

Uterine Function and Embryo Survival In Cattle

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INTRODUCTION

Fertilization rates are high in cattle, when pregnancy fails to occur a major contributor is uterine deficiency resulting in embryonic mortality. Unequivocally, proper uterine function is required for the establishment and maintenance of pregnancy. Unlike in primates and mice, in cattle attachment of the conceptus and placentation does not occur immediately following fertilization, but rather the conceptus spends a prolonged period within the uterine lumen before attaching to the uterine endometrium (Bazer et al., 2009). During this period, the embryo is dependent upon the uterus and its secretions to provide an adequate microenvironment for continued development and eventual attachment (Bazer, 1975). Optimal uterine function requires specific and sequential changes in the uterine gene expression (transcriptome) to ensure the uterine endometrium can produce the requisite secretions and is receptive to conceptus adhesion, attachment, and eventual placentation. To facilitate the progression of events obligatory for conceptus survival and attachment, coordination and communication amongst the ovarian steroids estradiol and progesterone, the uterine endometrium, and elongating conceptus

are required.

Unfortunately, uterine dysfunction occurs in cattle and the instances of uterine deficiencies appear to be exacerbated in certain populations. Most notably, both beef and dairy cattle that have reduced serum concentrations of estradiol prior to ovulation and(or) fail to have a rapid increase of progesterone following ovulation appear to have altered uterine function that results in embryonic mortality. The objective of this review is to discuss causes of these insufficiencies and explore how uterine deficiencies impede pregnancy establishment in livestock, with emphasis on cattle.

UTERINE CONTRIBUTIONS TO PREGNANCY FAILURE IN LIVESTOCK

Pregnancy failure in cattle can be due to either failure to fertilize the oocyte or pregnancy loss during gestation. Although fertilization rates in beef and dairy cattle have varied widely across reports, when insemination occurs at the appropriate time relative to ovulation, fertilization failure appears to represent only a minor proportion of pregnancy failure. In an early review in beef cattle, Ayalon (1978) summarized that fertilization failure ranged between 0.0 to 3.4% in first service heifers and between 15.0 to 17.0% in normal cycling cows. Several studies have since reported fertilization rates in beef cattle ranging from 76 to 94% (Diskin and Sreenan, 1980; Smith et al., 1982; Maurer and Chenault, 1983; Ahmad et al., 1995). In dairy cattle, fertilization failure is slightly greater than in beef cattle. Various studies have reported fertilization rates in lactating and non-lactating dairy cattle from 67 to 90% (Dejarnette et al., 1992; Dalton et al., 2001; Sartori et al., 2002; Cerri et al., 2009), with the exception of heat stressed dairy cattle (55.3%; Sartori et al., 2002). Collectively, these data indicate that fertilization rates in both beef and dairy cows are high and likely represent only a small proportion of pregnancy failure.

Although fertilization rates are approximately 90% in beef cows and between 75 to 85% in dairy cows, by d 30 of gestation viable embryos are present in only 50 to 60% of beef and 30 to 40% of dairy cows that are inseminated following an estrous and(or) ovulation synchronization program. This indicates that significant embryonic loss is occurring in both classes of animals. The exact timing of early embryonic loss and the proportion of pregnancies that fail due to early embryonic loss varies greatly between reports, and severity is dependent upon numerous factors such as animal age, parity, lactational status, stress, and type of cattle (i.e., beef or dairy). A variety of factors contribute to embryonic losses in livestock. Indeed, genetic defects, reproductive diseases, animal health, and heat stress all influence the probability of embryonic survival. Furthermore, a considerable proportion of losses occur prior to blastocyst hatching. In lactating dairy cows, pre-hatching embryos are often compromised, albeit the proportion of non-viable embryos collected varied considerably between studies (Sartori et al., 2010). In a summary of various studies (Sartori et al., 2010), the percentage of non-viable embryos collected from d 5 to 7 of gestation ranged from 33.3 to 71.4%. Ovulation of a compromised oocyte and(or) insufficient oviductal support of the developing embryo likely contributed to these losses. Although pre-hatching embryonic death or compromised development is high in lactating dairy cows following blastocyst hatching, during conceptus elongation, and through the period of conceptus attachment to the uterine endometrium, additional embryonic loss occurs in cattle that can be attributed to inadequate uterine function. Moreover, if uterine deficiencies were not prominent, placing a good quality d 7 embryo into a selected recipient would alleviate embryonic losses, due to embryo transfer overcoming the deficiencies in fertilization, oocyte viability, and oviductal function. Unfortunately, considerable pregnancy loss still occurs in cattle following embryo transfer. Therefore, not to negate the

importance of oocyte maturation and oviductal function, the focus of this review is on how deficient uterine function results in these losses and the mechanisms by which they are possibly occurring. Likely avenues for uterine deficiencies include: 1) failure of ovarian steroids to elicit the proper sequential regulation of factors in the uterine endometrium, 2) alterations in steroid receptor populations and the progressive up- and down-regulation of genes in the uterine endometrium responsible for delivering the nutrients and signals to the developing conceptus and preparing the endometrium to be receptive to the attaching conceptus, and 3) the inability of the uterus to respond to embryonic cues related to maternal recognition of pregnancy or elicit the production of various uterine gene products at the appropriate time.

ENDOCRINE AND UTERINE DYNAMICS REQUIRED FOR PREGNANCY ESTABLISHMENT

To recognize uterine deficiencies resulting in embryonic mortality, understanding the normal progression of events required for proper uterine receptivity to the implanting conceptus is required. The ewe has been the livestock model most commonly used to investigate the uterine factors involved in pregnancy establishment. Recently however, more information regarding uterine biology in cattle has become available. Therefore, inferences to the uterine biology of the ewe and the cow will be used in this review, recognizing some differences in uterine biology exist between these species. Furthermore, the intent of this review is not to outline in great detail the dynamic changes that occur in the uterine endometrium during early gestation that are mandatory for pregnancy establishment. This area has been previously reviewed by Spencer et al. (2004) and Bazer et al. (2009). Rather, this review will provide a brief synopsis of those events necessary to establish a baseline from which dysfunction can be identified.

Hormonal Requirements for Pregnancy Establishment

The sequential exposure to progesterone before estrus, elevated estradiol concentrations at estrus, and sufficient progesterone concentrations in the subsequent estrous cycle are necessary to establish the appropriate uterine environment for conceptus survival (Miller and Moore, 1976a,b, 1983; Moore, 1985; Wilmut et al., 1986). Estradiol, during the preovulatory period, is believed to ‘program’ the uterus in preparation for the conceptus by modifying cellular morphology, preparing secretory organelles, and regulating steroid receptor amounts and localization. Early experiments with ovariectomized ewes (Miller and Moore, 1976a,b) established the importance of adequate preovulatory estradiol for embryonic survival and uterine function. In steroid-treated ovariectomized ewes, when estradiol concentrations equivalent to those achieved at estrus were omitted, normal embryos were not recovered 21 d following synchronous embryo transfer (Miller and Moore, 1976a). Omitting estradiol also decreased uterine weight, rate of uterine protein synthesis, and the ratio of total RNA to total DNA in the uterus compared with animals that received adequate estradiol (Miller and Moore, 1976b). Recently, Roberts et al. (2012) demonstrated similar results with ovariectomized cows. Ovariectomized cows that did not receive estradiol to simulate the preovulatory period were able to maintain pregnancy through d 21 at a similar proportion of gestation as those that received either estradiol cypionate or estradiol benzoate to simulate the preovulatory period, but by d 29, cows not receiving estradiol had decreased pregnancy rates. Several studies in cattle have also associated the amount of estradiol received during the preovulatory period with subsequent fertility. A positive relationship between estradiol concentrations at induced ovulation and pregnancy success in beef cattle and dairy cattle (Vasconcelos et al., 2001; Perry et al., 2005; Lopes et al., 2007) have been reported. Various other reports have also demonstrated that

induced ovulation of smaller than normal follicles in cattle result in a reduction in pregnancy success (Pohler et al., 2012). Given the strong correlation between follicular diameter and follicular estradiol production (Ireland and Roche, 1982; Kruip and Dieleman, 1985), it is likely that in association with reduced follicle size, a reduction of preovulatory estradiol concentrations contributed to the reduction in fertility observed. It must be recognized that increases in ovulatory follicle diameter and circulating concentrations of estradiol prior to ovulation influence progesterone concentrations in the subsequent estrous cycle (Vasconcelos et al., 2001; Perry et al., 2005; Bridges et al., 2010), which can also contribute to embryonic survival.

