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Factors that affect fertility during oestrous cycles with short or normal luteal phases in postpartum cows

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We have used a model to study infertility in postpartum cows. In mated cows with short luteal phases, daily supplementation with progestagen, beginning on day 3, failed to maintain pregnancy, despite the fact that fertilization, early embryo development and transport of the embryo into the uterus appeared to be normal. Normal embryos were transferred, on day 7 after oestrus, into cows with short luteal phases that received daily supplementation with progestagen, and embryos from cows with short luteal phases were transferred, on day 6 after mating, into cows with normal cycles. Pregnancy rates in each case were about half those achieved using progestagen-pretreated cows with normal luteal phases. In mated cows with short luteal phases supplemented with progestagen, pregnancy rates were markedly improved by removal of the regressing corpus luteum on day 4 or 5 after mating. In cows with normal cycles, fertility was lower when preovulatory concentrations of oestradiol were high for more than 3 days. This may result from persistent follicles, which develop when progesterone is low and frequency of tonic pulses of LH is high. In cows with such follicles, oocytes may resume maturation before the surge of LH, and embryos died in the oviduct before the 16-cell stage. Finally, in both early postpartum cows with transferred embryos and cows with normal cycles, excessive follicular development and high oestradiol during the luteal phase were detrimental to embryo survival. Treatments designed to maximize fertility in early postpartum cows must provide high progesterone and low oestradiol before mating and not yield luteolytic influences such as $PGF_{2\alpha}$ and oestradiol, both early after mating and during maternal recognition of pregnancy.

Introduction

During the postpartum period, cows undergo a transition from an anovulatory, anoestrous, infertile animals into animals with normal ovulatory oestrous cycles and a high conception rate. Asdell (1953) listed "... breeding back too soon after calving" as a fault of management contributing to infertility. Casida *et al.* (1968) summarized results of their own and earlier studies in dairy and beef cows in which increasing fertility was observed with increasing interval after birth. Factors identified as causes of reduced fertility included ovulation adjacent to the previously pregnant uterine horn and diameter of that horn, as well as failure of ovulation at first oestrus in 10.5% of calving intervals. Ovulation without observed oestrus was detected in 46.5% of calving intervals. Fertility in dairy cows was not affected by genetic propensity for milk production, or by level of feeding concentrates (high versus average).

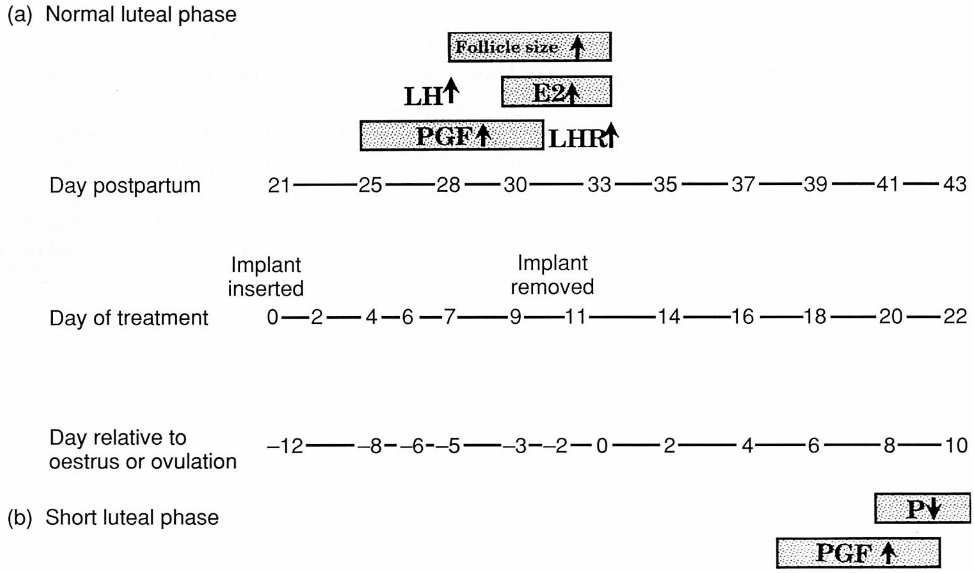

Fig. 1. Differences in the patterns of secretion of oestradiol (E2), LH, progesterone (P) and prostaglandin $F_{2\alpha}$ (PGF), follicle size and the presence of LH receptors (LHR) in postpartum cows induced to ovulate by weaning calves on day 28 postpartum and 2 days before removal of the norgestomet implant. The cows were divided into two groups and treated for 9 days with (a) a norgestomet implant or (b) untreated.

