

Preovulatory, postovulatory, and postmaternal recognition effects of concentrations of progesterone on embryonic survival in the cow^{1,2}

E. K. Inskeep³

Division of Animal and Veterinary Sciences, West Virginia University, Morgantown 26506-6108

ABSTRACT: Although fertilization rate usually is very high when male fertility is normal, pregnancy rates are below expectations when defined by the birth of live offspring in response to first service. Factors that affect establishment and retention of pregnancy include 1) preovulatory influences on the follicle and oocyte, 2) early postovulatory uterine and luteal function, 3) concentrations of hormones associated with trophoblastic and endometrial function during maternal recognition of pregnancy, and 4) less-well understood factors during the peri-attachment period. For example, decreased progesterone during preovulatory follicular development leads to a persistent follicle, premature resumption of meiosis, and a high incidence of embryonic death between the 2- and 16-cell stages. Elevated PGF_{2 α} during d 4 to 9 of the estrous cycle not only caused

luteolysis but also had a direct embryotoxic effect during the morula-to-blastocyst transition. Ideal conditions during placentation and attachment are not clearly defined. Late embryonic mortality might be increased after ovulation of persistent or immature follicles. Nominal increases in secretion of PGF_{2 α} between d 30 and 35 might be important for attachment and placentation. Lower survival of embryos from wk 5 to wk 7 to 9 of gestation in the cow was associated with lower circulating concentrations of progesterone on wk 5. To maximize embryonic survival in the cow, management must provide high progesterone before estrus, quality detection of estrus, and timely insemination. Luteolytic influences of estradiol-17 β or PGF_{2 α} must be minimized early after mating and during maternal recognition of pregnancy, and high progesterone is needed during the late embryonic/early fetal period.

Key Words: Cows, Embryonic Survival, Pregnancy Rate, Progesterone

©2004 American Society of Animal Science. All rights reserved.

J. Anim. Sci. 2004. 82(E. Suppl.):E24–E39

Introduction

As reviewed by numerous authors (Inskeep, 2002), much of the loss of potential offspring in cattle is concentrated in the embryonic period, the first 42 d after breeding. Luteal secretion of progesterone is essential to suc-

cessful gestation, for ovulation of a healthy oocyte, maintenance of uterine quiescence, nourishment and survival of the embryo/fetus, and normal parturition (Ulberg et al., 1951; McDonald et al., 1952). During luteal phases preceding and immediately after estrus in nonpregnant cows, progesterone regulates establishment and timing of mechanisms for luteal regression (Garrett et al., 1988a). By the same mechanisms, progesterone prepares the uterus for recognition of pregnancy (Vincent and Inskeep, 1986; Vincent et al., 1986). In addition, concentrations of progesterone regulate follicular development by negative-feedback control of pulse frequency of LH secretion (Kinder et al., 1996).

Concentrations of progesterone have been implicated in embryonic deaths during the following periods:

1. The early postovulatory period, before d 6 after mating, in cows in which persistent follicles developed under lower progesterone during the preceding luteal phase and most resultant embryos failed to reach 16 cells.
2. Days 4 through 9 after mating, when excessive secretion of PGF_{2 α} can be both embryotoxic and luteolytic; if progesterone was not present before estrus, the uterus lacks receptors for progesterone.

¹This article was presented at the 2003 ADSA-ASAS-AMPA meeting as part of the Triennial Reproduction symposium.

²The author is indebted to many others for assistance and guidance in the work that led to the invitation to prepare this review paper. Notably, R. L. Butcher, R. A. Dailey, P. E. Lewis, and A. W. Lishman have made long-term inputs, and R. A. Dailey provided a critical and helpful review of this manuscript. Data on effective practical utilization of important findings were shared by N. Schrick. Appreciation is expressed to numerous students and other colleagues whose works are cited herein. Support has come from Hatch Projects 321 (NE-161) and 427 (NE-1007) in the West Virginia Agric. and For. Exp. Stn. and several grants from the NRICGP, CSREES, USDA, as well as material support from Pharmacia Animal Health and Select Sires, Inc.

³Correspondence: G044 Agric. Sci. Bldg. (phone: 304-293-2406, ext. 4422; fax: 304-293-2232; e-mail: einskeep@wvu.edu).

Received July 10, 2003.

Accepted October 28, 2003.

3. Maternal recognition of pregnancy, d 14 through 17, when lower pregnancy rates have been associated with low progesterone and high estradiol-17 β .
4. The late embryonic period, d 28 to 42, when placentation and attachment are in progress. Low progesterone portends loss, but the embryo usually dies before luteal regression.

Low concentrations of progesterone lead to excessive concentrations of other hormones that may cause embryonic death. This review will be concerned with endocrine mechanisms involved in embryonic loss or survival and how understanding of those mechanisms has been developed.

Preovulatory Follicular Development in Relation to Circulating Concentrations of Progesterone, Estrogen, and Luteinizing Hormone

Kinder et al. (1996) summarized how pulsatile secretion frequency of gonadotropin releasing hormone (GnRH) from the hypothalamus is regulated by circulating concentrations of progesterone during the estrous cycle. Frequency of pulsatile secretion of luteinizing hormone from the anterior pituitary is determined, in turn, by the frequency of GnRH pulses. A high frequency of LH pulses stimulates continued growth of a dominant follicle (Taft et al., 1996), which secretes more estradiol-17 β and inhibin. A low frequency of LH pulses fails to support continued follicular growth and leads to atresia of the largest follicle, with a resultant decrease in secretion of estradiol-17 β and inhibin. Each time that the largest follicle of a wave stops growing, an increase in secretion of FSH stimulates development of a new cohort of follicles (Adams et al., 1992). Ginther et al. (1996) reviewed 1) the process of selection of the dominant follicle, 2) the roles of dominance loss by the largest follicle and increased secretion of FSH in the selection process, and 3) the acquisition of dependency on LH by the largest follicle of a cohort.

Extensive data support the sequence of hormonal events described above. Inskeep (2002) analyzed data pooled from nine studies in which progesterone, estradiol-17 β , and frequency of pulses of LH were measured. Concentrations of progesterone in peripheral circulation accounted for 37% of the variation in frequency of LH pulses and 38% of the variation in concentrations of estradiol. Luteinizing hormone pulse frequency accounted for 50% of the variation in concentrations of estradiol.

Workers utilizing repeated ultrasonic imaging to monitor ovarian follicles in different size categories (Pierson and Ginther, 1987) or individual follicles (Sirois and Fortune, 1988) have confirmed that growth of vesicular follicles in cattle occurs in a wavelike pattern (Rajakoski, 1960). Ultrasonographic observation of a wave begins with the emergence of a group or cohort of follicles ≥ 4 mm in diameter (Knopf et al., 1989) and a wave is characterized by continued development of a single follicle and regression of several subordinates

(Ginther et al., 1989a). The largest (dominant) follicle cannot ovulate during a luteal phase because the corpus luteum is dominant by virtue of its secretion of progesterone, which limits frequency of LH pulses, leading to atresia of the largest follicle. The largest follicle present at the onset of luteolysis may become dominant and ovulate during the ensuing follicular phase. Two (Ginther et al., 1989b; Knopf et al., 1989; Rajamahendran and Taylor, 1991), three (Savio et al. 1988; Sirois and Fortune, 1988), or even four (more frequently in Brahman cattle; Rhodes et al., 1995) waves occur during an estrous cycle. Waves emerged on d 0 (day of ovulation) and 10 of estrous cycles with two waves, and on d 0, 9, and 16 of cycles with three waves (Ginther et al., 1989b; Ahmad et al., 1997); thus, a follicular wave occurred about every 7 to 10 d. Ginther et al. (1996) and Ahmad et al. (1997) discussed the variation among herds in whether two or three follicular waves occurred during an estrous cycle in the majority of animals. In two studies, the proportions of animals with two or three waves varied with nutrition (Murphy et al., 1991) or body condition (Burke et al., 1998). In studies with larger numbers of animals, two waves (Ahmad et al., 1997; Townson et al., 2002) seemed to be the prevalent pattern.

Ulberg et al. (1951) recognized the larger size of follicles in animals completing treatment with low dosages of progesterone, but the phenomenon has been studied intensively only in the last decade. Concentrations of progesterone during the luteal phase clearly influence the persistence of a follicle and the number of follicular waves during an estrous cycle (Richards et al., 1990; Sanchez et al., 1993, 1995; Smith and Stevenson, 1995). Ahmad et al. (1997) collected peripheral blood samples every other day from beef animals with two and three waves during normal estrous cycles. Concentrations of progesterone and estradiol-17 β differed only in relation to the time that luteal regression occurred, not in mean concentrations during the luteal phase. Townson et al. (2002) found similar results for progesterone in lactating dairy cows. Thus, the length of the luteal phase appears to be the primary determinant of number of waves, unless very low progesterone leads to persistence of a dominant follicle.

With extensive studies of follicular growth by transrectal ultrasonography, the relationship of lower fertility to persistent large follicles began to be recognized (Savio et al., 1993a,b; Stock and Fortune, 1993; Wehrman et al., 1993). Breuel et al. (1993b) examined fertility of postpartum beef cows with normal luteal phases, after induction of estrus by weaning the calf. Cows with larger preovulatory follicles 5 d before the surge of LH had greater preovulatory concentrations of estradiol and a lower conception rate (36%) than those with smaller follicles at that time, which averaged 91% conception.

In retrospect, much of the variation in pregnancy rates at synchronized estrus in cattle can be accounted for by whether or not a dominant follicle became persis-

tent under conditions of low progesterone or progesterone. Oocytes from persistent follicles were likely to be at a more advanced stage of maturation than those from follicles of normal age and size (Revah and Butler, 1996; Mihm et al., 1999). Mihm et al. (1999) found that by 12 d duration of dominance, seven of eight oocytes had reached at least Metaphase I. In contrast, after 4 d of dominance, most oocytes were in nuclear stage II. Taft reduced circulating progesterone on d 6 of the estrous cycle (R. A. Taft, unpublished data, WV Agric. For. Exp. Stn.). He obtained preliminary evidence that changes in the oocyte characteristic of maturation began within 48 h of lowered progesterone (d 8), including advance to nuclear stage II, irregularity of the nuclear membrane, and degeneration of cumulus cell processes, as well as changes in shape and clumping of the mitochondria. By d 10 of the cycle, 1 d after emergence of the lead follicles of the second wave (Ginther et al., 1989b; Ahmad et al., 1997), similar changes were apparent in oocytes in the dominant follicles of the first wave in cows with normal concentrations of progesterone (R. A. Taft, unpublished data). Thus, changes characteristic of prematuration also are seen during the early stages of atresia. Although the oocyte from persistent follicles was fertilizable, development of the resultant zygote was retarded, and early embryonic death usually occurred before the 16-cell stage (Wishart, 1977; Ahmad et al., 1995). The sequence of relationships described above provides an explanation for the lowered fertility seen with low dosages of progestogens in programs for synchronization of estrus. Similarly, fertility was reduced when progesterone was low during the estrous cycle before breeding (Folman et al., 1973; Meisterling and Dailey, 1987) in untreated dairy cows.

The ovulatory follicle in cows with two waves of follicular development is older and larger than the ovulatory follicle in cows with three waves of follicular development during an estrous cycle (Ginther et al., 1989b; Ahmad et al., 1997; Townson et al., 2002). Given the greater secretion of estradiol-17 β from the ovulatory follicle, patterns of secretion of estradiol, before breeding, could contribute to embryonic losses during d 1 to 4 after breeding, before the 16-cell stage (Ahmad et al., 1995; Cooperative Regional Research Project, NE-161, 1996; Mihm et al., 1994, 1999; Revah and Butler, 1996). If exposure of the preovulatory oocyte to a longer duration of high concentrations of estrogen compromised embryo survival, then conception rates would be lower in those cows with two, rather than three, waves of follicular development. Indeed, conception rate to first service was reduced in lactating dairy cows in which the ovulatory follicle came from the second (63%) compared to the third (81%) wave of follicular development during the estrous cycle before insemination (Townson et al., 2002). The ovulatory follicles were older by 1.5 d and larger by 1.2 mm in cows that ovulated the dominant follicle from the second wave. Ahmad et al. (1997) found a similar trend in beef animals. Conception rates were 82% in 44 heifers and lactating cows in which the

ovulatory follicle came from the second wave and 100% in 8 heifers and cows in which the ovulatory follicle came from the third wave during the estrous cycle before insemination. In a replicate of that study, even fewer beef cows (6 of 67) had three waves of follicular development in the estrous cycle before insemination (H. Hernandez-Fonseca, unpublished data, WV Agric. For. Exp. Stn.). It was not possible to detect a difference in fertility due to number of follicular waves in the combined data from the two studies (83% for 108 cows with two waves vs. 92% for 14 cows with three waves). The low proportion of animals with three waves is discouraging to further work.