Physiologically, the minimum concentration of progesterone needed to support pregnancy has not been determined (Mann and Lamming, 1999), but maintenance of a functional corpus luteum is paramount to a successful pregnancy. Several observational studies have associated progesterone concentrations during early gestation with increased embryonic and conceptus development in cattle (Mann and Lamming, 2001; Green et al., 2005), with additional studies demonstrating that supplementation of progesterone during early gestation (i.e., d 1 to 9) advanced conceptus growth and affected uterine secretions (Garrett et al., 1988; Mann et al., 2006). Studies with dairy cows have demonstrated that progesterone concentrations during early gestation are positively associated with embryonic survival (McNeill et al., 2002; Stronge et al., 2005). Furthermore, Kenyon et al. (2012) demonstrated that change in progesterone concentrations from d 0 to 7 was associated with pregnancy outcomes following embryo transfer on d 28, 42, and 63 of gestation and progesterone change from d 7 to 14 was associated with pregnancy outcome on d 28 and 63 of gestation. More recently, Atkins et al. (2012) reported that progesterone concentrations at embryo recovery on d 7 after estrus directly influenced embryonic developmental stage. In ovariectomized ewes exogenously treated with steroids, excluding

progesterone during early gestation resulted in the recovery of no embryos, reduced uterine protein synthesis, and decreased the ratio of total RNA to total DNA in the uterus (Miller and Moore, 1976b). Indisputably, both elevated concentrations of estradiol around ovulation and adequate concentrations of progesterone during gestation are required for conceptus survival and optimal uterine function in all livestock species.

Although it has been observed that increased preovulatory estradiol concentrations and increased post-ovulatory progesterone concentrations are associated with enhanced fertility in cattle, studies reporting pregnancy success following supplementation of these reproductive steroids are equivocal. In lactating dairy cows, administering estradiol cypionate (ECP) prior to timed insemination has been associated with increased expression of estrus (Stevenson et al., 2004; Sellars et al., 2006). Although estrous expression at timed-AI has been associated with improved conception rates (Pancarci et al., 2003; Kasimanickam et al., 2005; Souza et al., 2007), ECP administration often fails to improve timed-AI pregnancy rates in lactating dairy cattle (Pancarci et al., 2002; Kasimanickam et al., 2005; Sellars et al., 2006; Hillegass et al., 2008). In beef cattle, ECP administration prior to timed-AI has been demonstrated to improve pregnancy success in cows induced to ovulate a small follicle (Jinks et al., 2013). Improvements in pregnancy success via direct progesterone supplementation or induction of ovulation with hCG or GnRH are mixed. While some investigators have observed improvements in fertility with progesterone supplementation during early embryonic development, others have failed to demonstrate a benefit (Mann and Lamming, 1999; Lamb et al., 2010; Wiltbank et al., 2012). Collectively, Mann and Lamming (1999) concluded that progesterone supplementation improved pregnancy success by 5% but this improvement was dependent upon days of progesterone supplementation and relative fertility of the treated herds. The reason for this discrepancy

between observational studies that have noted improved fertility in cattle with elevated estradiol and progesterone concentrations and the variable results in fertility when steroids are supplemented is not clear. Duration of exposure, differences in concentrations in circulation and those at targeted tissues and organs, and mode of delivery all may contribute to the variable responses in fertility when steroids are exogenously administered. It is also possible that stresses related to animal handling to administer supplemental progesterone could play a role in their intended success, as Geary et al. (2010) demonstrated handling beef heifers during early gestation (i.e., on approximately d 13) to administer the prostaglandin F inhibitor, flunixin meglumine, decreased pregnancy success.

Ovarian Steroid Receptor Regulation in Uterine Endometrium

Unraveling the complex embryonic-maternal communications and changes occurring in the endometrium required for pregnancy to occur and be maintained in cattle is critical for developing applied measures for improving reproductive efficiency. Recent evaluation of gene changes in the uterine endometrium of cattle (Bauersachs et al., 2005, 2006; Klein et al., 2006; Mitko et al., 2008; Shimizu et al., 2010; Forde et al., 2011; Bauersachs and Wolf, 2012; Forde and Lonergan, 2012) have demonstrated that gene expression is temporally modulated by circulating concentrations of progesterone and the presence of the conceptus (embryo + placental tissues), and these alterations have direct effects on the ability of the uterus to support embryonic development and maintain pregnancy. Collectively, these global gene expression evaluations have advanced the understanding of the dynamic changes occurring within the bovine endometrium during the estrous cycle and early pregnancy in response to various endocrine and embryonic cues. Future investigations can use these studies to further distinguish mechanisms by which alterations in reproductive steroids pre- and post-ovulation are causing uterine

deficiencies that ultimately result in conceptus death.

To maintain regular estrous cycles and establish a uterine environment suitable for conceptus development, the regulation of the expression and localization of progesterone (PGR), estradiol α (ESR1), and oxytocin receptors (OXTR) in the uterine endometrium is obligatory. Estradiol and progesterone coordinate the expression of these receptor populations (Kimmins and MacLaren, 2001; Meikle et al., 2001; Robinson et al., 2001; McNeill et al., 2006; Okumu et al., 2010). Simplistically, in cattle, the preovulatory rise in estradiol increases the expression of both PGR and ESR1 in the uterine endometrium. As serum concentrations of progesterone increase during the subsequent luteal phase, both receptor types decrease and reach nadir during the mid-luteal phase of the estrous cycle. Of interest, Okumu et al. (2010) and Bridges et al. (2012) observed decreased expression of PGR in the uterine endometrium on d 13 and 15.5 of the estrous cycle, respectively, in pregnant compared with non-pregnant cattle. In cyclic cattle, the decrease in PGR allows for an increase in OXTR, beginning approximately on d 16 of the estrous cycle. In pregnant ruminants, secretion of interferon-tau (IFNT) by the growing conceptus attenuates the increase in OXTR and subsequently prevents the pulsatile release of prostaglandin $F_{2\alpha}$ (PGF) from the uterine endometrium (Farin et al., 1990; Meyer et al., 1995). In non-pregnant ruminants, the increase in OXTR initiates the luteolytic mechanism and ultimately results in pulsatile PGF release from the uterus leading to luteolysis and the return to estrus (Silvia et al., 1991; McCracken et al., 1999).

In the pregnant cow, ewe, and pig, disappearance of the PGR in the uterine endometrium is a critical event required for proper uterine function during gestation; however, the extent of PGR loss in various cell types of the uterine endometrium varies by species (Geisert et al., 1994; Spencer and Bazer, 1995; Spencer et al., 1999, 2004; Mathew et al., 2011). In cattle, PGR is

absent in the luminal epithelium during early gestation; however, the extent of down-regulation of the PGR in the glandular epithelium is inconsistent across studies with reports of complete disappearance (Robinson et al., 1999, 2001; Kimmins and MacLaren, 2001) or moderate amounts of PGR (Guzeloglu et al., 2004; Bilby et al., 2006; Cruppe, 2011; Bridges et al., 2012). In all domestic livestock species, for proper expression of various proteins and secretions from the endometrium during gestation, the temporal and spatial regulation of the PGR is essential (Spencer et al., 1999; Carson et al., 2000; Johnson et al., 2000; Satterfield et al., 2006; Forde et al., 2009). Down-regulation of the PGR in the glandular epithelium may be required to permit, or be the result of, cell proliferation and differentiation in these cell types. It has been demonstrated that progesterone, acting via the PGR in these tissues, inhibits these processes (Tong and Pollard, 1999). Although progesterone is required to induce uterine secretions, the disappearance of the PGR is required for the increased secretion of uterine products required to support conceptus development. Among these include uterine histotroph. Uterine histotroph is composed of enzymes, cytokines, growth factors, ions, hormones, glucose, fructose, amino acids, transport proteins, and adhesion molecules (Bazer et al., 2012; Mullen et al., 2012). These secretions from the glandular epithelium are critical for conceptus growth and survival. In the ewe, since PGR are not present in the secretory cells but secretion is directed by progesterone, it has been speculated that during the pre-implantation period and throughout the remainder of pregnancy, progesterone directs histotroph secretions by acting through PGR in stromal cells of the uterine endometrium via paracrine actions of potential stroma-derived “progestamedins” (Spencer and Bazer, 2002; Spencer et al., 2004). Although these progestamedins have yet to be definitively identified, Spencer and Bazer (2002) suggested fibroblast growth factor-10, fibroblast growth factor-7, hepatocyte growth factor, Hoxa-10, and Indian Hedgehog signaling

molecule as potential candidates. It is unclear if a similar mechanism is responsible for progesterone signaling in the cow.