Conception rates in beef cows did not differ if fertility was measured at 3 days (fertilization) or 38–44 days (early fetal) after insemination at several different intervals after birth. This observation led Casida *et al.* (1968) to conclude that embryo death was a minimal contributor to early postpartum infertility in beef cows. However, if their data from slaughter at 3 days after insemination are combined over all postpartum intervals examined, fertilization rate was 52%, whereas pregnancy rate for cows slaughtered at 15 days or palpated at 38–44 days was 35%. Thus, estimated embryonic death would be 17% or nearly 33% of the embryos present at day 3 after insemination.

In this review, the physiological points at which fertility is compromised during the postpartum transition, specifically after cyclic occurrence of ovulation has been restored, will be discussed. Data from specific models designed to determine roles of selected endocrine events will be emphasized. Special attention will be given to factors that limit fertility during the first ovulatory and oestrous cycles and to the negative effects of persistent follicles and high preovulatory oestrogens. In addition, attention will be directed to the fact that high concentrations of oestrogens may compromise fertility during the period of maternal recognition of pregnancy.

The Short Luteal Phase: Role in Return to Normal Oestrous Cycles

The occurrence of a short luteal phase following first ovulation or first oestrus was first noted by Menge *et al.* (1962) and Morrow *et al.* (1966). With subsequent recognition that short-lived luteal structures were common to pubertal heifers and ewe lambs, the postpartum ewe and cow returning to ovulatory activity and the ewe returning from seasonal anoestrus, studies of the occurrence, regulation and effects of short luteal phases were begun. By 1985, sufficient data were available to permit extensive reviews by Lauderdale (1986) and by Garverick and Smith (1986).

A series of studies was conducted in which variables such as follicular development, pre- and post-ovulatory concentrations of gonadotrophins and luteal receptors for LH were shown to be responsible for some variations in luteal function (reviewed by Lishman and Inskeep, 1991; see Fig. 1), but not for its duration. Copelin *et al.* (1989) Peter *et al.* (1989) and Cooper *et al.* (1991) showed clearly that premature uterine secretion of $PGF_{2\alpha}$ was responsible for the short luteal phase (Fig. 1).

Several groups, beginning with Ramirez-Godinez *et al.* (1981), observed that pretreatment with a progestagen usually resulted in formation of a corpus luteum with a normal functional lifespan, in response to weaning or injection of gonadotrophins. Cooper *et al.* (1991) showed that secretion of $PGF_{2\alpha}$ rose during such progestagen treatment in the same manner as during a short luteal phase after injection of hCG in control cows (Fig. 1). Thus, it was deduced that the uterus that has not been exposed to progestagen increases secretion of $PGF_{2\alpha}$ prematurely in response to progesterone secreted by the first corpus luteum. Zollers *et al.* (1993) demonstrated that pretreatment with progestagen increased numbers of progesterone receptors in the uterus on day 5 after oestrus.

Secretion of and exposure to $PGF_{2\alpha}$ *per se*, before formation of the corpus luteum, does not appear to be an important component of the response. The lifespan of hCG-induced corpora lutea was not affected by either injection of $PGF_{2\alpha}$ in control cows or by blocking secretion of $PGF_{2\alpha}$ with flunixin meglumine in progestagen-pretreated cows, during the preovulatory follicular phase (Johnson *et al.*, 1992).

Examination of Factors Limiting Fertility in Cows with Short Luteal Phases

Johnson *et al.* (1992) observed that the secretion of $PGF_{2\alpha}$ stimulated by treatment with progestagen did not affect development of the ovulatory follicle before induced formation of corpora lutea. Earlier workers (Casida *et al.*, 1968; Ramirez-Godinez *et al.*, 1982) obtained evidence that ovulation and fertilization occurred at the expected time after oestrus preceding a short luteal phase in early weaned cows. Perhaps the only mechanism through which fertility was improved by pretreatment of the postpartum cow with progestagen was by preventing the shortened luteal phase. Bellows *et al.* (1974) showed that beef cows from which calves were weaned at about 35 days after birth would consistently exhibit oestrus in about 4 days and form corpora lutea. Thus the question of where fertility fails in the postpartum cow was approached in a model in beef cows (Fig. 2) in which calves were weaned at about 30 days after birth and half of the cows received progestagen treatment (6 mg norgestomet implants for 9 days, ending 2 days after the early weaning on day 7). With this model, control cows that had not formed corpora lutea before their calves were weaned were expected to have short luteal phases/oestrous cycles in all cases and cows pretreated with progestagen were expected to have normal luteal phases/oestrous cycles in an average of at least 80% of cases (Figs 1 and 2). Cows in both groups were at the same stage after birth when studied.

