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Management Strategies to Reduce Embryonic Loss

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INTRODUCTION

Embryonic loss may represent the single greatest economic loss for cow/calf producers. With 40,000,000 beef cows and heifers exposed to breeding each year in the U.S., annual losses exceed $1.2 billion. The mechanisms involved in pregnancy establishment and maintenance are complex, and based on the literature, we have made little progress reducing embryo wastage in the past 90 years. This paper will focus on when and why pregnancy failures occur and discuss some management practices that may alleviate these losses.

In beef cattle, it is generally accepted that fertilization rates to a single service are between 90 and 100% regardless of whether natural service or artificial insemination is utilized (Sreenan and Diskin, 1983). Yet, rarely more than 70% of matings result in a positive pregnancy diagnosed 30 days later and even fewer result in a live birth. Embryonic losses are defined as those losses that occur from fertilization until day 42 of pregnancy when differentiation and implantation has occurred. Losses after day 42 are generally referred to as fetal losses. Embryonic losses are further divided into two categories and classified as Early Embryonic Mortality (**EEM**; fertilization to day 27) and Late Embryonic Mortality (**LEM**; day 28 to 42). The majority of embryonic mortality is early embryonic mortality, with rates ranging from 20 to 44% reported in beef cattle (Humbolt, 2001). Late embryonic mortality occurs in 3 to 14% of beef cows and heifers (Humbolt, 2001; Perry et al., 2005; unpublished data). A wealth of information exists on embryonic mortality in dairy cattle, but very little information exists in beef cattle. Maurer and Chenault, (1983) suggest the type of pregnancy failures among beef cows and heifers differ. In that study, the majority of pregnancy failures in heifers were due to fertilization failure rather than embryonic loss. The embryo or unfertilized egg recovery rate was lower among heifers than cows, suggesting that perhaps a portion of heifers failed to ovulate after being in heat. Accurate measurement of embryonic mortality is complicated by the fact that we are unable to assess pregnancies until approximately day 27 (via ultrasound or pregnancy specific blood indicators) without harvesting and collecting reproductive tracts at slaughter.

No single factor has been proven to prevent early embryonic mortality. However, if we could prevent embryo wastage in just 5 out of every 100 cows, we would wean an additional 2,100 pounds per 100 cows.

UNDERSTANDING THE ESTROUS CYCLE AND EARLY PREGNANCY

A review of the estrous cycle (Figure 1) and early pregnancy (Figure 2) in a cow might be helpful. On day 0 or 21 of the estrous cycle, the female is in heat, and one of her ovaries has a blister-like structure called a follicle that contains a mature egg. This follicle is producing high levels of estrogen that cause the cow to display behavioral signs of heat. On day 1, approximately 28 hours after the onset of heat, this dominant follicle ruptures. The egg is released and enters the oviduct, where fertilization may occur.

If fertilization does not occur, the unfertilized egg will travel through the oviduct reaching the uterus by day 7. With the release of the egg from the ovary, cells of the ruptured follicle reorganize (luteinize) and become luteal cells which grow and divide over the next 5 days, filling the follicular cavity on the ovary to form a structure called the CL (corpus luteum). The CL produces progesterone, which prepares the uterus for a pregnancy. Note that progesterone levels gradually rise from day 1 to 5 which blocks the release of hormones (including GnRH, luteinizing hormone (LH) and follicle stimulating hormone (FSH)) that allow final maturation of new follicles on the ovaries. When an embryo is not present in the uterus on day 17 of the estrous cycle, the uterus produces the hormone prostaglandin, which destroys the CL, decreasing progesterone levels. Low progesterone levels allow GnRH, FSH & LH to increase, and stimulate follicles on the ovaries to develop and mature. One follicle becomes dominant and produces estrogen, resulting in the reoccurrence of estrus (day 21).

