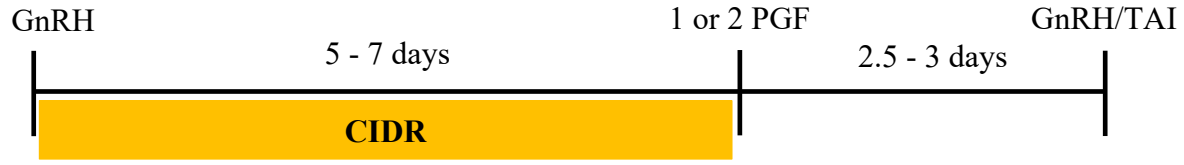


**Figure 1.3** CO-Synch with controlled internal drug release (CIDR) insert for use in timed artificial insemination (TAI) breeding systems. Gonadotropin-releasing hormone (GnRH) is initially administered to induce ovulation in the presence of a dominant follicle. In the CO-Synch + CIDR program, a CIDR insert is inserted vaginally to increase progesterone concentrations for 5 to 7 days between the first GnRH and prostaglandin $F_{2\alpha}$ (PGF) administration. On day 5 or 7, CIDR inserts are removed and one or two PGF doses are administered to regress any corpus luteum (CL) that may have developed in response to the first GnRH or that was already present on the ovary at CIDR insertion. A second GnRH injection is given 2.5 to 3 days later to induce ovulation and cows are concurrently subjected to TAI.

## Hormones Involved in Estrous Synchronization

### *Gonadotropin-Releasing Hormone*

Gonadotropin-releasing hormone is a neurohormone synthesized and secreted by the paraventricular nucleus of the hypothalamus. This decapeptide causes release of LH and FSH from the anterior pituitary (Senger, 2012). Under the influence of its basal production and marked pre-ovulatory surge on day 0 of each estrous cycle, GnRH is a fundamental regulator of ovarian cyclicity and gonadal function.

Since its discovery as the key regulator of reproductive cyclicity, GnRH has been isolated for use in estrous synchronization in livestock species. Commercially available GnRH agonists for use in cattle include, Cystorelin® (gonadorelin diacetate tetrahydrate; 100 μg/dose, Merial), Factrel® (gonadorelin hydrochloride; 100 μg/dose, Zoetis Inc.), Fertygyl® (gonadorelin acetate; 86 μg/dose, Merck Animal Health), and GONAbreed® (gonadorelin acetate; 100 μg/dose, Parnell). Agonists of GnRH can be administered exogenously either intramuscularly (i.m.) or subcutaneously (s.c.) to induce ovulation and (or) luteinization of dominant follicles (> 10 mm in diameter) and initiate a new follicular wave approximately 2 d later (Martinez et al., 1999). A single injection of GnRH is sufficient to cause ovulation in most cows when given in the mid to late luteal or follicular phases of the estrous cycle (Kesler and Constantaras, 2004).

Commonly, GnRH is given at the beginning of synchronization protocols to initiate a new follicle wave, as well as before (Ovsynch) or at (CO-Synch) the time AI to induce ovulation approximately 24 to 32 h later (Saumande and Humbolt, 2005). The addition of GnRH in synchronization programs helps to more effectively synchronize, and therefore predict, the time of ovulation and estrus. Gonadotropin-releasing hormone may also be used

to induce cyclicity in non-cycling animals such as pre-pubertal heifers or anestrous cows. However, the ovulation of a dominant follicle in response to GnRH relies on the acquisition of LH receptors and ovulatory capacity (Ginther et al., 1996). If ovulation does not occur in response to GnRH administration, partial luteinization may occur (Ginther et al., 1996), ultimately influencing follicle wave dynamics (Macmillan et al., 1985).

***Progesterone***

Progesterone is produced by luteal cells in the CL throughout the luteal phase of the estrous cycle. Sustained secretion of $P_4$ acts to prime the uterus and the hypothalamus to support embryonic development in the occasion of pregnancy. Elevations in $P_4$ concentrations during follicle development are important for oocyte quality and overall embryo survival and fertility in cattle (Wiltbank et al., 2014). Exogenous administration of $P_4$ during the estrous cycle may be implemented to prevent estrus and ovulation until a preferred time in synchronization protocols.

Many progestin sources are available on the market for use in estrous synchronization programs including melengestrol acetate (e.g. MGA®200; Zoetis Inc.), which is orally administered, and intravaginal inserts such as the PRID® delta (Ceva Inc.) and EAZI-breed® CIDR cattle insert (Zoetis Inc.). Controlled internal drug-releasing inserts are commonly used in synchronization programs for beef cows. These inserts contain 1.38 mg of $P_4$ and are introduced vaginally for prolonged periods of time where they gradually release $P_4$ into the surrounding mucous. Progesterone from the insert is then absorbed by the vaginal mucosa and diffuses into the blood circulation. Inserts are T-shaped to maximize contact with the surrounding vaginal tissues, as the amount of $P_4$ absorbed from the insert is dependent upon the area of contact between the CIDR and the vaginal mucous (Rathbone et al., 2002).

Progesterone from CIDR inserts has been shown to elevate blood $P_4$ concentrations by 0.8 ng/mL in cattle (Lima et al., 2009).

The $P_4$ released from a CIDR insert specifically acts to suppress estrus and spontaneous ovulation through negative feedback on the hypothalamus and inhibition of a preovulatory GnRH and LH surge. However, $P_4$ does not suppress FSH production, allowing for continued emergence of follicle waves in the presence of the exogenous progestin (Adams et al., 1992b). After removal of the CIDR insert, circulating $P_4$ concentrations diminish (within 1 h; Mann et al., 2001). In the absence of a preexisting CL, CIDR removal allows for pulsatile LH and FSH secretion, resumption of rapid follicular growth, increased $E_2$ production, estrus expression, and subsequent ovulation. In the presence of a CL at CIDR removal, PGF can be administered to regress the CL and reduce $P_4$ concentrations to allow follicle growth, maturation, and ovulation to occur.

***Progesterone Supplementation to Improve Estrous Synchronization***

Progestin inserts can be utilized in GnRH-based protocols to delay ovulation and estrus, as well as induce cyclicity in anestrous cows by increasing LH pulsatility after removal. In most synchronization protocols, the emergence of a new follicular wave is only synchronized when GnRH induces ovulation (Martinez et al., 2002). If the initial GnRH does not induce ovulation of a dominant follicle, emergence of a new follicular wave will not occur. Hence, ovulation to the second GnRH may be impaired, leading to poor P/AI. In addition, it is estimated that approximately 70% of cattle have an active CL at the start of any synchronization protocol (Fricke et al., 2003; Stevenson et al., 2008). The other 30% of animals without a CL before initiation of synchronization will go on to ovulate first-wave follicles that developed under the influence of low $P_4$ concentrations. Studies have shown that

P/AI is compromised in cows that develop follicles in the absence of a CL (Bisinotto et al., 2010; Denicol et al., 2012). Reduced $P_4$ concentrations during follicle development have been shown to increase follicle growth rate, which may impair oocyte quality (Cerri et al., 2011a,b; Rivera et al., 2011). A single CIDR insert provides sufficient $P_4$ to improve fertility by incrementally increasing blood concentrations during ovulatory follicle development (Bisinotto and Santos, 2011). Therefore, in an attempt to improve P/AI, oocyte quality, and synchrony of follicle wave emergence and ovulation, a progestin supplement in the form of a CIDR can be added to the Ovsynch and CO-Synch protocols between initial GnRH and PGF administration (Lamb et al., 2001; Martinez et al., 2002).

In CO-Synch protocols implemented without $P_4$ supplementation, approximately 20% of heifers show estrus before the PGF injection (Colazo et al., 2004). Likewise, DeJarnette et al. (2001) reported that spontaneous expression of estrus occurs in greater than 10% of cows prior to completion of Ovsynch. Therefore, addition of a $P_4$ source between GnRH and PGF administration can also help prevent premature estrus and ovulation during the period in which spontaneous luteolysis may occur in the small percentage of cows (10 to 30%) that failed to ovulate to the initial GnRH (Lima et al., 2009; Xu and Burton, 2000; Vasconcelos et al., 1999). Prevention of premature estrus expression and ovulation in progestin-based protocols has been shown to improve P/AI in cows bred at a fixed time (Stevenson et al., 2006). In fact, P/AI is increased 5 to 10 percentage points in beef cows when CIDR inserts are utilized in TAI protocols (Stevenson et al., 2006; Lamb et al., 2010). However, the benefit of improved P/AI with progesterone supplementation is predominantly observed in anestrous cattle, or cows lacking a CL at the time of protocol initiation (Lamb et al., 2001; Bisinotto et al., 2015). Conversely, some studies found no improvement in P/AI when CIDR inserts were

used in pre-synchronized cows (Colazo et al., 2013) or cows inseminated upon detection of estrus rather than at a fixed time (Chebel et al., 2013; Bisinotto et al., 2015). Thus, the benefit of adding a progestin source to FTAI protocols is seemingly observed mainly in anestrous cattle or cows failing to ovulate to initial GnRH.

***Prostaglandin $F_{2\alpha}$***

Prostaglandin $F_{2\alpha}$ is a lipid hormone derived from arachidonic acid that is produced throughout the body, but more specifically by the cells of the endometrium and partially by the ovary. Prostaglandins have various roles in reproduction including ovulation, maternal recognition, maintenance of gestation, parturition, and resumption of cyclicity, although their actions are more understood regarding luteal function (Weems et al., 2006).

Commercial introduction of exogenous PGF for induction of luteal regression was founded by its discovery as a luteolysin in ruminant species (Lauderdale, 1972; Rowson et al., 1972). Many PGF products are available on the market for exogenous administration in cattle; Lutalyse® (dinoprost tromethamine, 25 mg/dose; Zoetis Inc.), which is a naturally occurring PGF product, and Estrumate® (cloprostenol sodium, 500 μg/dose; Merck Animal Health,) and estroPLAN® (cloprostenol sodium; 250 μg/dose; Parnell) which are both synthetic PGF analogues. Dinoprost tromethamine has a short half-life of approximately 7-8 min and is metabolized similar to endogenous PGF (Kindahl et al., 1976). Cloprostenol sodium is more resistant to endogenous metabolism and lasts longer in circulation (half-life = 3 h) compared with dinoprost (Reeves, 1978). Mixed results have been seen regarding luteolytic response with the use of dinoprost versus cloprostenol (Martineau, 2003; Repasi et al., 2005; Martins et al., 2011); however, both have been shown to produce similar estrus

synchrony and P/AI when used in AI protocols (Salverson et al., 2002; Stevenson and Phatak, 2010).

More recently, a high-concentration PGF product, approved for both i.m. and s.c. administration, Lutalyse® *HighCon* (dinoprost tromethamine, 12.5 mg/mL; Zoetis Inc.), was developed. According to the manufacturer, this product reaches a greater maximum plasma concentration (Cmax) following administration of a standard 25 mg dose than the conventional Lutalyse product. When compared, the high-concentration and conventional dinoprost products contain the same PGF compound and only differ with respect to concentration and possibly the vehicle by which it is delivered in solution. While research does not imply any discrepancies in half-life or biological availability, it is possible that the greater Cmax associated with *HighCon* could be attributable to greater storage in lipid molecules and thus slower release following administration. Another PGF product, Bovaline® (cloprostenol sodium, 500 μg/dose; Syntex), was shown to have a longer biological activity compared with other PGF products, as it was stored in lipid molecules following administration (A. Tibary, personal communication, 2018). Therefore, *HighCon* may act under similar mechanisms as Bovaline, resulting in prolonged biological activity.

## TIMED-AI SYNCHRONIZATION PROTOCOLS

Estrous synchronization protocols that result in greater synchronized ovulation and P/AI help facilitate the use of AI and may increase its adoption among beef producers. Development of fixed-time AI (FTAI) protocols for reproductive management provides producers with a dependable method to synchronize ovulation and facilitate the use of AI without detecting estrus. Fixed-time protocols can incorporate the use of GnRH, progestins, and PGF (dinoprost) or its synthetic analogue (cloprostenol) to synchronize follicle wave

emergence, CL regression, and ovulation. Gonadotropin-releasing hormone-based protocols are the most commonly used in North America for estrous synchronization (Colazo and Mapletoft, 2014). Success of GnRH-based timed-AI protocols depends, in part, on the proper manipulation of physiological processes involved in ovulation, luteinization, and recruitment of new follicular waves with the use of GnRH. Stevenson (2016) proposed three other major factors that contribute to the success of FTAI protocols, which can explain 78% of variation in pregnancy risk to AI. These include 1) success of ovulation to GnRH administered at or near the time of AI; 2) high $P_4$ concentrations at the time of PGF injection; and 3) low $P_4$ concentrations within 48 h after PGF administration (Stevenson, 2016).

**CO-Synch**

Many protocols have been developed to synchronize timing of ovulation, several involving GnRH (Lamb et al., 2010; Lamb and Mercandante, 2016). In beef cattle, variations of the CO-Synch protocol, with or without the use of a CIDR between GnRH and PGF injections, is one popular method of synchronization for use of FTAI among beef producers (Geary and Whittier, 1998; Lamb et al., 2001). The CO-Synch protocol includes the administration of GnRH followed by one or two injections of PGF 5 or 7 days later, respectively, and concurrent GnRH and FTAI 56 to 72 h later (Figures 1.3 & 1.4). In cows, the first GnRH is administered at random stages of the estrous cycle. However, if first GnRH is administered at a time when circulating $P_4$ is low (i.e. the late luteal, follicular, or early luteal phase) a new follicular wave and dominant follicle development will occur under a prolonged period of low $P_4$ before ovulation. Research has implicated that low $P_4$ concentrations during final follicular wave development in the late luteal phase (Rosenburg et al., 1990) or before AI (Wiltbank et al., 2012) reduces conception rates by 10%.

Supplementation of progestins between GnRH and PGF improves synchrony of ovulation without prolonging a timed AI program, and has been shown to increase pregnancy risk and estrus response particularly in cows in late luteal phase at the beginning of the protocol (Xu et al., 1997; Larson et al., 2006). As indicated, addition of $P_4$ treatment reduces the incidence of estrus, premature ovulation, and spontaneous luteolysis during the protocol in cows with low endogenous $P_4$ (Vasconcelos et al., 1999) and has been shown to improve pregnancy success in previously anestrous beef cows (Lamb et al., 2001). The incidence of postpartum anestrous is greater in beef cows due to continual presence of a suckling calf, which delays the reinitiation of the estrous cycle (Williams, 1990; Stevenson et al., 2015). Exposure to $P_4$ is a prerequisite for first postpartum estrus behavior (Lamb et al., 2010), therefore, CIDR-based CO-Synch protocols (i.e. 5 d and 7 d CIDR) may help improve fertility in postpartum beef cows.


**Figure 1.4** 7-day CO-Synch with a controlled internal drug release (CIDR) insert for use in timed artificial insemination (TAI) breeding systems. Gonadotropin-releasing hormone (GnRH) is initially administered to induce ovulation in the presence of a dominant follicle. A CIDR insert is inserted vaginally to increase progesterone concentrations for 7 days between the first GnRH and prostaglandin $F_{2\alpha}$ (PGF) administration. On day 7, CIDR inserts are removed and one PGF dose is administered to regress any corpus luteum (CL) that may have developed in response to the first GnRH or that was already present on the ovary at CIDR insertion. A second GnRH injection is given 60 to 66 hours later to induce ovulation and cows are concurrently subjected to TAI.