Hormonal Mechanisms by Which Persistent Follicles Cause Low Fertility

The sequential relationship of low progesterone, increased frequency of pulses of LH, a persistent largest follicle, increased secretion of estradiol-17 β and decreased fertility (Savio et al., 1993a,b; Stock and Fortune, 1993; Wehrman et al., 1993) is widely accepted as one of causes and effects (Figure 1). However, it is not clear whether the reduction in fertility in a cow with a persistent follicle is due to effects of estrogens, LH, or both. Patterns of fertility in relation to concentrations of estradiol before breeding may be confusing. For example, concentrations of estradiol-17 β on the last day of treatment differed markedly (11 vs. 4 pg/mL) in beef cows and heifers in which the estrous cycle was extended by approximately 7 d with either low (3.7 ng/mL) or high (5.9 ng/mL) progesterone. Yet fertility was reduced equally (55 and 59%, compared to 84% in controls; Washburn and Keller, 1992). In another group of animals in that study, in which a norgestomet implant (6 mg) was used to delay estrus for 7 d, concentrations of estradiol-17 β were only slightly greater (14 vs. 11 pg/mL), yet fertility was reduced to 32%.

In one study, Revah and Butler (1996) treated cows with FSH to produce multiple follicles, which then persisted during low progesterone. However, concentrations of estradiol-17 β declined to very low values (<1 pg/mL) during the 7 d immediately before estrus. Even so, follicular oocytes were at a later stage of maturation (meiosis had resumed), just as in animals with high concentrations of estradiol-17 β from a single persistent follicle in another of their experiments and in the study by Mihm et al. (1999). Revah and Butler (1996) reviewed evidence that either LH or estrogens could be responsible for advances in oocyte maturation, and decreases in rates of fertilization, implantation, and embryo survival, as well as increased rates of embryonic and congenital anomalies in rats.

In most studies, comparisons were made between animals with persistent follicles or control follicles that were younger and smaller. Taft made contemporary comparisons of oocytes collected from follicles on d 8 and 10 of the estrous cycle, in cows with normal or lowered progesterone (R. A. Taft, unpublished data, WV

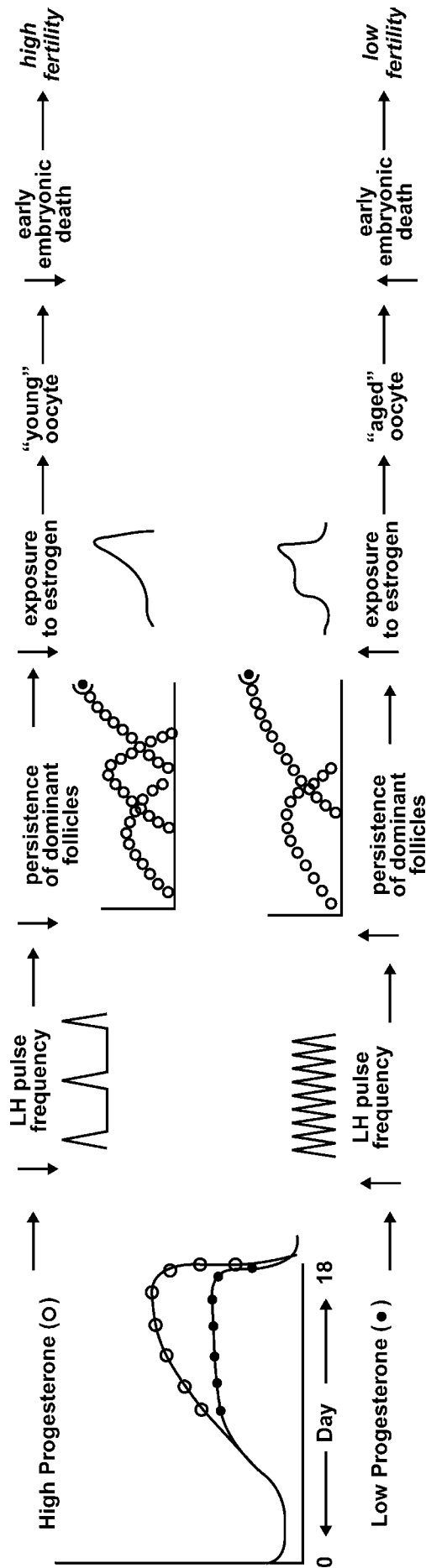


Figure 1. Effects of follicular development patterns on fertility in the cow.

Agric. For. Exp. Stn.). In treated animals (low progesterone), he regressed the corpus luteum with PGF_{2α} on d 6 and provided supplemental progesterone from previously used intravaginal inserts. Progesterone declined from 1.7 to 0.6 ng/mL during d 6 to 9 in treated animals, while increasing from 1.4 to 3.0 ng/mL in control animals. During the same time period, estradiol increased from 1.4 to 3.1 pg/mL in the treated animals, while decreasing from 2.2 to 1.1 pg/mL in the controls. As discussed earlier, more oocytes had advanced to stage II of meiosis by d 8 in the cows with low progesterone than in the controls. Thus, the changes that ultimately lead to lowered embryonic survival may begin very early in the exposure to lowered progesterone.

Shaham-Albalancy et al. (1997) showed that concentrations of progesterone before estrus altered endometrial morphology during the subsequent estrous cycle. Low concentrations of progesterone (2.1 to 2.3 ng/mL) during that period increased subsequent secretion of PGF_{2α} in response to oxytocin, as measured by its major metabolite (Shaham-Albalancy et al., 2001). These effects might lead to a decrease in fertility even though the original oocyte was healthy. However, Wehrman et al. (1996) have shown that development of a persistent follicle before synchronized estrus did not alter rate of survival of control embryos transferred into treated cows on d 7 after that estrus.

The sequence of relationships through which concentrations of progesterone during the preovulatory period lead to ovulation of oocytes of varying age and effects on fertility is summarized in Figure 1. In conclusion, although the effects of delayed ovulations on embryonic and fetal anomalies in the rat have been shown to be a result of prolonged exposure of the oocyte to estrogen (Butcher and Pope, 1979), data available at present do not allow one to differentiate clearly between effects of estrogen and direct effects of prolonged increases in LH in the cow.

Occurrence of Low Concentrations of Progesterone: The Lactating Dairy Cow as a Specific Case

Conceptually, lower progesterone can be due to reduced secretion by the corpus luteum or to increased metabolism of secreted progesterone. Feed intake, milk yield, and route of progesterone administration influenced metabolism and/or excretion of progesterone in lactating dairy cows in some studies (Wiltbank et al., 2000; Rabiee et al., 2001c), but neither feed intake nor metabolizable energy had an effect in others (Rabiee et al., 2002a). In heifers, greater feed intake increased (McCann and Hansel, 1986), decreased (Villa-Godoy et al., 1990), or had no effect on (Spitzer et al., 1978) plasma progesterone. Greater feed intake decreased plasma progesterone in nonlactating, intact (Rabiee et al., 2001b), or ovariectomized cows injected with progesterone (Rabiee et al., 2001a) or bearing intravaginal progesterone-releasing inserts (Rabiee et al., 2002b). High producing cows absorbed more progesterone from

an intravaginal insert and excreted more progesterone daily in the milk, but concentrations of progesterone in milk and plasma were not different from low producing cows (Rabiee et al., 2001c). Sartori et al. (2002a) found that concentrations of progesterone and estradiol-17 β in lactating dairy cows were lower than in heifers in summer and similar to dry cows in winter, despite the fact that they had larger ovulatory follicles and larger corpora lutea. They reviewed similar observations by several other workers.

Based on the earlier work of Parr et al. (1993a,b) and others in sheep, Sangsritavong et al. (2002) tested the hypothesis that increased liver blood flow, as a result of elevated feed intake, will increase steroid metabolism in lactating dairy cows. Liver blood flow and metabolic clearance rate of progesterone reached maximum at 2 h after feeding in lactating cows and persisted longer in cows given greater amounts of feed. In addition, metabolic clearance rate of progesterone was correlated ($r = 0.92$) with liver blood flow. Greater metabolism of progesterone might account for lower concentrations of progesterone and more frequent ovulation of persistent follicles in lactating cows than in heifers that received the same intravaginal dosage of progesterone (Cooperative Regional Research Project, NE-161, 1996). Similarly, it could be responsible, at least in part, for lower fertilization rates in lactating cows than in heifers and lower embryo quality in lactating cows than in heifers and dry cows (Sartori et al., 2002b)

Inadequate Follicular Development and Oocyte Maturation as Factors in Embryonic Mortality

Failure of normal luteal function when follicles were induced to ovulate prematurely has been documented in the earlier literature, especially in sheep (reviewed by Lishman and Inskeep, 1991). Benoit et al. (1992) observed a delay in initiation of luteal function when ewes were treated with an aromatase inhibitor and then treated with hCG to induce ovulation in the absence of an estrogen-induced LH surge. Mann and Laming (2000) modeled low, medium, and high patterns of estrogen secretion in ovariectomized cows to induce estrus and then gave increasing concentrations of progesterone on d 3 through 6 after estrus to mimic the luteal phase. Concentrations of 15-keto,13,14-dihydro-PGF_{2 α} in response to an oxytocin challenge on d 6 after estrus increased as pre-estrous concentrations of estradiol decreased. This result fits logically with the fact that estrogen at proestrus induces uterine progesterone receptors (Stone et al., 1978; Zelinski et al., 1982) and the observation by Zollers et al. (1993) that progesterone receptors were lower on d 5 after estrus in cows with short luteal phases (see discussion of short luteal phases in the next section).

Several authors have found evidence that inadequate follicular development reduced the ability of fertilized, cleaved oocytes to develop to the blastocyst stage. Ousaid et al. (1999) used a GnRH antagonist during the

follicular phase to produce that result in sheep. Treatment decreased concentrations of both LH and estradiol-17 β and increased FSH in plasma. As reviewed by Mermillod et al. (1999), the association of the ability of cattle oocytes to develop to the blastocyst stage after in vitro maturation and fertilization with the endocrine milieu of the follicle has been quite variable. Lower concentrations of progesterone, greater concentrations of estradiol, and more α -subunit of inhibin in follicular fluid sometimes appeared to have predictive value. Mermillod et al. (1999) concluded that the proportion of developmentally competent oocytes increased with increasing follicular size and that a competent oocyte retained its competence (to initiate development) during early stages of follicular atresia.

In a recent study, Perry et al. (2003) monitored pre-ovulatory follicular size in beef cows observed for estrus and inseminated 12 h later; conception rate, measured at d 25 to 39, averaged 72%. In contrast, conception rate in cows that underwent a timed insemination after a regimen of GnRH on d -9, PGF_{2 α} on d -2 and GnRH on d 0, at insemination, was 45%. Much of the difference in fertility was accounted for by the cows with follicles ≤ 11 mm in diameter. For cows with these small follicles, pregnancy rates were 79% in 14 cows inseminated 12 h after estrus but only 29% in 45 GnRH-treated, timed-insemination cows. Perry et al. (2002) had observed a similar trend for lowered fertility in cows induced to ovulate follicles ≤ 12 mm in an earlier study with fewer animals. In those cows, concentrations of progesterone rose at a slower rate than in cows induced to ovulate larger follicles. Thus, embryos might have been expected to be less advanced (Garrett et al., 1988b) and to produce less interferon- τ (Kerbler et al., 1997; Mann et al., 1999) in the cows that ovulated small follicles.

Early Embryonic Death Associated with Short Duration of the Luteal Phase in the Postpartum Beef Cow

The postpartum transition period, during which cyclic occurrence of ovulation is restored, provides an experimental situation in which to determine effects of selected endocrine events (reviewed by Inskeep 1995, 2002). A short luteal phase following first ovulation or first estrus is common in ruminants (Lauderdale, 1986; Garverick and Smith, 1986; Usmani et al., 1990), first reported in cattle by Menge et al. (1962). Obviously, a corpus luteum that regressed before d 14 could not support maternal recognition of pregnancy on d 14 to 17. Follicular development, pre- and postovulatory concentrations of gonadotropins, and luteal receptors for LH were shown to affect luteal function to some degree (Lishman and Inskeep, 1991) but not luteal life span. Copelin et al. (1987, 1989), Peter et al. (1989), and Cooper et al. (1991) eventually showed that premature uterine secretion of PGF_{2 α} was responsible for the short luteal phase.