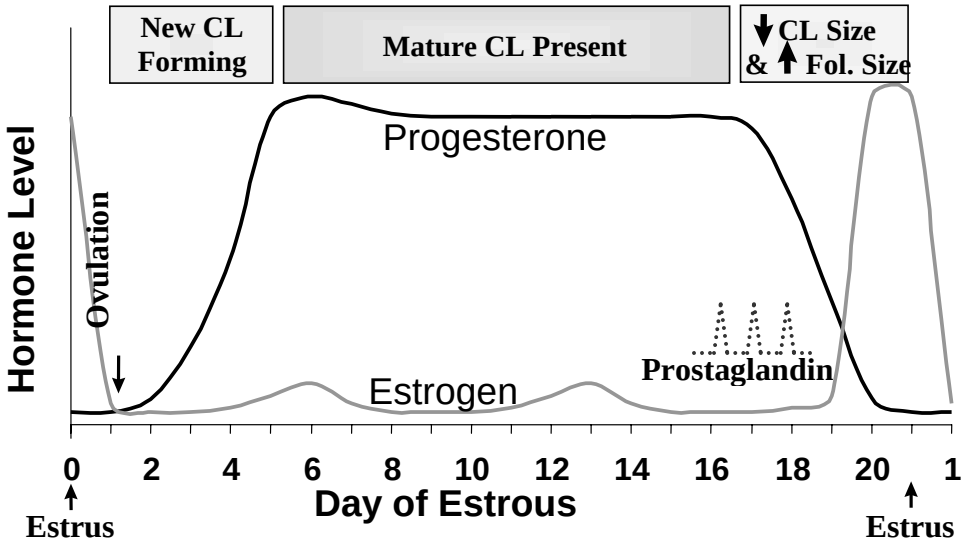

Figure 1. Illustration of the estrous cycle of a

If fertilization occurs, the fertilized embryo will divide and travel through the oviduct reaching the uterus by day 7, where an embryo will hopefully implant and develop into a calf. When an embryo reaches the uterus, it must continue to divide, elongate, and produce interferon-tau which blocks uterine prostaglandin production in a process referred to as maternal recognition of pregnancy. Embryos generally produce interferon-tau from day 14 to 23, but must at least produce sufficient quantities to prevent destruction of the corpus luteum by day 17 to 18 after estrus. Anything that impedes an embryo's growth or

production of interferon-tau or anything that interferes with the uterine ability to recognize this signal or block prostaglandin could disrupt maternal recognition of pregnancy and result in return to estrus, even though an embryo is present. If maternal recognition of pregnancy does not occur, the embryo will die and the cow will return to heat without any delay in the length of the estrous cycle.