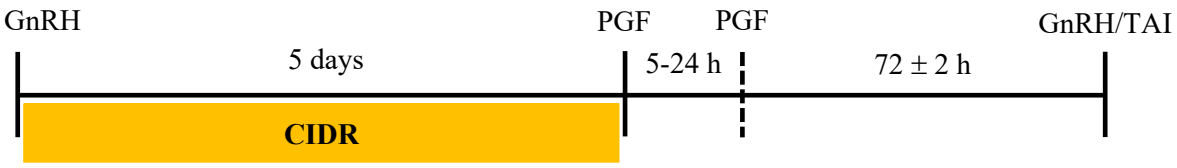


**Figure 1.5** 5-day CO-Synch with a controlled internal drug release (CIDR) insert for use in timed artificial insemination (TAI) breeding systems. Gonadotropin-releasing hormone (GnRH) is initially administered to induce ovulation in the presence of a dominant follicle. A CIDR insert is inserted vaginally to increase progesterone concentrations for 5 days between the first GnRH and prostaglandin $F_{2\alpha}$ (PGF) administration. On day 5, CIDR inserts are removed and two PGF doses (5 to 24 h apart) are administered to regress any corpus luteum (CL) that may have developed in response to the first GnRH or that was already present on the ovary at CIDR insertion. A second GnRH injection is given 72 ± 2 hours later to induce ovulation and cows are concurrently subjected to TAI.

**5-day vs. 7-day CO-Synch + CIDR**

Duration of follicular dominance under the influence of $P_4$ in CO-Synch + CIDR protocols may affect pregnancy response to AI. Santos et al. (2010) suggested prolonged follicular dominance may impair viability of oocytes ovulated around the time of insemination and reduce subsequent fertility. The logic behind reducing CIDR treatment from 7 to 5 days is to decrease the incidence of prolonged follicular dominance that normally results in ovulation of an aged oocyte. By shortening duration of $P_4$ exposure between initial GnRH and PGF, and increasing the time interval between PGF and AI/second GnRH, the follicular dominance period is reduced and the length of proestrus can be optimized allowing for increased follicular growth and ovulatory response in CO-Synch + CIDR protocols (Bridges et al., 2008; Lopes Jr. et al., 2013; Stevenson, 2016). In fact, a longer proestrus period in timed-AI protocols has been associated with increased dominant follicle diameter and $E_2$ concentrations at the time of AI, which have been shown to improve pregnancy rates (Perry et al., 2005).

Researchers hypothesized that P/AI would be similar or improved in CO-Synch + CIDR protocols if the interval from the initial GnRH to PGF was shortened from 7 to 5 days, and the time from PGF to the second GnRH extended to 72 h (Bridges et al., 2008; Lopes Jr. et al., 2009; Santos et al., 2010; Wilson et al., 2010; Whittier et al., 2013; Ahmadzadeh et al., 2015). This approach resulted in greater (Bridges et al., 2008; Santos et al., 2010; Whittier et al., 2013) or similar (Wilson et al., 2010; Gunn et al., 2011; Ahmadzadeh et al., 2015) P/AI compared with the 7-d CO-Synch + CIDR protocol. As indicated earlier, because delaying ovulation with supplemental $P_4$ prolongs the period of follicular dominance, shortening $P_4$ supplementation from 7 to 5 days may decrease the incidence of persistent follicles and aged

oocytes (Bridges et al., 2008; Cerri et al., 2009; Lopes Jr. et al., 2009; Santos et al., 2010). Ovulation of aged oocytes negatively impacts embryo quality and reduces subsequent P/AI (Cerri et al., 2004); therefore, reducing $P_4$ exposure may improve overall P/AI and viability of oocytes. Nonetheless, there is a limitation when the length of follicular dominance is shortened, as the success of synchronization relies on the ability of PGF to regress a newly formed (GnRH-induced) CL and reduce $P_4$ concentrations by the time of AI. Failure to induce complete luteolysis, and subsequently decrease in $P_4$ concentrations by the time of AI, may negatively affect fertility.

Recent studies involving the 5-day CO-Synch protocol have implied that ovulation to the initial GnRH will produce a new CL that is refractory to PGF at the time of CIDR removal five days later. It was not known whether a single dose of PGF would be sufficient to induce complete luteolysis in a GnRH-induced CL in this protocol (Bridges et al., 2008); therefore it was proposed that this lack of luteolytic response could partially be overcome by introducing a second injection of PGF 5 h ( Whittier et al., 2010) to 24 h (Chebel et al., 2008; Santos et al., 2010) after the initial PGF injection. It has been argued that the addition of the second PGF dose might imitate the natural pulsatile secretion of PGF from the bovine endometrium during luteolysis, thus resulting in improved luteal regression (Ginther et al., 2009). However, the added labor, treatment costs, and cattle handling associated with the second PGF was a point of concern for cattle producers. Therefore, some studies (Kasimanickam et al., 2009; Rabaglino et al., 2010; Ahmadzadeh et al., 2011; Peterson et al., 2011; Bridges et al., 2012; Ribeiro et al., 2012; Lima et al., 2013; Kasimanickam et al., 2014; Say et al., 2016; White et al., 2016) have assessed whether a single PGF dose is capable of causing complete luteolysis and reducing $P_4$ concentrations by the time of AI in the 5-d CO-Synch + CIDR protocol.

## RATIONALE

### One vs. Two Injections of Prostaglandin $F_{2\alpha}$ in a 5-day protocol

Incomplete luteolysis before the time of AI is associated with impaired fertility in cattle (Souza et al., 2007; Brusveen et al., 2009). Ovulation induced by GnRH at protocol initiation may result in an immature CL that is refractory to standard PGF doses on day 5 of the 5-d CO-Synch + CIDR protocol. Thus, research has been conducted to assess the efficacy of different dosages and number of PGF injections on luteolysis. Using the 5-d CO-Synch + CIDR protocol in beef or dairy heifers, one injection of PGF was shown to produce similar pregnancy rates as two doses of PGF given 6 h (Peterson et al., 2011; Kasimanickam et al., 2014) or 12 h (Rabaglino et al., 2010) apart. In the study by Rabaglino et al. (2010), CL regression ($P_4$ < 1.0 ng/mL 24 h after PGF) was also similar between PGF treatment groups, indicating one PGF (25 mg) was as effective to induce luteolysis as two 25 mg PGF injections in dairy heifers. Lima et al. (2013) observed contrasting results in dairy heifers. In that study, two PGF (25 mg each) given at a 12 h interval improved luteolysis ($P_4$ < 0.5 ng/mL at AI) and P/AI compared with one 25 mg PGF injection. However, this improvement in P/AI with two PGF was not observed in heifers that did not receive GnRH at the beginning of synchronization, indicating a second PGF is only beneficial if ovulation occurs to the initial GnRH of the 5-d CO-Synch + CIDR protocol. Concurrent administration of PGF (one 50 mg dose) has also been compared with two 25 mg PGF injections in an attempt to reduce time and labor requirements for the 5-d protocol (Say et al., 2016; White et al., 2016). Such studies showed that two PGF (given at 6 h interval) resulted in greater P/AI than one or two concurrent PGF doses in beef and dairy heifers (Say et al., 2016; White et al., 2016).

Research in mature cows has been equally inconsistent. Results from lactating dairy cows indicate the use of two PGF injections administered 24 h apart improved CL regression and P/AI, especially in cows that ovulated to initial GnRH (Ribeiro et al., 2012). Santos et al. (2010) observed similar P/AI results in dairy cows, however, cows in this study were pre-synchronized and submitted to the 5-d CO-Synch (no CIDR), therefore the chance of cows ovulating to initial GnRH and possessing a young CL at PGF administration was greater. In another study using Ovsynch, Repasi et al. (2005) evaluated one or two doses (8 h apart) of both cloprostenol and dinoprost in dairy cow. In that study, no differences were found in regression of luteal tissue volume or $P_4$ concentration decline between PGF product or dose number; however, P/AI was improved by two doses of PGF. Again, cows in that study were pre-synchronized. In beef cows, Kasimanickam et al. (2009) observed similar pregnancy results in the 5-d CO-Synch + CIDR protocols, whereby P/AI was greater in cows receiving two PGF compared with cows receiving one PGF injection. Though, luteal regression in response to PGF treatment was not assessed in that study.

Other studies have also tested the efficacy of an increased PGF dose on luteolytic response (Ahmadzadeh et al., 2011; Bridges et al., 2012). Ahmadzadeh et al. (2011) investigated the effects of one (25 mg), one and a half (37.5 mg) or two (25 mg) doses of PGF given 7 h apart at CIDR removal on luteolysis ($P_4 < 0.5$ ng/mL at AI) in beef cows subjected to the 5-d CO-Synch + CIDR protocol. Results from that study revealed no difference in serum $P_4$ concentration at AI among any of the treatments (Ahmadzadeh et al., 2011). Similarly, Bridges et al. (2012) compared the effect of a single (25 mg), double (50 mg) and double-split (2 x 25 mg, 8 h apart) PGF dose on luteolysis ($P_4 \leq 1.0$ ng/mL at TAI) in beef cows synchronized with the 5-d CO-Synch + CIDR program. In that study, P/AI was greater

in cows receiving the double and double-split doses of PGF, but no differences in incidence of luteolysis were observed between treatments (Bridges et al., 2012).

Although multiple or increased dosages of PGF have been shown to successfully induce regression of immature CL (less than 5 d old), the use of two PGF injections in the 5-d CO-Synch + CIDR protocol have not always improved fertility (Cruppe et al., 2010; Rabaglino et al., 2010; Peterson et al., 2011; Kasimanickam et al., 2014). Furthermore, although some studies have proposed that the apparent improvement of P/AI in cows receiving two PGF was attributable to complete luteolysis, they did not directly assess $P_4$ response and luteal regression following PGF treatment. Therefore, the relationship between luteolysis and the improvements in P/AI with two PGF could not be demonstrated in these studies. Given the variable and incomplete results associated with two PGF doses regarding luteolytic response and P/AI, and the lack of data concerning $P_4$ profiles at AI, it is still not well understood whether the use of a single PGF injection is effective in a 5-d CO-Synch + CIDR protocol. The lack of research regarding the importance of luteolysis and low $P_4$ concentrations at AI (particularly in beef cattle) provides opportunity for further exploration. It should also be noted that all aforementioned studies utilized either conventional dinoprost tromethamine or cloprostenol sodium when comparing one versus two injections of PGF. Hence, the effectiveness of alternative PGF products to induce luteolysis in a young CL has yet to be investigated in beef cattle.

**High-Concentration vs. Conventional Prostaglandin $F_{2\alpha}$**

Recently, Zoetis developed an alternative high-concentration PGF, (12.5 mg/mL, Lutalyse® *HighCon*) which has a larger Cmax and therefore reaches a higher peak serum concentration following administration than conventional Lutalyse. This high-concentration

product reduces the required volume per 25 mg dose from 5 mL to 2 mL. In addition, the cost per dose for *HighCon* is $2.48 to $2.99, whereas conventional Lutalyse is more expensive and ranges from $2.89 to $3.42 per dose (Zoetis, 2019). Current information on the efficacy of *HighCon* compared with conventional Lutalyse on improving or maintaining P/AI is limited. Only three studies have evaluated *HighCon* in synchronization protocols, two of which were conducted in beef heifers and the other in beef and dairy cows. The two studies in beef heifers (Oosthuizen et al., 2017; Lansford et al., 2018) found a single injection of *HighCon* to be equally effective as one conventional Lutalyse dose. Nonetheless, it should be noted that *HighCon* was administered s.c, whereas conventional Lutalyse was given i.m, which may have confounded the results observed in these studies.

Previous studies within our laboratory investigated the effect of one dose of *HighCon* and one or two doses of conventional Lutalyse in a 5-day CO-Synch + CIDR protocol on $P_4$ profiles and luteolysis in lactating beef and dairy cows (Spencer et al., 2017; 2018). In beef cows, $P_4$ concentrations at AI and the incidence of complete luteolysis, defined by the authors as $P_4 \leq 0.5$ ng/mL at the time of AI, were similar between all treatments (Spencer et al., 2018). In contrast, one conventional Lutalyse injection was not as effective as two conventional injections at reducing $P_4$ and causing complete luteal regression by the time of AI in lactating dairy cows. However, one injection of *HighCon* reduced $P_4$ concentrations as effectively as two conventional doses. Therefore, data from these studies suggest a single high-concentration PGF may be as effective as two conventional doses of PGF at inducing complete luteolysis and lowering $P_4$ concentrations by AI in beef and dairy cows (Spencer et al., 2017). It should be noted that fertility of beef and dairy cows in the above two studies was not examined.

**Practical Concerns with Two $PGF_{2\alpha}$ Injections**

Two methods have been employed to overcome luteal refractoriness in the 5-d CO-Synch + CIDR protocol: increasing the number of injections and increasing the dose of PGF. However, both of these resolutions, at the very least, increase the overall cost of synchronization or require two handlings of cattle within 24 hours, leading to increased time and labor requirements and stress on the animals. In addition, adding a second injection or increasing the dose of PGF (resulting in an increased volume delivered) can potentially decrease protocol compliance, thus reducing the number of animals successfully synchronized. The additional intramuscular injection may also impair beef quality through a rise in serum creatinine kinase levels (Fajt et al., 2011) as well as increase the prevalence of injection-site lesions, which can persist until slaughter (George et al., 1995).

The concept of testing reproductive performance of beef cows administered one or two doses of PGF in a 5-d CO-Synch + CIDR synchronization protocol was developed with the intention of weighing the potential losses in P/AI against the additional cattle handling and expenses associated with a second PGF injection. Given the variable results on the necessity of two PGF injections in a 5-d synchronization program, it is essential to investigate the differences in P/AI between two conventional PGF and one high-concentration PGF injection. If the use of a single high-concentration PGF is as effective as two conventional PGF injections, the 5-d protocol may be altered to reduce cattle handling, lower labor and drug costs, and possibly improve protocol compliance. Thus, our laboratory proposed to answer the question: Can the use of a single dose of high-concentration PGF be implemented in a 5-d CO-Synch + CIDR protocol without compromising fertility in postpartum beef cows? With this question in mind, the following objective was developed:

## GENERAL OBJECTIVE

To examine the effects of one dose of high concentration PGF (Lutalyse *HighCon;* 1 x 25 mg) versus two doses of conventional PGF (Lutalyse; 2 x 25 mg, 6 to 8 h apart) after CIDR removal on luteolysis, $P_4$ concentration at AI, and P/AI in a 5-d CO-Synch + CIDR protocol in postpartum beef cows.

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# CHAPTER TWO

Pregnancy and Hormonal Response in Postpartum Beef Cows Administered High-Concentration or Conventional Prostaglandin $F_{2\alpha}$ in a 5-day CO-Synch + CIDR Program

## ABSTRACT

The objectives of this study were to examine the effects of a single high-concentration dose of prostaglandin $F_{2\alpha}$ (PGF) in a 5-d CO-Synch + CIDR protocol on serum progesterone ($P_4$) concentration and pregnancy per AI (P/AI) in postpartum beef cows. Furthermore, the threshold concentration of $P_4$ at the time of AI, at which probability of pregnancy is optimized, was determined. Angus-Hereford crossbred cows (n=404) were synchronized with the 5-d CO-Synch + CIDR protocol and randomly assigned to receive either one injection of high-concentration PGF (HICON; 12.5 mg/mL; 1 x 25 mg; i.m; n=203) at CIDR removal or two injections (8 h apart) of conventional PGF (2PGF; 5 mg/mL, 2 x 25 mg, i.m; n=201), at CIDR removal (Day 5). Estrual behavior was monitored using estrous detection aids and visual observation thrice daily from Day 5 until AI (Day 8). All cows were inseminated at a fixed time 72 ± 2 h after PGF treatment. Pregnancy was determined by ultrasound 55 days after AI. To quantify $P_4$ concentrations, blood samples were collected seven days before protocol initiation (Day -7), on the day of protocol initiation (Day 0), and at AI. Samples taken on Day -7 and 0 were used to determine cyclicity (serum $P_4 \geq 1$ ng/mL). Cyclicity status and proportion of cows with a CL at protocol initiation did not differ between treatments ($P > 0.53$). Proportion of cows detected in estrus was greater ($P = 0.01$) for 2PGF (63.1%) than HICON (49.3%). Treatment did not affect P/AI ($P = 0.87$), as mean P/AI was 51% vs. 52% for HICON and 2PGF, respectively. Cows detected in estrus had increased ($P < 0.01$) P/AI (62.4 vs. 40.5%), and cyclic cows tended ($P = 0.09$) to have greater P/AI compared with non-cyclic cows (57.0 vs. 46%). Serum $P_4$ at AI also affected ($P < 0.01$) P/AI; as $P_4$ concentration

increased, P/AI decreased. A ROC curve analysis showed a greater P/AI was achieved when serum $P_4$ was ≤ 0.43 ng/mL, with a sensitivity and specificity of 96.8% and 23.0%, respectively. A lesser proportion ($P < 0.01$) of HICON cows had serum $P_4$ at AI ≤ 0.43 ng/mL than 2PGF cows (84.0 and 97.0%, respectively). Mean serum $P_4$ concentrations at AI were 0.36 ± 0.03 and 0.13 ± 0.03 ng/mL for HICON and 2PGF, respectively. In summary, although two doses of conventional PGF more effectively reduced $P_4$ to ≤ 0.43 ng/mL by the time of AI than one dose of high-concentration PGF, subsequent P/AI was not different between treatments.