Ramirez-Godinez et al. (1981, 1982b) and Sheffel et al. (1982) observed that pretreatment with a progestogen usually led to formation of a corpus luteum with a normal functional life span, in response to weaning or injection of gonadotropins. During treatment of anestrus cows with progestogen for 9 d, secretion of $\text{PGF}_{2\alpha}$ rose on d 6 through 1 before withdrawal (Cooper et al., 1991). Thus, if the uterus had not been exposed recently to progestogen, secretion of $\text{PGF}_{2\alpha}$ increased prematurely when the first corpus luteum began to secrete progesterone. However, secretion of $\text{PGF}_{2\alpha}$ during treatment was not necessary for treatment with progestogen to normalize the subsequent luteal phase (Johnson et al., 1992). The effect of progestogen was apparently mediated by an increase in numbers of receptors for progesterone in the uterus on d 5 after estrus (Zollers et al., 1993). Using ovariectomized postpartum beef cows as a test animal, Kieborz-Loos et al. (2003) recently showed that prevention of the early secretion of $\text{PGF}_{2\alpha}$ in response to progesterone required a sequence of exposure to progesterone and estrogen before the test exposure to progesterone. That is what happens in the progestogen-pretreated intact cow because follicular development and estrogen secretion follow withdrawal of progestogen.

A Model for Study of Fertility in Contemporary Groups of Cows with Short and Normal Cycles. Bellows et al. (1974) found that beef cows from which calves were weaned at about 35 d postpartum consistently exhibited estrus in 4 to 5 d and formed corpora lutea. Casida et al. (1968) and Ramirez-Godinez et al. (1982a) obtained evidence that ovulation and fertilization occurred at the expected time after estrus preceding a short luteal phase in early-weaned cows. Logically, fertility should be improved by pretreatment of the postpartum cow with progestogen because of the prevention of the shortened luteal phase described in the previous paragraph. A model was developed with which to determine the point(s) at which fertility fails in early-weaned postpartum beef cows. Calves were weaned at about 30 d postpartum, and half the cows received progestogen treatment (6-mg norgestomet implants for 9 d, ending 2 d after early weaning). Control cows that had not formed corpora lutea before calves were weaned were expected to have short luteal phases/estrous cycles in all cases. Cows pretreated with progestogen were expected to have normal luteal phases/estrous cycles in an average of at least 80% of cases. Cows in both groups were at the same stage postpartum when studied.

Breuel et al. (1993b) compared components of fertility in cows with short or normal luteal phases. First, they removed and flushed oviducts from cows in each group at d 3 after breeding. Fertilization rate (68%), development of fertilized oocytes to the four- to eight-cell stage (100%) and embryo quality did not differ between cows with short or normal luteal phases. When uteri were flushed nonsurgically on d 6, fertilization rate (82%) and development to at least the four-cell stage (90%) again did not differ (Breuel et al., 1993b). If loss of the

embryo was a consequence of early luteal regression, supplemental treatment with progestogen should maintain pregnancy. Progestogen therapy, either as a daily supplement of MGA in feed or as injections of 200 mg of progesterone daily, beginning on d 4 after breeding (Breuel et al., 1993b), did not maintain pregnancy in cows with short luteal phases. In contrast, 41% of all norgestomet-pretreated cows and 50% of those cows that had normal luteal phases maintained pregnancy regardless of whether or not they received MGA. Twelve of 13 cows that were deleted from these experiments because they had a spontaneous short luteal phase before breeding conceived at the postweaning estrus, at an average of only 33 d postpartum.

Whether the oocytes in cows with short luteal phases were inherently defective or the uteri of such cows were hostile to embryo survival was addressed in two experiments utilizing embryo transfer. First, two good-quality frozen-thawed embryos were transferred on d 7 after estrus into the uteri of postpartum cows expected to have short (control) or normal (norgestomet pretreated) luteal phases. All cows received 200 mg/d of supplemental progesterone, subcutaneously, beginning on d 4 after estrus. Pregnancies were maintained in 28% of control cows compared to 58% of norgestomet-pretreated cows (Butcher et al., 1992). Second, oocytes/embryos were flushed from the uteri of control and norgestomet-pretreated cows on d 6 after breeding, and, if viable, transferred into the uteri of nonlactating, cycling recipients on d 6. Survival rates for embryos deemed 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 (number of recipients pregnant divided by the number of experimental cows from which an embryo or oocyte was recovered on d 6) was 13% for cows with a short luteal phase compared to 32% for cows with a normal luteal phase. Likewise, embryonic survival of fertilized oocytes on d 6 for cows with a short luteal phase (23%) was half that for cows with a normal luteal phase (47%).

Evidence That $\text{PGF}_{2\alpha}$ Is Embryotoxic. The apparent timing of embryo loss, around d 5 through 8, was strikingly similar to the timing of increased uterine secretion of $\text{PGF}_{2\alpha}$ on d 4 through 9 after estrus in cows with short luteal phases (Cooper et al., 1991). Moreover, Schrick et al. (1993) had observed that $\text{PGF}_{2\alpha}$ concentrations in uterine flushings of cows with short luteal phases were more than double those from cows with normal luteal phases (636 ± 82 and 288 ± 90 pg/mL, respectively). Embryo quality tended to be correlated negatively with concentrations of $\text{PGF}_{2\alpha}$ in uterine flushings ($r = -0.42$). Because embryo quality was lower on d 6 (Schrick et al., 1993) than on d 3 (Breuel et al., 1993b), the specific problem in short luteal phase cows was likely to have occurred after the embryo entered the uterus. A direct embryotoxic effect of $\text{PGF}_{2\alpha}$ had been suggested for mouse (Harper and Skarnes, 1972) and shown for rabbit (Maurer and Beier, 1976) and rat (Breuel et al., 1993a) embryos.

Effects of $\text{PGF}_{2\alpha}$ on embryo survival were examined in cows in which daily supplemental progestogen was provided to replace the regressed corpus luteum. Buford et al. (1996) showed that $\text{PGF}_{2\alpha}$ was detrimental to embryos when given to normally cycling beef cows during d 4 to 7 after estrus and insemination, an interval similar to that during which high embryo mortality had been observed in cows with short luteal phases. Buford et al. (1996) tested whether embryonic survival in early-weaned cows was improved when the luteolytic rise in $\text{PGF}_{2\alpha}$ was reduced by treatment with flunixin meglumine, an inhibitor of prostaglandin G/H synthase (PGHS). All cows received 300 mg/d of progesterone in corn oil (s.c.) from d 3.5 after mating until pregnancy determination at d 30. Cows were allotted at random among three treatments: saline, flunixin meglumine, and flunixin meglumine plus removal of the corpus luteum (lutectomy). The latter treatment was intended to answer the secondary question, whether luteal maintenance per se, if it should occur in the group treated with flunixin meglumine, affected embryonic survival. Flunixin meglumine was given at 1 g every 8 h on d 4 through 9 and lutectomy was performed on d 7. Pregnancy rate was increased only when flunixin meglumine was combined with lutectomy. Therefore, the regressing corpus luteum appeared to be a component of the embryotoxic effect of $\text{PGF}_{2\alpha}$.

Buford et al. (1996) confirmed that the corpus luteum was required for the embryotoxic effect of $\text{PGF}_{2\alpha}$ in nonlactating, cycling cows supplemented with progestogen. From these data, it seemed possible that even subluteolytic concentrations of $\text{PGF}_{2\alpha}$ (Schramm et al., 1983) could play a role in embryonic loss during early development via release of an embryotoxin from the corpus luteum. Shelton et al. (1990) observed that peripheral concentrations of progesterone increased more slowly after estrus in subfertile dairy cows than in heifers, and several authors have reported that progesterone increased more slowly in cows subsequently diagnosed nonpregnant than in those diagnosed pregnant.

Secretion of $\text{PGF}_{2\alpha}$ might be especially important during cycles that are of relatively normal duration but have lowered concentrations of progesterone (Lishman and Inskeep, 1991). Robinson et al. (1976) observed that secretion of progesterone increased after removal of the uterine caruncles early in the estrous cycle in the ewe. In cows, concentrations of $\text{PGF}_{2\alpha}$ fall to basal values at estrus; slight increases on d 5 (as determined by concentrations of 15-keto,13,14-dihydro- $\text{PGF}_{2\alpha}$) were associated with metestrous bleeding (Kindahl et al., 1976). Schallenberger et al. (1989) observed an increase in concentrations of $\text{PGF}_{2\alpha}$ until d 6 after estrus and artificial insemination. The majority of embryonic mortality in subfertile dairy cows occurred 6 to 7 d after estrus (Ayalon, 1978), when the morula was developing into the blastocyst. Maurer and Chenault (1983) observed that 67% of embryonic mortality had occurred or was occurring by d 8 of gestation in beef cows. Seals et al. (1998) showed that premature luteal regression

by $\text{PGF}_{2\alpha}$ on d 5 through 8 caused embryo death in cows supplemented with progestogen (confirming the results of Buford et al., 1996), but treatment on either d 10 through 13 or 15 through 18 of pregnancy was not effective. Either the embryo was susceptible only until about d 8 or older regressing corpora lutea did not produce or promote production of the embryotoxic factor. Hockett et al. (1998) showed that treatment with $\text{PGF}_{2\alpha}$ on d 5 through 8 reduced quality and delayed or stopped development of embryos recovered on d 8. Scenna et al. (2002) confirmed that $\text{PGF}_{2\alpha}$ decreased the rate of hatching of bovine blastocysts in culture.

Bovine corpora lutea can secrete prostaglandins (Shemesh and Hansel, 1975). Synthesis of $\text{PGF}_{2\alpha}$ by luteal cells in vitro varied with stage of the estrous cycle (Milvae and Hansel, 1983) but was not affected by LH (Pate and Condon, 1984). Rexroad and Guthrie (1979) showed that corpora lutea of ewes secreted more $\text{PGF}_{2\alpha}$ after treatment with prostaglandins to induce luteolysis. Recently, Tsai and Wiltbank (1997, 1998) proposed that secretion of $\text{PGF}_{2\alpha}$ from the corpus luteum amplified the luteolytic signal from the uterus in an autocrine or paracrine manner. A single injection of $\text{PGF}_{2\alpha}$ upregulated mRNA encoding PGHS-2 in mid- and late-cycle ovine and bovine corpora lutea but was not effective in animals on d 4 of the estrous cycle. In subsequent studies, Sayre et al. (2000) showed that the early corpus luteum can become sensitive with repeated exposure to $\text{PGF}_{2\alpha}$. Treatment every 8 h beginning on d 4 upregulated mRNA for both PGHS-2 and PGF synthase within 24 h. Hu et al. (1990) observed that short-lived corpora lutea produced more $\text{PGF}_{2\alpha}$ than did corpora lutea with a normal life span.

Concentrations of $\text{PGF}_{2\alpha}$ can be increased locally in the ovarian artery by venoarterial diffusion from the uterine vein (Ginther 1974; Bonnin et al., 1999). Therefore, Hernandez-Fonseca et al. (2000) transferred an embryo to each uterine horn to test whether a luteal embryotoxin might be delivered locally to the uterine horn adjacent to the regressing corpus luteum. The reduction in survival of embryos in ipsilateral and contralateral uterine horns did not differ; thus, the effect was systemic or through the uterine lumen. Using ewes with corpora lutea in only one ovary as a result of unilateral ovariectomy after breeding, Costine et al. (2001) ligated both uterine horns and treated with $\text{PGF}_{2\alpha}$. Pregnancy rates were reduced equally in the uterine horns ipsilateral and contralateral to the regressing corpora lutea, so they ruled out intraluminal transfer and concluded that a local effect was not required.

Overall, the preponderance of evidence is that chronic high concentrations of $\text{PGF}_{2\alpha}$ are toxic to the very early embryo in cows and ewes (Figure 2). The most recent data support the concept that the effect is direct and does not require local transfer from the ovary to the uterus, but that a regressing corpus luteum can be a significant source of the $\text{PGF}_{2\alpha}$ involved in the effect.