In the first experiment to examine factors affecting fertility with the short versus normal oestrous cycle model, Breuel *et al.* (1993a) removed and flushed the oviducts from cows in each group at day 3 after breeding. They found that 100% of the cows had formed corpora lutea, and that recovery of oocytes/embryos (86%), fertilization (68%), development of fertilized oocytes to the four- to eight-cell stage and embryo quality did not differ between cows with short and normal luteal phases. These data confirmed the early observations of Casida *et al.* (1968) and Ramirez-Godinez *et al.* (1982) that fertilization could occur after early weaning in beef cows. When uteri were flushed non-surgically on day 6, recovery of oocytes/embryos (79%), fertilization rate (82%) and development to at least the four-cell stage (90%) again did not differ (Breuel *et al.*, 1993a). Thus, it was concluded that losses of embryos must be related to early luteal regression, so that supplemental treatment with progestagen should maintain the pregnancy.

When this idea was tested by providing a daily supplement of melengestrol acetate (MGA) in feed, beginning on day 4 after mating (Breuel *et al.*, 1993a), two significant observations were made. First, no pregnancies were maintained in cows with short luteal phases, whereas 41% of all norgestomet-pretreated cows and 50% of those with normal luteal phases maintained pregnancy, regardless of whether they received MGA. Second, when cows with normal luteal phases, regardless of treatment, were examined separately, those with larger preovulatory follicles 5 days before the surge of LH (12.9 ± 0.9 mm) had a lower conception rate (36%) and higher preovulatory concentrations of oestradiol than did those with smaller follicles (7.5 ± 1.8 mm), which averaged 91% conception. No pregnancies

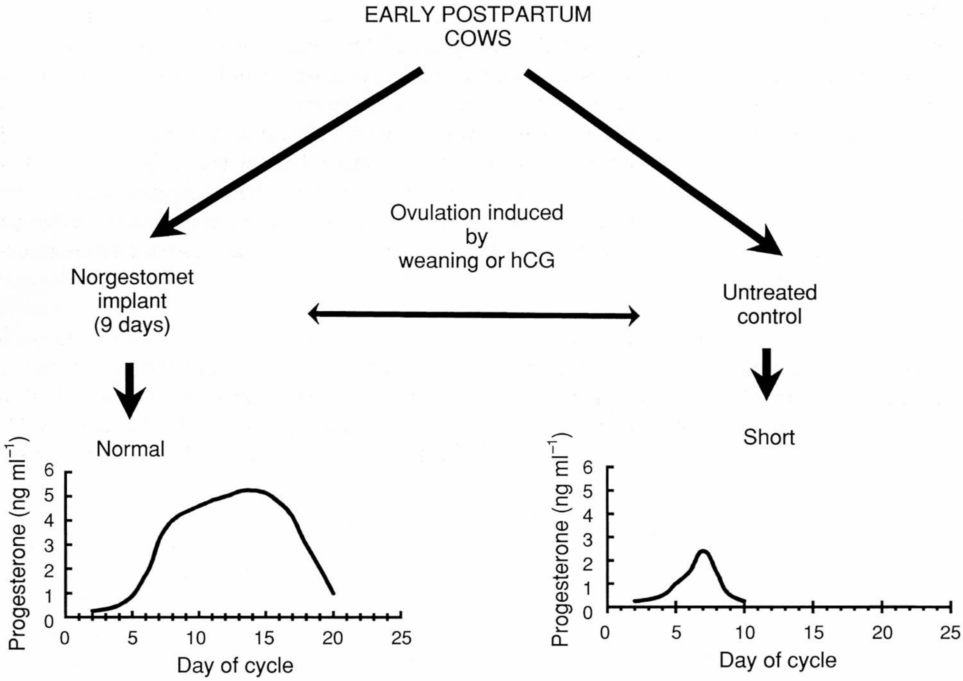

Fig. 2. Experimental model used to compare factors that might affect fertility during normal and short oestrous cycles in postpartum beef cows. Cows were either treated with norgestomet or not treated, then induced to ovulate, by weaning their calves at an average of day 28 postpartum (2 days before removal of the norgestomet implant) or, in some studies of hormonal patterns, by treatment with hCG 2 days after removal of the norgestomet implant. Profiles of progesterone depicted in the inset graphs show that pretreatment with norgestomet was followed by a normal luteal phase (Normal), while untreated cows formed a corpus luteum that regressed prematurely (Short). .