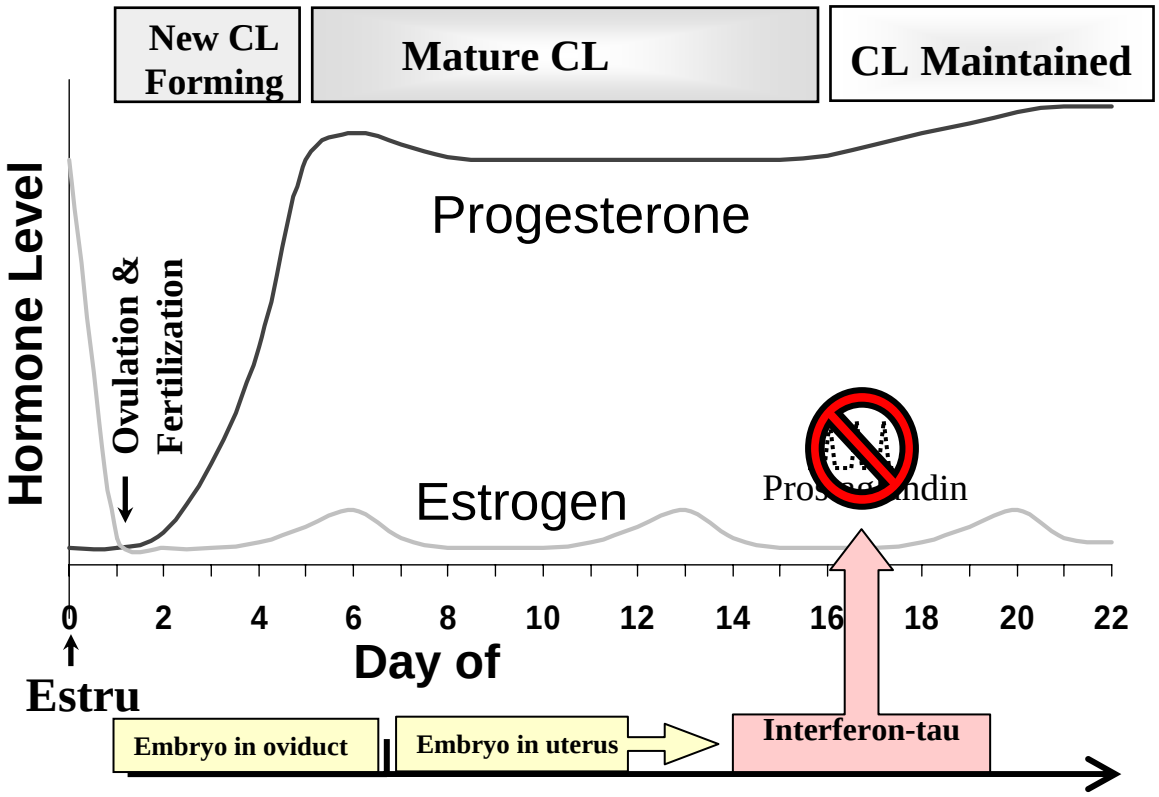

Figure 2. Illustration of early pregnancy in a cow.

GENETIC FACTORS AFFECTING EMBRYONIC LOSS

Genetic abnormalities account for approximately 10% of the embryonic losses and generally result in pregnancy failure within the first two weeks (King et al, 1990). Expression of lethal genes can cause death of the embryo within the first 5 days of pregnancy. Another genetic disorder contributing to embryonic death is an abnormal chromosomal number in some or all of the embryonic cells. This is the most common genetic abnormality and generally results in embryonic death. Causes of abnormal chromosome numbers include polyspermy (when more than one sperm fertilizes an egg), and meiotic errors within the gametes or developing embryo. Higher incidences of polyspermy can occur when artificial inseminations occur nearer the time of ovulation as opposed to the optimal 12 h after the onset of estrus (Saacke et al., 2000). Insemination nearer the time of ovulation actually results in higher fertilization rate, but poor embryo quality. Inseminations nearer the onset of estrus result in lower fertilization rates, but higher embryonic survival. Together, this suggests that some natural selection for fertilization by the “fittest” sperm is

important. Higher incidences of polyspermy and increased chromosomal numbers have also been reported following insemination of super-ovulated cows (King, 1985).

Genetic abnormalities can also cause fetal mortality (after day 42 of gestation). Fetal mortality was increased among inbred relative to non-inbred Hereford cattle. Further, as the level of inbreeding increased, so did the level of fetal mortality (MacNeil et al., 1989). The exact stage of pregnancy when fetal mortality occurred in the above study is not known. Cundiff et al., (1974) reported that calf crop was 6.4% greater for crossbred cows than for straight-bred cows with this advantage resulting from differences in pregnancy and first service conception rates. Both lines of evidence suggest that crossbreeding should, and probably does, result in increased embryonic and fetal survival.

