

## The Physiology of Pregnancy Loss; Female Factors

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Patterns of prenatal mortality vary among species, breeds, and herds and with nutritional factors and body condition within herds. Species variation is readily illustrated by the greater losses in lactating dairy cows than in heifers, dry dairy cows or lactating beef cows. In cattle, most losses occur within the first eight days after mating (Maurer and Chenault, 1983; Inskeep and Dailey, 2005) and the majority of the remainder by day 45, whereas losses in the sheep continue throughout pregnancy (Dixon et al., 2007) and recent data indicate that losses in goats are concentrated in the last third of gestation (Aimee Wurst, unpublished data, Lincoln Univ, Jefferson City, MO). One estimate of the distribution of pregnancy failures in cattle is illustrated in table 1.

**Table 1. Distribution of Pregnancy Failures in Cattle<sup>a</sup>**

Timing or Cause	Percentage of Losses
Fertilization failure	
Male factors	10
Ovum transport, etc.	8
Embryonic	57
Lethal genes	5
Rebred by mistake	1
Placentation	
Early	4
Late	12
Fetal	3

<sup>a</sup>From Inskeep and Dailey (2005).

The later in gestation that a loss occurs, the greater the economic loss associated with it. Total losses of no more than 4 to 5% can be expected in beef cattle or dairy heifers after diagnosis of pregnancy at 28 to 35 days, whereas lactating dairy cows lose an average of 12 to 14% between 28 and 45 days (Thatcher et al., 1994) or 30 and 60 days (Bamber et al., 2009). In the latter study, losses within 8 herds utilizing OvSynch protocols varied from 7.6 to 21.6%. Manipulations involved in protocols for synchronization of estrus and especially for synchronization of ovulation and fixed time insemination can increase losses in some cases in beef cattle, as well.

Several authors have reviewed the literature on early and late embryonic losses, as well as fetal losses, in cattle in the last several years (e.g., Thatcher et al., 1994; Inskeep, 2004; Inskeep and Dailey, 2005; Diskin et al., 2011; Geary et al., 2013). Rather than reiterate details of the many studies, this presentation will summarize the critical periods for loss and known causes of loss during those periods. The assumption will be made that bull fertility and technician competence are high, so that fertilization rate is high, exceeding 90% (Diskin et al., 2011). In several cases, the causes will be manifested during periods before the time of loss. Such cases likely involve genetic defects in the oocyte or early embryo. Bamber et al. (2009) estimated the heritability of pregnancy loss (from a sire-maternal grandsire model) as 0.49. Thus selection for survival after 30 days should be an effective tool. Many of the losses can be related to deficiencies or excesses of the steroid hormones, estradiol and progesterone, sometimes occurring naturally, and sometimes as a result of hormonal manipulations used to synchronize estrus or ovulation.

It is well established that progesterone is essential for both initiation and maintenance of pregnancy in the cow. Progesterone regulates the ovulation of a healthy oocyte, maintains uterine quiescence, and provides for nourishment and survival of the embryo and fetus. Its timely withdrawal allows both estrus and ovulation and normal parturition. Regulation of ovulation is accomplished by fine tuning of follicular development via negative feedback controlling pulse frequency of secretion of luteinizing hormone (LH). By timing uterine secretion of prostaglandins  $F_2\alpha$  and  $E_2$ , progesterone regulates luteal regression in a non-pregnant cycle (thus cycle length) and prepares the uterus for the establishment of pregnancy.

In a 2004 review, Inskeep summarized the relationship of embryonic losses to progesterone as follows: “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  $PGF_2\alpha$  can be both embryotoxic and luteolytic; if progesterone was not present before estrus, the uterus lacks receptors for progesterone.
3. Maternal recognition of pregnancy, d 14 through 17, when lower pregnancy rates have been associated with low progesterone and high estradiol- $17\beta$ .
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.”