ENDOCRINE INDUCED UTERINE DYSFUNCTION

As previously indicated, beef or dairy females induced to ovulate small follicles following ovulation synchronization and dairy cows due to steroid catabolism (Sangsrivong et al., 2002; Vasconcelos et al., 2003) have erroneous endocrine profiles and reduced fertility (Lamb et al., 2001; Lucy et al., 2001; Perry et al., 2005, 2007). In these steroid deficient animals, previous studies in beef (Mussard et al., 2003; Bridges et al., 2010, 2012; Atkins et al., 2012) and lactating dairy cows (Sartori et al., 2006) indicate that deficient uterine function contributes to this increased occurrence of pregnancy failure. This is not surprising given the numerous functions that estradiol and progesterone have in dictating uterine environment and function.

Role of Preovulatory Estradiol Concentrations on Uterine Receptivity to the Conceptus and Conceptus Survival

In recent years, several studies have been conducted utilizing either an animal model that varied the functional status, diameter, and age of follicle from which ovulation was induced (Mussard 2003a,b, 2007) and later, an animal model that allowed follicle age and diameter to remain constant between treatments but altered interval of proestrus and subsequently preovulatory estradiol concentrations (Figure 1; Bridges et al., 2010, 2012). Collective interpretation from these experiments summarized in Table 1 indicated that: 1) the reduction in fertility associated with ovulation from 'immature' follicles is governed by the steroidogenic capacity of the follicle during proestrus and the resultant corpus luteum rather than directly by

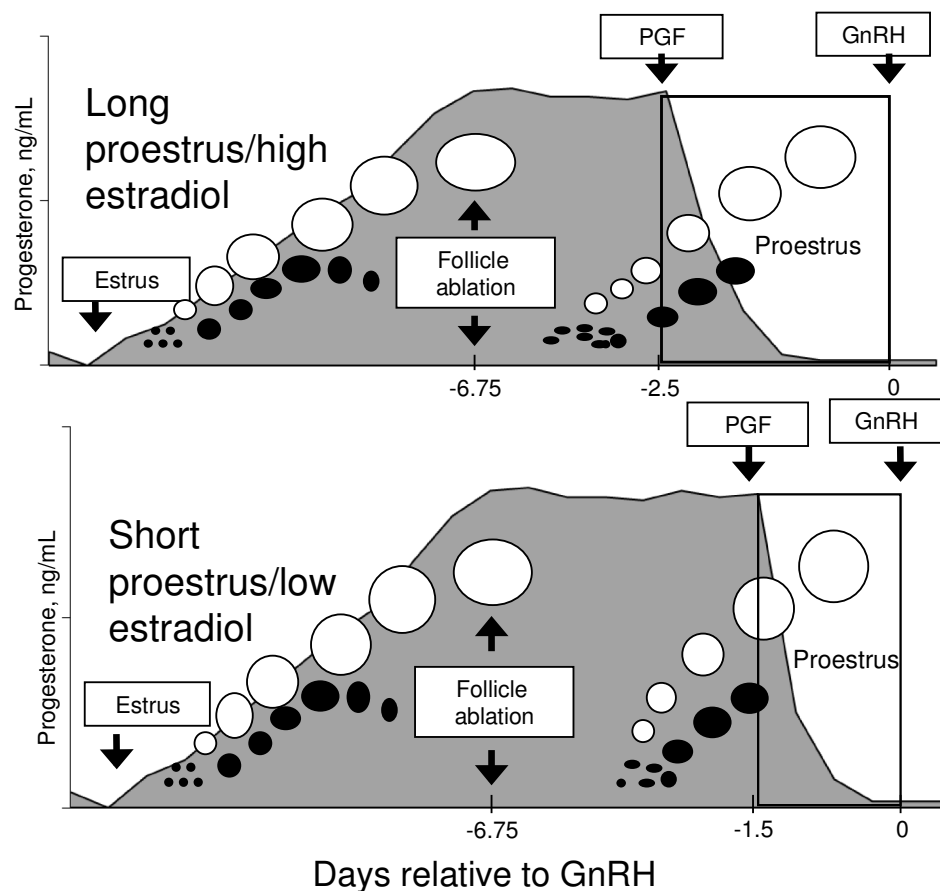


Figure 1. Animal model used in a series of experiments cited to result in treatments that varied in length of proestrus and prevovulatory estradiol concentrations. Animals were synchronized to a common day of estrus prior to ovarian follicular aspiration. Ovarian follicular aspiration resulted in emergence of a new wave of follicles either 1 or 2 d after aspiration. In the long proestrus/high estradiol treatment, PGF was administered approximately 4 d after aspiration and GnRH given 2.5 d later. In the short proestrus/low estradiol treatment, PGF was given 5 d after emergence and GnRH administered 1.5 d later.

diameter or age of the follicle and 2) this aberrant steroid hormone milieu leads to a decreased ability of the uterus to support an embryo. This conclusion is based on results of Mussard et al. (2003b) where embryo transfer in lieu of AI still resulted in reduced pregnancy rates in cows with a decreased duration of proestrus.

Table 1. Conception rate, diameter and age of the ovulatory follicle, duration of proestrus, and number of cows included from a series of experiments investigating the effect of follicle maturity on fertility.

Conception rate, % ¹	Follicle diameter at ovulation, mm ²	Duration of proestrus, d ³	n	Experiment
4	11.1 ± 0.2	1.0 ± 0.1	45	Mussard et al., 2003a ⁴
8	11.1 ± 0.2	1.0 ± 0.1	12	Mussard et al., 2003b ⁵
10	12.6 ± 0.2	1.25	10	Bridges et al., 2010 ⁶
57	13.6 ± 0.2	2.2 ± 0.1	54	Mussard et al., 2003a ⁴
67	13.7 ± 0.2	2.0 ± 0.1	12	Mussard et al., 2003b ⁵
71	12.9 ± 0.2	2.25	28	Bridges et al., 2010 ⁶
76	10.7 ± 0.1	3.3 ± 0.1	29	Mussard et al., 2007 ⁷
100	12.0 ± 0.3	4.7 ± 0.2	24	Mussard et al., 2007 ⁷

¹Percentage of animals determined to be pregnant following insemination. Pregnancy determination was conducted via ultrasonography at approximately 30 days post-insemination.

²Diameter of the largest ovulatory follicle as determined by ultrasonography conducted either at GnRH administration or estrus.

³Interval from PGF_{2α} until GnRH administration.

⁴Cows were induced to ovulate either a small (~11 mm) or large (~13 mm) ovarian follicle with GnRH. Animals were inseminated 12 h following GnRH administration.

⁵Cows were induced to ovulate either a small (~11 mm) or large (~13 mm) ovarian follicle with GnRH. Embryo from non-treated cows were then transferred 7 days after GnRH.

⁶Cows were induced to ovulate an ovarian follicle of similar diameter with GnRH either 1.25 or 2.25 days following PGF_{2α} administration. Animals were inseminated 12 h following GnRH administration. Includes only cows with a luteal phase of normal length.

⁷Cows were either induced with GnRH to ovulate a small (~11 mm) follicle or allowed to spontaneously exhibit estrus. Cows were inseminated 12 hours following estrus or GnRH.

Further supporting the notion that uterine dysfunction is responsible for decreased pregnancy rates in animals with decreased preovulatory estradiol concentrations is recent

research using a single ovulation reciprocal embryo transfer protocol (Atkins et al., 2012; Jinks et al., 2013). Within these studies, single embryos were collected from cows induced with GnRH to ovulate a small dominant follicle and transferred into cows induced with GnRH to ovulate a small or large follicle. Similarly, single embryos from cows that were induced with GnRH to ovulate a large follicle were transferred into cows induced with GnRH to ovulate a small or large follicle. Initial evaluation demonstrated that donor serum concentration of estradiol significantly contributed to the likelihood of fertilization and estradiol concentrations of the embryo recipient cow at GnRH induced ovulation was among the greatest factors affecting the likelihood of pregnancy (Atkins et al., 2012). Jinks et al. (2013) retrospectively divided the embryo donor and embryo recipient cows each into two groups of low estradiol (i.e., < 8.4 pg/mL) or high estradiol (i.e., ≥ 8.4 pg/mL) based on serum concentration of estradiol at induced ovulation. Fertilization success was greater in embryo donor cows with greater serum concentration of estradiol at GnRH-induced ovulation. However, results indicated that circulating concentrations of estradiol at GnRH-induced ovulation in the recipient cows, not the donor cows, was predictive of pregnancy success at d 27 of gestation. These results indicate that the primary benefit of increased preovulatory estradiol is mediated through alterations in the maternal environment of the recipient cows. An additional study by Jinks et al. (2013) demonstrated that administering ECP 24 h before AI increased pregnancy rates in cows induced to ovulate a small dominant follicle (< 12.2 mm). It is likely that ECP increased circulating concentrations of estradiol in cows induced to ovulate a small follicle, thus improving uterine functionality and thereby increasing pregnancy success. Collectively, these studies demonstrate that pregnancy success is diminished in cows with decreased concentrations of estradiol prior to ovulation and the inability of the uterus to support pregnancy establishment is responsible for the

reduction in fertility.

