SUPPLEMENTATION OF PROGESTERONE ON ESTABLISHMENT OF
PREGNANCY, RESYNCHRONIZATION OF ESTRUS, AND DEVELOPMENT OF
IN VITRO-PRODUCED BOVINE EMBRYOS

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ABSTRACT

Progesterone is necessary for the maintenance of pregnancy and has been associated with embryonic mortality. Five experiments were conducted to discover the role of progesterone on establishment of pregnancy, resynchronization of estrus, and development of in vitro produced bovine embryos. The objective of experiment 1 was to determine whether an ovulatory estrus could be resynchronized in previously synchronized artificially inseminated (AI) nonpregnant cows without compromising pregnancy from the previous synchronized ovulation or to those inseminated at the resynchronized estrus. Treatments consisted of a vaginal insert containing progesterone administered after a timed AI (TAI) at different timepoints, and for differing durations. It was determined that pregnancy rates to the initial TAI were similar and the resynchronization of estrus was successful in two treatments, however, fertility in those treatments was decreased. In experiment 2, we established the concentrations of progesterone in the lumen of the uterine horn and oviduct to establish a base concentration to be used in further experiments. Using uterine flushes of estrus synchronized heifers and fluid collection of reproductive tracts post-slaughter; we determined that, while concentration of progesterone was highly variable in females, the mean was nearly 1 ng/mL. The objectives of experiments 3 and 4 were to determine the effects of culturing in vitro produced embryos with or without a co-culture of bovine oviductal epithelial cells (BOEC) with or without two concentrations of progesterone on embryo development. We determined that co-culture of bovine embryos with BOEC decreased development and the addition of progesterone decreased development of

embryos. The objective of experiment 5 was to determine if the addition of two concentrations of progesterone at two stages of culture impacted embryo development, embryo metabolism and numbers of cells in each embryo. We determined that while some characteristics changed slightly with the addition of progesterone, overall viability was not increased or decreased dramatically. Our conclusion was that although we do not fully understand the role of progesterone on embryonic mortality, we were unable to affect pregnancy rate or embryonic development with the concentrations and timing of progesterone employed in these experiments.

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CHAPTER 1

The role of progesterone on pregnancy, resynchronization of the estrous cycle, and embryonic mortality in cattle

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LITERATURE REVIEW

Defining progesterone

Progesterone is a steroid hormone produced by the corpus luteum (CL), the placenta, and the adrenal cortex. Progesterone binds to receptors in the reproductive tract and the hypothalamo-pituitary axis and promotes endometrial gland growth and stimulates secretory activity of the oviduct and endometrial glands of the uterus (Maslar et al., 1986). Progesterone also changes the proteins secreted by the endometrial cells in the uterus (Strinden and Shapiro, 1983; Maslar et al., 1986). Elevated concentrations of progesterone inhibit mitosis in the endometrium (Padykula et al., 1989). These functions collectively serve to provide nutrients to the developing embryo prior to implantation.

Progesterone down-regulates the receptors for estradiol (Brenner et al., 1974; West et al., 1987; Iwai et al., 1995); therefore, blocking many of estradiol's actions, including preventing contractions of the uterus by blocking estradiol's ability to induce α-adrenergic receptors that, when activated, cause the uterus to contract (Bottari et al., 1983). If an embryo is not present, a release of $PGF_{2\alpha}$ from the uterus causes luteolysis of the CL (Lauderdale, 1972; Hansel et al., 1973).

Synchronization and resynchronization of the estrous cycle

Manipulation of the estrous cycle by exogenous hormones was initiated with the use of progestins (Hansel and Malven, 1960; Zimbelman, 1963; Hansel et al., 1966; Zimbelman and Smith, 1966) and increased shortly after the discovery of the luteolytic effects of $PGF_{2\alpha}$ (Lauderdale, 1972; Liehr et al., 1972; Louis et al., 1972; Hansel et al., 1973). Synchronizing the estrous cycle and/or ovulation provides a labor-efficient way

to incorporate artificial insemination (AI) into cattle operations which allows for the potential genetic improvement AI brings. Synchronization of AI has the potential to shorten the breeding season (Short et al., 1990) which can lead to a shorter calving season, allowing more calves to be born near the beginning of the calving season (Lamb et al., 2006; Larson et al., 2006). In order to increase the percentage of cows that conceive to AI in a breeding season, the reinsemination of females that did not conceive to the first AI at the first eligible return estrus can be facilitated by resynchronization of the estrous cycle (Van Cleeff et al., 1996). In addition, increasing the percentage of cows that conceive to AI can increase profitability of the operation due to a decrease in the number of bulls necessary for clean-up pregnancies (Rodgers, 2008). Additional hormonal control of the estrous cycle allows for a second AI to occur with efficient utilization of labor.

Resynchronization with a progestin increased synchronized return rates of nonpregnant females (Stevenson et al., 2003; Colazo et al., 2006). Resynchronization with progesterone and estradiol cypionate or estradiol benzoate, however, decreased subsequent conception rates (Stevenson et al., 2003). Supplementation with progesterone on d 5 post-AI enhanced pregnancy rates in Holstein cows (Villarroel et al., 2004), but suppressed fertility when administered within 2 d of first insemination (Van Cleeff et al., 1996). It is clear that supplementation of progesterone may be used to resynchronize cattle but that the timing of supplementation may impact the viability of previously established pregnancies and fertility of the following ovulation of the subsequent return estrus.

Impact of embryonic mortality

Progesterone is essential for the maintenance of pregnancy and insufficient concentrations of progesterone have been speculated to enhance embryonic mortality. Embryonic loss has substantial economic effects in both dairy and beef cattle operations because of loss of milk production, a decrease in the number of calves that can be sold or used as replacement heifers, and an increase in culling rate. While it is difficult to establish the economic impact of embryonic loss to cattle producers. Thurmond and Picanso (1990) estimated the cost at $640 and Eicker and Fetrow (2003) estimated $600 to $800 per pregnancy loss in dairy females. De Vries (2006) valued a pregnancy loss at $555 in an economic evaluation of pregnancy in dairy females. Annual economic impact of infertility (including embryonic loss) to U.S. beef producers was estimated to be greater than $1.06 billion (Lamb et al., 2008).

Despite an increased understanding of reproduction in the dairy cow, reproductive efficiency continues to decline. Increases in embryo transfer applications and early pregnancy diagnosis resulted in the discovery that less than 50% of the viable embryos establish pregnancy by 27 to 30 d after ovulation in lactating dairy cows (Drost et al., 1999; Sartori et al., 2003). Declining fertility is an economic burden and that embryonic mortality has a significant role in producing a live calf and enhancing the economic success of beef and dairy producers.

Terminology and definitions

Definitions according to the Committee on Bovine Reproductive Nomenclature (1972) used throughout this thesis:

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Embryonic period: from conception to the end of the differentiation stage, approximately d 45 of gestation.

Early embryonic loss: pregnancy loss prior to d 24 after fertilization.

Late embryonic loss: pregnancy loss between d 24 and d 45 after fertilization.

Fetal period: from approximately d 45 of gestation to delivery.

Fetal loss: pregnancy loss after d 45 after fertilization.

Definitions of other terms used throughout this thesis are:

Synchronization rate: Proportion of females detected in estrus to total number treated.

Conception rate: Proportion of females becoming pregnant to those exhibiting estrus and inseminated during the synchronized period (measured at differing points in time).

Pregnancy rate: Proportion of females becoming pregnant to total number treated (measured at differing points in time).

The method used to determine these rates also is critical for consideration and may include flushing of the preimplantation embryo, slaughter and uterus retrieval, return to estrus, determination of the concentration of progesterone in milk, determination of bovine pregnancy-associated glycoprotein (Zoli et al., 1992), palpation per rectum, transrectal ultrasonography, and calving, all with varying advantages and disadvantages (Ludwick and Rader, 1968; Skemish et al., 1973; Garrett et al., 1988; Szenci et al., 1998; Whisnant et al., 2001; Fricke, 2002). The timing of the determination should be noted as embryonic or fetal mortality after fertilization can affect the outcome at accumulating rates as pregnancy continues.

Overview of embryonic mortality

An early review, indicated that fertilization failure rate ranged from close to zero in first-service heifers and upwards of 40% in repeat-breeder heifers and cows when assessed 3 d after mating (Ayalon, 1978). In studies using dairy cows that were inseminated and then flushed for collection of embryos on d 6 or 7, fertilization rates were similar in lactating and nonlactating cows, averaging 76.2% (ranging from 55.3 to 87.8%) and 78.1% (ranging from 58.0 to 98.0%), respectively (Dalton et al., 2001; DeJarnette et al., 1992; Sartori et al., 2002). However, by d 27 to 31 after AI, conception rates decreased to 35 to 45% in lactating dairy cows. By d 5 to 6 after AI, only 65% of the embryos were considered viable (Dalton et al., 2001; DeJarnette et al., 1992; Sartori et al., 2002). There are several factors in dairy cows that account for these perceived pregnancy rates. In a meta-analysis combining early and late embryonic loss with fetal loss, it was estimated that about 60% of all pregnancies are lost in high-producing dairy cows (Santos et al., 2004b), with most losses occurring in the first 42 d of pregnancy. Fertility in dairy cows has been decreasing throughout the U.S. and internationally (Royal et al., 2000; Lucy, 2001; Stevenson, 2001, Zeron et al., 2001; López-Gatius, 2003). This decline in fertility of dairy cows has become a primary focus of many scientists. In the 1950s conception rates of dairy cows inseminated after detected estrus were approximately 55% (Casida, 1961). However, recently published conception rates are approximately 45% (Dransfield et al., 1998), determined by palpation per rectum 35 to 75 d after AI, for detected estrus and 35% or less with timed AI (Schmitt et al., 1996; Pursley et al., 1997a, b, 1998; Cartmill et al., 2001; Portaluppi and Stevenson, 2005), determined by palpation per rectum 25 to 49 d after AI.

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Factors associated with embryonic mortality

An explanation for declining pregnancy rates is difficult, and the explanation has been multi-faceted, because numerous factors contribute to embryonic loss. A major contributor is thought to be diminished maternal recognition of pregnancy. A lack of communication between the conceptus and the endometrial epithelial cells leads to the secretion of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) which causes regression of the CL (Thatcher et al., 1986; Mann and Lamming, 2001; Thatcher et al., 2001). The CL releases progesterone, which, among other things, enhances conceptus development (Garrett et al., 1988). Progesterone also is produced by the placenta and this progesterone source takes over the maintenance of pregnancy at approximately d 200 of gestation in the bovine (Estergreen et al., 1967; Chew et al., 1979). Small luteal cells of thecal origin respond to a number of hormones, primarily luteinizing hormone (LH; Marsh et al., 1966), and it is these cells that control progesterone production and secretion. Progesterone blocks surges of gonadotropin releasing hormone (GnRH; Attardi and Happe, 1986; Kasa-Vubu et al., 1992), and down-regulates estrogen receptors (Brenner et al., 1974; Evans and Leavitt, 1980; Spencer et al., 1995a, b; Wathes et al., 1996), thus creating a quiescent uterus for pregnancy (Bottari et al., 1983), and healthy conceptus maturation.

Conceptus growth leads to stimulation of interferon-tau (IFNτ) secretion (Mann and Lamming, 2001). Interferon-τ is produced by the mononuclear cells of the trophectoderm (Thatcher et al., 2001) beginning on d 12 of pregnancy (Farin et al., 1990). The effects of IFNτ are antiluteolytic due to the inhibition of endometrial expression of oxytocin receptors, thus inhibiting the release of $PGF_{2\alpha}$ (Robinson et al.,

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1999). The inability of the conceptus to suppress the luteolytic cascade during CL maintenance is a cause of embryonic loss, furthermore, progesterone is essential for the embryo and trophectoderm to have the capability to secrete IFNτ and halt luteolysis. Sustaining embryos to the stage where they are able to produce IFNτ by d 12 of pregnancy is critical to reducing embryonic loss, for which progesterone is an important component.

Lack of maternal recognition of pregnancy obviously indicates that the embryo will not survive; however, reasons why embryos do not induce maternal recognition is multifaceted. There are three primary events that create a suboptimum situation to decrease embryonic survival: an abnormal embryo (McFeely and Rajakoski, 1968; Hare et al., 1980; Gayerie de Abreu et al., 1984; King, 1990), an abnormal reproductive tract environment (Adler 1959; Brenner and Maslar, 1988; Verhage et al., 1990; Murray, 1992; Buhi, 1997; Hugentobler et al., 2007a,b), or an inappropriate relationship between the embryo and dam. The third reason listed occurs more frequently in litter-bearing species and can occur when follicles ovulate at differing intervals, causing a range of development stages among embryos or when uterine capacity is reached (Wilmut et al., 1986).

Chromosomal abnormalities are a source of embryonic and fetal death and may be a signal against maternal recognition of pregnancy. When cows were superovulated, only 1.9% of blastocysts were chromosomaly abnormal (Hare et al., 1980) whereas a second study demonstrated that 10% of morulae and blastocysts were cytogenetically abnormal (King 1990). Several studies involving non-superovulated cattle indicated a frequency of anomalies in 7.5% of embryos (McFeely and Rajakoski, 1968; Gayerie de

Abreu et al., 1984). Few calves with chromosomal abnormalities are born, indicating a high level of embryonic or fetal death in these calves. Chromosomal abnormalities may be transmitted from the sire or dam to the offspring or result from an error in the process of gamete formation or at fertilization and early development. It should be noted that humans suffer the greatest incidence of chromosomal abnormality, generally between 50 and 60% of spontaneous human abortuses of the first and second trimester (Boué and Lazar, 1975; Hassold et al., 1980); thus it is inappropriate to extrapolate from humans to other species (Wilmut et al., 1986).

Maternal disease state may affect embryonic survival. Trichomonas foetus and Vibrio fetus have known implications on embryo viability (Adler, 1959). Mastitis also has been associated with lower pregnancy rates (Hansen et al., 2004) due to an increased immune response, likely through the involvement of cytokines. Gröhn and Rajala-Schultz (2000) stated a hazard ratio indicating that retained placenta, metritis, and ovarian cysts all decreased the risk of conception. Disease likely affects embryonic mortality; however, the majority of cows are not classified as diseased at the time of insemination and therefore other factors are likely more crucial to embryonic survival.

Stress, including the environmental factors, may influence embryonic health. Heat stress has a large impact on overall reproduction in cattle (Thatcher, 1974; Ealy et al., 1993) and specifically decreases oocyte viability (Biggers et al., 1987; Hansen et al., 2001; Al-Katanami et al., 2002). Furthermore, climatic stress intended to mimic that of rainfall caused a reduction in prenatal survival in ewes when applied after mating (Griffiths et al., 1970).

The reproductive tract environment is critically important to embryonic viability. The oocyte is susceptible to poor viability from before ovulation until after fertilization in the oviduct; the oviduct and uterus appear to be involved in the promotion or demise of embryo growth. The oviduct consists of three specific regions: the infundibulum, ampulla, and isthmus. Each region has different characteristics that are believed to be designed to provide for the changing needs of the oocyte, spermatozoa, and developing embryo (Nayak and Zimmerman, 1971; Buhi et al., 1997). Two cell types, ciliated and nonciliated (secretory) cells, make up the epithelial lining and change during the estrous cycle. While sows are in estrus, the high concentrations of estrogen promote ciliation and secretory granules (Nayak and Zimmerman, 1971) in the ampulla and isthmus.

During diestrus, when progesterone increases, the secretory capabilities of both the ampulla and isthmus decrease and ciliation decreases in the ampulla (Buhi et al., 1997). The biosynthetic activity (the hormones and proteins that are released) of the oviduct is also under hormonal control. During times of estrogen influence, highly developed secretory organelles are present and disappear during times of progesterone influence (Brenner and Masler, 1988; Murray, 1992; Verhage et al., 1990). Oviductal cells secrete proteins, cytokines, and growth factors, and while the functions of many secretions remain unknown, their ability to improve embryo development has been shown. In vitro fertilization and embryo development are enhanced in several species, including bovine (Eyestone and First, 1989), by the addition of oviduct epithelial cells. Oviductal and uterine fluids also are the source of amino acids for the developing oocyte or embryo (Hugentobler et al., 2007a). Regulation of the concentrations of

important amino acids is critical but is not suspected to be a major factor in embryonic mortality (Hugentobler et al., 2007a). Similarly, concentrations of ions in the oviduct and uterus are important for embryo viability but they are not believed to be associated with embryonic mortality and they are not affected by progesterone (Hugentobler et al., 2007b). Proteins, often referred to as uterine milk proteins, are secreted in the uterus and are under progesterone control, increasing during early pregnancy or under supplemental progesterone treatment (Ing et al., 1989). These proteins may serve a function as support of the conceptus during pregnancy.

Declining pregnancy rates may be directly or indirectly related to the increase in milk production of dairy cows. Cows had reduced fertility when they were in a negative energy balance, and they partitioned more nutrients towards milk synthesis, which reduced embryo quality and viability (Sartori et al., 2002). In contrast, several studies have demonstrated no connection between milk production, embryonic loss, or 30 to 31 d pregnancy rates (López-Gatius et al., 2002; Silke et al., 2002; Cerri et al., 2003; Santos et al., 2004a). The connection between an increase in milk production and a decline in fertility is not well established.

Additional evidence indicates that greater milk production is causing a decrease in blood progesterone concentrations. In 1964, the University of Minnesota established two genetic lines of dairy cows. A control line was maintained at 1964 genetics by using semen from bulls with an average predicted transmitting ability (PTA) for milk yield at that time (**1964**). A select line was developed by using semen from the four highest bulls for PTA for milk yield each year (**select**). When comparing the 1964 herd to the select herd, the select cows had lower concentrations of progesterone than the

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1964 herd (Lucy et al., 1998), establishing a link between genetic selection of milk production and lower concentrations of progesterone. Concentrations of circulating progesterone are maintained by a balance of secretion by the CL and liver clearance rate. Cleavage rate and the number of oocytes developing into blastocysts were lower for cows with high milk-producing genetics compared with cows with medium milk-producing genetics (Snijders et al., 2000).

Greater feed intake resulted in lower circulating concentrations of progesterone (Rabiee et al., 2000), and increased liver blood flow, thus increasing metabolism of progesterone (Sangsritavong et al., 2000, 2002). This observation was confirmed in ewes, in which the decrease in circulating progesterone with increased feed intake was due to an increase in clearance and not a reduction of synthesis rate (Smith et al., 2006). A slower rise in progesterone during early diestrus could compromise early embryonic development (Mann and Lamming, 1999) thereby reducing conception rates (Darwash and Lamming, 1998; Wathes et al., 2003). Concentrations of plasma progesterone increase from a near zero concentration at the time of estrus, to approximately 1 ng/mL three days after estrus and then to over 2 ng/mL after four days (McNeill et al., 2006a). Progesterone peaks around d 8 of the estrous cycle at plasma concentrations of approximately 4 ng/mL, though there is a wide range of concentrations among cows, and will continue to increase throughout pregnancy or will decline after luteolysis if maternal recognition fails to occur (Starbuck et al., 2006).

Henricks et al. (1971) investigated concentrations of progesterone before estrus and after mating. In 12 heifers that conceived, a steep rise in progesterone was seen until d 12 after mating where it reached 9.9 ng/mL and remained stable until d 33 and

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then increased to 13.9 ng/mL by d 39. Eight heifers did not conceive (assessed by palpation per rectum on d 42); five of these had a slow rise in progesterone (to about 7 ng/mL) by d 12 followed by a decrease after d 15. One heifer had a slow rise to d 18 and then an abrupt decrease by d 21. Two heifers followed the trend of the pregnant heifers until d 18 but then began to slowly decrease, registering below 1 ng/mL between d 26 and 30. The authors suggested these two heifers had either abnormal estrous cycles or were pregnant and suffered embryonic mortality.

A delay of 1 to 1.7 d in the post-ovulatory rise of progesterone was associated with decreased pregnancy rate in cows (Darwash and Lamming, 1998; Starbuck et al., 1999, 2001). In ewes, the conceptus was particularly sensitive to reductions in plasma progesterone over a 48-h period on d 11 and 12 of pregnancy (Parr, 1992). In mice selected for small-litter size, daily injections of progesterone during the early post-implantation period increased the number of offspring to control levels although concentrations of progesterone in the blood were not different (Michael et al., 1975).

Progesterone is required for pregnancy to be maintained, but a direct link between concentrations of progesterone and embryonic loss has not been validated. No reports indicate a specific nadir for circulating progesterone concentration to maintain pregnancy. It is clear that low concentrations of progesterone or a delay in the rise of systemic progesterone during the early post-ovulatory period is associated with low embryo survival in cattle (Lamming et al., 1989; Lamming and Darwash, 1995; Darwash and Lamming, 1998; Starbuck et al., 1999, 2001; Hommeida et al., 2004).

Progesterone is transferred locally from the ovarian/oviductal venous drainage to the uterine artery (Weems et al., 1988). The local transfer results in higher

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concentrations of progesterone within the uterus ipsilateral to the CL (Pope et al., 1982). This may render concentrations of progesterone in the blood (where it is measured and is subject to clearance) irrelevant in understanding maintenance of pregnancy. In spite of this, the concentration of progesterone in milk from d 4 to 7 after ovulation is positively correlated with embryo survival in dairy cows (Stronge et al., 2005; McNeill et al., 2006a). Similar results were noted in studies measuring concentration of progesterone in plasma of heifers (Diskin et al., 2002; McNeill et al., 2002).

The immune system has an important role in embryo survival (Stites and Siiteri, 1983). Half the genome in an embryo is derived from the male, thus embryos are considered foreign to the uterus. It is essential that an immune response does not occur that would create a suboptimal uterine environment or cause the embryo to be rejected. Sperm express some histocompatable antigens (Erickson, 1977; Erickson et al., 1981) and do not go undetected in the female reproductive tract. Seminal plasma contains potent immunosuppressive substances which may protect them from local immunologic attack (Lord et al., 1977). The female's role in immunosuppression remains somewhat unknown; however, it is clear that progesterone is integrally involved (Black et al., 1953; Simmons et al., 1968; Watnick and Russo, 1968; Munroe, 1971; Siiteri et al., 1977; Kovats et al., 1990; Yie et al., 2006). A decreased resistance to infection was noted in pseudopregnant rabbit uteri compared to those in estrus (Black et al., 1953) indicating, in this study, that progesterone and not estrogen may be an important immunorepressive hormone. The HLA-G gene is one gene that is involved in maternal-fetal immune tolerance (Kovats et al., 1990) and is expressed after a progesterone response element binds in the HLA-G promoter region (Yie et al., 2006). Results vary

in several studies using synthetic or supplemental progesterone. Krohn (1954) was unable to prolong rabbit skin allograft survival in animals injected with progesterone, while a combination of progesterone and estrogen did moderately prolong graft survival in mice (Simmons et al., 1968), rats (Watnick and Russo, 1968) and monkeys (Munroe, 1971). In addition, Moriyama and Sugawa (1972) demonstrated that survival of xenogeneic cell lines in the uterus of hamsters was prolonged with progesterone. Siiteri et al. (1977) hypothesized that a high local concentration of progesterone was a key factor in the immunosuppression at the maternal-placental interface.

Many studies have been conducted to elicit details on the role of progesterone as an immunosuppressant; most of them indicating effects on lymphocyte activation by mitogens, antigens, and allogeneic cells (Siiteri and Stites, 1982). More specifically, progesterone inhibits T lymphocyte activation in humans (Stites and Siiteri, 1983). As concentrations of progesterone decrease before parturition, there is an increase in uterine monocytes (Padykula and Tansey, 1979). This may indicate that a withdrawal of progesterone, or low concentrations of progesterone, may be associated with immune rejection and tissue inflammation. Further research is warranted to determine associations between embryonic mortality and immunosuppression.

Circulating progesterone may play a role in affecting gonadotropin secretion, which alters follicular growth patterns in dairy cows. The uterine environment changes, both physically (Buhi et al., 1997) and biochemically (Garrett et al., 1988; Geisert et al., 1992; Mann et al., 1998; Hugentobler et al., 2007a,b), in response to progesterone stimulation in order to create an environment that can sustain the embryo. Progesterone stimulates the production and secretion of endometrial proteins that are critical to

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embryonic growth (Garrett et al., 1988; Geisert et al., 1992; Mann et al., 1998).

Therefore, lower concentrations of progesterone could impair the production and

secretion of endometrial proteins creating a sub-optimal uterine environment, which

may be a factor in embryonic death.

