# ESTROGEN AND PROSTAGLANDIN $F_{2\alpha}$ CONCENTRATIONS DURING THE POSTPARTUM PERIOD AND THE ESTROUS CYCLE

W.W. Thatcher, MS and PhD; F. Silvestre, DVM; J. Bartolome, DVM, MS
Department of Animal Sciences, IFAS, University of Florida,
Gainesville, FL, 32611-0920

## Normal Bovine Estrous Cycle

*Ovarian Follicular Waves and Plasma Estradiol:*
The ability to monitor ovarian follicular development in cattle on a daily basis with the use of ultrasound has caused a quantum leap in our understanding of follicular dynamics during the estrous cycle. In a companion paper at this conference, Dr. Wiltbank has reviewed the physiological and hormonal factors regulating the normal estrous cycle. After ovulation of the follicle responsible for estrus, there is recruitment of a new wave of small follicles that are 2 to 5 mm in diameter. Among this pool of small follicles of the first wave, a single follicle is selected to become the dominant and largest follicle growing at a rate of ~1.5 mm per day. The time that the dominant follicle begins to differ in its growth rate from the second largest follicle is defined as the point of follicular deviation. This occurs when the dominant follicle of the wave reaches approximately 8.5 mm (1). After deviation of the dominant follicle, the smaller subordinate follicles decrease their growth rate and then actually decrease in size or undergo atresia (i.e., follicular turnover). Following the period of linear growth, the dominant follicle enters a plateau phase where size does not change. During this period, the dominant follicle loses its dominance and recruitment of a second follicular wave occurs. The period of follicular dominance during the first wave extends until there is recruitment of a second wave of follicles (e.g., at approximately day 11 of the cycle). Re-occurring processes of recruitment, deviation and dominance characterize the second follicular wave. Dairy cattle may have either two or three follicular waves during the estrous cycle with either the second or third follicular wave evolving as the ovulatory follicle, depending upon whether the cycle is comprised of two or three follicular waves. The estrous cycle is slightly longer in cattle expressing three versus two follicular waves, respectively. Evidence indicates that fertility to insemination at estrus following a three-wave cycle is greater than that of a previous two-wave cycle.

Ovarian follicular dynamics and function of the CL are controlled by precise changes in hormonal secretions involving the hypothalamic, pituitary, ovarian and ovarian axis. Following ovulation of the dominant follicle that induced estrous behavior, there is an increase in FSH concentration in plasma that induces recruitment of the first wave dominant follicle. Indeed a peak in FSH secretion occurs before each wave of follicular development. These timely increases in FSH are due to an inability of the previous dominant follicle to suppress FSH secretion from the pituitary. Collectively, the

hormones inhibin and estradiol produced by the dominant follicle are able to inhibit FSH secretion. As the functional competence of the dominant follicle is altered due to ovulation, luteinization or atresia, the clamp on FSH secretion is removed, and enhanced FSH secretion initiates recruitment of a new wave of follicles. As the newly selected dominant follicle reduces FSH secretion, continued growth of subordinate follicles is arrested and the subordinate follicles undergo atresia or turnover.

Associated with growth of the dominant follicles is the potential of dominant follicles to produce estradiol. The preovulatory follicle produces an appreciable amount of estradiol that is responsible for inducing estrous behavior in a low progesterone environment. Estradiol concentrations decrease rapidly following the estradiol induced pre-ovulatory surge of LH. The first wave dominant follicle has the capability to produce estradiol when the follicle is approximately 10 mm in size. Concentrations of estradiol associated with development of the first wave dominant follicle are not as high as at estrus because of the increasing concentrations of progesterone associated with development of the CL. Research studies indicate that the first wave dominant follicle undergoes turnover or atresia because the high concentrations of progesterone induce a low frequency of LH secretion such that LH concentrations are inadequate to support continued growth of the follicle and atresia ensues. In contrast, the second wave dominant follicle, of a two wave follicular cycle, continues to grow and becomes the ovulatory follicle. The reason for sustained growth of the second wave dominant follicle is the drop in progesterone concentrations due to uterine induced regression of the CL in response to the luteolytic agent, $PGF_{2\alpha}$. It is clear that the LH pulse frequency has increased, and the rise in plasma LH concentrations causes a copious secretion of estradiol. Estradiol priming in the presence of low progesterone concentrations induces estrous behavior and a massive pre-ovulatory surge of LH that occurs at the onset of estrus. This is a finely regulated system in that the LH surge induces oocyte maturation, terminates estradiol secretion and causes ovulation of the oocyte at ~ 30 h after the preovulatory peak of LH.

