TRIENNIAL REPRODUCTION SYMPOSIUM:
Deficiencies in the uterine environment and failure to support embryonic development[1]

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ABSTRACT: Pregnancy failure in livestock can result from failure to fertilize the oocyte or embryonic loss during gestation. The focus of this review is on cattle and factors affecting and mechanisms related to uterine insufficiency for pregnancy. A variety of factors contribute to embryonic loss and it may be exacerbated in certain animals, such as high-producing lactating dairy cows, and in some cattle in which estrous synchronization and timed AI was performed, due to reduced concentrations of reproductive steroids. Recent research in beef cattle induced to ovulate immature follicles and in lactating dairy cows indicates that deficient uterine function is a major factor responsible for infertility in these animals. Failure to provide adequate concentrations of estradiol before ovulation results in prolonged effects on expression and localization of uterine genes and proteins that participate in regulating uterine functions during early gestation. Furthermore, progesterone concentrations during early gestation affect embryonic growth, interferon-tau production, and uterine function. Therefore, an inadequate uterine environment induced by insufficient steroid concentrations before and after ovulation could cause early embryonic death either by failing to provide an adequate uterine environment for recognition of embryo signaling, adhesion, and implantation or by failing to support appropriate embryonic growth, which could lead to decreased conceptus size and failed maternal recognition of pregnancy.

Key words: cattle, embryonic loss, estradiol, progesterone, uterus

INTRODUCTION

Given that fertilization rates are high in most livestock species, 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 most ruminant livestock species attachment of the conceptus and placentation does not occur immediately after fertilization, but rather the conceptus spends a prolonged period within the uterine lumen before developing a definitive attachment to the uterine endometrium (Bazer et al., 2009). During this period, the embryo and eventual conceptus is dependent on the uterus and its secretions to provide an adequate mircoenvironment for continued development and eventual attachment (Bazer, 1975). Optimal uterine function requires specific and sequential changes in the uterine 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 among the ovarian steroids estradiol and progesterone, the uterine endometrium, and elongating conceptus are required.

Unfortunately, uterine dysfunction occurs in livestock and the instances of uterine deficiencies appear to be exacerbated in certain populations of animals. Most notably, both beef and dairy cattle that have reduced

serum concentrations of estradiol before ovulation and/or fail to have a rapid increase of progesterone after 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 livestock 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 nonlactating 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 and 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 after 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 on 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 before blastocyst hatching. In lactating dairy cows, prehatching embryos are often compromised albeit the proportion of nonviable embryos collected varied considerably between studies (Sartori et al., 2010). In a summary of various studies (Sartori et al., 2010), the percentage of nonviable 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 prehatching embryonic death or compromised development is high in lactating dairy cows after 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 after 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 downregulation 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. In recent years 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.

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 holistic transcriptome profiling of the bovine uterine endometrium (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) assists in delineating the dynamic changes occurring within the uterus during the estrous cycle and in response to the presence of the conceptus. Not surprisingly, distinct transcriptome profiles are found in the uterus during the follicular and luteal phases of the estrous cycle (Bauersachs et al., 2005; Mitko et al., 2008) representing the differential biological processes that occur during each period. Likely, these differential transcriptome profiles are due to signals from the predominant reproductive hormones in circulation at the respective times of the estrous cycle. Estradiol, progesterone, and the combination of progesterone pre-exposure followed by estradiol result in differential gene expression within the bovine endometrium (Shimizu et al., 2010). Estradiol and progesterone provoke transcriptome changes within the uterine endometrium, with response to estradiol being greater when the uterus was preexposed to progesterone (Shimizu et al., 2010). Beyond differences in the transcriptome profiles during the estrous cycle, the presence of the conceptus after maternal recognition of pregnancy also induces distinct gene expression within the bovine uterus. Bauersachs et al. (2006) and Klein et al. (2006) reported 109 and 87 genes to be upregulated, respectively, in the bovine endometrium on d 18 of gestation in response to embryonic cues. Both studies reported that the majority of these genes were stimulated by type I interferons and contributed to several biological processes within the endometrium, such as modulation of the maternal immune system, cellular communication, cell adhesion, cell growth and differentiation, and endometrial remodeling. As will be further reviewed subsequently in this manuscript, Forde et al. (2011, 2012) demonstrated that the bovine transcriptome is temporally modulated by circulating concentrations of progesterone, and these alterations have direct effects on the ability of the uterus to support embryonic development and maintain pregnancy. Collectively, these transcriptome 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 postovulation are causing uterine deficiencies that ultimately result in conceptus death.

