Effect of Progesterone Pre- and Post-Insemination on Pregnancy Success on Cattle

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Introduction

Pregnancy failure can be caused by several factors; however, the most common cause of pregnancy failure is embryonic loss during early gestation. Two primary factors influencing the probability of embryonic loss are quality of the oocyte ovulated and uterine support of embryonic development. The ovulation of a poor quality oocyte often leads to early embryonic mortality (prior to conceptus elongation), albeit fertilization of the oocyte is often not inhibited. Likewise, insufficient uterine function during early gestation can lead to embryonic death. In both scenarios, oocyte quality and uterine function may be regulated by circulating concentrations of progesterone prior to and following ovulation, respectively. Progesterone concentrations prior to ovulation can influence the release of luteinizing hormone (LH), which can subsequently affect oocyte maturation and follicular growth and estradiol production (Rahe et al., 1980; Schallenberger et al., 1985; Savio et al., 1993; Gong et al., 1995). Alterations in oocyte maturation or limiting preovulatory estradiol concentrations can result in a reduction in fertility. Moreover, sensitivity to progesterone concentrations during follicular development may vary between breeds of cattle *(Bos indicus* vs. *Bos taurus*; beef vs. dairy). Following ovulation, progesterone concentrations during early gestation can impact uterine function and rate of embryonic growth (Garrett et al., 1988; Satterfield et al., 2006). It has been demonstrated in numerous studies that progesterone concentrations and the rate of progesterone increase following ovulation impacts pregnancy success (Johnson, 1958; Mauer and Echternkamp, 1982; Robinson et al., 1989; Starbuck et al., 2004). Therefore, the focus of this review is to outline potential mechanisms by which progesterone prior to ovulation and after ovulation can impact pregnancy success in cattle.

Importance of Progesterone Pre-Insemination

Circulating concentrations of progesterone during the estrous cycle regulate the secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus (Rahe et al., 1986; Schallenberger et al., 1985; Kinder et al., 1996) and the secretion of the gonadotropins (LH and follicle stimulating hormone, FSH) from the anterior pituitary (Savio et al., 1993; Gong et al., 1995). The release of gonadotropins from the anterior pituitary regulates follicular development, influences oocyte maturation, and is essential for production of steroid hormones. Follicle stimulating hormone (FSH) is necessary for follicular wave recruitment (Ginther, 2000) while luteinizing hormone (LH) drives the final stages of follicular growth (Savio et al., 1993) and oocyte maturation (Gong et al., 1995). Furthermore, LH promotes estradiol production and secretion from the dominant follicle (Schallenberger et al., 1984). Elevated progesterone concentrations results in decreased LH pulsatility, but pulses have high amplitude. In contrast, when progesterone concentrations are low, such as following luteolysis, LH pulse frequency increases (Rahe et al., 1980). Following luteolysis, when progesterone concentrations are low, increased LH pulsatility stimulates continued growth of a dominant follicle (Taft et al., 1996), increased estradiol production, and eventual estrus and ovulation. During periods of high

progesterone, low frequency pulses of LH on the contrary, do not support sustained follicular growth, resulting in eventual atresia of the dominant follicle Adams et al., 1992). Several investigators have used this principle to alter the frequency of release of LH by administering exogenous progesterone. Progesterone has a dose dependent effect on LH secretion (Kinder et al., 1996). Kojima and colleagues (1992) demonstrated that administration of exogenous sub-luteal doses of progesterone in the absence of a CL resulted in a pattern of LH secretion in cattle similar to the follicular phase. The differential regulation of LH release by progesterone may affect the development and future capacity of the oocyte to be fertilized and result in a successful pregnancy. It is clear that when this period of sub-luteal progesterone exposure is extended for a long duration, persistent follicles develop and fertility is drastically reduced (Kinder et al., 1996). However, the impact of a finite period of low progesterone on LH secretion, follicular growth, oocyte competence, and ultimately fertility is not clear.

Progesterone concentrations during follicular development and pregnancy success

With exception of studies investigating the phenomenon of persistent follicles and infertility, few studies have directly assessed the effect of progesterone concentrations, and subsequent LH concentrations, during the development of the follicular wave on quality of the oocyte induced to ovulate and the resulting impact on pregnancy success. Ancillary studies investigating progesterone concentrations during estrous synchronization protocols indicate that progesterone concentrations during the development of the ovulatory follicle wave can influence fertility. However, optimal progesterone concentrations needed to enhance fertility appear to vary between cattle species; i.e. *Bos indicus* versus *Bos taurus* and dairy cattle versus beef cattle.