NUTRITIONAL FACTORS AFFECTING EMBRYONIC LOSS

Effects of Energy and Protein. Gross nutritional deficiencies in diets of breeding animals are likely to have detrimental effects on fertility. There is very little experimental data dealing with nutrition which sorts out cycling status from fertilization rate from embryonic mortality. However, we know that dietary energy and protein levels play a role in pregnancy success. It has long been known that cows bred when they are gaining weight (increasing body condition score) have higher pregnancy rates than cows bred when they are losing weight (Wiltbank et al., 1962). Decreased progesterone levels following breeding may be responsible for the decreased fertility / increased embryonic mortality among cows bred during a negative energy balance (Folman et al., 1973). Cows can be managed to be gaining body condition by scoring cows at or shortly after calving and adjusting diets accordingly. Cows with high milk production may experience negative energy balance and increased embryonic mortality when forage conditions are limiting, thus producers should be careful not to select for excess milk in their cowherds.

Excess protein (especially rumen degradable protein) has been demonstrated to increase embryonic mortality in cows (Blanchard et al., 1990; Elrod and Butler, 1993; Elrod et al., 1993). Increased blood urea nitrogen and ammonia are the results of increased protein metabolism by the rumen, and decreased uterine pH on day 7 following estrus. These studies suggest that both fertilization rates and early embryo survival (before day 17) are compromised and that altered hormone production (primarily progesterone) and a more acidic uterine pH are the likely causes. Cattle on pasture are rarely exposed to excess protein in their diets. Extreme cases of excess protein may occur when cattle graze alfalfa or wheat pastures. Beneficial effects of protein supplementation have been reported for cattle fed rumen by-pass protein during the breeding season (Patterson et al., 2003; Wamsley et al., 2005). The exact mechanism(s) by which increased undegradable intake protein improves fertility is unknown, but is likely related to decreased embryonic mortality. Feeding fishmeal has also been demonstrated to suppress oxytocin induced prostaglandin secretion in heifers with low progesterone concentrations suggesting it may improve an embryo's ability to signal maternal recognition of pregnancy (Wamsley et al., 2005).

Effects of Toxins. Some of the more common plant toxins in our area that can cause reproductive problems include mycotoxins, endophyte infected fescue, nitrates, locoweed,

and ponderosa pine. Mycotoxins can occur in moldy feed and one mycotoxin, zearalenone, is suspected to cause abortions in cattle by decreasing progesterone concentrations. Avoiding or limiting the amount of moldy feed in the diet of pregnant cows is the simplest way to avoid this problem.

Fescue toxicity is not as prevalent in our area, but is a common problem for cattle producers south and east of here. The ergot alkaloid, ergovaline is the primary compound in endophyte infected tall fescue and is reported to delay onset of estrus, and reduce embryonic survival by inhibiting progesterone synthesis sufficiently to affect pregnancy maintenance (Porter and Thompson, 1992). Producers are advised to avoid grazing high endophyte infected fescue pastures before and during the breeding season.

Nitrate itself is not particularly toxic to animals. Nitrates consumed by ruminants are normally reduced to ammonia and then absorbed and excreted, thus converted by bacteria into bacterial protein. Nitrate poisoning occurs when nitrate consumption in feed and water is sufficient such that nitrates are converted to nitrites. Nitrites bind to hemoglobin and decrease its oxygen carrying capacity, which could lead to embryonic death in less severe cases. Because of the mechanism by which nitrate poisoning occurs, cattle that are suspected to have been poisoned should be handled as little and as quietly as possible to minimize their oxygen needs. Elevated nitrates are often found in plants that are stressed by poor growing conditions such as drought, cool and cloudy weather, hail damage and frost. Plants such as oats, millet, sorghum, sudan, and corn are especially susceptible to high nitrates and should be tested for nitrate content before grazing/feeding. Diets of pregnant cows should not exceed 5,000 ppm nitrates on a dry matter basis (Brownson and Zollinger, 2003).