*Persistent Dominant Follicles and Sustained Estradiol*

The ovulatory follicle generated in the last follicular wave does not turn over, but ovulates in a low progesterone ($P_4$) environment. Turnover of the dominant follicle (DF) is associated with high concentrations of $P_4$, typical of mid-cycle, which lowers LH pulse frequency. Turnover of the first wave DF can be blocked by exogenous progestins and injection of $PGF_{2\alpha}$. The resulting sub-luteal concentration of progestin in plasma permits an increase in LH pulse frequency, which sustains growth of the DF. This "persistent" DF (PDF) is estrogenic based upon elevated plasma estradiol, and subsequent fertility, as measured by conception rate at first service, is lower compared to animals bearing a normal DF. Indeed development of a PDF is not uncommon during normal synchronization programs involving uses of progestins and $PGF_{2\alpha}$. However if the PDF is turned over and a freshly recruited follicle is allowed to ovulate then fertility after AI is restored. Possible explanations for reduced fertility include alterations in the oocyte and /or in the oviductal environment. In a study by Ahmad et al. (2), cows ovulating a PDF had embryos that at day 6 of pregnancy were less developed (i.e., were less able to reach the 16-cell stage) than embryos from cows ovulating a fresh (F) DF. In addition, Revah and Butler (3) showed that oocytes recovered from the PDF showed expanded cumulus

cells and condensed chromatin dispersed in their ooplasm. In contrast, compact cumulus cells and intact germinal vesicles were found in oocytes from FDF. Recently, Mihm and coworkers (4) demonstrated that final oocyte maturation leading to metaphase I is initiated before the preovulatory LH surge in most dominant follicles with a dominance period greater than 9 days. This is associated with a reduction in dominant follicle health and subsequent ovulation of aged oocytes. Collectively these results indicate that the PDF may affect oocyte maturation, which could affect early embryonic development and decrease fertility. Furthermore, PDF's also appear to alter the oviductal microenvironment, which may contribute to low fertility. Fertility studies indicate that if the duration of follicular dominance is greater than 5 days, than conception rates are reduced following ovulation and insemination to such follicles.

*Lactating Dairy Cows and Estradiol*

We have reported that concentrations of progesterone in the luteal phase and estradiol in the proestrus phase of the estrous cycle are lower for multiparous lactating dairy cows compared to multiparous non-lactating cows (5). Furthermore, a greater occurrence of follicles larger than 15 mm occurred during the proestrus period in lactating cows. Recent investigations by Wiltbank and coworkers (6) clearly indicate that lactating dairy cows have a marked increase in liver blood flow following feeding and that this contributes to a greater metabolism of progesterone and estradiol. These alterations due to lactation likely account for the lower estradiol concentrations and follicular dynamics in the proestrus period of lactating cows compared to dairy heifers (7). Lactational status and high milk production have profound effects on the occurrence of multiple ovulations, twins, reduced estrus activity and subsequent fertility (6,7). Perhaps one use of the Heatsynch program for controlling occurrence of ovulation is that the injection of ECP (1mg) at 24 h after injection of $PGF_{2\alpha}$ corrects the sub-optimal concentrations of estradiol. Cows treated with the Heat Synch program have a high occurrence of detected heats, and cows not expressing estrus are more likely to be anestrus or anovulatory. Cows not expressing estrus clearly have a lower conception rate when timed inseminated (8).

## The Peripartum and Postpartum Period

Restoration of ovarian function is a multifactoral process involving coordination between the hypothalamus, pituitary ovarian and uterine axis. With delivery of calf and fetal membranes, there is a precipitous decline in plasma concentrations of progesterone, estrogens and corticosteroids.