occurred in cows with short-lived corpora lutea when the study was repeated in control cows given 200 mg progesterone daily as the supplemental progestagen instead of MGA. In both experiments, control cows that had shown a spontaneous short luteal phase before mating at postweaning oestrus had a high conception rate (11 of 13 cows) even though they were mated at an average of only 33 days post partum.

Embryo Transfer as a Method to Determine Time of Embryo Death

Two experiments involving embryo transfer were performed to determine whether oocytes in cows that showed short luteal phases were inherently defective or whether the uteri of such cows were hostile to embryo survival. First, two good quality frozen–thawed embryos were transferred on day 7 into the uteri of early postpartum cows expected to have short (control) or normal (norgestomet pretreated) luteal phases. All cows received 200 mg supplemental progesterone per day, subcutaneously, beginning on day 4 after oestrus. Pregnancies were maintained in 28% of control cows compared with 58% of norgestomet-pretreated cows (Butcher *et al.*, 1992). Second, oocytes/embryos were flushed from the uteri of control and norgestomet-pretreated cows on day 6 after mating, and, if viable, transferred into the uteri of non-lactating, cyclic recipients on day 6. Survival rates for embryos that were considered fit to transfer did not differ with source (50% and 73% for cows with short and normal luteal phases, respectively; Schrick *et al.*, 1993). However, pregnancy rate, determined as the number of recipients pregnant divided by the number of experimental cows from which an embryo or oocyte was recovered on day 6, was 13% for cows with a short luteal phase compared with 32% for cows with a normal luteal phase. Likewise, embryonic survival for all fertilized oocytes on day 6 was 23% for cows with a short luteal phase and 47% for cows with a normal luteal phase. The values for cows with a normal luteal phase are comparable to pregnancy rates obtained in early weaned, mated cows with normal luteal phases (33%, Ramirez-Godinez *et al.*, 1981; 50%, Breuel *et al.*, 1993a).

The combined results of Butcher *et al.* (1992), Breuel *et al.* (1993a) and Schrick *et al.* (1993) are summarized in Fig. 3. From these data, it is concluded that about half of the difference in ability to maintain pregnancy between cows with short and normal luteal phases (when supplemental progestagen was provided) can be attributed to events that affect the oocyte or embryo before day 7 after oestrus and the other half to a hostile uterine environment on or after day 7. In anoestrous ewes induced to ovulate at 21 to 35 days post partum, Wallace *et al.* (1989a, b) obtained results similar to those in the above studies in cows.

Although negative effects that led to embryonic losses before day 7 could have occurred while the oocyte was still in the follicle (Butcher and Page, 1981) or during transport through the oviduct, there was a striking similarity between the apparent timing of embryo loss in cows with short luteal phases and the timing of increased uterine secretion of $PGF_{2\alpha}$ on days 4 through to day 9 after oestrus (Cooper *et al.*, 1991), which had been shown to be responsible for the short luteal phase. Moreover, Schrick *et al.* (1993) observed that concentrations of $PGF_{2\alpha}$ in flushings from the uterine lumen were more than twice as high in cows with short luteal phases as in those with normal luteal phases (636 ± 82 and 288 ± 90 pg ml^{-1}, respectively). Embryo quality tended to be correlated negatively (-0.42) with concentrations of uterine luminal $PGF_{2\alpha}$. Because embryo quality was lower on day 6 (Schrick *et al.*, 1993) than on day 3 (Breuel *et al.*, 1993a), it was proposed that the specific problem in short luteal phase cows was likely to have occurred after the embryo entered the uterus. A direct embryotoxic effect of $PGF_{2\alpha}$ seemed possible since that had been shown for rabbit (Maurer and Beier, 1976) and rat (Breuel *et al.*, 1993b) embryos *in vitro*.

Does $PGF_{2\alpha}$ Cause Death of Bovine Embryos?