In recent years, numerous studies in Nelore cattle have demonstrated that reducing progesterone concentrations during the final stages of follicular growth improves pregnancy rates; albeit this relationship in *Bos taurus* cattle has not been established. This difference amongst cattle species may be explained by a study conducted by Carvalho et al. (2008), who compared the interval from CIDR insertion and estradiol treatment to follicular wave emergence in *Bos indicus*, *Bos indicus* X *Bos taurus*, and *Bos taurus* heifers. Even though the time of follicle emergence after estradiol administration was not different between groups (3.2 days), purebred *Bos indicus* heifers had the smallest dominant follicles at the end of the CIDR treatment. This slowed growth rate of the dominant follicle in *Bos indicus* heifers was also associated with greater blood progesterone concentrations during estrous synchronization, suggesting that *Bos indicus* cattle are more sensitive to the actions of progesterone and elevated progesterone prior to ovulation may be harmful to follicular development in *Bos indicus* cattle.

To increase timed-AI (TAI) pregnancy rates in *Bos indicus* cattle, several approaches to reduce progesterone concentrations during follicular development and/or enhance gonadotropin concentrations during an ovulation synchronization protocol have been investigated (Claro et al., 2010; Meneghettii et al., 2009; Sá Filho et al., 2009; Peres et al., 2009; Dias et al., 2009). One method for increasing gonadotrophic support of the dominant follicle is a lengthened proestrus period, or a greater interval between luteal regression and the LH surge. It has been demonstrated in both *Bos indicus* and *Bos taurus* cattle that a longer proestrus period resulted in greater pregnancy success (Vasconcelos et al., 2001; Mussard et al., 2007; Bridges et al., 2008; Perez et al., 2009). Another approach to reduce progesterone concentrations during follicular development is administration of a luteolytic dose of prostaglandin $F_{2\alpha}$ (PGF) prior to CIDR removal. This has been demonstrated to improve fertility in *Bos indicus* females (Peres et al.,

2009). Furthermore, equine chorionic gonadotropin (eCG) has been shown to increase pregnancy success to TAI when given at the end of a CIDR treatment in anestrous cows. Administration of eCG may increase pregnancy success by allowing for the ovulation of a larger dominant follicle which results in a larger CL and greater circulating progesterone concentrations 12 days following ovulation (Vasconcelos et al., 2001; Baruselli et al., 2003; Peres et al., 2009; Sá Filho et al., 2009). It is unclear if eCG administration can have a direct effect on oocyte competence. However, when eCG stimulation was compared to no stimulation prior to OPU, those cows receiving eCG treatment yielded more than twice the number of viable oocytes than those not treated while the number of follicles aspirated remained the same (Aller et al., 2012).

Another management strategy used to increase gonadotrophic support of the developing ovulatory follicle in ovulation synchronization protocols is the use of a previously used CIDR. If *Bos indicus* cattle are more sensitive to circulating concentrations of progesterone, using a CIDR with lesser progesterone concentrations may reduce the progesterone-induced reduction in LH secretion and improve follicular growth and estradiol production, oocyte quality, and fertility. Claro et al. (2010) demonstrated that pregnancy success in prepubertal Nelore heifers was greater in heifers that were synchronized using a previously used CIDR (resulting in serum progesterone levels of 1.20 ± 0.11 ng/mL; pregnancy success = 47.7%) versus a new CIDR (serum P4 = 2.31 ± 0.11; pregnancy success = 39.2%). A separate study conducted in pubertal Nelore heifers demonstrated that using a previously used CIDR to reduced progesterone concentrations resulted in greater diameter of the ovulatory follicle and increased TAI pregnancy rates (Dias et al., 2009). Collectively, these studies demonstrated that *Bos indicus* cattle have the greatest pregnancy success when presented with a low progesterone environment prior to ovulation. Although the exact mechanisms by which the low progesterone environment is improving fertility has not been conclusively determined, several factors could be contributing. The low progesterone concentrations could enhance LH secretion, thus increase follicular growth and estradiol production; both of these factors influence pregnancy success in cattle (Vasconcelos et al., 2001, Perry et al., 2005, Bridges et al., 2010). Furthermore, the ovulation of a larger follicle often results in the development of a large CL, capable of producing increase concentrations of progesterone. As will be discussed later, progesterone concentrations following ovulation are critical for embryonic survival. In addition, increased LH may promote the ovulation of an oocyte of greater quality.

In *Bos taurus* breeds of cattle, the implications of low progesterone concentrations during follicular development are not as clear, and fewer studies have investigated these interactions. However, a recent study using crossbred beef heifers did indicate that reducing circulating concentrations of progesterone during the development of the follicular wave resulted in a larger dominant follicle at CIDR removal (Con = 10.05 ± 0.34 mm; LowP4 = 11.17 ± 0.38) presumably due to increased LH secretion as a result of reduced circulating progesterone (Sparks et al., 2012). Sparks and colleagues (2012) also found that progesterone concentrations in the subsequent estrous cycle were increased in heifers in the low progesterone treatment. This was likely due to the ovulation of a larger follicle. However, there was no increase in AI pregnancy rates in *Bos taurus* heifers when estrous was synchronized using a protocol to reduce progesterone concentrations during development of the follicular wave. This suggests that progesterone concentrations prior to ovulation may not impact AI pregnancy rates in *Bos taurus* cattle as observed in *Bos indicus* breeds, however additional investigations are warranted.