#### *Follicle size, age and estrogen secretion*

The recent review by Geary et al. (2013) provides a thorough discussion of how age and diameter of the ovulatory follicle and its secretion of estrogen can affect fertility and early embryonic survival. Persistent follicles develop when progesterone is low because the pulses of LH from the anterior pituitary are very frequent, about 1 per hour, whereas the frequency of

those pulses is only about 1 per 4 hours during the normal mid-luteal phase when progesterone concentrations are relatively high. When these follicles ovulate, the oocyte is older than normal, and has been subjected to high concentrations of estradiol for a longer period than normal. The oocyte is highly fertilizable, but the resultant embryo is subject to death before the 16-cell stage (Ahmad et al., 1995; Figure 1). These findings led to the question, whether fertility might be greater in cows with three follicular waves during an estrous cycle, rather than the more frequent pattern of two waves. Such an effect was significant only in the period equivalent to a cycle after breeding in beef cows (Ahmad et al., 1997). In lactating dairy cows, pregnancy rate was 81% in those with three waves and only 63% in those with two waves, which ovulated larger and older follicles (Townson et al., 2002). Unfortunately, wave pattern was not repeatable from one cycle to the next in the beef cattle studies, thus selection for cows with three waves would not be effective.

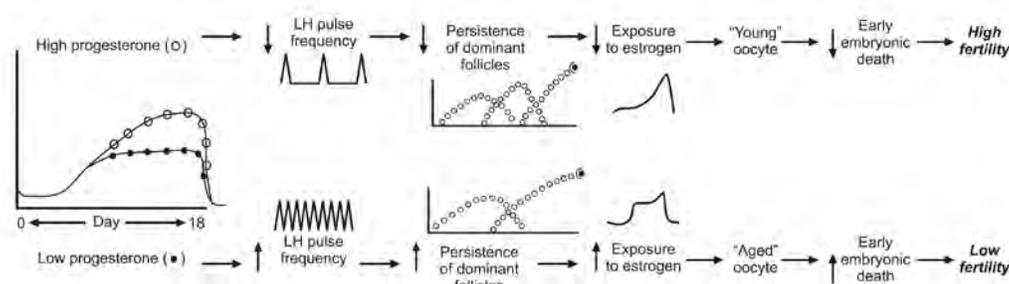


Figure 1. Negative effects of persistent follicles and the resultant greater exposure to estrogen on embryonic survival.

The negative effect of older follicles is why previous speakers at this meeting have emphasized techniques to insure ovulation of a relatively young oocyte at synchronized estrus. That is often very important in lactating dairy cows. However, the problem is rare in beef cows compared to lactating dairy cows, because milk production and associated feed consumption are lower, so that blood flow through the liver and metabolism of progesterone are less. Thus the need for pretreatment with gonadotropin releasing hormone (GnRH) to ovulate older follicles at the beginning of a synchronization protocol is limited in beef cows (and can be counterproductive in heifers) and the expected increase in conception rate from that practice is minimal if progesterone treatment is for only 5 to 7 days with prostaglandin F<sub>2α</sub> on the next to last day.

The high concentrations of LH and estrogen associated with low progesterone advance the stage of development/maturation of the oocyte and Israeli workers (Shaham-Albalancy et al., 2001) saw altered endometrial morphology and uterine hormone secretion in cows with low progesterone before estrus. However, Wehrman et al. (1996) did not find reduced survival of transferred embryos in recipients that had ovulated persistent follicles. Conversely, the oocyte and the reproductive tract need to see adequate estrogen to reach optimal maturity and receptivity. Status has been characterized in different studies by follicle size at ovulation or duration of proestrus. Studies by Perry and others at Missouri and Miles City have shown that smaller follicles ovulated naturally did not reduce fertility, whereas smaller follicles induced to ovulate prematurely by GnRH led to greater loss of early embryos (Perry et al., 2005). When

follicles ovulated by GnRH were smaller and produced less estrogen before ovulation, fewer of the cows expressed estrus, and the corpora lutea formed produced less progesterone than in cows induced to ovulate larger follicles or cows that ovulated small or large follicles spontaneously (Busch et al., 2008) (Figure 2).