Three experiments were conducted to examine the effects of $PGF_{2\alpha}$ on embryo survival in cows in which daily progestagen supplements were provided to replace the regressed corpora lutea. The first was a preliminary experiment (Buford *et al.*, 1994) to test whether either $PGF_{2\alpha}$ or the prostaglandin endoperoxide synthase inhibitor flunixin meglumine was detrimental to embryos when given to normally cyclic beef cows during days 4–7 after oestrus. This period corresponded to a major portion of the interval during which high embryo mortality had been observed in cows with short luteal phases. Non-lactating beef cows were mated to bulls of known high fertility and artificially inseminated (AI) 12 h later to a high fertility bull to maximize fertility. All cows were supplemented with progesterone (300 mg day^{-1} s.c. in corn oil) from day 3.5 to day 30 after oestrus. They were allocated randomly to three groups and received intramuscular injections three times a day (06:00 h, 14:00 h and 22:00 h) of either 3 ml saline, 1 g flunixin meglumine, or 15 mg $PGF_{2\alpha}$ on days 4 to 7. Pregnancy rates at ultrasonography on day 30 were 75%, 67% and 0%, respectively. Therefore, flunixin meglumine was considered safe to use in the next experiment and further evidence was obtained that $PGF_{2\alpha}$ was somehow detrimental to the embryo.

The second experiment (F. N. Schrick, R. L. Butcher and E. K. Inskeep, 1993, unpublished) was conducted in postpartum beef cows expected to have short luteal phases. The purpose was to test whether embryonic survival would be improved when the rise in $PGF_{2\alpha}$ which caused luteal regression was reduced by treatment with flunixin meglumine. A second question was whether luteal maintenance *per se* was important to any effect of the flunixin meglumine. Calves were weaned at about 28 days after birth to induce oestrous behaviour and ovulation with expected short luteal phases. Cows were mated at observed oestrus by natural service and 12 h later by AI to bulls of high fertility. All cows received 300 mg progesterone day^{-1} in corn oil (s.c.) from day 3.5 after mating until pregnancy determination at day 30. Cows were allotted at random within calving date among three treatments: saline ($n = 19$), flunixin meglumine ($n = 15$), and flunixin meglumine plus lutectomy ($n = 15$). Flunixin meglumine, 1 g, was given every 8 h on days 4–9 and lutectomy was performed on day 7. Pregnancy rates were 21, 27

