

Infertility associated with the duration of luteal phase in postpartum cows

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ABSTRACT. After first postpartum ovulation most cows have a short luteal phase due to a premature secretion of PGF₂ α from the uterus. Pregnancy rate in estrous cycles with short luteal phases is essentially nil. The lack of exposure of the uterus to P₄ and estrogen prior to first postpartum ovulation decreases the concentration of P₄ receptors and up regulates oxytocin receptors. The inadequate number of P₄ receptors and presence of endometrial oxytocin receptors earlier in cows with short than normal luteal phases allows premature release of PGF₂ α and the resultant regression of the CL. During luteolysis, uterine and luteal PGF₂ α cause death of the embryos mainly during the transition from morula to blastocyst when the embryos are more susceptible to embryotoxic effect.

Key words: Cattle, First ovulation, Infertility, Postpartum, Short luteal phase

Infertilidad asociada con la duración de la fase luteal en vacas postparto

RESUMEN. Luego de la primera ovulación postparto, la mayoría de las vacas presentan un ciclo estrual corto debido a la secreción anticipada de PGF₂ α desde el útero. La tasa de preñez en ciclos estrales con una fase luteal corta es prácticamente cero. La falta de exposición del útero a la P₄ y estrógenos antes de la primera ovulación postparto, disminuye la concentración de receptores de P₄ y estimula la expresión de receptores de oxitocina. El número inadecuado de receptores uterinos de P₄ y la presencia de receptores endometriales de oxitocina más temprano en vacas con ciclos estrales cortos que con ciclos estrales normales, permite la secreción prematura de PGF₂ α y la resultante regresión del cuerpo lúteo. Durante la luteólisis, la PGF₂ α de origen uterino y luteal causan la muerte embrionaria principalmente durante la transición de morula a blastocito, cuando los embriones son más susceptibles al efecto embriotóxico.

Palabras clave: Postparto, Primera ovulación, Fase luteal corta, Infertilidad, Ganado vacuno.

Introduction

An important goal of cow-calf beef operations and dairying is to achieve calving and lactation once yearly, which allows maximum expression of biological productivity, leading to greatest milk and beef sales (Perea *et al.*, 2002). In order to accomplish this goal, conception, should occur before Day 90 postpartum. However, several environmental, genetic, and physiologic factors can contribute to low fertility and losses once cows reinstate reproductive cyclicity (Perea *et al.*, 2005).

During the postpartum transition period the reproductive axis recovers after gestation to progressively reach the anatomical and functional status needed to initiate a new pregnancy. The presence of dominant follicles in the ovaries, as well as an adequate pattern of LH secretion, leads to the first postpartum ovulation a few weeks after parturition (Murphy *et al.*, 1990; Rajamahendran and Taylor, 1990; Perea *et al.*, 1998). Fertility at this first estrous cycle is lower

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than in subsequent cycles, mainly due to embryonic death during the first two weeks after first insemination (Wells *et al.*, 1985; Inskeep, 2002). Premature secretion of PGF₂ α and early regression of the corpus luteum (CL) are responsible for the reduced conception rate (Cooper *et al.*, 1991; Zollers *et al.*, 1993; Inskeep and Dailey, 2004). Thus, maternal recognition of pregnancy is prevented, and a few days later the onset of a subsequent estrous cycle occurs, which has a normal conception rate (Odde *et al.*, 1980; Ramirez-Godinez *et al.*, 1981). In fact, as will be documented in this review, the premature secretion of PGF₂ α in the first cycle is embryotoxic and the embryo dies even if exogenous progesterone is provided.

Short estrous cycles with sub-functional CL are frequent before the first ovulatory estrus in periparturient heifers (Berardinelli *et al.*, 1979), in postpartum dairy (Menge *et al.*, 1962; Morrow *et al.*, 1966) and beef (Corah *et al.*, 1974) cows and as a consequence of early weaning of beef calves (Bellows *et al.*, 1974; Odde *et al.*, 1980). Treatment with progesterone (P₄) prior to the first ovulation induces a CL with normal lifespan (Ramirez-Godinez *et al.*, 1981; Zollers *et al.*, 1993; Fike *et al.*, 1997) in a large proportion of cows. An experimental model for comparisons of normal or short estrous cycles at the same stage postpartum was developed based on these facts. For short cycles, either early weaning or treatment with GnRH, hCG or estradiol will suffice; whereas for normal estrous cycles, these same treatments can be preceded by treatment with a progestogen. The model has been used extensively to study the mechanisms involved in short cycles following the first postpartum ovulation (Sheffell *et al.*, 1982; Buford *et al.*, 1996; Hernandez-Fonseca *et al.*, 2000), as well as to find therapeutic alternatives to improve reproductive efficiency during postpartum anestrus (Butcher *et al.*, 1992; Breuel *et al.*, 1993; Schrick *et al.*, 1993; Buford *et al.*, 1996; Fike *et al.*, 1997).