Previous experiments using the experimental model depicted in Figure 1 demonstrated that by d 30 of gestation, pregnancy rates were reduced in beef cattle with reduced preovulatory estradiol concentrations (Bridges et al., 2010). In addition, it was demonstrated that manipulating estradiol concentrations prior to ovulation altered OXTR and cyclooxygenase-2 gene expression in the uterine endometrium on d 5 of the estrous cycle (Bridges et al., 2005). These results and those outlined previously led Bridges et al. (2012) to investigate the effect of altering preovulatory estradiol on conceptus development and IFNT production, steady-state uterine concentrations of mRNA for steroid receptors and IFN-stimulated genes (ISG), and localization of the PGR in the uterine endometrium. Beef heifers received an embryo 7 d after induced ovulation following either elevated (HiE2) or reduced (LoE2) preovulatory concentrations of estradiol (Figure 1). On d 15.5 of gestation, heifers were slaughtered, conceptuses were collected, and the reproductive tract processed for future mRNA and immunohistochemical evaluation.

As previously demonstrated (Bridges et al., 2010; Cruppe et al., 2010; Souto et al., 2010), this experimental model resulted in two treatment groups that differed in preovulatory estradiol (Figure 2) concentrations while diameter of the ovulatory follicle at GnRH-induced ovulation was similar. Peripheral progesterone concentrations in the subsequent estrous cycle did not differ between estradiol treatments, but were greater in pregnant heifers, irrespective of treatment, on d 4, 7, and 15.5 of gestation and tended to be greater on d 6. Interestingly, concentration of preovulatory estradiol did not affect conceptus development, IFNT production, and ISG expression. However, PGR staining intensity (Figure 3) in the deep glandular epithelium and ESR1 mRNA in the uterine endometrium were increased in the heifers with

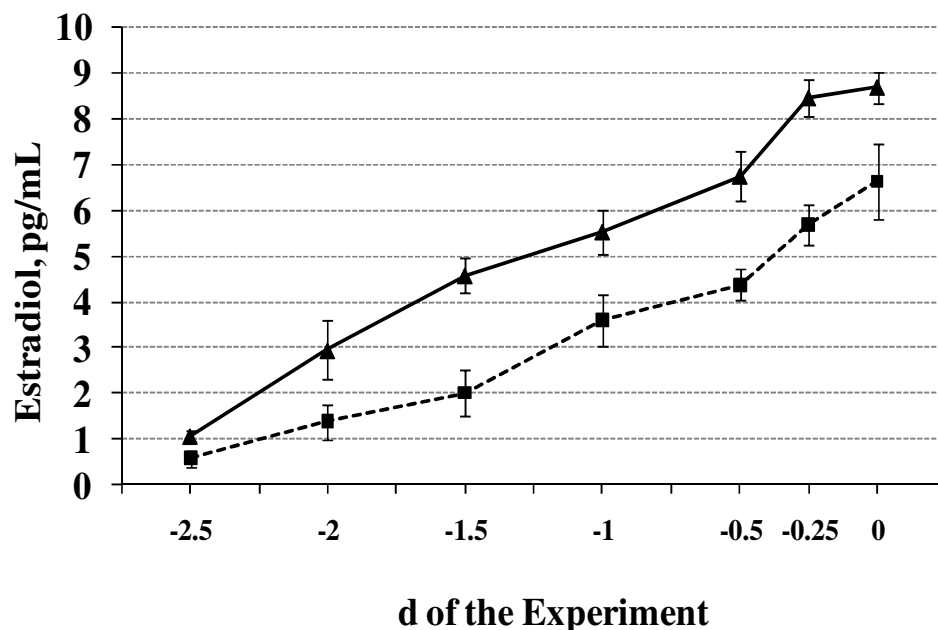


Figure 2. Circulating concentrations of estradiol during the preovulatory period in heifers manipulated to have either elevated (HiE2; ▲) or reduced (LoE2; ■) preovulatory estradiol concentrations using the animal model described in Figure 1 (Bridges et al., 2010). Heifers were administered PGF on d -2.5 (HiE2) or d -1.5 (LoE2), all heifers received GnRH on d 0 to induce ovulation [Treatment, $P < 0.05$; treatment x time interaction, $P > 0.05$].

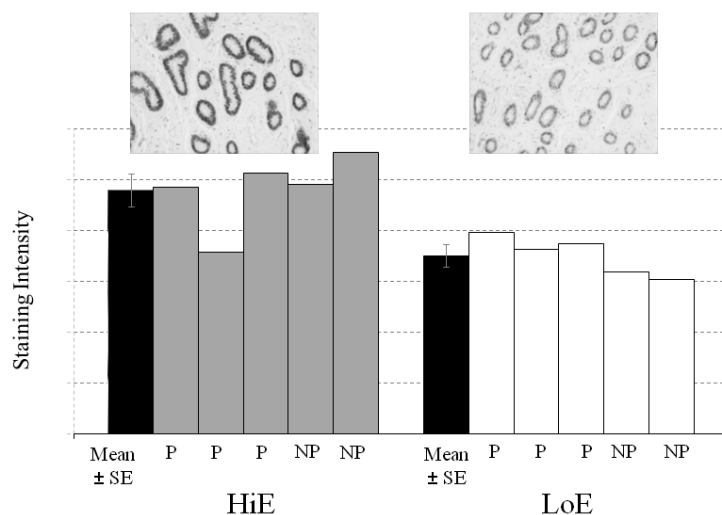


Figure 3. Nuclear progesterone receptor in the deep glandular epithelium in cattle on day 15.5 of the estrous cycle in pregnant (P) and nonpregnant (NP) heifers that were induced to ovulate a similar sized follicle following either a long proestrus (2.5 days) that increased preovulatory estradiol concentrations (HiE) or a shortened proestrus (1.5 days) that resulted in reduced (LoE) preovulatory concentrations (Bridges et al., 2012). Representative sections from females in each treatment are shown above the respective treatment. Staining intensity differed between treatment, $P < 0.05$.

elevated estradiol concentrations. The increased concentrations of estradiol prior to ovulation likely caused increased expression of ESR1 and greater PGR protein immediately following

ovulation, which were still evident at d 15.5 of the estrous cycle. Although specific changes were observed in the uterine endometrium, conceptus development, IFNT production, or responsiveness of the uterine endometrium to conceptus signals had not yet been impacted. These findings, combined with previous research using this animal model (Bridges et al., 2010), indicated that the defects in uterine function that lead to embryonic mortality in cattle experiencing low preovulatory estradiol concentration are manifested after d 15.5 of gestation but before d 30. Subsequent studies by Cruppe (2011) and Souto (2011), where a similar animal model was used (Figure 1) but conceptuses and reproductive tissues were evaluated on d 17.5 of gestation also failed to clearly determine the impact of preovulatory estradiol concentrations on uterine function and conceptus survivability. On d 17.5 of gestation, conceptuses from cows with elevated preovulatory estradiol concentrations tended to produce greater amounts of IFNT in the uterine lumen but other indicators of functional competence of the conceptus were not impacted by preovulatory estradiol concentrations (Souto, 2011). Cruppe (2011) assessed the impact of preovulatory estradiol concentrations on amounts of various proteins and mRNA in the uterine endometrium on d 17.5 of gestation. Preovulatory estradiol concentrations did not impact ISG expression in the uterine endometrium. Relative amounts of mRNA for secreted phosphoprotein 1, a uterine secreted protein associated with conceptus attachment in the ewe (Johnson et al., 2003), were variable between replications and, thus, did not associate with different preovulatory estradiol concentrations. However, amounts of PGR in the deep glandular epithelium were reduced in cows with elevated concentrations of estradiol prior to ovulation, in contrast to that reported by Bridges et al. (2012). The reason for the difference in PGR expression between studies is not clear but perhaps the continued down-regulation of the PGR in the deep glandular epithelium from d 15.5 to 17.5 of gestation is requisite for adequate uterine

function to support the developing conceptus.