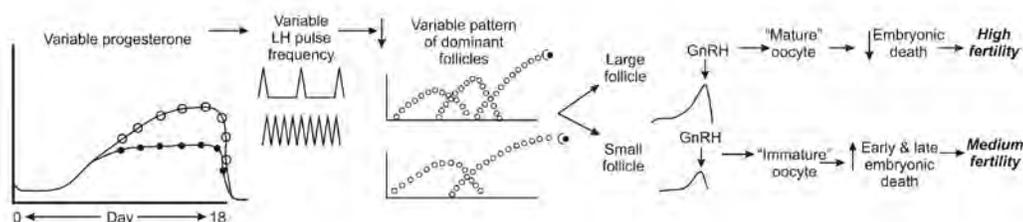


Figure 2. Negative effect of ovulating small follicles before cows exhibit estrus after a synchronization treatment.

Embryo transfer has been a useful tool to study the basis for losses in relation to whether they can be related to the oocyte, the fertilizing sperm, or the environment of the reproductive tract. For example, Farin et al. (2001) examined the fate of in vitro-produced embryos and concluded that loss after embryo transfer occurred often prior to day 21 and that losses after 2 months were relatively low (2 to 5%). Jinks et al. (2013) recently measured preovulatory follicle size and serum concentrations of estradiol in estrous synchronized, suckled beef cows that received GnRH after progesterone and were used as embryo donors or recipients in a factorial experiment. Serum estradiol was related positively to follicle size and to concentrations of progesterone at transfer. Transfers occurred from donors with high or low estradiol into recipients with high or low estradiol, creating 4 groups. Pregnancy rates were high, 61 to 63%, when estradiol was high in recipients and low, 43 to 45%, when estradiol was low in recipients: The only effect of concentrations of estradiol in the donors was that those with greater estradiol were more likely to yield an embryo than an unfertilized oocyte.

In a second experiment, Jinks et al. (2013) found that an injection of 500 micrograms of estradiol cyclopentyl-propionate one day before GnRH and AI increased pregnancy rate by approximately 10 percentage points in cows that were induced to ovulate a follicle less than 12.2 mm in diameter. In contrast with the earlier work by Wehrman et al. (1996), the effect of the estrogen appeared to become negative when the follicle exceeded 16 mm in diameter. There is probably an ideal amount of estradiol to prepare the uterus to receive an embryo. Too much estrogen may have more detrimental effects on the oocyte itself, based on earlier work in rats and the study by Ahmad et al. (1995) mentioned above.

Atkins et al. (2013) carried out an extensive analysis using a technique known as path analysis to examine factors in both the donor cows and the recipients that could influence success of transferred embryos in suckled beef cows subjected to fixed time AI at synchronized ovulation without exhibited estrus. As would be expected from the above discussion, greater concentrations of progesterone before luteal regression and greater ovulatory follicle size in the donor cow increased viability of embryos at transfer on day 7. Embryonic survival to day 27 was

greater in recipient cows with increased estradiol at the time of injection of GnRH and increased progesterone on day 7, but surprisingly was reduced by greater follicular diameter in the recipient cow at GnRH. Overall, the variables studied accounted for less than 12% of the variation in pregnancy at 27 days post ovulation. Simply put, there is still a lot to be learned about preovulatory factors that influence embryonic survival.