Characteristics of postpartum cattle

Resumption of ovarian cyclicity in *Bos taurus* dairy and beef cows takes place by around 30 or 60 days following parturition, respectively (Roche *et al.*, 1992), although this period is longer in tropical crossbred cows (*Bos taurus* \times *Bos indicus*) (Perea *et al.*, 2002, 2005; Gonzalez *et al.*, 2003). The occurrence of a short interovulatory interval (7 to 12 d) is frequent after first postpartum ovulation (Macmillan *et al.*, 1971; Odde *et al.*, 1980; Ramirez-Godinez *et al.*, 1982; Copelin *et al.*, 1989; Zollers *et al.*, 1989; Cooper *et al.*, 1991; Zollers *et al.*, 1993). In different studies the incidence of short estrous

cycles in postpartum cows ranged from 7 to 100% (Macmillan *et al.*, 1971; Odde *et al.*, 1980; Corah *et al.*, 1974; Wells *et al.*, 1985; Murphy *et al.*, 1990; Savio *et al.*, 1990; Stagg *et al.*, 1995; Mackey *et al.*, 2000). However, in cows induced to ovulate by early weaning or treatment with GnRH or hCG, the occurrence ranged between 60 and 80% (Odde *et al.*, 1980; Kesler *et al.*, 1980; Ramirez-Godinez *et al.*, 1981; Copelin *et al.*, 1988; Johnson *et al.*, 1992). Fertility associated with first estrous cycles was lower than in subsequent cycles, which had normal lifespan of the CL (Odde *et al.*, 1980; Ramirez-Godinez *et al.*, 1982; Breuel *et al.*, 1993).

Physiologic bases of short luteal phase

In initial studies, short luteal phase was not associated with either decreased luteotropic support by LH (Garcia-Winder *et al.*, 1986; Copelin *et al.*, 1988) or greater luteal sensitivity to PGF₂ α (Copelin *et al.*, 1988; Figure 1). Non-suckled hysterectomized cows weaned at 23 to 30 d postpartum did not return to estrus following first estrus, while intact cows had estrous cycles averaging 8.8 d (Copelin *et al.*, 1987; Figure 2). Although concentrations of P₄ were similar from estrus to Day 5, secretion of P₄ decreased after day 5 of the first cycle, but not after second estrus in intact cows or following the first or a second estrus (induced by PGF₂ α) in hysterectomized cows (Copelin *et al.*, 1987; Figures 1 and 2). Patterns of P₄ before and after Day 5 postestrus in postpartum cows expected to have CL of normal or short lifespan had similar profiles (Zollers *et al.*, 1989, 1991, 1993). Active immunization of anestrous beef cows against PGF₂ α extended the lifespan and secretory capacity of CL (Copelin *et al.*, 1989). The physiologic phenomenon has been described as a subnormal luteal phase occurring due to a premature release of PGF₂ α from the uterus and concomitant early CL regression (Copelin *et al.*, 1987; Cooper *et al.*, 1991). Presumably, lack of P₄ caused death of the embryo and the onset of a new estrous cycle.

During normal estrous cycles, luteal phase P₄ allows a rise in estrogen to promote synthesis of P₄ receptors (Zollers *et al.*, 1993) and to inhibit synthesis of uterine receptors for oxytocin through the mid-luteal phase (McCracken *et al.*, 1984; Kieborz-Loos *et al.*, 2003). However, the lack of exposure of the uterus to P₄ followed by estrogen prior to first postpartum ovulation decreases the concentration of P₄ receptors and up regulates oxytocin receptors, which allows premature secretion of PGF₂ α (Cooper *et al.*, 1991). Although embryonic death was presumably due to a lack of P₄ support, exogenous P₄ did not prevent it and evidence began