*Peripartum Hormonal Changes:*

The endocrine state of the dairy cow has been altered markedly throughout pregnancy due to the array of pregnancy specific hormones that has inhibited pituitary secretion of FSH and LH. Progesterone and increasing concentrations of estrogens (estrone, estradiol and estrone sulfate) coupled with mammogenic placental hormones lead to growth of the mammary gland. With the impending maturation and arrival of the fetus, there are dynamic changes in hormones that culminate in parturition, initiation of lactation and

regression of the uterus. For the last 2 weeks of gestation, concentrations of progesterone decline gradually in association with increases in free estrone and estradiol produced by placentomes of the placenta. The high concentrations of free estrogens and lowered concentrations of progesterone lead to an elevation of prolactin that is critical to initiating milk secretion (lactogenesis). The final precipitous decline in progesterone is due to enhanced secretion of $PGF_{2\alpha}$ from the placenta. The progesterone block of pregnancy is removed, and, with a uterus that is extremely responsive to oxytocin, the calf is delivered. With delivery of the calf and fetal membranes, there is a precipitous decline in estrogens, and the cow re-bounds from the estrogenic negative feedback effects of pregnancy and develops the potential to secrete FSH and LH in the early postpartum period. Secretion of $PGF_{2\alpha}$ continues into the postpartum period since the major site of secretion is the maternal uterine caruncle that is not shed with the placenta during the first 24 h after parturition. Concentrations of 13,14-dihydro-15-keto $PGF_{2\alpha}$ (PGFM) in plasma decline as the uterus decreases in size and return to low basal concentrations by ~14 days postpartum. Concentrations of PGFM are highly correlated with uterine production rate at this time. High basal concentrations of PGFM beyond this period are associated with localized uterine infections. These orchestrated changes in hormones lead to a successful completion of pregnancy with delivery of the newborn calf that has a readily available supply of nutrients and immune-globulins delivered via colostrums/milk. From the perspective of the cow, the periparturient time is a delicate transition period in which her feed intake is decreased during the pre-partum period, in association with increases in estradiol, and she initiates a major mobilization of nutrients from adipose tissue to meet her subsequent energy needs for lactation. This delicate hormonal transition is further confounded by the stress of the calving process.

*Restoration of Postpartum Ovarian Activity and Energy Status:*
The dairy cow is amazing in that ovarian follicular activity resumes early in the pospartum period. With the drop in plasma concentrations of estradiol following delivery of calf and fetal membranes, inhibition of FSH secretion is removed and early increases in plasma FSH begin to stimulate follicle development as early as 7 days after parturition. A dominant follicle develops in response to FSH; however, the follicle does not produce estradiol and this appears to be associated with an inadequate amount of plasma LH. A low pulse frequency of LH leads to inadequate LH availability to support continued growth of the follicle, and the follicle turns over. Also during this period, there may be an inadequate amounts of IGF-I secreted to synergize with LH. Both LH and IGF-I are necessary for full functional development of an estrogenic follicle. Dr. Ron Butler's laboratory at Cornell University has demonstrated that the cow appears to sense its energy balance such that when the cow reaches her nadir in negative energy status and begins to have an increase in energy status, although still negative, LH secretion begins to increase as characterized by a higher LH pulse frequency. As a consequence, the next follicular wave induced by FSH produces a dominant follicle that continues to grow in response to greater availability of LH and produces estradiol. The cow now develops a positive feedback response to estradiol and induces a pre-ovulatory surge of LH that causes ovulation. It is not unusual for cows to have their first ovulation as early as 14 days postpartum. In many instances the first ovulation is not associated with estrus, and lifespan of the CL is shortened to 6 to 10 days. Early first ovulations usually occur on the

ovary contralateral to the previous uterine horn of pregnancy. As uterine regression is completed (about 30 days postpartum), frequency of subsequent ovulations returns to a normal frequency with a slightly higher rate of ~55% on the right ovary. It is clear that restoration of normal follicular and CL activity is tightly controlled.

Postpartum dairy cows undergo a marked change in energy status preceding the time they are returning to normal restoration of ovarian cycles. Energy status has been defined as the net energy intake of the animal minus the net energy required for maintenance and minus the net energy necessary for milk production. Dairy cattle undergo a period of negative energy status in early lactation because energy output via milk production exceeds energy intake via feed consumption. Negative energy states are associated with certain profiles of blood hormones and metabolites. Postpartum reproductive function can be divided into recrudescence of ovarian follicular activity, induction of a spontaneous LH surge that induces ovulation, and formation of fully functional corpora lutea that will maintain a pregnancy.