Supplemental progesterone

Increasing concentrations of progesterone in the uterus has the potential to

increase embryonic survival. A meta-analysis of 17 progesterone supplementation

studies indicated that progesterone treatment during the first week of pregnancy resulted

in an overall increase in pregnancy rate; however, treatment during the second or third

week of pregnancy did not significantly change pregnancy rates (Mann and Lamming,

1999). In addition to the studies used in this meta-analysis, Table 1 includes more

recent studies, in which supplementation of progesterone was used after an initial

insemination. Overall, pregnancy rate of treated animals was 47.6 % (4130/8675) and

49.1 % (3345/6808) in control animals. Many of these studies did not reach statistical

significance individually; however, when studies were grouped under categories,

several trends were apparent.

The earlier studies using orally administered or injected progesterone

demonstrated promising results. In this subgroup, pregnancy rates of treated animals

was 57.79% (204/353) and control animals was 44.05% (148/336; Table 1). These

early studies also administered progesterone within 10 d after initial AI. However,

when taking all studies into consideration, pregnancy rates were decreased by 1.5

percentage points with treatment of supplemental progesterone (Table 1); however,

dosage, timing, and mode of action of progesterone's effects on fertility were not elucidated.

Supplemental progesterone during the first 4 days after AI increased morphological development and biosynthetic activity of day 14 conceptuses (Garrett et al., 1988). In addition, supplemental progesterone increased conception rates when administered to lactating dairy cows within 6 d after AI (Mann and Lamming, 1999). Timing of supplemental progesterone is critical, ideally around d 4 or 5 of gestation, because during this period progesterone may alter the secretory activity of the endometrium, thus influencing embryonic growth (Garrett et al., 1988; Geisert et al., 1992). This is the period of the normal increase in the concentration of progesterone and, as previously mentioned, a delay in the increase could impair embryonic growth. Progesterone supplemented immediately after AI caused an increase in the occurrence of short estrous cycles (Van Cleeff et al., 1996; Garrett et al., 1988; Lynch et al., 1999) which may pose some practical limitations for its use. In dairy cows that received an embryo, the average time for progesterone to reach 1 ng/mL after ovulation was 4.1 d for cows with detectable INF-τ on d 16 or 5.6 d for those with no detectable INF-τ on d 16 (Mann and Lamming, 2001).

The quantity of supplemented progesterone also appears to be critical. High concentrations of progesterone results in down-regulation of progesterone receptors (Clarke 1990; Spencer and Bazer 1995; Spencer et al., 1995b) and may down-regulate endogenous production of progesterone (Mann et al., 2001). The combination of these two factors may result in insufficient progesterone or reduced receptor activity after

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treatment with exogenous progesterone. Therefore, the exact timing and amount of supplemental progesterone is critical and needs to be further investigated.

Data from a second meta-analysis (Mann 2008) concluded that while progesterone treatment after either a natural or synchronized estrus improved pregnancy rates by 4.9%, treatment after a synchronized ovulation did not improve pregnancy rates. In addition, cows treated with supplemental progesterone after a nonsynchronized ovulation between d 3 to 9 had an 8.5% increase in pregnancy rate. Cows treated with supplemental progesterone from d 0 to 2 or after d 10 failed to improve fertility.

A recent study in dairy cows utilized an injection of either hCG or GnRH to induce the formation of an ancillary CL and increase concentrations of progesterone (Stevenson et al., 2008). Pregnancy losses were not affected by treatment or number of induced luteal structures but were nearly 9-fold greater in females in which induced luteal structures regressed (4 vs. 38%). In addition, cows that had greater serum concentrations of progesterone at the initial pregnancy diagnosis, regardless of treatment, were less likely to have pregnancy loss (Stevenson et al., 2008).

Collectively, these data indicate that a complex relationship exists between the induction of ovulation, concentrations of progesterone, and the likelihood of an embryo to survive and result in a successful pregnancy.

LITERATURE CITED

Adler, H. C. 1959. Genital vibriosis in the bovine. An experimental study on the influence on early embryonic mortality. Acta. Vet. Scand. 1:1-11.

Al-Katanami, Y. M., F. F. Paula-Lopes, and P. J. Hansen. 2002. Effect of season and exposure to heat stress on oocyte competence in Holstein cows. J. Dairy Sci. 85:390-396.

Attardi, B. and H. K. Happe. 1986. Modulation of the estradiol-induced luteinizing hormone surge by progesterone or antiestrogens: effects on pituitary gonadotropin-releasing hormone receptors. Endocrinology 119:274-283.

Ayalon, N. 1978. A review of embryonic mortality in cattle. J. Reprod. Fertil. 54:483-493.

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

Black, W. G., J. Simon, S. H. McNutt, and L. E. Cassida. 1953. Investigations on the physiological basis for the differential response of estrous and pseudopregnant rabbit uteri to induced infection. Am. J. Vet. Res. 11:318-323.

Bottari, S. P., A. Vokaer, E. Kaivez, J. P. Lescrainer, and G. P. Vauquelin. 1983. Differential regulation of alpha-adrenergic receptor subclasses by gonadal steroids in human myometrium. J. Clin. Endocrinol. Metab. 57:937-941.

Boué, J., A. Boué, and P. Lazar. 1975. Retrospective and prospective epidemiological studies of 1500 karyotyped spontaneous human abortions. Teratology 12:11-26.

Brenner, R. M. and I. A. Maslar. 1988. The primate oviduct and endometrium.

Pages 303-329 in Physiology of Reproduction. K. Knobil, J. D. Neill, L. L. Ewing, C. L. Markert, G. S. Greenwald, and D. W. Pfaff, eds. Raven Press, Ltd., New York.

Brenner, R. M., J. A. Resko, and N. B. West. 1974. Cyclic changes in oviductal morphology and residual cytoplasmic estradiol binding capacity induced by sequential estradiol-progesterone treatment of spayed Rhesus monkeys. Endocrinology 95:1094-1104.

Buhi, W. C., I. M. Alvarez, and A. J. Kouba. 1997. Oviductal regulation of fertilization and early embryonic development. J. Reprod. Fertil. 52:285-300.

Cartmill, J. A., S. Z. El-Zarkouny, B. A. Hensley, G. C. Lamb, and J. S. Stevenson. 2001. Stage of cycle, incidence, and timing of ovulation, and pregnancy rates in dairy cattle after three timed breeding protocols. J. Dairy Sci. 84:1051-1059.

Casida, L. E. 1961. Present status of the repeat-breeder cow problem. J. Dairy Sci. 44:2323-2329.

Cerri, R. L. A., K. N. Galvão, S. O. Juchem, R. C. Chebel, and J. E. P Santos. 2003. Timed AI (TAI) with estradiol cypionate (ECP) or insemination at detected estrus in lactating dairy cows. J. Dairy Sci. 86(Suppl. 1):181. (Abstr.)

Chebel, R. C., J. E. Santos, R. L. Cerri, K. N. Galvao, S. O. Juchem, and W. W. Thatcher. 2003. Effect of resynchronization with GnRH on day 21 after artificial insemination on pregnancy rate and pregnancy loss in lactating dairy cows. Theriogenology 60(8):1389-1399.

Chenault, J. R., J. F. Boucher, K. J. Dame, J. A. Meyer, and S. L. Wood-Follis. 2003. Intravaginal progesterone insert to synchronize return to estrus of previously inseminated dairy cows. J. Dairy Sci. 86:2039-2049.

Chew, B. P., R. E. Erb, J. F. Fessler, C. J. Callahan, and P. V. Malven. 1979. Effects of ovariectomy and of prematurely induced parturition on progesterone, estrogens, and calving traits. J. Dairy Sci. 62:557-566.

Clarke, C. L. 1990. Cell-specific regulation of progesterone receptor in the female reproductive system. Mol. Cell. Endocrinol. 70:C29-C33.

Colazo, M. G., J. P. Kastelic, R. C. Mainar-Jaime, Q. A. Gavaga, P. R. Whittaker, J. A. Small, M. F. Martinez, R. E. Wilde, D. M. Veira, and R. J. Mapletoft. 2006. Resynchronization of previously timed-inseminated beef heifers with progestins. Theriogenology 65:557-572.

Committee on Bovine Reproductive Nomenclature, 1972. Recommendations for standardizing bovine reproductive terms. Cornell Vet. 62:216-237.

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.

Darwash, A. O. and G. E. Lamming. 1998. The importance of milk progesterone concentrations during early pregnancy in the cow. J. Anim. Breed. 2:41-43.

Dawson, F. L. M. 1954. Progesterone in functional infertility of cattle. Vet. Rec. 66:324-326.

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 number in artificially inseminated cattle. J. Anim. Sci. 70:484-491.

De Vries, A. 2006. Economic value of pregnancy in dairy cattle. J. Dairy Sci. 89:3876-3885.

Diskin, M. G., D. A. Kenny, L. D. Dunne, and J. M. Sreenan. 2002. Systemic

   progesterone pre- and post-AI and embryo survival in heifers. Page 27 in Irish

   Agricultural Research Forum. M. D. Diskin, ed. Tullamore, Co. Offaly,

   Ireland.

Dransfield, M. B., R. L. Nebel, R. E. Pearson, and L. D. Warnick. 1998. Timing of

   insemination for dairy cows identified in estrus by a radiotelemetric estrus detection

   system. J. Dairy Sci. 81:1874-1882.

Drost, M., J. E. Ambrose, M-J Thatcher, C. K. Cantrell, K. E. Wolsdorf, J. F. Hansen,

   and W. W. Thatcher. 1999. Conception rates after artificial insemination or embryo

   transfer in lactating dairy cows during summer in Florida. Theriogenology 52:1161-

   1176.

Ealy, A. D., M. Drost, and P. J. Hansen. 1993. Developmental changes in embryonic

   resistance to adverse effects of maternal heat stress in cows. J. Dairy Sci. 76:2899-

   2905.

Eicker, S. and J. Fetrow. 2003. New tools for deciding when to replace used dairy cows.

   Pages 33-46 in Proc. Kentucky Dairy Conf., Cave City, KY. Univ. Kentucky,

   Lexington.

Erickson, R. P. 1977. Differentiation and other alloantigens of spermatozoa. Pages 85-

   94 in Immunology of Gametes. M. Edidin and M. H. Johnson, eds. Oxford

   University Press, London.

Erickson, R. P., S. E. Lewis, and M. Butley. 1981. Is haploid gene expression possible

   for sperm antigens? J. Reprod. Immunol. 3:195-219.

Estergreen, V. L., O. L. Frost, W. R. Gomes, R. E. Erb, and J. F. Bullard. 1967. Effect

of ovariectomy on pregnancy maintenance and parturition in dairy cow. J. Dairy Sci. 50:1293-1295.

Evans, R. W. and W. W. Leavitt. 1980. Progesterone inhibition of uterine nuclear estrogen receptor: dependence on RNA and protein synthesis. Proc. Natl. Acad. Sci. USA 77:5856-5860.

Eyestone, W. H. and N. L. First. 1989. Co-culture of early cattle embryos to the blastocyst stage with oviductal tissue or in conditioned medium. J. Reprod. Fertil. 85:715-720.

Farin, G. 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.

Fricke, P. M. 2002. Scanning the future-Ultrasonography as a reproductive management tool for dairy cattle. J. Dairy Sci. 85:1918-1926.

Funston, R. N., R. J. Lipsey, T. W. Geary, and A. J. Roberts. 2005. Effect of administration of human chorionic gonadotropin after artificial insemination on concentrations of progesterone and conception rates in beef heifers. J. Anim. Sci. 83:1403-1405.

Garrett, J. E., R. D. Geisert, M. T. Zavy, and G. L. Morgan. 1988. Evidence for maternal regulation of early conceptus growth and development in beef cattle. J. Reprod. Fertil. 84:437-446.

Gayerie de Abreu, F., G. E. Lamming, and R. C. Shaw. 1984. A cytogenetic investigation of early stage bovine embryos; relation with embryos mortality. Proc. 10th Int. Congr. Anim. Reprod. and A.I., Urbana. 2:82.

Geisert, R. D., G. L. Morgan, E. C. Short, and M. T. Zavy. 1992. Endocrine events associated with endometrial function and conceptus development in cattle. Reprod. Fertil. Dev. 4:301-305.

Griffiths J. G., R. G. Gunn, and J. M. Doney. 1970. Fertility in Scottish Blackface ewes as influenced by climatic stress. J. Agric. Sci., Camb. 75:485.

Gröhn, Y. T. and P. J. Rajala-Schultz. 2000. Epidemiology of reproductive performance in dairy cows. Anim. Reprod. Sci. 60-61:605-614.

Hansel, W., P. W. Concannon, and J. H. Lukaszewska. 1973. Corpora lutea of the large domestic animals. Biol. Reprod. 8:222-245.

Hansel, W., L. E. Donaldson, W. C. Wagner, and M. A. Brunner. 1966. A comparison of estrous cycle synchronization methods in beef cattle under feedlot conditions. J. Anim. Sci. 25:497-503.

Hansel, W. and P. V. Malven. 1960. Estrus cycle regulation in beef cattle by orally active progestational agents. J. Anim. Sci. 19:1324. (Abstr.)

Hansen, P. J., M. Drost, R. M. Rivera, F. F. Paula-Lopes, Y. M. Al-Katanani, C. E. Krininger 3rd, and C. C. Chase, Jr. 2001. Adverse impact of heat stress on embryo production: causes and stratagies for mitigation. Theriogenology 55(1):91-103.

Hansen, P. J., P. Soto, and R. P. Natzke. 2004. Mastitis and fertility in cattle – Possible involvement of inflammation or immune activation in embryonic mortality. Am. J. Reprod. Immunol. 51(4):294-301.

Hassold, T., N. J. Chen, J. Funkhouser, T. Jooss, B. Manuel, J. Matsura, A. Matsuyarma, C. Wilson, J. A. Yamane, and P. A. Jacobs. 1980. A cytogenetic study

of 1000 spontaneous abortions. Annls. Hum. Genet. 44:151-178.

Hare, W. C. D., E. L. Singh, K. J. Betteridge, M. D. Eaglesome, G. C. B. Randall, D. Mitchell, R. J. Bilton, and A. O. Trounson. 1980. Chromosomal analysis of 159 bovine embryos collected 12-18 days after estrus. Can. J. Genet. Cytol. 22:615-626.

Henricks, D. M., D. R. Lamond, J. R. Hill, and J. F. Dickey. 1971. Plasma progesterone concentrations before mating and in early pregnancy in the beef heifer. J. Anim. Sci. 33:450-454.

Herrick, J. B. 1953. Clinical observation of progesterone therapy in repeat breeding heifers. Vet. Med. 48:498-490.

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.

Hugentobler, S. A., M. G. Diskin, H. J. Leese, P. G. Humpherson, T. Watson, J. M. Sreenan, and D. G. Morris. 2007a. Amino acids in oviduct and uterine fluid and blood plasma during the estrous cycle in the bovine. Mol. Reprod. Devl. 74:445-454.

Hugentobler, S. A., D. G. Morris, J. M. Sreenan, and M. G. Diskin. 2007b. Ion concentrations in oviduct and uterine fluid and blood serum during the estrous cycle in the bovine. Theriogenology 68:538-548.

Ing, N. H., H. Francis, J. J. McDonnell, J. F. Amann, and R. M. Roberts. 1989. Progesterone induction of the uterine milk proteins: Major secretory proteins of sheep endometrium. Biol. Reprod. 41:643-654.

Iwai, M., H. Kanzaki, M. Fujimoto, K. Kojima, H. Hatayama, T. Inoue, T. Higuchi, H.

Nakayama, T. Mori, and J. Fujita. 1995. Regulation of sex steroid receptor gene expression by progesterone and testosterone in cultured human endometrial stromal cells. J. Clin. Endocrinol. Metab. 80:450-454.

Johnson, K. R., R. H. Ross, and D. L. Fourt. 1958. Effect of progesterone administration on reproductive efficiency. J. Anim. Sci. 17:386-390.

Kasa-Vubu, J. Z., G. E. Dahl, N. P. Evans, L. A. Thrun, S. M. Moenter, V. Padmanabhan, and F. J. Karsch. 1992. Progesterone blocks the estradiol-induced gonadotropin discharge in the ewe by inhibiting the surge of gonadotropin-releasing hormone. Endocrinology 131:208-212.

King, W. A. 1990. Chromosome abnormalities and pregnancy failure in domestic animals. Adv. Vet. Sci. Comp. Med. 34:229-250.

Kovats, S., E. K. Main, C. Librach, M. Stubblebine, S. J. Fisher, and R. DeMars. 1990. A class I antigen HLA-G, expressed in human trophoblasts. Science 248:220-223.

Krohn, P. L. 1954. The effect of steroid hormones on the survival of skin homografts in the rabbit. J. Endocrinol. 11:78-82.

Lamb, G. C., C. R. Dahlen, and M. Maddox. 2008. What is the economic impact of infertility in beef cattle? University of Florida, IFAS Florida Coop. Ext. Serv., Animal Science Dept., EDIS Publication AN208.

Lamb, G. C., J. E. Larson, T. W. Geary, J. S. Stevenson, S. K. Johnson, M. L. Day, R. P. Ansotegui, D. J. Kesler, J. M. DeJarnette, and D. G. Landblom. 2006. Synchronization of estrus and artificial insemination in replacement beef heifers using gonadotropin-releasing hormone, prostaglandin F2{alpha}, and progesterone. J. Anim. Sci. 84:3000-3009.

Lamming, G. E. and A. O. Darwash. 1995. Effect of inter-luteal interval on subsequent luteal phase length and fertility in post partum dairy cows. Biol. Reprod. 52(Suppl. 1):72.

Lamming, G. E., A. O. Darwash, and H. L Back. 1989. Corpus luteum function in dairy cows and embryo mortality. J. Reprod. Fertil. 37:245-252.

Larson, S. F., W. R. Butler, and W. B. Currie. 1995. Progesterone supplementation increases pregnancy rates in lactating dairy cattle. J. Reprod. Fertil. Abst. Series 15:63. (Abstr.)

Larson, J. E., G. C. Lamb, J. S. Stevenson, S. K. Johnson, M. L. Day, T. W. Geary, D. J. Kesler, J. M. DeJarnette, F. N. Schrick, A. DiCostanzo, and J. D. Arseneau. 2006. Synchronization of estrus in suckled beef cows for detected estrus and artificial insemination and timed artificial insemination using gonadotropin-releasing hormone, prostaglandin $F_{2\alpha}$, and progesterone. J. Anim. Sci. 84:332-342.

Larson, J. E., K. N. Thielen, B. J. Funnel, J. S. Stevenson, D. J. Kesler, and G. C. Lamb. 2009. Influence of a controlled internal drug release after fixed-time artificial insemination on pregnancy rates and returns to estrus of nonpregnant cows. J. Anim. Sci. 87:914-921.

Lauderdale, J. W. 1972. Effects of $PGF_{2\alpha}$ on pregnancy and estrous cycle of cattle. J. Anim. Sci. 35:246. (Abstr.)

Liehr, R. A., G. B. Marion, and H. H. Olson. 1972. Effects of prostaglandin on cattle estrous cycle lengths. J. Anim. Sci. 35:247. (Abstr.)

López-Gatius, F. 2003. Is fertility declining in dairy cattle? A retrospective study in northeastern Spain. Theriogenology 60:89-99.

López-Gatius, F., P. Santolaria, J. Yániz, J. Rutllant, and M. López-Béjar. 2002. Factors affecting pregnancy loss from gestation day 38 to 90 in lactating dairy cows from a single herd. Anim. Reprod. Sci. 57:1251-1261.

Lord, E. M., G. F. Sensabaugh, and D. P. Stites. 1977. Immunosuppressive activity of human seminal plasma. I. Inhibitory in vitro lymphocyte activation. J. Immunol. 118: 1704-1711.

Louis, T. M., H. D. Hafs, and D. A. Morrow. 1972. Estrus and ovulation after uterine $PGF_{2\alpha}$ in cows. J. Anim. Sci. 35:247. (Abstr.)

Lucy, M. C. 2001. Reproductive loss in high-producing dairy cattle: where will it end? J. Dairy Sci. 84:1277-1293.

Lucy, M. C., W. J. Weber, L. H. Baumgard, B. S. Seguin, A. T. Koenigsfeld, L. B. Hansen, H. Chester-Jones, and B. A. Crooker. 1998. Reproductive endocrinology of lactating dairy cows selected for increased milk production. J. Anim. Sci. 76(Suppl. 1):296. (Abstr.)

Ludwick, T. M. and E. R. Rader. 1968. Diagnosis of early pregnancy in cattle by ovarian analysis. J. Dairy Sci. 51:74-77.

Lynch, P. R., K. L. Macmillan, and V. K. Taufa. 1999. Treating cattle with progesterone as well as GnRH analogue affects oestrous cycle length and fertility. Anim. Reprod. Sci. 56:189-200.

MacMillan, K. L., V. K. Taufu, A. M. Day, and A. J. Peterson. 1991. Effects of supplemental progesterone on pregnancy rates in cattle. J. Reprod. Fertil. (Suppl.)43:304. (Abstr.)

Mann, G. E. 2008. Meta-analysis of progesterone supplementation during early

pregnancy in cattle. J. Anim. Sci. (Suppl.)86:387. (Abstr.)

Mann, G. E. and G. E. Lamming. 1999. The influence of progesterone during early pregnancy in cattle. Reprod. Domest. 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. Reproduction 121:175-180.

Mann, G. E., G. E. Lamming, and P. A. Fisher. 1998. Progesterone control of embryonic interferon tau production during early pregnancy in the cow. J. Reprod. Fertil. 19:132. (Abstr.)

Mann, G. E., P. Merson, M. D. Fray, and G. E. Lamming. 2001. Conception rate following progesterone supplementation after second insemination in dairy cows. Vet. J. 162:161-162.

Marsh, J. M., R. W. Butcher, K. Savard, and E. W. Sutherland. 1966. The stimulatory effect of luteinizing hormone on adenosine 3',5'-monophosphate accumulation in corpus luteum slices. J. Biol. Chem. 241:5436-5440.

Maslar, I. A., P. Powers-Craddock, and R. Ansbacher. 1986. Decideral prolactin production by organ cultures of human endometrium: effects of continuous and intermittent progesterone treatment. Biol. Reprod. 34:741-750.

McFeely, R. A. and E. Rajakoski. 1968. Chromosome studies on early embryos of the cow. Proc. 6th Int. Cong. Reprod. Anim. and A.I., Paris. 2:905-907.

McNeill, R. E., M. G. Diskin, and J. M. Sreenan. 2002. Progesterone and early embryo survival in dairy cows. Page 23 in Irish Agricultural Research Forum. M. D. Diskin, ed. Tullamore, Co. Offaly, Ireland.

McNeill, R. E., D. G. Morris, M. G. Diskin, and J. M. Sreenan. 2006a. Associations between milk progesterone concentration on different days and with embryo survival during the early luteal phase in dairy cows. Theriogenology 65:1435-1441.

McNeill, R. E., J. M. Sreenan, M. G. Diskin, M. T. Cairns, R. Fitzpatrick, T. J. Smith, and D. G. Morris. 2006b. Effect of systemic progesterone concentration on the expression of progesterone-responsive genes in the bovine endometrium during the early luteal phase. Reprod. Fertil. Devl. 18:573-583.

Michael, S. D., I. I. Geschwind, G. E. Bradford, and G. H. Stabenfeldt. 1975. Pregnancy in mice selected for small little size: Reproductive hormone levels and effect of exogenous hormones. Biol. Reprod. 12:400-407.

Moriyama, I. and T. Sugawa. 1972. Progesterone facilitates implantation of xenogeneic cultured cells in hamster uterus. Nat. New. Biol. 236:150-152.

Munroe, J. P. 1971. Progesteroids as immunosuppressive agents. J. Reticuloendothel. Soc. 9:361-375.

Murray, M. K. 1992. The biosynthesis, immunocytochemical localization of an estrogen-dependent glycoprotein and associated morphological alterations in the sheep ampulla oviduct. Biol. Reprod. 47:889-902.

Nayak, R. K. and D. R. Zimmerman. 1971. Ultrastructural changes in porcine oviduct epithelium during the estrous cycle. J. Anim. Sci. 33:262.

Nebel, R. L., J. M DeJarnette, D. A. Whitlock, C. E. Marshall, M. R. Mink, and R. Kasimanickam. 2007. Effect of human chorionic gonadotropin or gonadotropin releasing hormone injected 5 or 7 days after 72 h Co-Synch on first service pregnancy rates in lactating dairy cows. J. Dairy Sci. 90(Suppl. 1): 327. (Abstr.)

Padykula, H. A., L. G. Coles, W. C. Okulicz, S. I. Rapaport, J. A. McCracken, N. W. King, Jr., C. Longcope, and I. R. Kaiserman-Abramof. 1989. The basalis of the primate endometrium: a bifunctional germinal compartment. Biol. Reprod. 40:681-690.