Locoweed is a common weed in our area that can have detrimental effects on all aspects of livestock production. Ingestion of locoweed by all classes of livestock is common because of its palatability. The toxin in locoweed (swainsonine) prevents cellular carbohydrate metabolism and acts as a vasoconstrictor decreasing blood flow to the uterus. Exact mechanisms by which it causes embryonic loss are not known, but it likely affects all aspects of reproduction. Cattle are most likely to consume locoweed during early summer when succulent pods appear on the plants. Producers should avoid grazing locoweed infested areas if possible and monitor calves of cows for signs of locoweed consumption and toxicity of their dams as swainsonine is concentrated in milk.

Pine needle abortion is common in western rangelands when pregnant cattle eat the pine needles of ponderosa pine trees. Isocupressic acid has been identified as the abortifacient in pine needles and pine bark that cows sometimes consume during late fall, winter and early spring storms. Cattle can develop a taste for the pine needles. The mechanism by which isocupressic acid induces abortions is by decreasing blood flow to the uterus and inducing parturition similar to a natural delivery (Ford et al., 1992). While the abortions generally occur during the third trimester of pregnancy, sufficient consumption of ponderosa pine needles can cause abortion during the second trimester as well (Short et al., 1992). Cattle fed high protein diets did not change their consumption of pine needles, but the incidence of abortion was reduced (Short et al., 1994). These researchers also reported that cows fed corn silage did not consume pine needles. As with all toxic plants, it is generally

best to develop a grazing plan that allows cattle to avoid consumption of the plants when their toxins are highest or the plants themselves are more palatable and likely to cause detrimental effects.

ENVIRONMENTAL INFLUENCES ON EMBRYO MORTALITY

Numerous studies have focused on the effects of heat stress on reproduction in dairy cows (especially high producing dairy cows). Heat stress is not consistently defined in the literature, but generally refers to temperatures between 90 and 110° F and relative humidity above 40%. Heat stress is something that has not been studied much in beef cattle and we may not need to be too concerned about it in this area. However, cattle in our area are occasionally exposed to these types of temperatures for at least 8 hours per day, and effects such as longer hair coats, thicker and darker hides than Holsteins, along with higher elevations, direct sunlight, and later breeding seasons may result in heat stress conditions in some cow herds. In dairy cattle, short term heat stress at the time of breeding or within the 1st week after breeding appears to be the most deleterious time for elevated temperatures, as it results in delayed embryonic development that eventually becomes "out of sync" with its maternal environment. Cows exposed to heat stress from day 8 to 16 after breeding had decreased progesterone concentration and increased uterine prostaglandin secretion (Biggers et al., 1987; Geisert et al., 1988).

Handling stress may be something more beef cattle are exposed to during the breeding season in this area than heat stress. Producers who use AI, for example, sometimes transport cattle to pastures after breeding, which may affect pregnancy establishment (Harrington et al., 1995). Heifers that were transported for 6 hours either 8 to 12 or 29 to 33 days after AI had lower pregnancy rates than heifers that were transported 1 to 4 days after AI, suggesting that the transportation stress resulted in embryonic loss. We have conducted 3 additional studies in this area to determine the mechanism by which transportation stress is creating embryonic loss and prevention of that loss. Because placentation is well under way by day 29 after breeding, elevated prostaglandin as a result of transportation stress seemed like the most logical method by which this stress might affect embryonic survival during day 8 to 12 and 29 to 33 pregnancies. A single administration of the prostaglandin inhibitor Flunixin Meglumine (active ingredient in Banamine) to cows and heifers that received transportation stress approximately 13 d after AI resulted in suppressed prostaglandin in the bloodstream and increased pregnancy rates (Merrill et al., 2003; 2004). Administration of Flunixin Meglumine to cows in the absence of transportation stress approximately 13 days after AI did not increase pregnancy rates, while administration of Flunixin Meglumine to heifers in the absence of transportation stress approximately 13 days after AI decreased pregnancy rates (Geary et al., 2005). Our best interpretation of this data is that gathering and handling cattle through working facilities to administer an injection is perceived as being more stressful by heifers than by cows. Whether handling heifers to move them to a new pasture during the breeding season creates enough stress to affect embryonic mortality is unknown, but is probably also dependent on the stage of pregnancy for the majority of heifers in the herd. It is likely, however, that working heifers through a chute to conduct an early pregnancy diagnosis (for example 35 days after a synchronized AI) may affect

embryonic mortality of especially those heifers that may have conceived to a return estrus (anticipated to be approximately 14 days pregnant).