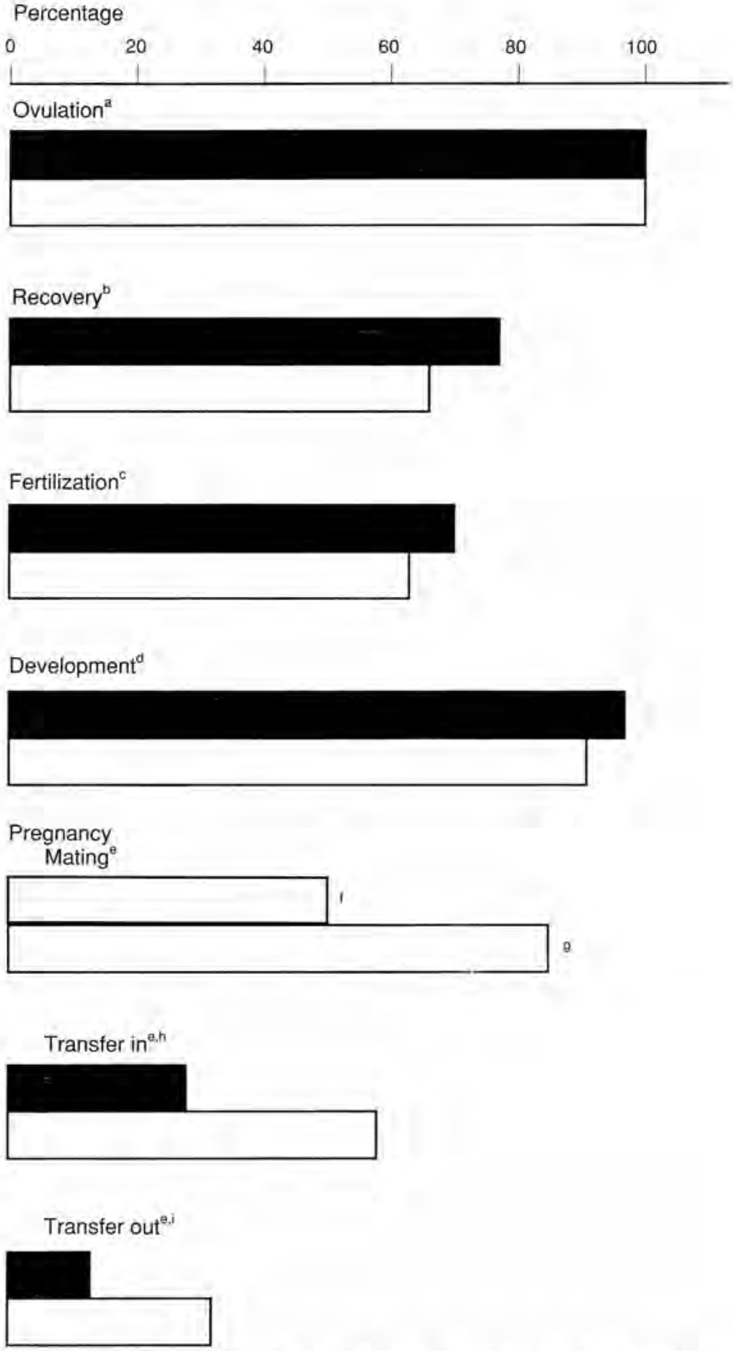

Fig. 3. Summary of comparisons of components of fertility in early postpartum beef cows. Values for each variable (percentage) as defined below. Solid bars depict results for cows with short luteal phases and open bars depict results for cows with normal luteal phases (as a result of norgestomet pretreatment except as noted in g below). [a] Of cows in oestrus after calves were weaned at 24–30 days post partum. [b] Of an oocyte or embryo, from cows flushed without technical difficulties on day 3 (oviduct) or 6 (uterus). [c] Of total oocytes recovered. [d] Beyond four-cell stage, of fertilized oocytes. [e] With supplemental progestagen, beginning on day 4 after oestrus. [f] With norgestomet pretreatment for 9 days. [g] With prior spontaneous luteal phase. [h] Two good quality frozen embryos into experimental cows on day 7 after oestrus. [i] Into nonlactating recipients after recovery from the uterus of experimental cows on day 6 after oestrus.

and 53%, respectively. The unexpected finding was that pregnancy rate was increased by lutectomy, thus indicating that the regressing or partially regressing corpus luteum (the corpus luteum was maintained to some degree to at least day 15 in 9 of 15 intact cows treated with flunixin meglumine) was a component of the embryotoxic effect of $PGF_{2\alpha}$.

A third experiment was performed in nonlactating cyclic cows to confirm that the corpus luteum was required for the embryotoxic effect of $PGF_{2\alpha}$ in the early period after mating, as indicated by data from cows expected to have short luteal phases (Buford *et al.*, 1994). It was designed to confirm the results of the first experiment, that $PGF_{2\alpha}$ reduced pregnancy rate, and to test whether lutectomy could abrogate this effect. Twenty-eight nonlactating beef cows were mated, supplemented with progestagen as before and assigned at random to three groups. Cows in group 1 ($n = 9$) received saline, while those in groups 2 ($n = 9$) and 3 ($n = 10$) received 15 mg $PGF_{2\alpha}$, every 8 h on days 5–8 after oestrus. In group 3, the cows were lutectomized on day 5. Pregnancy rates were 67, 22 and 80%, respectively, at ultrasonography on day 30. Thus, lutectomy prevented the negative effects of $PGF_{2\alpha}$ on the early embryo in both early postpartum cows (endogenous $PGF_{2\alpha}$) and nonlactating, cyclic cows (exogenous $PGF_{2\alpha}$). On the basis of data from groups 2 and 3, the lower embryo survival during luteolysis was not due to increased concentrations of $PGF_{2\alpha}$ *per se* (both groups received exogenous $PGF_{2\alpha}$). Rather, because lutectomy removed the effect, lower embryo survival appeared to be brought about by the regressing corpus luteum. From these data, it seems possible that even subluteolytic concentrations of $PGF_{2\alpha}$ (Schramm *et al.*, 1983) could play a role in embryonic loss during this early period of development via release of an embryotoxin from the corpus luteum.