Padykula, H. A. and T. R. Tansey. 1979. The occurrence of uterine stromal and intraepithelial monocytes and heterophils during normal late pregnancy in the rat. Anat. Rec. 193(3):329-356.

Parr, R. A. 1992. Nutrition-progesterone interactions during early pregnancy in sheep. Reprod. Fertil. Dev. 4:297-300.

Pope, W. F., R. R. Maurer, and F. Stormshak. 1982. Distribution of progesterone in the uterus, broad ligament, and uterine arteries of beef cows. Anat. Rec. 203:245-250.

Portaluppi, M. A. and J. S. Stevenson. 2005. Pregnancy rates in lactating dairy cows after presynchronization of estrous cycles and variations of the Ovsynch protocol. J. Dairy. Sci. 88:914-921.

Pursley, J. R., M. R. Kosorok, and M. C. Wiltbank. 1997a. Reproductive management of lactating dairy cows using synchronization of ovulation. J. Dairy Sci. 80:301-306.

Pursley, J. R., R. W. Silcox, and M. C. Wiltbank. 1998. Effect of time of artificial insemination on pregnancy rates, calving rates, pregnancy loss, and gender ratio after synchronization of ovulation in lactating dairy cows. J. Dairy Sci. 81:2139-2144.

Pursley, J. R., M. C. Wiltbank, J. S. Stevenson, J. S. Ottobre, H. A. Garverick, and L. L. Anderson. 1997b. Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized estrus. J. Dairy Sci.

80:295-300.

Rabiee, A. R., K. L. Macmillan, and F. Schwarzenberger. 2000. Effect of level of feed intake on plasma progesterone concentrations in desolrelin-implanted dairy cows treated with a CIDR device. J. Anim. Sci. 78(suppl. 1):221. (Abstr.)

Rivera, H., H. Lopez, and P. M. Fricke. 2005. Use of intravaginal progesterone-releasing inserts in a synchronization protocol before timed AI and for synchronizing return to estrus in Holstein heifers. J. Dairy Sci. 88:957-968.

Robinson, N. A., K. E. Leslie, and J. S. Walton. 1989. Effect of treatment with progesterone on pregnancy rate and plasma concentrations of progesterone in Holstein cows. J. Dairy Sci. 72:202-207.

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. Endocrinology 160:21-33.

Rodgers, J. C. 2008. An economic evaluation of estrous synchronization and timed artificial insemination in beef cows. Master's Thesis. University of Minnesota, Saint Paul.

Royal, M., G. E. Mann, and A. P. Flint. 2000. Strategies for reversing the trend towards subfertility in dairy cattle. Vet. J. 160:53-60.

Sangsritavong, S., D. K. Combs, R. Sartori, L. E. Amentano, and M. C. Wiltbank. 2002. High feed intake increases liver blood flow and metabolism of progesterone and estradiol-17β in dairy cattle. J. Dairy Sci. 85:2831-2842.

Sangsritavong, S., D. K. Combs, R. F. Sartori, and M. C. Wiltbank. 2000. Liver blood flow and steroid metabolism are increased by both acute feeding and hypertrophy of

the digestive tract. J. Anim. Sci. 78(Suppl. 1):221. (Abstr.)

Santos, J. E. P., S. O. Juchem, R. L. A. Cerri, K. N. Galνγo, R. C. Chebel, W. W. Thatcher, C. Dei, and C. Bilby. 2004a. Effect of bST and reproductive management on reproductive and lactational performance of Holstein dairy cows. J. Dairy Sci. 87:868-881.

Santos, J. E. P., W. W. Thatcher, R. C. Chebel, R. L. A. Cerri, and K. N. Galνγo. 2004b. The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. Anim. Reprod. Sci. 82-83:513-535.

Santos, J. E. P., W. W. Thatcher, L. Pool, and M. W. Overton. 2001. Effect of human chorionic gonadotropin on luteal function and reproductive performance of high-producing lactating Holstein dairy cows. J. Anim. Sci. 79:2881-2894.

Sartori, R., A. Gómen, J. N. Guenther, A. H. Souza, and M. C. Wiltbank. 2003. Comparison of artificial insemination (AI) versus embryo transfer (ET) in lactating dairy cows. J. Dairy Sci. 86(Suppl. 1):238. (Abstr.)

Sartori, R., R. Sartor-Bergfelt, S. A. Mertens, J. N. Guenther, J. J. Parrish, 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.

Schmitt, E. J., T. Diaz, M. drost, and W. W. Thatcher. 1996. Use of a gonadotropin-releasing hormone agonist or human chorionic gonadotropin for timed insemination in cattle. J. Anim. Sci. 74:1084-1091.

Short, R. E., R. A. Bellows, R. B. Staigmiller, J. G. Berardinelli, and E. E. Custer. 1990. Physiological mechanisms controlling anestrus and infertility in postpartum beef

cattle. J. Anim. Sci. 68:799-816.

Siiteri, P. K., F. Febres, L. E. Clemens, R. J. Chang, B. Gondos, and D. Stites. 1977. Progesterone and the maintenance of pregnancy: Is progesterone nature's immunosuppressant? Ann. N.Y. Acad. Sci. 286:384-397.

Siiteri, P. K. and D. P. Stites. 1982. Immunologic and endocrine interrelationships in pregnancy. Biol. Reprod. 26:1-14.

Silke, V., M. G. Diskin, D. A. Kenny, M. P. Boland, P. Dillon, J. F. Mee, and J. M. Sreenan. 2002. Extent, pattern, and factors associated with late embryonic losses in dairy cows. Anim. Reprod. Sci. 71:1-12.

Simmons, R. L., A. L. Price, and A. J. Ozerkis. 1968. The immunologic problem of pregnancy. V. The effect of estrogen and progesterone on allograft survival. Am. J. Obstet. Gynecol. 100:908-911.

Skemesh, M., N. Ayalon, and H. R. Lindner. 1973. Early pregnancy diagnosis based upon plasma progesterone levels in the cow and ewe. J. Anim. Sci. 36:726-729.

Smith, D. L., B. M. Stinefelt, K. P. Blemings, and M. E. Wilson. 2006. Diet-induced alterations in progesterone clearance appear to be mediated by insulin signaling in hepatocytes. J. Anim. Sci. 84:1102-1109.

Snijders, S. E., P. Dillon, D. O. O'Callaghan, and M. P. Boland. 2000. Effect of genetic merit, milk yield, body condition and lactation number on in vitro oocyte development in dairy cows. Theriogenology 53:981-989.

Spencer, T. E. and F. W. Bazer. 1995. Temporal and spatial alterations in uterine estrogen receptor expression and progesterone receptor gene expression during the estrous cycle and early pregnancy in the ewe. Biol. Reprod. 53:1527-1543.

Spencer, T. E., W. C. Becker, P. George, M. A. Mirando, T. F. Ogle, and F. W. Bazer. 1995a. Ovine interferon-τ inhibits estrogen receptor up-regulation and estrogen-induced luteolysis in cyclic ewes. Endocrinology 136:4932-4944.

Spencer, T. E., W. C. Becker, P. George, M. A. Mirando, T. F. Ogle, and F. W. Bazer. 1995b. Ovine interferon-τ regulates expression of endometrial receptors for estrogen and oxytocin but not progesterone. Biol. Reprod. 53:732-745.

Sreenan, J. M. and M. G. Diskin. 1983. Early embryonic mortality in the cow: its relationship with progesterone concentration. Vet. Rec. 112:517-521.

Starbuck, G. R., A. O. Darwash, and G. E. Lamming. 1999. The importance of progesterone during early pregnancy in the dairy cow. Cattle Practice 7:397-399.

Starbuck, G. R., A. O. Darwash, G. E. Mann, and G. R. Lamming. 2001. The detection and treatment of post-insemination progesterone insufficiency in dairy cows. Pages 447-450 in Fertility in the High Producing Dairy Cow. M. G. Diskin, ed. BSAS Occasional Publication No. 26(2).

Starbuck, G. R., C. G. Gutierrez, A. R. Peters, and G. E. Mann. 2006. Timing of follicular phase events and the postovulatory progesterone rise following synchronization of oestrus in cows. Vet. J. 172:103-108.

Sterry, R. A., M. L. Welle, and P. M. Fricke. 2006. Treatment with gonadoptropin-releasing-hormone after first timed artificial insemination improves fertility in noncycling lactating dairy cows. J. Dairy Sci. 89:4237-4245.

Stevenson, J. S. 2001. Reproductive management of dairy cows in high milk-producing herds. J. Dairy Sci. 84(E. Suppl.):E128-E143.

Stevenson, J. S., S. K. Johnson, M. A. Medina-Britos, A. M. Richardson-Adams, and G.

C. Lamb. 2003. Resynchronization of estrus in cattle of unknown pregnancy status using estrogen, progesterone, or both. J. Anim. Sci. 81:1681-1692.

Stevenson, J. S. and M. O. Mee. 1991. Pregnancy rate of Holstein cows after post-insemination treatment with a progesterone-releasing intra-vaginal device. J. Dairy Sci. 74:3849-3856.

Stevenson, J. S., M. A. Portaluppi, D. E. Tenhouse, A. Lloyd, D. R. Eborn, S. Kacuba, and J. M. DeJarnette. 2007. Interventions after artificial insemination: conception rates, pregnancy survival, and ovarian responses to gonadotropin-releasing hormone, human chorionic gonadotropin, and progesterone. J. Dairy Sci. 90:331-340.

Stevenson, J. S., S. M. Tiffany, and E. K. Inskeep. 2008. Maintenance of pregnancy in dairy cattle after treatment with human chorionic gonadotropin or gonadotropin-releasing hormone. J. Dairy Sci. 91:3092-3101.

Stites, D. P. and P. K. Siiteri. 1983. Steroids as immunosuppressants in pregnancy. Immunol. Rev. 75:117-138.

Strinden, S. T. and S. S. Shapiro. 1983. Progesterone-altered secretory proteins from cultured human endometrium. Endocrinology 112:862-870.

Stronge, A. J. H., J. M. Sreenan, M. G. Diskin, J. F. Mee, D. A. Kenny, and D. G. Morris. 2005. Post-insemination milk progesterone concentration and embryo survival in dairy cows. Theriogenology 64:1212-1224.

Szenci, O., J. F. Beckers, P. Humblot, J. Sulon, G. Sasser, M. A. M. Taverne, J. Varga, R. Baltusen, and G. Schekk. 1998. Comparison of ultrasonography, bovine

pregnancy-specific protein B, and bovine pregnancy-associated glycoprotein 1 tests for pregnancy detection in dairy cows. Theriogenology 50(1):77-88.

Thatcher, W. W. 1974. Effects of season, climate, and temperature on reproduction and lactation. J. Dairy Sci. 57:360-368.

Thatcher, W. W., F. W. Bazer, D. C. Sharp, and R. M. Roberts. 1986. Interrelationships between uterus and conceptus to maintain corpus luteum function in early pregnancy: sheep, cattle, pig and horses. J. Anim. Sci. 62(Suppl. 2):25-46.

Thatcher, W. W., A. Guzeloglu, R. Mattos, M. Binelli, T. R. Hansen, and J. K. Pru. 2001. Uterine-conceptus interactions and reproductive failure in cattle. Theriogenology 56:1435-1450.

Thurmond, M. C. and J. P. Picanso. 1990. A surveillance system for bovine abortion. Prev. Vet. Med. 9:41-53.

Van Cleeff, J., M. Drost, and W. W. Thatcher. 1991. Effects of post-insemination progesterone supplementation on fertility and subsequent estrous responses of dairy heifers. Theriogenology 36:795-807.

Van Cleeff, J., K. L. Macmillan, M. Drost, and W. W. Thatcher. 1996. Effects of administering progesterone at selected intervals after insemination of synchronized heifers on pregnancy rates and resynchronization of returns to estrus. Theriogenology 4:1117-1130.

Verhage, H. G., P. A. Mavrogianis, M. L. Boice, W. Li, and A. T. Fazleabas. 1990. Oviductal epithelium of the baboon: Hormonal control and the immuno-gold localization of oviduct-specific glycoproteins. Am. J. Anat. 187:81-90.

Villarroel, A., A. Martino, R. H. BonDurant, F. Deletang, and W. M. Sischo. 2004.

Effect of post-insemination supplementation with PRID on pregnancy in repeat-breeder Holstein cows. Theriogenology 61:1513-1520.

Walker, R. S., P. D. Burns, T. E. Engle, G. E. Sides, and D. D. Zelesky. 2005. Effects of human chorionic gonadotrophin administration on artificial insemination pregnancy rates in beef heifers. Prof. Anim. Sci. 21:361-364.

Walton, J. S., G. W. Halbert, N. A. Robinson, and K. E. Leslie. 1990. Effects of progesterone and human chorionic gonadotrophin administration five days post-insemination on plasma and milk concentrations of progesterone and pregnancy rates of normal and repeat breeder dairy cows. Can. J. Vet. Sci. 54:305-308.

Wathes, D. C., G. E. Mann, J. H. Payne, P. R. Riley, K. R. Stevenson, and G. E. Lamming. 1996. Regulation of oxytocin, oestradiol, and progesterone receptor concentrations in different uterine regions by oestradiol, progesterone and oxytocin in ovariectomised ewes. Endocrinology 151:375-393.

Wathes, D. C., V. J. Taylor, Z. Cheng, and G. E. Mann. 2003. Follicle growth, corpus luteum function and their effect on embryo development in postpartum dairy cows. Reproduction Suppl. 61:219-237.

Watnick, A. S. and R. A. Russo. 1968. Survival of skin homografts in uteri of pregnant and progesterone-estrogen treated rats. Proc. Soc. Exp. Biol. Med. 128:1-4.

Weems, C. W., C. N. Lee, Y. S. Weems, and D. L. Vincent. 1988. Distribution of progesterone to the uterus and associated vasculature of cattle. Endocrinol. Jpn. 35:625-630.

West, N. B., M. C. McClellan, M. D. Sternfeld, and R. M. Brenner. 1987.

Immunocytochemistry versus binding assays of the estrogen receptor in the reproductive tract of spayed and hormone-treated macaques. Endocrinology 121:1789-1800.

Whisnant, C. S., L. A. Pagels, and M. G. Daves. 2001. Case study: Effectiveness of a commercial early conception factor test for use in cattle. Prof. Anim. Sci. 17:51-53.

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, J. N., H. W. Hawk, H. E. Kidder, W. G. Black, L. C. Ulberg, and L. E. Casida. 1956. Effect of progesterone therapy on embryo survival in cows of lowered fertility. J. Dairy Sci. 39:456-461.

Xu, Z. Z., L. J. Burton, and K. L Macmillan. 1997. Reproductive performance of lactating dairy cows following estrus synchronization regimens with PGF2alpha and progesterone. Theriogenology 47(3):687-701.

Yie, S. M, R. Xiao, and C. L. Librach. 2006. Progesterone regulates HLA-G gene expression through a novel progesterone response element. Human Reprod. 21:2538-2544.

Zeron, Y., A. Ocheretny, O. Kedar, A. Borochov, D. Sklan, and A. Arav. 2001. Seasonal changes in bovine fertility: relation to developmental competence of oocytes, membrane properties and fatty acid composition of follicles. Reproduction 121:447-454.

Zimbelman, R. G. 1963. Determination of the minimal effective dose of 6a-methyl-17α-acetoxyprogesterone for control of the estrual cycle in cattle. J. Anim. Sci. 22:1051-1058.

Zimbelman, R. G. and L. W. Smith. 1966. Control of ovulation in cattle with
melengestrol acetate. I. Effect of dosage and route of administration. J. Reprod.
Fertil. 11:185-191.

Zoli, A. P., L. A. Guilbault, P. Dhlahaut, W. B. Ortiz, and J. F. Beckers. 1992.
Radioimmunoassay of a bovine pregnancy-associated glycoprotein in serum: Its
application for pregnancy diagnosis. Biol. Reprod. 46:83-92.

Table 1. Summary of fertility from experiments conducted using supplementation of progesterone or induction of secondary corpora lutea after insemination.

Study	Cattle type	Type of progesterone	Time of supplementation	Control	Treated	Overall effect[1]
				——————— % (no.) —————————		
Herrick 1953	Dairy and beef	Repositol, 500 mg i.m.	d 0	5.0 (1/20)	35.0 (7/20)	↑ 30.0
Dawson 1954	Dairy	100 mg tablet	d 4	16.7 (3/18)	46.8 (22/47)	↑ 30.1
Wiltbank et al., 1956	Dairy	50 mg or 200 mg daily injection	d 3 to 34	29.9 (20/67)	41.8 (28/67)	↑ 11.9
Johnson et al., 1958	Dairy	Repositol, 100 mg i.m.	d 2, 3, 4, 6 and 9	37.7 (26/69)	70.0 (49/70)	↑ 32.3
Sreenan and Diskin 1983	Beef	100 mg daily	d 5	45.0 (9/20)	73.7 (14/19)	↑ 18.7
Sreenan and Diskin 1983	Dairy	100 mg daily	d 10 to 20	63.0 (89/142)	65.0 (84/130)	↑ 2.0
Robinson et al., 1989	Dairy	PRID[2], 1.55 g	d 5 to 12	30.0 (9/30)	60.7 (17/28)	↑ 30.7
Robinson et al., 1989	Dairy	PRID[2], 1.55 g	d 10 to 17	30.0 (9/30)	59.3 (16/27)	↑ 29.3
Walton et al., 1990	Dairy	PRID[2], 1.55 g	d 5 to 12	57.1 (8/14)	68.0 (17/25)	↑ 10.9
Macmillan et al., 1991	Dairy	CIDR[3], 1.9 g	d 10, 12, 14 or 16; 6 d duration	67.0 (421/628)	64.3 (317/493)	↓ 2.7
Macmillan et al., 1991	Dairy	CIDR[3], 1.9 g	d 14-17 to 21	63.6 (300/472)	64.0 (329/514)	↑ 0.4
Macmillan et al.,	Dairy	CIDR[3], 1.9 g	d 4 to 9	66.3 (309/466)	74.6 (344/461)	↑ 8.3

1991

Reference						
Stevenson and Mee, 1991	Dairy	PRID[2]	d 5 to 13	42.4 (39/92)	36.4 (16/44)	↑ 7.6
Stevenson and Mee, 1991	Dairy	PRID[2]	d 13 to 21	42.4 (39/92)	50.0 (18/36)	↑ 7.6
Van Cleef et al., 1991	Dairy	CIDR[3], 1.9 g	d 7 to 13	53.6 (83/155)	57.9 (92/159)	↑ 4.3
Larson et al., 1995	Dairy	CIDR[3], used previously	d 3 to 10	34.9 (22/63)	47.8 (32/67)	↑ 12.9
Xu et al., 1997	Dairy	CIDR[3], 1.9 g	d 16 to 21	59.7 (354/593)	65.1 (396/608)	↑ 5.4
Mann et al., 2001	Dairy	CIDR[3], 0.95 g	d 10 to 17	53.4 (72/135)	56.0 (75/134)	↑ 2.7
Santos et al., 2001[4]	Dairy	hCG, 3,300 IU	d 5	38.7 (79/203)	45.8 (93/203)	↑ 7.1
Chebel et al., 2003[4]	Dairy	GnRH	d 21	26.8 (79/295)	27.0 (78/290)	↑ 0.2
Chenault et al., 2003	Dairy	CIDR[3], 1.38 g	d 14 to 21	36.7 (317/863)	32.7 (288/881)	↓ 4.0
Funston et al., 2005[4]	Beef	hCG, 3,333 IU	d 5 or 6	62.9 (229/364)	61.5 (216/351)	↑ 1.4
Rivera et al., 2005	Dairy	CIDR[3], 1.38 g	d 14 to 20	29.5 (28/95)	31.9 (30/94)	↑ 2.4
Walker et al., 2005[4]	Beef	hCG, 3,300 IU	d 5	64.9 (24/37)	66.7 (26/39)	↑ 1.8
Sterry et al., 2006[5]	Dairy	GnRH, 100 μg	d 5	50.4 (193/383)	53.1 (205/386)	↑ 2.7
Sterry et al., 2006	Dairy	CIDR[3], 1.38 g	d 5 to 12	49.8 (111/223)	46.8 (102/218)	↓ 3.0
Sterry et al., 2006[5]	Dairy	GnRH, 100 μg	d 7	51.3 (82/160)	52.2 (85/163)	↓ 0.9

Nebel et al., 2007[4]	Dairy	hCG, 3,300 IU	d 5	42.3 (108/256)	38.4 (99/257)	↓ 3.9
Stevenson et al., 2007[6]	Dairy	CIDR[3], 1.38 g	between d 4 and 9 for 7 d	28.3 (200/708)	32.7 (232/711)	↑ 4.4
Stevenson et al., 2007[6]	Dairy	GnRH, 100 µg	between d 4 and 9	28.3 (200/708)	28.1 (202/719)	↓ 0.2
Stevenson et al., 2007[4]	Dairy	hCG, 3,300 IU	between d 4 and 9	28.3 (200/708)	33.6 (240/714)	↑ 5.3
Larson et al., 2009	Beef	CIDR[3], 1.38 g	d 5 to 14	54.9 (130/237)	53.8 (126/234)	↓ 1.1
Larson et al., 2009	Beef	CIDR[3], 1.38 g	d 14 to 21	54.9 (130/237)	47.8 (111/232)	↓ 7.1
Larson et al., 2009	Beef	CIDR[3], 1.38 g	d 5 to 21	54.9 (130/237)	53.0 (124/234)	↓ 1.9

[1] Overall difference in pregnancy rates between control and treated animals.

[2] Progesterone releasing internal device (PRID) containing 1.55 g progesterone.

[3] Controlled internal drug release (CIDR) containing either 0.95, 1.38, or 1.9 g progesterone, as specified.

[4] Circulating concentrations of progesterone did increase after treatment with GnRH or hCG.

[5] Blood samples were not collected, therefore, it was unknown whether circulating concentrations of progesterone increased with treatment.

[6] Circulating concentrations of progesterone did not increase after treatment with GnRH or hCG.

CHAPTER 2

Influence of a CIDR after fixed-time AI on pregnancy rates and returns to estrus

of nonpregnant cows[1]

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ABSTRACT

We determined whether an ovulatory estrus could be resynchronized in previously synchronized artificially inseminated (AI) nonpregnant cows without compromising pregnancy from the previous synchronized ovulation or to those inseminated at the resynchronized estrus. Ovulation was synchronized in 937 suckled beef cows at 6 locations using a CO-Synch + progesterone insert (controlled internal drug release; CIDR) protocol (a 100-µg injection of GnRH at the time of progesterone insert, followed in 7 d by a 25-mg injection of $PGF_{2\alpha}$ at insert removal. At 60 h after $PGF_{2\alpha,}$ cows received a fixed-time AI [TAI] plus a second injection of GnRH). After initial TAI cows were assigned randomly to 4 treatments: 1) untreated (control; n = 237); 2) progesterone insert at 5 d after TAI and removed 14 d after TAI (CIDR5-14; n = 234); 3) progesterone insert placed at 14 d after TAI and removed 21 d after TAI (CIDR14-21; n = 232); or 4) progesterone insert at 5 d after TAI and removed 14 d after TAI and then a new CIDR inserted at 14 d and removed 21 d after TAI (CIDR5-21; n = 234). After TAI, cows were observed twice daily until 25 d after TAI for estrus and inseminated according to the AM-PM rule. Pregnancy was determined at 30 and 60 d after TAI to determine conception to the first and second AI. Pregnancy rates to TAI were similar for control (55%), CIDR5-14 (53%), CIDR14-21 (48%), and CIDR5-21 (53%). A greater ($P < 0.05$) proportion of nonpregnant cows were detected in estrus in the CIDR5-21 (76/110, 69%) and CIDR14-21 (77/120, 64%) treatments than in controls (44/106, 42%) and CIDR5-14 (39/109, 36%) cows. Although overall pregnancy rates after second AI service were similar, combined conception rates of treatments without a

CIDR from d 14 to 21 (68.7% [57/83]; Control and CIDR5-14 treatments) were greater

(P = 0.03) than those with a CIDR during that same interval (53.5% [82/153]; CIDR5-

21 and CIDR14-21 treatments). We conclude that placement of a progesterone insert 5

d after a TAI did not compromise or enhance pregnancy rates to TAI, however,

conception rates of nonpregnant cows inseminated after a detected estrus were

compromised when resynchronized with a CIDR from d 5 or 14 until 21 d after TAI.