**Keywords:** beef cows, pregnancy, progesterone, prostaglandin $F_{2\alpha}$, 5-day CO-Synch + CIDR

## INTRODUCTION

Estrous synchronization (ES) and fixed-time AI (FTAI) programs are useful management tools for beef producers to improve reproductive efficiency and rapidly introduce superior genetics into their herd. Nonetheless, the current adoption of synchronization protocols in the beef industry is less than 10% in the United States, as cow-calf producers have been hesitant to implement these reproductive technologies, partially because of cost, facilities, and labor and time constraints (Elliott et al., 2013). The development of ES protocols that limit cost and labor inputs may therefore improve the adoption of FTAI within the industry.

The 5 and 7-d, progesterone-based, CO-Synch protocols have been used to promote the use of AI and produce acceptable pregnancy rates in beef cows (Bridges et al., 2008; Ahmadzadeh et al., 2015; Kasimanickam et al., 2009). Addition of progestins, in the form of a CIDR, to CO-Synch protocols has been shown to improve synchrony of ovulation and overall fertility to FTAI, particularly in anestrous cows (Lamb et al., 2001; Gunn et al., 2016). Santos

et al. (2010), however, suggested that prolonged $P_4$ exposure in the 7-d CO-Synch + CIDR protocol may increase risk of persistent follicles and aged oocytes. By shortening the duration of CIDR exposure (i.e. from 7 to 5 d), duration of follicular dominance can be decreased resulting in improved oocyte quality and P/AI (Bridges et al., 2008; Cerri et al., 2011a,b; Santos et al., 2010). However, when the interval between GnRH and PGF administration is shortened, the success of synchronization relies on the ability of PGF to regress a newly formed, GnRH-induced corpus luteum (CL), and reduce circulating $P_4$ concentrations by the time of AI.

It is known that new and immature (≤ 5 d old) bovine CL are refractory to the luteolytic actions of PGF (Lauderdale, 1972; Tsai and Wiltbank, 1998; Miyamoto et al., 2009); therefore, one luteolytic dose of PGF may not be sufficient to cause complete luteolysis. Incomplete luteal regression after treatment with PGF decreases fertility in FTAI protocols (Stevenson and Phatak, 2010). To overcome variations in luteolysis and reliably induce CL regression, researchers have recommended that two 25 mg PGF injections, given 5 to 24 h apart at CIDR removal, should be utilized in a 5-d CO-Synch + CIDR protocol (Bridges et al., 2008; Chebel et al., 2008; Kasimanickam et al., 2009; Whittier et al., 2010; Santos et al., 2010). Although two PGF injections have been shown to improve luteolytic response in dairy cows (Santos et al., 2010) and P/AI in beef cows (Kasimanickam et al., 2009; Bridges et al., 2012), the addition of a second PGF involves additional handling of cattle, increases drug cost, labor and time requirements, and potentially decreases protocol compliance.

A recent study evaluated the ability of a high-concentration PGF product (*dinoprost tromethamine,* 12.5 mg/mL) to establish complete luteolysis compared with that of two

injections of conventional PGF (*dinoprost tromethamine,* 5 mg/mL) in the 5-d CO-Synch + CIDR protocol (Spencer et al., 2017). This study revealed that one 25 mg dose of high-concentration PGF, administered at CIDR removal, is as effective as two doses of conventional PGF in inducing luteolysis and reducing serum $P_4$ concentrations (≤ 0.5 ng/mL) by the time of AI in beef cows. However, there is a lack of information on the effect of this high-concentration PGF product in a 5-d CIDR protocol on P/AI in postpartum beef cows.

## OBJECTIVES

The objectives of this study were to examine the effects of administering a single high-concentration dose of PGF at CIDR removal on 1) serum $P_4$ concentration at the time of fixed-time artificial insemination and 2) pregnancy rates to artificial insemination in postpartum crossbred beef cows synchronized with a 5-d CO-Synch + CIDR protocol. An ancillary objective was to determine a cutoff concentration of $P_4$ at the time of AI at which probability of pregnancy is optimized.

## HYPOTHESIS

Based on previous results from Spencer et al. (2017) that showed the effectiveness of a single injection of high-concentration PGF in causing luteal regression and reducing circulating $P_4$ by AI, we hypothesized that serum $P_4$ concentrations at the time of insemination and P/AI would not differ between cows receiving one high-concentration dose or two conventional doses of PGF at CIDR removal.

## MATERIALS AND METHODS

All procedures and protocols in this experiment were in compliance with the University of Idaho, Animal Care and Use Committee (Protocol #IACUC-2016-17; Appendix 1).

This study was conducted in two consecutive years at the Nancy M. Cummings Research, Extension and Education Center in Carmen, ID. Four-hundred and four primiparous (n=60) and multiparous (n=344) Angus-Hereford crossbred cows were used. Cows were maintained on irrigated pasture and supplemented with alfalfa hay before and at the time of experiment. Body condition scores (BCS; 1 to 9 scale, 1= emaciated, 9=obese) and body weights (BW) were determined at initiation of the experiment. At initiation, cows were approximately 67 days postpartum (DPP) and had an average BCS of 5.5 (Table 2.1).

**Experimental Design and Treatments**

Seven days before (Day -7) and on the day of experiment initiation (Day 0), blood samples were collected from all cows to determine cyclicity status (Figure 2.1). On Day 0, GnRH (100 μg, i.m.; Factrel®; Zoetis Inc., Kalamazoo, MI) was administered to all animals and, using aseptic techniques, a controlled internal drug release insert (1.38 g [$P_4$]; EAZI-breed CIDR cattle insert; Zoetis Inc.) was inserted vaginally (Figure 2.1). On Day 5, CIDR inserts were removed and cows were stratified by BCS, BW, age, post-TAI grazing location and DPP, and assigned randomly to receive either one luteolytic dose of high-concentration PGF i.m. (HICON n=203; 12.5 mg/mL, 25 mg, Lutalyse® *HighCon*, Zoetis Inc., Parsippany, NJ; Figure 2.1) or two i.m. doses of conventional PGF, 8 h apart (2PGF n=201; 5 mg/mL, 50 mg, Lutalyse®, Zoetis Inc., Parsippany, NJ; Figure 2.1). All injections were administered in the neck region. Cows in both treatment groups were separated from their calves before the initial PGF injection and remained apart (7 to 11 h) until after the second PGF was given to cows in the 2PGF group. On Day 8, an additional blood sample was collected for $P_4$ analysis (Figure 2.1). Subsequently, all cows received a second GnRH (100 μg) and were artificially inseminated by one of two technicians (same technicians in both years) at a fixed time (72 ± 2

hours after treatments) with semen from one of six sires in 2018 and nine sires in 2019. After insemination, cows were split into two grazing groups (irrigated pasture or rangeland). Within five days of insemination, rangeland cows (n=171) were transported off-site to their post-TAI grazing location and exposed to herd bulls seven days after TAI. Remaining cows (n=233) were kept on irrigated pasture and exposed to herd bulls approximately 14 d after TAI. Cows in both treatment groups were evenly distributed across post-TAI grazing location.

**Cyclicity Status, Estrous Detection and Pregnancy Diagnosis**

To determine whether cows were cyclic or acyclic before initiation of the experiment, serum $P_4$ concentration was quantified using blood samples collected on Day -7 and Day 0. Cows with serum $P_4 \geq 1.0$ ng/mL on one or both sampling days were considered cyclic, whereas animals with $P_4 < 1.0$ ng/mL on both days were categorized as anestrous.

For detecting estrus, Estrotect heat detection-aid patches (Rockaway Inc. Spring Valley, WI) were applied to the tailhead of all cows on Day 5 and cows were visually observed for estrual behavior three times daily until Day 8 (Figure 2.1). Animals were considered to have been in estrus before TAI if visually observed standing to be mounted or if estrus patches were ≥ 50% activated (at least half of the patch had changed color) at the time of insemination. Cows with missing patches were considered to have been in estrus only if previously observed standing to be mounted.

Pregnancy status was confirmed by examination of the uterine contents using transrectal ultrasonography 48 to 63 d after TAI (ReproScan XTC, ReproScan, Winterset, IA or Ibex Evo, E.I. Medical Imaging, Loveland, CO). On average, pregnancy diagnosis in cows on rangeland was performed within 7 d after cows on irrigated pasture. In the second year,

initial pregnancy diagnosis was conducted later after TAI than in the first year because of management choices of the operation.

**Blood Sampling and Progesterone Quantification**

Blood samples were collected by venipuncture of the coccygeal artery or vein using a 10 mL vacutainer tube (Coviden® LLC, Mansfield, MA). All samples were placed on ice immediately after collection and then stored at 4°C for 24 h. Samples were centrifuged at 2,400 x g at 4°C for 20 minutes and serum was harvested and stored at -20°C until assayed for $P_4$ quantification.

Serum $P_4$ concentration was analyzed using a solid phase single antibody-coated tube RIA (ImmuChem® Coated Tube Progesterone $^{125}$I RIA Kit, MP Biomedicals, Costa Mesa, CA) in a single assay under equilibrium conditions. Assay standards were modified per the procedure developed by Scarpa et al. (2019). Briefly, $P_4$ (minimum 99%, SIGMA-ALDRICH, Inc., St. Louis, MO) was dissolved in absolute ethanol for a working $P_4$ stock solution (50 μg/mL). Progesterone standards were prepared by serial dilution of the $P_4$ stock solution in fetal bovine serum (VWR Seradigm LIFE SCIENCE®, VWR International, LLC, Radnor, PA). Fetal bovine serum was used as a diluent to account for matrix effects observed in previous assays using original standards (with human serum as the diluent) provided by the manufacturer.

Serum samples collected on Day 8 were run in duplicate, whereas Day -7 and Day 0 samples were run as singles to establish high (≥ 1 ng/mL) or low (< 1 ng/mL) $P_4$ concentration for determination of cyclicity status. Cows were considered to be cycling if at least one of two blood samples (Day -7 or 0) had concentrations of $P_4 \geq 1.0$ ng/mL. The standard curve ranged from 0.25 ng/mL to 20 ng/mL, and the lowest limit of detection was

determined as 0.10 ng/mL, based on the concentration at which 90% of tracer was bound to antibody. Intra-assay coefficient of variation was 7.3%.

**Statistical Analyses**

Continuous data including descriptive variables (BCS, BW, age, and days postpartum), and $P_4$ concentrations on Day -7, 0 and Day 8 were analyzed by ANOVA using the GLM procedure of SAS version 9.4 (SAS Inst. Inc., Cary, NC, USA) to compare treatment groups. The final model used to analyze descriptive data included the effects of treatment, year, and treatment by year interaction. The differences in mean $P_4$ on Day -7 and 0 were analyzed using the same model. To examine the effect of treatment and pregnancy status on $P_4$ concentration on Day 8, the final model included treatment (HICON vs. 2PGF), P/AI (pregnant vs. non-pregnant) and its interaction with treatment.

Categorical data including cyclicity status, presence of an active CL at protocol initiation (Day 0), progesterone status at AI ($P_4 \leq$ or $>$ 1.0, 0.5, and 0.43 ng/mL), estrus expression, and P/AI and breeding season pregnancy rates were analyzed by logistic regression using the GLIMMIX procedure of SAS. The effects of treatment and year were kept in all final models, but additional effects and their interaction with treatment and year were sequentially removed from the model if $P > 0.20$.

Original models for testing the difference in presence of a CL on Day 0 and cyclicity status before the breeding season between treatments included the effects of treatment, year, parity, BCS, days postpartum and all appropriate interactions. The final models for both of the variables above included the effects of treatment, year, days postpartum, and treatment by year interaction. To determine the difference in $P_4$ status between treatments at AI ($P_4 \leq$ or $>$ 0.5, 1.0 and 0.43 ng/mL) and estrus expression before AI, the original model included the

covariates of $P_4$ on Day 0 and -7, cyclicity, CL presence on Day 0, parity (primi- or multiparous), days postpartum (< 60 or ≥ 60 d) and all necessary two-way interactions with treatment and year. Both the final models for progesterone status at AI and estrus expression were reduced to the effects of treatment, year, and their interaction.

To determine differences in P/AI between treatment groups, the original model included the effects of treatment, year, post-AI grazing location (rangeland or irrigated pasture), BCS (score 1 to 9), days postpartum (< 60 or ≥ 60 d), presence of a CL on Day 0, AI sire, estrus expression before AI, cyclicity (acyclic or cyclic), parity (primi- or multiparous), $P_4$ concentration at AI (ng/mL), AI technician (Tech 1 or Tech 2) and all two-way interactions with year and treatment. The reduced model for P/AI contained the effects of treatment, year, estrus expression before AI, cyclicity, parity, $P_4$ concentration at AI, and AI technician and its interaction with year. Two cows in the HICON group were missing at the time of pregnancy diagnosis and one cow from the 2PGF group was not inseminated at the time of breeding because of an injury; thus, these cows were not included in the final analysis for P/AI.

Predicted probabilities of pregnancy were computed using the LOGISTIC procedure in SAS and the effect of serum progesterone at TAI on P/AI was determined. The logistic regression equation for the prediction model for probability of pregnancy took the form of:

$$\ln [P / 1 - P] = \beta_0 + \beta_1 x_1$$

x = serum progesterone concentration cutoff

$\beta_0$ = intercept

$\beta_1$ = slope

Using the intercept and the coefficient estimates from the logistic regression equation, receiver operating characteristic (ROC) curve analysis was performed in SAS to compute the

optimal cutoff concentration of $P_4$ at TAI to achieve greater probability of pregnancy. The ideal $P_4$ cutoff was selected based on the greatest combined sensitivity and specificity using the maximum Youden Index value (sensitivity + [specificity – 1]) to predict pregnancy in beef cows (Unal, 2017). Sensitivity was the probability of pregnancy accurately predicted as a pregnancy (true positive), and specificity was the probability of non-pregnancy accurately predicted as a non-pregnancy (true negative; Hart, 2016).

All means are expressed as least square means ± SEM. A probability of 0.05 or less was considered significant, and a probability between 0.05 and 0.10 was considered as a tendency.