Possible Mechanisms for Products of Complete or Partial Luteal Regression to Cause Embryonic Death

After oestrus, concentrations of $PGF_{2\alpha}$ fall to basal values with slight increases on day 5 (as determined by concentrations of 15-keto,13,14-dihydro-$PGF_{2\alpha}$), which were associated with metoestrous bleeding in cows (Kindahl *et al.*, 1976). Schallenberger *et al.* (1989) observed an increase in concentrations of $PGF_{2\alpha}$ until day 6 after oestrus following artificial insemination. In the light of these data and the findings in postpartum cows, the effect of naturally occurring early increases in concentrations of $PGF_{2\alpha}$ on development of the embryo and establishment of pregnancy must be determined in cyclic cows. Secretion of $PGF_{2\alpha}$ may be particularly important during cycles that are of normal duration but have lowered patterns of secretion of progesterone (see Lishman and Inskeep, 1991, for review). Moreover, Ayalon (1978) reported that most embryonic mortality in subfertile dairy cows occurred 6–7 days after oestrus, when the morula was developing into the blastocyst. Maurer and Chenault (1983) observed that 67% of embryonic mortality had occurred or was occurring by day 8 of gestation in beef cows.

There are many possible products of luteolysis or partial luteolysis that could play a role in embryo loss. One of these is oxytocin, which is released by $PGF_{2\alpha}$ (Schallenberger *et al.*, 1984) and can also release $PGF_{2\alpha}$ (Newcomb *et al.*, 1977) during the bovine oestrous cycle. Involvement of luteal prostaglandins is worth further evaluation; Hu *et al.* (1990) observed that corpora lutea that were destined to be short-lived produced more prostaglandins *in vitro*, particularly $PGF_{2\alpha}$, than did normal corpora lutea. In the study by Buford *et al.* (1994), plasma oxytocin was increased by treatment with $PGF_{2\alpha}$ and that increase was abrogated by lutectomy, but neither replacement of oxytocin in lutectomized animals nor direct treatment with oxytocin during days 4–7 has been examined in that model. Oxytocin provided for the first 12–14 days of pregnancy in ewes reduced pregnancy rate, but in some cases of pregnancy loss, luteal function was maintained (Wathes *et al.*, 1991). Exogenous oxytocin injected for the first 14 days of pregnancy resulted in only 18 of 43 cows maintaining normal embryos to day 15 in comparison with 74% of control animals (Staples and Hansel, 1961). The remaining oxytocin-treated cows either had regressed corpora lutea or had normal corpora lutea without the presence of an embryo. None of the animals in these studies was provided with exogenous progestagen, so it was not possible to determine whether death of the embryo occurred before or after luteal regression.

Embryo Loss During Oestrous Cycles of Normal Duration

The topic of embryonic mortality was recently reviewed by Thatcher *et al.* (1994). The purpose here is to examine only a few specific aspects that relate to postpartum cows.

In the study by Breuel *et al.* (1993a) reviewed above, it was noted that in early-weaned cows with normal cycles, fertility was lower when preovulatory follicles were larger 5 days before the ovulatory surge of LH (36%, compared with 91% for cows with smaller follicles), and that the larger follicles secreted high concentrations of oestradiol. On the basis of many reports (for example, Sirois and Fortune, 1990; Thatcher *et al.*, 1994), the norgestomet implants used routinely in postpartum cows to initiate a normal luteal phase probably allow the development of persistent follicles in many cases. From both older (Wishart, 1977) and recent (Ahmad *et al.*, 1994) data, it appears that embryonic losses in animals with persistent follicles occur during the early cleavage stages, when zygotes are still in the oviduct. In fact, the oocytes, although still fertilizable at a normal rate, probably have begun maturation and experienced germinal vesicle breakdown while still in the follicles (Mihm *et al.*, 1994; I. Revah and W. R. Butler, 1994, personal communication). Whether these changes result from stimulation by more frequent pulses of LH during periods of low progestagen (Ireland and Roche, 1982 and subsequent studies from several groups), by higher secretion of oestradiol from the persistent follicle (Sirois and Fortune, 1990 and several other authors), or by both LH and oestradiol, remains to be determined.

On the basis of these findings, treatments designed for induction of oestrus and ovulation with progestagen must either use sufficiently high doses, or include some other treatment that will assure follicular turnover, if high fertility is to be achieved. Such a treatment could be either an additional injection of progesterone late in the treatment period (Anderson and Day, 1994), an injection of oestrogen early during the regimen of progestagen (Bo *et al.*, 1994; Vasoconcelos *et al.*, 1994) or treatment with a GnRH agonist (Schmitt *et al.*, 1994). The oestrogen would have to be provided in a short-acting preparation. Follicular turnover occurs with the Synchro-Mate B system, but McGuire *et al.* (1990) have shown that sufficient oestrogen from the initial injection of oestradiol valerate remains after implant removal 9 days later to induce oestrus in ovariectomized animals. Such high oestrogen could jeopardize viability of oocytes in intact animals.