Key Words: Estrus Synchronization, Resynchronization, CIDR, Artificial Insemination

INTRODUCTION

Reproduction is the main factor limiting production efficiency of beef cattle

(Short et al., 1990). Artificial insemination (AI) provides an economically viable

technique to introduce desired genetics into a herd, whereas synchronization of estrus,

ovulation, or both provides a more labor-efficient way to incorporate AI into

management practices. Synchronization of AI also shortens the calving season,

allowing more calves to be born near the beginning of the calving season (Larson et al.,

2006). Reinsemination of nonpregnant cows at the first eligible estrus can be facilitated

by resynchronization of the estrous cycle (Van Cleeff et al., 1996). With additional

hormonal control of the estrous cycle, a second AI is possible. Resynchronization with

a progestin increased synchronized return rates of nonpregnant females (Stevenson et

al., 2003; Colazo et al., 2006), thereby increasing the number of animals that conceive

to AI while maintaining efficient use of labor. Resynchronization with progesterone

and estradiol cypionate or estradiol benzoate, however, decreased subsequent

conception rates (Stevenson et al., 2003). Supplementation with progesterone on d 5

post-AI enhanced pregnancy rates in Holstein cows (Villarroel et al., 2004), but suppressed fertility when administered within 2 d of first insemination (Van Cleeff et al., 1996). In addition, heifers that received progesterone on d 2 after AI had shorter estrous cycles than controls (Lynch et al., 1999).

Therefore, the objectives of this study were to determine whether resynchronization of an ovulatory estrus could be accomplished in previously inseminated nonpregnant cows without compromising pregnancy in cows pregnant from a previous synchronized ovulation and whether insertion of a CIDR at 5 or 14 d after TAI would alter pregnancy rates.

MATERIALS AND METHODS

Locations and Cows

During the spring breeding seasons (April 1 to June 30) of 2004 and 2005, beef cattle used in this study were managed at 6 locations, located in 3 states. Herd size ranged from 113 to 248 cows. A total of 937 suckled beef cows were submitted for treatment consisting of British, Continental, and British × Continental breed types. Mean days postpartum at the beginning of the breeding season (d 0) were 58 with a range of 19 to 102 d. Average parity was 3.0 ± 1.6 (mean $\pm$ SD) with a range of 1 to 10. Body condition scores (scale of 1 to 9; Whitman, 1975) were determined by an experienced, but not the same, individual at each location on d –20 relative to $PGF_{2\alpha}$ (PG), with a mean BCS of 4.8 ± 0.6 (mean $\pm$ SD) and a range of 3 to 6.5. Individual location data are summarized in Table 1.

Treatments

Ovulation was synchronized with the CO-Synch + progesterone insert (controlled internal drug release; CIDR) protocol (Larson et al., 2006). Cows received a CIDR insert containing 1.38 g of progesterone (Pfizer Animal Health, New York, NY) and a 100 μg injection of GnRH (OvaCyst; IVX Animal Health, St. Joseph, MO) on d −9. On d −2 the insert was removed and cows received a 25 mg of PG (Lutalyse, dinoprost tromethamine, Pfizer Animal Health), followed in 60 h by a second injection of GnRH and fixed-time AI (TAI).

After the initial TAI cows were assigned randomly (for IL1 and IL2 locations) or stratified by BCS and days postpartum (for KS1, KS2, MN1, and MN2 locations) and then assigned to 4 resynchronization protocols (Figure 1): 1) untreated (control; n = 237); 2) progesterone insert at 5 d after TAI and removed 14 d after TAI (CIDR5-14; n = 234); 3) progesterone insert placed at 14 d after TAI and removed 21 d after TAI (CIDR14-21; n = 232); or 4) progesterone insert at 5 d after TAI and removed 14 d after TAI and then a new CIDR inserted at 14 d and removed 21 d after TAI (CIDR5-21; n = 234). A minimum of 2 daily visual observations (at least 45 min each) for estrus began on d 5 and continued until d 26 after TAI. To assist in detection of estrus cows were fitted with heatmount detectors (Kamar Inc., Steamboat Springs, CO), which were affixed midline to the rump of each cow between the tailhead and the tuber coxae (hook bones). Detectors were placed on all cows on d 5 after TAI. Cows detected in estrus received an AI 9 to 14 h after the first detected estrus (AM-PM rule). At 2 of the locations (KS1 and KS2), cows were visually observed for estrus between d 21 and 25 after TAI and no heatmount detectors were applied.

Pregnancy was diagnosed by transrectal ultrasonography (5-MHz or 7.5-MHz intrarectal transducer, Aloka 500V, Corometrics, Wallingford, CT) on d 30 after AI to determine the presence of a viable embryo thereby assessing TAI pregnancy rates. A second pregnancy diagnosis was performed at 60 d after TAI to determine cumulative first and second service pregnancy rates, plus to establish pregnancy loss of cows that conceived to TAI but lost the pregnancy after d 30. Clean-up bulls were not introduced until d 26 after TAI and remained with the cows for the remainder of the breeding season.

Blood Collection and Radioimmunoassay

At the KS1, KS2, and MN2 locations blood samples were collected via tail venipuncture on d -19 and -9 relative to the TAI. In addition, at the IL1 and IL2 locations, blood samples were collected by tail venipuncture on d 11, whereas blood samples were collected at the MN1 location on d 14. Blood was centrifuged at $1,500 \times$ *g* for 10 to 15 min and serum was recovered and stored at $-20°$C until RIA. Blood serum was analyzed for concentrations of progesterone in the laboratory of individual investigators, according the validation procedures of the progesterone RIA in each laboratory.

For the MN and KS locations, concentrations of progesterone in samples were analyzed by radioimmunoassay using progesterone kits (Coat-A-Count; Diagnostic Products Corp. Los Angeles, CA). The assay kit was validated for bovine serum (Kirby et al., 1997) using an assay volume of 100 μl. Assay tubes for the standard curve contained 0.01, 0.025, 0.05, 0.2, 0.5, 1, 2, and 4 ng/tube. Assay sensitivity for a 100-μl

sample was 0.1 ng/mL. Pooled samples revealed that the intra- and inter-assay coefficient of variation were 5.4 and 7.2%, respectively.

Serum progesterone concentrations for samples collected at the IL locations were quantified by radioimmunoassay (Kesler, et al., 1990) with intra- and inter-assay coefficients of variation of 2.4 and 4.6%, respectively.

For samples collected on d -19 and d -9, when at least 1 of 2 blood samples had concentrations of progesterone $\geq$ 1 ng/mL, the cow was considered to be cycling at the initiation of treatments (Perry et al., 1991). Serum samples collected on d 11 or 14 were used to compare differences in serum concentrations of cycling cows during diestrus or early pregnancy with or without a CIDR.

Statistical Analyses

Procedures GLM and CATMOD of SAS (SAS Inst. Inc., Cary, NC) were used to analyze all categorical data, and procedure GLM was used to analyze noncategorical data. Means were separated by using the least significant difference (LSD) in procedure GLM when a protected F test (P $\leq$ 0.05) was detected by ANOVA.

Proportions of cows cycling at the onset of treatments were analyzed for KS1, KS2, and MN2 locations with location as a fixed effect, and days postpartum and BCS as regression covariables. The model excluded the IL1, IL2, and MN1 locations because blood samples were not collected on d -19 and -9 at those 2 locations. The model used to analyze pregnancy rates to TAI, second service conception rates, cumulative pregnanices after two AI, and pregnancy loss included treatment and location and the treatment × location interaction, with days postpartum as a regression covariable. Because breed composition, AI sires, and AI technicians were confounded with

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location, they were not included in the model, but reasonable attempts were made to ensure that these variables were distributed evenly among treatments at each location.

Models used to analyze concentrations of progesterone on d 11 (at the IL1 location), d 14 (at MN1 location), percentage of nonpregnant cows returning to estrus, and percentage of nonpregnant cows returning to estrus during the 48-h interval from d 22 to 23 included treatment in the model. Orthogonal contrasts were used to compare concentrations of progesterone between cows that had no CIDR from d 5 to 14 (control and CIDR14-21 treatments) and cows that had a CIDR from d 5 to 14 (CIDR5-14 and CIDR5-21 treatments).

Within each model, analyses were conducted to ensure that no biases existed among treatments based on days postpartum, parity, BCS, and cycling status.

RESULTS

Cycling Status

Based on concentrations of progesterone from blood samples collected from cows at the KS1, KS2, and MN2 locations at d –19 and –9 we determined that an average of 54.4% (296 of 544) of cows were cycling before initiation of the CO-Synch + CIDR estrous synchronization protocol. Proportions of cycling cows among the three locations ranged from 40 to 65% and were influenced by location and BCS (Table 1). Pregnancy rates to TAI for cycling cows (48.1%; 142 of 297) did not differ ($P = 0.33$) from noncycling cows (52.2%; 129 of 247). Similarly, cumulative pregnancies after 2 AI were similar between cycling (60.9%; 181 of 297) and noncycling (63.7%; 156 of 245) cows.

For every unit increase in BCS over the range 3.0 to 6.5, the proportion of cows cycling increased ($P < 0.01$) by $11.9 \pm 3.8\%$. Days postpartum did not affect cycling status at the beginning of the breeding season.

Fertility to Fixed-Time AI

Treatment with progesterone beginning on d 5 or 14 after TAI did not alter pregnancy rates to the initial TAI. Pregnancy rates determined on d 30 after TAI were 55% for control, 53% for CIDR5-14, 48% for the CIDR14-21, 53% for the CIDR5-21 treatments (Table 2).

No location × treatment interaction was detected; however, when pregnancy rates among treatments were combined within each location, a location effect ($P < 0.01$) on AI pregnancy rates was observed. Pregnancy rates among locations ranged from 40% (MN2 location) to 68% (IL1 location), whereas those at the remaining 4 locations were intermediate (Table 2). Pregnancy rates were greater ($P < 0.05$) for cows that calved > 50 d before the onset of the breeding season (53.3%) than for those that calved ≤ 50 d before the onset of the breeding season (46.0%). In addition, for every unit increase in BCS over the range 3.0 to 6.5, pregnancy rate increased ($P < 0.01$) by $9.7 \pm 3.6\%$. Regardless of treatment, no interaction was detected between stage postpartum at the onset of treatments or BCS and treatment.

Detection of Estrus and Fertility after Resynchronization

Pregnancy diagnosis at d 30 revealed that 106, 109, 120, and 110 cows were not pregnant for the control, CIDR5-14, CIDR14-21, and CIDR5-21 treatments, respectively. Of these nonpregnant cows, a greater ($P < 0.01$) percentage of CIDR14-21 (64%) and CIDR5-21 (69%) were detected in estrus than control (42%) and CIDR5-

14 (36%). When contrasting treatments with a CIDR from d 14 to 21 (CIDR5-21 and CIDR14-21 treatments) to those without a CIDR during that same interval (control and CIDR5-14), the return estrus for those cows treated with progesterone was more synchronous than those that were treated with progesterone during that time (Figure 2).

Conception rates to the return estrus tended ($P = 0.069$) to be greater in the CIDR5-14 (72%) treatment than the CIDR14-21 (53%) and CIDR5-21 (54%) treatments, whereas controls (66%) were intermediate (Table 2). In contrast, combined conception rates in cows treated with progesterone from d 14 to 21 (CIDR5-21 and CIDR14-21 treatments) were greater ($P = 0.03$) than those not treated with progesterone during that same interval (control and CIDR5-14).

No treatment differences were detected for cumulative pregnancies after 2 AI, but number of pregnancies were different among locations with MN1 (83%) experiencing the greatest proportion and KS2 (51%) the poorest. In addition, pregnancy loss for cows diagnosed pregnant to the initial TAI on d 30 but diagnosed nonpregnant on d 60 was similar among treatments, demonstrating that treatment with progesterone beginning 5 d after TAI failed to enhance conception rates.

Concentrations of Progesterone

A tendency ($P = 0.11$) was detected for concentrations of progesterone to be greater in CIDR5-14 cows (4.28 ± 0.24 ng/mL) than in controls (3.50 ± 0.23 ng/mL) on d 11 after TAI. The CIDR5-21 (4.08 ± 0.23 ng/mL) and CIDR14-21 (3.81 ± 0.25 ng/mL) treatments were intermediate. Significant differences ($P = 0.03$) were noted in progesterone concentrations when contrasting treatments that received progesterone

from d 5 to 14 (CIDR5-14 and CIDR5-21) and those that did not receive progesterone (control and CIDR14-21; Figure 3).

Similarly, on d 14 after TAI, concentrations of progesterone were greater (P = 0.03) for the CIDR5-14 (7.21 ± 0.41 ng/mL) and CIDR5-21 (7.19 ± 0.39 ng/mL) treatments than controls (5.99 ± 0.38 ng/mL), and tended (P = 0.07) to be greater than the CIDR 14-21 (6.21 ± 0.39 ng/mL) treatment. When contrasting treatments that received progesterone from d 5 to 14 (CIDR5-14 and CIDR5-21) and those that did not (control and CIDR14-21), progesterone-treated cows had greater ($P < 0.01$) concentrations of progesterone than those not receiving progesterone (Figure 3).

DISCUSSION

When managing reproduction in beef cattle to improve the genetic base of the calf crop efficiently, the percentage of cows conceiving to AI must be increased. The two-fold purpose of this study was to determine: 1) whether increasing progesterone in early diestrus (d 5 after TAI) would reduce pregnancy loss and increase pregnancy rates to TAI; and 2) if the interval required for detection of the first eligible estrus of previously inseminated nonpregnant cows could be reduced and effectively resynchronized without interfering with established pregnancies resulting from an earlier TAI. Early progesterone supplementation via a CIDR insert failed to enhance pregnancy rates to TAI and we found no evidence that implementation of the resynchronization protocols was disruptive to established pregnancies. In contrast, resynchronization of estrus with progesterone between d 14 and 21 after TAI effectively

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resynchronized nonpregnant cows, but conception rates to the resynchronized estrus were compromised resulting in no benefit to overall pregnancy rates.

In dairy cows, providing supplemental P4 by CIDR (containing 1.9 g progesterone) increased or tended to increase pregnancy rates when treatments were initiated no earlier than d 3 after AI (Robinson et al., 1989; Van Cleeff et al., 1996; Stevenson et al., 2007), but not consistently (Stevenson and Mee, 1991). Supplemental progestin during the luteal phase tended to increase conception rates (Wilmut et al., 1986) or calving rates of beef heifers (Favero et al., 1993). In addition, supplementing exogenous P4 may preclude low concentrations of P4 from occurring in the maternal circulation and prevent pregnancy losses in dairy cows (Stevenson and Mee, 1991; El-Zarkouny and Stevenson, 2004; Stevenson et al., 2007) and dairy heifers (Van Cleeff et al., 1996). In contrast, treatment with progesterone beginning on d 5 after TAI in the present study failed to enhance pregnancy rates to TAI.

In lactating dairy cattle, studies have demonstrated that steroid metabolism reduces the concentration of circulating progesterone compared with nonlactating cows or heifers (Sangsritavong et al., 2002; Sartori et al., 2002). Therefore, lactating dairy cows may benefit from treatments that enhance progesterone after AI. In contrast, in beef cows, the concentration of circulating progesterone shortly after TAI is likely sufficient for pregnancy establishment and maintenance. Mean concentrations of progesterone on d 11 after TAI were 3.93 ng/mL and addition of the CIDR treatment provided 4.66 ng/mL of progesterone, only a 0.62 ng/mL increase in serum progesterone compared with cows without a CIDR. Further, mean concentrations of progesterone on d 14 after TAI were 6.10 ng/mL and addition of a CIDR provided 7.20

ng/mL of progesterone, only a 1.10 ng/mL increase in serum progesterone compared with cows without a CIDR. In addition, there was no difference in concentrations of progesterone between cows that became pregnant and those that failed to become pregnant.

Previous studies demonstrated that pregnancy survival in cows established after the TAI were unaffected by resynchronization treatments. Pregnancies were unaffected when injections of estradiol benzoate (EB) was administered on d 12, 13, or 14 after AI (Macmillan et al., 1999). or when injections of EB or estradiol cypionate (ECP) administered at insertion and removal of a used progesterone CIDR insert on d 11 to 18 or 13 to 20 after TAI (Stevenson et al., 2003). Our results indicate that administering progesterone via a CIDR insert also was effective in preventing the occurrence of spontaneous estrus before its removal.

Concentrating the distribution of estrus into a short, predictable time frame provides advantages for an AI program. Detection of estrus is both time consuming and labor intensive, especially for repeat periods of estrus after a failed AI because interval to estrus is more variable compared with noninseminated females (Van Cleeff et al., 1996). Therefore, another purpose of these studies was to determine whether the protocols employed effectively increased percentages of nonpregnant females returning to estrus. When a CIDR was present between d 14 and 21, percentages of eligible nonpregnant females returning to estrus increased, which concurs with our previous report (Stevenson et al., 2003). In addition, distribution patterns of estrus for our cattle receiving a CIDR between d 14 and 21 after TAI were consistent with previous studies when beef cows given a single injection of EB on d 13 (CIDR insertion) and on d 20 at

CIDR removal (Stevenson et al., 2003), or dairy cattle (Macmillan et al., 1999) in which 43% were in estrus on d 1 and 42% on d 2 after CIDR removal.

Despite a greater proportion of cows resynchronized with a CIDR insert expressing estrus, conception rates were compromised resulting in similar overall pregnancy rates after 2 AI to cows that were not resynchronized. These results agree with our previous report (Stevenson et al., 2003) when the use of progesterone or melengestrol acetate (MGA) plus ECP either tended to reduce or reduced fertility at the resynchronized estrus, respectively. Other research (Purvis and Whittier, 1997) has shown that conception rate of beef heifers after resynchronization with MGA did not differ from controls, but was numerically less in MGA-treated heifers. Lower conception rates in MGA-treated (Chenault et al., 1990) or CIDR-treated females (Macmillan and Peterson, 1993) indicated that some persistent follicles might have developed in heifers assigned to those treatments. This very likely could be the reason for the reduction in fertility in our study, because no treatment was used to initiate a new follicular wave at CIDR insertion. When EB was administered at CIDR insertion for resynchronization of estrus, fertility increased in dairy cows as a consequence of promoting a third follicular wave (Macmillan et al., 1999). This was evident when conception rates were less in cows in which fertilized oocytes were derived from the second (58%) compared with the third (95%) follicular wave of the estrous cycle in beef (Ahmad et al., 1997) and dairy cows (30 vs. 68%; Townson et al., 2002).

Enhancing fertility of estrus- or ovulation synchronization protocols facilitates the potential increased usage of AI. We demonstrated that supplementation of progesterone via a CIDR insert to postpartum suckled beef cows on d 5 after TAI failed

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to enhance fertility. In addition, resynchronization of estrus in nonpregnant cows with a CIDR enhanced synchrony of estrus and increased the proportion of cows detected in estrus, but had a negative impact on conception rates. Therefore, further research is required to seek a protocol that successfully resynchronizes previously inseminated nonpregnant females while maintaining satisfactory conception rates after detected estrus.

LITERATURE CITED

Ahmad, N., E. C. Townsend, R. A. Dailey, and E. K. Inskeep. 1997. Relationships of hormonal patterns and fertility occurrence of two or three waves of ovarian follicles, before and after breeding, in beef cows and heifers. Anim. Reprod. Sci. 49:13-28.

Chenault, J. R., J. F. McAllister, and C. W. Kasson. 1990. Synchronization of estrus with melengestrol acetate and prostaglandin $F_{2\alpha}$ in beef and dairy heifers. J. Anim. Sci. 68:296-303.

Colazo, M. G., J. P. Kastelic, R. C. Mainar-Jaime, Q. A. Gavaga, P. R. Whittaker, J. A. Small, M. F. Martinez, R. E. Wilde, D. M. Veira, and R. J. Mapletoft. 2006. Resynchronization of previously timed-inseminated beef heifers with progestins. Theriogenology 65:557-572.

El-Zarkouny, S. Z., and J. S. Stevenson. 2004. Resynchronization of estrus with progesterone or progesterone plus estrogen in lactating dairy cows of unknown pregnancy status. J. Dairy Sci. 87:3306-3321.

Favero, R. J., D. B. Faulkner, and D. J. Kesler. 1993. Norgestomet implants synchronize estrus and enhance fertility in beef heifers subsequent to timed artificial insemination. J. Anim. Sci. 71:2594-2600.

Kesler, D. J., H. Khazali, and R. J. Favero. 1990. Quantification of steroids via a polymer linked second antibody enzyme immunoassay system: Methods of linking anti-rabbit IgG to polystyrene. Pages 157-170 in: Progress in Biomedical Polymers. Ed., C.G. Gebelein and R.L. Dunn, Plenum Publishing Co., New York, NY, pp. 157-170.

Kirby C. J., M. F. Smith, D. H. Keisler, and M. C. Lucy. 1997. Follicular function in lactating dairy cows with sustained-release bovine somatotropin. J. Dairy Sci. 80:273-285.

Larson, J. E., G. C. Lamb, J. S. Stevenson, S. K. Johnson, M. L. Day, T. W. Geary, D. J. Kesler, J. M. DeJarnette, F. N. Schrick, A. DiCostanzo, and J. D. Arseneau. 2006. Synchronization of estrus in suckled beef cows for detected estrus and artificial insemination and timed artificial insemination using gonadotropin-releasing hormone, prostaglandin $F_{2\alpha}$, and progesterone. J. Anim. Sci. 84:332-342.

Lynch, P. R., K. L. Macmillan, and V. K. Taufa. 1999. Treating cattle with progesterone as well as a GnRH analogue affects oestrous cycle length and fertility. Anim. Reprod. Sci. 56:189-200.

Macmillan, K. L., and A. J. Peterson. 1993. A new intravaginal progesterone releasing device for cattle (CIDR-B) for oestrous synchronisation, increasing pregnancy rates and the treatment of post-partum anoestrus. Anim. Reprod. Sci. 33:1-25.

Macmillan, K. L., V. K. Taufa, A. M. Day, and V. M. Eagles. 1999. Some effects of post-oestrous hormonal therapies on conception rates and resubmission rates in lactating dairy cows. Pages 195-208 in Fertility in the High Producing Dairy Cow, Vol. 1. Occasional pub. No. 26. Br. Soc. Anim. Sci., Penicuik, Scotland.

Perry, R. C., L. R. Corah, R. C. Cochran, W. E. Beal, J. S. Stevenson, J. E. Minton, D. D. Simms, and J. R. Brethour. 1991. Influence of dietary energy on follicular development, serum gonadotropins, and first postpartum ovulation in suckled beef cows. J. Anim. Sci. 69:3762-3773.

Purvis, H. T., and J. C. Whittier. 1997. Use of short-term progestin treatment to

    resynchronize the second estrus following synchronized breeding in beef heifers.

    Theriogenology 48:423-434.

Robinson, N. A., K. E. Leslie, and J. S. Walton. 1989. Effect of treatment with

    progesterone on pregnancy rate and plasma concentrations of progesterone in

    Holstein cows. J. Dairy Sci. 72:202-207.

Sangsritavong, 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-17ß in dairy cattle. J. Dairy Sci. 85:2831-2842.

Sartori, R., G. J. M. Rosa, and M. C. Wiltbank. 2002. Ovarian structures and circulating

    steroids in heifers and lactating cows in summer and lactating and dry cows in

    winter. J. Dairy Sci. 85:2813-2822.

Short, R. E., R. A. Bellows, R. B. Staigmiller, J. G. Berardinelli, and E. E. Custer. 1990.

    Physiological mechanisms controlling anestrus and infertility in postpartum beef

    cattle. J. Anim. Sci. 68:799-816.

Stevenson, J. S., S. K. Johnson, M. A. Medina-Britos, A. M. Richardson-Adams, and G.

    C. Lamb. 2003. Resynchrnonization of estrus in cattle of unknown pregnancy status

    using estrogen, progesterone, or both. J. Anim. Sci. 81:1681-1692.

Stevenson, J. S., and M. O. Mee. 1991. Pregnancy rates of Holstein cows after

    postinsemination treatment with a progesterone-releasing intravaginal device. J.

    Dairy Sci. 74:3849-3856.

Stevenson, J. S., M. A. Portaluppi, D. E. Tenhouse, A. Lloyd, D. R. Eborn, S. Kacuba,

    and J. M. DeJarnette. 2007. Interventions after artificial insemination: Conception

rates, pregnancy survival, and ovarian responses to gonadotropin-releasing hormone, human chorionic gonadotropin, and progesterone. J. Dairy Sci. 90:331-340.

Townson, D. H., P. C. W. Tsang, W. R. Butler, M. Frajblat. L. C. Griel Jr., C. J. Johnson, R. A. Milvae, and J. L. Pate. 2002. Relationship of fertility to ovarian follicular waves before breeding in dairy cows. J. Anim. Sci. 80:1053-1058.