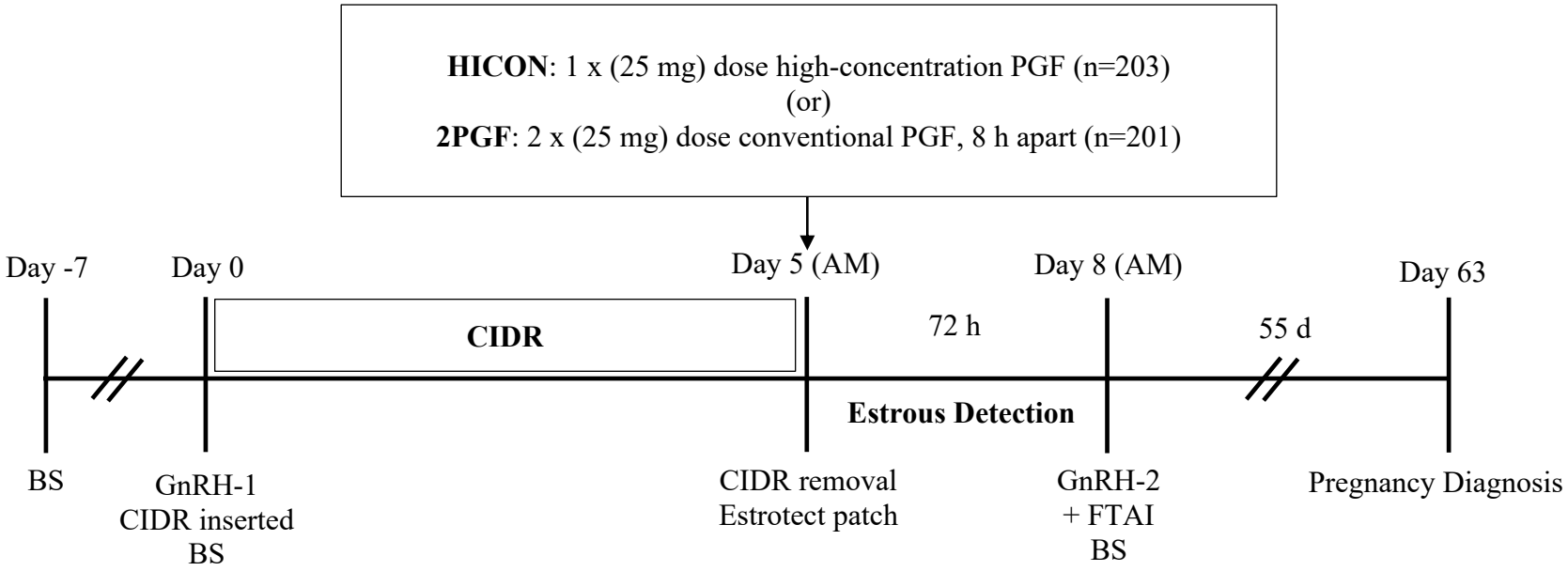



**Figure 2.1.** Schematic of experimental design to examine the effect of one high-concentration PGF (HICON; n=203; 25 mg, i.m. [12.5 mg/mL]) or two conventional PGF (2PGF; n=201; 50 mg, i.m. [5 mg/mL]) on progesterone concentrations and AI pregnancy rates in Angus-Hereford crossbred beef cows subjected to a 5-d CO-Synch + controlled internal drug release (CIDR) protocol. To quantify serum progesterone, blood samples (BS) were collected on Day -7, 0 and 8 via venipuncture of the coccygeal vein. Pregnancy diagnosis using ultrasonography was conducted 55 d after fixed-time AI (FTAI). Herd bulls were introduced to cows either 5 (n=171) or 14 (n=233) days following FTAI in both years

## RESULTS

### Descriptive Data

There were no differences in mean DPP ($P = 0.65$), cow age ($P = 0.88$), BW ($P = 0.77$), or BCS ($P = 0.34$) between treatments at initiation of the experiment (Table 1). Similarly, means for age ($P = 0.12$), BW ($P = 0.51$), and BCS ($P = 0.25$) did not differ between years. However, DPP at the start of the experiment differed ($P < 0.01$) between years, as average DPP at the initiation of the experiment was 7 d greater in 2019 than in 2018. (Table 2.1). Overall, cows were 67 ± 0.7 days postpartum, 6.0 ± 0.2 years of age, 581.2 ± 4.3 kg in BW, and had a BCS of 5.5 ± 0.1 at initiation of the protocol (Table 2.1).

### Cyclicity and Estrus Response

Based on $P_4$ concentrations > 1.0 ng/mL on Day -7 and Day 0, 73.3% of all cows were cyclic before synchronization, and 55% had an active CL on the day of initiation (Table 2.2). Proportion of cows cyclic ($P = 0.99$) or with an active CL on Day 0 ($P = 0.48$) were not different between treatments, indicating that, on average, cows in both treatment groups were in similar reproductive states (at the ovarian level) at the beginning of the experiment (Table 2.2). Interestingly, more ($P < 0.01$) cows were cyclic before synchronization and a greater ($P < 0.01$) proportion had a CL on Day 0 in 2018 than 2019 (Table 2.2).

There was an effect of DPP on cyclicity status ($P < 0.01$), as a greater proportion of cows ≥ 60 DPP were cyclic than cows that were < 60 DPP (82.9 vs. 54.1%). Similarly, more ($P < 0.01$) cows that were ≥ 60 DPP had an active CL on Day 0 than cows that were < 60 DPP (61.2 vs. 41.8%). No significant two-way interactions were detected among DPP, treatment or year for cyclicity or presence of a CL on Day 0.

There was no effect of year by treatment interaction on estrus response; however, estrus response differed between treatments ($P = 0.01$) and year ($P < 0.01$). Regarding treatments, fewer cows in the HICON group were detected in estrus than in the 2PGF group (50.7 vs. 63.1%; Table 2.2). In addition, fewer cows were detected in estrus in 2018 than in 2019 (64.1% vs. 49.5; Table 2.2). Regardless of year, CL presence on Day 0 affected estrual behavior, in that cows without an active CL on Day 0 had greater ($P < 0.01$) estrus response before AI (65.5%) than cows with a CL on Day 0 (49.7%).

**Progesterone at the Time of Insemination**

For all cows, serum $P_4$ concentrations at TAI ranged from 0.10 to 5.89 ng/mL, with an overall mean concentration of 0.22 ± 0.03 ng/mL. A total of 34 cows had $P_4 > 0.5$ ng/mL (HICON: n=29; 2PGF: n=5) and 21 cows had $P_4 > 1.0$ ng/mL (HICON: n = 20; 2PGF: n = 1) at the time of AI. There was an effect of treatment on serum $P_4$ at TAI, as mean concentration at TAI was greater ($P < 0.01$) in HICON (0.36 ± 0.03 ng/mL) than 2PGF cows (0.13 ± 0.03 ng/mL; Table 2.3). Across both treatments, $P_4$ concentrations on Day 8 also tended ($P = 0.08$) to be lesser in 2019 than in 2018 (Table 2.3). In addition, there was a tendency ($P = 0.06$) for a year by treatment interaction, whereby HICON cows in 2018 had greater $P_4$ at TAI (0.44 ± 0.05 ng/mL) than in 2019 (0.28 ± 0.05 ng/mL). In contrast, $P_4$ concentration in 2PGF cows was the same between years (0.13 ± 0.05 ng/mL). This may be attributable to the fact that two HICON cows in 2018 had $P_4 \geq 4.0$ ng/mL at AI, whereas all cows, regardless of treatment in 2019 had $P_4 \leq 2.5$ ng/mL at AI.

Lack of an active CL at TAI was defined using two different $P_4$ cutoff points. Specifically, cows with $P_4 \leq 0.5$ or $\leq 1.0$ ng/mL were considered to have no functional luteal tissue at the time of insemination. Proportion of cows with $P_4 \leq 1.0$ ng/mL at timed-AI were

not different (100 vs. 91.6% for 2PGF and HICON, respectively; $P = 0.97$) between treatment groups (Table 2.3). However, a greater ($P < 0.01$) proportion of cows in the 2PGF group had $P_4$ concentrations ≤ 0.5 ng/mL at TAI than cows in the HICON group (98.6% and 86.8%, respectively; Table 2.3). There was no effect of year or year by treatment interaction on the proportion of cows with $P_4 \leq 0.5$ or ≤ 1.0 ng/mL at TAI.

Regardless of treatment, mean $P_4$ concentrations in cows that became pregnant to AI were lesser ($P < 0.01$) at TAI (0.13 ± 0.03 ng/mL) than in non-pregnant cows (0.36 ± 0.04 ng/mL). A pregnancy status by treatment interaction was also detected ($P < 0.01$) for $P_4$ concentration at the time of insemination. Non-pregnant cows in the HICON group had greater serum $P_4$ at AI (0.59 ± 0.05 ng/mL) than pregnant cows (0.13 ± 0.04 ng/mL), whereas pregnant and non-pregnant cows in the 2PGF group had similar mean $P_4$ concentrations (0.13 ± 0.04 ng/mL and 0.13 ± 0.05 ng/mL, respectively).

**Pregnancy Response to AI**

There was no effect ($P = 0.87$) of treatment on P/AI. Mean (adjusted) P/AI for HICON and 2PGF was 51% and 52%, respectively (Table 2.4). The unadjusted proportion of cows pregnant to AI for HICON was 58.4% and 65.8% for 2PGF. Although there was no treatment or treatment by year effect on pregnancy response, P/AI was greater ($P = 0.04$) in 2018 (58.6%) than in 2019 (45.4%; Table 2.4). Cyclicity status (based on $P_4$ concentrations before experiment initiation) tended to affect ($P = 0.09$) P/AI, regardless of treatment. As would be expected, cows that were cyclic tended to have a greater pregnancy response to AI than acyclic cows (57% and 46%, respectively; Table 2.4). In addition, there was an effect ($P = 0.03$) of parity on P/AI, as primiparous cows had lesser P/AI than multiparous cows (42.9% and 69.5%, respectively; Table 2.4). To no surprise, P/AI was greater ($P < 0.01$) in cows

detected in estrus before or at the time of AI (62.4%) than in non-estrus cows (40.5%; Table 2.4). No treatment or year by estrus expression interactions were detected. Insemination technician also had an effect on pregnancy outcome, as cows bred by Tech 1 had greater ($P$ = 0.04) P/AI than cows bred by Tech 2 (55.4% vs. 45.5%; Table 2.4). Likewise, there was an interaction between AI technician and year for P/AI. For Tech 2, P/AI differed ($P$ = 0.02) between 2018 (58.6%) and 2019 (33.1%), whereas Tech 1 produced consistent P/AI across years (56.5% and 58.4%; Table 2.5). No additional two-way interactions between AI technician and other independent variables (i.e. estrus expression, cyclicity, CL on Day 0, DPP, BCS, parity, $P_4$ status at AI, or AI sire) were detected that could have described the discrepancy in P/AI between years for Tech 2.

Sires used to inseminate cows for AI were distributed equally across treatments. Pregnancy rates among AI sires range from 40% to 75%. Further data on frequency of use and pregnancy success for each sire can be found in Appendix 2. Average breeding season pregnancy rate after exposure to herd bulls was 89.5%. Less ($P$ = 0.03) cows were pregnant at the end of the breeding season in the HICON group (87.5%) than in the 2PGF group (93.8%). By year, breeding season pregnancy rate was greater ($P$ < 0.01) in 2018 than 2019 (95.8 and 84.3, respectively).

**Relationship between P/AI and Progesterone**

Serum $P_4$ concentration at TAI affected ($P$ < 0.01) P/AI. In general, as $P_4$ concentration increased, the probability of pregnancy decreased. Cows with $P_4 \leq 0.5$ ng/mL at AI (n=273) had 65.5% P/AI, whereas cows with $P_4 > 0.5$ (n=30) achieved 20%. Receiver operator characteristic curve analysis revealed a nonlinear relationship between $P_4$ at TAI and P/AI, whereby the optimal threshold for $P_4$ at TAI was ≤ 0.43 ng/mL, with a specificity and

sensitivity of 23.0 and 96.8, respectively (Figure 2.2). This implies that 96.8% of cows pregnant to AI had $P_4 \leq 0.43$ ng/mL, and 23.0% of nonpregnant cows had $P_4 > 0.43$ ng/mL (Figure 2.3). At the cutoff concentration of 0.43 ng/mL, the area under the ROC curve was 0.59 (95% CI = 0.545 to 0.629; $P < 0.01$), indicating that $P_4$ concentration at TAI correctly classifies randomly selected pairs of non-pregnant and pregnant cows 59% of the time (Figure 2.2). Using this cutoff to model P/AI, it was determined that the odds of pregnancy decreased ($P < 0.01$) in cows with $P_4$ concentration > 0.43 ng/mL (OR = 7.72; 95% CI: 3.44 to 17.30).

Total percentage of animals with serum $P_4 \leq 0.43$ ng/mL at TAI was 87.9%. Proportion of cows with $P_4 \leq 0.43$ ng/mL at TAI was greater ($P < 0.01$) in the 2PGF group than the HICON group (97.0% vs. 84.0%, respectively; Table 2.3).

**Table 2.1.** Least square means (± SEM) for days postpartum, age, body weight (BW), and body condition scores (BCS) for postpartum suckled beef cows subjected to a 5-d CO-Synch + CIDR protocol given different doses of prostaglandin $F_{2\alpha}$ (PGF) at CIDR removal.

| Item | Treatment[1] | | Year[2] | | Overall (n=404) | *P*-value | |
|---|---|---|---|---|---|---|---|
| | HICON (n=203) | 2PGF (n=201) | 2018 (n=200) | 2019 (n=204) | | *Treatment* | *Year* |
| Days Postpartum[3] | 66.67 ± 1.0 | 67.33 ± 1.0 | 63.14 ± 1.0 | 70.85 ± 1.0 | 67.0 ± 0.7 | 0.65 | < 0.01 |
| Age, y[4] | 5.84 ± 0.2 | 5.79 ± 0.2 | 6.08 ± 0.2 | 5.54 ± 0.2 | 6.0 ± 0.1 | 0.88 | 0.12 |
| BW, kg[5] | 579.87 ± 6.1 | 582.37 ± 6.1 | 578.26 ± 61. | 583.97 ± 6.1 | 581.16 ± 4.3 | 0.77 | 0.51 |
| BCS[6] | 5.47 ± 0.1 | 5.54 ± 0.01 | 5.55 ± 0.1 | 5.46 ± 0.1 | 5.5 ± 0.0 | 0.34 | 0.25 |

[1] Cows were assigned to receive either one high-concentration PGF (HICON, 25 mg [12.5 mg/mL] per dose) or two conventional PGF (2PGF, 25 mg [5 mg/mL] per dose) 8 h apart at CIDR removal (Day 5).
[2] Cows from the same herd were used in two consecutive years.
[3] Days from last calving to the initiation of the protocol (Day 0).
[4] Age of cow at beginning of breeding season.
[5] Body weight on day of protocol initiation (Day 0).
[6] Body condition score (scale 1-9; 1 = emaciated 9 = obese) on day of protocol initiation (Day 0).

**Table 2.2.** Percentage of cows cyclic before estrous synchronization[1], with luteal tissue on Day 0 of the experiment, and detected in estrus by the time of AI within treatment and year.

| | Treatment[2] | | Year | | | *P*-value | |
|---|---|---|---|---|---|---|---|
| Item | HICON | 2PGF | 2018 | 2019 | Overall | *Treatment* | *Year* |
| Cyclic (%)[3] | 70.5 (149/202) | 70.5 (146/200) | 81.7 (161/198) | 56.1 (134/204) | 73.3 (295/402) | 0.99 | < 0.01 |
| CL on Day 0 (%)[4] | 53.4 (115/203) | 49.7 (107/201) | 62.0 (126/199) | 40.9 (96/204) | 55.0 (222/403) | 0.48 | < 0.01 |
| Estrus (%)[5] | 50.7 (103/203) | 63.1 (126/201) | 49.5 (99/200) | 64.1 (130/204) | 56.7 (229/404) | 0.01 | < 0.01 |

[1] The 5-day CO-Synch + CIDR protocol was used to synchronize cows as follows: GnRH + CIDR (Day 0), CIDR removal and treatment (Day 5), GnRH + AI (Day 8).
[2] Cows were assigned to receive either one high-concentration PGF (HICON, 25 mg [12.5 mg/mL] per dose) or two conventional PGF (2PGF, 25 mg [5 mg/mL] per dose) 8 h apart at CIDR removal.
[3] Cows with $P_4$ > 1.0 ng/mL on Day -7 and/or Day 0 were considered cyclic.
[4] Cows with $P_4$ > 1.0 ng/mL on Day 0 were considered to have an active CL.
[5] Cows detected in estrus before and/or at the time of AI using visual observation and heat detection aid patches.