The effect of energy status upon integrated ovarian activity during early lactation was assessed by examination of plasma progesterone profiles of 54 multiparous Holstein cows (9). Twenty-eight percent of the cows (n=15) were anestrus (no ovarian cycles) for the 9-week postpartum period based on their plasma $P_4$ concentrations (<1 ng/ml). These cows were compared with two cycling groups: a group of 25 cows showing corpus luteum (CL) activity within 40 days after parturition and a second group of 14 cows showing CL activity between 40 and 63 days postpartum. The energy status during the first 2 weeks postpartum was very important. Both the later cycling and non-cycling cows were in progressively negative energy states, that is, they continued to be in a more negative energy state in the second week than in the first week. This was especially true for the anestrus cows. Intake of feed by anestrous cows continually lagged behind that of cycling cows. Not only did anestrus cows eat less at week 1 postpartum, but the difference between them and cycling cows became greater as time went on. On the average, anestrous cows ate between 2.5 and 3.6 kg less feed per day than cycling cows. The cows returning to CL activity the earliest started their recovery to a positive energy state immediately after the first week. It was interesting in that cows cycling earlier had the greatest milk production. The movement back toward positive energy status appears to be important in initiation of estrus. Concentrations of IGF-I in plasma were related closely to the recrudescence of CL activity among the three groups (10). Cows that cycled early in the postpartum period experienced an early increase in IGF-I concentrations (2 wk postpartum), whereas cows that were anestrous through 8 wk of lactation did not exhibit a rise in plasma IGF-I concentrations until 5 to 6 wk postpartum. Cows beginning to cycle between 40 and 63 days postpartum showed an intermediate pattern in IGF-I concentrations in plasma. It is our contention that these changes in IGF-I were related to metabolic differences among cows that began to cycle at different times and were critical to follicle development and subsequent formation of the CL. The marked deficit in early energy status for the anestrus cows exerted a marked carryover effect on conception. Only 33% (5/15) of anestrous cows eventually conceived compared to 84% (21/25) and 93% (13/14) for early and late cycling cows, respectively.

Recovery of daily energy status from its most negative nadir appears to be an important signal for initiation of ovarian activity. Indeed the relationship in lactating dairy cows between days to first ovulation postpartum (Y) and interval in days from the energy nadir to ovulation (X) is described by the following linear regression ($R^2 = 0.77$), $Y = 10.3 + 1.2(X)$ (11). Bean and Butler (12,13) conducted a series of elegant experiments to actually characterize follicle dynamics in a large number of lactating dairy cows during the early postpartum period and related the dynamics of follicle development to hormonal and metabolic statuses of the cows. Among 45 postpartum cows, follicular development prior to first ovulation was characterized by either ovulation of the first dominant follicle (n=10), one or more waves of nonovulatory dominant follicles, n=18) or the formation of a follicular cyst (n=8). The follicular dynamics of these three categories and associated concentrations of plasma estradiol is depicted in Figure 1 (12). Cows displayed an increase in plasma FSH between 1 to 5 days postpartum following the abrupt decline in plasma estradiol from day 1 postpartum. All cows displayed a subsequent wave of follicular development characterized by emergence of a large dominant follicle during the second week postpartum. This phase of dominant follicle development occurred during the early period of negative energy status, and it would appear that the early increase in plasma FSH and emergence of dominant follicles are not influenced by negative energy status.

From an endocrine perspective, follicular development can be divided into stages of: gonadotropin independence (follicles < 3mm in size), dependence on FSH (follicles 3 to 10 mm), dependence on pulsatile LH (10 mm to preovulatory size) and dependence on a preovulatory surge of LH for ovulation. It appears that the two latter LH dependent stages, interacting with metabolic hormones, are associated with patterns of follicle development. For example, the early first wave of follicle development in cow # 4690 (Figure 1) was associated with a substantial rise in estradiol that led to ovulation of the first dominant follicle. In contrast cows with re-occurring follicular waves prior to first ovulation (e.g., Cows #4440, Figure 1) did not have parallel increases in estradiol. The follicles appeared to be incompetent to secrete estradiol although follicle development to the preovulatory stage occurred before turnover or atresia. Based upon our understanding of follicle development, these cows should have had sufficient pulsatile LH secretion to develop preovulatory size follicles. In cows with early dominant follicles that produce estradiol and ovulate, concentrations of plasma IGF-1 are higher compared to cows that develop non-ovulatory follicles during the same time period. The majority of these estrogenic and ovulatory follicles emerged after the energy balance nadir. IGF-1