MISCELANEOUS FACTORS AFFECTING EMBRYONIC LOSS

Role of Progesterone. Progesterone is absolutely essential for the establishment and maintenance of pregnancy. If a cow or heifer does not produce enough progesterone, the pregnancy will be lost. There are two conditions when cows produce lower levels of progesterone after being in heat. The first condition is with a shortened estrous cycle (usually 8 to 12 days) in which cows have a short-lived CL that regresses before and embryo would have time to signal its presence. Most cows have a short lived CL following their first ovulation after calving. The only proven way to avoid short cycles is to mimic this event using progesterone (such as with a CIDR) or GnRH about one week before an injection of prostaglandin to induce cyclicity. The other incidence of reduced progesterone occurs in cows that have a normal length estrous cycle, but lower levels of progesterone, and whose uterus is less responsive to the embryonic signal for maternal recognition and more sensitive to the release of prostaglandin. Recent studies suggest that low progesterone concentrations during the previous estrous cycle precondition the uterus to release prostaglandin even in the presence of a developing embryo (Shaham-Albalancy et al., 2001). In addition, lower concentrations of progesterone during the previous estrous cycle may increase the incidence of ovulation of persistent follicles, whose eggs are "aged". These eggs are fertilizable, but early embryonic death is high and usually occurs before the 16-cell stage (Ahmad et al., 1995). Ovulation of "less mature" eggs or ovulation of smaller follicles can also result in reduced pregnancy rates and subsequent progesterone concentrations (Perry et al., 2005). New CIDRs used for synchronization of estrus generally release sufficient progesterone to create follicular turnover and ovulation of a healthy follicle, but **"used"** CIDRs release less progesterone that may not cause follicular turnover and result in ovulation of persistent follicles. Several studies have been aimed at increasing progesterone concentrations after breeding with a CIDR insert or administration of GnRH or hCG to create an accessory CL. Most of these techniques have been successful at increasing blood progesterone concentrations, but their ability to improve embryo survival and pregnancy rates have been inconsistent. A part of the variability in pregnancy response is that only cows with low endogenous progesterone concentrations would be expected to benefit from these treatments.

Effect of Age. A lot of factors may be lumped into an effect of age on embryonic loss. Heifers are generally considered to have higher pregnancy rates, and this increase seems to be associated with less embryonic mortality than cows. Among dairy cattle, both early and late embryonic losses increased among cows with increasing age (Humbolt, 2001; Starbuck et al., 2004). This increase in embryonic loss was also associated with increased milk production and decreased circulating concentrations of progesterone. Increased milk production in dairy cows is often associated with increased feed intake and metabolic clearance of progesterone, which are not likely major occurrences among beef cows in range environments. Thirty years ago, Erickson et al., (1976) reported no change in beef cow fertility with age. However, with increased demands placed on beef cattle in some environments today, they may respond with decreased fertility more like dairy cows.

Effect of the Male. There is known variation in fertility among sires, most of which we are unable to detect with traditional breeding soundness exams. A portion of this variation appears to be related to bulls with higher incidences of embryonic mortality rather than fertilization ability of their sperm. Use of bulls that have uncompensable semen traits such as misshapen heads or nuclear vacuoles results in reduced embryo cleavage rates and increased embryonic mortality (Saacke et al., 2000). Most uncompensable semen traits would not be detected during a routine breeding soundness exam, but semen collection studs these traits to identify bulls with these traits, as they cannot compensate for decreased fertility among these bulls by adding more spermatozoa into AI straws.