*Early secretion of prostaglandin F<sub>2α</sub> and embryotoxicity*

Work that has been reviewed in detail by Inskeep (1995, 2004, 2010) in several venues over the last two decades has led to a clear understanding of how progesterone regulates the length of the estrous cycle in ruminants and provides support for the early embryo. Not only does progesterone regulate pulse frequency of LH secretion and allow the rise in estrogen to trigger an LH surge when it is withdrawn by regression of the corpus luteum, but also it sets the timing for increased secretion of PGF<sub>2α</sub> by the uterus that leads to luteal regression. The short cycles that are common as ruminants begin to ovulate and show estrous cycles at puberty or during return from seasonal, postpartum, or lactational anestrus, allowed development of a model to compare factors affecting fertility. By pre-treating postpartum beef cows with progesterone, then weaning their calves or injecting human chorionic gonadotropin (hCG) or GnRH (hCG was more effective) at 30 days postpartum, the first cycle usually was normal in length, with a normal luteal phase and reasonably normal fertility. If cows were weaned or given hCG without pre-treatment with progesterone, a short luteal phase ensued and fertility was nil, despite the fact that the weaned cows showed estrus and ovulated 4 days after weaning (Figure 3). Transfers of embryos from cows with short or normal cycles into recipients with short or normal luteal phases revealed that there were negative effects on the embryos both before and after transfer. Thus uterine function was involved.

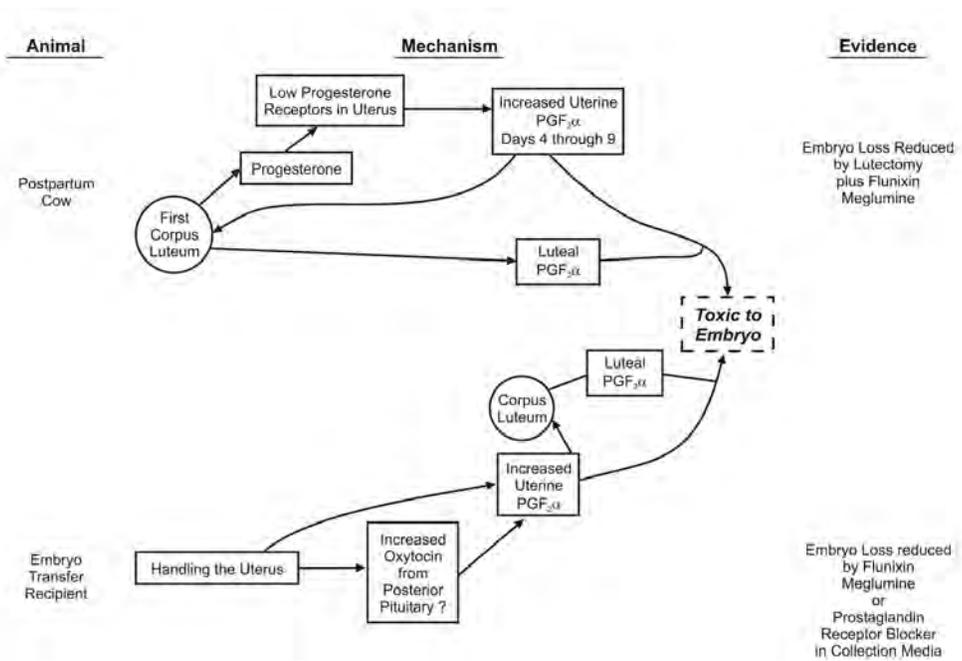


Figure 3. Embryotoxic effects of PGF<sub>2α</sub> during the morula to blastocyst transition are prevented by blocking uterine and luteal secretion of PGF<sub>2α</sub> or blocking receptors for PGF<sub>2α</sub> on the embryo itself.

From these studies, it was ultimately learned that premature uterine secretion of  $\text{PGF}_{2\alpha}$  was responsible for the short luteal phase and that supplementation with progesterone or other progestogens could not salvage the embryo unless the increase in  $\text{PGF}_{2\alpha}$  on days 4 through 9 after ovulation also was blocked by treatment with flunixin meglumine. Neal Schrick's group at Tennessee found that the embryotoxic effect of  $\text{PGF}_{2\alpha}$  was specific to the morula to blastocyst transition during days 5 to 8 and did not occur at later stages in cows supplemented with progestogen (reviewed by Inskip, 2004, 2010). Manipulation of the uterus, including handling it for embryo transfer, stimulates secretion of  $\text{PGF}_{2\alpha}$ . Further studies revealed a benefit in survival of transferred embryos from treatment with flunixin meglumine at transfer, and led to addition of a prostaglandin receptor inhibitor to the media used in transferring embryos to improve pregnancy rate (Roper et al., 2008; Scenna et al., 2008; Figure 3).