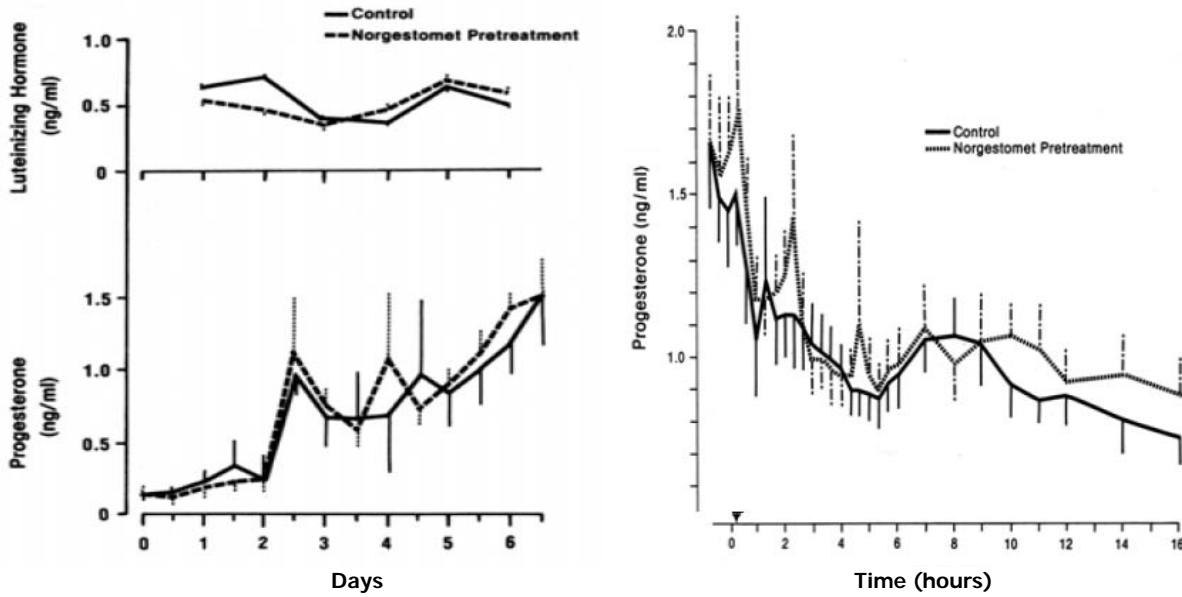


Figure 1. Pattern of plasma concentrations of LH (Days 1 to 6) and progesterone (Days 0 to 16) after GnRH injection (Day 0) in control (C; anticipated to have short luteal phase) and norgestomet pretreated hysterectomized (NH; anticipated to have normal luteal phase) cows (panel A). Mean (\pm SE) concentrations of progesterone following injection of 10 mg of PGF₂ α on Day 7 in C and NH cows (panel B). Cows in NH group received a norgestomet implant for 9 d beginning 17 to 21 d post-calving. All cows were injected with 100 μ g GnRH 28 to 32 d post-calving to induce ovulation, and in NH group, hysterectomy was performed 3 d later (reproduced from Copelin *et al.*, 1988 with permission).

to accumulate that PGF₂ α might act as an embryo-toxin (Butcher *et al.*, 1992; Breuel *et al.*, 1993). During luteolysis both uterine and luteal PGF₂ α cause death of the embryos mainly during the transition from morula to blastocyst when the embryos are more susceptible to embryotoxins (Buford *et al.*, 1996; Seals *et al.*, 1998; Hockett *et al.*, 2004). Furthermore,

the process of embryo transfer stimulates uterine secretion of PGF₂ α and success rates have been improved by treatment with inhibitors of prostaglandin synthesis at or near the time of transfer (Scenna *et al.*, 2005).

Mean concentrations of PGF₂ α in vena caval plasma on Days 4 to 9 after injection of hCG were

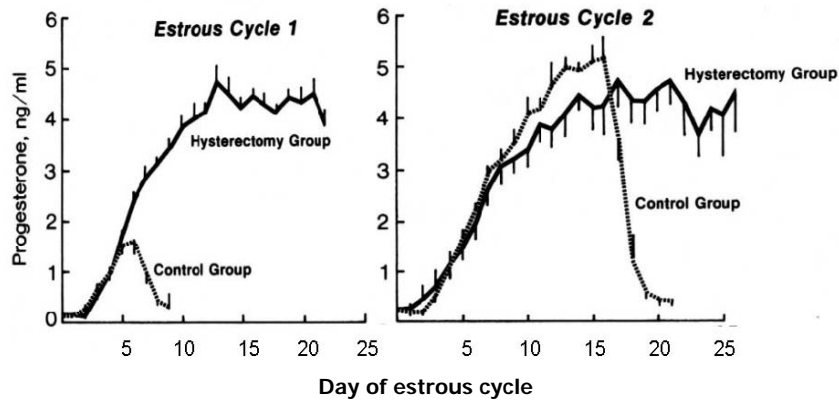


Figure 2. Pattern of plasma concentration of progesterone (mean \pm SE) in control and hysterectomized beef cows during the first and second estrous cycle. Calves were weaned at 23 to 33 d postpartum and cows were allotted to a control (sham surgery, n = 8) or a hysterectomy (n = 7) group (HG), with surgery performed at weaning. Cows in HG were injected with 25 mg of PGF₂ α approximately 20 d after first estrus (Day 0). Blood samples were collected daily from surgery until completion of two estrous cycles (control) or until 24 d of the second estrous cycle (HG group) (reproduced from Copelin *et al.*, 1987 with permission).

greater in postpartum beef cows undergoing premature luteal regression than in those with a normal luteal phase (Cooper *et al.*, 1991). Peter *et al.* (1989) found increased concentrations of oxytocin in association with increased concentrations of PGFM in dairy cows with short luteal phases, but Cooper *et al.* (1991) did not find consistent increases in oxytocin in association with increases in $\text{PGF}_{2\alpha}$ in plasma from the inferior vena cava of beef cows. Daily administration of oxytocin from Day 2 postestrus in beef cows markedly reduced length of the estrous cycle, but it did not appear to influence the rate at which P_4 concentration increased during the first days after estrus (Donaldson *et al.*, 1970). In postpartum cows, oxytocin induced a release of $\text{PGF}_{2\alpha}$ on Day 5 of a short but not a normal estrous cycle, and the oxytocin-induced release of $\text{PGF}_{2\alpha}$ on Day 5 of the short estrous cycle was similar to that near to the time of luteolysis (day 16; Zollers *et al.*, 1989). Moreover, *in vitro* secretion of PGF from endometrial explants on Day 5 was greater for cows anticipated to have a short than a normal luteal phase (Zollers *et al.*, 1991). Concomitantly, endometrial concentration of oxytocin receptors was greater and mean number of P_4 receptors per cell was lower on Day 5 after estrus in postpartum beef cows expected to have short luteal phase (Figure 3) than in cows receiving P_4 pre-treatment and expected to have a normal luteal phase (Zollers *et al.*, 1993). Although P_4 pre-

treatment is necessary for normal luteal lifespan and timing of uterine secretion of $\text{PGF}_{2\alpha}$ following first ovulation in cattle, early evidence indicated that oxytocin may be involved in premature secretion of $\text{PGF}_{2\alpha}$ in cows with short estrous cycles.