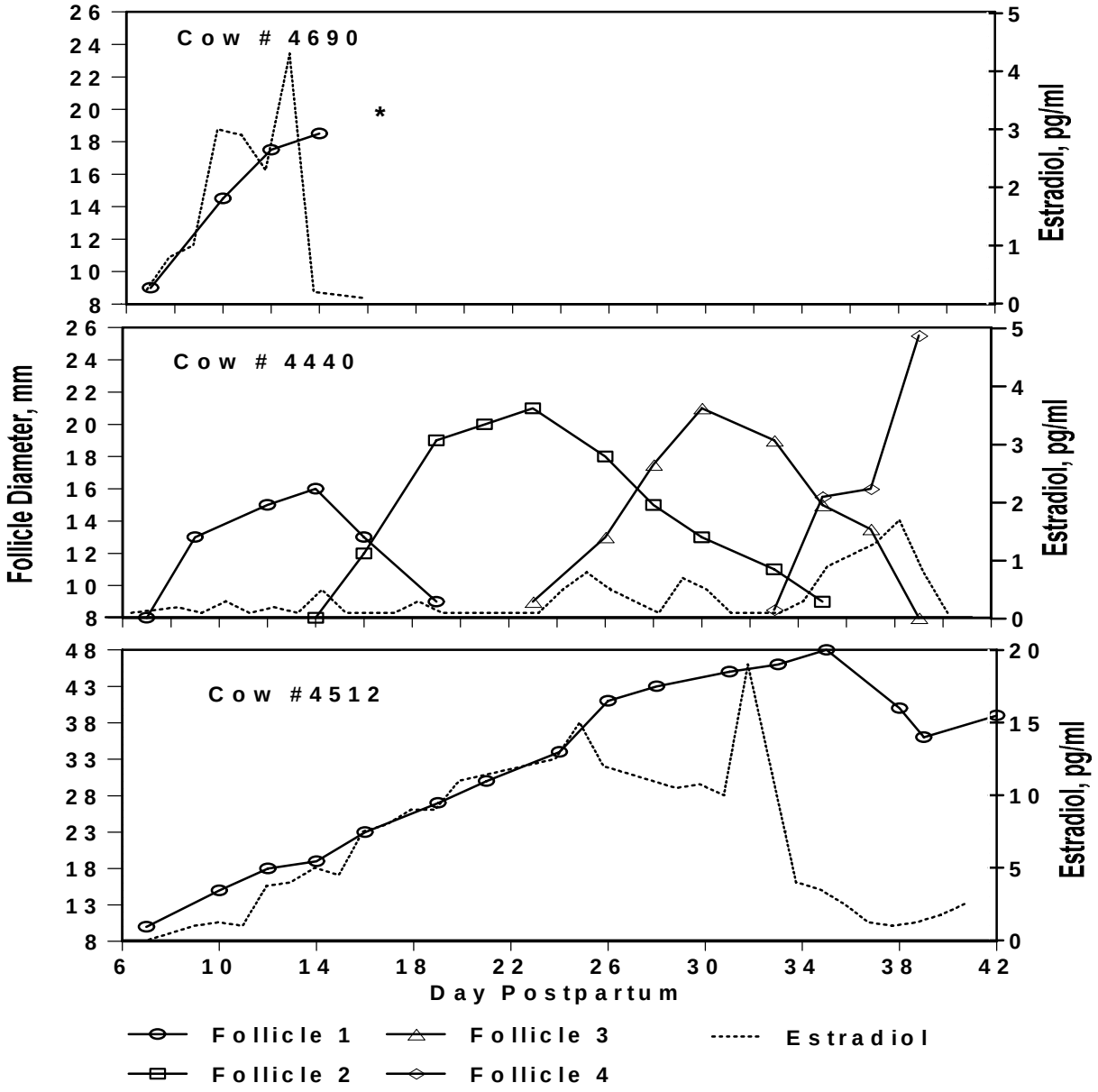


Figure 1. Patterns of dominant follicle growth and estradiol levels in three representative Holstein cows that 1) ovulated the dominant follicle of the first follicular wave PP, 2) experienced multiple waves of nonovulatory dominant follicle development and low plasma estradiol, and 3) developed an estrogen-active follicular cyst. * Denotes ovulation.