**Table 2.3.** Least square means (± SEM) of serum progesterone ($P_4$) concentrations and percentage of cows with $P_4$ below different concentration thresholds at the time of AI (Day 8) between treatments[1].

| | Treatment | | Year | | | *P*-value | |
|---|---|---|---|---|---|---|---|
| Item | HICON (n=203) | 2PGF (n=201) | 2018 (n=200) | 2019 (n=204) | Overall (n=404) | *Treatment* | *Year* |
| $P_4$ (ng/mL) | 0.36 ± 0.03 | 0.13 ± 0.03 | 0.28 ± 0.03 | 0.20 ± 0.03 | 0.22 ± 0.03 | < 0.01 | 0.08 |
| $P_4$ cutoff at AI (%) | -- | -- | -- | -- | -- | -- | -- |
| ≤ 1.0 ng/mL[2] | 91.6 | 100.0 | 99.9 | 97.2 | 95.3 | 0.97 | 0.98 |
| ≤ 0.5 ng/mL[3] | 86.8 | 98.6 | 96.0 | 95.1 | 92.6 | < 0.01 | 0.76 |
| ≤ 0.43 ng/mL[4] | 84.0 | 97.0 | 91.9 | 93.8 | 87.9 | < 0.01 | 0.54 |

[1] Cows were assigned to receive either one high-concentration PGF (HICON, 25 mg [12.5 mg/mL] per dose) or two conventional PGF (2PGF, 25 mg [5 mg/mL] per dose) 8 h apart at CIDR removal.
[2] Proportion of cows with $P_4 \leq 1.0$ ng/mL at AI.
[3] Proportion of cows with $P_4 \leq 0.5$ ng/mL at AI.
[4] Proportion of cows with $P_4 \leq 0.43$ ng/mL at AI, based on cutoff defined by ROC analysis.

**Table 2.4.** Pregnancy per artificial insemination (P/AI) in postpartum suckled beef cows subjected to a 5-d CO-Synch + CIDR protocol given different doses of prostaglandin $F_{2\alpha}$ (PGF) at CIDR removal.

| Item | Adjusted mean %[2] | Unadjusted mean %[3] | n/n | *P*-value[4] |
|---|---|---|---|---|
| Treatment[1] | | | | 0.87 |
| HICON | 51.0 | 58.4 | 118/202 | |
| 2PGF | 52.0 | 65.8 | 131/199 | |
| Year | | | | 0.04 |
| 2018 | 57.6 | 66.5 | 133/200 | |
| 2019 | 45.4 | 57.7 | 116/201 | |
| Cyclicity status | | | | 0.09 |
| Cyclic | 57.0 | 64.5 | 189/293 | |
| Acyclic | 46.0 | 55.7 | 59/106 | |
| Parity | | | | 0.03 |
| Primiparous | 42.9 | 51.7 | 31/60 | |
| Multiparous | 69.5 | 63.9 | 218/341 | |
| Estrus expression | | | | < 0.01 |
| Estrus | 62.4 | 71.1 | 162/228 | |
| Non-estrus | 40.5 | 50.3 | 87/173 | |
| AI technician | | | | 0.04 |
| Tech 1 | 57.4 | 68.0 | 142/206 | |
| Tech 2 | 45.5 | 54.9 | 107/195 | |

[1] Cows were assigned to receive either one high-concentration PGF (HICON, 25 mg) or two conventional PGF (2PGF, 25 mg each dose) 8 h apart at CIDR removal.
[2] Adjusted mean proportion derived from the logistic regression analysis.
[3] Unadjusted mean proportion calculated by number of pregnant cows over total number of cows within each category.
[4] *P*-value pertaining to the adjusted means derived from the logistic regression analysis.

**Table 2.5.** Pregnancy per AI for technician by year.

| Item | 2018 | 2019 |
|---|---|---|
| Technician 1 | 56.5[ax] (73/107) | 58.4[ax] (69/99) |
| Technician 2 | 58.6[ax] (60/93) | 33.1[by] (47/102) |

[a,b] Values without a common superscript within row differ ($P = 0.02$).
[x,y] Values without a common superscript within column differ ($P = 0.02$).

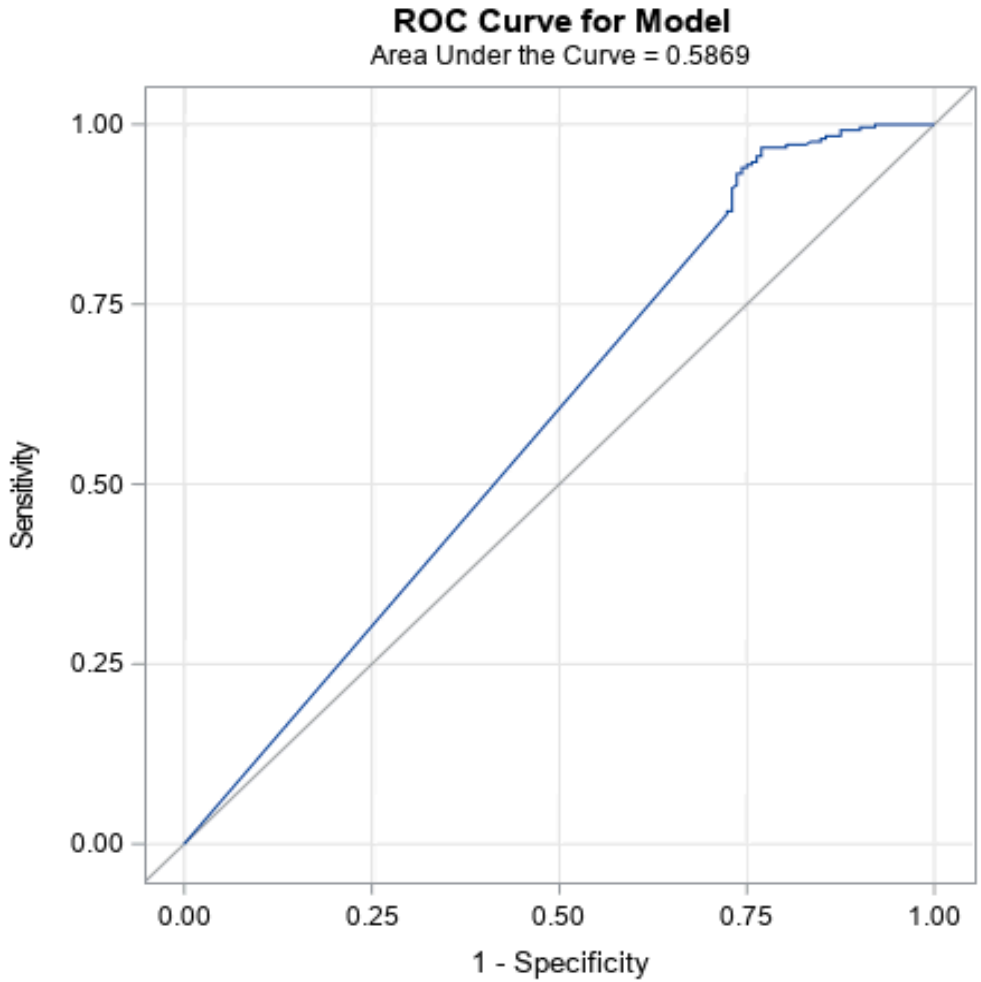



**Figure 2.2.** Receiver operating characteristic (ROC) curve for serum concentration of progesterone at AI that predicts pregnancy with the highest accuracy. Progesterone concentration of 0.43 ng/mL best predicted pregnancy. At progesterone concentration 0.43 ng/mL, sensitivity was 96.8% and specificity was 23.0%. Area under the curve was 0.59 (95% CI = 0.55 to 0.63; $P < 0.01$).

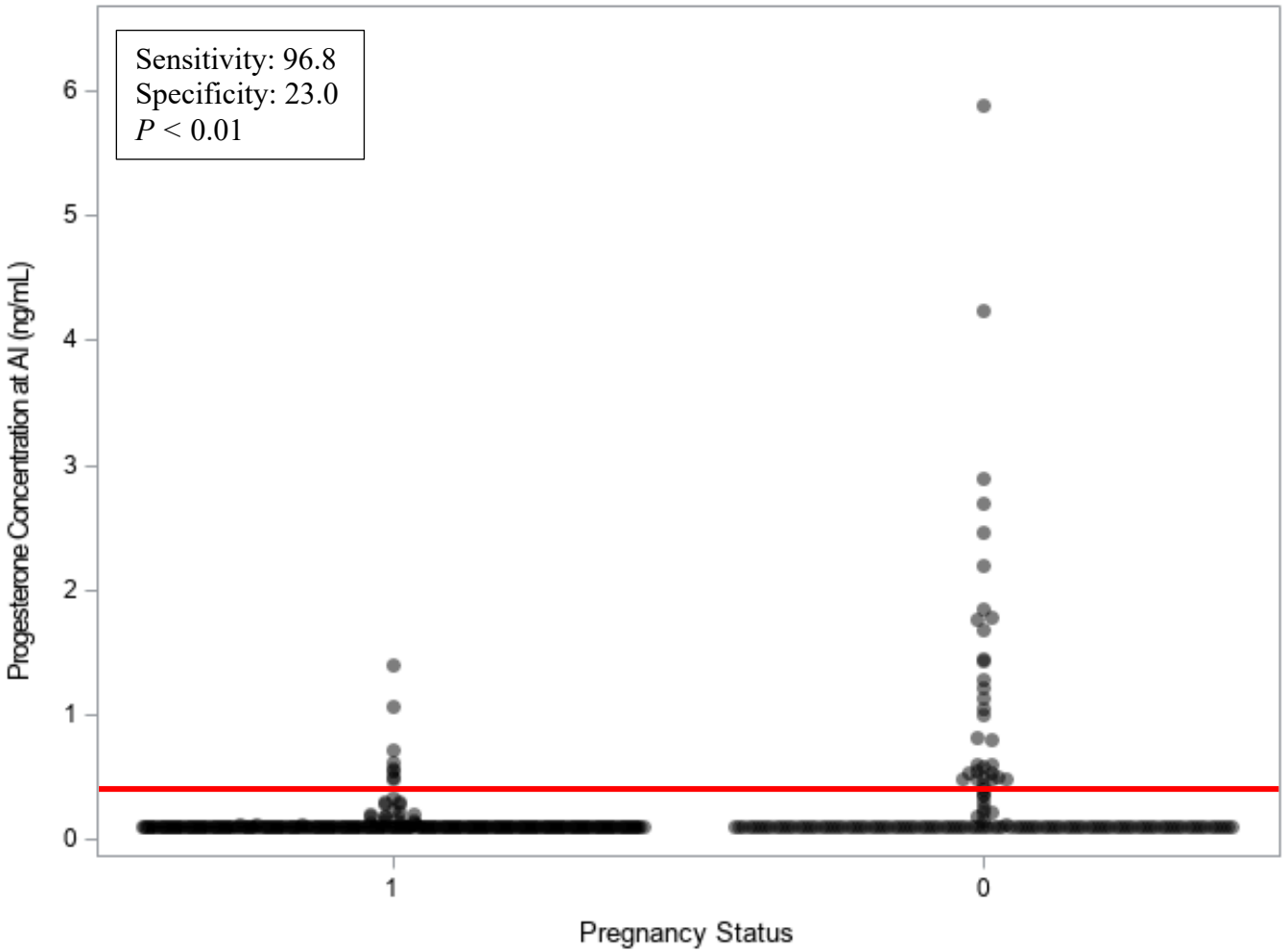
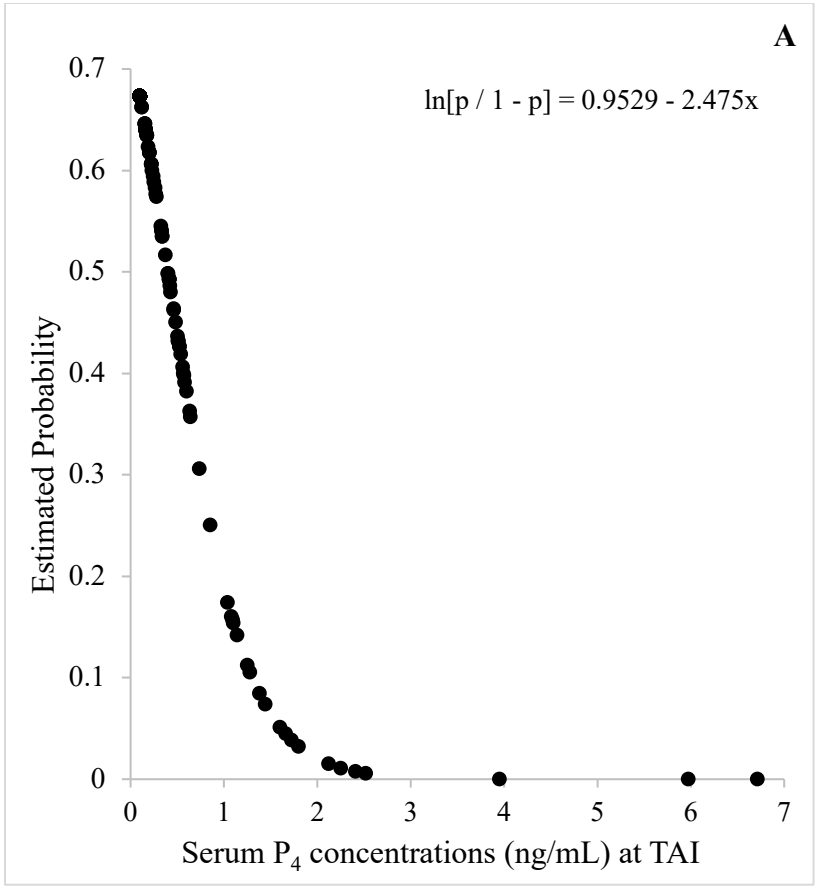



**Figure 2.3.** Dot plot for the distribution of serum progesterone concentrations at AI and pregnancy outcome (1 = pregnant; 0 = nonpregnant). The horizontal bar indicates the cut-off point level calculated by the receiver operating characteristics (ROC) analysis (≤ 0.43 ng/mL).



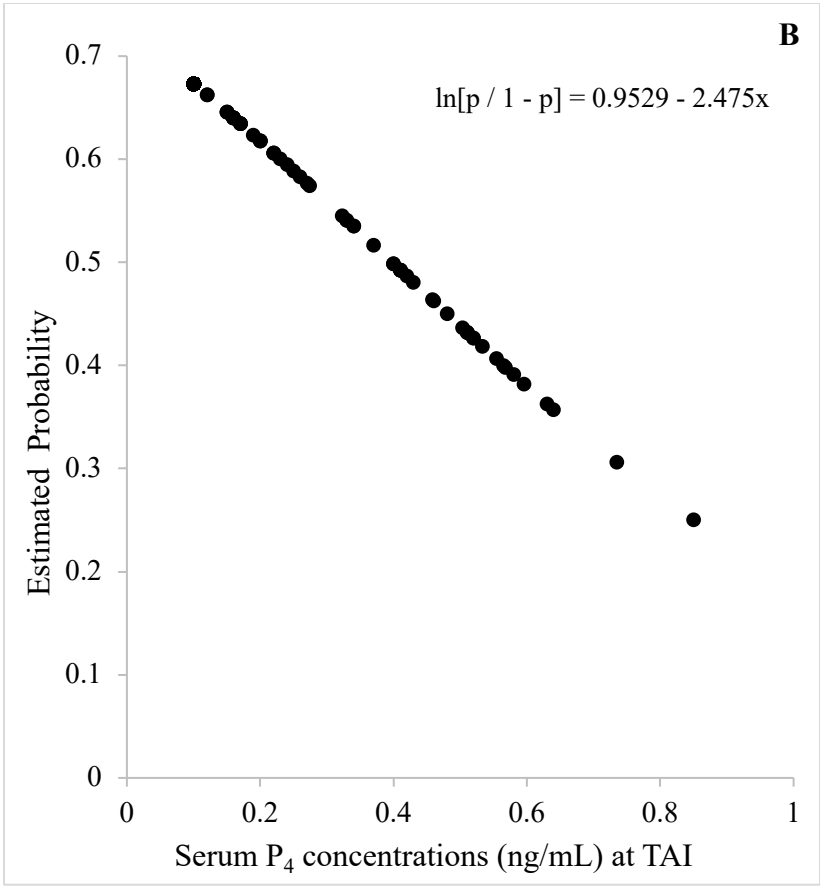




**Figure 2.4.** A) Relationship between serum progesterone ($P_4$) concentrations (ng/mL) at TAI and predicted probability of pregnancy (data from 403 cows were used). B) Relationship between serum $P_4$ concentrations at TAI and predicted probability of pregnancy including only cows with $P_4$ between 0.0 and 1.0 ng/mL at TAI (n=382). Cows with serum $P_4$ > 0.43 ng/mL had reduced ($P < 0.01$) chances to become pregnant.