Luteolysis during the estrous cycle in ruminants was believed to occur as a consequence of a reciprocal positive feedback mechanism between luteal oxytocin and uterine $\text{PGF}_{2\alpha}$ (McCracken *et al.*, 1984; Lafrance and Goff, 1988; Lamsa *et al.*, 1989). This mechanism was proposed to be responsible for the episodic secretion of $\text{PGF}_{2\alpha}$ necessary to complete luteal regression (Flint *et al.*, 1990); furthermore, this process only occurs at a specific time of the estrous cycle (Lafrance and Goff, 1988; Silvia *et al.*, 1991; Kieborz-Loos *et al.*, 2003). It was believed that P_4 suppressed secretion of $\text{PGF}_{2\alpha}$ when more P_4 receptors were present, presumably by inhibition of synthesis of oxytocin receptors early in the estrous cycle when the uterus was more responsive to P_4 (McCracken *et al.*, 1984; Hazzard *et al.*, 1998; Kieborz-Loss *et al.*, 2003). However, as the estrous cycle progressed, concentrations of P_4 receptors decreased, which in turn allowed the synthesis of estrogen and oxytocin receptors (McCracken *et al.*, 1984). As a result, the uterus became more responsive to oxytocin and a positive feedback mechanism was apparently established between oxytocin and endometrial $\text{PGF}_{2\alpha}$.

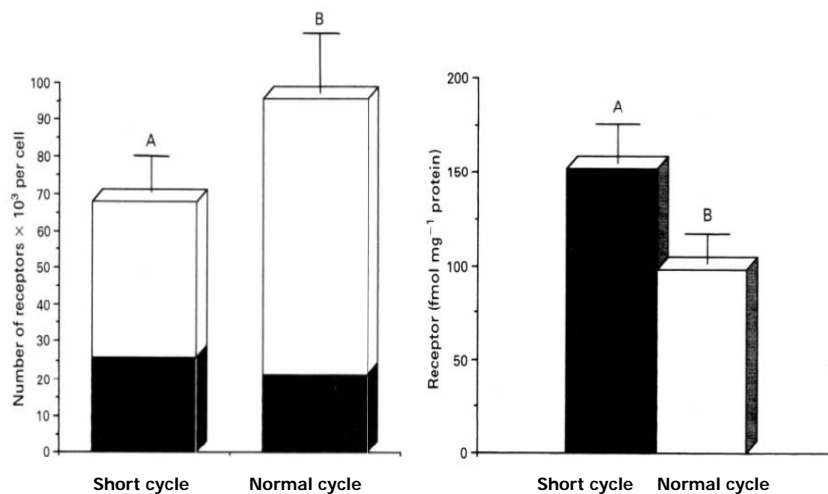


Figure 3. Number of progesterone receptors per cell (left panel) and oxytocin receptor content (right panel) in bovine endometrial tissue (\square cytosolic; \blacksquare nuclear) on Day 5 of a short or normal estrous cycle. Calves from cows in the short cycle group were weaned at 30-32 d postpartum, while cows in the short cycle group received norgestomet implant for 9 d at 21-23 d and their calves were weaned at implant insertion. On Day 5 following first postpartum ovulation, two groups of cows were hysterectomized and endometrial tissue was collected for measurement of oxytocin and progesterone receptors (reproduced from Zollers *et al.*, 1993 with permission).

In the normal estrous cycle of ruminants, a period of progestational influence appears to be important in the timing of luteal regression. Treatment with exogenous P₄ on Day 0 or 1 after estrus advanced the occurrence of the first peak of PGF₂α and shortened the length of the estrous cycle in sheep (Days 8 and 11 for P₄-treated ewes and Days 12.3 and 17 for control ewes; Ottobre *et al.*, 1980). In addition, the blockade of P₄ receptors by mifepristone (RU 486) during the early to mid-luteal phase inhibited luteolysis and preserved a functional corpus luteum beyond Day 20, presumably due to insufficient PGF₂α secretion from the endometrium (Morgan *et al.*, 1993). Abolition of P₄ source on Days 12 or 14 by removal of either ovaries or CL in sheep, resulted in a temporal increase of PGF₂α followed by a gradual decline (Ottobre *et al.*, 1984; Vincent *et al.*, 1986), suggesting that continued secretion of P₄ is necessary for secretion of PGF₂α to continue beyond Day 14 of the estrous cycle. Apparently, P₄ regulates secretion of PGF₂α by timing the initial peaks of secretion early in the estrous cycle, and thereafter, by modulating secretion of PGF₂α until luteal regression is completed. However, in post-partum cows and anestrus ewes the absence of a previous exposure of the uterus to P₄ followed by estrogen prior to first ovulation alters the physiological conditions of the uterus and as a consequence, the timing of luteolysis.

Endocrine events conducive to short or normal luteal phase

Preovulatory follicles from anestrus beef cows pre-treated with the synthetic progestogen, norgestomet, were larger and contained more estradiol-17β than those of control cows with short estrous cycles (Sheffel *et al.*, 1982; Garcia-Winder *et al.*, 1986). Furthermore, greater concentrations of receptors for LH, in both granulosa and theca, and greater concentrations of estradiol were detected in the largest preovulatory follicles collected from postpartum cows treated with norgestomet than from control cows (Inskip *et al.*, 1988; Table 1). In addition, postpartum anestrus cows treated with a subcutaneous ear implant containing estradiol-17β for 40 d after calving had approximately 40% fewer short estrous cycles during the time of estradiol exposure than non-treated cows (Day *et al.*, 1990). Increased concentrations of estradiol have been associated with induction of endometrial P₄ receptors (Zelinski *et al.*, 1980; Ing *et al.*, 1997). Therefore, lower concentrations of estradiol secreted by preovulatory follicles from cows that would have a short luteal phase (Sheffel *et al.*, 1982; Garcia-Winder *et al.*, 1986) could reduce uterine P₄ receptor synthesis (Zollers *et al.*, 1993) and consequently the first rise in P₄ is followed by early secretion of PGF₂α (Cooper *et al.*, 1991), which shortened the luteal phase.