In the lactating dairy cow, ample evidence suggests that greater progesterone concentrations during follicular development are required to maximize fertility (Cerri et al., 2009. 2011; Denicol et al., 2012). Recently, it was demonstrated in lactating dairy cows that use of two CIDRs resulted in greater circulating concentrations of progesterone prior to ovulation and resulted in greater pregnancy success (Denicol et al., 2012). These investigators concluded that estrus synchronization protocols for lactating dairy cows must be designed to ensure that progesterone concentrations are >2 ng/mL during follicular development to maximize the probability of pregnancy to AI. In addition, beginning an estrous synchronization protocol during the second follicular wave, when progesterone concentrations are greater, improves TAI pregnancy rates in lactating dairy cows (Bisinotto et al., 2010). Although the reasons for improved fertility in lactating dairy cattle with elevated progesterone concentrations during estrous synchronization are not clear, it may involve progesterone regulation of LH release. Due to increased feed intake, increased liver blood flow, and increased steroid catabolism progesterone concentrations in the lactating dairy cow are considerably less than concentrations observed in the non-lactating dairy cow or dairy heifer (Sangsritavong et al., 2002; Sartori et al., 2004). The potential exists that low progesterone concentrations during estrous synchronization are promoting abnormal LH secretion and subsequently affecting the quality of the oocyte induced to ovulate. These are only speculations and further research is required to conclusively determine why pregnancy rates are reduced in lactating dairy cows when ovulation synchronization is conducted during periods of reduced progesterone concentrations.

Function of progesterone concentrations during follicular development on oocyte competence

A recent area of research amongst our lab groups has been investigating how progesterone concentrations during the development of the follicular wave impacts oocyte competence. Reducing circulating progesterone concentrations during the follicular wave may allow for increased gonadotropin support of the ovulatory follicle (Robinson et al., 1989; Dias et al., 2009), increase follicular estradiol production (Sirois and Fortune, 1990) and directly affect oocyte maturation and competence (Driancourt et al., 1998; va de Leemput et al., 1998). It has been previously demonstrated that bovine oocytes obtained from preovulatory follicles with greater estradiol concentrations were more likely to develop into blastocysts in an *in vitro* production system (van de Leemput et al., 1998). Moreover, inhibition of LH pulsatility and subsequent decline in follicular estradiol production caused a decreased proportion of ovine oocytes to cleave and reach the blastocysts stage (Oussaid et al., 1999). Given the role of LH on maturation of the oocyte (Savio et al., 1993; Gong et al., 1995) and its ability to cause increased estradiol production by the dominant follicle (Schallenberger et al, 1984), increasing LH pulsatility via lowered progesterone may benefit oocyte competence. Therefore, our laboratory recently performed two experiments in *Bos taurus* beef heifers and cows with the objective to determine the impact of circulating concentrations of progesterone on follicular dynamics and oocyte competence. We implemented transvaginal ultrasound-guided oocyte collection (OPU) to collect oocytes from females with differing progesterone concentrations and evaluated oocyte quality and the ability of the oocyte to develop into a blastocyst stage embryo following *in vitro* embryo production (IVP) procedures. We hypothesized that reducing concentrations of progesterone during follicular growth would result in increased LH concentration and therefore promote development of more competence oocyte that had a greater probability developing into blastocysts.