The Missouri group addressed the mechanism by which pre-treatment with progesterone regulated the first postpartum cycle to normal length in a series of experiments. They showed that the sequence of progesterone, progesterone withdrawal, and a rise in estrogen secretion at estrus induced uterine receptors for progesterone. These receptors were essential for the rising progesterone after ovulation to prevent the increased secretion of  $\text{PGF}_{2\alpha}$  in the early period of days 4 to 9, and set it to occur at the normal time of days 14 to 17 in the cow. Thus we now understand why conception rate is not severely compromised in beef cows that are in good body condition and induced to show estrus and ovulate by progesterone treatment for 5 days or more, after 30 days postpartum, relative to cows that are cycling. Still cows that have cycled more times before breeding are more fertile (Inskip, 2002), and in fixed-time insemination programs, cows or heifers that have shown estrus have greater pregnancy rates than those that are not in estrus.

#### *The “maternal recognition” period, days 14 to 17 post-estrus*

There has been a huge research effort over the last 5 decades to understand how the presence of an embryo signals the uterus and ovary to prevent luteal regression. We now know that the trophoblast of the embryo secretes a protein named interferon tau that causes a myriad of changes in secretion of other proteins by the uterus and ovaries that contribute to this process. In addition, there is a major increase in secretion of prostaglandins E (especially  $\text{PGE}_2$  has been measured), which begins around day 14 and helps to overcome the luteolytic effect of  $\text{PGF}_{2\alpha}$ . In sheep, that increase in  $\text{PGE}_2$  requires the presence of both progesterone and an embryo (Vincent and Inskip, 1986). When considered in relation to earlier data in various species, probably both  $\text{PGE}_2$  and  $\text{PGF}_{2\alpha}$  are required for the attachment and implantation processes. In fact, one interpretation would be that the intended function of  $\text{PGF}_{2\alpha}$  is to support attachment and placentation, and it becomes luteolytic only when an embryo is not present.

In fact, embryonic mortality during the maternal recognition period is relatively rare. It does occur when progesterone is low and allows increased follicular development so that there is increased secretion of estrogen during maternal recognition (reviewed by Inskip, 2004). In some studies in dairy cows, pregnancy rate was sometimes increased by ovulation or luteinization of the largest follicle, but treatment with GnRH or hCG during this period was not routinely valuable. High concentrations of estrogen were seen as contributors to loss of pregnancy in postpartum beef cows that had short luteal phases and were supplemented with

minimal dosages of progestogens after transfer of embryos on day 7. Pregnancy rates were inversely correlated with concentrations of estradiol on days 14 through 17 in lactating beef cows that had been inseminated at synchronized estrus. Further circumstantial evidence comes from the fact that fertility was greater in beef females with a three-wave pattern of follicular development during the equivalent of an estrous cycle after insemination than in those with a two-wave pattern during that period (Ahmad et al., 1997). Overall, one can conclude that there is an association of pregnancy loss with greater circulating concentrations of estrogen during maternal recognition, but neither the exact timing nor the mechanism by which estrogen interferes with the developing embryo have been established.