appears to play a regulatory role in follicle development in that it will stimulate steroidogenesis and proliferation of follicular cells (e.g., theca and granulosa cells). Perhaps, increased concentrations of IGF-1 in early postpartum cows increases sensitivity of the developing dominant follicles to FSH and LH. It is clear across several laboratories that higher postpartum plasma concentrations of IGF-1 are associated positively with both postpartum energy status and ovarian activity as measured by earlier development of competent dominant ovulatory follicles and earlier occurrence of CL. An additional example of the association of IGF-I concentrations with follicular

development was the observation that an experimentally induced, acute reduction in energy status (from +3.7 to -7.3 Mcal/day) during a 4-day period of terminal development of the preovulatory follicle reduced follicular growth rate (14). Associated with the decrease in DF function was a decrease in IGF-I concentrations in plasma.

The third pattern of postpartum follicle development, associated with development of an estrogen active follicular cyst (Figure 1, cow #4512), implies an additional dysfunction to control of ovarian follicular development. In this scenario, a functionally active dominant follicle that produces estradiol developed during the first 2 weeks postpartum, but the cow appeared incapable of inducing a preovulatory surge of LH to induce ovulation. Apparently, there is some inherent dysfunction of the hypothalmic-pituitary axis such that the cow did not respond to the positive feedback effect of estradiol to induce a preovulatory like surge of LH. Perhaps this is due to metabolic and stress responses associated with lactation and nutrient partitioning which predisposes certain animals to this condition. Fate of the first wave dominant follicle had a significant impact on the postpartum anovulatory interval. Regression of the first-wave dominant follicle or formation of a follicular cyst prolonged substantially the interval to first ovulation (approximately 50 days) versus 20 days for cows that ovulated the first wave dominant follicle.

*Estradiol, PGFM and Progesterone Associations with Postpartum Uterine Function and Health*

Lewis et al. (15) indicated that most cows develop a mild nonpathological endometritis during the early puerperal phase of the post-partum period. Uterine fluids (lochia) are usually voided during the first 2 weeks postpartum. Indeed the uterine immune system seems to up-regulate during the periparturient period and remains so until progesterone from the first post-partum ovulation down-regulates the uterine immune system. With the increase in progesterone, cows susceptible to nonspecific uterine infections are likely to develop uterine infections.

Spontaneous or induced uterine infections increase concentrations of PGFM in plasma (15). A recent study examined the relationship of PGFM concentrations in Holstein cows with the occurrence of endometritis during the postpartum period (16). Onset of endometritis was associated with an increase in progesterone concentrations. Cows that developed endometritis had dynamic changes in PGFM concentrations with lower PGFM concentrations during the early postpartum period (i.e., 0 to 14 days postpartum). However, during the period when endometritis was detected, cows had higher concentrations of PGFM. These workers speculated that changes in uterine prostaglandin production might affect the ability of the uterus to prevent and/or manage infections. For example, reduced PGFM concentrations early in the postpartum period may increase the likelihood of developing endometritis. Treatment of cows, diagnosed with endometritis, with a single injection of $PGF_{2\alpha}$ (25 mg; Lutalyse; Pharmacia Animal Health, Kalamazoo, MI) did not affect interval from parturition to first detected estrus (29.5 days) or to AI (73.3 days). Strategies to increase the availability of prostaglandins as a means to possibly enhance neutrophil function is an attractive means to possibly manage uterine

infections. Ways to enhance uterine secretion or to administer $PGF_{2\alpha}$ that causes sustained exposure warrant investigation.