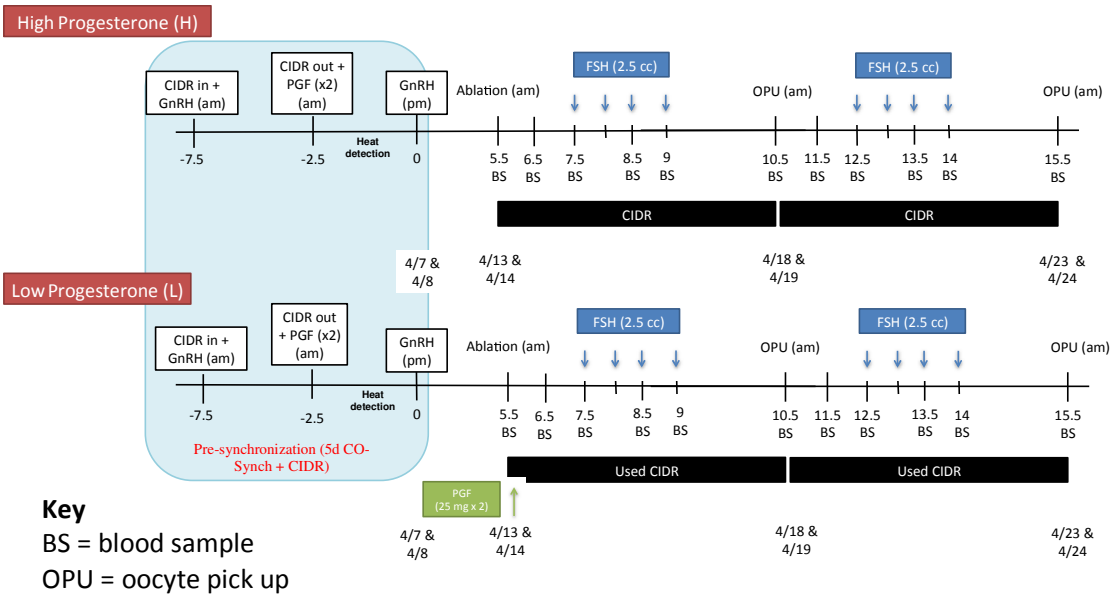
The first experiment was performed using yearling Charolais heifers (n = 36). All heifers were pre-synchronized to a common day of the estrous cycle (estrus = experimental day 0; Figure 1) and allotted to one of two treatments; 1) high progesterone, (H) or 2) low progesterone (L). Follicular ablation was performed on day 5.5 to reset and standardize follicular growth. Immediately following ablation, heifers in the L treatment received a used CIDR and two, 25 mg injections of PGF given 6 to 8 hours apart to induce luteolysis. Hence, the only progesterone in circulation in the L treatment was from the used CIDR. At follicular ablation, heifers in the H treatment received a new CIDR and did not receive PGF. Follicular growth was stimulated via administration of 40 mg FSH on days 7.5, 8, 8.5, and 9 and oocyte were collected from all visible follicles via OPU on day 10.5. Following the initial OPU, heifers received another new (H treatment) or used (L treatment) CIDR and were again administered 40 mg of FSH on days 12.5, 13, 13.5, and 14 to stimulate follicular growth. A second OPU was conducted on day 15.5 to collect oocytes from all visible follicles. Circulating concentrations of progesterone were assessed throughout the trial. At OPU, the number of follicles aspirated was recorded. Oocytes collected were immediately transported to the laboratory and graded (as described by Blondin et al., 1996; Table 1). Once graded, oocytes were pooled by treatment and placed in maturation media. Unfortunately, due to unforeseen circumstances in the IVP laboratory, IVP embryo production was insufficient to draw accurate conclusions in regards to effect of treatment on oocyte viability *in vitro*. Hence, only follicular number, oocyte collection, and oocyte quality parameters could be assessed in this study.

Table 1. Classification of bovine cumulus-oocyte complexes (COC)[a,b]

Class of Oocyte	No. of cumulus layers	Expansion of cumulus	Texture of ooplasm
1	≥ 5	Compact	Homogeneous
2	≥ 5	Compact	Dark zone around periphery
3	≥ 5	Slight expansion in outer layers	Slight granulations
4	≥ 5	Full expansion with dark clumps	Heavy granulations
5	1	Only corona radiata	Variable
6	0	No cumulus	Variable

[a]Adapted from Blondin et al., 1996.
[b] Oocytes that did not correspond to these classifications were omitted.

Figure 1. Design of Experiment 1



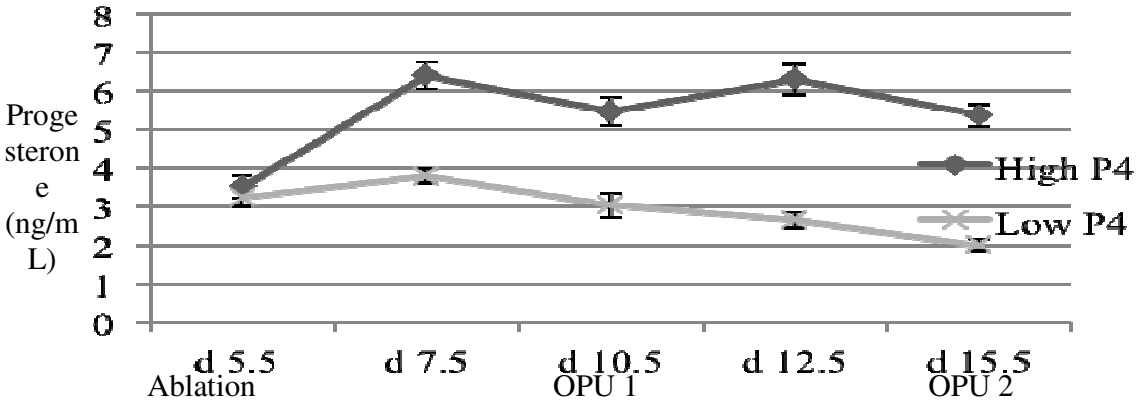
As designed, progesterone concentrations differed ($P < 0.01$; Figure 2) at every sampling point after ablation between treatments during follicular growth. Heifers in the L treatment produced a greater number of follicles ($P = 0.03$; Table 3) than those in the H treatment and also yielded a greater number of oocytes recovered ($P = 0.02$; Table 2). Furthermore, heifers in the L treatment yielded more grade 1-3 oocytes per animal ($P = 0.02$; Table 3) than heifers in the H treatment.