Although it is clear that reductions in preovulatory estradiol concentrations affect fertility through altering the ability of the uterus to support conceptus development, these series of studies were unable to specifically identify gene targets or proteins within the uterine endometrium responsible for the deficient uterine environment. It appears that when estradiol concentrations are inadequate prior to ovulation, although marginal differences in uterine steroid receptor populations are observed, conceptus development is not inhibited through maternal recognition of pregnancy. Therefore, it is likely that the negative effects of reduced estradiol on uterine function are occurring at later stages of conceptus attachment. This is supported by ancillary data, where return to estrus interval in cows with a shortened proestrus was extended to approximately 25 d, indicating that the ability of the embryos to progress through maternal recognition of pregnancy but unable to survive to d 30 of gestation (M. L. Mussard and M. L. Day, unpublished results). Furthermore, Roberts et al. (2012) demonstrated that in ovariectomized cows, there was no difference in the proportion of cows having elevated ISG products in circulation (indicating presence of an conceptus) between cows administered or not administered estradiol to simulate the preovulatory period through d 21 of gestation; yet by d 29, cows not receiving estradiol had reduced pregnancy rates. Various other reports have demonstrated that preovulatory estradiol concentrations can have long-term impacts on the expression of various genes within the uterine endometrium (Ing et al., 2006; Schiefelbein et al., 2008; Perry et al., 2009). Therefore, additional research is warranted during the peri-implantation period of gestation to definitively determine the specific deficiencies in uterine function that result in conceptus death in cattle lacking sufficient preovulatory estradiol concentrations.

Luteal Progesterone Concentrations, Uterine Functionality, and Conceptus Survival

It is theorized that increasing concentrations of progesterone during early gestation facilitates conceptus growth indirectly by accelerating the down-regulation of the PGR in the uterine endometrium to allow earlier and increased secretion of uterine histotroph through up regulation of specific nutrient transport pathways (Forde et al., 2009, 2010). In the pregnant ewe, exogenous progesterone increased various amino acids (i.e., arginine, serine, glutamine, and lysine) and tended to increase glucose concentrations in the uterine histotroph (Satterfield et al., 2010). Similarly in the cow, supplementing progesterone during early gestation advanced endometrial expression of numerous genes associated with histotroph production or components to advance conceptus development (Forde et al., 2009, 2011).

More pertinent to the focus of this paper is the effect of a reduction of circulating concentrations of progesterone on uterine function and conceptus development. As outlined previously, certain populations of both beef and dairy cattle have reduced progesterone concentrations during early gestation. Just as increased progesterone concentrations early in gestation are associated with increased conceptus development, reduced progesterone concentrations during early gestation was demonstrated to decrease conceptus growth and IFNT secretion, which may interfere with maternal recognition of pregnancy (Mann and Lamming, 2001; Mann et al., 2006). Atkins et al. (2012) recently demonstrated using path analysis that in embryo recipient beef cows, progesterone concentration at transfer on d 7 was the variable that best predicted the probability of pregnancy success at d 27 of gestation. Moreover, several observational studies have reported that cows diagnosed to be non-pregnant have reduced concentrations of progesterone compared with those diagnosed as pregnant (Lukaszewska and Hansel, 1980; Shelton et al., 1990; Mann et al., 1995; Kerbler et al., 1997; Perry et al., 2005;

Mann and Lamming, 2001; Hommeida et al., 2004). Although this is an associative response, it supports the hypothesis that reduced progesterone during early gestation impedes fertility.

The most comprehensive assessment of the impacts of reduced circulating concentrations of progesterone on changes in the uterine transcriptome, temporal pattern of endometrial gene expression, and ultimate effects on conceptus development in cattle was reported recently by Forde et al. (2011) and (2012). Using a unique animal model, these investigators were able to reduce circulating concentrations of progesterone and then compare uterine and conceptus changes with those observed in heifers with normal patterns of progesterone secretion. Heifers with reduced versus normal progesterone concentrations following ovulation had 498 differentially expressed genes on d 7 of the estrous cycle and 351 differentially expressed genes on d 13 (Forde et al., 2012). Reducing progesterone concentrations resulted in a delayed down-regulation of the PGR in the glandular and luminal epithelium (Forde et al., 2011). The reduction in progesterone concentrations, in concert with alteration in the temporal regulation of the PGR in heifers with low progesterone, altered the temporal expression of several genes associated with histotroph composition, selected transporters, cellular proliferation and migration, protein remodeling, and genes that likely contribute to conceptus elongation (Forde et al., 2011, 2012). Such alterations in uterine function by reducing circulating concentrations of progesterone were demonstrated to result in retarded conceptus growth in embryos following embryo transfer (Forde et al., 2011). Of interest, compared with previous evaluations in heifers with elevated progesterone concentrations following ovulation (Forde et al., 2011), genes altered by low progesterone were distinct from those genes affected by high progesterone (Forde et al., 2012). Collectively, the observed changes in the uterine transcriptome elicited by reduced progesterone concentrations resulted in a dysfunctional uterus that was unable to support

conceptus elongation.

SUMMARY AND CONCLUSIONS

Although multiple factors (Atkins et al., 2012), including follicular characteristics as summarized by Geary et al. (2012), impact embryo survival, it is evident that uterine deficiencies are a major contributor to infertility in cattle. This review focused primarily on the role that ovarian steroids play in establishing proper uterine function to support conceptus growth and implantation, and how deficiencies in estradiol prior to ovulation and progesterone post-ovulation can create an inadequate uterine microenvironment that ultimately results in conceptus death. Although, it is clear that decreased serum concentrations of preovulatory estradiol create uterine deficiencies that prevent the maintenance of pregnancy, the exact alteration within the uterine endometrium responsible for deficient uterine function have yet to be definitively identified. The uterine deficiencies in response to reduced post-ovulatory progesterone concentrations have recently been partially defined. Reducing progesterone concentrations in early gestation alters the normal regulation of PGR and results in numerous alterations in the transcriptome of the endometrium, specifically those genes involved with histotroph production and composition, and preparation of the uterus for conceptus attachment. Ultimately these changes translate into retarded conceptus development and, in many instances, conceptus death. From a practical standpoint, animal scientists must be cognizant of these potential uterine deficiencies induced by alterations in reproductive steroid concentrations when developing and implementing reproductive management technologies and strategies in cattle.

LITERATURE CITED

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.
- Atkins, J. A., M. F. Smith, K. J. Wells, and T. W. Geary. 2010a. Factors affecting preovulatory follicle diameter and ovulation rate after gonadotropin-releasing hormone in postpartum beef cows. Part I: Cycling Cows. *J. Anim. Sci.* 88:2300-2310.
- Atkins, J. A., M. F. Smith, K. J. Wells, and T. W. Geary. 2010b. Factors affecting preovulatory follicle diameter and ovulation rate after gonadotropin-releasing hormone in postpartum beef cows. Part II: Anestrous Cows. *J. Anim. Sci.* 88:2311-2320.
- Atkins, J. A., M. F. Smith, M. D. MacNeil, E. M. Jinks, F. M. Abreu, L. J. Alexander, and T. W. Geary. 2012. Pregnancy establishment and maintenance in cattle. *J. Anim. Sci.* 91: [Epub ahead of print November 12, 2012] doi: 10.2527/jas.2012-5368.
- Ayalon, N. 1978. A review of embryonic mortality in cattle. *J. Reprod. Fertil.* 54:483-493.
- Bartol, F. F., A. A. Wiley, J. G. Floyd, T. L. Ott, F. W. Bazer, C. A. Gray, and T. E. Spencer. 1999. Uterine differentiation as a foundation for subsequent fertility. *J. Reprod. Fertil. Suppl.* 54:287-302.
- Bauersachs, S., S. E. Ulbrich, K. Gross, S. E. M. Schmidt, H. H. D. Meyer, R. Einspanier, H. Wenigerkind, M. Vermehren, H. Blum, F. Sinowatz, and E. Wolf. 2005. Gene expression profiling of bovine endometrium during the oestrous cycle: detection of molecular pathways involved in functional changes. *J. Mol. Endocrinol.* 34:889-908.
- Bauersachs, S., S. E. Ulbrich, K. Gross, S. E. M. Schmidt, H. H. D. Meyer, H. Wenigerkind, M. Vernehen, F. Sinowatz, H. Blum, and E. Wolf. 2006. Embryo-induced transcriptome changes in bovine endometrium reveal species-specific and common molecular markers of uterine receptivity. *Reproduction* 132:319-331.