Table 1. Concentrations of LH and FSH receptors, weights of follicular components and amounts of estradiol in follicular fluid from largest follicles of postpartum cows treated or not with a progestogen and expected to have a normal or short lifespan of the corpora lutea (Modified from Inskip *et al.*, 1988)

Variables	Treatment		Significance
	Control	Norgestomet	
Number of cows	11	11	
LH receptors (fmol/mg):			
<i>Granulosa</i>	11 1.16 ± 0.25	11 1.78 ± 0.16	<i>P</i> < 0.01
<i>Theca</i>	0.31 ± 0.04	0.73 ± 0.11	<i>P</i> < 0.01
Weight			
<i>Entire follicle (g)</i>	1.26 ± 0.10	2.66 ± 0.29	<i>P</i> < 0.01
<i>Theca (mg)</i>	136.1 ± 8.3	171.8 ± 17.8	<i>P</i> < 0.10
<i>Granulosa (mg)</i>	65.4 ± 4.8	101.4 ± 19.1	<i>P</i> < 0.10
<i>Fluid (g)</i>	1.06 ± 0.09	2.38 ± 0.26	<i>P</i> < 0.01
Estradiol in fluid: <i>Content (ng/follicle)</i>	4.28 ± 1.71	23.43 ± 6.49	<i>P</i> < 0.05
<i>Concentration (ng/g fluid)</i>	3.87 ± 1.54	9.36 ± 2.19	<i>P</i> < 0.05

A confirmatory study of previously documented findings was carried out by Kieborz-Loos *et al.* (2003). Postpartum anestrous cows exposed or not to different combinations and/or sequences of exogenous estradiol-17b and P₄ were ovariectomized on Day 23 or 32 after calving. Oxytocin was injected at the end of each treatment to test the ability of the postpartum uterus to secrete PGF₂α. Oxytocin-induced secretion of PGF₂α was greater on Day 16 than on Day 6 of a simulated normal luteal phase, implying that steroid treatment (P₄ followed by estradiol) stimulated the uterine responsiveness to oxytocin similar to that observed at the end of a normal estrous cycle (Lafrance and Goff, 1988). However, neither P₄ alone nor estradiol-17b followed by P₄ treatment were capable of reducing PGF₂α secretion on Day 6 of a simulated estrous cycle in ovariectomized cows. Only when estradiol-17b/P₄ treatment was preceded by a period of P₄ priming, was exogenous oxytocin unable to provoke an increased secretion of PGF₂α on Day 6 of the simulated cycle (Kieborz-Loos *et al.*, 2003). In postpartum anestrous cows, comparatively more animals ovulated and formed CL with typical lifespan after treatment with a P₄ releasing intravaginal device alone or in combination with estradiol benzoate (EB) than those treated with only EB or untreated controls (C) (Fike *et al.*, 1997). The addition of EB enhanced the proportion of cows with normal length of the estrous cycle (P₄+EB: 76%; P₄: 57%; EB: 24%; C: 19%).

What is the source of the steroids that assure a normal lifespan of the CL in those cows during spontaneous ovulatory cycles after calving?

In prepubertal heifers, Berardinelli *et al.* (1979) showed that small bits of luteal tissue embedded in the ovarian stroma and surrounded by connective tissue were responsible for increases in P₄ before first normal ovulatory cycles. In addition, a gradual rise in P₄ prior to the first postpartum estrus has been reported in cattle (Donaldson *et al.*, 1970; Corah *et al.*, 1974; Perry *et al.*, 1991; Dimmick *et al.*, 1991; Breuel *et al.*, 1993). Increased P₄ 3 days before first postpartum estrus was detected in primiparous cows that conceived, while no elevation prior to estrus was detected in those cows that failed to conceive and had a short estrous cycle (Corah *et al.*, 1974). Also, Ramirez-Godinez *et al.* (1981) reported that seven cows with estrous cycles of at least 17 d had a significant rise in serum progesterone prior to the first detected estrus.

The occurrence of ovulations in absence of clinical signs of estrus frequently observed in postpartum cows spontaneously reinitiating ovarian activity might account for the particular sequence of endocrine events required for a normal luteal phase (Stevenson and Britt, 1979; Fike *et al.*, 1997; Henao *et al.*, 2000). In this regard, Castenson *et al.* (1976) reported that the increase in P₄ prior to first postpartum estrus was produced by a short-lived CL, presumably from ovulation because of the presence of a stigma. More recently a study using ultrasonography supported those findings (Perry *et al.*, 1991).