Table 2. Effects of progesterone concentration on number of follicles aspirated, oocytes recovered, and number of grade 1 to 3 oocytes collected per heifer in Experiment 1[1]

Progesterone	**High (n = 31)**	**Low (n = 37)**
Follicles Aspirated	12.1 ± 1.0[a]	15.3 ± 1.1[b]
Oocytes Recovered	6.4 ± 0.8[a]	9.9 ± 1.2[b]
Grade 1 to 3 Oocytes	4.8 ± 0.7[a]	7.8 ± 1.0[b]

[1]LS means reported
[a,b] $P < 0.03$

Figure 2. Circulating concentrations of progesterone (ng/mL) in heifers in the high or low treatment in Experiment 1. Progesterone concentrations differed ($P < 0.01$) at every sampling point following ablation (day 5.5)



The second experiment was conducted in mature Angus cows (n = 56). The experimental design was similar to experiment 1, with the exception that an alternative objective included in experiment 2 to compare the effects of progesterone concentrations on oocyte quality with or without FSH stimulation to induce follicular growth (Figure 3). To accomplish this, all cows were synchronized to a common day of the estrous cycle (day 0 = estrus), follicular ablation was used to reset follicle waves (day 5.5), and ovarian stimulation using 40 mg of FSH was conducted on day 7.5, 8, 8.5, and 9 prior to OPU collection of all visible follicles on day 10.5. The second wave of follicular growth was not stimulated with FSH. Hence, OPU was conducted on day 14.5, 4 days following the previous OPU session. Therefore, this experiment was a 2 x 2 factorial with the main effects of progesterone concentration (L vs. H) and FSH (+/-). In experiment 2, all oocytes collected successfully went through an IVP system, allowing for evaluation of cleavage rate, blastocyst rate, and visual assessment of embryo quality. Furthermore, epifluorescent microscopy was used to determine the number of total and dead blastomeres.

Figure 3. Design of Experiment 2

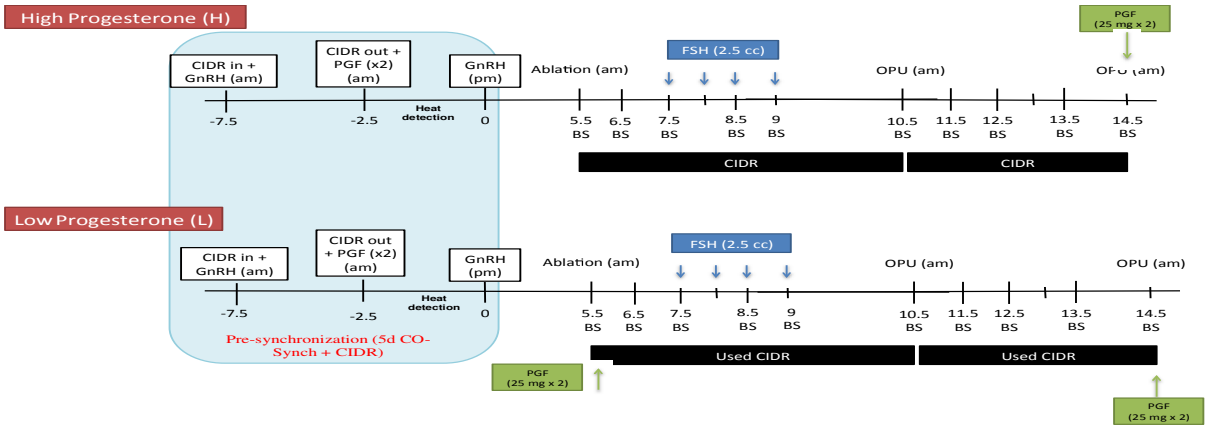
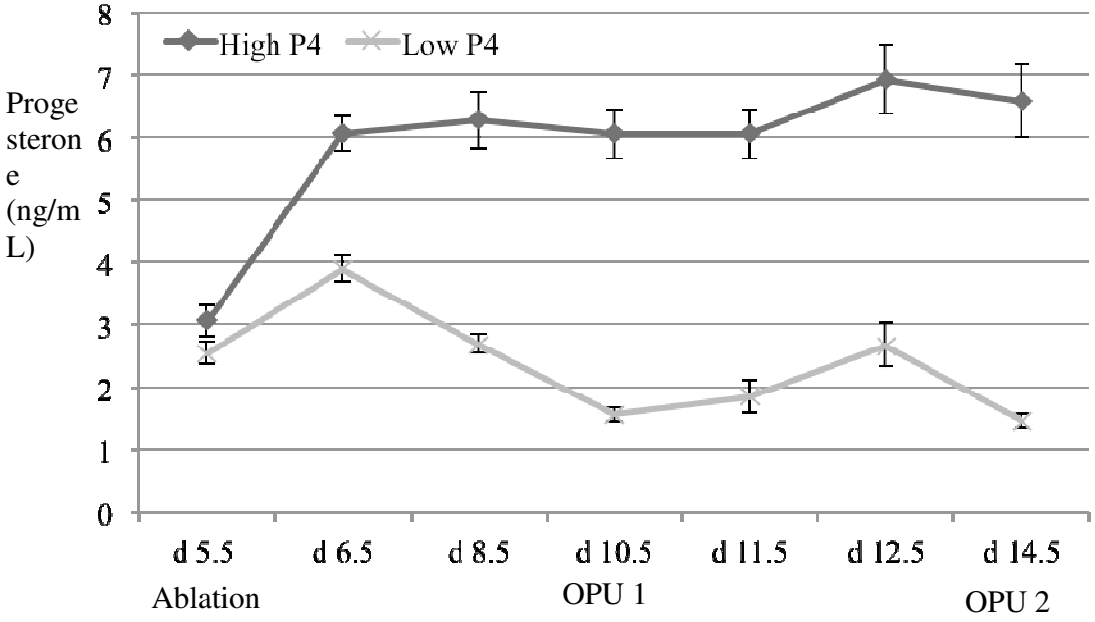