Hormonal Requirements for Pregnancy Establishment

The sequential exposure to progesterone before estrus, increased 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, 1976, 1983; Moore and Miller, 1976; 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, 1976; Moore and Miller, 1976) 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 after synchronous embryo transfer (Miller and Moore, 1976). 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 (Moore and Miller, 1976). 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 (**ECP**) 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) has 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 before 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., 2006; 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 after 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. (2013) 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 (Moore and Miller, 1976). Indisputably, both increased 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 postovulatory progesterone concentrations are associated with enhanced fertility in cattle, studies reporting pregnancy success after supplementation of these reproductive steroids are equivocal. In lactating dairy cows, administering ECP before 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 (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 before 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. Whereas 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 on 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 increased 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

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 (**OXTR**) receptors 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 midluteal 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 nonpregnant cattle. In cyclic cattle, the decrease in PGR allows for an increase

in OXTR beginning approximately on d 16 of the estrous cycle. In sheep, the increase in OXTR is preceded by an increase in ESR1 (Flint et al., 1992; Spencer and Bazer, 1996); however, this relationship was not evident in cattle (Robinson et al., 1999). In pregnant ruminants, secretion of interferon-tau (**IFNT**) by the growing conceptus attenuates the increase in OXTR and subsequently prevents the pulsatile release of $PGF_{2\alpha}$ from the uterine endometrium (Farin et al., 1990; Meyer et al., 1995). In nonpregnant ruminants, the increase in OXTR initiates the luteolytic mechanism and ultimately results in pulsatile $PGF_{2\alpha}$ 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. In the ewe, PGR is not detectable in the luminal epithelium and is largely downregulated or absent from the glandular epithelium after d 11 of pregnancy; however, after d 11 of the estrous cycle, PGR is present in the stroma and myometrium (Spencer and Bazer, 1995; Spencer et al., 2004). Similar to the ewe, PGR is absent in the luminal epithelium during early gestation in the cow; however, the extent of downregulation 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 the pregnant sow, PGR is completely downregulated in the luminal epithelium but remains in greater amounts in the glandular epithelium than in the ewe or cow (Geisert et al., 1994; Mathew et al., 2011).

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 (Carson et al., 2000; Johnson et al., 2000; Satterfield et al., 2006; Forde et al., 2009). Downregulation 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). In the ewe, it has been speculated that during the preimplantation period and throughout the remainder of pregnancy, progesterone directs endometrial 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.

Disappearance of the PGR allows for the increased secretion of uterine products required to support conceptus development. Such as 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. The most compelling evidence for the role of uterine histotroph in directing embryo elongation is from uterine gland knockout ewes that lack endometrial gland development and subsequently lack proper histotroph secretion (Bartol et al., 1999; Gray et al., 2000, 2001, 2002). Even after repeated matings, uterine gland knockout ewes fail to maintain pregnancy through maternal recognition of pregnancy and conceptus development past d 11 of gestation is severely retarded (Gray et al., 2001).

Endocrine Induced Uterine Dysfunction

As previously indicated, beef or dairy females induced to ovulate small follicles after ovulation synchronization and dairy cows due to steroid catabolism (Sangsritavong et al., 2002; Vasconcelos et al., 2003) have erroneous endocrine profiles and reduced fertility (Lamb et al., 2001; Lucy, 2001; Perry et al., 2005, 2007). In these steroid deficient animals, previous studies in beef (Mussard et al., 2003a,b; Bridges et al., 2010, 2012; Atkins et al., 2013) 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 using either an animal model that varied the functional status, diameter, and age of follicle from which ovulation was induced (Mussard et al., 2003a,b, 2007) or, 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 (Fig. 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"

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]

[1]Percentage of animals determined to be pregnant after insemination. Pregnancy determination was conducted via ultrasonography at approximately 30 d postinsemination

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

[3]Interval from $PGF_{2\alpha}$ until GnRH administration

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

[5]Cows were induced to ovulate either a small (~11 mm) or large (~13 mm) ovarian follicle with GnRH. Embryos from nontreated cows were then transferred 7 d after GnRH

[6]Cows were induced to ovulate an ovarian follicle of similar diameter with GnRH either 1.25 or 2.25 d after $PGF_{2\alpha}$ administration. Animals were inseminated 12 h after GnRH administration. Includes only cows with a luteal phase of normal length

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

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 before 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, $PGF_{2\alpha}$ (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.

follicles is governed by the steroidogenic capacity of the follicle during proestrus and the resultant corpus luteum rather than directly by 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.