Infectious Agents. Today, producers are more aware of infectious agents that cause abortions or decreases in fertility. This paper could adequately cover all of the causative organisms, their etiology, or their net effects on a herd. Appropriate vaccination and herd health programs vary too greatly from one operation to the next, and producers should work with local veterinarians to determine the best program for your operation.

SUMMARY

Regretfully, there are no "silver bullets" for eliminating embryonic mortality. Hopefully, a better understanding of some of the factors involved and the likely causes of embryonic mortality will enable us to limit its effect in our herds. The most important concept here is that anything that can affect early embryo divisions and growth will likely affect its synchrony with its maternal environment and decrease its ability to produce adequate signal in time for maternal recognition of pregnancy. In addition, we have preached for years the principle founded by Byerley et al., (1987) that the fertility of a heifer's third estrus is greater than her first estrus. This principal is probably also true for cows following calving, that it takes a female's entire reproductive system a couple of estrous cycles to be fully prepared for establishment and maintenance of a pregnancy.

LITERATURE CITED

Ahmad, N., F. N. Schrick, R. L. Butler, and E. K. Inskeep. 1995. Effect of persistent follicles on early embryonic losses in beef cows. Biol. Reprod. 52:1129-1135.

Biggers, B. G., R. D. Geisert, R. P. Wetteman, and D. S. Buchanan. 1987. Effect of heat stress on early embryonic development in the beef cow. J. Anim. Sci. 64:1512-1518.

Blanchard, T., J. Ferguson, L. Love, T. Takeda, B. Henderson, J. Hasler, and W. Chalupa. 1990. Effect of dietary crude-protein type on fertilization and embryo quality in dairy cattle. Am. J. Vet. Res. 51:905-908.

Brownson, R. and B. Zollinger. 2003. Nitrates in cattle feed and water (CL355) In Cow-Calf Management Guide and Cattle Producer's Library, 2nd edition. University of Idaho, Moscow.

Byerley, D. J., R. B. Staigmiller, J. G. Berardinelli, and R. E. Short. 1987. Pregnancy rates of beef heifers bred either on puberal or third estrus. J. Anim. Sci. 65:645-650.

Cundiff, L. V., K. E. Gregory, and R. M. Koch. 1974. Effects of heterosis on reproduction in Hereford, Angus and Shorthorn cattle. J. Anim. Sci. 38:711-727.