Figure 4. Circulating concentrations of progesterone (ng/mL) in cows in the high and low treatment in Experiment 2. Progesterone concentrations differed ($P < 0.01$) at every sampling point following ablation (day 5.5)



As designed, circulating concentrations of progesterone differed ($P < 0.01$; Figure 4) at every sampling following ablation between treatments. Reducing progesterone concentrations during follicular development resulted in increased numbers of follicles at the time of OPU, independent of FSH treatment (Table 3). However, progesterone concentrations did not impact the number of oocytes recovered, the quality of these oocytes as assessed via oocyte visual assessment (Table 4), nor the ability of the oocytes to undergo cleavage and develop into a blastocyst stage embryo following IVP (Table 4). Although blastocyst yield was not affected by progesterone concentration, blastocyst stage embryos derived from oocytes collected from cows with reduced progesterone concentrations were advanced in stage ($P < 0.05$) and had a greater number of total cells ($P < 0.05$) when compared to blastocyst stage embryos derived from oocytes collected from cows that were exposed to elevated progesterone concentrations (Table 5). In regards to the second objective, cows treated with FSH produced a greater number of follicles ($P < 0.001$) and had more oocytes recovered ($P < 0.001$; Table 3). Furthermore, FSH treatment, resulted in more grade 1-3 oocytes per cow ($P < 0.001$; Table 4) and tended ($P = 0.06$) increase the percentage of grade 1-3 oocytes per cow.

Table 3. Effect of Progesterone and FSH on Follicles Aspirated and Oocyte Recovered per Cow in Experiment 2[1]

Treatment	Progesterone		FSH	
	High (n = 50)	**Low (n = 57)**	**Yes (n = 53)**	**NO (n = 54)**
Follicles aspirated, n	19.0 ± 1.41[a]	23.2 ± 1.31[b]	25.1 ± 1.33[c]	16.9 ± 1.32[d]
Oocytes recovered, n	11.6 ± 3.85	13.0 ± 3.55	16.1 ± 3.75[c]	8.5 ± 3.65[d]

[1]LS means reported
[a,b] $P = 0.03$ [c,d] $P < 0.001$

Table 4. Effect of Progesterone and FSH on Oocyte Quality and Embryo Production in Experiment 2

Treatment	Progesterone		FSH	
	High	**Low**	**Yes**	**NO**
% Ooctye Grading 1 to 3	63.6 (328/516)	65.5 (443/676)	67.0[c] (531/792)	60.0[d] (240/400)
Grade 1 to 3 Oocytes per Cow	6.5 ± 0.69	7.7 ± 0.76	9.9 ± 0.94[a]	4.3 ± 0.42[b]
Cleavage, %	56.7% (244/430)	61.9% (337/544)	60.2% (380/631)	58.6% (201/343)
Blastocyst, %	19.3% (83/430)	22.1% (120/544)	22.2% (140/631)	18.4% (63/343)

[a,b] $P < 0.001$ [c,d] $P = 0.06$

Table 5. Effect of Progesterone and FSH on Embryo Characteristics in Experiment 2[1]

Treatment	Progesterone		FSH	
	High (n = 59)	**Low (n = 57)**	**Yes (n = 76)**	**NO (n = 40)**
Embryo Stage	5.1 ± 0.22[a]	5.5 ± 0.18[b]	5.3 ± 0.16	5.4 ± 0.24
Embryo Quality	1.5 ± 0.10	1.3 ± 0.09	1.5 ± 0.08	1.4 ± 0.11
Dead Cells, n	5.1 ± 1.43	4.6 ± 1.11	5.3 ± 1.02	4.4 ± 1.54
Total Cells, n	78.6 ± 3.60[a]	90.4 ± 3.31[b]	80.6 ± 2.87	89.3 ± 4.18
Live, %	92.9 ± 2.13	94.7 ± 1.54	92.8 ± 1.44	94.7 ± 2.24