**DISCUSSION**

The main objective of the current study was to compare the effects of administering a single injection of high-concentration PGF (12.5 mg/mL, 25 mg total) or two conventional doses (8 h apart, 5 mg/mL, 50 mg total) on serum $P_4$ concentrations at the time of AI and on P/AI in beef cows synchronized with the 5-d CO-Synch + CIDR protocol. Such a modification in PGF delivery in this protocol would reduce the number of animal handlings, cost associated with treatment and labor, and consequently increase the practicality of this approach to ovulation synchronization. Because $P_4$ is an important determining factor of fertility in FTAI protocols, a secondary objective was to assess the relationship between $P_4$ concentrations at the time of insemination and probability of pregnancy to AI.

Estrous synchronization protocols have become useful management tools in the beef industry to improve reproductive performance and reduce the number of days to first AI. The use of a CIDR, particularly in the 7-d or 5-d CO-Synch + CIDR protocols, has allowed for tight synchronization of ovulation, implementation of timed AI, and improved P/AI compared with Ovsynch (Xu and Burton, 2000; Larson et al., 2006; Bridges et al., 2008; Kasimanickam et al., 2009). However, researchers have reported improved P/AI when the interval from the initial GnRH to CIDR withdrawal was shortened from 7 to 5 days (Bridges et al., 2008; Kasimanickam et al., 2009). While reducing the period of $P_4$ exposure in the CO-Synch protocol may improve P/AI, it has been concluded that two injections of PGF are needed in the 5-d CIDR protocol to cause luteolysis of a newly formed GnRH-induced CL, reliably reduce $P_4$ concentrations by the time of AI, and optimize P/AI. (Bridges et al., 2008). Despite having specific receptors for PGF (Wiltbank et al., 1995), the bovine CL is resistant to the actions of PGF before day 5 of the estrous cycle (Lauderdale et al., 1974). While the

mechanisms associated with luteal resistance to PGF before day 5 are still not well understood (Pate, 2003; Meidan et al., 2017), it was hypothesized that two doses of PGF (administered 7 to 24 h apart) would overcome this refractoriness. Specifically, it was thought that a second PGF would improve luteal regression of a GnRH-induced CL by mimicking the natural pulsatile release of PGF from the endometrium during spontaneous luteolysis (Ginther et al., 2009).

Currently, there is limited information on the effectiveness of one or two PGF injections on $P_4$ concentrations in beef cows subjected to a 5-d CO-Synch + CIDR protocol. In addition, the importance of circulating $P_4$ concentrations before and at the time of AI in beef cows has not been reported on as extensively as in lactating dairy cattle. Stevenson and Lamb (2016) suggested that $P_4$ concentrations at the time of PGF administration in FTAI protocols is less important in beef breeds for subsequent luteolysis and fertility than it is in dairy cows. Though, the ability of PGF to cause luteolysis and reduce circulating $P_4$ below 0.5 ng/mL by the time of AI helps optimize P/AI in both beef and dairy cows (Stevenson and Lamb, 2016). Besides the current research, few studies in beef cattle have evaluated the use of one or two PGF injections in the 5-d CO-Synch + CIDR protocol (Kasimanickam et al., 2009; Souto et al., 2009; Bridges et al. 2012; Spencer et al., 2018), of which only one used a high-concentration dinoprost and studied $P_4$ profiles between PGF and AI (Spencer et al., 2018).

The current study provides the first evidence in postpartum beef cows that one high-concentration dose of PGF used in a 5-d CO-Synch + CIDR protocol results in similar P/AI as two conventional PGF doses; however, two PGF appeared to reduce $P_4$ concentrations more reliably by the time of AI. Inconsistent with our findings, Kasimanickam et al. (2009) found that P/AI was greater in cows receiving two injections of PGF (dinoprost) 7 h apart (69%)

compared with either one injection of dinoprost (52%) or a PGF analogue (cloprostenol; 54.3%). As a result, these researchers postulated that the apparent improvement of P/AI in cows that received two PGF was attributable to the effectiveness of a double-injection scheme to cause complete luteolysis and achieve optimal $P_4$ concentrations by the time of AI. However, $P_4$ decline and luteal regression following PGF treatment was not assessed in their study; therefore, the relationship between luteolysis and the improvements in P/AI with two PGF could not be demonstrated. When the effectiveness of inducing luteolysis using a single dose of cloprostenol or two doses of dinoprost given 12 h apart was compared by Souto et al. (2009), results provided evidence that a single administration of PGF did not cause complete luteal regression in all cows. Specifically, 65% of cows receiving a single cloprostenol (n=23) and 96% administered two dinoprost (n=25) underwent complete luteolysis. Though, it should be noted that the sample size used in their study was small. Another study by Bridges et al. (2012) evaluated luteolysis and P/AI in beef cows receiving one PGF, two concurrent PGF (50 mg), or two split PGF doses given 8 h apart following CIDR removal. These researchers defined luteolysis as $P_4$ concentrations > 2 ng/mL at PGF treatment and ≤ 1 ng/mL at the time of AI. Among cows receiving one, two, or two concurrent PGF doses, luteal regression did not differ (Bridges et al., 2012). Similar to these results, in the present study, proportion of cows with $P_4$ concentrations less than 1.0 ng/mL at the time of AI was not different between 2PGF and HICON groups. It should be noted, however, that a distinction exists between the current study, and those discussed above, in the type of dinoprost product administered to cows in a single injection, as the high-concentration dinoprost used in the present study may act differently at a physiological level.

It was formerly hypothesized that a single high-concentration product may have a prolonged physiological effect that could induce endogenous PGF pulsatility similar to two doses of conventional PGF (Spencer et al., 2017; 2018). This high concentrate dinoprost product, Lutalyse *HighCon,* has an apparent pharmokinetic advantage over its conventional counterpart, as it reaches a greater concentration in circulation (Cmax) following administration (Zoetis Inc., 2015). Former speculation has suggested that the vehicle by which this product is delivered in solution may differ from the conventional dinoprost product (A. Tibary, personal communication, 2018). While research does not imply any discrepancies in half-life or biological availability, it is possible that the greater Cmax associated with *HighCon* could be attributable to greater storage in lipid molecules and thus slower release following administration. Another PGF product, Bovaline® (cloprostenol sodium, 500 µg/dose; Syntex), was shown to have a longer biological activity compared with other PGF products, as it was stored in lipid molecules following administration (A. Tibary, personal communication, 2018). Therefore, *HighCon* may also become stored in lipids in circulation, resulting in prolonged biological activity. A recent study (Spencer et al., 2018) in our laboratory evaluated the effects of one high-concentration (Lutalyse *HighCon*) and one or two conventional dinoprost doses (12 h apart) in the 5-d CIDR protocol in Charolais beef cows (n=53). The authors demonstrated similar declines in circulating $P_4$ across PGF treatments, suggesting that one injection of high-concentration PGF is as effective as two conventional PGF in causing complete luteolysis ($P_4 \leq 0.5$ ng/mL; Spencer et al., 2018). Our research showed contrasting results to the study by Spencer et al. (2018), as 2PGF increased the proportion of cows with $P_4 \leq 0.5$ ng/mL at AI when compared with cows receiving HICON. However, if luteolysis is defined as $P_4 \leq 1.0$ ng/mL, our data correspond with previous

findings (Bridges et al., 2012) in that a similar proportion of cows receiving two or one PGF injection(s) had serum $P_4$ concentrations less than 1.0 ng/mL by AI.

Incomplete luteolysis is a limiting factor for achieving pregnancy in FTAI protocols (Souza et al., 2007; Brusveen et al., 2009). Complete luteal regression in response to exogenous PGF has been defined in various ways. In general, cows with elevated $P_4$ at the time of PGF administration (> 1.0 ng/mL) followed by reduced $P_4$ by 48 to 72 h later are considered to have undergone functional luteolysis. Multiple studies have defined luteolysis as a reduction in $P_4$ concentrations below 1.0 ng/mL at the time of AI (Stevenson and Phatak, 2010; Bridges et al., 2012; Hill et al., 2014), whereas others have suggested complete luteolysis does not occur unless $P_4$ is reduced to < 0.5 ng/mL (Martins et al., 2011; Colazo et al., 2017) or less (< 0.24 ng/mL; Santos et al., 2010). In response to the Ovsynch protocol, Martins et al. (2011) classified dairy cows as having complete ($P_4$ < 0.5 ng/mL), incomplete (0.5 < $P_4$ < 1.0 ng/mL) or no luteolysis ($P_4$ > 1.0 ng/mL) at 56 h after PGF treatment. In that study, cows with incomplete or no luteolysis had significantly reduced chances of becoming pregnant compared with cows with complete luteolysis. In the present study, by the definition of Martins et. al. (2011), the proportion of cows with complete luteolysis ($P_4$ < 0.5 ng/mL) 72 h following PGF treatment was greater in 2PGF than HICON; however, no effect of treatment was detected for the number of cows with $P_4$ concentrations > 1.0 ng/mL (no luteolysis) at AI.

It should be noted that we could not properly determine the risk of luteolysis in response to PGF treatment, as serum $P_4$ concentrations nor presence of luteal tissue at PGF administration were assessed. Nonetheless, because an equal proportion of animals in both treatment groups in the current study had luteal tissue present at CIDR insertion (56.9 and 53.7%, respectively), it could be postulated that a similar number of cows between treatments

also had a CL present at the time of CIDR removal and PGF administration. Moreover, although presence of new CL at PGF treatment was not evaluated, based on previous studies (Vasconcelos et al., 1999; Stevenson, 2016), it can also be speculated that, of the cows without luteal tissue at experiment initiation, approximately 50 to 60% responded to GnRH-1, and therefore would have possessed a new CL at the time of PGF treatment. Thus, the treatment effect on proportion of cows with low $P_4$ concentration at AI ($P_4 \leq 0.5$ ng/mL) could potentially represent a difference in effectiveness of 2PGF in causing luteolysis compared with the HICON group. Nonetheless, because it is unknown which cows responded to GnRH-1 and (or) had luteal tissue at PGF administration, the difference in incidence of luteolysis and the physiological reasoning behind the observed discrepancy between the two treatments could not be definitively assessed.

Kasimanickam et al. (2009) detected a greater incidence of estrus behavior in cows receiving two PGF injections than cows given one PGF. On the contrary, Bridges et al. (2012) did not observe an improvement in tail paint scores between cows receiving one or two PGF. Similar to results from Kasimanickam et al. (2009), estrus response was greater in cows that received 2PGF in the current study. The difference in estrus response between treatments may be explained, in part, by the fact that more cows in the 2PGF group had $P_4 \leq 0.5$ ng/mL at AI than cows in the HICON group. In other words, greater risk of presumed luteolysis in cows receiving 2PGF may explain the improved estrus response in this group. In our study, there was also an effect of estrus on P/AI across treatments. Cows detected in estrus had increased P/AI (62.4%) compared with non-estrus cows (40.5%). Several studies (Bridges et al., 2008; Dobbins et al., 2009; Kasimanickam et al., 2009; Whittier et al., 2010; Bridges et al., 2012) have demonstrated that FTAI pregnancy rates are improved in cows expressing estrus

behaviors near or at the time of AI. In addition, it has also been shown that multiparous beef cows often respond more effectively to FTAI protocols than primiparous cows (Bridges et al., 2012). As suggested, parity affected pregnancy outcome across treatments in the current study, as multiparous cows had greater P/AI than primiparous cows. Though, it should be noted that only 60 primiparous cows were used in our study.

Researchers have shown that anestrous cows do not respond as well as cyclic cows to estrous or ovulation synchronization and AI (Lamb et al., 2001; Larson et al., 2006). In the present study, cows that failed to establish an estrous cycle prior to synchronization tended to have lesser P/AI than cyclic cows. We also observed an effect of year on P/AI in that pregnancy response to TAI was greater in 2018 compared with 2019. It was previously demonstrated that cows with greater days postpartum at the beginning of the breeding season are more likely to become pregnant to AI (Larson et al., 2006; Bridges et al., 2012). One could argue that the difference in average day postpartum between the years may explain the discrepancy in P/AI. However, that is not the case, as cows in 2019 were, on average, 7 d more advanced postpartum at the start of synchronization than in 2018. Cows more advanced in postpartum are also more likely to be cyclic at the beginning of the breeding season (Stevenson et al., 2003). Interestingly, in this study, proportion of cyclic cows in 2019 was considerably lesser compared to cows in 2018 (56.1 vs. 81.7%), which does not align with previous concepts suggesting cows later in postpartum have a greater chance of resuming estrous cyclicity before the breeding season. Nonetheless, the decreased performance of one of the AI technicians in the 2019 breeding season should not be overlooked when considering the effect of year on P/AI.

In the present study, $P_4$ concentrations at the time of insemination affected P/AI. The relationship between fertility and $P_4$ at AI was negative, and risk of pregnancy was dramatically decreased in cows with $P_4 > 0.43$ ng/mL at AI. Others have also found a negative relationship between circulating $P_4$ 48 to 72 h after PGF treatment on the probability of pregnancy in dairy cows (Santos et al., 2010; Colazo et al., 2017). As in the current study, Brusveen et al. (2009) observed a decline in P/AI in lactating dairy cows with $P_4$ concentrations above 0.4 ng/mL at insemination, whereas others have determined 0.5 ng/mL as the optimal cutoff near the time of GnRH administration in the 7-d CO-Synch (Colazo et al., 2017) and Ovsynch (Souza et al., 2007; Martins et al., 2011) protocols. In fact, Souza et al., (2007) indicated that cows with $P_4 > 0.5$ ng/mL 48 h after PGF administration had a 50% decrease in P/AI compared with cows with $P_4 \leq 0.5$ ng/mL. Interestingly, although we observed that mean concentrations of $P_4$ at AI for both treatments were less than 0.43 ng/mL ($0.13 \pm 0.03$ and $0.36 \pm 0.03$ ng/mL), a greater proportion of cows had concentrations ≤0.43 ng/mL in 2PGF than HICON. Regardless, the P/AI outcome did not differ between treatments in the current study. Mean $P_4$ concentration at AI also differed between cows pregnant and not pregnant to AI in our study. Cows that became pregnant to AI had lesser $P_4$ concentrations at AI ($0.13 \pm 0.03$ ng/mL) than cows that failed to conceive ($0.36 \pm 0.04$ ng/mL). Likewise, Ambrose et al. (2015) recently reported lower mean circulating $P_4$ at AI in lactating dairy cows that became pregnant versus cows that did not ($0.23 \pm 0.07$ vs. $0.63 \pm 0.05$ ng/mL).