Oxytocin is released from the corpus luteum by $\text{PGF}_{2\alpha}$ (Schallenberger et al., 1984) and can increase uterine

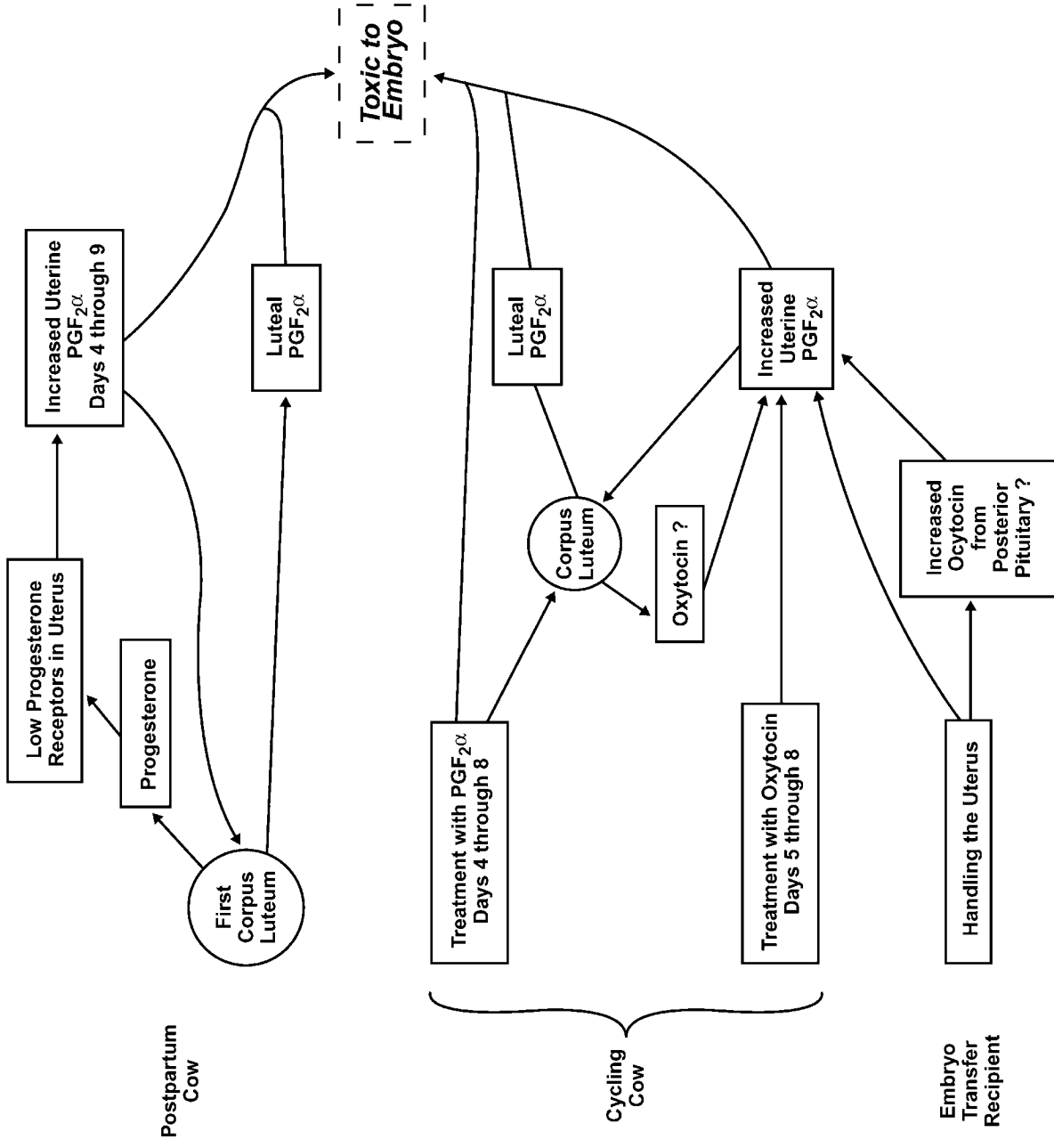
Evidence

Embryo Loss Reduced by Lutectomy plus Flunixin Meglumine

Embryo Loss Blocked by Lutectomy

Embryo Loss Blocked by Flunixin Meglumine

Embryo Loss reduced by Flunixin Meglumine



Animal

Figure 2. Proposed mechanisms for embryonic loss during estrous cycles with a short luteal phase or impaired early luteal function. The suggested known and possible mechanisms are based on studies in postpartum and cycling cows that were supplemented with progesterone in order to study effects of luteolysis or luteolytic agents. Supporting data are presented in detail in the text.

secretion of PGF_{2α} (Newcomb et al., 1977; Milvae and Hansel, 1980). Buford et al. (1996) observed that injections of PGF_{2α} increased concentrations of oxytocin in serum of intact, but not luteotomized, nonlactating cows. Lemaster et al. (1999) treated with oxytocin in the same type of experimental design used by Buford et al. (1996) with PGF_{2α}. Treatment on d 5 through 8 reduced pregnancy rate from 80 to 33%, but the effect of oxytocin was blocked by concurrent treatment with flunixin meglumine (80% pregnancy rate). Thus, if oxytocin was involved in the embryotoxic effect, it was to increase secretion of PGF_{2α}, not a direct effect on the embryo.

On the other hand, oxytocin, injected to cause milk letdown or released by uterine manipulation (Roberts et al., 1975) associated with embryo transfer, might play a significant role in early embryonic death in cattle, with or without causing complete luteolysis. In fact, Schrick et al. (2001) found that treatment with flunixin meglumine improved pregnancy rates to nonsurgical embryo transfer. Other factors that increase secretion of PGF_{2α}, such as heat stress (Malayer et al., 1990), uterine infection (Manns et al., 1985), or mastitis (Barker et al., 1998; Cullor, 1990; Stewart et al., 2003), might cause very early embryonic death through this mechanism (reviewed by Zavy, 1994).

Role of Follicular Secretion of Estradiol in Embryonic Mortality During Maternal Recognition of Pregnancy

Patterns of follicular development and resultant secretion of estradiol-17β during d 14 to 17 after breeding may be important in embryonic loss during maternal recognition of pregnancy. This concept was originally presented by Macmillan et al. (1986) and has been supported in subsequent studies by Thatcher et al. (1989) and others, in which ovulation or atresia of the largest follicle during the mid-luteal phase sometimes increased pregnancy rate. Attention was brought to this period as a result of observations made during preliminary studies of the survival of embryos when transferred into postpartum cows with short luteal phases that were supplemented with progestogens (Butcher et al., 1992). Attempts were made to provide supplemental progestogen by silastic implants containing up to 25 mg of norgestomet. However, large follicles developed in the ovaries during d 12 to 20 and embryos transferred on d 7 failed to survive. Therefore high dosages of injected progesterone or flurogestone acetate were used in subsequent studies in which embryo survival was increased (Butcher et al., 1992). Kastelic et al. (1991) concluded that luteal regression preceded death of the embryo in losses that occurred before d 25 of gestation. Even short periods of deprivation of progesterone can decrease embryo survival during the maternal recognition period. Lulai et al. (1994b) studied the effects of initiation of luteal regression on d 15, either 24 or 36 h before beginning replacement therapy with norgestomet. Embryo survival was 84% in control heifers and

cows, but was reduced to 45 or 13%, respectively, when replacement therapy was delayed for 24 or 36 h.

Evidence for association of embryonic loss with excessive secretion of estrogen during maternal recognition of pregnancy in beef cows was obtained by Pritchard et al. (1994). They sampled concentrations of progesterone and estradiol in peripheral blood during d 14 to 17 after breeding in over 100 lactating beef cows. Cows were divided into three groups according to concentrations of estradiol-17β, the lower quarter, middle half, and upper quarter, which averaged 1.6, 2.1, and 3.1 pg/mL of estradiol, respectively, during the 4-d sampling period. Conception rate to first service by artificial insemination declined as concentration of estradiol increased. Mean conception rates were 77, 60, and 42%, respectively. Numerous studies have been done in which GnRH or hCG have been used to ovulate or luteinize large follicles during this stage after breeding, with considerable variation in response. Based on the review by Lewis et al. (1990), one cannot conclude that these treatments are routinely valuable.

Given the above information, workers in the NE - 161 regional research project proposed that secretion of estrogen from a large follicle during d 14 through 17 (or beyond) after breeding can compromise embryo survival, either directly or through interference with mechanisms of maternal recognition of pregnancy/luteal maintenance. Further, they proposed that cows with two waves of follicular development during the equivalent of an estrous cycle after breeding would have such a follicle. Ahmad et al. (1997) found that fewer animals conceived among those that had two (70%) rather than three (96%; $P < 0.05$) waves of follicular development during the equivalent of one estrous cycle after insemination. Surprisingly, however, concentrations of estrogen in peripheral blood were not greater on d 14 after estrus and insemination in animals with two waves than in those with three waves.

It is clear that there is an association of pregnancy loss during maternal recognition with higher circulating estrogen in some cases. However, neither the exact timing of an estrogen effect nor the mechanism by which estrogen may interfere with the developing embryo have been established.

Late Embryonic/Early Fetal Mortality: A Significant Factor in Lactating Dairy Cows During the Peri-Attachment Period

With the recognition that duration of follicular development was a significant factor in determining pregnancy rate at synchronized estrus (reviewed by Inskeep, 2002), numerous protocols to regulate follicular development have been devised. With such protocols, it has been possible to program the time of AI, without detection of estrus and with greater initial rates of pregnancy than previous systems (Burke et al., 1996; Thatcher et al., 1996; Stevenson et al., 1996; Pursley et al., 1997a,b). However, researchers have noted high

frequencies of late embryonic and early fetal losses in several studies, both before (Van Cleeff et al., 1991) and after d 25 of gestation (Schallenberger et al., 1989; Kastelic et al., 1991; Van Cleeff et al., 1991; Wolff, 1992; Smith and Stevenson, 1995). Based on return intervals exceeding 27 d after breeding, Thatcher et al. (1994) estimated that late embryonic death rate was 10.6% in heifers bred at estrus after synchronization with two injections of PGF_{2 α} 11 d apart.

Attachment of the embryo in the uterus is initiated around d 30 in the cow, with marked development of the placentomes between d 30 and 40 (Melton et al., 1951; King et al., 1982). In cows with viable embryos, imaged ultrasonographically at 25 to 32 d after timed AI, pregnancy losses until subsequent diagnosis at 50 to 98 d ranged from 14 to 40% (Vasconcelos et al., 1999; Cartmill et al., 2001; Moreira et al., 2001). Vasconcelos et al. (1997) found that 10.5% of lactating dairy cows that were pregnant at 28 d after timed breeding had lost the pregnancy by d 42 and another 9.7% were lost by d 98.

Drost et al. (1999) used embryo transfer from super-ovulated donors to attempt to overcome effects of heat stress in lactating dairy cows in summer in Florida. They estimated late embryonic mortality from the difference in pregnant cows at d 42 and cows with high progesterone on d 22, which was 65% in cows inseminated artificially and 41% in cows with transferred embryos.

There are limited data on embryonic death in beef cows after fixed-time insemination. Bridges et al. (1999) found that only 1 of 71 cows pregnant at d 39 failed to calve. They had used timed breeding after progesterone, PGF_{2 α} , and estradiol benzoate. The observations by Perry et al. (2003) allow consideration of the possibility that follicle size in cows that underwent timed insemination after GnRH might affect late embryonic losses, but the data are tenuous. Thirteen of 43 timed-insemination cows with small follicles were pregnant at d 27, but 5 of the 13 (38.5%) lost pregnancy by d 68, leaving a net pregnancy rate of 19%, whereas no late losses occurred in 57 pregnant cows with larger follicles (overall loss, 6.7%). In the cows inseminated at estrus, 3 of 127 (2.4%) lost pregnancies from first to second diagnosis, but their follicle sizes were 12, 14, and ≥ 16 mm. These data illustrate that very large numbers of cows must be sampled to elucidate causes of late embryonic mortality.

Four studies have been done in animals that were inseminated 12 h after detection of estrus. In Brahman crossbred heifers, fertilization rate was 93% of intact ova, 78% had intact embryos and 10% had degenerating embryos on d 16, and 72% were pregnant on d 35 (Smith et al., 1982). Beal et al. (1992) diagnosed pregnancy by ultrasonography at 25, 45, and 65 d in 205 beef cows that initially had 138 viable embryos. Losses were 6.5% from d 25 to 45 and another 1.5% to d 65. Lamb et al. (2002) measured embryo mortality in *Bos taurus* heifers on three ranches with 169 to 439 heifers per ranch.

These heifers had been inseminated 12 h after they were first detected to be in estrus in response to an injection of PGF_{2 α} 17 d after withdrawal of MGA, which had been fed at 0.5 mg/d for 14 d. Conception rates as determined by ultrasonography at 29 to 33 d after insemination ranged from 44 to 67%. Of 525 pregnant heifers, 4.2% did not have viable embryos at palpation 60 to 90 d after the end of the breeding season. Dunne et al. (2000) measured embryo survival at slaughter on d 14 as 68%. By ultrasonography at d 30, their estimate was 76% pregnant, whereas at full term, 71.8% calved, so that the late embryonic and fetal loss was 4.2 percentage points. Thus, they concluded that most losses occurred before d 14 and that losses after d 30 were approximately 5.5%.