- Bauersachs, S., and E. Wolf. 2012. Transcriptome analyses of bovine, porcine and equine endometrium during the pre-implantation phase. *Anim. Reprod. Sci.* 134:84-94.
- Bazer, T.W. 1975. Uterine protein secretions: relationship to development of the conceptus. *J. Anim. Sci.* 41:1376-1382.
- Bazer, T. W., T. E. Spencer, G. A. Johnson, R. C. Burghardt, and G. Wu. 2009. Comparative aspects of implantation. *Reproduction* 138:195-209.
- Bazer, F. W., G. Wu, T. E. Spencer, G. A. Johnson, R. C. Burghardt, and K. Bayless. 2010. Novel pathways for implantation and establishment and maintenance of pregnancy in mammals. *Mol. Human Reprod.* 16:135-152.
- Bazer, F. W., G. Song, J. Kim, D. W. Erikson, G. A. Johnson, R. C. Burghardt, H. Gao, M. C. Satterfield, T. E. Spencer, and G. Wu. 2012. Mechanistic mammalian target of rapamycin (MTOR) cell signaling: Effects of select nutrients and secreted phosphoprotein 1 on development of mammalian conceptuses. *Mol. Cell. Endocrinol.* 354:22-33.
- Bilby, T. R., A. Guzeloglu, L. A. MacLaren, C. R. Staples, and W. W. Thatcher. 2006. Pregnancy, bovine somatotropin, and dietary n-3 fatty acids in lactating dairy cows: II. Endometrial gene expression related to maintenance of pregnancy. *J. Dairy Sci.* 89:3375-3385.
- Bridges, G. A., M. L. Mussard, J. L. Winkler, C. L. Gasser, D. E. Grumm, J. L. Pate, and M. L. Day. 2005. Influence of duration of proestrus on preovulatory estradiol concentrations and uterine gene expression following induced ovulation in cattle. *Proceedings of the 38th Annual Meeting of the Society for the Study of Reproduction (Abstr. M451).*
- Bridges, G. A., M. L. Mussard, C. R. Burke, and M. L. Day. 2010. Influence of the length of proestrus on fertility and endocrine function in female cattle. *Anim. Reprod. Sci.*

117:208-215.

Bridges G. A, M. L. Mussard, J. L. Pate, T. L. Ott, T. R. Hansen, and M. L. Day. 2012. Impact of preovulatory estradiol concentrations on conceptus development and uterine gene expression. *Anim. Reprod. Sci.* 133:16-26.

Carson, D. D., I. Bagchi, S. K. Dey, A. C. Enders, A. T. Fazleas, B. A. Lessey, and K. Yoshinaga. 2000. Embryo implantation. *Develop. Biol.* 223:217-237.

Cerri, R. L. A., S. O. Juchem, R. C. Chebel, H. M. Rutigliano, R. G. S. Bruno, K. N. Galvão, W. W. Thatcher, and J. E. P. Santos. 2009. Effects of fat source differing in fatty acid profile on metabolic parameters, fertilization, and embryo quality in high-producing dairy cows. *J. Dairy Sci.* 92:1520-1531.

Cruppe, L. H. 2011. The effect of preovulatory concentrations of estradiol and length of proestrus on fertility in beef cattle. MS Thesis. The Ohio State Univ., Columbus.

Dalton, J. C., S. Nadir, J. H. Bame, M. Noftsinger, R. L. Nebel, and R. G. Saacke. 2001. Effect of time of insemination on number of accessory sperm, fertilization rate, and embryo quality in nonlactating dairy cattle. *J. Dairy Sci.* 84:2413-2418.

DeJarnette, J. M., R. G. Saacke, J. Bame, and C. J. Vogler. 1992. Accessory sperm: Their importance to fertility and embryo quality, and attempts to alter their numbers in artificially inseminated cattle. *J. Anim. Sci.* 70:484-491.

Diskin, M. G., and J. M. Sreenan. 1980. Fertilization and embryonic mortality rates in beef heifers after artificial insemination. *J. Reprod. Fertil.* 59:463-468.

Farin, C. E., K. Imakawa, T. R. Hansen, J. J. McDonnell, C. N. Murphy, P. W. Farin, and R. M. Roberts. 1990. Expression of trophoblastic interferon genes in sheep and cattle. *Biol. Reprod.* 43:210-218.

- Flint, A. P., H. J. Stewart, G. E. Lamming, and J. H. Payne. 1992. Role of the oxytocin receptor in the choice between cyclicity and gestation in ruminants. *J. Reprod. Fertil. Suppl.* 45:53-58.
- Forde, N., F. Carter, T. Fair, M. A. Crowe, A. C. O. Evans, T. E. Spencer, T. W. Bazer, R. McBride, M. P. Boland, P. O’Gaora, P. Lonergan, and J. F. Roche. 2009. Progesterone-regulated changes in endometrial gene expression contribute to advanced conceptus development in cattle. *Biol. Reprod.* 81:784-794.
- Forde, N., T. E. Spencer, F. W. Bazer, G. Song, J. F. Roche, and P. Lonergan. 2010. Effect of pregnancy and progesterone concentrations on expression of genes encoding for transporters or secreted proteins in the bovine endometrium. *Physiol. Genomics* 41:53-62.
- Forde, N., M. E. Beltman, G. B. Duffy, P. Duffy, J. P. Mehta, P. O’Gaora, J. F. Roche, P. Lonergan, and M. A. Crowe. 2011. Changes in the endometrial transcriptome during the bovine estrous cycle: Effect of low circulating concentrations of progesterone and consequences for conceptus elongation. *Biol. Reprod.* 84:266-278.
- Forde, N., J. P. Mehta, M. Minten, M. A. Crowe, J. F. Roche, T. E. Spencer, and P. Lonergan. 2012. Effects of low progesterone on the endometrial transcriptome in cattle. *Biol. Reprod.* 87:1-11.
- Forde, N., and P. Lonergan. 2012. Transcriptomic analysis of the bovine endometrium: what is required to establish uterine receptivity to implantation in cattle? *J. Reprod. Dev.* 58:189-195.
- Geary, T. W., R. P. Ansotegui, M. D. MacNeil, A. J. Roberts, and R. C. Waterman. 2010. Effects of flunixin meglumine on pregnancy establishment in beef cattle. *J. Anim. Sci.* 88:943-949.

- Geary, T. W., M. F. Smith, M. D. MacNeil, M. L. Day, G. A. Bridges, G. A. Perry, F. M. Abreu, J. A. Atkins, K. G. Pohler, E. M. Jinks, and C. A. Madsen. 2012. Influence of follicular characteristics at ovulation on early embryo survival. *J. Anim. Sci.* 91: [Epub ahead of print December 10, 2012] doi: 10.2527/jas.2012-5887.
- Geisert, R. D., T. N. Pratt, F. W. Bazer, J. S. Mayes, and G. H. Watson. 2004. Immunocytochemical localization and changes in endometrial progesterin receptor protein during the porcine estrous cycle and early pregnancy. *Reprod. Fertil. Dev.* 6:749-760.
- Gray, C. A., F. F. Bartol, K. M. Taylor, A. A. Wiley, W. S. Ramsey, T. L. Ott, F. W. Bazer, and T. E. Spencer. 2000. Ovine uterine gland knock-out model: effects of gland ablation on the estrous cycle. *Biol. Reprod.* 62:448-456.
- Gray, C. A., K. M. Taylor, W. S. Ramsey, J. R. Hill, F. W. Bazer, F. F. Bartol, and T. E. Spencer. 2001. Endometrial glands are required for preimplantation conceptus elongation and survival. *Biol. Reprod.* 64:1608-1613.
- Gray, C. A., R. C. Burghardt, G. A. Johnson, F. W. Bazer, and T. E. Spencer. 2002. Evidence that absence of endometrial gland secretions in uterine gland knockout ewes compromises conceptus survival and elongation. *Reproduction* 124:289-300.
- Green, M. P., M. G. Hunter, and G. E. Mann. 2005. Relationships between maternal hormone secretion and embryo development on day 5 of pregnancy in dairy cows. *Anim. Reprod. Sci.* 88:179-189.
- Guzeloglu, A., T. R. Bilby, A. Meikle, S. Kamimura, A. Kowalski, F. Michel, L. A. MacLaren, and W. W. Thatcher. 2004. Pregnancy and bovine somatotropin in nonlactating dairy cows: II. Endometrial gene expression related to maintenance of pregnancy. *J. Dairy Sci.* 87:3268-3279.