Twenty % of cows with $P_4 > 0.5$ ng/mL and 11% with $P_4 > 1.0$ ng/mL still became pregnant to AI, which appears to contradict the notion that elevated $P_4$ or incomplete/partial luteolysis impairs pregnancy establishment. Similar results were seen in a study by de Silva et al. (1981) whereby P/AI ranged from 10 to 20% in cows with $P_4$ between 0.8 and 1.0 ng/mL

at the time of AI. It should be noted that the exact mechanism by which sub-luteal $P_4$ concentrations (0.5 to 1.0 ng/mL) decrease P/AI is not entirely clear. Contrary to previous suggestions, decreased P/AI may not be related to lack of ovulation to GnRH in FTAI protocols, as studies have shown that cows have a high ovulatory response (> 90%) to the final GnRH of Ovsynch (Pursley et al., 1995), even under the influence of elevated (> 0.5 ng/mL) $P_4$ concentrations (Bello et al., 2006). In the current study, although proportion of cows with low $P_4$ at AI was greater in 2PGF, this did not translate to an advantage in P/AI. Additionally, using the cutoff value from the ROC analysis, 77% of non-pregnant cows had $P_4$ below 0.43 ng/mL at AI. This suggests that, while low $P_4$ is important for achieving optimal fertility, it is not the only factor determining pregnancy response to AI. It has been shown that fertility is influenced by many factors other than $P_4$. Previous reports have indicated that beef cows with increased circulating concentrations of $E_2$ before GnRH-induced ovulation have a greater chance of luteolysis and pregnancy to AI (Jinks et al., 2013; Madsen et al., 2015). However, the benefits of $E_2$ is dependent upon the size of the preovulatory follicle (Bello et al., 2006). According to Perry et al. (2005) cows with follicles > 11.5 mm that are induced to ovulate have improved pregnancy rates. Although $E_2$ and ovulatory follicle size were not measured in the current study, it may explain why cows with greater $P_4$ concentrations at AI still became pregnant (or why cows with low $P_4$ did not). While it cannot be derived from our study, it can be hypothesized that cows pregnant to AI, with incomplete or no luteolysis, had greater $E_2$ concentrations and ovulatory follicle diameters than cows not pregnant to AI with elevated $P_4$. Likewise, non-pregnant cows that had reduced $P_4$ by the time of AI could have failed to ovulate to GnRH-2 because of insufficient follicle size and $E_2$:$P_4$ concentration ratios.

The concept of testing the reproductive performance of beef cows administered one or two doses of PGF in the 5-d CO-Synch + CIDR synchronization protocol was developed with the intention of weighing the influence on P/AI against the additional cattle handling and expenses associated with a second PGF injection. Given the variable results on the necessity of two PGF injections in this protocol, it was essential to investigate the differences in P/AI when compared with one high-concentration PGF injection. Our research showed that the double-dose PGF scheme in the 5-d CO-Synch + CIDR protocol may reduce circulating $P_4$ concentrations by the time of AI more effectively than one high-concentration PGF dose. Nonetheless, results from the current experiment confirm our hypothesis that a single high-concentration dose of PGF given at CIDR removal would lead to similar P/AI as two conventional PGF doses (given 8 h apart) in postpartum beef cows. Future research in beef cow-calf herds of differing regions, climates, and breed is needed to further evaluate the effectiveness of a single high-concentration PGF on P/AI and luteolysis in the 5-d CO-Synch + CIDR protocol. The current data also substantiate additional evidence that low $P_4$ concentrations at insemination in FTAI protocols are positively associated with probability of pregnancy in beef cattle. However, the optimal cutoff for $P_4$ at AI is still arguable for multiple reasons: 1) current definitions of complete luteolysis are variable; 2) reports in beef cows are limited; and 3) the factor of variations in sensitivity of different types of $P_4$ assays across studies must also be considered. Further investigation on the relationship between $P_4$ concentration at the time of insemination and P/AI in beef cows is therefore necessary.

**IMPLICATIONS**

If additional research can establish that a single high-concentration PGF dose produces similar or improved P/AI in beef cattle synchronized with a 5-d CO-Synch + CIDR protocol,

it may be a viable alternative to the original double-dose scheme. Added benefits of this potential modification to the 5-d CO-Synch + CIDR synchronization program would include reduced animal handling, drug costs (approximately $3.00 less per head), and labor as well as improved protocol compliance. Currently, the adoption rate of estrous synchronization and AI by beef producers is less than 10% in the U.S partially due to cost, limited facilities, and labor and time constraints (Elliott, et al., 2013). Therefore, the development of an effective estrous synchronization protocol that limits cost and labor inputs may help to increase the implementation of FTAI within the industry.

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# CHAPTER THREE

Subcutaneous Administration of High-Concentration Prostaglandin $F_{2\alpha}$ and Incidence of Luteolysis in Charolais Cows Synchronized with a 5-Day CO-Synch + CIDR program


## ABSTRACT

The objective of this study was to evaluate the efficacy of a single subcutaneous (s.c) injection of high-concentration prostaglandin $F_{2\alpha}$ (PGF) on progesterone ($P_4$) concentration and incidence of luteolysis by the time of AI in a CIDR-based estrous synchronization program. Charolais beef cows (n=39) were synchronized with the 5-d CO-Synch + CIDR protocol. On Day 0, all cows received 100 μg of GnRH i.m., and a CIDR insert for 5 d. On Day 5, CIDR inserts were removed and one hour later, a blood sample was collected. Upon CIDR removal, cows randomly received one of two treatments: one s.c. dose of high concentration (12.5 mg/mL) dinoprost tromethamine (HICON-SC; n=19) or two intramuscular (i.m.) doses (given 6 h apart) of conventional (5 mg/mL) dinoprost tromethamine (2PGF-IM; n=20). A second GnRH was administered (i.m.) 72 ± 2 h later and another blood sample was collected for $P_4$ quantification. Following GnRH administration, cows either received fixed-time AI (FTAI) or embryo transfer (ET) upon detection of a CL 7 days later. Pregnancy was diagnosed via transrectal ultrasound 30 to 35 d after FTAI or 23 to 28 d following ET. Mean $P_4$ concentrations decreased over time ($P < 0.01$) between PGF administration and AI in both treatments. Progesterone concentrations on Day 5 did not differ ($P = 0.44$) between treatments and were 1.14 ± 0.13 and 1.29 ± 0.13 for 2PGF-IM and HICON-SC, respectively. Treatment did not affect ($P = 0.66$) mean $P_4$ concentration on Day 8, as concentrations were 0.10 ± 0.13 and 0.18 ± 0.13 for 2PGF-IM and HICON-SC, respectively. Overall, mean $P_4$ concentration decreased from 1.21± 0.13 ng/L on Day 5 to on 0.14 ± 0.13 ng/mL Day 8 in response to treatments. Functional luteolysis ($P_4 < 0.5$ ng/mL at

AI) occurred in 100% of 2PGF-IM cows and 91.9% of HICON-SC cows. Pregnancy per AI (P/AI) was 80% (2PGF-IM) and 50% (HICON-SC), and pregnancy per ET (P/ET) was 66.7% (2PGF-IM) and 75.0% (HICON-SC). In summary, a single subcutaneously administered dose of high-concentration PGF reduced $P_4$ with similar efficacy as two i.m. doses of conventional PGF in beef cows.

**Key words:** administration route, beef cow, luteolysis, progesterone, prostaglandin $F_{2\alpha}$

## INTRODUCTION

Prostaglandin $F_{2\alpha}$ has a relatively short half-life; following absorption into the bloodstream, it is rapidly metabolized by oxidation (approximately 90%) after a single passage through the lungs (Kindahl, 1980). Exogenous administration of PGF results in regression of a CL between d 5 and 16 of the estrous cycle (Rowson et al., 1972), followed by estrus within approximately 48 to 72 h (Tervit et al., 1973). The luteolytic effect of various exogenous forms of PGF have been assessed in ruminants. Many studies detected no difference in ability of naturally derived (dinoprost tromethamine) or synthetic (cloprostenol sodium) PGF to reduce $P_4$ concentrations (Schams and Karg, 1982) or induce estrus (Salverson et al., 2002; Martineau, 2003) in cattle. Both products have also been shown to effectively induce luteolysis (Martineau, 2003; Stevenson and Phatak, 2010) and both are used to effectively manipulate the estrous cycle for AI in cattle.

Numerous routes of PGF administration have been used in beef and dairy cows to examine their effectiveness in decreasing $P_4$. While i.m. and s.c. routes are most commonly used in livestock, studies have reported that PGF is also luteolytic when administered to cattle by intrauterine (Louis et al., 1974), intraovarian (Rayos et al., 1990), intravulvosubmucosal (i.v.s.m.; Rovani et al., 2012) and intravenous routes (i.v.; Maurer et

al., 1989; Martineau, 2003). When compared with i.m. routes in cattle, i.v. and i.v.s.m. administration of PGF resulted in similar incidence of luteolysis (Rovani et al., 2012) and pregnancy rates (Martineau, 2003). However, these methods are substantially more difficult to employ than i.m. or s.c. injections and are therefore impractical for widespread use in production systems.

Many PGF product labels recommend i.m. administration, though, some studies have suggested that it should be avoided in beef cattle to minimize injection site lesions (Van Donkersgoed et al., 2000). According to Roeber et al. (2001) one of the top five meat quality challenges in both beef and dairy market animals is the incidence of injection-site lesions in muscle. Employing a s.c. route of administration may decrease the incidence of blemishes on beef carcasses (Powell, 2013) and reduce monetary losses per head at slaughter (Hilton, 2004). The option of administering PGF subcutaneously in breeding programs has been investigated in dairy animals (Colazo et al., 2002a; Chebel et al., 2007), and it is clear CL regression and substantial declines in serum $P_4$ concentrations occur following a s.c. injection of PGF. Comparisons between i.m and s.c. injections of PGF in beef heifers (Colazo et al., 2002b) and cows (Muth-Spurlock et al., 2016) have also been made, the results of which showed similar intervals from administration to estrus and comparable ovulation rates between routes. These studies provide evidence s.c. injections are a viable alternative route to i.m. injections for administration of reproductive hormones such as PGF in estrous synchronization programs.

Recently, the U.S. Food and Drug Administration approved a high-concentration PGF product, Lutalyse *HighCon* (12.5 mg/mL of dinoprost tromethamine; Zoetis Animal Health). Per label instructions, Lutalyse *HighCon* may be administered by i.m. or s.c. routes in cattle.

Studies in beef heifers (Lansford et al., 2018; Oosthuizen et al., 2018) showed a single s.c. dose of Lutalyse *HighCon* results in similar estrus response and P/AI as an i.m. dose of conventional Lutalyse (5 mg/mL of dinoprost tromethamine; Zoetis Animal Health). In addition, it has been demonstrated that the pharmacokinetics of the high-concentration product slightly differ from that of its conventional counterpart, as the PGF metabolite, PGFM, reaches a greater Cmax in circulation following administration (Zoetis Inc., 2015).

Recent studies (Spencer et al., 2017, 2018) have evaluated the luteolytic effectiveness of a single i.m. dose of Lutalyse *HighCon* in a 5-d CO-Synch + CIDR protocol in beef and dairy cows. In both studies, the results showed no differences in luteal regression or decline in $P_4$ concentrations following administration of two conventional Lutalyse or a single Lutalyse *HighCon* injection. In beef cows subjected to the 5-d CO-Synch + CIDR protocol, Corpron et al. (2019) found a single i.m. dose of Lutalyse *HighCon*, given at CIDR removal, produced similar P/AI in cows compared to those that received two doses of conventional Lutalyse (given 8 h apart). However, *HighCon* in this study appeared to reduce $P_4$ by the time of AI less effectively than a double dose of conventional Lutalyse, as the proportion of cows with $P_4$ < 0.5 ng/mL at AI was less in those receiving *HighCon* (i.m.). Currently, no studies have assessed the efficacy of this high concentrate product to induce luteolysis when administered subcutaneously in beef cows subjected to a 5-d FTAI protocol. More research is needed to further evaluate the use of a single injection of this high-concentration PGF product in the 5-d CO-Synch + CIDR synchronization protocol, as the use of s.c. in place of i.m. administration could help improve carcass quality and reduce injection site lesions in cattle subjected to synchronization programs. In addition, by reducing the number of injections in FTAI breeding

programs may help reduce labor and costs requirements, improve protocol compliance, and potentially increase the adoption of estrous synchronization and AI in the beef industry.

## OBJECTIVE

Given the increased potential for injection site lesions with i.m. administration of reproductive hormones in estrous synchronization programs, the use and effectiveness of an alternative administration route should be considered. Currently, research has yet to evaluate the efficacy of a single s.c. injection of Lutalyse *HighCon* to induce luteal regression in the 5-d CO-Synch + CIDR synchronization program. Therefore, in an effort to reduce the number of injections and potentially decrease the incidence of beef carcass damage per BQA recommendations, the objective of this study was to assess the effectiveness of s.c. administration of Lutalyse *HighCon*, to reduce $P_4$ concentration by the time of insemination in a 5-d CO-Synch + CIDR protocol.

## HYPOTHESIS

We hypothesized that a single s.c. injection of high-concentration PGF (Lutalyse *HighCon;* 25 mg) would not affect serum $P_4$ concentrations at the time of AI when compared with the administration of two i.m. injections of conventional PGF (Lutalyse; 2 x 25 mg, given 6 h apart) in postpartum beef cows.

## MATERIALS AND METHODS

All procedures and protocols in this experiment were approved by the University of Idaho, Animal Care and Use Committee (Protocol #IACUC-2019-25; Appendix 3).

### Experimental Design and Treatments

This study was conducted at the University of Idaho Beef Center, Moscow, ID. Thirty-nine multiparous Charolais cows were synchronized with the 5-d CO-Synch + CIDR protocol.

On day of protocol initiation (Day 0), GnRH (GnRH-1; 100 μg, i.m.; Factrel®; Zoetis Inc., Kalamazoo, MI) was administered to all animals and, using aseptic techniques, a controlled internal drug release insert (1.38 g [$P_4$]; EAZI-breed CIDR cattle insert; Zoetis Inc.) was inserted vaginally (Figure 3.1). On Day 5, CIDR inserts were removed and a blood sample was collected 1 h later. Cows were then stratified by breeding method (ET or FTAI), and assigned randomly to receive either one s.c. dose of high-concentration PGF (HICON-SC n=19; 25 mg; Lutalyse® *HighCon*, Zoetis Inc., Parsippany, NJ) or two i.m. doses of conventional PGF, 6 h apart (2PGF-IM n=20; 2 x 25 mg, Lutalyse®, Zoetis Inc., Parsippany, NJ; Figure 3.1) in the neck region. Subsequently, all cows received a second GnRH (GnRH-2; 100 μg) and were either inseminated at a fixed-time 72 ± 2 hours after treatment (n=19) or received ET (n=20) upon detection of an active CL 7 d after GnRH-2 (Day 15) via transrectal ultrasound (Figure 3.1). An additional blood sample was collected from all cows just before GnRH-2 administration for quantification of $P_4$.

**Estrous Detection and Pregnancy Diagnosis**

For purposes of monitoring estrus, paint was applied to the tail head of all cows at CIDR removal and estrual behavior was visually observed twice daily until Day 8 (Figure 3.1). Animals were considered to have been in estrus if observed standing to be mounted or if tail paint was rubbed off at the time of GnRH-2 administration. Pregnancy status was confirmed by examination of the uterine contents using transrectal ultrasonography 30 to 35 d after FTAI or 23 to 28 d after ET.

**Blood Sampling and Progesterone Quantification**

Blood samples were collected by venipuncture of the coccygeal artery or vein using a 21” gauge needle and a 10 mL vacutainer tube (Coviden® LLC, Mansfield, MA). All samples

were placed on ice immediately after collection and then stored at 4°C for 24 h. Samples were centrifuged at 2,400 x g at 4°C for 20 minutes and serum was harvested and stored at -20°C until assayed for $P_4$ quantification.