Because initial reports of high rates of late embryonic and early fetal mortality were in dairy cows inseminated at predetermined times, without detection of estrus, it seemed important to determine amounts of pregnancy loss in dairy animals that were inseminated in relation to observed estrus. Starbuck et al. (2004) studied lactating dairy cows and heifers, including Holstein and Ayrshire breeds, on two farms on which animals were inseminated approximately 12 h after observed onset of estrus or bred naturally. Eleven percent of 211 animals that were pregnant at ultrasonography during the fifth week after breeding (d 28 to 36) lost the pregnancy by wk 9, with 65% of those losses having occurred by wk 7. No losses were seen in 22 heifers in that study; after data from three more groups of heifers were added (M. J. Starbuck, unpublished data, WV Agric. For. Exp. Stn.), only 2 of 97 heifers lost pregnancy during d 30 to 60. In a larger study of 862 Holstein heifers pregnant at d 30 after breeding at estrus synchronized by PGF_{2 α} , Alexander et al. (1995) found an average of 5.3% lost pregnancy by d 60, with no effect of rectal palpation on pregnancy retention. Rivera et al. (2003a,b) reported 7 to 10% losses from 30 to 65 or 75 d in smaller groups of Holstein heifers. Finally, in a study of lactating Holstein cows in California, Cerri et al. (2003) found no difference in loss rates from 30 to 58 d of gestation between timed-insemination cows (11%) and those inseminated at detection of estrus (12.4%). When all of these studies are considered together, the problem appears to be most prevalent or of greater magnitude in lactating dairy cows.

In seven of eight heifers in which embryonic death occurred between d 25 and 40 postbreeding, Kastelic et al. (1991) found that the onset of luteal regression, as detected by ultrasonography, began at least 3 d after embryonic death, as indicated by loss of heartbeat. In another study utilizing 70 cows pregnant at d 35 after breeding, seven pregnancies were lost by d 42. Embryonic death in each of the seven cows preceded luteal regression, detected by ultrasonography and by declining concentrations of progesterone in milk (Wolff, 1992). Although late embryonic loss preceded luteolysis, the possibility that luteal function was compromised before embryos were lost was not ruled out. Schallenberger et

Hormonal Concentrations Day 30 to 35 of Pregnancy

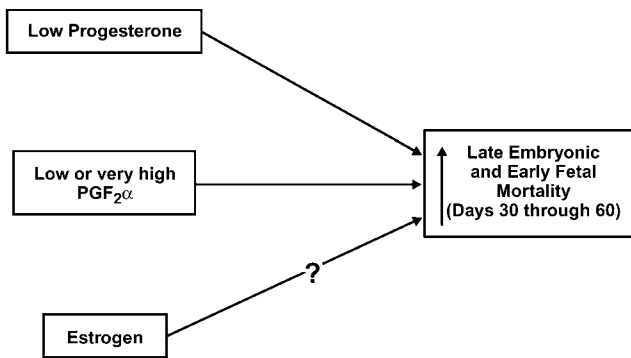


Figure 3. Summary of current knowledge of endocrine factors in late embryonic and early fetal loss in the cow.

al. (1989) observed increased secretion of $\text{PGF}_{2\alpha}$ between d 30 and 36 in pregnant heifers, one of which had extremely high values and lost the pregnancy. The placenta may be the source of increased $\text{PGF}_{2\alpha}$ because Eley et al. (1979) found higher total $\text{PGF}_{2\alpha}$ in allantoic fluid on d 33 than on d 27, 30, 40, 50, 60, or 70.

Lulai et al. (1994a) induced a new corpus luteum (CL) on d 36 to 40 of pregnancy, during progestogen treatment and after induced regression of the original CL. When the induced CL was on the ovary adjacent to the pregnant uterine horn, it was maintained after progestogen withdrawal and supported the pregnancy. When the induced CL was on the contralateral ovary, pregnancy was lost in four of five animals. In later work, maintenance of pregnancy was examined after induction of a new CL between d 27 and 54 after breeding (Bridges et al., 2000) in cows in which original CL had either regressed or been removed earlier and pregnancy had been maintained with an exogenous progestogen. After induction of new CL, progestogen was withdrawn gradually. Pregnancy was maintained only if the new CL was induced on the ovary adjacent to the embryo. When the new CL was induced later than d 36 after mating, 21 of 21 pregnancies were maintained. However, when the CL was induced on or before d 36 after mating, only 15 of 30 pregnancies were maintained.

In one study, Bridges et al. (2000) removed the original CL on d 26 of pregnancy, induced a new CL between d 28 and 31, and examined patterns of secretion of $\text{PGF}_{2\alpha}$, progesterone, and estradiol-17 β during d 31 through 35. Surprisingly, in cows with greater concentrations of $\text{PGF}_{2\alpha}$, an induced CL secreted more progesterone and maintenance of pregnancy tended to be higher. In addition, there was a tendency for more pregnancies to continue when concentrations of estradiol were lower (Figure 3).

The role of prostaglandins in embryonic attachment has not been elucidated in ruminants, but $\text{PGF}_{2\alpha}$ is

important in the comparable process of implantation in rodents. In the rat, implantation is initiated on d 5 of pregnancy and uterine $\text{PGF}_{2\alpha}$ and PGE were increased at that time (Novaro et al., 1996). Treatment with indomethacin on d 5 of pregnancy in the rat decreased the weight of implantation sites and extended the length of pregnancy (Kennedy, 1977); thus, it was suggested that treatment with indomethacin delayed the process of implantation. Similar effects of indomethacin were seen in mice and hamsters (Rankin et al., 1979; Evans and Kennedy, 1978). The effects of prostaglandins seem to be due, in part, to changes in endometrial capillary permeability, because inhibition of prostaglandin secretion reduced uterine uptake of dye in the rat and the hamster (Kennedy, 1977; Evans and Kennedy, 1978).

Because lowered concentrations of progesterone and greater concentrations of estradiol appeared to limit successful maintenance of pregnancy by a replacement CL (Bridges et al., 2000), concentrations of progesterone and estradiol were studied as predictors of pregnancy maintenance in dairy cows (Starbuck et al., 2004). Serum was assayed for progesterone to determine if the CL was functional at the time nearest to detection of embryonic mortality. In most cases in which cows lost pregnancy, the CL was functional at the last collection before embryonic or fetal death was detected, so it was suggested that the embryo died before the CL regressed, in agreement with Kastelic et al. (1991) and Wolff (1992). However, pregnancy loss before d 45 (20%) was greater in cows with the lowest 25% of serum concentrations of progesterone at 28 to 37 d of gestation than in cows in the middle 50% (3.8 to 5.9 ng/mL) or upper 25%, each of which had only 8% loss. Logistic regression analysis confirmed this relationship. Pregnancy loss after d 45 was not related to concentrations of progesterone on either d 28 to 37 or d 45 to 51.

Based on classification of concentrations of estradiol into the upper 25, middle 50, and lower 25% of observed values, and analysis by χ^2 , retention of pregnancy to wk 7 of gestation increased with increasing concentrations of estradiol at wk 5 (Starbuck et al., 2004). Retention of pregnancy to wk 9 was not associated with concentrations of estradiol at wk 5 or 7. When evaluated by logistic regression, retention of pregnancy was not associated with concentrations of estradiol at either wk 5 or 7. These data are in contrast to the relationship reported by Bridges et al. (2000) in cows with replacement corpora lutea.

Thus, embryonic mortality after maternal recognition of pregnancy and during placentation is a significant problem in the dairy cow. It is associated with lower progesterone during d 28 to 37 (Figure 3). Lower progesterone could be due to reduced secretion of progesterone by the CL, or to greater metabolism of progesterone, as pointed out earlier.

Conclusions

Embryonic mortality is a factor that significantly limits the success of establishment and maintenance of

pregnancy in cattle. Some causes and mechanisms involved in loss of embryos have been elucidated and much of success or failure appears to depend on circulating concentrations of progesterone at specific time points and changes in other hormones as a consequence of patterns of progesterone. These factors are expressed in the luteal phase immediately before estrus, during d 4 through 8 after estrus, during maternal recognition of pregnancy on d 14 through 17 after estrus, and during the late embryonic/early fetal period, between d 28 and 42 to 50. Early embryonic, late embryonic, and early fetal losses appear to be greater in lactating dairy cows than in beef cattle and dairy heifers.

Implications

Both producers and researchers must be aware that the management required to maximize fertility in the cow is not simple. Very high fertility was observed in all animals in studies that involved frequent ultrasonographic scanning, careful observation of the cows, and breeding in relation to observed estrus. Based on these data, the continuing value of close observation for estrus in an artificial insemination program should be emphasized in educational programs. Treatments for synchronization of estrus must provide high progesterone, keep luteinizing hormone and estradiol low during treatment, and lead to development of a highly functional corpus luteum after mating. The reduction of luteolytic influences—such as excesses of prostaglandin $F_{2\alpha}$ or estradiol- 17β early after mating, during maternal recognition of pregnancy, and during the late embryonic period—is important. Management systems designed to limit the metabolism of progesterone are needed during the estrous cycle before breeding as well as in these periods.

Literature Cited

- Adams, G. P., R. L. Matteri, and O. J. Ginther. 1992. Effect of progesterone on ovarian follicles, emergence of follicular waves and circulating follicle-stimulating hormone in heifers. *J. Reprod. Fertil.* 96:627–640.
- Ahmad, N., E. C. Townsend, R. A. Dailey, and E. K. Inskeep. 1997. Relationships of hormonal patterns and fertility to occurrence of two or three waves of ovarian follicles, before and after breeding, in beef cows and heifers. *Anim. Reprod. Sci.* 49:13–28.
- Ahmad, N., F. N. Schrick, R. L. Butcher, and E. K. Inskeep. 1995. Effect of persistent follicles on early embryonic losses in beef cows. *Biol. Reprod.* 52:1129–1135.
- Alexander, B. M., M. S. Johnson, R. O. Guardia, W. L. Van de Graff, P. L. Senger, and R. G. Sasser. 1995. Embryonic loss from 30 to 60 days post breeding and the effect of palpation per rectum on pregnancy. *Theriogenology* 43:551–556.
- Ayalon, N. 1978. A review of embryonic mortality in cattle. *J. Reprod. Fertil.* 54:483–493.
- Barker, A. R., F. N. Schrick, M. J. Lewis, H. H. Dowlen, and S. P. Oliver. 1998. Influence of clinical mastitis during early lactation on reproductive performance of Jersey cows. *J. Dairy Sci.* 81:1285–1290.
- Beal, W. E., R. C. Perry, and L. R. Corah. 1992. The use of ultrasound in monitoring reproductive physiology of beef cattle. *J. Anim. Sci.* 70:924–929.
- Bellows, R. A., R. E. Short, J. J. Urlick, and O. F. Pahnish. 1974. Effects of early weaning on postpartum reproduction of the dam and growth of calves born as multiples or singles. *J. Anim. Sci.* 39:589–600.
- Benoit, A. M., E. K. Inskeep, and R. A. Dailey. 1992. Effect of a nonsteroidal aromatase inhibitor on in vitro and in vivo secretion of estradiol and on the estrous cycle in ewes. *Domest. Anim. Endocrinol.* 9:313–327.
- Bonnin, P., L. Huynh, R. L'Haridon, N. Chene, and J. Martal. 1999. Transport of uterine $PGF_{2\alpha}$ to the ovaries by systemic circulation and local lymphovenous-arterial diffusion during luteolysis in sheep. *J. Reprod. Fertil.* 116:199–210.
- Breuel, K. F., A. Fukuda, and F. N. Schrick. 1993a. Effects of prostaglandin $F_{2\alpha}$ on development of 8-cell rat embryos *in vitro*. *Biol. Reprod.* 48(Suppl. 1):173.
- Breuel, K. F., P. E. Lewis, F. N. Schrick, A. W. Lishman, E. K. Inskeep, and R. L. Butcher. 1993b. Factors affecting fertility in the postpartum cow: role of the oocyte and follicle in conception rate. *Biol. Reprod.* 48:655–661.
- Bridges, P. J., P. E. Lewis, W. R. Wagner, and E. K. Inskeep. 1999. Follicular growth, estrus and pregnancy after fixed-time insemination in beef cows treated with intravaginal progesterone inserts and estradiol benzoate. *Theriogenology* 52:573–583.
- Bridges, P. J., D. J. Wright, W. I. Buford, N. Ahmad, H. Hernandez-Fonseca, M. L. McCormick, F. N. Schrick, R. A. Dailey, P. E. Lewis, and E. K. Inskeep. 2000. Ability of induced corpora lutea to maintain pregnancy in beef cows. *J. Anim. Sci.* 78:2942–2949.
- Buford, W. I., N. Ahmad, F. N. Schrick, R. L. Butcher, P. E. Lewis, and E. K. Inskeep. 1996. Embryotoxicity of a regressing corpus luteum in beef cows supplemented with progestogen. *Biol. Reprod.* 54:531–537.
- Burke, J. M., R. L. de La Sota, C. A. Risco, C. R. Staples, E. J. P. Schmitt, and W. W. Thatcher. 1996. Evaluation of timed insemination using a gonadotropin-releasing hormone agonist in lactating dairy cows. *J. Dairy Sci.* 79:1385–1393.
- Burke, J. M., J. H. Hampton, C. R. Staples, and W. W. Thatcher. 1998. Body condition influences maintenance of a persistent first wave dominant follicle in dairy cattle. *Theriogenology* 49:751–760.
- Butcher, R. L., and R. S. Pope. 1979. Role of estradiol during prolonged estrous cycle of the rat on subsequent death or development. *Biol. Reprod.* 21:491–495.
- Butcher, R. L., J. E. Reber, A. W. Lishman, K. F. Breuel, F. N. Schrick, J. C. Spitzer, and E. K. Inskeep. 1992. Maintenance of pregnancy in postpartum beef cows that have short-lived corpora lutea. *J. Anim. Sci.* 70:3831–3837.
- Cartmill, J. A., S. Z. El-Zarkouny, B. A. Hensley, T. G. Rozell, J. F. Smith, and J. S. Stevenson. 2001. An alternative AI breeding protocol for dairy cows exposed to elevated ambient temperatures before and after calving or both. *J. Dairy Sci.* 84:799–806.
- Casida, L. E., W. E. Graves, E. R. Hauser, J. W. Lauderdale, J. W. Riesen, S. Saiduddin, and W. J. Tyler. 1968. Studies on the postpartum cow. *Res. Bull.* 270, Univ. Wisconsin, Madison.
- Cerri, R. L. A., K. N. Galvao, S. O. Juchem, R. C. Chebel, and J. E. P. Santos. 2003. Timed AI (TAI) with estradiol cypionate (ECP) or insemination at detected estrus in lactating dairy cows. *J. Anim. Sci.* 81(Suppl. 1):181. (Abstr.)
- Cooperative Regional Research Project, NE-161. 1996. Relationship of fertility to patterns of ovarian follicular development and associated hormonal profiles in dairy cows and heifers. *J. Anim. Sci.* 74:1943–1952.
- Cooper, D. A., D. A. Carver, P. Villeneuve, W. J. Silvia, and E. K. Inskeep. 1991. Effects of progestagen treatment on concentrations of prostaglandins and oxytocin in plasma from the posterior vena cava of *post-partum* beef cows. *J. Reprod. Fertil.* 91:411–421.
- Copelin, J. P., M. F. Smith, D. H. Keisler, and H. A. Garverick. 1989. Effect of active immunization of *pre-partum* and *post-partum* cows against prostaglandin $F_{2\alpha}$ on lifespan and progesterone secretion of short-lived corpora lutea. *J. Reprod. Fertil.* 87:199–207.