#### *Late embryonic and early fetal mortality; placentation as a critical period*

The high rate of late embryonic/early fetal loss in lactating dairy cows, mentioned above, has been seen within varying periods, depending on times of pregnancy diagnosis, but appears to be initiated during placentation and to be associated with lower concentrations of progesterone on days 28 to 37 (Inskeep, 2004). As pointed out by Dr. Michael Smith earlier in this conference (Smith et al., 2013), GnRH-induced ovulation of small dominant follicles led to not only greater early loss of embryos, but also an increased late embryonic/early fetal mortality in postpartum beef cows (Perry et al., 2005). Poorer quality embryos, younger cows, lower body condition, ovulation of premature oocytes, and manipulations associated with assisted reproductive technologies can all contribute to late embryonic/early fetal mortality. In a study in which new corpora lutea were induced during the placentation period, in cows in which the original corpora lutea had been removed on day 26 and pregnancy maintained in the interim by treatment with a progestogen, pregnancies were more likely to be maintained beyond that point if the new corpora lutea were induced after day 36 (Bridges et al., 2000). When the new corpora lutea were induced on days 28 to 31, they secreted more progesterone and tended to maintain more pregnancies when concentrations of PGF<sub>2</sub>α were greater and concentrations of estradiol were lower.

Losses after day 28 are the most costly because of the long delay until rebreeding. That means a younger, lighter weight calf at market and lower income with no reduction, or even an increase, in input costs. Therefore, knowing your herd performance before implementing a synchronization program, as pointed out earlier by Dr. Smith, is essential. It pays to know whether your cows are ready for a synchronized AI program and if there is any real need for a complex program. Simpler short-term treatment protocols with progesterone or PGF<sub>2</sub>α, or a combination of the two, and fewer times to handle the cows, are most often adequate in beef animals.

#### *Conclusions and recommendations; important management concepts*

Embryonic and early fetal losses are a significant factor in cattle reproductive management programs. To minimize such losses, it is important to have cows or heifers in good body condition, be well organized to conduct the treatments and inseminations, and know that facilities and labor are adequate to maintain the schedule and handle all animals efficiently. The guidelines for a successful program that were provided yesterday by Dr. Smith need to be followed carefully. Some important things to remember relative to embryonic and early fetal mortality in beef females are highlighted in Table 2.

**Table 2. Take-home Messages Relative to Embryonic and Fetal Mortality.**

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*Physiological factors associated with follicular development*

- Females that are in estrus are more fertile than those that are not, in fixed-time insemination programs.
- Concentrations of estrogen just before and at time of insemination affect pregnancy success.
- Diameter of the ovulatory follicle and duration of proestrus are indicators of estrogen exposure.
- Follicles that are “persistent” and too old secrete detrimental amounts of estrogen and yield defective oocytes that are fertilizable, but embryos die before the 16-cell stage.
- Ideal estrogen is secreted by a growing follicle that induces estrous behavior.
- Longer durations of progestogen treatment (e.g., 14 days or more) can lead to persistent follicles. Breeding at second estrus, especially in heifers, will yield greater fertility.
- High concentrations of estrogen during maternal recognition of pregnancy in females with a two-wave-type pattern of follicular development after breeding may contribute to losses.

*Preparation of both the uterus and ovaries in anestrous cows or prepubertal heifers*

- Treatment with progesterone or other progestogens for 5 to 7 days can induce estrus and a normal luteal phase in anestrous females. This treatment prevents early embryonic loss due to short luteal phases and embryotoxic effects of PGF<sub>2</sub> $\alpha$  that occur during the morula to blastocyst transition. From a management standpoint, this means that exposure of prepubertal heifers and anestrous cows to a progestogen will induce normal luteal function, so that a developing embryo is able to signal its presence and establish pregnancy.
- Animals must be in good body condition to respond to progestogen and show estrus.
- Programs without progestogen pretreatment or prior successful induction of a functional corpus luteum will not be effective in anestrous females.

*Placentation is a critical period*

- Elevated estrogen during days 30 to 35 of gestation can contribute to pregnancy loss.
- Late embryonic losses are increased in animals that were induced to ovulate a small follicle by treatment with GnRH.

*Fetal losses*

- Losses after onset of placentation are high in lactating dairy cows, but minimal in beef animals that are free of disease, except in reported cases of inappropriate vaccinations.

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