Fertility and embryo viability in cows with short luteal phase

As stated above, the first spontaneous or induced ovulation after calving is associated with a CL of short lifespan (for review see: Inskeep, 1995; 2002; Inskeep and Dailey, 2004). Even when first ovulation is preceded by estrus, fertility in beef cows is lower than in first normal length or subsequent estrous cycle (Ramirez-Godinez *et al.*, 1981; Breuel *et al.*, 1993). Fertilized oocytes in different stages of development were recovered from oviduct and uterus (on Days 3 and 6 after first estrus, respectively) from cows with a short-lived CL (Ramirez-Godinez *et al.*, 1982; Breuel *et al.*, 1993; Schrick *et al.*, 1993). The failure to sustain pregnancy was presumed to be due to early luteal regression, as indicated by serum concentrations of P₄ (Ramirez-Godinez *et al.*, 1982). Surprisingly, neither daily provision of melengestrol acetate nor twice daily injection of P₄ from Day 4 to 35 after estrus, maintained pregnancy in cows with short-lived CL (Breuel *et al.*, 1993). Thus, premature regression of the CL was not the only factor responsible for the low fertility associated with the short luteal phase.

Two good-quality embryos were transferred on Day 7 into cows induced to have short or normal luteal phases and supplemented with daily doses of P₄ from day 4 to 35. Pregnancy rates were 28% and 56% for short and normal luteal phases, respectively (Butcher *et al.*, 1991). In a later study, uterine horns ipsilateral to the CL from cows induced to have short or normal luteal phases were flushed non-surgically and embryos were transferred into recipient cows with normal estrous cycles (Schrick *et al.*, 1993). Quality and stage of development of embryos on Day 6 did not differ among groups. Pregnancy rate did not differ after embryos were transferred into cycling recipients (50 vs 72% for embryos from short and normal cycles, respectively). However, on the

basis of donor animals, only 23% of recovered embryos from short cycles resulted in ultimately successful pregnancies compared to 46% from normal cycles. Apparently, both detrimental factors that affected the oocytes or embryos before Day 7 and an improper uterine environment after Day 7 could contribute to early embryonic death (Butcher *et al.*, 1991; Schrick *et al.*, 1993).

Embryotoxic effect of prostaglandin F_{2α}

Inskeep (1995) stated that subluteolytic concentrations of PGF_{2α} could play a role in embryonic death during early development via release of an embryotoxin from the CL. In characterizing such embryotoxic factors, Schrick *et al.* (1993) found concentrations of PGF_{2α} in uterine flushings to be greater in cows with short than normal luteal phases on Day 6; the most relevant finding was that the more PGF_{2α} in the uterine fluid the lower the quality of the embryos ($r = -0.42$; $P < 0.07$). Therefore, a detrimental uterine environment appeared to be a major cause of low fertility in cows with short luteal phases after first ovulation (Schrick *et al.*, 1993).

A series of experiments was designed to study the role of the PGF_{2α} in reducing fertility in cows with short luteal phases. Cycling cows were injected every 8 h with saline (control), PGF_{2α} or flunixin meglumine (an inhibitor of prostaglandin endoperoxide synthetase), on Days 4 through 7 or

5 through 8, and supplemented with exogenous P4 twice daily from Day 3 until pregnancy diagnosis was performed. In one group of PGF_{2α}-treated cows, the CL was removed on day 7. As expected, pregnancy rate was lower in cows treated with PGF_{2α} compared with those that received saline or flunixin meglumine (Figure 4), but surprisingly, pregnancy was restored to normal when treatment with PGF_{2α} was accompanied by lutectomy (Buford *et al.*, 1996).

Cows induced to have short estrous cycles by weaning at 25 to 31 d postpartum were treated with saline (S), flunixin meglumine (F), flunixin meglumine plus lutectomy or lutectomy only (L), on Days 4 through 9, and supplemented with exogenous progesterone twice daily from Day 3 until pregnancy diagnosis. Pregnancy rate did not differ among S, F and L groups ($< 21\%$). However, pregnancy rate was higher (50%; Figure 4) when lutectomized cows were treated with flunixin meglumine (Buford *et al.*, 1996). That treatment would have reduced secretion of PGF_{2α}. Moreover, in ewes treated with PGF_{2α} on Days 4 through 7 after mating and supplemented with an intravaginal progestogen, lutectomy increased pregnancy rate (59%) compared with sham lutectomy (33%; Costine *et al.*, 2001).

In summary, lutectomy and the inhibition of prostaglandin synthetase prevented the negative