- Copelin, J. P., M. F. Smith, H. A. Garverick, and R. S. Youngquist. 1987. Effect of the uterus on subnormal luteal function in anestrus beef cows. *J. Anim. Sci.* 64: 1506–1511.
- Costine, B. A., B. L. Sayre, and E. K. Inskeep. 2001. Embryotoxicity of a regressing corpus luteum in ewes. *Reproduction* 122:883–887.
- Cullor, J. S. 1990. Mastitis and its influence upon reproductive performance in dairy cattle. Pages 176–180 in *Internatl. Symp. Bovine Mastitis*, Indianapolis, Indiana.
- Drost, M., J. D. Ambrose, M.-J. Thatcher, C. K. Cantrell, K. E. Wolfendorf, J. F. Hasler, and W. W. Thatcher. 1999. Conception rates after artificial insemination or embryo transfer in lactating dairy cows during summer in Florida. *Theriogenology* 52:1161–1167.
- Dunne, L. D., M. G. Diskin, and J. M. Sreenan. 2000. Embryo and foetal loss in beef heifers between day 14 of gestation and full term. *Anim. Reprod. Sci.* 58:39–44.
- Eley, R. M., W. W. Thatcher, and F. W. Bazer. 1979. Hormonal and physical changes associated with bovine conceptus development. *J. Reprod. Fertil.* 55:181–190.
- Evans, C. A., and T. G. Kennedy. 1978. The importance of prostaglandin synthesis for the initiation of blastocyst implantation in the hamster. *J. Reprod. Fertil.* 54:255–261.
- Folman, Y., M. Rosenberg, Z. Herz, and M. Davidson. 1973. The relationship between plasma progesterone concentrations and conception in postpartum dairy cows maintained on two levels of nutrition. *J. Reprod. Fertil.* 34:267–278.
- Garverick, H. A., and M. F. Smith. 1986. Mechanisms associated with subnormal luteal function. *J. Anim. Sci.* 62(Suppl. 2):2–105.
- Garrett, J. E., R. D. Geisert, M. T. Zavy, L. K. Gries, R. P. Wettemann, and D. S. Buchanan. 1988a. Effect of exogenous progesterone on prostaglandin $F_{2\alpha}$ release and the interestrous interval in the bovine. *Prostaglandins* 36:85–96.
- Garrett, J. E., R. D. Geisert, M. T. Zavy, and G. L. Morgan. 1988b. Evidence for maternal regulation of early conceptus growth and development in beef cattle. *J. Reprod. Fertil.* 84:437–446.
- Ginther, O. J. 1974. Internal regulation of physiological processes through venoarterial pathways: A review. *J. Anim. Sci.* 39:550–564.
- Ginther, O. J., J. P. Kastelic, and L. Knopf. 1989a. Composition and characteristics of follicular waves during the bovine estrous cycle. *Anim. Reprod. Sci.* 20:187–200.
- Ginther, O. J., L. Knopf, and J. P. Kastelic. 1989b. Temporal associations among ovarian events in cattle during oestrous cycles with two and three follicular waves. *J. Reprod. Fertil.* 87:223–230.
- Ginther, O. J., M. C. Wiltbank, P. M. Fricke, J. R. Gibbons, and K. Kot. 1996. Selection of the dominant follicle in cattle. *Biol. Reprod.* 55:1187–1194.
- Harper, M. J. K., and R. C. Skarnes. 1972. Inhibition of abortion and fetal death produced by endotoxin or prostaglandin $F_{2\alpha}$. *Prostaglandins* 2:295–309.
- Hernandez-Fonseca, H. J., B. L. Sayre, R. L. Butcher, and E. K. Inskeep. 2000. Embryotoxic effects adjacent and opposite to the early regressing bovine corpus luteum. *Theriogenology* 54:83–91.
- Hockett, M. E., N. R. Rohrbach, and F. N. Schrick. 1998. Effect of administration of PGF $_{2\alpha}$ on embryonic development and quality in cows supplemented with exogenous progestogen. *J. Anim. Sci.* 76(Suppl. 1):241. (Abstr.)
- Hu, Y., J. D. H. Sanders, S. G. Kurz, J. S. Ottobre, and M. L. Day. 1990. *In vitro* prostaglandin production by bovine corpora lutea destined to be normal or short-lived. *Biol. Reprod.* 42:801–807.
- Inskeep, E. K. 1995. Factors that affect fertility during oestrous cycles with short or normal luteal phases in postpartum cows. *J. Reprod. Fertil. Suppl.* 49:493–503.
- Inskeep, E. K. 2002. Factors that affect embryonic survival in the cow: Application of technology to improve calf crop. Pages 255–279 in *Factors Affecting Calf Crop: Biotechnology of Reproduction*. M. J. Fields, R. S. Sand, and J. V. Yelich, ed. CRC Press, Boca Raton, FL.
- Johnson, S. K., R. P. Del Vecchio, E. C. Townsend, and E. K. Inskeep. 1992. Role of prostaglandin $F_{2\alpha}$ in follicular development and subsequent luteal life span in early postpartum beef cows. *Domest. Anim. Endocrinol.* 9:49–56.
- Kastelic, J. P., D. L. Northey, and O. J. Ginther. 1991. Spontaneous embryonic death on days 20 to 40 in heifers. *Theriogenology* 35:351–363.
- Kennedy, T. G. 1977. Evidence for a role for prostaglandins in the initiation of blastocyst implantation in the rat. *Biol. Reprod.* 16:286–291.
- Kerbler, T. L., M. M. Buhr, L. T. Jordan, K. E. Leslie, and J. S. Walton. 1997. Relationship between maternal plasma progesterone concentration and interferon- γ synthesis by the conceptus in cattle. *Theriogenology* 47:703–714.
- Kieborz-Loos, K. R., H. A. Garverick, D. H. Keisler, S. A. Hamilton, B. E. Salfen, R. S. Youngquist, and M. F. Smith. 2003. Oxytocin-induced secretion of prostaglandin $F_{2\alpha}$ in postpartum beef cows: Effects of progesterone and estradiol-17 β treatment. *J. Anim. Sci.* 81:1830–1836.
- Kindahl, H., L.-E. Edqvist, A. Bane, and E. Granstrom. 1976. Blood levels of progesterone and 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$ during the normal estrous cycle and early pregnancy in heifers. *Acta Endocrinol.* 82:134–149.
- Kinder, J. E., F. N. Kojima, E. G. M. Bergfeld, M. E. Wehrman, and K. E. Fike. 1996. Progestin and estrogen regulation of pulsatile LH release and development of persistent ovarian follicles in cattle. *J. Anim. Sci.* 74:1424–1440.
- King, G. J., B. A. Atkinson, and H. A. Robertson. 1982. Implantation and early placentation in domestic ungulates. *J. Reprod. Fertil. Suppl.* 31:17–30.
- Knopf, L., J. P. Kastelic, E. Schallenberger, and O. J. Ginther. 1989. Ovarian follicular dynamics in heifers: test of two-wave hypothesis by ultrasonically monitoring individual follicles. *Domest. Anim. Endocrinol.* 6:111–119.
- Lamb, G. C., C. R. Dahlen, and D. R. Brown. 2002. Reproductive ultrasound technology for monitoring ovarian structure development, fetal development, embryo survival, and the incidence of twinning in beef cows. *Prof. Anim. Sci.* 19:135–143.
- Lauderdale, J. W. 1986. A review of patterns of change in luteal function. *J. Anim. Sci.* 62(Suppl. 2):79–91.
- Lemaster, J. W., R. C. Seals, F. M. Hopkins, and F. N. Schrick. 1999. Effects of administration of oxytocin on embryonic survival in progestogen-supplemented cattle. *Prostaglandins Other Lipid Mediat.* 57:259–268.
- Lewis, G. S., D. W. Caldwell, C. E. Rexroad, Jr., H. H. Dowlen, and J. R. Owen. 1990. Effects of gonadotropin-releasing hormone and human chorionic gonadotropin on pregnancy rate in dairy cattle. *J. Dairy Sci.* 73:66–72.
- Lishman, A. W., and E. K. Inskeep. 1991. Deficiencies in luteal function during re-initiation of breeding activity in beef cows and in ewes. *S. Afr. J. Anim. Sci.* 21:59–74.
- Lulai, C., I. Dobrinski, J. P. Kastelic, and R. J. Mapletoft. 1994a. Induction of luteal regression, ovulation and development of new luteal tissue during early pregnancy in heifers. *Anim. Reprod. Sci.* 35:163–172.
- Lulai, C., J. P. Kastelic, T. D. Carruthers, and R. J. Mapletoft. 1994b. Role of luteal regression in embryo death in cattle. *Theriogenology* 41:1081–1089.
- Macmillan, K. L., V. K. Taufa, and A. M. Day. 1986. Effects of an agonist of gonadotropin releasing hormone (Buserelin) in cattle. III. Pregnancy rates after a post-insemination injection during metoestrus or dioestrus. *Anim. Reprod. Sci.* 11:1–10.
- Malayer, J. R., P. J. Hansen, T. S. Gross, and W. W. Thatcher. 1990. Regulation of heat shock-induced alterations in the release of prostaglandins by the uterine endometrium of cows. *Theriogenology* 34:219–230.
- Mann, G. E., and G. E. Lamming. 2000. The role of sub-optimal preovulatory estradiol secretion in the aetiology of premature luteolysis during the short oestrous cycle in the cow. *Anim. Reprod. Sci.* 64:171–180.
- Mann, G. E., G. E. Lamming, R. S. Robinson, and D. C. Wathes. 1999. The regulation of interferon- γ production and uterine hormone