We reported originally that cows having more detected heats during the postpartum period required fewer services per pregnancy and had a higher percent nonreturn to first service (17). Indeed most of the variation was associated with occurrence of heats during the first 30 days postpartum. One possible interpretation is that cows experiencing a greater turnover of CL would be less prone to develop sustained concentrations of progesterone that may predispose cows to uterine infections. Administration of a GnRH agonist (Deslorelin) implant is able to suppress follicular growth, reduce concentrations of estradiol, and delay both CL development and concentrations of progesterone in postpartum dairy cows (18). We hypothesized that administration of a Deslorelin implant immediately after parturition would eliminate ovulations and prevent a progestational induced down regulation in uterine immune function. We conducted an experiment to evaluate ovarian follicular activity, presence of a CL and uterine involution in cows treated with a non-degradable Deslorelin implant (5 mg; n = 10) or a control group (n = 9) that did not receive an implant (Silvestre F, Bartolome J and Thatcher W, unpublished observations). Cows received Deslorelin implants between 1d to 4d postpartum. All cows had normal parturitions without dystocia, retained fetal membranes, or milk fever. Ultrasound (US) was used to monitor number of ovarian follicles (Class 1, $\leq$ 5 mm; Class 2, 6-9 mm; Class 3 $\geq$ 10 mm, and number of CL) ipsilateral and contralateral to the previous pregnant horn (PH) on days 21, 28 and 35 after enrollment. We measured diameters of uterine horns by US at 4 cm past the intercornual ligament and cervix. Vaginal endoscopy evaluated cervical discharge and color on days 14, 21, 28 and 35 after enrollment. Ovarian activity was suppressed due to the Deslorelin implant group based upon a lack of Class 2 ($0.0 \pm 0.19 < 0.9 \pm 0.2$ $P<0.01$) and Class 3 ($0.0 \pm 0.19 < 1.3 \pm 0.20$; $P<0.01$) follicles that are dependent upon gonadotrophin secretion. The Deslorelin implant decreased the number of CL ($0.0 \pm 0.09 < 0.45 \pm 0.1$; $P <0.01$). The Deslorelin implant, inserted at 1-4 days postpartum, reduced size of the previous pregnant uterine horn (Figure 2) and lowered the frequency of a purulent discharge from the cervical os. Results from this experiment question the merits of stimulating early postpartum ovarian activity and the need of supplemental estrogens during the immediate postpartum period. However, this does not rule out the potential benefit of estrogen treatments that may temporarily delay postpartum ovulations. Not having ovarian activity during the period of uterine involution appears to be important to the involutionary process. Perhaps this is the reason that suckled beef cows experience less postpartum problems involving the uterus. Treatments that delay ovarian activity or reduce sustained progesterone exposure warrant further study relative to improving uterine health and fertility in both clinically normal and abnormal lactating dairy cows.

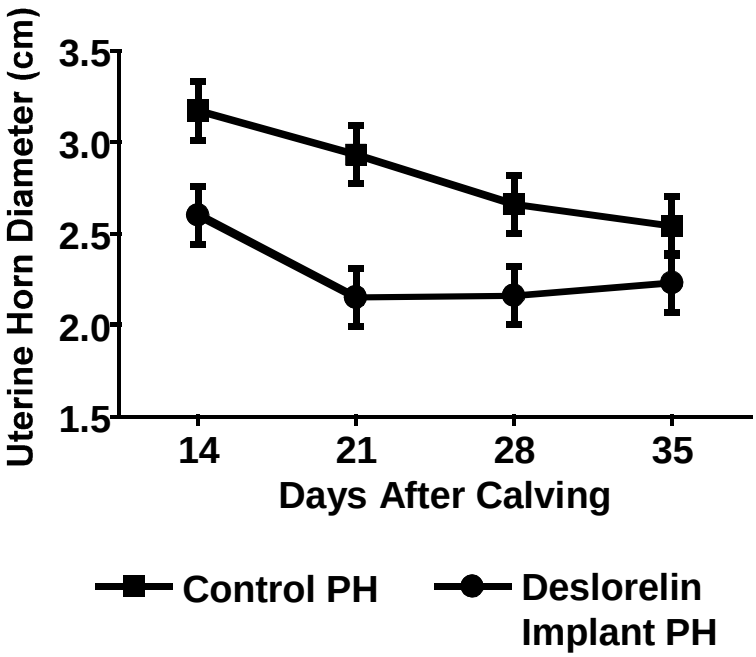


Figure 2. Size of previous pregnant uterine horn (PH) in lactating dairy cows that received Deslorelin implant or no implant within 1-4 days postpartum.