Van Cleeff, J., K. L. Macmillan, M. Drost, M. C. Lucy, and W. W. Thatcher. 1996. Effects of administering progesterone at selected intervals after insemination of synchronized heifers on pregnancy rates and resynchronization of returns to service. Theriogenology 46:1117-1130.

Villarroel, A., A. Martino, R. H. BonDurant, F. Deletang, W. M. Sischo. 2004. Effect of post-insemination supplementation with PRID on pregnancy in repeat-breeder Holstein cows. Theriogenology 61:1513-1520.

Whitman, R. H. 1975. Weight changes, body condition and beef-cow reproduction. Ph.D. Dissertation. Colorado State University, Fort Collins.

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.

Table 1. Characteristics of cows at each location including number of cows treated, breed composition, days postpartum, parity, body condition score and estrous cycling percentage

Location[d]	N	Breed origin	Days postpartum[a], mean (range)	Parity, mean (range)	BCS[b], mean (range)	Cyclicity[c], % (no./no.)
IL1	120	British, Continental	-	-	-	-
IL2	113	British, Continental	-	-	-	-
KS1	248	British, Continental, British x Continental	$61\ (23\text{-}96)^x$	$4.9\ (1\text{-}10)^x$	$5.0\ (3.0\text{-}6.5)^x$	$65\ (161/248)^x$
KS2	143	British, Continental	$49\ (19\text{-}78)^y$	$3.7\ (1\text{-}8)^y$	$4.2\ (3.0\text{-}6.0)^y$	$40\ (57/143)^y$
MN1	160	British, Continental	$59\ (19\text{-}83)^x$	$1.9\ (1\text{-}9)^z$	$5.1\ (4.0\text{-}6.0)^z$	-
MN2	153	British, Continental	$61\ (22\text{-}102)^x$	-	$5.0\ (3.5\text{-}6.5)^{xz}$	$60\ (92/153)^x$
Overall	937	-	$58\ (19\text{-}102)$	$3.0\ (1\text{-}10)$	$4.8\ (3.0\text{-}6.5)$	$57\ (310/544)$

[a] Days postpartum at initiation of the breeding season (d 0).

[b] Body condition score on 1 to 9 scale (1 = emaciated and 9 = obese; Whitman, 1975).

[c] Percentage of cows cycling at initiation of treatments (d −7).

[d] Location abbreviations refer to each of 6 herds among three states. Days postpartum and BCS was not recorded at IL1 and IL2 locations. Parity was not recorded at IL1, IL2, and MN2. Blood samples were not collected at IL1, IL2, and MN1 locations to determine cyclicity.

[xyz] Means within a column differ ($P < 0.05$).

Table 2. Fertility of previously inseminated suckled beef cows whose first eligible estrus was resynchronized with or without progesterone via a CIDR insert after an initial fixed-time AI.

Item	Control	CIDR5-14	CIDR-14-21	CIDR5-21
	Treatment[a]			
	-------------------n (%)--------------------			
Pregnancy rates to TAI[b]:				
IL1	20/30 (67)	24/30 (80)	18/30 (60)	20/30 (67)
IL2	10/29 (34)	13/28 (46)	11/26 (42)	15/30 (50)
KS1	36/62 (58)	38/63 (60)	32/62 (52)	34/61 (56)
KS2	22/35 (63)	18/36 (50)	17/37 (46)	14/35 (40)
MN1	26/41 (63)	18/39 (46)	21/40 (53)	26/40 (65)
MN2	17/40 (43)	16/38 (42)	13/37 (35)	15/38 (39)
Overall	131/237 (55)	125/234 (53)	112/232 (48)	124/234 (53)
Return to estrus[c]:	44/106 (42)^{w}	39/109 (36)^{w}	77/120 (64)^{x}	76/110 (69)^{x}
Conception rates[d]:	29/44 (66)^{yz}	28/39 (72)^{y}	41/77 (53)^{z}	41/76 (54)^{z}
Cumulative pregnancy rates[e]:				

IL1	23/30 (76)	24/30 (80)	25/30 (83)	23/30 (77)
IL2	16/29 (55)	18/28 (64)	12/26 (46)	18/30 (60)
KS1	36/62 (58)	38/63 (60)	40/62 (65)	47/61 (77)
KS2	22/35 (63)	18/36 (50)	22/36 (61)	14/35 (40)
MN1	32/41 (78)	34/39 (87)	32/40 (80)	34/40 (85)
MN2	29/40 (73)	26/38 (68)	23/37 (62)	25/37 (68)
Overall	158/237 (67)	158/234 (68)	154/231 (67)	161/233 (69)
Pregnancy loss[f]:	9/130 (7)	6/126 (5)	6/111 (5)	6/124 (5)

[a] Cows were assigned to treatments according to Figure 1.

[b] Timed AI = fixed-time AI; pregnancy rates were determined on d 30.

[c] Percentage of nonpregnant females returning to estrus d 21 to 26 after TAI.

[d] Percentage of cows pregnant after AI for cows returning to estrus.

[e] Overall pregnancy rates after 2 AI as determined on d 60.

[f] Pregnancy loss of TAI pregnancies between d 30 and 60.

[wx] Means within a row differ ($P < 0.05$).

[yz] Means within a row differ ($P < 0.10$).

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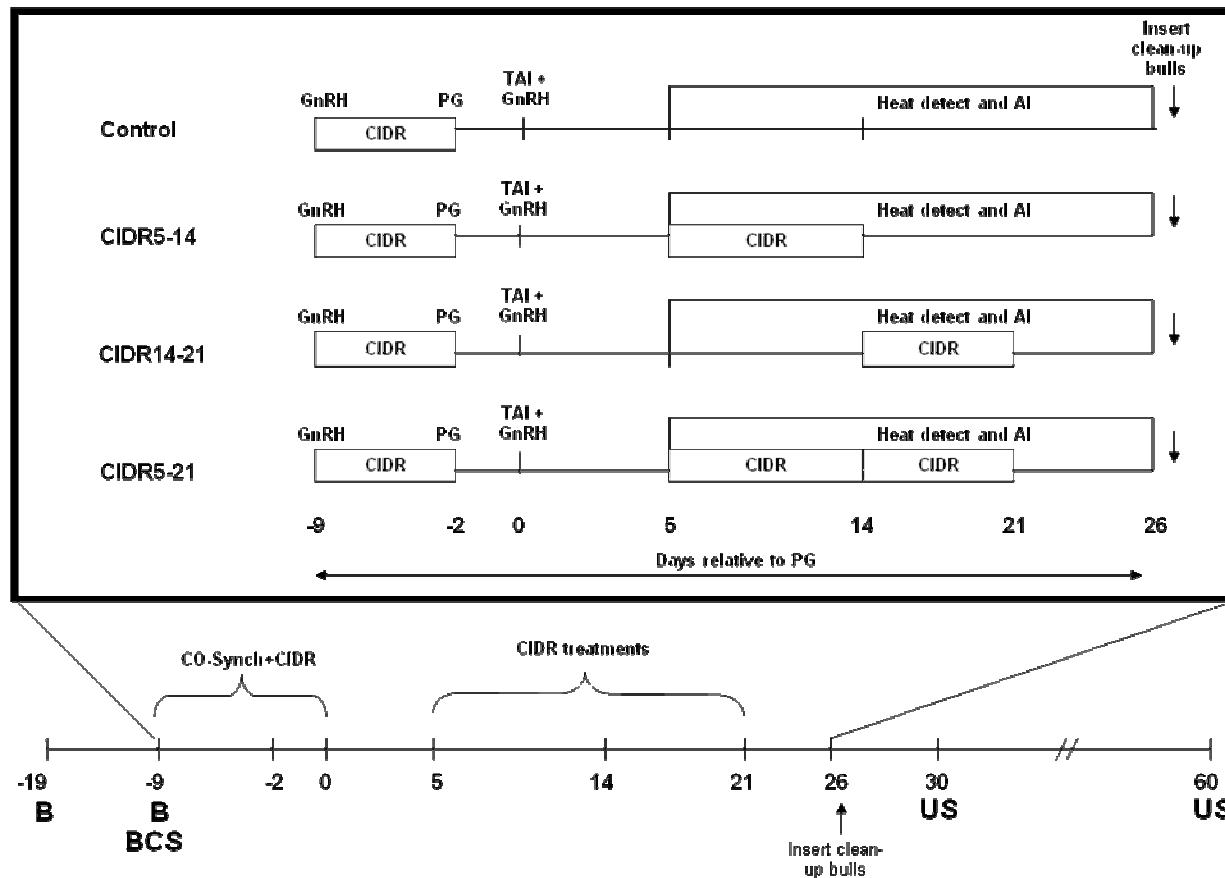
Figure 1. Experimental protocol for treatments to synchronize ovulation and resynchronize estrus of previously inseminated cows.

Blood (B) samples were collected on d −19, and −9. BCS = body condition score; GnRH = gonadotropin-releasing hormone; CIDR = controlled internal device release; AI = artificial insemination; TAI = fixed-timed AI; US = ultrasonography.
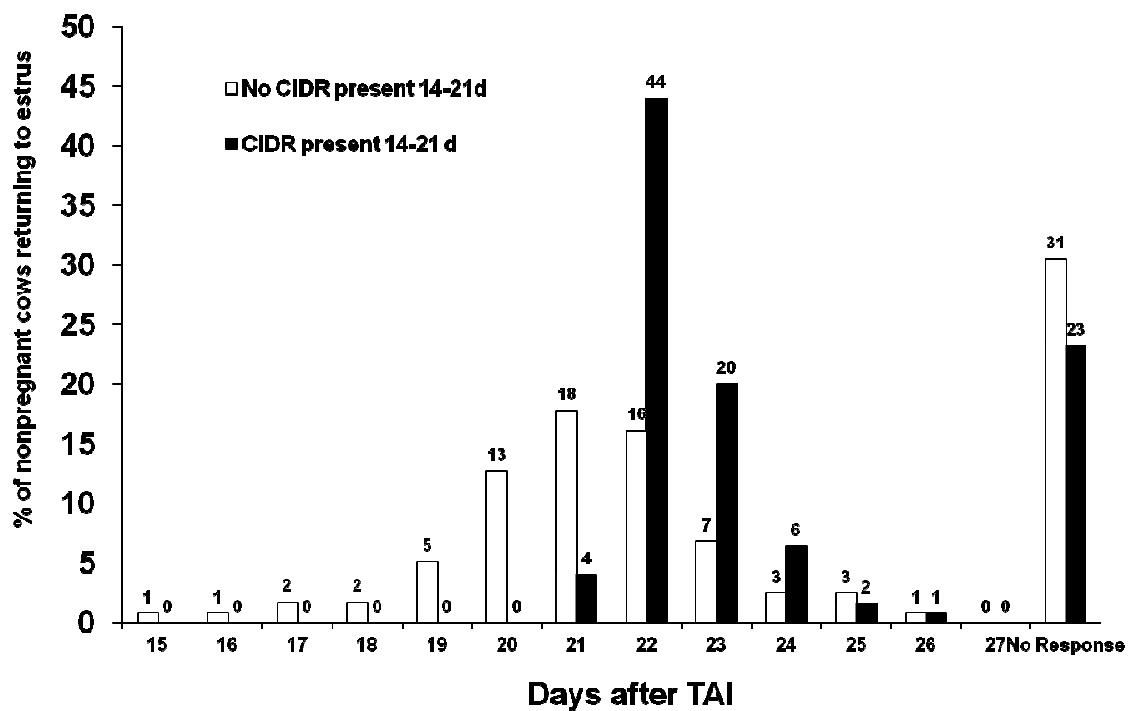
Figure 2. Distribution of estrus of nonpregnant cows after either receiving progesterone

via a CIDR insert from d 14 to 21 after fixed-time AI (solid bars) or not receiving

progesterone from d 14 to 21 after fixed-time AI (open bars).
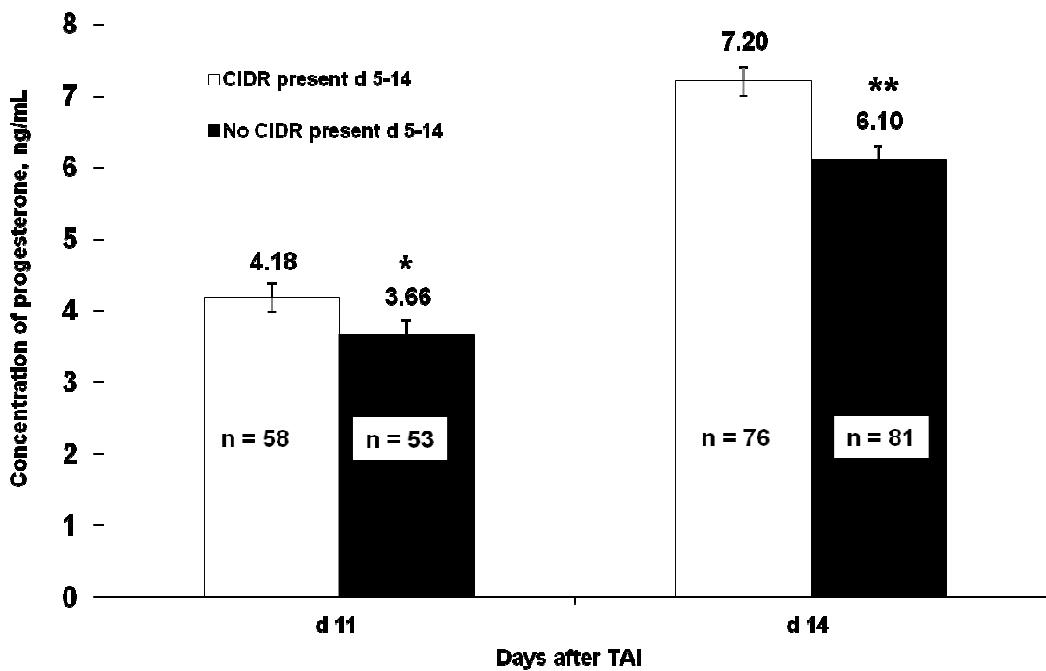
Figure 3. Concentrations of progesterone on d 11 (IL1 location) and d 14 (MN1 location) between cows receiving progesterone via a CIDR from d 5 to 14 after TAI (CIDR5-14 and CIDR5-21 treatments) and those not treated with progesterone from d 5 to 14 after TAI (control and CIDR14-21 treatments). * Concentrations of progesterone differ ($P = 0.03$) on d 11. ** Concentrations of progesterone differ ($P < 0.01$) on d 14.

CHAPTER 3

Concentrations of progesterone in the bovine reproductive tract

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ABSTRACT

Progesterone is necessary for maintenance of pregnancy and stimulates conceptus development. To accurately simulate reproductive tract environments in order to study the in vitro interaction of progesterone and embryos, an accurate concentration of progesterone must be known. Our objective was to establish the concentration of progesterone in the uterine horn and oviduct to provide a base concentration to be used in further experiments. The estrous cycles of 13 Angus heifers were synchronized with the CO-Synch + CIDR protocol and inseminated artificially at a fixed-time. Nine heifers were then subjected to a uterine horn/oviduct flush using standard embryo transfer equipment and PBS as the flushing agent on d 3 (n = 4) or d 7 (n = 5) post-AI. Blood samples were collected on d -9, -2 and 0 relative to TAI and 2× daily beginning 24 h after AI until 156 h after TAI. To further assess concentrations of progesterone in the reproductive tract, concentrations of progesterone were verified in uterine fluid from the reproductive tracts of eleven females collected from an abattoir. Uterine flush, blood serum, and uterine fluid samples were analyzed for concentration of progesterone using radioimmunoassay. Concentrations of progesterone from flushes on d 3 (0.68 ± 0.31 ng/mL) were similar ($P > 0.05$) to those on d 7 (0.47 ± 0.27 ng/mL) and total progesterone from flushes on d 3 (20.37 ± 9.41 ng) was similar to those on d 7 (11.79 ± 8.42 ng). Concentrations of progesterone in serum increased from shortly after AI (0.14 ng/mL, 48 h post-AI) until after 156 h post-AI (2.88 ng/mL). Five reproductive tracts collected had a CL, and concentrations of progesterone were 0.16, 0.30, 0.58, 1.75 and 9.94 ng/mL for those tracts (mean ± sd = 2.55 ± 4.18 ng/mL). There appears to be significant variability in the concentration of progesterone among the reproductive tract

of beef females within one week of estrus with the approximate concentration ranging from 0.50 and 1.00 ng/mL.

Key Words: Concentration of progesterone, Reproductive tract, Bovine embryo

INTRODUCTION

The concentration of progesterone in blood serum has frequently been used to represent the amount of progesterone available for reproductive processes. Progesterone is transferred locally from the ovarian/oviductal venous drainage to the uterine artery (Weems et al., 1988). The local transfer results in greater concentrations of progesterone within the uterine horn ipsilateral to the corpus luteum (CL; Pope et al., 1982). Concentrations of progesterone also were greater in uterine blood near the luteal tissue compared to systemic circulating blood (Cicinelli et al., 2004). This may indicate that blood serum or plasma concentrations of progesterone are irrelevant in understanding maintenance of pregnancy. In addition, to ensure accurate simulation in an in vitro progesterone model of oviduct and uterine conditions, accurate concentrations of progesterone are necessary.

Progesterone is necessary for maintenance of pregnancy and stimulates conceptus development. Proteins are secreted in the uterus and are under control of progesterone, increasing during early pregnancy or under treatment of supplemental progesterone (Ing et al., 1989). These proteins may serve a function as support of the conceptus during pregnancy. Low concentrations of progesterone or a delay in the rise of systemic progesterone during the early post-ovulatory period is associated with low embryo survival in cattle (Lamming et al., 1989; Lamming and Darwash, 1995;

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Darwash and Lamming, 1998; Starbuck et al., 1999, 2001; Hommeida et al., 2004).
Therefore, our goals were to establish the concentration of progesterone in the uterine
horn and oviduct to gain knowledge and provide a base concentration to be used in
further experiments.

MATERIALS AND METHODS

Uterine horn/oviductal flush

The estrous cycles of 13 Angus heifers were synchronized with the CO-Synch +
CIDR protocol (Lamb et al., 2006) and heifers were inseminated artificially at a fixed-
time. Heifers received a CIDR insert containing 1.38 g of progesterone (Pfizer Animal
Health, New York, NY) and a 100 μg injection of GnRH (OvaCyst; IVX Animal
Health, St. Joseph, MO) on d -9. On d -2 the insert was removed and heifers received
25 mg of $PGF_{2\alpha}$ (Lutalyse, dinoprost tromethamine, Pfizer Animal Health), followed in
54 h by a second injection of GnRH and fixed-time AI (TAI). Nine heifers were then
subjected to a uterine horn/oviduct flush using standard embryo transfer equipment and
PBS as the flushing agent on d 3 (n = 4) or d 7 (n = 5) post-AI. On d 3 and 7 after TAI,
the uterine horn ipsilateral to the CL was flushed. In addition, four heifers were not
flushed, since heifers failed to respond to estrous synchronization and the cervix of two
heifers were too small to safely pass the catheter.

Of the four heifers flushed on d 3, three were flushed with 30 mL PBS and one
was flushed with 15 mL PBS due to capacity of uterine horn and oviduct. Of the five
heifers flushed on d 7, four were flushed with 20 mL PBS and one was flushed with 30
mL PBS due to capacity of uterine horn and oviduct. A standard 56 cm, 16 Fr. embryo

collection catheter containing a 5 mL cuff (High Recover Catheter, AB Technology, Pullman, WA), which was inflated in the uterine horn, was used on all d 3 flushes and one d 7 flush. An infusion pipette was used for the remaining four d 7 flushes. During the flushes using an infusion pipette, the uterine horn contralateral to the CL was manually crimped to limit PBS from entering the horn. Regardless of flushing method, PBS was massaged in the uterine horn and oviduct for approximately 2 min and then as much as possible was collected and the quantity was recorded. Fluid was then vortexed and allowed to settle and aliquoted into vials prior to freezing at -20°C and later analyzed for concentration of progesterone.

Blood was collected from all heifers via venipuncture of the tail vein using 10 mL vacutainer tubes containing no additive. Samples were collected on d -9, -2 and 0 relative to TAI and 2x daily beginning 24 h after AI until 156 h after TAI. Blood samples were refrigerated and then centrifuged at $1,500 \times g$ for 20 min within 24 h. Serum was collected and frozen at -20°C until concentration of progesterone was determined using radioimmunoassay (RIA).

Uterine fluid collection

To verify concentrations of progesterone in the uteri of heifers, concentrations of progesterone were verified in uterine fluid from the reproductive tracts of eleven females collected from an abattoir and transported immediately to the laboratory. An incision of the reproductive tract was made beginning at the cervix/uterine body junction to the end of each uterine horn, and all fluid and mucous was collected via a syringe and placed in 50 mL vials. Fluid was measured and frozen at -20°C until RIA analysis. When fluid was thawed for analysis, samples with more than 5 mL of fluid

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were centrifuged at $2{,}000 \times$ g for 20 min to attain a diluted fluid to facilitate pipetting. Ovarian and uterine characteristics, including structures on the ovary, were recorded to approximate phase of the estrous cycle.

Radioimmunoassay

The RIA procedure of Coat-A-Count (Diagnostic Products Corp., Los Angeles, CA) was used to determine concentration of progesterone in thawed serum, uterine flushings, and uterine fluid samples. The assay kit was validated for bovine serum (Kirby et al., 1997) using an assay volume of 100 μl. Assay tubes for the standard curve contained 0.01, 0.025, 0.05, 0.2, 0.5, 1, 2, and 4 ng/tube. Assay sensitivity for a 100-μl sample was 0.1 ng/mL. Intra-assay coefficient of variation for pooled samples was $< 10\%$.

Statistical analyses

The concentration of progesterone in each sample was calculated based on a standard curve generated by regressing the standard assay tubes along with a total count standard and a non-specific binding standard. Total progesterone was calculated using the concentration of progesterone recorded in each sample multiplied by the total amount of PBS flushed into the uterus.

Statistical models to analyze concentration of progesterone in uterine flushings as well as total progesterone consisted of the variable, d of flush (3 or 7) and data were analyzed using the GLM Procedure of SAS (SAS Inst., Inc., Cary, NC). Statistical models to analyze concentration of progesterone in serum samples consisted of the variable date of sample collection and data were analyzed using the MIXED Procedure of SAS (SAS Inst., Inc.) with blood sample as a repeated variable. Means were

separated by using the least significant difference in the GLM or MIXED procedure of SAS (SAS Inst., Inc.), respectively, when a protected F-test ($P \leq 0.05$) was detected by ANOVA. Standard errors are presented.

The statistical model to analyze concentrations of progesterone in the uterine fluid from reproductive tracts consisted of reproductive tract and data were analyzed using the GLM Procedure of SAS (SAS Inst., Inc).

RESULTS AND DISCUSSION

Uterine horn/oviductal flush

Concentrations of progesterone from flushes on d 3 (0.68 ± 0.31 ng/mL) were similar ($P > 0.05$) to those on d 7 (0.47 ± 0.27 ng/mL; Table 1). Similarly, total progesterone from flushes on d 3 (20.37 ± 9.41 ng) was similar to those on d 7 (11.79 ± 8.42 ng; Table 1). We hypothesized that an increase in concentration of progesterone would be noted between d 3 and d 7 due to growth of a forming CL. However, variability of values was large in these samples generating large standard errors and individual animal variation may have had a greater impact on concentrations of progesterone than progesterone within the flushes.

In an experiement (Abecia et al., 1999), endometrial concentrations of progesterone were measured in ewes by homogenizing endometrial tissue in tissue culture media. On d 9 post-mating, concentrations of progesterone (presented as mean $\pm$ sd) ranged from 37.9 ng/mg in a nonpregnant ewe to 57.3 ± 14.6 ng/mg in pregnant ewes (n=6). On d 15 post-mating, concentrations of progesterone in endometrial tissue of different ewes were 26.2 ± 3.2 ng/mg (n=6). Ovarian venous blood samples also

were assessed on d 9 and 15 post-mating. Concentrations of progesterone in these samples were 5567 ng/mL in the nonpregnant ewe and 1607 ± 797 ng/mL in the pregnant ewes on d 9 and 1761 ± 795 ng/mL on d 15 in pregnant ewes. When corpus lutea were removed from the ovaries of these ewes on d 9 and incubated in tissue culture media to measure progesterone production, concentrations of progesterone released from luteal tissue per hour was 3385 ng/mg in the nonpregnant ewe and 3139 ± 563 ng/mg in the pregnant ewes. On d 15, concentrations of progesterone were 2016 ± 599 ng/mg in pregnant ewes. It is evident that a large quantity of progesterone is released from luteal tissue but little of this remains in ovarian venous blood and less is detected in endometrial tissue.