- receptors during early pregnancy. *J. Reprod. Fertil. Suppl.* 54:317–328.
- Manns, J. G., J. R. Nkuuhe, and F. Bristol. 1985. Prostaglandin concentrations in uterine fluid of cows with pyometra. *Can. J. Comp. Med.* 49:436–438.
- Maurer, R. R., and H. M. Beier. 1976. Uterine proteins and development in vitro of rabbit preimplantation embryos. *J. Reprod. Fertil.* 48:33–41.
- Maurer, R. R., and J. R. Chenault. 1983. Fertilization failure and embryonic mortality in parous and nonparous beef cattle. *J. Anim. Sci.* 56:1186–1189.
- McCann, J. P., and W. Hansel. 1986. Relationship between insulin and glucose metabolism and pituitary ovarian functions in fasted heifers. *Biol. Reprod.* 34:630–641.
- McDonald, L. M., R. E. Nichols, and S. H. McNutt. 1952. Study of corpus luteum ablation and progesterone replacement therapy in the cow. *Am. J. Vet. Res.* 13:446–451.
- Meisterling, E. M., and R. A. Dailey. 1987. Use of concentrations of progesterone and estradiol-17 β in milk in monitoring postpartum ovarian function in dairy cows. *J. Dairy Sci.* 70:2154–2161.
- Melton, A. A., R. O. Berry, and O. D. Butler. 1951. The interval between the time of ovulation and attachment of the bovine embryo. *J. Anim. Sci.* 10:993–1005.
- Menge, A. C., S. E. Mares, W. J. Tyler, and L. E. Casida. 1962. Variation and association among postpartum reproduction and production characteristics in Holstein-Friesian cattle. *J. Dairy Sci.* 45:233–241.
- Mermillod, P., B. Oussaid, and Y. Cognie. 1999. Aspects of follicular and oocyte maturation that affect the developmental potential of embryos. *J. Reprod. Fertil. Suppl.* 54:449–460.
- Mihm, M., A. Baguisi, M. P. Boland, and J. F. Roche. 1994. Association between the duration of dominance of the ovulatory follicle and pregnancy rate in beef heifers. *J. Reprod. Fertil.* 102:123–130.
- Mihm, M., N. Curran, P. Hyttel, P. G. Knight, M. P. Boland, and J. F. Roche. 1999. Effect of dominant follicle persistence on follicular fluid oestradiol and inhibin and on oocyte maturation in beef heifers. *J. Reprod. Fertil.* 116:293–304.
- Milvae, R. A., and W. Hansel. 1980. Concurrent uterine venous and ovarian arterial prostaglandin F concentrations in heifers treated with oxytocin. *J. Reprod. Fertil.* 60:7–15.
- Milvae R. A., and W. Hansel. 1983. Prostacyclin, prostaglandin F_{2 α} and progesterone production by bovine luteal cells during the estrous cycle. *Biol. Reprod.* 29:1063–1068.
- Moreira, F., C. Orlandi, C. A. Risco, R. Mattos, F. Lopes, and W. W. Thatcher. 2001. Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed AI protocol in lactating dairy cows. *J. Dairy Sci.* 84:1646–1659.
- Murphy, M. G., W. J. Enright, M. A. Crowe, K. McConnell, L. J. Spicer, M. P. Boland, and J. F. Roche. 1991. Effect of dietary intake on pattern of growth of dominant follicles during the oestrus cycle in beef heifers. *J. Reprod. Fertil.* 92:333–338.
- Newcomb, R., W. D. Booth, and L. E. A. Rowson. 1977. The effect of oxytocin treatment on the levels of prostaglandin F in the blood of heifers. *J. Reprod. Fertil.* 49:17–24.
- Novaro, V., V. Rettori, E. T. Gonzalez, A. Jawerbaum, A. Faletti, G. Canteros, and M. A. de Gimeno. 1996. Interaction between uterine PGE and PGF_{2 α} production and the nitridergic system during embryonic implantation in the rat. *Prostaglandins* 51:363–376.
- Oussaid, B., J. C. Mariana, N. Poulin, J. Fontaine, P. Lonergan, J. F. Beckers, and Y. Cognie. 1999. Reduction of the developmental competence of sheep oocytes by inhibition of LH pulsed during the follicular phase with a GnRH antagonist. *J. Reprod. Fertil.* 117:71–77.
- Parr, R. A., I. F. Davis, M. A. Miles, and T. J. Squires. 1993a. Feed intake affects metabolic-clearance rate of progesterone in sheep. *Res. Vet. Sci.* 55:306–310.
- Parr, R. A., I. F. Davis, M. A. Miles, and T. J. Squires. 1993b. Liver blood flow and metabolic clearance rate of progesterone in sheep. *Res. Vet. Sci.* 55:311–316.
- Pate, J. L., and W. A. Condon. 1984. Effects of prostaglandin F_{2 α} on agonist-induced progesterone production in cultured bovine luteal cells. *Biol. Reprod.* 31:427–435.
- Peter, A. T., W. K. Bosu, R. M. Liptrap, and E. Cummings. 1989. Temporal changes in serum prostaglandin F_{2 α} and oxytocin in dairy cows with short luteal phases after the first postpartum ovulation. *Theriogenology* 32:277–284.
- Perry, G. A., T. W. Geary, M. C. Lucy, and M. F. Smith. 2002. Effect of follicle size at the time of induced ovulation on luteal function and fertility. *Proc. West. Sect. Am. Soc. Anim. Sci.* 55:45–48.
- Perry, G. A., M. F. Smith, M. C. Lucy, A. J. Roberts, M. D. MacNeil, and T. W. Geary. 2003. Effect of ovulatory follicle size at the time of GnRH injection or standing estrus on pregnancy rates and embryonic/fetal mortality in beef cattle. *J. Anim. Sci.* 81 (Suppl. 1):52. (Abstr.)
- Pierson, R. A., and O. J. Ginther. 1987. Follicular populations during the estrous cycle in heifers. I. Influence of day. *Anim. Reprod. Sci.* 14:165–176.
- Pritchard, J. Y., F. N. Schrick, and E. K. Inskeep. 1994. Relationship of pregnancy rate to peripheral concentrations of progesterone and estradiol in beef cows. *Theriogenology* 42:247–259.
- Pursley, J. R., M. R. Kosorok, and M. C. Wiltbank. 1997a. Reproductive management of lactating dairy cows using synchronization of ovulation. *J. Dairy Sci.* 80:301–306.
- Pursley, J. R., M. C. Wiltbank, J. S. Stevenson, J. S. Ottobre, H. A. Garverick, and L. L. Anderson. 1997b. Pregnancy rates per AI for cows and heifers inseminated at synchronized ovulation or synchronized estrus. *J. Dairy Sci.* 80:295–300.
- Rabiee, A. R., D. Dalley, J. M. Borman, K. L. Macmillan, and F. Schwarzenberger. 2002a. Progesterone clearance rate in lactating dairy cows with two levels of dry matter and metabolisable energy intakes. *Anim. Reprod. Sci.* 72:11–25.
- Rabiee, A. R., K. L. Macmillan, F. Schwarzenberger, and P. J. Wright. 2002b. Effects of level of feeding and progesterone dose on plasma and faecal progesterone in ovariectomised cows. *Anim. Reprod. Sci.* 73:185–195.
- Rabiee, A. R., K. L. Macmillan, and F. Schwarzenberger. 2001a. Evaluating progesterone metabolism in ovariectomized non-lactating Holstein Friesian cows treated with progesterone with two levels of feed intake. *Anim. Reprod. Sci.* 66:35–36.
- Rabiee, A. R., K. L. Macmillan, and F. Schwarzenberger. 2001b. Evaluating the effect of feed intake on progesterone clearance rate by measuring faecal progesterone metabolites in grazing dairy cows. *Anim. Reprod. Sci.* 67:205–214.
- Rabiee, A. R., K. L. Macmillan, and F. Schwarzenberger. 2001c. Excretion rate of progesterone in milk and faeces in lactating dairy cows with two levels of milk yield. *Reprod. Nutr. Dev.* 41:309–319.
- Rajakoski, E. 1960. The ovarian follicular system in sexually mature heifers with special reference to seasonal, cyclical, and left-right variations. *Acta Endocrinol. Suppl.* 52:7–68.
- Rajamahendran, R., and C. Taylor. 1991. Follicular dynamics and temporal relationships among body temperature, oestrus, the surge of luteinizing hormone and ovulation in Holstein heifers treated with norgestomet. *J. Reprod. Fertil.* 92:461–467.
- Ramirez-Godinez, J. A., G. H. Kiracofe, D. L. Carnahan, M. F. Spire, K. B. Beeman, J. S. Stevenson, and R. R. Schalles. 1982a. Evidence for ovulation and fertilization in beef cows with short estrous cycles. *Theriogenology* 17:409–414.
- Ramirez-Godinez, J. A., G. H. Kiracofe, R. R. Schalles, and G. D. Niswender. 1982b. Endocrine patterns in the postpartum beef cow associated with weaning: a comparison of the short and subsequent normal cycles. *J. Anim. Sci.* 55:153–158.
- Ramirez-Godinez, J. A., G. H. Kiracofe, R. M. McKee, R. R. Schalles, and R. J. Kittok. 1981. Reducing the incidence of short estrous cycles in beef cows with norgestomet. *Theriogenology* 15:613–623.
- Rankin, J. C., B. E. Ledford, H. E. Johnson, Jr., and B. Baggett. 1979. Prostaglandins, indomethacin and the decidual cell reaction in the mouse uterus. *Biol. Reprod.* 20:399–404.