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., 2013; 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 est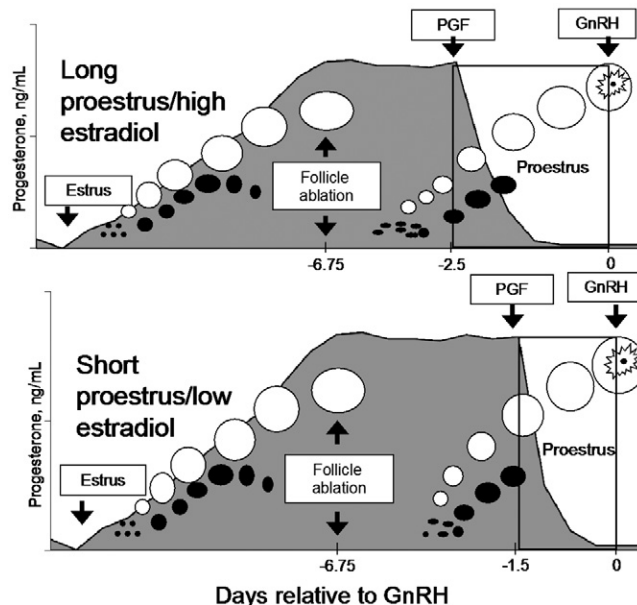radiol significantly contributed to the likelihood of fertilization and estradiol concentrations of the embryo recipient cow at GnRH induced ovulation was among the greatest factors affecting the likelihood of pregnancy (Atkins et al., 2013). Jinks et al. (2013) retrospectively divided the embryo donor and embryo recipient cows each into 2 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 before 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 Fig. 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 before 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 IFNT-stimulated genes (**ISG**), and localization of the PGR in the uterine endometrium. Beef heifers received an embryo 7 d after induced ovulation after either increased or reduced preovulatory concentrations of estradiol (Fig. 1). On d 15.5 of gestation, heifers were slaughtered, conceptuses were collected, and the reproductive tract was processed for future mRNA and immunohistochemical evaluation.

As anticipated and previously demonstrated (Bridges et al., 2010; Cruppe et al., 2011; Souto et al., 2011), this experimental model resulted in 2 treatment groups that differed in preovulatory estradiol concentrations whereas 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 in the deep glandular epithelium and ESR1 mRNA in the uterine endometrium were increased in the heifers with increased estradiol concentrations. The increased concentrations of estradiol before ovulation likely caused increased expression of ESR1 and greater PGR protein immediately after 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 (Fig. 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 increased 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 increased concentrations of estradiol before 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 downregulation 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 before 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 the ability of the conceptus to progress through maternal recognition of pregnancy but unable to survive to d 30 of gestation (M. L. Day, unpublished data). Furthermore, Roberts et al. (2012) demonstrated that in ovariectomized cows, there was no difference in the proportion of cows having increased 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). Also of interest, when evaluating functional annotation of the transcriptome profiles of the bovine endometrium, Shimizu et al. (2010) reported that the term "decidualization" was prevalent among the estrogen responsive genes. Although decidualization does not occur in cattle, this may provide additional evidence of the role of estradiol on preparing the bovine endometrium for attachment of the conceptus. 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 in the ewe (Satterfield et al., 2006, 2010) and cow (Forde et al., 2009, 2010) facilitates conceptus growth indirectly by accelerating the downregulation of the PGR in the uterine endometrium to allow earlier and increased secretion of uterine histotroph through upregulation of specific nutrient transport pathways. In the pregnant ewe, exogenous progesterone increased various AA (i.e., arginine, serine, glutamine, and lysine) and tended to increase glucose concentrations in the uterine histotroph (Satterfield et al., 2010). The increased availability of these nutrients was associated with a progesterone-induced upregulation of the sodium-dependent (**SLCA1**) and facilitative (**SLC2A1**) glucose transporters as well as the cationic amino acid (arginine and lysine) transporter (**SLC7A2B**). Previous work by this group also demonstrated that supplementing additional progesterone to ewes during early gestation reduced tight junction-associated proteins in the luminal epithelium to allow increased serum- and/or stromal-derived molecules to enter the uterine lumen (Satterfield et al., 2007). 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. (2013) 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 nonpregnant 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.

Administration of progesterone antagonists during early conceptus development in the ewe (Satterfield et al., 2006) and sow (Mathew et al., 2011) disrupted downregulation of PGR in the uterine endometrium and retarded conceptus growth. 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, 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 after 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 downregulation 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 decreased progesterone altered the temporal expression of several genes associated with histotroph composition, selected transporters, cellular proliferation and migration, and 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 after embryo transfer (Forde et al., 2011). Of interest, compared with previous evaluations in heifers with increased progesterone concentrations after ovulation (Forde et al., 2011), genes altered by reduced progesterone were distinct from

those genes affected by increased 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., 2013), including follicular characteristics as summarized by Geary et al. (2013), 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 before ovulation and progesterone postovulation 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 postovulatory 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 reduced 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 livestock species.

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