Effects of Oestrogens After Mating

Ayalon (1978) reviewed data on profiles of steroids during the early luteal phase in pregnant and nonpregnant cows and reported that concentrations of oestrogen in plasma were higher on the day of oestrus and on days 3 and 4 after insemination in cows with degenerating embryos than in cows with normal embryos. Continuous infusions or twice daily injections of $PGF_{2\alpha}$ increased the size and number of large follicles in postpartum cows (Villeneuve *et al.*, 1988). Villeneuve (1990) found increased concentrations of oestradiol after treatment of early postpartum cows with $PGF_{2\alpha}$. Therefore, the increase in $PGF_{2\alpha}$ from day 4 to day 9 after oestrus may increase concentrations of oestradiol in serum during that period.

Higher oestradiol concentrations may be expected to have a detrimental effect on the embryo during and after the period of maternal recognition of pregnancy. Administration of agonists of GnRH 11–13 days after oestrus, which resulted in follicular atresia, increased pregnancy rates of cows inseminated at the previous oestrus (Macmillan *et al.*, 1986). When inadequate amounts of exogenous progestagen were provided to postpartum cows with transferred embryos, Butcher *et al.* (1992) observed increased serum oestradiol and failure to maintain pregnancy. Lower doses of progesterone were required to maintain pregnancy in bilaterally ovariectomized heifers than in unilaterally ovariectomized heifers with the corpus luteum removed (McDonald *et al.*, 1952). Likewise, Zimbelman and Smith (1966) reported that 1 mg MGA each day was adequate to maintain pregnancy when both ovaries were removed at day 56 of gestation, but failed when one ovary without a corpus luteum remained. They concluded that follicular size and oestrogen production increased during treatment with MGA in the absence of a corpus luteum and that the increased oestrogenic activity of the remaining ovary may have caused the fetal loss. In rabbits, injections of oestradiol from day 6 to day 11 after mating resulted in 100% fetal death, which could not be reversed by simultaneous injections of progesterone, even though abortion was prevented (Stormshak and Casida, 1965).

In lactating, cyclic beef cows, Pritchard *et al.* (1994) examined the effects on conception rate to first service of mean concentrations, slopes (linear regressions on day) and ratios of oestradiol and progesterone during days 4–7 and 14–17 after AI at synchronized oestrus. They used concentrations of steroids or regressions as independent variables, dividing each variable into three classes, defined as the upper 25%, the middle half and the lower 25% of values. Days 4–7 were chosen because that is the period during which rising concentrations of progesterone set up the timing for luteolytic secretion of $PGF_{2\alpha}$ by the uterus beginning on days 14–17 (Garrett *et al.*, 1988). Days 14–17 were chosen because they are critical for the maternal recognition of pregnancy (Thatcher *et al.*, 1994). Conception rates decreased linearly as relative mean concentrations of oestradiol increased on days 14–17, but were not affected by any of the other hormonal variables during either period. This appeared to be an independent effect of oestradiol, because conception rate was not affected by the ratio of oestradiol to progesterone and retrospective analysis did not detect any effects of pregnancy on concentrations of oestradiol. On the basis of these results, using a method to limit follicular development and secretion of oestradiol during maternal recognition of pregnancy may have the potential to enhance efficiency of mating as proposed by Thatcher *et al.* (1989).

Conclusions

Fertility in postpartum cows is limited by the occurrence of short luteal phases in early oestrous cycles. Even when luteal phases are of normal duration or progestagen supplements are provided, fertility may be limited by higher preovulatory concentrations of oestradiol, and perhaps by higher secretion of $PGF_{2\alpha}$ and resultant partial luteal regression in the early period after ovulation. Higher than normal secretion of oestradiol in either the early part of the luteal phase or during maternal recognition of pregnancy may compromise fertility in cows with oestrous cycles of normal duration. Treatments to induce oestrus and ovulation in postpartum cows should be designed to ovulate rapidly growing follicles and yield a luteal phase of normal duration and function if maximum fertility is to be attained. If an adequate luteal phase is achieved, effects of other limiting factors will be minimized.

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