Concentration of progesterone in serum

As expected, concentrations of progesterone in serum increased from shortly after AI (0.14 ng/mL, 48 h post-AI) until after 156 h post-AI (2.88 ng/mL; Figure 1). Results from 84 h to 156 h post-AI only include heifers flushed on d 7. Similar results were recorded by Sartori et al. (2004), where serum concentrations of progesterone in heifers increased from < 0.5 ng/mL at the time of ovulation to approximately 2.8 ng/mL on d 7 of the estrous cycle. In this same study, luteal tissue volume increased from approximately 1000 mm^3 on d 3 to 5,500 mm^3 on d 7 of the estrous cycle. Luteal tissue volume reached a plateau on approximately d 7 or 8 whereas concentrations of progesterone in serum continued to rise until d 14 of the estrous cycle (Sartori et al., 2004).

In humans, serum concentrations of progesterone were significantly lower in the systemic circulation compared to the arterial/venous uterine blood (Cicinelli et al.,

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2004). Concentrations of progesterone in serum from women in the follicular phase of the menstrual cycle were 1.21 ± 1.02, 0.86 ± 0.49, and 0.75 ± 0.51 (ng/mL; mean $\pm$ sd) in the uterine vessels, radial artery (systemic blood), and antecubital vein (systemic blood), respectively. In contrast, concentrations of progesterone for women in the luteal stage of the menstrual cycle were 23.67 ± 13.11, 10.39 ± 3.69, and 9.81 ± 3.38 (ng/mL; mean $\pm$ sd) in the uterine vessels, radial artery (systemic blood), and antecubital vein (systemic blood), respectively (Cicinelli et al., 2004).

In beef cows, progesterone also was detected at a lower concentration in uterine tissue contralateral to the CL compared to uterine tissue ipsilateral to the CL (Pope et al., 1982). These data indicate the potential existence of mechanisms of local distribution of ovarian steroids and that measurement of progesterone in the systemic system may not accurately portray the concentration of progesterone available to the embryonic environment.

Uterine fluid collection

Initially, 11 reproductive tracts were recovered for fluid collection, however, fluid collection was only successful in 6 of 11 tracts. Of those where fluid was not collected, one tract contained a fetus and there was not sufficient fluid surrounding the placenta for collection. Two tracts did not have sufficient fluid for collection; one tract had pyometria and one tract had 15 mL of thick mucous which restricted pipetting and therefore concentration of progesterone could not be measured. Of the tracts from which fluid was collected, one tract appeared to be during estrus and contained 104 mL of thick mucus with a concentration of progesterone of 0.07 ng/mL. The remaining five tracts all contained small and medium follicles and a CL. Concentrations of

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progesterone were 0.16, 0.30, 0.58, 1.75 and 9.94 ng/mL for the five tracts, respectively. Mean concentrations of progesterone of the five tracts containing a CL were 2.55 ± 4.18 ng/mL (mean $\pm$ sd).

In conclusion, there appears to be significant variability in concentration of progesterone among the reproductive tract of beef females within one week of estrus, which concurs with data in humans and sheep. The primary goal of this study was to establish in vivo uterine concentrations of progesterone for subsequent in vitro experiments. The mean (0.61 ng mL) we established was based on concentrations of progesterone in the reproductive tracts of 13 females with a CL, with the exception of the tract with a 9.94 ng/mL value since this value apparently was excessively high.

LITERATURE CITED

Abecia, J. A., F. Forcada, and J. M. Lozano. 1999. A preliminary report on the effect of dietary energy on prostaglandina F2α production in vitro, interferon-tau synthesis by the conceptus, endometrial progesterone concentration of days 9 and 15 of pregnancy and associated rates of embryo wastage in ewes. Theriogenology 52:1203-1213.

Cicinelli, E., N. Einer-Jensen, M. Cignarelli, L. Mangiacotti, D. Luisi, and S. Schonauer. 2004. Preferential transfer of endogenous ovarian steroid hormones to the uterus during both the follicular and luteal phases. Hum. Reprod. 19(9):2001-2004.

Darwash, A. O. and G. E. Lamming. 1998. The importance of milk progesterone concentrations during early pregnancy in the cow. J. Anim. Breed. 2:41-43.

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., H. Francis, J. J. McDonnell, J. F. Amann, and R. M. Roberts. 1989. Progesterone induction of the uterine milk proteins: Major secretory proteins of sheep endometrium. Biol. Reprod. 41:643-654.

Kirby C. J., M. F. Smith, D. H. Keisler, and M. C. Lucy. 1997. Follicular function in lactating dairy cows with sustained-release bovine somatotropin. J. Dairy Sci. 80:273-285.

Lamb, G.C., J.E. Larson, T.W. Geary, J.S. Stevenson, S.K. Johnson, M.L. Day, R. P. Ansotegui, D. J. Kesler, J.M. DeJarnette, and D. Landblom. 2006. Synchronization

of estrus and artificial insemination in replacement beef heifers using GnRH, PGF$_{2\alpha}$ and progesterone. J. Anim. Sci. 84:3000-3009.

Lamming, G. E. and A. O. Darwash. 1995. Effect of inter-luteal interval on subsequent luteal phase length and fertility in post partum dairy cows. Biol. Reprod. 52 (Suppl. 1):72.

Lamming, G. E., A. O. Darwash, and H. L Back. 1989. Corpus luteum function in dairy cows and embryo mortality. J. Reprod. Fertil. 37: 245-252.

Pope, W. F., R. R. Maurer, and F. Stormshak. 1982. Distribution of progesterone in the uterus, broad ligament, and uterine arteries of beef cows. Anat. Rec. 203:245-250.

Sartori, R., J. M. Haughian, R. D. Shaver, G. J. M. Rosa, and M. C. Wiltbank. 2004. Comparison of ovarian function and circulating steroids in estrous cycles of Holstein heifers and lactating cows. J. Dairy Sci. 87:905-920.

Starbuck, G. R., A. O. Darwash, and G. E. Lamming. 1999. The importance of progesterone during early pregnancy in the dairy cow. Cattle Practice 7:397-399.

Starbuck, G. R., A. O. Darwash, G. E. Mann, and G. R. Lamming. 2001. The detection and treatment of post-insemination progesterone insufficiency in dairy cows. Pages 447-450 in Fertility in the High Producing Dairy Cow. M.G. Diskin, ed. BSAS Occasional Publication No. 26(2).

Weems, C. W., C. N. Lee, Y. S. Weems, and D. L. Vincent. 1988. Distribution of progesterone to the uterus and associated vasculature of cattle. Endocrinol. Jpn. 35:625-630.

Table 1. Flushing information and progesterone (P4) data for each heifer.

Day of flush	Fluid in, mL	Fluid out[1], mL	P4[2], ng/mL	P-value	SEM	Total P4[3], ng	P-value	SEM
3	30	26.2	1.68	0.618	0.31	50.35	0.519	9.41
3	30	24.1	0.04			1.12		
3	30	18.1	0.98			29.45		
3	15	13.2	0.04			0.54		
7	20	12.5	0.30	0.519	0.27	5.97		8.42
7	20	13.3	0.40			8.09		
7	20	11.6	0.18			3.51		
7	20	11.3	0.27			5.47		
7	30	20.8	1.20			35.89		
d 7 mean			0.47			11.79		

[1] Quantity of fluid retrieved from oviduct

[2] Quantity of progesterone (P4) per mL of fluid retrieved from oviduct

[3] Quantity of progesterone (P4) retrieved (concentration of progesterone measured in ng/mL of fluid multiplied by total mL fluid infused)
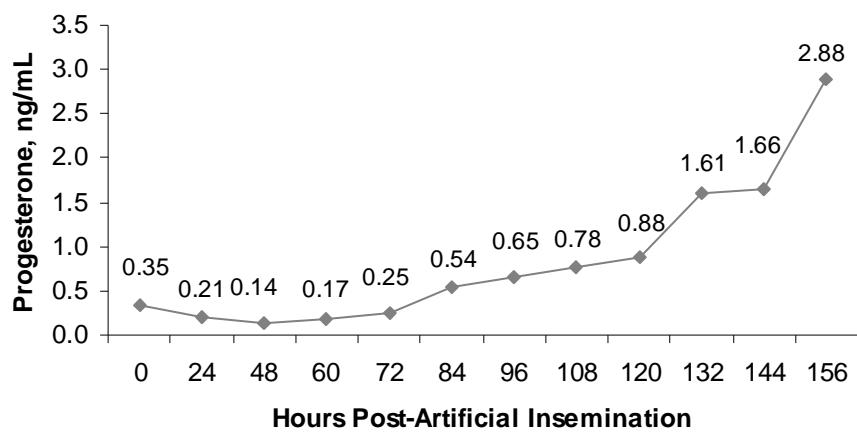
Figure 1. Concentration of progesterone in serum samples collected post-artificial insemination from heifers.

CHAPTER 4

Development of in vitro produced bovine embryos cultured with differing concentrations of progesterone in medium, with or without a co-culture of bovine oviductal epithelial cells

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ABSTRACT

Progesterone promoted development of bovine conceptuses, in vivo. An increased understanding of the factors affecting development of embryos is imperative to determine how to influence embryonic survival. Therefore, the objectives of this study were: 1) to determine whether a co-culture of bovine oviductal epithelial cells (BOEC) during in vitro culture of in vitro produced embryos affects embryo development; and 2) to determine the effects of progesterone in culture media during the in vitro culture of in vitro-produced embryos on embryo development. Bovine oocytes were purchased, matured, and fertilized, in vitro, and treatments were applied during the culture-phase of in vitro production. In Experiment 1, 311 oocytes in 33 droplets were used in three replicates. A 3 × 2 factorial arrangement of treatments was used. Droplets were assigned to receive one of three concentrations of progesterone: 1) embryos were exposed to 0 ng/mL (**CON**; n = 10); 2) embryos were exposed to 1 ng/mL (**LO**; n = 11); and, 3) embryos were exposed to 100 ng/mL (**HI**; n = 12). In addition, droplets were assigned to be cultured in the presence (**COCULT**; n = 17) or absence (**CON COCULT**; n = 16) of a co-culture of BOEC. In Experiment 2, 825 oocytes in 84 droplets were used in eight replicates. Droplets were assigned to receive one of three concentrations of progesterone: 1) embryos were exposed to 0 ng/mL (**CON**; n = 32); 2) embryos were exposed to 1 ng/mL (**LO**; n = 34); and, 3) embryos were exposed to 100 ng/mL (**HI**; n = 35). In Experiments 1 and 2, the percentage of oocytes that cleaved was not affected by the co-culture of BOEC or progesterone treatment (P > 0.10). In Experiment 1, the co-culture of embryos with BOEC significantly decreased development (47.0 ± 27.7% vs. 68.2 ± 17.5%) of those oocytes that cleaved to the

morula stage or greater ($P = 0.023$) and to the blastocyst stage ($7.5 \pm 13.5\%$ vs. $31.4 \pm$ 20.2%; $P < 0.001$). In Experiment 1, concentrations of progesterone did not affect embryo development to the morula stage or greater ($P = 0.945$) or to the blastocyst stage ($P = 0.107$). In Experiment 2, concentrations of progesterone did not affect embryo development to the morula stage or greater of development ($P = 0.857$), but tended to impact embryo development to the blastocyst stage of development ($P = 0.056$). The percentage of embryos that developed to the blastocyst stage was greatest for the CON group with $26.2 \pm 20.2\%$, lowest for the HI group with $18.8 \pm 14.7\%$, and intermediate for the LO group with $22.4 \pm 18.6\%$. In conclusion, development decreased with the addition of BOEC to the culture of embryos produced in vitro. Embryo development also tended to decrease with the addition of 100 ng/mL of progesterone.

Key Words: Progesterone, Embryo Development, Co-culture, Bovine

INTRODUCTION

Embryonic loss contributes to substantial economic losses in both dairy and beef cattle operations because of loss of milk production, a decrease in numbers of calves that can be sold or used as replacement heifers, and an increase in culling rate. In dairy cows, fertilization rates have been similar in lactating (mean = 76.2%; range = 55.3 to 87.8%) and nonlactating (mean = 78.1%; range = 58.0 to 98.0%) cows (Dalton et al., 2001; DeJarnette et al., 1992; Sartori et al., 2002). However, by d 27 to 31 after AI, conception rates decreased to 35 to 45%. By d 5 to 6 after AI, only 65% of the

fertilized oocytes were considered viable (Dalton et al., 2001; DeJarnette et al., 1992; Sartori et al., 2002).

Supplemental progesterone during the first 4 days after AI increased morphological development and biosynthetic activity of d 14 conceptuses (Garrett et al., 1988). In addition, supplemental progesterone during the first week of pregnancy increased pregnancy rates (Mann and Lamming, 1999) in some studies. However, progesterone cannot be supplemented immediately after AI because it increases the occurrence of short estrous cycles (Garrett et al., 1988; Van Cleeff et al., 1996; Lynch et al., 1999). High concentrations of progesterone may result in the down-regulation of progesterone receptors (Clarke 1990; Spencer and Bazer 1995; Spencer et al., 1995b), and may down-regulate endogenous production of progesterone (Mann et al., 2001). In dairy cows that received an embryo, the average interval after ovulation until progesterone achieved a 1 ng/ml concentration was 4.1 days for cows with detectable INF-τ and 5.6 days for those with no detectable INF-τ on d 16 (Mann and Lamming, 2001). Timing of supplemental progesterone is important, in part, because it is thought to alter the secretory activity of the endometrium, thus influencing embryonic growth (Garrett et al., 1988; Geisert et al., 1992). In addition, progesterone appears to have a direct effect on oocyte maturation or competence. In monkeys, supplemental progesterone during the maturation phase of in vitro-produced embryos improved the developmental competence of oocytes (Zheng et al., 2003) indicating that progesterone acts directly on oocytes or surrounding cumulus cells. Therefore, supplementation of progesterone during early stages of embryo development may enhance the ability of the embryo to survive to maternal recognition of pregnancy and needs further investigation.

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An increased understanding of the factors affecting viability of embryos is imperative to determine how to influence embryonic survival. Therefore, the objectives of this study were: 1) to determine whether a co-culture of bovine oviductal epithelial cells during in vitro culture of in vitro produced embryos affects embryo development; and 2) to determine the effects of progesterone in culture media during the in vitro culture of in vitro-produced embryos on embryo development.

MATERIALS AND METHODS

Substrates, chemicals, and media preparation

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless specified otherwise. All-in-one synthetic oviductal fluid (SOF) medium for bovine (Gandi et al., 2000) was used for the in vitro fertilization and culture of oocytes and embryos. Stock solutions were prepared with 18.2 MΩ water and stored at 4°C for either 1 wk (bicarbonate, pyruvate, glutamine/alanine), 1 mo (calcium, glucose, EDTA, taurine) or 3 mo (HEPES, basil salt solution). Stock solutions of penicillin/streptomycin/ amphotericin (PSA; Invitrogen Corp., Grand Island, NY), amino acids (minimal essential medium concentrations; MP Biomedicals, LLC, Solon, OH) and vitamins (minimal essential medium concentrations; MP Biomedicals, LLC) were stored according to manufacturers' suggestions and were used directly for medium preparation. Amounts of stock solutions as well as BSA in working solutions are described in Table 1. Working solutions were prepared for each replicate, filtered (0.22 μm; MillexGV; Millipore, Billerica, MA) and equilibrated in the appropriate gas atmosphere.

Oocytes and in vitro maturation

Bovine oocytes were purchased (Ovitra Biotechnology, Amarillo, TX) where they were aspirated from abattoir ovaries. Oocytes with at least three layers of compact cumulus cells and even cytoplasm were placed in maturation media containing 10% FBS. Fifty oocytes in 10 mL maturation media were placed in each vial and vials were flushed with 5% CO_2 in air before being capped. Oocytes were shipped overnight at 39° C in temperature-controlled incubators.

In vitro fertilization

Oocytes were allowed to mature for 22-24 h and were then washed twice in HEPES buffered medium and once in SOF fertilization medium (Table 1). Nine hundred eighty five (eight replicates with approximately 125 oocytes per replicate) oocytes were then placed in groups of 10, in 44 μl droplets of SOF fertilization medium under 10 mL washed mineral oil in 10×60 mm plastic dishes (Falcon 1007; Becton Dickinson Labware, Franklin Lakes, NJ). Mineral oil was washed by adding 1 part D-PBS (Invitrogen Corp.) without calcium to 3 parts mineral oil and then shaken vigorously and allowed to separate.

Frozen semen from the same bull for each replicate was thawed for 1 min in a 37° C water bath. Sperm were capacitated by centrifugation through a 90% and 45% Percoll (GE Healthcare Bio-sciences Corp., Piscataway, NJ) gradient for 20 min at 700 $\times$ g and washed by centrifugation in sperm wash medium (Table 1) at $700 \times$ g twice for 5 minutes to remove any remaining semen extender. Insemination was carried out by adding 2 μL of the spermatozoa and fertilization media mixture, to reach a final concentration of 0.75×10^6 spermatozoa/mL in the droplet, 2 μL of 4 µg/mL heparin,

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and 2 µL PHE (Ball et al., 1983; Parrish et al., 1985, 1988: penicillamine, 20 µmol/l; hypotaurine, 10 µmol/l; epinephrine, 1 µmol/l). The final droplet volume was 50 µL. Oocytes were co-incubated with spermatozoa for 18 h at 38.7°C with 6.3% CO_2 in atmosphere.

In vitro culture

After incubation with sperm, all presumptive zygotes were placed into 0.5 mL SOF-HEPES with 0.11 mg/mL hyaluronidase and vortexed for 3 min to remove cumulus cells, followed by an additional wash in SOF-HEPES and a wash in culture-1 medium. No selection was performed after fertilization; all presumptive zygotes were moved in groups of 10 into 50 µL droplets of culture-1 medium under 10 mL washed mineral oil. Embryo cultures were performed at 38.7° C with 5% CO_2: 10% O_2: 85% N_2 in atmosphere. After 72 h of culture, all embryos were removed from culture drops, and while being maintained in their experimental group of 10, examined for cleavage, washed 2 times in SOF-HEPES and once in culture-2 medium, and placed into culture-2 medium drops in the same conditions as culture-1 for an additional 96 h. After embryos were in culture for a total of 7 d, further measurements were taken.

Treatments

In Experiment 1, 311 oocytes in 33 droplets were used in three replicates. A 3 × 2 factorial arrangement of treatments was used. Droplets were assigned to receive one of three concentrations of progesterone: 1) embryos were exposed to 0 ng/mL (**CON**; n = 10); 2) embryos were exposed to 1 ng/mL (**LO**; n = 11); and, 3) embryos were exposed to 100 ng/mL (**HI**; n = 12). In addition, droplets were assigned to be cultured in the presence (**COCULT**; n = 17) or absence (**CON COCULT**; n = 16) of a co-culture

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of bovine oviductal epithelial cells (BOEC). Treatments were randomly assigned to each dish containing approximately 3 droplets. A droplet served as the experimental unit.

In Experiment 2, 825 oocytes in 84 droplets were used in eight replicates. Droplets were assigned to receive one of three concentrations of progesterone: 1) embryos were exposed to 0 ng/mL (**CON**; n = 32); 2) embryos were exposed to 1 ng/mL (**LO**; n = 34); and, 3) embryos were exposed to 100 ng/mL (**HI**; n = 35). A droplet served as the experimental unit.

Embryos exposed to progesterone treatment received treatment when embryos were moved into droplets to ensure that the concentration was at treatment concentrations when embryos were introduced.

Embryos exposed to BOEC during the culture phase (Eyestone and First, 1989) received BOEC isolated from oviducts collected from an abattoir and transported to the laboratory in 0.9% NaCl within 30 to 60 min after evisceration. Oviducts were rinsed and placed in sterile plastic dishes, grasped with forceps at the isthmic end and scraped gently toward the infundibulum with a glass microscope slide to squeeze out oviductal cells. Cells were transferred to a 12-mL conical tube with 10 mL HEPES medium and washed through 5 changes of HEPES medium by centrifugation ($700 \times$ g for 5 min). The tissue pellet was resuspended in 10 mL Medium 199 (Mediatech, Inc., Herndon, VA) supplemented with 10% heat-treated fetal calf serum (Invitrogen Corp., Grand Island, NY) and 50 µg gentamicin/mL. Tissue suspension was cultured in 6-well culture plates (2 mL suspension/well) at 39° with 5% CO_2 in the air. Cells were established for 2 to 14 d before being added to embryo culture droplets. Cells were

collected, washed through HEPES medium and were allowed to settle. One µL of concentrated BOEC was added to each droplet (Xu et al., 1992).

Determination of embryo development

Embryos were visually assessed for cleavage on d 4 of culture (when moved into culture-2 medium). After embryos had been in culture for 7 d, oocytes or embryos were visually assessed and assigned a developmental stage according to standards set forth by the International Embryo Transfer Society (Savoy, IL). For statistical analyses, a category was assigned to each oocyte/embryo dependent on stage of development: 1) < morula (1 to < 32 cells), 2) morula 3) early or expanded blastocyst, 4) hatched blastocyst.

Statistical analyses

Hypothesis

Factor 1: co-culture with BOEC

H_0: $\tau_1 = \tau_2$

H_1: $\tau_1 \neq \tau_2$

Factor 2: concentration of progesterone

H_0: $\tau_1 = \tau_2 = \tau_3$

H_1: $\tau_1 \neq \tau_2 \neq \tau_3$

Interaction of factors

H_0: Interaction Factor 1 and Factor 2 = 0

H_1: Interaction Factor 1 and Factor 2 $\neq$ 0

Statistical significance of results was obtained using an alpha error of 0.05, a beta error of 0.20 and a Power of 80%. Values were considered statistically different

when $P \leq 0.05$ and a tendency for statistical difference was considered when $0.05 \leq P \geq 0.10$.

Procedure GENMOD of SAS (SAS Inst., Inc., Cary, NC) using a probit model with a binary distribution was used to analyze data. Means were separated by using the MEANS procedure of SAS when a protected F-test ($P \leq 0.05$) was detected. Standard deviations are presented. For Experiment 1, the percentage of oocytes that cleaved and the percentage of cleaved embryos reaching the morula stage or further or the blastocyst stage of development were analyzed using a model that included replicate, progesterone treatment, co-culture treatment, and all two-way interactions. For Experiment 2, the percentage of oocytes that cleaved and the percentage of cleaved embryos reaching the morula stage or further or the blastocyst stage of development were analyzed using a model that included replicate, progesterone treatment, and the replicate $\times$ treatment interaction.

RESULTS AND DISCUSSION

Embryonic loss contributes to substantial economic losses in both dairy and beef cattle operations (Thurmond and Picanso, 1990; Eicker and Fetrow, 2003; De Vries, 2006); mechanisms to decrease embryonic loss would benefit beef and dairy producers. Some of these potential mechanisms have been researched and typically involve the use of a progestin. Results have been variable (Mann and Lamming, 1999; Mann 2008) and the exact mechanism behind treatments with progestins have not been elucidated. Progesterone, a steroid hormone produced by the CL and placenta, blocks surges of GnRH (Attardi and Happe, 1986; Kasa-Vubu et al., 1992) and down-regulates estrogen

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receptors (Brenner et al., 1974; Evans and Leavitt, 1980; Spencer et al., 1995a, b; Wathes et al., 1996), thus creating a quiescent uterus for pregnancy (Bottari et al., 1983), and healthy conceptus maturation. Progesterone enhances conceptus development in vivo and promotes the secretion of interferon tau (IFN$_\tau$; Garrett et al., 1988), which is necessary for the maintenance of pregnancy.

The reproductive tract environment is critically important to embryonic viability. The oviduct consists of regions which have different characteristics that are controlled, in part, by progesterone (Brenner and Masler, 1988; Verhage et al., 1990; Murray, 1992; Buhi et al., 1997). In vitro fertilization and embryo development are enhanced in several species (including the bovine; Eyestone and First, 1989), by the addition of oviduct epithelial cells. Oviductal and uterine fluids also are the source of amino acids for the developing oocyte or embryo (Hugentobler et al., 2007). Therefore, we elucidated whether progesterone directly influenced early embryonic development in vitro or, by using a BOEC co-culture system, whether progesterone acted upon BOEC which could influence embryonic development in vitro.