- Hillegass, J., F. S. Lima, M. F. Sá Filho, and J. E. P. Santos. 2008. Effect of time of artificial insemination and supplemental estradiol on reproduction of lactating dairy cows. *J. Dairy Sci.* 91:4226-4237.
- Hommeida, A., T. Nakao, and H. Kubota. 2004. Luteal function and conception in lactating cows and some factors influencing luteal function after first insemination. *Theriogenology* 62:217-225.
- Ing N. H., T. E. Spencer, and F. W. Bazer. 1996. Estrogen enhances endometrial estrogen receptor gene expression by a posttranscriptional mechanism in the ovariectomized ewe. *Biol. Reprod.* 54:591-599.
- Ing, N. and Y. Zhang. 2004. Cell-specific expression of estrogen-responsive genes in the uteri of cyclic, early pregnant and ovariectomized ewes. *Theriogenology* 62:403-414.
- Ing, N. H., R. L. Wolfskill, S. Clark, J. A. DeGraauw, and C. A. Gill. 2006. Steroid hormones acutely regulate expression of a nudix protein-encoding gene in the endometrial epithelium of sheep. *Mol. Reprod. Dev.* 73:967-976.
- Ireland, J. J. and J. F. Roche. 1982. Development of antral follicles in cattle after prostaglandin-induced luteolysis: changes in serum hormones, steroids in follicular fluid, and gonadotropin receptors. *Endocrinology* 111:2077-2086.
- Jinks, E. M., M. F. Smith, J. A. Atkins, K. G. Pohler, G. A. Perry, M. D. MacNeil, A. J. Roberts, R. C. Waterman, L. J. Alexander, and T. W. Geary. 2013. Preovulatory estradiol and the establishment and maintenance of pregnancy in suckled beef cows. *J. Anim. Sci.* 91: (Accepted) doi:10.2527/jas.2012-5611.
- Johnson, G. A., T. E. Spencer, R. C. Burghardt, K. M. Taylor, C. A. Gray, and F. W. Bazer. 2000. Progesterone modulation of osteopontin gene expression in the ovine uterus. *Biol.*

- Reprod. 62:1315-1321.
- Johnson, G. A., R. C. Burghardt, F. W. Bazer, and T. E. Spencer. 2003. Osteopontin: Roles in implantation and placentation. *Biol. Reprod.* 69:1458-1471.
- Kasimanickam, R., J. M. Cornwell, and R. L. Nebel. 2005. Fertility following fixed-time AI or insemination at observed estrus in Ovsynch and Heatsynch programs in lactating dairy cows. *Theriogenology* 63:2550-2559.
- 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-tau synthesis by the conceptus in cattle. *Theriogenology* 47:703-714.
- Kenyon, A. G., L. G. D. Mendonca, G. Lopes Jr., J. R. Lima, J. E. P. Santos, and R. C. Chebel. 2012. Minimal progesterone concentration required for embryo survival after embryo transfer in lactating Holstein cows. *Anim. Reprod. Sci.* [Epub ahead of print October 29, 2012] doi: 10.1016/j.anireprosci.2012.10.014.
- Kimmins, S., and L. A. MacLaren. 2001. Oestrous cycle pregnancy effects on the distribution of oestrogen and progesterone receptors in bovine endometrium. *Placenta* 22:742-748.
- Lukaszewska J. and W. Hansel. 1980. Corpus luteum maintenance during early pregnancy in the cow. *J. Reprod Fertil.* 59:485-493.
- Klein, C., S. Bauersachs, S. E. Ulbrich, R. Einspanier, H. H. D. Meyer, S. E. M. Schmidt, H. D. Reichenbach, M. Vermehren, F. Sinowatz, H. Blum, and E. Wolf. 2006. Monozygotic twin model reveals novel embryo-induced transcriptome changes of bovine endometrium in the preattachment period. *Biol. Reprod.* 74:253-264.
- Kruip, T. A., and S. J. Dieleman. 1985. Steroid hormone concentrations in the fluid of bovine follicles relative to size, quality and stage of the oestrus cycle. *Theriogenology* 24:395-

- Lamb, G. C., J. S. Stevenson, D. J. Kesler, H. A. Garverick, D. R. Broan, and B. E. Salfen. 2001. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin F_{2α} for ovulation control in postpartum suckled beef cows. *J. Anim. Sci.* 79:2253-2259.
- Lamb, G. C., C. R. Dahlen, J. E. Lason, G. Marquezini, and J. S. Stevenson. 2010. Control of the estrous cycle to improve fertility for fixed-time artificial insemination in beef cattle: a review. *J. Anim. Sci.* 88(E-Suppl.):E181-E192.
- Lopes, A. S., S. T. Butler, R. O. Gilbert, and W. R. Butler. 2007. Relationship of pre-ovulatory follicle size, estradiol concentrations and season to pregnancy outcome in dairy cows. *Anim. Reprod. Sci.* 99:34-43.
- Lucy, M. C. 2001. Reproductive loss in high-producing dairy cattle: where will it end? *J. Dairy Sci.* 84:1277-1293.
- Lukaszewska J. and W. Hansel. 1980. Corpus luteum maintenance during early pregnancy in the cow. *J. Reprod Fertil.* 59:485-493.
- Mann, G. E., G. E. Lamming, and M. D. Fray. 1995. Plasma oestradiol and progesterone during early pregnancy in the cow and the effects of treatment with buserelin. *Anim. Reprod. Sci.* 37:121-131.
- Mann, G. E. and G. E. Lamming. 1999. The influence of progesterone during early pregnancy in cattle. *Reprod. Dom. Anim.* 34:269-274.
- Mann, G. E. and G. E. Lamming. 2001. Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. *Reprod.* 121:175-180.
- Mann, G. E., M. D. Fray, and G. E. Lamming. 2006. Effects of time of progesterone

- supplementation on embryo development and interferon- τ production in the cow. *Vet. J.* 171:500-503.
- 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.
- Mathew, D. J., E. M. Sellner, J. C. Green, C. S. Okamura, L. L. Anderson, M. C. Lucy, R. D. Geisert. 2011. Uterine progesterone receptor expression, conceptus development, and ovarian function in pigs treated with RU 486 during early pregnancy. *Biol. Reprod.* 84:130-139.
- McCracken, J. A., E. E. Custer, and J. C. Lamsa. 1999. Luteolysis: A neuroendocrine-mediated event. *Physiol. Rev.* 79:263-324.
- McNeill, R. E., M. G. Diskin, J. M. Sreenan, and D. G. Morris. 2006. Associations between milk progesterone concentrations on different days and with embryo survival during the early luteal phase in dairy cows. *Theriogenology* 65:1435-1441.
- Meikle, A., L. Sahlin, A. Ferraris, B. Masironi, J. E. Blanc, M. Rodriguez-Irazaqui, M. Rodriguez-Pinon, H. Kindahl, and M. Forsberg. 2001. Endometrial mRNA expression of oestrogen receptor α , progesterone receptor and insulin-like growth factor-I (IGF-I) throughout the bovine oestrous cycle. *Anim. Reprod. Sci.* 68:45-56.
- Meyer, M. D., G. D. Desnoyers, B. Oldick, W. W. Thatcher, M. Drost, T. K. Schalue, and R. M. Roberts. 1996. Treatment with recombinant bovine interferon- τ in utero attenuates secretion of prostaglandin F from cultured endometrial epithelial cells. *J. Dairy Sci.* 79:1375-1384.
- Miller, B. G. and N. W. Moore. 1976a. Effects of progesterone and oestradiol on endometrial metabolism and embryo survival in the ovariectomized ewe. *J. Reprod. Fertil.* 46:535-536.

- Miller, B. G. and N. W. Moore. 1976b. Progesterone and oestrogen requirements for the survival of embryos in the ovariectomized ewe. *J. Reprod. Fertil.* 46:536-537.
- Miller, B. G., and N. W. Moore. 1983. Endometrial protein secretion during early pregnancy in entire and ovariectomized ewes. *Reprod. Fertil.* 68:137-144.
- Mitko, K., S. E. Ulbrich, H. Wenigerkind, F. Sinowatz, H. Blum, E. Wolf, and S. Bauersachs. 2008. Dynamic changes in messenger RNA profiles of bovine endometrium during the estrous cycle. *Reproduction* 135:225-240.
- Moore, N. W., 1985. The use of embryo transfer and steroid hormone replacement therapy in the study of prenatal mortality. *Theriogenology* 23:121-129.
- Mullen, M., G. Elia, M. Hilliard, M. H. Parr, M. G. Diskin, A. C. O. Evans, and M. A. Crow. 2012. Proteomic characterization of histotroph during the preimplantation phase of the estrous cycle in cattle. *J. Proteome Res.* 11:3004-3018.
- Mussard, M. L., C. R. Burke, E. J. Behlke, C. L. Gasser, A. R. Robison, J. E. Kinder, and M. L. Day. 2002. Influence of premature induction of an LH surge with GnRH on ovulation, luteal function and fertility in cattle. *Biol. Reprod.* 66:(Suppl. 1):266. (Abstr.)
- Mussard, M. L., C. R. Burke, and M. L. Day. 2003a. Ovarian follicle maturity at induced ovulation influences fertility in cattle. Pages 179-185 in *Proc. Ann. Conf. Soc. Theriogenol.*, Columbus, OH.
- Mussard, M. L., C. R. Burke, C. L. Gasser, E. J. Behlke, K. A. Colliflower, D. E. Grum, and M. L. Day. 2003b. Ovulatory response, luteal function and fertility in cattle induced to ovulate dominant follicles of early or late maturity. *Biol. Reprod.* 68(Suppl. 1):332. (Abstr.)
- Mussard, M. L., C. R. Burke, E. J. Behlke, C. L. Gasser, and M. L. Day. 2007. Influence of