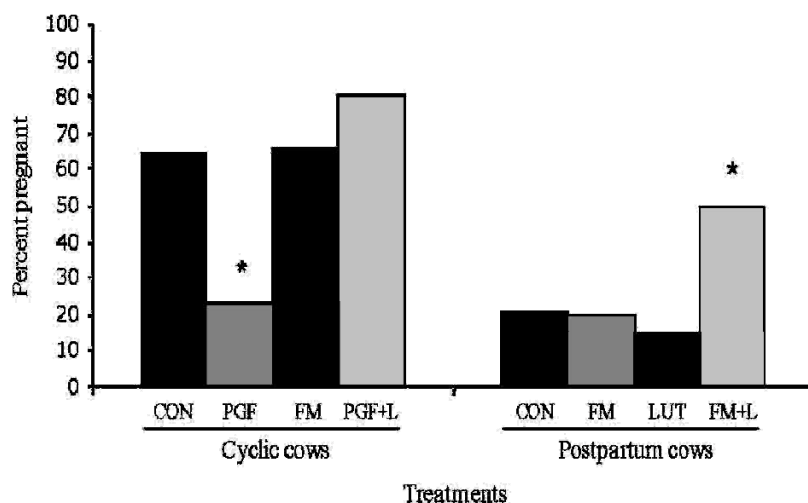


Figure 4. Pregnancy rates in cyclic (left) and postpartum (right) beef cows at 30 d of induced estrus. Exogenous progesterone was injected twice daily from Day 3 or 4 until pregnancy diagnosis was performed. Cows were mated and injected every 8 h with saline (CON), prostaglandin F_{2α} (PGF), flunixin meglumine (FM), and PGF and lutectomy (PGF+L) on Days 4 through 7 or 5 through 8 postestrus (cyclic cows); or saline, FM, L, and FM+L on Days 4 through 9 postestrus (postpartum cows). Treatment differed ($P < 0.05$) within type of cow if an asterisk is present (adapted from Buford *et al.*, 1996 with permission).

effects of endogenous PGF_{2α} on the early embryonic stage in postpartum cows (Buford *et al.*, 1996), whereas lutectomy alone was effective in nonlactating cycling cows (Buford *et al.*, 1996), and ewes (Costine *et al.*, 2001) treated with exogenous PGF_{2α}. Therefore, the lower embryo survival in postpartum cows appeared to be due to the combined effect of uterine PGF_{2α} secreted on Days 4 through 9 (Cooper *et al.*, 1991) and the luteal secretion of PGF_{2α} in response to the uterine signal (Buford *et al.*, 1996).

In subsequent studies pregnancy rate was lowered by daily dosage with PGF_{2α}, on Days 5 through 8 (23%) but not on Days 10 to 13 (50%) or 15 to 18 (60%) after mating of P₄-supplemented cows (Seals *et al.*, 1998). Treatment of P₄-supplemented cows with PGF_{2α} on Day 5 through 8 reduced quality and retarded development of embryos (Hockett *et al.*, 2004). Also, PGF_{2α} *in vitro* reduced the ability of 26 to 32-cell bovine embryos to develop to blastocysts (Scenna *et al.*, 2004). Therefore, PGF_{2α} may interfere with the process of compaction of the embryo at the morula stage (Hockett *et al.*, 2004), probably by alterations in gap junctions and cell adhesion molecules, induction of apoptosis mechanisms or alterations of gene transcription during embryonic development (Scenna *et al.*, 2004).

In order to test whether the luteal source of the embryotoxin was delivered by a systemic or a local pathway, beef heifers and cows received one good quality embryo into each uterine horn on Day 6 or 7 of the estrous cycle, and P₄ replacement therapy from transfer through Day 35 (Hernandez-Fonseca *et al.*, 2000). Each female was injected with three daily therapeutic doses of PGF_{2α} or saline from Day 5 through 8. Survival of embryos placed into the uterine horn adjacent or opposite to the CL was not different and no evidence was found to support the existence of a local embryotoxic effect originated from the regressing CL. Therefore, embryotoxicity probably involved compounds that either act systemically or were transported via the uterine lumen to the uterine horn contralateral to regressing CL (Hernandez-Fonseca *et al.*, 2000). In a similar experiment in sheep, the embryotoxic effect of the regressing CL also was demonstrated; again, there was evidence that the effect occurred through a systemic pathway (Costine *et al.*, 2001).

Is oxytocin involved as an embryotoxic component of the regressing corpus luteum?

At the time of Inskeep's review in 1995, oxytocin was included in the list of factors that could play a role in embryonic death in cows with short luteal phases. As was previously pointed out, luteal oxytocin and uterine prostaglandin were believed to participate in luteolysis through a reciprocal positive feedback mechanism (McCracken *et al.*, 1984). Concentrations of endometrial oxytocin receptors were greater at Day 5 after estrus in cows with short lifespan of the CL than in cows receiving P₄ pretreatment and expected to have a normal luteal phase (Zollers *et al.*, 1993). Greater concentrations of oxytocin were evident 30 minutes after first injection of PGF_{2α} in P₄-supplemented cows treated three times daily with PGF_{2α} on Day 5 to 8 after estrus compared with cows injected with PGF_{2α} and lutectomized (Buford *et al.*, 1996). Lemaster *et al.* (1999) tested the possibility of a role for oxytocin. Administration of oxytocin on Day 5 through 8 decreased the embryonic survival in progesterone-supplemented beef (pregnancy rate: 33.3%) even when it was accompanied by lutectomy (30%). However, pregnancy rate was restored to normal (80%) if oxytocin-treated cows also received a prostaglandin synthesis inhibitor. Therefore, the oxytocin acted through stimulation of uterine secretion of PGF_{2α}. Given that a regressing CL was required to manifest the negative effect of PGF_{2α} on embryo survival (Buford *et al.*, 1996; Seals *et al.*, 1998), it could be concluded removal of luteal tissue reduced the lethal effect by interruption of the modulatory effect of oxytocin on uterine PGF_{2α} secretion (Lemaster *et al.*, 1999) and by abolition of PGF_{2α} secreted from CL once luteolysis was initiated (Rexroad and Guthrie, 1979). Therefore, a direct embryotoxic effect of oxytocin on embryo development is improbable.