Replicate differences

Oocytes and embryos are sensitive to temperature fluctuations (Schumacher and Fischer, 1988; Rivera and Hansen, 2001) and pH changes (Ocon and Hansen, 2003) and therefore, researchers are limited in the number of oocytes that can successfully be handled at one time. To combat this, experiments of this manner are conducted in replicates. In addition, when acquiring oocytes from unknown cows in an abattoir, oocyte quality is beyond laboratory control and replicates balance the factors that could be involved such as breed, age, disease and nutritional status of cows, heat stress the

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oocyte may have been exposed to, and handling of ovaries after evisceration. As a result of these factors, replication was a significant source of variation in this experiment.

Three replicates of in vitro produced embryos were included in Experiment 1 whereas eight replicates were included in Experiment 2. Each replicate consisted of approximately 123 oocytes (range 85 to 151) and approximately 13 droplets (range 9 to 15). Each droplet contained approximately 10 oocytes or embryos (range 7 to 11). Replication was a significant source of variation on cleavage rate and development of cleaved embryos to the morula or blastocyst stage ($P < 0.05$; Table 2). The percentage of oocytes that cleaved, per replicate, ranged from $66.5 \pm 14.6\%$ to $84.9 \pm 15.2\%$. The percentage of cleaved embryos that reached the morula stage or greater ranged from $48.1 \pm 33.7\%$ to $80.5 \pm 31.7\%$ and that reached the blastocyst stage ranged from $14.6 \pm 14.4\%$ to $30.1 \pm 15.7\%$. Similar differences among replicates have been recorded in our laboratory in other experiments even though extensive attempts were made to control variables.

Incidence of oocyte cleavage

In Experiment 1, the percentage of oocytes that cleaved was not affected by the co-culture of BOEC or progesterone treatment ($P > 0.10$; Table 3). For oocytes without co-culture (n = 16), $73.3 \pm 11.9\%$ cleaved whereas $75.6 \pm 16.9\%$ of oocytes with co-culture (n = 17) cleaved. For progesterone treatment, $74.6 \pm 12.1\%$, $70.0 \pm 16.7\%$, and $78.5 \pm 14.1\%$ cleaved in CON (n = 10), LO (n = 11) and HI (n = 12) treatment groups, respectively.

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In Experiment 2, the percentage of oocytes that cleaved among the treatment concentrations of progesterone were similar ($P = 0.169$; Table 5). The percentage of oocytes that cleaved were $82.1 \pm 13.1\%$, $76.5 \pm 15.4\%$, and $80.9 \pm 16.4\%$ for the CON (n = 32), LO (n = 34), and HI (n = 35) progesterone treatment groups.

These results were expected because co-culture and progesterone treatments were not administered until presumptive zygotes were moved into the culture-1 phase of in vitro production. At this point, many oocytes were nearing, or even beginning, the first division of mitosis.

Embryo development and co-culture with BOEC

In Experiment 1, the co-culture of embryos with BOEC significantly decreased development of those oocytes that cleaved to the morula stage or greater ($P = 0.023$) and to the blastocyst stage ($P < 0.001$; Table 3). For oocytes that cleaved, $68.2 \pm 17.5\%$ of those without co-culture developed to the morula stage or greater whereas $47.0 \pm 27.7\%$ of those with co-culture developed to the morula stage or greater. Similarly, of oocytes that cleaved, $31.4 \pm 20.2\%$ of those without co-culture developed to the blastocyst stage whereas $7.5 \pm 13.5\%$ of those with co-culture developed to the blastocyst stage (Table 3).

In Experiment 1, an interaction tended to occur between the co-culture treatment and progesterone treatments on the development of cleaved oocytes to the morula stage or greater ($P = 0.062$) and also on the development to the blastocyst stage ($P = 0.098$; Table 4). In droplets where co-culture was present, development was decreased; however, development to the morula stage or greater was decreased less when progesterone was present at either LO or HI concentrations (Table 4). In assessing

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development to the blastocyst stage, development was decreased most significantly in the LO progesterone treatment and decreased less in the NO and HI concentrations of progesterone (Table 4). Perhaps progesterone influenced BOEC slightly to enhance their capacity to secrete factors to improve embryonic growth. However, since all treatments with co-culture had decreased development compared to treatments without co-culture, it was clear these embryonic factors were not sufficient to overcome the negative factors of co-culture on embryo development.

In most cell culture environments, serum (fetal calf serum, heat-treated estrus cow serum, or pregnant mare serum) usually is included in media as a protein source for cellular growth (Bavister, 1995). Serum also contains beneficial factors that enhance cell proliferation (Eppig et al., 1992), many of which are unknown, but may also be toxic (Maurer, 1992). These factors may differ among serum sources as well as within lots of similar sources (Bavister, 1995). For these reasons, it is ideal to conduct in vitro embryo experiments in the absence of serum, but use purified bovine serum albumin, which was used the current experiments (Table 1). The resulting media was more defined and repeatable, even though the viability of cell cultures was likely compromised. Serum was not used in these experiments; therefore, many of the live BOEC placed into co-culture may have undergone apoptosis during the culture phase. Therefore, the nonliving cells may have emitted factors that compromised embryonic development.

Embryo development and treatment with progesterone

In Experiment 1, concentrations of progesterone did not affect embryo development to the morula stage or greater ($P = 0.945$) or to the blastocyst stage ($P = $

0.107; Table 3). For oocytes that cleaved, $57.0 \pm 32.7\%$, $56.2 \pm 23.2\%$, and $58.5 \pm$

22.5% developed to the morula stage or greater for the CON, LO, and HI progesterone

treatments, respectively. In oocytes that cleaved, $27.7 \pm 23.6\%$, $15.5 \pm 22.4\%$, and 15.1

$\pm 15.4\%$ developed to the blastocyst stage for CON, LO and HI PG treatments,

respectively (Table 3).

In Experiment 2, concentrations of progesterone did not affect embryo

development to the morula stage or greater of development ($P = 0.857$; Table 5). The

percentage of embryos that developed to the morula stage or greater was $62.4 \pm 24.4\%$,

$62.2 \pm 20.0\%$, and $66.8 \pm 27.9\%$ for the CON, LO and HI progesterone treatments,

respectively. Concentrations of progesterone tended to impact embryo development to

the blastocyst stage of development ($P = 0.056$; Table 5). The percentage of embryos

that developed to the blastocyst stage was greatest for the CON group with $26.2 \pm$

20.2%, lowest for the HI group with $18.8 \pm 14.7\%$, and intermediate for the LO group

with $22.4 \pm 18.6\%$. Administering supplmental progesterone, in vivo, from d 1 to 4 of

pregnancy advanced development of d 14 conceptuses (Garrett et al., 1988). The

current experiment is the first known attempt to determine whether progesterone acts

directly on the embryo and influences early embryonic development without a co-

culture present. Our results are similar to those from Pereira et al., (2009) in that

supplementation of progesterone during the culture phase decreased embryo

development when a co-culture of either BOEC or granulosa cells was used with 10%

superovulated oestrus cow serum present in medium. We determined that progesterone,

at either 1 ng/mL or 100 ng/mL, did not significantly affect embryo development in

vitro. Progesterone may likely affect embryo development in vivo by causing changes

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in the oviductal and uterine environment (Brenner and Masler, 1988; Verhage et al., 1990; Murray, 1992; Buhi et al., 1997). When progesterone was added, in vitro, to oocyte maturation media, embryo development was advanced (Zheng et al., 2003). However, cumulus cells were surrounding oocytes during maturation and these were likely affected by progesterone which acted on the oocytes (Zheng et al., 2003).

In conclusion, these results indicated that development decreased with the addition of BOEC to the culture of embryos produced in vitro. Embryo development also tended to decrease with the addition of 100 ng/mL of progesterone. While progesterone may slightly decrease the negative impact of the co-culture of embryos with BOEC, it was insufficient to produce a positive outcome on development.

LITERATURE CITED

Attardi, B. and H. K. Happe. 1986. Modulation of the estradiol-induced luteinizing hormone surge by progesterone or antiestrogens: effects on pituitary gonadotropin-releasing hormone receptors. Endocrinology 119:274-283.

Ball, G. D., M. L. Leibfried, R. W. Lenz, R. L. Ax, B. D. Bavister, and N. L. First. 1983. Factors affecting successful in vitro fertilization of bovine follicular oocytes. Biol. Reprod. 28:717-725.

Bavister, B. D. 1995. Culture of preimplantation embryos: facts and artifacts. Hum. Reprod. Update 1(2):91-148.

Bottari, S. P., A. Vokaer, E. Kaivez, J. P. Lescrainer, and G. P. Vauquelin. 1983. Differential regulation of alpha-adrenergic receptor subclasses by gonadal steroids in human myometrium. J. Clin. Endocrinol. Metab. 57:937-941.

Brenner, R. M. and I. A. Maslar. 1988. The primate oviduct and endometrium. In: Physiology of Reproduction. K. Knobil, J.D. Neill, L.L. Ewing, C.L. Markert, G.S. Greenwald, and D.W. Pfaff, eds. Raven Press, Ltd., New York, pp. 303-329.

Brenner, R. M., J. A. Resko, and N. B. West. 1974. Cyclic changes in oviductal morphology and residual cytoplasmic estradiol binding capacity induced by sequential estradiol-progesterone treatment of spayed Rhesus monkeys. Endocrinology 95:1094-1104.

Buhi, W. C., I. M. Alvarez, and A. J. Kouba. 1997. Oviductal regulation of fertilization and early embryonic development. J. Reprod. Fertil. 52:285-300.

Clarke, C. L. 1990. Cell-specific regulation of progesterone receptor in the female reproductive system. Mol. Cell. Endocrinol. 70:C29-C33.

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 number in artificially inseminated cattle. J. Anim. Sci. 70:484-491.

De Vries, A. 2006. Economic value of pregnancy in dairy cattle. J. Dairy Sci. 89:3876-3885.

Eicker, S. and J. Fetrow. 2003. New tools for deciding when to replace used dairy cows. Pages 33-46 in Proc. Kentucky Dairy Conf., Cave City, KY. Univ. Kentucky, Lexington.

Eppig, J. J., K. Wigglesworth, and M. J. O'Brien. 1992. Comparison of embryonic developmental competence of mouse oocytes grown with and without serum. Mol. Reprod. Dev. 32:33-40.

Evans, R. W. and W. W. Leavitt. 1980. Progesterone inhibition of uterine nuclear estrogen receptor: dependence on RNA and protein synthesis. Proc. Natl. Acad. Sci. USA 77:5856-5860.

Eyestone, W. H. and N. L. First. 1989. Co-culture of early cattle embryos to the blastocyst stage with oviductal tissue or in conditioned medium. J. Reprod. Fertil. 85:715-720.

Gandi, A. P., M. Lane, D. K. Gardner, and R. L. Krisher. 2000. A single medium supports development of bovine embryos throughout maturation, fertilization and culture. Hum. Reprod. 15:395-401.

Garrett, J. E., R. D. Geisert, M. T. Zavy, and G. L. Morgan. 1988. Evidence for

    maternal regulation of early conceptus growth and development in beef cattle. J.

    Reprod. Fertil. 84:437-446.

Geisert, R. D., G. L. Morgan, E. C. Short, and M. T. Zavy. 1992. Endocrine events

    associated with endometrial function and conceptus development in cattle. Reprod.

    Fertil. Dev. 4:301-305.

Hugentobler, S. A., M. G. Diskin, H. J. Leese, P. G. Humpherson, T. Watson, J. M.

    Sreenan, and D. G. Morris. 2007. Amino acids in oviduct and uterine fluid and

    blood plasma during the estrous cycle in the bovine. Mol. Reprod. Devl. 74:445-

    454.

Kasa-Vubu, J. Z., G. E. Dahl, N. P. Evans, L. A. Thrun, S. M. Moenter, V.

    Padmanabhan, and F. J. Karsch. 1992. Progesterone blocks the estradiol-induced

    gonadotropin discharge in the ewe by inhibiting the surge of gonadotropin-releasing

    hormone. Endocrinology 131:208-212.

Lynch, P. R., K. L. Macmillan, and V. K. Taufa. 1999. Treating cattle with

    progesterone as well as GnRH analogue affects oestrous cycle length and fertility.

    Anim. Reprod. Sci. 56:189-200.

Mann, G. E. 2008. Meta-analysis of progesterone supplementation during early

    pregnancy in cattle. J. Anim. Sci. (Suppl.)86:387. (Abstr.)

Mann, G. E. and G. E. Lamming. 1999. The influence of progesterone during early

    pregnancy in cattle. Reprod. Domest. 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. Reproduction 121:175-180.

Mann, G. R., P. Mierson, M. D. Fray, and G. E. Lamming. 2001. Conception rate

following progesterone supplementation after second insemination in dairy cows.

Vet. J. 162:161-162.

Maurer, H. R. 1992. Towards serum-free, chemically defined media for mammalian cell

culture. In Freshney, R. I. (ed), Animal Cell Culture: A Practical Approach, 2nd ed.

Oxford Univ. Press, Oxford. Pp. 15-46.

Murray, M. K. 1992. The biosynthesis, immunocytochemical localization of an

estrogen-dependent glycoprotein and associated morphological alterations in the

sheep ampulla oviduct. Biol. Reprod. 47:889-902.

Ocon, O. M. and P. J. Hansen. 2003. Disruption of bovine oocytes and preimplatation

embryos by urea and acidic pH. J. Dairy Sci. 86:1194-1200.

Parrish, J. J., J. L. Susko-Parrish, and N. L. First. 1985. Effect of heparin and

chondroitin sulfate on the acrosome reaction and fertility of bovine sperm *in vitro*.

Theriogenology 24:537-549.

Parrish, J. J., J. Susko-Parrish, M. A. Winer, and N. L. First. 1988. Capacitation of

bovine sperm by heparin. Biol. Reprod. 38:1171-1180.

Pereira, R. M., C. C. Marques, M. C. Baptista, M. I. Vasques, and A. E. Horta. 2009.

Embryos and culture cells: a model for studying the effect of progesterone. Anim.

Reprod. Sci. 111(1):31-40.

Rivera, R. M. and P. J. Hansen. 2001. Development of cultured bovine embryos after exposure to high temperatures in the physiological range. Reproduction 121:107-115.

Sartori, R., R. Sartor-Bergfelt, S. A. Mertens, J. N. Guenther, J. J. Parrish, 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.

Schumacher, A. and B. Fischer. 1988. Influence of visible light and room temperature on cell proliferation in preimplantation rabbit embryos. J. Reprod. Fert. 84:197-204.

Spencer, T. E. and F. W. Bazer. 1995. Temporal and spatial alterations in uterine estrogen receptor expression and progesterone receptor gene expression during the estrous cycle and early pregnancy in the ewe. Biol. Reprod. 53:1527-1543.

Spencer, T. E., W. C. Becker, P. George, M. A. Mirando, T. F. Ogle, and F. W. Bazer. 1995a. Ovine interferon-τ inhibits estrogen receptor up-regulation and estrogen-induced luteolysis in cyclic ewes. Endocrinology 136:4932-4944.

Spencer, T. E., W. C. Becker, P. George, M. A. Mirando, T. F. Ogle, and F. W. Bazer. 1995b. Ovine interferon-τ regulates expression of endometrial receptors for estrogen and oxytocin but not progesterone. Biol. Reprod. 53:732-745.

Thurmond, M. C. and J. P. Picanso. 1990. A surveillance system for bovine abortion. Prev. Vet. Med. 9:41-53.

Van Cleeff, J., K. L. Macmillan, M. Drost, and W. W. Thatcher. 1996. Effects of administering progesterone at selected intervals after insemination of synchronized heifers on pregnancy rates and resynchronization of returns to estrus.

Theriogenology 4:1117-1130.

Verhage, H. G., P. A. Mavrogianis, M. L. Boice, W. Li, and A. T. Fazleabas. 1990. Oviductal epithelium of the baboon: Hormonal control and the immuno-gold localization of oviduct-specific glycoproteins. Am. J. Anat. 187:81-90.

Wathes, D. C., G. E. Mann, J. H. Payne, P. R. Riley, K. R. Stevenson, and G. E. Lamming. 1996. Regulation of oxytocin, oestradiol, and progesterone receptor concentrations in different uterine regions by oestradiol, progesterone and oxytocin in ovariectomised ewes. Endocrinology 151:375-393.

Xu, K. P., B. K. Yadav, R. W. Rorie, L. Plante, K. J. Betteridge, and W. A. King. 1992. Development and viability of bovine embryos derived from oocytes matured and fertilized in vitro and co-cultured with bovine oviduct epithelial cells. J. Reprod. Fert. 94:33-43.

Zheng, P., S. Wei, B. D. Baviser, J. Yang, C. Ding, and W. Ji. 2003. 17β-Estradiol and progesterone improve in-vitro cytoplasmic maturation of oocytes from unstimulated prepubertal and adult rhesus monkeys. Human Reprod. 18:2137-2144.

Table 1. Composition of synthetic oviductal fluid (SOF) medium used to fertilize oocytes, wash spermatozoa and culture bovine zygotes and embryos in vitro[1]

Reagent (mmol/L)	Fertilization	Sperm Wash	Culture-1	Culture-2
Sodium pyruvate	0.33	1.0	0.33	0.33
Alanine/Glutamine	-	-	1.0	1.0
Glucose	-	-	1.5	3.0
Sodium lactate	-	18.3	-	-
HEPES	-	12.5	-	-
Bovine serum albumin[2]	6 mg/mL	3 mg/mL	8 mg/mL	8 mg/mL
EDTA	-	-	0.1	-
Taurine	-	-	0.1	-
NEAA[3]	2×	-	1×	1×
EAA[4]	-	-	1×	2×
Vitamins	-	-	-	1×
PSA[5]	-	10 μL/mL	-	-

[1] Supplements added to a base SOF medium consisting of 99.70 mmol/L NaCl, 7.16 mmol/L KCl, 1.19 mmol/L KH_2PO_4, 0.49 $MgCl_2 \cdot 6H_2O$, 3.30 mmol/L lactic acid (L) free acid (MP Biomedicals, LLC), sodium salt (60% syrup), i.e. sodium lactate, 25.07 mmol/L $NaHCO_3$, 1.71 $CaCl_2 \cdot 2H_2O$.

[2] Bovine serum albumin used in fertilization was Fatty Acid Free (Sigma A7511), used in sperm wash was Fraction V (Sigma A3311), and used in culture-1 and culture-2 was crystallized (Cat. No. 810012; MP Biomedicals, LLC).

[3] Non-essential amino acids (MP Biomedicals, LLC).

[4] Essential amino acids (MP Biomedicals, LLC).

[5] Pennicillin/streptomycin/amphotericin (PSA; Invitrogen Corp., Grand Island, NY).

Table 2. Percentage of oocytes that were fertilized and cleaved and percentage of cleaved embryos that developed to morula or greater, or blastocysts by replicate.

			Percentage of cleaved embryos that developed to	
			$\geq$ Morula	Blastocyst
Replicate	No. Droplets	Percentage cleaved (no.)[1]		
		---------------------------------- Mean $\pm$ sd ----------------------------------		
1	9	$73.1^{abc} \pm 12.3$ (85)	$68.7^{cd} \pm 19.8$	$26.2^{cd} \pm 20.8$
2	12	$83.5^{d} \pm 11.2$ (108)	$57.9^{abc} \pm 16.0$	$14.7^{a} \pm 19.5$
3	12	$66.5^{a} \pm 14.6$ (118)	$48.1^{a} \pm 33.7$	$18.0^{abc} \pm 22.1$
4	15	$78.7^{bcd} \pm 14.1$ (144)	$50.2^{ab} \pm 20.8$	$14.6^{ab} \pm 14.4$
5	13	$80.0^{cd} \pm 12.9$ (128)	$74.2^{d} \pm 16.3$	$22.7^{bcd} \pm 18.4$
6	15	$84.9^{d} \pm 11.8$ (151)	$60.5^{bc} \pm 17.2$	$26.5^{d} \pm 12.8$
7	11	$83.6^{d} \pm 21.1$ (107)	$80.5^{d} \pm 31.7$	$27.2^{cd} \pm 19.0$
8	14	$84.9^{d} \pm 15.2$ (144)	$74.7^{d} \pm 17.8$	$30.1^{d} \pm 15.7$

[1] no. = Number of oocytes that were fertilized and cleaved.

abcd Within a column, means without a common superscript letter differ ($P \leq 0.10$).

Table 3. Percentage of oocytes that were fertilized and cleaved and percentage of cleaved embryos from Experiment 1 that developed to morula or greater or blastocysts by main effect.

Treatment	No. Droplets	Percentage cleaved (no.)[1]	Percentage of cleaved embryos that developed to	
			$\geq$ Morula	Blastocyst
		---------------------------------- Mean $\pm$ sd ----------------------------------		
CON P4[2]	10	74.6 $\pm$ 12.1 (95)	57.0 $\pm$ 32.7	27.7 $\pm$ 23.6
LO P4[3]	11	70.0 $\pm$ 16.7 (110)	56.2 $\pm$ 23.2	15.5 $\pm$ 22.4
HI P4[4]	12	78.5 $\pm$ 14.1 (106)	58.5 $\pm$ 22.5	15.1 $\pm$ 15.4
CON COCULT[5]	16	73.3 $\pm$ 11.9 (151)	68.2[b] $\pm$ 17.5	31.4[b] $\pm$ 20.2
COCULT[6]	17	75.6 $\pm$ 16.9 (160)	47.0[a] $\pm$ 27.7	7.5[a] $\pm$ 13.5

[1] no. = Number of oocytes that cleaved.

[2] CON P4 included embryos exposed to no progesterone (CON).

[3] LO P4 included embryos exposed to 1 ng/mL progesterone (LO).

[4] HI P4 included embryos exposed to 100 ng/mL progesterone (HI).

[5] CON COCULT included embryos exposed to no co-culture with bovine oviductal epithelial cells (CON COCULT).

[6] COCULT included embryos exposed to co-culture with bovine oviductal epithelial cells (COCULT).

[ab] Within a column for each factor, means without a common superscript letter differ ($P \leq 0.05$).

Table 4. Percentage of cleaved embryos in Experiment 1 that developed to morula or greater, or blastocysts by co-culture and progesterone treatment.

Treatment Co-culture/Progesterone	No. Droplets	Percentage of cleaved embryos that developed to	
		$\geq$ Morula	Blastocyst
		------------------ Mean $\pm$ sd ------------------	
CON/CON[1]	5	75.7 ± 8.3^{c}	45.2 ± 13.5^{d}
COCULT/CON[2]	5	38.2 ± 38.2^{a}	10.2 ± 17.3^{ab}
CON/LO[3]	5	66.3 ± 15.4^{bc}	31.3 ± 25.4^{cd}
COCULT/LO[4]	6	47.9 ± 26.6^{ab}	2.4 ± 5.8^{a}
CON/HI[5]	6	63.7 ± 24.3^{b}	19.9 ± 14.5^{bc}
COCULT/HI[6]	6	53.4 ± 21.5^{ab}	10.3 ± 16.1^{ab}

[1] CON/CON included embryos not exposed to co-culture (CON) with bovine oviductal epithelial cells (BOEC) or to progesterone (CON).

[2] COCULT/CON included embryos exposed to BOEC (COCULT) but not exposed to progesterone (CON).

[3] CON/LO included embryos not exposed to BOEC (CON) but exposed to 1 ng/mL of progesterone (LO).

[4] COCULT/LO included embryos exposed to BOEC (COCULT) and exposed to 1 ng/mL of progesterone (LO).

[5] CON/HI included embryos not exposed to BOEC (CON) but exposed to 100 ng/mL of progesterone (HI).

[6] COCULT/HI included embryos exposed to BOEC (COCULT) and exposed to 100 ng/mL of progesterone (HI).

[abcd] Within a column, means without a common superscript letter differ ($P \leq 0.10$).

Table 5. Percentage of oocytes that were fertilized and cleaved and percentage of cleaved embryos in Experiment 2 that developed to morula or greater, or blastocysts by progesterone treatment.

| Treatment | No. Droplets | Percentage cleaved (no.)[1] | Percentage of cleaved embryos that developed to | |
			≥ Morula	Blastocyst
			------------------------------- Mean ± sd -------------------------------	
CON P4[2]	32	82.1 ± 13.1 (267)	62.4 ± 24.4	26.2 ± 20.2[b]
LO P4[3]	34	76.5 ± 15.4 (281)	62.2 ± 20.0	22.4 ± 18.6[ab]
HI P4[4]	35	80.9 ± 16.4 (277)	66.8 ± 27.9	18.8 ± 14.7[a]

[1] no. = Number of oocytes that were fertilized and cleaved.

[2] CON P4 included embryos exposed to no progesterone (CON).

[3] LO P4 included embryos exposed to 1 ng/mL progesterone (LO).