Serum $P_4$ concentration was analyzed using a solid phase single antibody-coated tube RIA (ImmuChem® Coated Tube Progesterone $^{125}$I RIA Kit, MP Biomedicals, Costa Mesa, CA) in an equilibrium condition. Assay standards were modified per the procedure developed by Scarpa et al. (2019). Briefly, $P_4$ (minimum 99%, SIGMA-ALDRICH, Inc., St. Louis, MO) was dissolved in absolute ethanol for a working $P_4$ stock solution (50 μg/mL). Progesterone standards were prepared by serial dilution of the $P_4$ stock solution in fetal bovine serum (VWR Seradigm LIFE SCIENCE®, VWR International, LLC, Radnor, PA). Fetal bovine serum was used as a diluent to account for matrix effects observed in previous assays using original standards (with human serum as the diluent) provided by the manufacturer.

All serum samples were run in duplicate in a single assay. The standard curve ranged from 0.25 ng/mL to 20 ng/mL, and the lowest limit of detection was determined as 0.10 ng/mL, based on the concentration at which 90% of tracer was bound to antibody. Intra-assay coefficient of variance was 3.56%.

**Statistical Analyses**

Data for mean serum $P_4$ concentration on Day 5 and 8 was analyzed using a generalized linear model analysis of variance procedure in SAS (v. 9.4; SAS Institute Inc. 2015). The model included the fixed effects of treatment, time, and treatment by time interaction. Significance was declared at $P < 0.05$ and $0.05 < P < 0.1$ was considered a tendency.

Pregnancy per AI and P/ET were not analyzed between treatments because the number of observations were not sufficient to appropriately detect a statistical difference. However, the proportion of cows pregnant to AI or ET are reported.

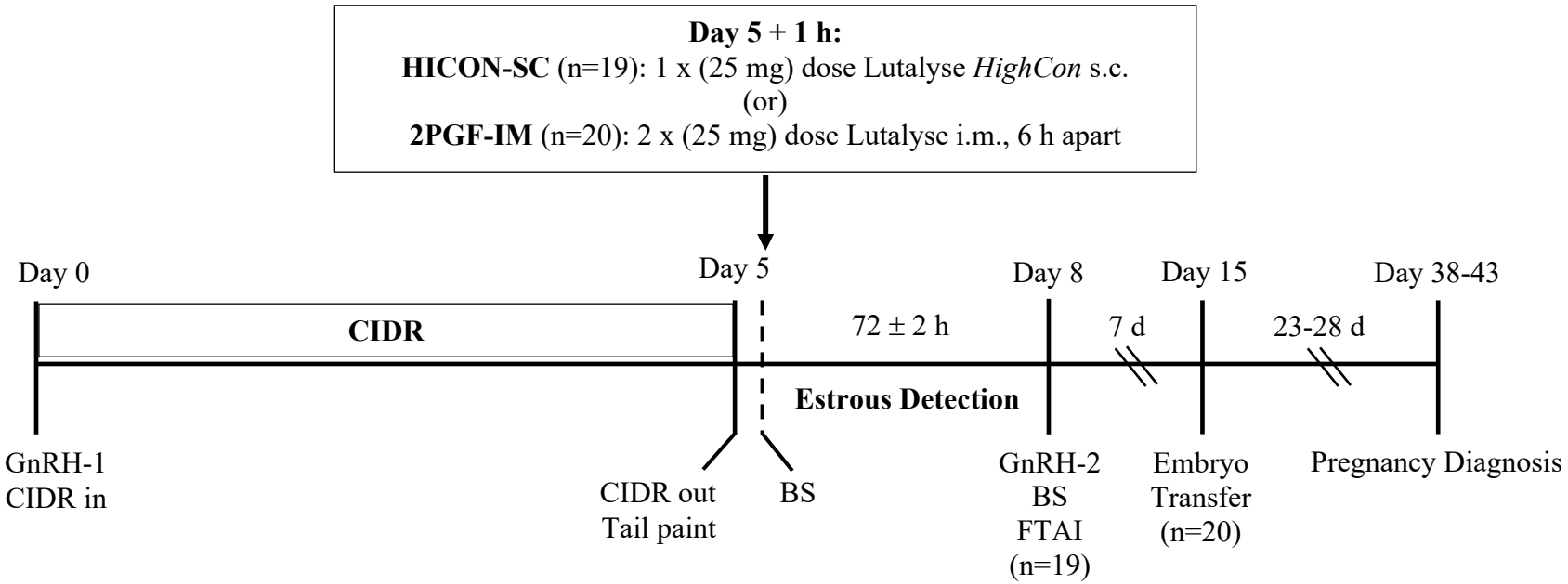


**Figure 3.1.** Experimental design for Charolais cows (n=39) synchronized with a 5-d CO-Synch + CIDR protocol. Day 0, all cows received an injection of GnRH (100 μg) and a controlled internal drug release (CIDR) insert. Five days later, CIDR was removed and tail paint applied to the tail-head of all cows for heat detection purposes. One h following CIDR removal, a blood sample (BS) was collected and cows randomly received one of two treatments: One s.c. dose of Lutalyse *HighCon* (HICON-SC; n=19) or two doses (6 h apart) of conventional Lutalyse (2PGF-IM; n=20). Estrus was detected twice daily until Day 8 (72 ± 2 h after CIDR removal) and all cows received a second i.m. dose of GnRH (100 μg) and a BS was collected. Cow were randomly assigned either be inseminated at a fixed time (FTAI; n=19) on Day 8 or receive embryo transfer (ET; n=20) upon detection of an active corpus luteum 7 d later by transrectal ultrasonography. Pregnancy was diagnosed via ultrasound of uterine contents 30 to 35 d after Day 8.

## RESULTS

### Progesterone Concentrations

Mean $P_4$ concentrations did not differ ($P = 0.44$) between treatments on Day 5. Progesterone concentrations on Day 5 were 1.14 ± 0.13 and 1.29 ± 0.13 ng/mL for 2PGF-IM and HICON-SC, respectively (Table 3.1). As expected, mean $P_4$ concentrations decreased ($P < 0.01$) over time between Day 5 and Day 8 following PGF administration in both treatments. Overall, mean $P_4$ concentration decreased from 1.21 ± 0.13 ng/mL on Day 5 to 0.14 ± 0.13 ng/mL on Day 8 in response to treatments. There was no effect of treatment ($P = 0.39$) or treatment by time ($P = 0.81$) on $P_4$ concentration on Day 5 or Day 8 (Table 3.1). Mean $P_4$ concentration on Day 8 did not differ ($P = 0.66$) between treatment, as concentrations were 0.10 ± 0.13 and 0.18 ± 0.13 for 2PGF-IM and HICON-SC, respectively (Table 3.1). This indicates that $P_4$ decreased similarly following PGF administration in both treatments.

### Luteolysis

Twenty-three cows had an active CL on Day 5 ($P_4 > 1.0$ ng/mL). The proportion of cows with a CL on Day 5 were 60% (12/20) and 57.9% (11/19) for 2PGF-IM and HICON-SC, respectively (Table 3.2). Cows with a CL on Day 5 were considered eligible to undergo luteolysis in response to PGF treatment. Functional luteolysis was defined as having an active CL on Day 5 ($P_4 > 1.0$ ng/mL), followed by a decrease in serum $P_4$ below 0.5 ng/mL on Day 8. Of the cows eligible on Day 5, 95.7% (22/23) underwent luteolysis in response to PGF. Functional luteolysis occurred in 100% (12/12) of 2PGF-IM cows and 91.9% (10/11) of HICON-SC cows (Table 3.2).

**Pregnancy per AI and ET**

Of the cows that received FTAI (n=18; 1 cow culled before pregnancy diagnosis), 66.7% became pregnant. Pregnancy per AI (P/AI) was 80% (8/10) and 50% (4/8) for 2PGF-IM and HICON-SC, respectively. Seventeen out of 20 cows in the ET group received embryos based on the detection of an active CL 7 d after GnRH-2 administration. Among the cows exposed to ET, 70.6% became pregnant. Between treatments, pregnancy per ET (P/ET) was 66.7% (6/9) and 75.0% (6/8) for 2PGF-IM and HICON-SC, respectively.

**Table 3.1** Least square means (± SEM) for serum progesterone concentrations 1 h following controlled internal drug release (CIDR) insert removal (Day 5) and at GnRH-2 (Day 8) in cows administered HICON-SC or 2PGF-IM[1] in a 5-d CO-Synch + CIDR protocol.

| | 2PGF-IM | HICON-SC | Overall |
|---|---|---|---|
| Progesterone (ng/mL) | -- | -- | -- |
| Day 5[1] | $1.14 \pm 0.13^{a*}$ | $1.29 \pm 0.13^{a*}$ | $1.21 \pm 0.13$ |
| Day 8[2] | $0.10 \pm 0.13^{b*}$ | $0.18 \pm 0.13^{b*}$ | $0.14 \pm 0.13$ |

[a,b] Values without a common superscript within column differ ($P < 0.01$).
[*] Values within row between treatment did not differ ($P > 0.44$).
[1] Cows were assigned to receive either one s.c. high-concentration PGF (HICON-SC, 25 mg) or two conventional PGF i.m. (2PGF-IM, 25 mg each dose) 6 h apart at CIDR removal.
[2] Serum progesterone concentration one hour following CIDR removal and before treatment administration.
[3] Serum progesterone concentration 72 ± 2 h after CIDR removal.

**Table 3.2** Proportion of cows with an active corpus luteum (CL)[1] at controlled internal drug release (CIDR) insert removal (Day 5) and that underwent functional luteolysis[2] in response to treatment.

| Item | 2PGF-IM | HICON-SC |
|---|---|---|
| CL on Day 5 (%) | 60.0 (12/20) | 57.9 (11/19) |
| Luteolysis (%) | 100 (12/12) | 91.9 (10/11) |

[1] Cows with $P_4$ > 1.0 ng/mL 1 h following CIDR removal were considered to have an active CL on Day 5.
[2] Cows with an active CL on Day 5 ($P_4$ > 1.0 ng/mL) followed by $P_4$ below 0.5 ng/mL on Day 8 were considered to have undergone luteal regression following PGF administration.

**IMPLICATIONS**

Intramuscular routes of drug administration increase the incidence of injection-site lesions and tissue damage associated with reduced beef carcass quality and tenderness (Boleman et al., 1998). The use of a s.c. route of administration is therefore favorable to reduce carcass damage. In the current study, a single s.c. injection of Lutalyse *HighCon* reduced serum $P_4$ concentrations at the time of AI as effectively as two i.m. injections of conventional Lutalyse. Interestingly, a s.c. route of administration of this high-concentration PGF product appears to effectively reduce $P_4$ by the time of AI, whereas a single i.m. injection does not, as observed in our previous research (Corpron et al., 2019). More research with a larger sample size needs to be conducted to make direct comparisons between either i.m. or s.c. administration of a single injection of Lutalyse *HighCon* on the incidence of functional luteolysis and pregnancy responses in postpartum beef cows. If a single injection of *HighCon* can induce luteolysis and produce P/AI similar to the original double-dose scheme in the 5-d CO-Synch + CIDR protocol, the number of injections, and therefore animal handlings, labor, and cost of synchronization may all be reduced. By decreasing the amount of labor, expenses, and handling requirements associated with estrous synchronization, the adoption of reproductive technologies such as FTAI can potentially be increased in the beef industry. In addition, the use of s.c. administration of *HighCon* in breeding programs into the neck region would hold the added benefit of reducing carcass damage normally induced by the persistent use of i.m. injections.

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# APPENDIX 1

## **Chapter Two:** Animal Care and Use Committee Approval

University of Idaho
Office of Research Assurances
Institutional Animal Care and Use Committee
875 Perimeter Drive, MS 3010
Moscow, ID 83844-3010
Phone: 208-885-6162
Fax: 208-885-6014
Email: iacuc@uidaho.edu

**Date:** March 19, 2019

**To:** Amin Ahmadzadeh

**From:** University of Idaho Institutional Animal Care and Use Committee

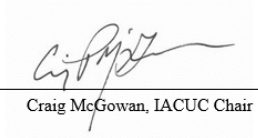
**Re:** Protocol IACUC-2016-17 *The use of two injections of prostaglandin F2αin a 5-d CIDR timed-AI breeding protocols*

Your requested renewal of the animal care and use protocol listed above was reviewed and approved by the Institutional Animal Care and Use Committee on: 03/19/2019.

This renewal was originally submitted for review on: 03/07/2019 09:51:48 AM PST

The original approval date for this protocol was: 04/27/2016
This approval will remain in effect until: **04/27/2019**

Federal laws and guidelines require that institutional animal care and use committees review ongoing projects annually. For the first two years after initial approval of the protocol you will be asked to submit an annual update form describing any changes in procedures or personnel. The committee may, at its discretion, extend approval for the project in yearly increments until the third anniversary of the original approval of the project. At that time, the protocol must be replaced by an entirely new submission.



Craig McGowan, IACUC Chair

# APPENDIX 2

**Chapter Two:** Additional Tables

**Appendix Table 2.1** Breed, fertility, and number of semen units used per AI sire in cows receiving 2PGF or HICON.

| | | | | Treatment[4] | |
|---|---|---|---|---|---|
| Sire | Breed[1] | Fertility (%)[2] | n[3] | 2PGF | HICON |
| 1 | Simmental | 60.0 | 35 | 20 | 15 |
| 2 | Sim/Angus | 73.3 | 30 | 14 | 16 |
| 3 | Simmental | 55.2 | 29 | 14 | 15 |
| 4 | Simmental | 40.0 | 10 | 5 | 5 |
| 5 | Sim/Angus | 40.0 | 20 | 10 | 10 |
| 6 | Simmental | 66.7 | 3 | 1 | 2 |
| 7 | Angus | 40.0 | 15 | 8 | 7 |
| 8 | Simmental | 60.0 | 30 | 17 | 13 |
| 9 | Simmental | 60.0 | 30 | 17 | 13 |
| 10 | Simmental | 65.5 | 30 | 16 | 14 |
| 11 | Simmental | 68.4 | 38 | 16 | 22 |
| 12 | Simmental | 65.7 | 35 | 17 | 18 |
| 13 | Sim/Angus | 45.0 | 20 | 7 | 13 |
| 14 | Simmental | 73.0 | 37 | 19 | 18 |
| 15 | Sim/Angus | 75.0 | 40 | 20 | 20 |

[1] Sires used for AI in this study were either full Angus or Simmental, or a Simmental-Angus cross (Sim/Angus).
[2] Pregnancy per AI of cows bred to each sire at AI.
[3] Number of semen units used per sire to AI cows.
[4] Distribution of AI sire semen between cows that received either one high-concentration (HICON; 25 mg) or two conventional doses (2PGF; 25mg/dose; 8 h apart) of prostaglandin $F_{2\alpha}$ in a 5-d CO-Synch + CIDR protocol.

# APPENDIX 3

## **Chapter Three:** Animal Care and Use Committee Approval

University of Idaho
Office of Research Assurances
Institutional Animal Care and Use Committee
875 Perimeter Drive, MS 3010
Moscow, ID 83844-3010
Phone: 208-885-6162
Fax: 208-885-6014
Email: iacuc@uidaho.edu

**Date:** April 15, 2019

**To:** Amin Ahmadzadeh

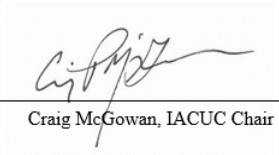
**From:** University of Idaho Animal Care and Use Committee

**Re:** Protocol IACUC-2019-25 *The use of two injections of prostaglandin F2α in a 5-d CIDR timed-AI breeding protocols*

Your animal care and use protocol for the project shown above was reviewed and approved by the Institutional Animal Care and Use Committee on 04/15/2019.

This protocol was originally submitted for review on: 03/20/2019 09:52:02 AM PDT
The original approval date for this protocol is: 04/15/2019
This approval will remain in effect until: 04/14/2020
The protocol may be continued by annual updates until: 04/14/2022

Federal laws and guidelines require that institutional animal care and use committees review ongoing projects annually. For the first two years after initial approval of the protocol you will be asked to submit an annual update form describing any changes in procedures or personnel. The committee may, at its discretion, extend approval for the project in yearly increments until the third anniversary of the original approval of the project. At that time, the protocol must be replaced by an entirely new submission.



Craig McGowan, IACUC Chair