Niswender *et al.* (2007) summarized evidence that luteal PGF_{2α} is involved in luteolysis in sheep. Once the uterine signal (PGF_{2α}) initiates increased luteal secretion of PGF_{2α}, it triggers mobilization of Ca⁺⁺ in large luteal cells and secretion of oxytocin by these cells. Oxytocin in turn, triggers mobilization of Ca⁺⁺ in small luteal cells. Apoptosis follows Ca⁺⁺ mobilization in both cell types. Thus, secretions from the CL are involved in its demise, acting within the CL itself.

As conclusion, Figure 5 indicates the sequence of endocrine events conducive to embryonic death or survival in short and normal estrous cycles.

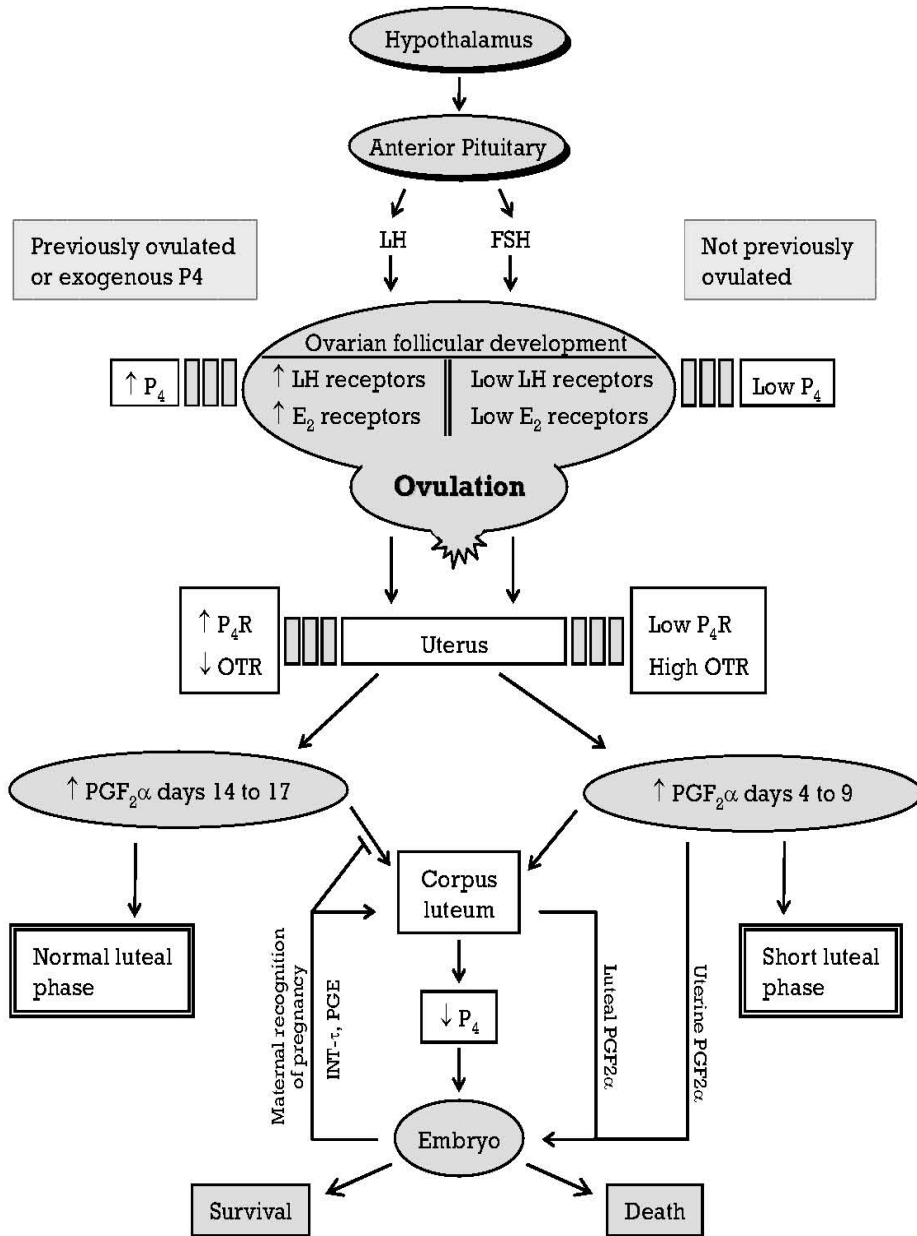


Figure 5. Sequence of endocrine events conducive to embryonic death or survival in short and normal estrous cycles. After parturition, the hypothalamic-pituitary axis stimulates progressively ovarian follicular development, estradiol production and expression of LH receptors in the follicle. Without a previous cycle or exposure to P₄ followed by estrogen (E₂), uterine P₄ receptors (P₄R) are low and the increase in P₄ after ovulation initiates immediate secretion of uterine prostaglandin (PG) F₂α which causes luteolysis and, if the animal has been mated, is embryotoxic. Exposure of the uterus to P₄ followed by E₂ prior to first postpartum ovulation up regulates uterine P₄R and contributes to establishment of P₄ dominance of the uterus. As the estrual cycle progresses, P₄ binding to its receptors delays uterine secretion of PGF₂α. If the animal is not mated/pregnant luteolysis is initiated by uterine secretion of PGF₂α that starts around Day 14 to 17 and decreases P₄ production. If pregnant, the embryo produces interferon-τ (INT-τ), the uterus increases secretion of PGE and the corpus luteum is maintained allowing the embryo to survive. This process is called maternal recognition of pregnancy.

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