- premature induction of a luteinizing hormone surge with gonadotropin-releasing hormone on ovulation, luteal function and fertility in cattle. *J. Anim. Sci.* 85:937-943.
- Okumu, L. A., N. Forde, A. G. Fahey, E. Fitzpatrick, J. F. Roche, M. A. Crowe, and P. Lonergan. 2010. The effect of elevated progesterone and pregnancy status on mRNA expression and localization of progesterone and oestrogen receptors in the bovine uterus. *Reproduction* 140:143-153.
- Pancarci, S. M. E. R. Jordan, C. A. Risco, M. J. Schouten, F. L. Lopes, F. Moreira, and W. W. Thatcher. 2002. Use of estradiol cypionate in a presynchronized time artificial insemination program for lactating dairy cattle. *J. Dairy Sci.* 85:122-131.
- 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. Natl. Acad. Sci. USA* 102:5268-5273.
- Perry, G. A., M. F. Smith, A. J. Roberts, M. D. MacNeil, and T. W. Geary. 2007. Relationship between size of the ovulatory follicle and pregnancy success in beef heifers. *J. Anim. Sci.* 85:684-689.
- Perry, G. A., B. L. Perry, and R. A. Cushman. 2009. Association between preovulatory concentrations of estradiol and expression of uterine milk protein precursor, inhibin beta A, period 1, proenkephalin, and receptors for oxytocin, progesterone, and estradiol. *Biol. Reprod.* 79 (Suppl. 1). 308. (Abstr.)
- Pohler, K. G., T. W. Geary, J. A. Atkins, G. A. Perry, E. M. Jinks, and M. F. Smith. 2012. Follicular determinants of pregnancy establishment and maintenance. *Cell. Tissue Res.* 349:649-664.
- Roberts, C. A., G. A., Perry, M. D. MacNeil, M. A. Minten, and T. W. Geary. 2012. Effects of

- preovulatory estradiol concentrations on embryo survival and pregnancy establishment in beef cows. *J. Anim. Sci.* 90 (Suppl. 3):155. (Abstr.)
- Robinson, R. S., G. E. Mann, G. E. Lamming, and D. C. Wathes. 1999. The effect of pregnancy on the expression of uterine oxytocin, oestrogen and progesterone receptors during early pregnancy in the cow. *J. Endocrinol.* 160:21-33.
- Robinson, R. S., G. E. Mann, G. E. Lamming, and D. C. Wathes. 2001. Expression of oxytocin, oestrogen and progesterone receptors in uterine biopsy samples throughout the oestrous cycle and early pregnancy in cows. *Reproduction* 122:965-979.
- Sangsrivong, 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-17 β in dairy cattle. *J. Dairy Sci.* 85:2831-2842.
- Sartori, R., R. Sartor-Bergfelt, S. A. Mertens, J. N. Guenther, J. J. Parish, and M. C. Wiltbank. 2002. 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.
- Sartori, R., A. Gumen, J. N. Guenther, A. H. Souza, D. X. Caraviello, and M. C. Wiltbank. 2006. Comparison of artificial insemination versus embryo transfer in lactating dairy cows. *Theriogenology* 65:1311-1321.
- Sartori, R., M. R. Bastos, and M. C. Wiltbank. 2010. Factors affecting fertilization and early embryo quality in single- and superovulated dairy cattle. *Reprod. Fertil. Dev.* 22:151-158.
- Satterfield, M. C., F. W. Bazer, and T. E. Spencer. 2006. Progesterone regulation of preimplantation conceptus growth and galectin 15 (LGALS15) in the ovine uterus. *Biol. Reprod.* 75:289-296.
- Satterfield, M. C., K. A. Dunlap, K. Hayashi, R. C. Burghardt, T. E. Spencer, and F. W. Bazer.

- Tight and adherens junctions in the ovine uterus: differential regulation by pregnancy and progesterone. *Endocrinology* 148:3922-3931.
- Satterfield, M. C., H. Gao, X. Li, G. Wu, G. A. Johnson, T. E. Spencer, and F. W. Bazer. 2010. Select nutrients and their associated transporters are increased in the ovine uterus following early progesterone administration. *Biol. Reprod.* 82:224-231.
- Schiefelbein, A. K., B. L. Perry, and G. A. Perry. 2008. Association between preovulatory concentrations of estradiol and expression of uterine receptors for oxytocin, progesterone, and estradiol. *Biol. Reprod.* 78 (Suppl. 1):362. (Abstr.)
- Sellars, C. B., J. C. Dalton, R. Manzo, J. Day, and A. Ahmadzadeh. 2006. Time and incidence of ovulation and conception rates after incorporating estradiol cypionate into a timed artificial insemination protocol. *J. Dairy Sci.* 89:620-626.
- Shelton, K., M. F. Gayerie de Abreu, M. G. Hunter, T. J. Parkinson, and G. E. Lamming. 1990. Luteal inadequacy during the early luteal phase of subfertile cows. *J. Reprod. Fertil.* 90:1-10.
- Shimizu, T., S. Krebs, S. Bauersachs, H. Blum, E. Wolf, and A. Miyamoto. 2010. Actions and interactions of progesterone and estrogen on transcriptome profiles of the bovine endometrium. *Physiol. Genomics* 42:290-300.
- Silvia, W. J., G. S. Lewis, J. A. McCracken, W. W. Thatcher, and L. Wilson Jr. 1991. Hormonal regulation of uterine secretion of prostaglandin $F_{2\alpha}$ during luteolysis in ruminants. *Biol. Reprod.* 45:655-663.
- Smith, M. F., 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.

- Souto, L. A. 2011. The effect of length of the preovulatory period on mechanisms regulating embryonic survival in beef cattle. MS Thesis. The Ohio State Univ., Columbus.
- Souza, A. H., A. Gumen, E. P. B. Silva, A. P. Cunha, J. M. Guenther, C. M. Peto, D. Z. Caraviello, and M. C. Wiltbank. 2007. Supplementation with estradiol-17 β before the last gonadotropin-releasing hormone injection of the Ovsynch protocol in lactating dairy cows. *J. Dairy Sci.* 90:4623-4634.
- Spencer, T. E., and F. W. Bazer. 1995. Temporal and spatial alterations in uterine estrogen receptor and progesterone receptor gene expression during the estrous cycle and early pregnancy in the ewe. *Biol. Reprod.* 53:1527-1543.
- Spencer, T. E., and F. W. Bazer. 1996. Ovine interferon tau suppression transcription of the estrogen receptor and oxytocin receptor genes in the ovine endometrium. *Endocrinology* 137:1144-1147.
- Spencer, T. E., and F. W. Bazer. 2002. Biology of progesterone action during pregnancy recognition and maintenance of pregnancy. *Front. Biosci.* 7:d1879-d1898.
- Spencer, T. E., G. A. Johnson, F. W. Bazer, and R. C. Burghardt. 2004. Implantation mechanisms: insights from the sheep. *Reproduction* 128:657-668.
- Stevenson, J. S., S. M. Tiffany, and M. C. Lucy. 2004. Use of estradiol cypionate as a substitute for GnRH in protocols for synchronizing ovulation in dairy cattle. *J. Dairy Sci.* 87:3298-3305.
- Stronge, A. J., J. M. Sreenan, M. E. Diskin, J. F. Mee, D. A. Kenny, and D. G. Morris. 2005. Post-insemination milk progesterone concentrations and embryo survival in dairy cows. *Theriogenology* 64:1212-1224.
- Tong, W., and J. W. Pollard. 1999. Progesterone inhibits estrogen-induced cyclin D1 and cdk4

nuclear translocation, cyclin E- and cclinA-cdk2 kinase activation, and cell proliferation in uterine epithelial cells in mice. *Mol. Cell. Biol.* 19:2251-2264.

Vasconcelos, J. L. M., R. Sartori, H. N. Oliveira, J. G. Guenther, and M. C. Wiltbank. 2001.

Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. *Theriogenology* 56:307-314.

Vasconcelos, J. L., S. Sangritavong, S. J. Tsai, and M. C. Wiltbank. 2003. Acute reduction in

serum progesterone concentrations after feed intake in dairy cows. *Theriogenology* 60:795-807.

Wilmut, I., D. I. Sales, and C. J. Ashworth. 1986. Maternal and embryonic factors associated

with prenatal loss in mammals. *J. Reprod. Fertil.* 76:851-864.

Wiltbank, M. C., A. H. Souza, P. D. Carvalho, R. W. Bender, and A. B. Nascimento. 2012.

Improving fertility to timed artificial insemination by manipulation of circulating progesterone concentrations in dairy cattle. *Reprod. Fertil. Dev.* 24:238-243.