[1]LS means reported
[a,b] $P < 0.05$

In both Experiments 1 (heifers) and 2 (cows), low progesterone concentrations during follicular development resulted in an increase in the number of follicles present on the ovaries at OPU. The reason for the increase in follicular number is not completely understood, but we propose that the reduction in progesterone may have resulted in greater follicular development by either enhancing FSH release and thus allowing more follicles to be recruited or the low progesterone resulted in greater LH secretion that reduced the number of follicles undergoing atresia following recruitment. The increased number of grade 1 to 3 oocytes observed in heifers receiving low progesterone was more a function of increased follicular number present on the ovary at time of OPU rather than an improvement in quality of oocytes collected as a result of decreased progesterone concentrations. This is concluded because the proportion of Grade 1-3 oocytes of total oocytes collected did not differ between treatments in either study. Although this may not support our hypothesis that low progesterone concentrations would increase oocyte quality, it is important from a practical sense as practitioners conducting OPU desire more total numbers of grade 1 to 3 oocytes.

In experiment 2, oocyte cleavage rate and blastocyst development was not impacted by progesterone concentration. This may be due in part to the limitations of the use of an IVP system to measure oocyte competence. All oocytes collected were included in the IVP system in this study. Typical production practices would limit oocytes to grades 1-3 since oocytes of grade 4-6 typically have blastocysts rates between 0 – 6% (Blondin et al., 1996). The reason that all oocytes were included was to avoid biasing one treatment over the other if oocyte grade differences had occurred between treatments. Although cleavage and blastocyst rates did not differ between treatments, embryos derived from cows in the low progesterone treatment were more advanced in stage and had greater cell numbers on d 7. This may reflect a marginal improvement in oocyte quality in females in the low progesterone treatment. Although studies making similar comparisons to this are limited, using an animal model somewhat similar to ours, Chaubal et al. (2007) did report that in cows treated with LH prior to OPU, oocytes from cows without a CIDR generated nearly twice as many blastocysts than oocytes from cows that did have a CIDR. Although these results differ from ours, this further evidence that a low progesterone environment prior to OPU and during follicular development may improve oocyte quality and increase the number of blastocysts generated. Furthermore, in the study by Chaubal et al. (2007) follicles aspirated and oocytes collected did not differ in cows with or without a CIDR. Hence, increased total number of blastocyst generated following IVP was likely due to improved oocyte quality in those cows where a CIDR was not used. The reasons for the different results between the current study and those reported by Chaubal et al (2007) are not clear. Additional studies directly investigating the role of progesterone and gonadotropin concentrations on oocyte quality are needed.

From an applied standpoint, results from these experiments have implications in terms of IVP embryo production and transfer. *In vitro*-produced bovine embryos can be generated inexpensively in comparison to *in vivo*-derived counterparts, and are in demand to create pregnancies in both beef and dairy cattle. In addition, the transfer of non-frozen IVP embryos results in acceptable pregnancy rates (53.8%, 1,220/2,268; Hasler et al., 1995). According to the most recent reports by the International Embryo Transfer Society, the total number of transferrable bovine IVP embryos worldwide has increased since 2000 (Stroud, 2010), with growth of 19.7% from 2009 to 2010. As technologies such as oocyte pick-up and IVP continue to be perfected, the number of IVP transfers is expected to continue to grow. Therefore,

implications of this study could affect management of OPU donors. In beef cattle, it may be advisable to develop OPU protocols that promote a low progesterone endocrine profile during development of the follicular wave. Our results and those from others (Chaubal et al., 2007) suggest that such an approach may increase the number of oocytes recovered and increase blastocyst yield and/or quality. More research in this area is needed to confirm these speculations. Furthermore, Brazil leads the global field of IVP embryo production and transfer, responsible for nearly 80% of worldwide IVP embryo activities. Keeping in mind that the current results were generated in *Bos taurus* cattle, it is anticipated that similar results may be seen in *Bos indicus* cattle and should be further investigated as these cows are more often used for OPU and IVP embryo production.

Importance of Progesterone Post-Insemination

The role of progesterone following fertilization on promoting embryonic growth and survival has been well established. It has been repeatedly demonstrated that progesterone concentrations during early gestation are greater in cows eventually diagnosed pregnant than those diagnosed non-pregnant (Johnson, 1958; Robinson et al., 1989; Starbuck et al., 2001). Furthermore, progesterone concentrations during early gestation can directly influence the probability of embryonic survival or embryonic loss (Starbuck et al., 2004). In beef heifers, Diskin et al. (2006) demonstrated a positive linear and quadratic relationship between blood progesterone concentrations and embryo survival. A classic study performed by Garrett et al. (1988) demonstrated that progesterone concentrations affect conceptus growth, with greater progesterone concentrations promoting a larger conceptus. Increasing progesterone concentrations and subsequent conceptus development during early gestation may increase pregnancy success by improving the ability of the conceptus to signal maternal recognition of pregnancy (MRP). Insufficient production of interferon-tau (IFNt) by the conceptus has been implicated as a main reason for early embryonic failure in cattle (Mann et al., 1999). This is critical as IFNt is the conceptus-produced factor that signals MRP. It has been demonstrated that circulating progesterone concentrations and IFNt secretion by the conceptus are highly correlated (Kerbler et al., 1997). Furthermore, both Mann and Lamming (2001) and Mann et al. (2006) observed that larger conceptuses produced increased concentrations of IFNt. Additionally, Mann et al. (2006) demonstrated a significant association between embryo length and uterine IFNt concentrations ($R^2 = 0.83$). As conceptus development proceeds during elongation, mRNA expression of IFNt per trophoblast cell does not increase. Conversely as the conceptus elongates, greater numbers of trophoblast cells allow for increased total IFNt amounts in the uterus (Robinson et al., 2006).