**Selected References**


1. Wiltbank MC, Gumen A, Sartori R. Physiological classification of anovulatory conditions in cattle. Theriogenology 2002; 57: 21-52.
2. Ahmad N, Schrick FN, Butcher RL, Inskeep EK. Effects of persistent follicles on early embryonic losses in beef cows. Biol. Reprod. 1995; 52:1129-1135.
3. Revah I., Butler WR. Prolonged dominance of follicles and reduced viability of bovine oocytes. J. Reprod. Fertil. 1996; 106: 39-47.
4. Mihm M, Curran N, Hyttel P, Knight PG, Boland MP, Roche JF. Effect of dominant follicle persistence on follicular fluid oestradiol and inhibin and on oocyte maturation in heifers. J. Reprod. Fertil. 1999; 116: 293-304.
5. De La Sota RL, Lucy MC, Staples CR, Thatcher WW. Effects of recombinant bovine somatotrophin (Sometribove) on ovarian function in lactating and nonlactating dairy cows. J. Dairy Sci. 1993; 76:1002-1013.
6. Wiltbank MC, Fricke PM, Sangsritavong S, Sartori R, Ginther OJ. Symposium: Physiology, Lactation, and Reproduction. Mechanisms that prevent and produce double ovulations in dairy cattle. J Dairy Sci 2000; 83:2998-3007.
7. Sartori R, Haughian J, Rosa GJ, Shaver RD, Wiltbank MC. Differences between lactating cows and nulliparous heifers in follicular dynamics, luteal growth, and serum steroid concentrations. J. Anim Sci 2000; 78 Suppl 1/ J Dairy Sci 2000; 83 Suppl 1:212.
8. Pancarci SM, Jordan ER, Risco CA, Schouten MJ, Lopes FL, Moreira F, Thatcher WW. Use of estradiol cypionate in a pre-synchronized timed artificial insemination program for lactating dairy cattle. J Dairy Sci 2002; 85:122-131.

9. Staples CR, Thatcher WW, Clark JH. Relationship between ovarian activity and energy status during the early postpartum period of high producing dairy cows. J Dairy Sci 1990; 73:938-947.
10. Thatcher WW, De La Sota RL, Schmitt E J-P, Diaz T, Badinga L, Simmen FA, Staples CR, Drost M. Control and management of ovarian follicles in cattle to optimize fertility. Reprod. Fertil Dev1996; 8:203-217.
11. Canfield RW, Butler WR. Energy balance and pulsatile LH secretion in early postpartum dairy cattle. Dom Animal Endoc 1990; 7:323-330.
12. Beam SW, Butler WR. Energy balance, and ovarian follicle development prior to the first ovulation postpartum in dairy cows receiving three levels of dietary fat. Biol Reprod 1997; 56:133-142.
13. Beam SW, Butler WR. Energy balance, metabolic hormones and early postpartum follicular development in dairy cows fed prilled lipid. J Dairy Sci. 1998; 81: 121-131.
14. Lucy MC, Beck J, Staples CR, Head HH, De La Sota RL, Thatcher WW. Follicular dynamics, plasma metabolites, hormones and insulin-like growth factor I (IGF-1) in lactating cows with positive or negative energy balance during the preovulatory period. Reprod Nut Dev 1992; 32:331-341.
15. Lewis GS. Symposium: Health problems of the post-partum cow. Uterine health and disorders. J. Dairy Sci. 1997; 80: 984-994.
16. Seals RC, Matamoros I, Lewis GS. Relationship between postpartum changes in 13, 14-dihydro-15-keto-$PGF_{2\alpha}$ concentrations in Holstein cows and their susceptibility to endometritis. J Anim Sci 2002; 80:1068-1073.
17. Thatcher WW, Wilcox CJ. Postpartum estrus as an indicator of reproductive status in the dairy cow. J Dairy Sci1973; 56:608-610.
18. Mattos R, Orlandi C, Williams J, Staples CR, Trigg T, Thatcher WW. Effect of an implant containing the GnRH agonist Deslorelin on secretion of LH, ovarian activity and milk yield of postpartum dairy cows. Theriogenology 2001; 56:371-386.