Elrod, C. C. and W. R. Butler. 1993. Reduction of fertility and alteration of uterine pH in heifers fed excess ruminally degradable protein. J. Anim. Sci. 71:694-701.
Elrod, C. C., M. Van Amburgh, and W. R. Butler. 1993. Alterations of pH in response to increased dietary protein in cattle are unique to the uterus. J. Anim. Sci. 71:702-706.
Erickson, B. H., B. H. Reynolds, and R. L. Murphree. 1976. Ovarian characteristics and reproductive performance of the aged cow. Biol. Reprod. 15:555-560.
Folman, Y., M. Rosenberg, Z Herz, and M. Davidson. 1973. The relationship between plasma progesterone concentration and conception in postpartum dairy cows maintained on two levels of nutrition. J. Reprod. Fert. 34:267-278.
Ford, S. P., L. K. Christenson, J. P. Rosazza, and R. E. Short. 1992. Effects of ponderosa pine needle ingestion on uterine vascular function in late-gestation beef cows. J. Anim. Sci. 70:1609-1614.
Geary, T. W., R. P. Ansotegui, A. J. Roberts, R. C. Waterman, M. D. MacNeil, E. E. Grings, B. D. Thompson, and R. J. Lipsey. 2005. Effects of Flunixin Meglumine on pregnancy establishment in beef cattle. Proc. West. Sec. Am. Soc. Anim. Sci. 56:309-311.
Geisert, R. D., M. T. Zavy, and B. G. Biggers. 1988. Effect of heat stress on conceptus and uterine secretion in the bovine. Theriogenology 29:1075-1082.
Harrington, T.E., M.E. King, H.E. Mihura, D.G. LeFever, R. Hill, and K.G. Odde. 1995. Effect of transportation time on pregnancy rates of synchronized yearling beef heifers. Colorado State University Beef Program Report. Fort Collins, CO.
King, W. A. 1985. Intrinsic embryonic factors that may affect survival after transfer. Theriogenology 23:161-174.
Humbolt, P. 2001. Use of pregnancy specific proteins and progesterone assays to monitor pregnancy and determine the timing, frequencies and sources of embryonic mortality in ruminants. Theriogenology 56:1417-1433.
MacNeil, M. D., D. D. Dearborn, L. V. Cundiff, C. A. Dinkel, and K. D. Gregory. 1989. Effects of inbreeding and heterosis in Hereford females on fertility, calf survival and preweaning growth. J. Anim. Sci. 67:895-901.
Maurer, R. R. and J. R. Chenault. 1983. Fertilization failure and embryonic mortality in parous and nonporous beef cattle. J. Anim. Sci. 56:1186-1189.
Merrill, M. L., R. P. Ansotegui, N. E. Wamsley, P. D. Burns, and T. W. Geary. 2003. Effects of flunixin meglumine on embryonic loss in stressed beef cows. Proc. West. Sec. Am. Soc. Anim. Sci. 54:53-56.
Merrill, M. L., R. P. Ansotegui, J. A. Paterson, and T. W. Geary. 2004. Effect of Flunixin Meglumine on early embryonic mortality in stressed beef females. Proc. West. Sec. Am. Soc. Anim. Sci. 55:304-307.
Perry, G. A., M. F. Smith, M. C. Lucy, J. A. Green, T. E. Parks, M. D. MacNeil, A. J. Roberts, and T. W. Geary. 2005. Relationship between follicle size at insemination and pregnancy success. Proc. Nat. Acad. Sci. 102:5268-5273.
Porter, J. K. and F. N. Thompson. 1992. Effects of fescue toxicosis on reproduction in livestock. J. Anim. Sci. 70:1594-1603.
Saacke, R. G., J. C. Dalton, S. Nadir, R. L. Nebel, and J. H. Bame. 2000. Relationship of seminal traits and insemination time to fertilization rate and embryo quality. Anim. Reprod. Sci. 60-61:663-677.

Shaham-Albalancy, A. Y. Folman, M. Kaim, M. Rosenberg, and D. Wolfenson. 2001. Delayed effect of low progesterone concentrations on bovine uterine PGF2a secretion in the subsequent oestrous cycle. Reproduction 122:643-648.

Short, R. E., L. F. James, K. E. Panter, R. B. Staigmiller, R. A. Bellows, J. Malcolm, and S. P. Ford. 1992. Effects of feeding ponderosa pine needles during pregnancy: Comparitive studies with bison, cattle, goats, and sheep. J. Anim. Sci. 70:3498-3504.

Short, R. E., R. A. Bellows, R. B. Staigmiller, and S. P. Ford. 1994. Pine needle abortion in cattle: Effects of diet variables on consumption of pine needles and parturition response. J. Anim. Sci. 72:805-810.

Starbuck, M. J., R. A. Dailey, and E. K. Inskeep. 2004. Factors affecting retention of early pregnancy in dairy cattle. Anim. Reprod. Sci. 84:27-39.

Wamsley, N. E., P. D. Burns, T. E. Engle, and R. M. Enns. 2005. Fish meal supplementation alters uterine prostaglandin $F_{2\alpha}$ synthesis in beef heifers with low luteal-phase progesterone. J. Anim. Sci. 83:1832-1838.

Wiltbank, J. N., W. W. Rowden, J. E. Ingalls, K. E. Gregory, and R. M. Koch. 1962. Effect of energy level on reproductive phenomena of mature Hereford cows. J. Anim. Sci. 21:219-225.