Recently, investigators have demonstrated that progesterone receptor mRNA is present at all stages of embryo development (Clemente et al., 2009). However, the actions of progesterone to increase embryonic development do not appear due to direct effects on the embryo. Rather, increasing progesterone concentrations act on the uterus to enhance the secretion of various factors, termed histotroph, to increase embryonic development (Spencer and Bazer, 2002; Satterfield et al., 2006). The uterine endometrium produces and secretes a wide array of factors necessary for embryo development and survival. These factors promote embryonic development, alterations in cellular membranes that facilitate conceptus and endometrial adhesion, coordinate the attachment of the conceptus to the endometrium, and modulate the mother's immune response to accept the developing conceptus (Spencer and Bazer, 2002;

Spencer et al., 2004). The production and secretion of these factors is regulated by the expression and down-regulation of various genes in the uterus. Progesterone concentrations during early gestation affect the global gene expression, termed transcriptome, in the uterine endometrium (Bauersachs et al., 2006). Alterations in progesterone concentrations therefore cause alterations in the uterine transcriptome that impede uterine function and result in a decreased chance of conceptus survival by altering uterine secretions (NcNeill et al., 2006; Forde et al., 2011). Hence, if progesterone concentrations during early gestation are manipulated, such as by affecting the size and quality of the ovulatory follicle and subsequent CL, this can have direct effects on the probability of embryonic survival.

Given the importance of elevated progesterone concentrations during early pregnancy on embryonic growth, MRP, and ultimately embryonic survival, numerous studies have been conducted to investigate the ability of exogenously administered progesterone to improve pregnancy rates in cattle. The ultimate aim of these studies was to increase circulating concentrations of progesterone. Several approaches have been taken including direct administration of daily injections of progesterone, insertion of progesterone releasing devices such as a CIDR, and inducing the ovulation of a second follicle and formation of a second CL via administration of GnRH, eCG, or hCG to increase progesterone concentrations in cattle. Over fifty years ago, Johnson (1958) was able to increase first service conception rates of dairy cows from 42% to 68% following 100 mg injections of progesterone on d 2, 3, 4, 6, and 9 following AI. A similar increase was observed when Holstein cows were treated with a progesterone releasing intrauterine device on days 5 to 12 or 10 to 17 following AI (Robinson et al., 1989). Starbuck and colleagues (2001) also demonstrated that progesterone supplementation of dairy cows at risk embryonic loss due to progesterone insufficiency improved embryo survival rates. Although some investigators have observed improvements in fertility with progesterone supplementation during early embryonic development, others have failed to demonstrate a benefit (Mann and Lamming, 1999; Lamb et al., 2010; Wiltbank et al., 2012). Collectively, Mann and Lamming (1999) concluded that progesterone supplementation improved pregnancy success by 5% but this improvement was dependent upon days of progesterone supplementation and relative fertility of the treated herds. The reason for this discrepancy between observational studies that have noted improved fertility in cattle with elevated progesterone concentrations and the variable results in fertility when progesterone is supplemented is not clear. Duration of exposure, differences in concentrations in circulation and those at targeted tissues and organs, and mode of delivery all may contribute to the variable responses in fertility when progesterone is exogenously administered.

Summary

Circulating concentrations of progesterone prior to and following ovulation can impact fertility in cattle. The role of progesterone concentrations prior to ovulation and implications on fertility requires additional investigations. In *Bos indicus* cattle, it appears that fertility is improved when progesterone concentrations are reduced during the development of the ovulatory follicle. Decreased progesterone concentrations likely promote increased LH release, enhanced follicular development and estradiol production, and may directly affect the quality of the oocyte. In *Bos taurus* beef cattle, the benefits of decreased progesterone prior to ovulation are not as defined. However, our lab groups have been investigating the potential benefits. Following ovulation, elevated progesterone concentrations during early embryonic development are critical.

Progesterone concentrations affect uterine function and thereby affect embryonic growth. A greater understanding of the roles of progesterone both prior to and after insemination is paramount in reducing early embryonic loss and generating therapeutic strategies involving the use of exogenous progesterone to ensure optimal oocyte competence at ovulation, appropriate conceptus development, and maintenance of pregnancy.

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