- Revah, I., and W. R. Butler. 1996. Prolonged dominance of follicles reduces the viability of bovine oocytes. *J. Reprod. Fertil.* 106:39–47.
- Rexroad, C. E., Jr., and H. D. Guthrie. 1979. Prostaglandin $F_{2\alpha}$ and progesterone release in vitro by ovine luteal tissue during induced luteolysis. *Adv. Exp. Med. Biol.* 112:639–644.
- Rhodes, F. M., G. De'ath, and K. W. Entwistle. 1995. Animal and temporal effects on ovarian follicular dynamics in Brahman heifers. *Anim. Reprod. Sci.* 38:265–277.
- Richards, M. W., R. D. Geisert, L. J. Dawson, and L. E. Rice. 1990. Pregnancy response after estrus synchronization of cyclic cows with or without a corpus luteum prior to breeding. *Theriogenology* 34:1185–1193.
- Rivera, H., H. Lopez, and P. M. Fricke. 2003a. Use of CIDR devices in a synchronization of ovulation protocol using GnRH and PG $F_{2\alpha}$ for first AI service and for resynchronizing return to estrus for second AI service in Holstein dairy heifers. *J. Anim. Sci.* 81(Suppl. 1):181. (Abstr.)
- Rivera, H., H. Lopez, and P. M. Fricke. 2003b. Reproductive management of dairy heifers using synchronization of ovulation and fixed-time artificial insemination (TAI) artificial insemination after removed tail chalk. *J. Anim. Sci.* 81(Suppl. 1):182. (Abstr.)
- Roberts, J. S., B. Barcikowski, L. Wilson, Jr., R. C. Skarnes, and J. A. McCracken. 1975. Hormonal and related factors affecting the release of prostaglandin $F_{2\alpha}$ from the uterus. *J. Steroid Biochem.* 6:1091–1097.
- Robinson, J. S., J. R. G. Challis, B. J. A. Furr, T. M. Louis, and G. D. Thorburn. 1976. Is the sheep corpus luteum subject to tonic inhibition during the luteal phase of the estrous cycle? *Eur. J. Obstet. Gynecol. Reprod. Biol.* 6:191–195.
- Sanchez, T., M. E. Wehrman, E. G. Bergfeld, K. E. Peters, F. N. Kojima, A. S. Cupp, V. Mariscal, R. J. Kittok, R. J. Rasby, and J. E. Kinder. 1993. Pregnancy rate is greater when the corpus luteum is present during the period of progestin treatment to synchronize time of estrus in cows and heifers. *Biol. Reprod.* 49:1102–1107.
- Sanchez, T., M. E. Wehrman, F. N. Kojima, A. S. Cupp, E. G. Bergfeld, K. E. Peters, V. Mariscal, R. J. Kittok, and J. E. Kinder. 1995. Dosage of the synthetic progestin, norgestomet, influences luteinizing hormone pulse frequency and endogenous secretion of 17β -estradiol in heifers. *Biol. Reprod.* 52:464–469.
- Sangsrivavong, S., D. K. Combs, R. Sartori, L. E. Armentano, and M. C. Wiltbank. 2002. High feed intake increases liver blood flow and metabolism of progesterone and estradiol- 17β in dairy cattle. *J. Dairy Sci.* 85:2831–2842.
- Sartori, R., G. J. M. Rosa, and M. C. Wiltbank. 2002a. Ovarian structures and circulating steroids in heifers and lactating cows in summer and lactating and dry cows in winter. *J. Dairy Sci.* 85:2813–2822.
- Sartori, R., R. Sartori-Bergfeld, S. A. Mertens, J. N. Guenther, J. J. Parrish, and M. C. Wiltbank. 2002b. Fertilization and early embryonic development in heifers and lactating cows in summer and lactating and dry cows in winter. *J. Dairy Sci.* 85:2803–2812.
- Savio, J. D., L. Keenan, M. P. Boland, and J. F. Roche. 1988. Pattern of growth of dominant follicles during the oestrous cycle in heifers. *J. Reprod. Fertil.* 83:663–671.
- Savio, J. D., W. W. Thatcher, L. Badinga, R. L. de la Sota, and D. Wolfenson. 1993a. Regulation of dominant follicle turnover during the oestrous cycle in cows. *J. Reprod. Fertil.* 97:197–203.
- Savio, J. D., W. W. Thatcher, G. R. Morris, K. Entwistle, M. Drost, and M. R. Mattiacci. 1993b. Effects of induction of low plasma progesterone concentrations with a progesterone-releasing intravaginal device on follicular turnover and fertility in cattle. *J. Reprod. Fertil.* 98:77–84.
- Sayre, B. L., R. Taft, E. K. Inskeep, and J. Killefer. 2000. Increased expression of insulin-like growth factor binding protein-1 during induced regression of bovine corpora lutea. *Biol. Reprod.* 63:21–29.
- Scenna, F. N., M. E. Hockett, H. E. Blackmon, and F. N. Schrick. 2002. Development of in vivo-derived bovine embryos cultured with prostaglandin $F_{2\alpha}$. *Theriogenology* 57:512. (Abstr.)
- Schallenger, E., D. Schams, B. Bullermann, and D. L. Walters. 1984. Pulsatile secretion of gonadotrophins, ovarian steroids and ovarian oxytocin during prostaglandin-induced regression of the corpus luteum in the cow. *J. Reprod. Fertil.* 71:493–501.
- Schallenger, E., D. Schams, and H. H. D. Meyer. 1989. Sequences of pituitary, ovarian and uterine hormone secretion during the first 5 weeks of pregnancy in dairy cattle. *J. Reprod. Fert. Suppl.* 37:277–286.
- Schramm, W., L. Bovaird, M. E. Glew, G. Schramm, and J. A. McCracken. 1983. Corpus luteum regression induced by ultralow pulses of prostaglandin $F_{2\alpha}$. *Prostaglandins* 26:347–364.
- Schrick, F. N., M. E. Hockett, T. M. Towns, A. M. Saxton, N. E. Wert, and M. E. Wehrman. 2001. Administration of a prostaglandin inhibitor immediately prior to embryo transfer improves pregnancy rates in cattle. *Theriogenology* 55:370. (Abstr.)
- Schrick, F. N., E. K. Inskeep, and R. L. Butcher. 1993. Pregnancy rates for embryos transferred from early postpartum beef cows into recipients with normal estrous cycles. *Biol. Reprod.* 49:617–621.
- Seals, R. C., J. W. Lemaster, F. M. Hopkins, and F. N. Schrick. 1998. Effects of elevated concentrations of prostaglandin $F_{2\alpha}$ on pregnancy rates in progestogen-supplemented cattle. *Prostaglandins Other Lipid Mediat.* 56:377–389.
- Shaham-Albalancy, A., Y. Folman, M. Kaim, M. Rosenberg, and D. Wolfenson. 2001. Delayed effect of low progesterone concentrations on bovine uterine PGF $_{2\alpha}$ secretion in the subsequent oestrous cycle. *Reproduction* 122:643–648.
- Shaham-Albalancy, A., A. Nyska, M. Kaim, M. Rosenberg, Y. Folman, and D. Wolfenson. 1997. Delayed effect of progesterone on endometrial morphology in dairy cows. *Anim. Reprod. Sci.* 48:159–174.
- Sheffel, C. E., B. R. Pratt, W. L. Ferrell, and E. K. Inskeep. 1982. Induced corpora lutea in the postpartum beef cow. II. Effects of treatment with progestogen and gonadotropins. *J. Anim. Sci.* 54:830–836.
- Shelton, K., M. F. Gayerie de Abreu, M. G. Hunter, T. J. Parkinson, and G. E. Lammung. 1990. Luteal inadequacy during the early luteal phase of subfertile cows. *J. Reprod. Fertil.* 90:1–10.
- Shemesh, M., and W. Hansel. 1975. Stimulation of prostaglandin synthesis in bovine ovarian tissues by arachidonic acid and luteinizing hormone. *Biol. Reprod.* 13:448–452.
- Sirois, J., and J. E. Fortune. 1988. Ovarian follicular dynamics during the estrous cycle in heifers monitored by real-time ultrasonography. *Biol. Reprod.* 39:308–317.
- Smith, M. W., K. J. Nix, D. C. Kraemer, M. S. Amoss, M. A. Herron, and J. N. Wiltbank. 1982. Fertilization rate and early embryonic loss in Brahman crossbred heifers. *J. Anim. Sci.* 54:1005–1011.
- Smith, M. W., and J. S. Stevenson. 1995. Fate of the dominant follicle, embryonal survival, and pregnancy rates in dairy cattle treated with prostaglandin $F_{2\alpha}$ and progestins in the absence or presence of a functional corpus luteum. *J. Anim. Sci.* 73:3743–3751.
- Spitzer, J. C., G. D. Niswender, G. E. Seidel, and J. N. Wiltbank. 1978. Fertilization and blood levels of progesterone and LH in beef heifers on a restricted energy diet. *J. Anim. Sci.* 46:1071–1077.
- Starbuck, M. J., R. A. Dailey, and E. K. Inskeep. 2004. Factors affecting retention of early pregnancy in dairy cattle. *Anim. Reprod. Sci.* (In press)
- Stevenson, J. S., Y. Kobayashi, M. P. Shipka, and K. C. Rauchholz. 1996. Altering conception of dairy cattle by gonadotropin-releasing hormone preceding luteolysis induced by prostaglandin $F_{2\alpha}$. *J. Dairy Sci.* 79:402–410.
- Stewart, A. B., E. K. Inskeep, E. C. Townsend, and R. A. Dailey. 2003. Effects of gram-positive bacterial pathogens in ewes: Peptidoglycan as a potential mediator of interruption of early pregnancy. *Reproduction (Camb)* 125:295–299.
- Stock, A. E., and J. E. Fortune. 1993. Ovarian follicular dominance: relationship between prolonged growth of the ovulatory follicle and endocrine parameters. *Endocrinology* 132:1108–1114.
- Stone, G. M., L. Murphy, and B. G. Miller. 1978. Hormone receptor levels and metabolic activity in the uterus of the ewe: Regulation by oestradiol and progesterone. *Aust. J. Biol. Sci.* 31:395–403.

- Taft, R., N. Ahmad, and E. K. Inskeep. 1996. Exogenous pulses of luteinizing hormone cause persistence of the largest bovine ovarian follicle. *J. Anim. Sci.* 74:2985–2991.
- Thatcher, W. W., R. L. de La Sota, E. J. P. Schmitt, T. C. Diaz, L. Badinga, F. A. Simmen, C. R. Staples, and M. Drost. 1996. Control and management of ovarian follicles in cattle to optimize fertility. *Reprod. Fertil. Dev.* 8:203–217.
- Thatcher, W. W., K. L. Macmillan, P. J. Hansen, and M. Drost. 1989. Concepts for regulation of corpus luteum function by the conceptus and ovarian follicles to improve fertility. *Theriogenology* 31:149–164.
- Thatcher, W. W., C. R. Staples, G. Danet-Desnoyers, B. Oldick, and E. P. Schmitt. 1994. Embryo health and mortality in sheep and cattle. *J. Anim. Sci.* 72(Suppl. 3):16–30.
- Townson, D. H., P. C. W. Tsang, W. R. Butler, M. Frajblat, L. C. Griel, Jr., C. J. Johnson, R. A. Milvae, G. M. Niksic, and J. L. Pate. 2002. Relationship of fertility to ovarian follicular waves before breeding in dairy cows. *J. Anim. Sci.* 80:1053–1058.
- Tsai, S. J., and M. C. Wiltbank. 1997. Prostaglandin $F_{2\alpha}$ induces expression of prostaglandin G/H synthase-2 in the ovine corpus luteum: a potential positive feedback loop during luteolysis. *Biol. Reprod.* 57:1016–1022.
- Tsai, S. J., and M. C. Wiltbank. 1998. Prostaglandin $F_{2\alpha}$ regulates distinct physiological changes in early and mid-cycle bovine corpora lutea. *Biol. Reprod.* 58:346–352.
- Ulberg, L. C., R. E. Christian, and L. E. Casida. 1951. Ovarian response of heifers to progesterone injections. *J. Anim. Sci.* 10:752–759.
- Usmani, R. H., R. A. Dailey, and E. K. Inskeep. 1990. Effects of limited suckling and varying prepartum nutrition on postpartum reproductive traits of milked buffaloes. *J. Dairy Sci.* 73:1564–1570.
- Van Cleeff, J. K., M. Drost, and W. W. Thatcher. 1991. Effects of postinsemination progesterone supplementation on fertility and subsequent estrous responses of dairy heifers. *Theriogenology* 36:795–807.
- Vasconcelos, J. L. M., R. L. Silcox, J. A. Lacerda, J. R. Pursley, and M. C. Wiltbank. 1997. Pregnancy rate, pregnancy loss and response to heat stress after AI at 2 different times from ovulation in dairy cows. *Biol. Reprod.* 56(Suppl. 1):140.
- Vasconcelos, J. L. M., R. W. Silcox, G. J. M. Rosa, J. R. Pursley, and M. C. Wiltbank. 1999. Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. *Theriogenology* 52:1067–1078.
- Villa-Godoy, A., T. I. Hughes, R. S. Emery, W. J. Enright, A. D. Ealy, S. A. Zinn, and R. L. Fogwell. 1990. Energy balance and body condition influence luteal function in Holstein heifers. *Domest. Anim. Endocrinol.* 7:135–148.
- Vincent, D. L., and E. K. Inskeep. 1986. Role of progesterone in regulating utero-ovarian venous concentrations of $PGF_{2\alpha}$ and PGE_2 during the estrous cycle and early pregnancy in ewes. *Prostaglandins* 31:715–733.
- Vincent, D. L., S. Meredith, and E. K. Inskeep. 1986. Advancement of uterine secretion of prostaglandin E_2 by treatment with progesterone and transfer of asynchronous embryos. *Endocrinology* 119:527–529.
- Washburn, S. P., and M. L. Keller. 1992. Fertility of beef cattle when estrous cycles are extended with progestogens. *J. Anim. Sci.* 70(Suppl. 1):255. (Abstr.)
- Wehrman, M. E., K. E. Fike, E. J. Melvin, E. G. M. Bergfeld, and J. E. Kinder. 1996. Development of a persistent ovarian follicle during synchronization of estrus does not alter conception rate after embryo transfer in cattle. *Theriogenology* 45:291. (Abstr.)
- Wehrman, M. E., M. S. Roberson, A. S. Cupp, F. N. Kojima, T. T. Stumpf, L. A. Werth, M. W. Wolfe, R. J. Kittok, and J. E. Kinder. 1993. Increasing exogenous progesterone during synchronization of estrus decreases endogenous 17- β estradiol and increases conception in cows. *Biol. Reprod.* 49:214–220.
- Wiltbank M. C., P. M. Fricke, S. Sangsritavong, R. Sartori, and O. J. Ginther. 2000. Mechanisms that prevent and produce double ovulation in dairy cattle. *J. Dairy Sci.* 83:2998–3007.
- Wishart, D. F. 1977. Synchronization of oestrus in heifers using steroid (SC 5914, SC 9880 and SC 21009) treatment for 21 days: The effect of treatment on the ovum collection and fertilization rate and the development of the early embryo. *Theriogenology* 8:249–269.
- Wolff, N. 1992. [Detection of embryonic mortality in cattle using sonography]. *Tieraerztl. Prax.* 20:373–380.
- Zavy, M. T. 1994. Embryonic mortality in cattle. Chapter 5 in *Embryonic Mortality in Domestic Species*. M. T. Zavy and R. D. Geisert, ed. CRC Press, Boca Raton, FL.
- Zelinski, M. B., P. Noel, D. W. Weber, and F. Stormshak. 1982. Characterization of cytoplasmic progesterone receptors in the bovine endometrium during proestrus and diestrus. *J. Anim. Sci.* 55:376–383.
- Zollers, W. G., H. A. Garverick, M. F. Smith, R. J. Moffatt, B. E. Salfen, and R. S. Youngquist. 1993. Concentrations of progesterone and oxytocin receptors in endometrium of *postpartum* cows expected to have a short or normal oestrous cycle. *J. Reprod. Fertil.* 97:329–337.