[4] HI P4 included embryos exposed to 100 ng/mL progesterone (HI).

[ab] Within a column, means without a common superscript letter differ ($P \leq 0.10$).

CHAPTER 5

Effects of supplemental progesterone during two phases of culture on the development, metabolism, and numbers of cells of bovine embryos produced by in vitro maturation, fertilization, and culture

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ABSTRACT

Supplemental progesterone may influence embryonic survival in cattle. An increased understanding of the factors affecting viability of embryos is imperative to determine how to influence embryonic survival. The objectives of this experiment were to determine whether the supplementation of progesterone during either the first or second phases of culture of in vitro-produced embryos alters embryo development, embryonic cell metabolism, or cell number. Oocytes were purchased, matured, and fertilized in vitro; treatments were applied during the culture phase of in vitro production. Five treatments including a control were randomly assigned to a dish and each dish contained 6 droplets with each droplet assigned the same treatments. Treatments were: 1) control (**CON**; n = 23); 2) 1 ng/mL progesterone in culture-1 medium (**LONO**; n = 21); 3) 100 ng/mL progesterone in culture-1 medium (**HINO**; n = 24); 4) 1 ng/mL progesterone in culture-2 medium (**NOLO**; n = 23); and, 5) 100 ng/mL progesterone in culture-2 medium (**NOHI**; n = 23). Culture-1 consisted of the first 72 h of culture, and culture-2 consisted of h 73 to 168. The percentage of oocytes that cleaved, the percentage of cleaved embryos that developed to the morula stage or greater, the blastocyst stage or greater, or the hatched blastocyst stage were similar among treatments. Quantities of glucose metabolized by blastocyst, per hour were similar, but when metabolism data was normalized for numbers of cells in each blastocyst, differences were detected ($P = 0.03$; 61.3 ± 7.7 fmols, 59.7 ± 6.7 fmols, 66.4 ± 8.0 fmols, 84.8 ± 7.6 fmols, 52.1 ± 8.3 fmols of glucose in CON, LONO, HINO, NOLO, and NOHI, respectively). Embryos receiving LO progesterone had greater ($P = 0.085$) metabolism of glucose compared to embryos receiving HI progesterone. Quantities of

pyruvate oxidized by blastocyst, per hour and quantity per cell were similar among treatments. Embryos exposed to progesterone during Culture-1 tended to have increased oxidation of pyruvate per embryo ($P = 0.089$) and per cell ($P = 0.091$) compared to embryos exposed to progesterone in Culture-2. The number of cells in each blastocyst were similar among treatments; however, blastocysts in the CON group had 100.8 ± 5.55 cells compared to 89.2 ± 2.74 ($P = 0.039$) cells per blastocyst in P4 treated groups. In conclusion, supplementation of progesterone to in vitro produced embryos failed to directly enhance cleavage rates, development to blastocysts, and metabolic characteristics.

Key Words: Progesterone, Embryo Development, Embryo metabolism, Bovine

INTRODUCTION

Embryonic loss contributes to substantial economic effects in both dairy and beef cattle operations. Fertilization rates average approximately 75 to 80% (Dalton et al., 2001; DeJarnette et al., 1992; Sartori et al., 2002); however, by d 5 to 6 after AI, only 65% of the fertilized oocytes were considered viable (Dalton et al., 2001; DeJarnette et al., 1992; Sartori et al., 2002).

Supplemental progesterone during the first 4 days after AI has increased morphological development and biosynthetic activity of day 14 conceptuses (Garrett et al., 1988). In addition, supplemental progesterone during the first week of pregnancy increased pregnancy rates (Mann and Lamming, 1999) in some studies. In dairy cows that received an embryo, the average interval after ovulation until progesterone achieved a 1 ng/ml concentration was 4.1 days for cows with detectable INF-τ and 5.6

days for those with no detectable INF-τ on d 16 (Mann and Lamming, 2001). Timing of supplemental progesterone is important, in part, because it is thought to alter the secretory activity of the endometrium, thus influencing embryonic growth (Garrett et al., 1988; Geisert et al., 1992). The reproductive tract environment is critically important to embryonic viability. The oviduct consists of regions which have different characteristics that are controlled, in part, by progesterone (Brenner and Masler, 1988; Verhage et al., 1990; Murray, 1992; Buhi et al., 1997).

In addition, progesterone appears to have a direct effect on oocyte maturation or competence. In monkeys, supplemental progesterone during the maturation phase of in vitro-produced embryos improved the developmental competence of oocytes (Zheng et al., 2003) indicating that progesterone acts directly on oocytes or surrounding cumulus cells. Therefore, supplementation of progesterone during early stages of embryo development may enhance the ability of the embryo to survive to maternal recognition of pregnancy and needs further investigation.

Traditionally, morphology and cell number have been used to determine embryo viability. Morphology is the best way to visually detect differences in development among embryos and increased cell numbers has been associated with increased competence. Measures of metabolic activity are another indicator of embryo viability. Glucose utilization increases as an embryo develops (Javed and Wright, 1991; Rieger et al., 1992; Thompson et al., 1996; Khurana and Niemann, 2000); and glycolysis becomes the predominant energy pathway used (Thompson, 1996) even though the oxidation of pyruvate, which is the predominant pathway used in the oocyte and embryo during its earliest stages of development, is still used. Embryos at a further stage of

development will utilize more glucose than embryos less developed, creating a measurable index of development. However, embryos that are stressed or that show characteristics associated with decreased viability have uncharacteristically high rates of glycolysis (Javed and Wright, 1991; Rieger et al., 1995; Lane and Gardner, 1996) requiring careful interpretation of results. The in vitro procedure causes sufficient stress to increase glycolysis in in vitro produced embryos as compared to in vivo embryo counterparts (Khurana and Niemann, 2000).

An increased understanding of the factors affecting viability of embryos is imperative to determine how to influence embryonic survival. Therefore, the objectives of this experiment were to determine whether the supplementation of progesterone during either the first or second phases of culture of in vitro-produced embryos alters embryo development, total cell number, or embryonic cell metabolism.

MATERIALS AND METHODS

Substrates, chemicals, and media preparation

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless specified otherwise. All-in-one synthetic oviductal fluid (SOF) medium for bovine (Gandi et al., 2000) was used for the in vitro fertilization and culture of oocytes and embryos. Stock solutions were prepared with 18.2 MΩ water and stored at 4°C for either 1 wk (bicarbonate, pyruvate, glutamine/alanine), 1 mo (calcium, glucose, EDTA, taurine) or 3 mo (HEPES, basil salt solution). Stock solutions of penicillin/streptomycin/ amphotericin (PSA; Invitrogen Corp., Grand Island, NY), amino acids (minimal essential medium concentrations; MP Biomedicals, LLC, Solon,

OH) and vitamins (minimal essential medium concentrations; MP Biomedicals, LLC) were stored according to manufacturers' suggestions and were used directly for medium preparation. Amounts of stock solutions as well as BSA in working solutions are described in Table 1. Working solutions were prepared for each replicate, filtered (0.22 μm; MillexGV; Millipore, Billerica, MA) and equilibrated in the appropriate gas atmosphere.

Oocytes and in vitro maturation

Bovine oocytes were purchased (Ovitra Biotechnology, Amarillo, TX) where they were aspirated from abattoir ovaries. Oocytes with at least three layers of compact cumulus cells and even cytoplasm were placed in maturation media containing 10% FBS. Fifty oocytes in 10 mL maturation media were placed in each vial and vials were flushed with 5% CO_2 in air before being capped. Oocytes were shipped overnight at 39°C in temperature-controlled incubators.

In vitro fertilization

Oocytes were allowed to mature for 22 to 24 h and were then washed two times in SOF-HEPES buffered medium and once in SOF fertilization medium (Table 1). One thousand one hundred thirty five (approximately 284 oocytes per replicate; four replicates over time) oocytes were then placed in groups of 10, in 44 μL droplets of SOF fertilization medium under 10 mL washed mineral oil in 10×60 mm plastic dishes (Falcon 1007; Becton Dickinson Labware, Franklin Lakes, NJ). Mineral oil was washed by adding 1 part D-PBS (Invitrogen, Corp.) without calcium to 3 parts mineral oil and then shaken vigorously and allowed to separate.

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Frozen semen from the same bull for each replicate was thawed for 1 min in a 37°C water bath. Sperm were capacitated by centrifugation through a 90% and 45% Percoll (GE Healthcare Bio-sciences Corp., Piscataway, NJ) gradient for 20 min at 700 $\times$ g and washed by centrifugation in sperm wash medium (Table 1) at 700 $\times$ g twice for 5 min to remove any remaining semen extender. Insemination was carried out by adding 2 µL of the spermatozoa and fertilization media mixture, to reach a final concentration of 0.75×10^6 spermatozoa/mL in the droplet, 2 µL of 4 µg/mL heparin, and 2 µL PHE (Ball et al., 1983; Parrish et al., 1985, 1988: penicillamine, 20 µmol/l; hypotaurine, 10 µmol/l; epinephrine, 1 µmol/l). The final droplet volume was 50 µL. Oocytes were co-incubated with spermatozoa for 18 h at 38.7°C with 6.3% CO_2 in atmosphere.

In vitro culture

After incubation with sperm, all presumptive zygotes were placed into 0.5 mL SOF-HEPES with 0.11 mg/mL hyaluronidase and vortexed for 3 min to remove cumulus cells, followed by an additional wash in SOF-HEPES and a wash in culture-1 medium. No selection was performed after fertilization; all presumptive zygotes were moved in groups of 10 into 50 µL droplets of culture-1 medium under 10 mL washed mineral oil. Embryo cultures were performed at 38.7°C with 5% CO_2: 10% O_2: 85% N_2 in atmosphere. After 72 h of culture, all embryos were removed from culture drops, and while being maintained in their experimental group of 10, examined for cleavage, washed 2 times in SOF-HEPES and once in culture-2 medium, and placed into culture-2 medium drops in the same conditions as culture-1 for an additional 96 h. After embryos were in culture for a total of 7 d, further measurements were taken.

Treatments

Five treatments including a control were randomly assigned to a dish and were applied during the in vitro culture phase. Each dish contained 6 droplets with each droplet assigned the same treatments. Treatments were: 1) control (**CON**; n = 23); 2) 1 ng/mL progesterone in culture-1 medium (**LONO**; n = 21); 3) 100 ng/mL progesterone in culture-1 medium (**HINO**; n = 24); 4) 1 ng/mL progesterone in culture-2 medium (**NOLO**; n = 23); and, 5) 100 ng/mL progesterone in culture-2 medium (**NOHI**; n = 23). Treatments were added to the droplets (containing approximately 10 embryos in each; n = 114); therefore, experimental unit was a droplet for development data. Culture-1 consisted of the first 72 h of culture and culture-2 consisted of hours 73 to 168 of culture.

In addition to the droplets of culture medium containing the treatment concentration of progesterone, the mineral oil overlay also was mixed with a matching concentration of progesterone to eliminate the progesterone exiting the droplets to equilibriate with the oil.

Determination of embryo development

Embryos were visually assessed for cleavage on d 4 of culture (when moved into culture-2 medium). After embryos had been in culture for 7 d, oocytes or embryos were visually assessed and assigned a developmental stage according to standards set forth by the International Embryo Transfer Society (Savoy, IL). For analyses, a category was assigned to each oocyte/embryo dependent on stage of development: 1) < morula (1 to < 32 cells), 2) morula 3) early or expanded blastocyst, 4) hatched blastocyst.

Determination of metabolic activity

All embryos placed into the early or expanded blastocyst or hatched blastocyst category (n = 194) underwent an analysis of metabolic activity to assess health and viability of the embryo. To determine the metabolic activity of embryos a modification of the hanging drop technique was used to measure metabolic metabolism of D-[5-^{3}H] glucose (specific activity 19.63 Ci/mmol; PerkinElmer LAS, Inc., Boston, MA) and [2-^{14}C] pyruvic acid, sodium salt (specific activity 15 mCi/mmol; American Radiolabeled Chemicals, Inc., St. Louis, MO).

Appropriate quantities of labeled substrates were dried under nitrogen gas and taken up in metabolism medium (SOF base with 0.099 mmol/L unlabeled sodium pyruvate, 1.5 mmol/L unlabeled glucose and 1 x 10^{-4}/mL polyvinyl alcohol). Metabolism medium was used within 24 h of preparation, and was pre-equilibrated at 38.7°C in 5% CO_2: 10% O_2:85% N_2 at least 8 hours before use. After assessment for development, all blastocysts were removed from culture-2 droplets and placed into a 50-µL drop of pre-equilibrated metabolism medium under washed mineral oil, with individual treatments in separate droplets. Each embryo was taken up in 2 µL of metabolism medium and placed in the cap of a 1.5-mL microcentrifuge tube together with 2 µL of pre-equilibrated metabolism medium containing the radiolabeled substrates. The caps were lightly placed over warm tubes containing 1.5 mL bicarbonate solution (25.07 mmol/L) and the airspace was flushed with a gas mixture (5% CO_2: 10% O_2:85% N_2) for 5 s and then caps were tightened over tubes and incubated at 38.7°C for 3 h. Four sham tubes and four total-count tubes were prepared in an identical manner but contained no embryo were prepared in duplicate, one set at

the beginning and one set at the end. Total-count tubes were shaken to mix the

metabolism medium with the bicarbonate solution before and after incubation.

At the end of the 3 h incubation period, caps were removed and 1 mL of

bicarbonate solution was removed from each tube and placed into a glass scintillation

vial containing 200 µL of 0.1 M NaOH solution to convert the dissolved CO_2 and

bicarbonate into carbonate. Each sample tube was examined to verify the presence of 1

embryo, and the embryos were recovered into individual numbered wells of 24-well

plates for counting of cell numbers. Scintillation vials were held at 4°C for 18 to 24 h,

and then 10 mL scintillation fluid (Ecolite (+)TM; MPBiomedicals, LLC) was added to

each vial. The vials were then held at room temperature in the dark for 12 h and then

counted for 4 min in a liquid scintillation counter programmed for dual-label counting.

The amount of glucose and pyruvate metabolized by each embryo was calculated as

described by Tiffin et al., (1991).

Determination of cell numbers

After embryos were incubated for determination of metabolism, they were

placed individually into 24-well plates containing 500 µL of 100% ethanol with 25

µg/mL bisbenzimide (Hoechst 33342) and were stored at 4°C in the dark for 12 h.

Embryos were then individually taken up with a minimal amount of the stain and

approximately 2 µL glycerol and placed on glass slides and covered with a coverslip.

Embryos were viewed and nuclei were counted using fluorescence microscopy.

Statistical analyses

Hypothesis

H_0: $\tau_1 = \tau_2 = \tau_3 = \tau_4 = \tau_5$

$$H_1: \tau_1 \neq \tau_2 \neq \tau_3 \neq \tau_4 \neq \tau_4$$

Data was collected in four replicates over time. A droplet was considered the experimental unit in developmental data and embryo was considered the experimental unit for metabolism and cell number data. Statistical significance of results was obtained using an alpha error of 0.05, a beta error of 0.20 and a Power of 80%. Values were considered statistically different when $P \leq 0.05$ and a statistical trend was considered when $0.05 \leq P \geq 0.10$.

Procedure GENMOD of SAS (SAS Inst., Inc., Cary, NC) using a probit model with a binary distribution was used to analyze data, including: the percentage of oocytes that cleaved and the percentage of cleaved embryos reaching the morula stage or further, the blastocyst stage or further, and the hatched blastocyst stage of development. Means were separated by using the MEANS procedure of SAS when a protected F-test ($P \leq 0.05$) was detected. Standard deviations are presented. Models included replicate, treatment and all two-way interactions.

Orthogonal contrasts compared droplets in the CON group with all treatments with progesterone (**P4**; NOLO, NOHI, LONO, and HINO), droplets receiving **LO** (NOLO and LONO) compared to those receiving **HI** (NOHI and HINO) concentrations of progesterone, and droplets receiving progesterone during culture-1 (**Culture-1**; LONO and HINO) compared to those receiving progesterone during culture-2 (**Culture-2**; NOLO and NOHI).

Procedure MIXED of SAS was used to analyze data, including: metabolism of glucose, oxidation of pyruvate, and number of cells in each embryo. To normalize metabolism data on a per cell basis, quantities of glucose and pyruvate metabolized

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were divided by the number of cells counted in each embryo. Means were separated by using the least significant difference in procedure MIXED when a protected F-test ($P \leq$ 0.05) was detected by ANOVA. Standard errors are presented. Models included replicate as a random variable and treatment as a fixed variable.

RESULTS AND DISCUSSION

Replicate differences

Oocytes and embryos are sensitive to temperature fluctuations (Schumacher and Fischer, 1988; Rivera and Hansen, 2001) and pH changes (Ocon and Hansen, 2003) and, therefore, researchers are limited in the number of oocytes that can successfully be handled at one time. To combat this, experiments are conducted in replicate. In addition, when acquiring oocytes from unknown cows in an abattoir, oocyte quality is beyond laboratory control and replicates balance the factors that could be involved such as breed, age, disease and nutritional status of cows, heat stress the oocyte may have been exposed to, and handling of ovaries after evisceration. Because of these factors, replicates were a significant source of variation in this experiment, as is common when researching with in vitro produced embryos.

Four replicates of in vitro produced embryos were included in this experiment. Each replicate consisted of approximately 284 oocytes (range 276 to 300) and approximately 28 droplets (range 28 to 30). Each droplet contained approximately 10 oocytes or embryos (range 8 to 11). Replicates were a significant source of variation on cleavage rate and development of cleaved embryos to the morula stage or greater and

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the blastocyst stage or greater ($P < 0.05$; Table 2). Replicate tended to affect embryos reaching the hatched blastocyst stage ($P < 0.10$; Table 2).

The percentage of oocytes that cleaved, per replicate, ranged from $49.2 \pm 11.8\%$ to $81.9 \pm 13.3\%$. The percentage of cleaved embryos that reached the morula stage or greater ranged from $20.4 \pm 17.0\%$ to $47.4 \pm 19.5\%$, and that reached the blastocyst stage or greater ranged from $18.6 \pm 15.4\%$ to $29.9 \pm 17.1\%$, and that reached the hatched blastocyst stage ranged from $4.2 \pm 6.3\%$ to $9.8 \pm 13.5\%$. Similar differences among replicates have been recorded in our laboratory in other experiments even though extensive attempts were made to control vaiables.

Incidence of oocyte cleavage

The percentage of oocytes that cleaved was not affected by progesterone treatment ($P > 0.10$; Table 3). Among treatments, $67.8 \pm 18.8\%$, $74.1 \pm 17.4\%$, $70.1 \pm 19.3\%$, $72.7 \pm 19.5\%$, and $69.6 \pm 19.1\%$ of oocytes cleaved for CON (n = 23), LONO (n = 21), HINO (n = 24), NOLO (n = 23), and NOHI (n = 23), respectively.

Cleavage rates were expected to be similar because treatments were not administered until presumptive zygotes were moved into the culture-1 phase of in vitro production. At this point, many oocytes were nearing, or even beginning, the first division of mitosis.

Embryo development

The percentage of cleaved embryos that developed to the morula stage or greater, the blastocyst stage, or the hatched blastocyst stage were not affected by progesterone treatment ($P > 0.10$; Table 3). Among treatments, $30.5 \pm 22.2\%$, $41.8 \pm 15.5\%$, $30.1 \pm 22.6\%$, $37.0 \pm 24.2\%$, and $38.1 \pm 24.9\%$ of embryos developed to the

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morula stage or greater for CON, LONO, HINO, NOLO, and NOHI, respectively. Among treatments, $26.8 \pm 20.5\%$, $33.0 \pm 16.7\%$, $20.9 \pm 17.6\%$, $26.6 \pm 20.1\%$, and $25.2 \pm 13.8\%$ of embryos developed to the blastocyst stage or greater for CON, LONO, HINO, NOLO, and NOHI, respectively. Among treatments, $7.2 \pm 9.0\%$, $8.1 \pm 9.4\%$, $4.0 \pm 6.7\%$, $8.7 \pm 13.5\%$, and $5.9 \pm 10.2\%$ of embryos developed to the hatched blastocyst stage for CON, LONO, HINO, NOLO, and NOHI, respectively.

All contrasts performed between CON versus P4, between LO versus HI concentrations of progesterone, and between treatment of progesterone during Culture-1 versus Culture-2 indicated similar development (Table 4). It is unknown whether embryos in the early stages of development have progesterone receptors on the surface of the zona pellucida. The lack of receptors may indicate that progesterone does not act directly on the embryo to affect embryonic development; rather, in in vivo situations, the progesterone acts on endometrial tissue, which does express progesterone receptors (McNeill et al., 2006b), to promote embryonic development (Garrett et al., 1988).

Metabolic activity

Metabolism characteristics of oocytes and embryos have been used to elucidate development and determine viability. There is a 150-fold increase in the incorporation of glucose between immature oocytes, which do not use glucose as an energy substrate, to the blastocyst stage (Javed and Wright, 1991; Rieger et al., 1992; Thompson, 1996; Khurana and Niemann, 2000). The first marked increase in glycolysis occurs between the 8- and 16-cell stages when the embryonic genome is activated (Telford et al., 1990). While an increase in glucose uptake has been associated with advanced development, extremely high rates of glycolysis have been associated with reduced embryo viability

in mice (Lane and Gardner, 1996). Embryos that have characteristics likely to cause

reduced viability, such as a slow rate of development, have higher rates of glycolysis

(Rieger et al., 1995). Glycolysis is a less efficient method to obtain energy than using

the pyruvate that is present in the media, so the unusually high use of glycolysis in

embryos with reduced viability could be the result of metabolic stress (Javed and

Wright, 1991) that accompanies in vitro production. Anaerobic fermentation, which

produces lactate, also is increased in embryos produced in vitro (Khurana and Niemann,

2000), supporting the notion that these embryos are under metabolic stress.

Pyruvate, which is the predominant energy substrate used by oocytes and

embryos in the early stages of development, also is oxidized at increasing rates as

bovine embryos develop into blastocysts (Rieger and Guay, 1988; Leese, 1992; Donnay

and Leese, 1999; Khurana and Niemann, 2000). While there are no strict standards set

for the ideal rate of metabolism in bovine embryos, abnormally high or abnormally low

rates of metabolism of glucose and pyruvate in treated embryos compared to those in

the control group could indicate embryo incompetence.

There were no significant differences in total amounts of glucose metabolized

per blastocyst (Table 5). Among treatments, 17.9 ± 2.4 pmols, 15.5 ± 2.2 pmols, $19.4 \pm$

2.5 pmols, 20.7 ± 2.4 pmols, and 14.0 ± 2.5 pmols of glucose was metabolized per

blastocyst in CON, LONO, HINO, NOLO, and NOHI treatments, respectively. There

were no differences in quantities of glucose metabolized per blastocyst, per hour (Table

5). Among treatments, 6.0 ± 0.8 pmols, 5.2 ± 0.7 pmols, 6.5 ± 0.8 pmols, 6.9 ± 0.9, and

4.7 ± 0.8 pmols of glucose was metabolized per blastocyst in each hour in CON,

LONO, HINO, NOLO, and NOHI treatments, respectively. When metabolism data was

normalized for number of cells in each blastocyst, significant differences were detected ($P = 0.03$; Table 5). Among treatments, 61.3 ± 7.7 fmols, 59.7 ± 6.7 fmols, 66.4 ± 8.0 fmols, 84.8 ± 7.6 fmols, 52.1 ± 8.3 fmols of glucose was metabolized per cell in each blastocyst, per hour in CON, LONO, HINO, NOLO, and NOHI treatments, respectively.

Orthogonal contrasts indicated the quantities of glucose metabolized per cell in each blastocyst for LO (n = 89) versus HI (n = 67) progesterone treatments tended to be different ($P = 0.085$; Table 6). Embryos in the LO group metabolized 72.33 ± 5.02 fmol/cell while 59.09 ± 5.74 fmol/cell was metabolized in the HI group.

Total quantities of pyruvate oxidized per blastocyst were similar among treatments (Table 5). Among treatments, 12.8 ± 4.6 pmols, 13.4 ± 4.2 pmols, 22.3 ± 4.6 pmols, 13.8 ± 4.5 pmols, and 9.2 ± 4.7 pmols of pyruvate was oxidized per blastocyst in CON, LONO, HINO, NOLO, and NOHI treatments, respectively. Similarly, quantities of pyruvate oxidized per blastocyst, per hour were similar (Table 5). Among treatments, 4.3 ± 1.5 pmols, 4.5 ± 1.4 pmols, 7.4 ± 1.5 pmols, 4.6 ± 1.5, and 3.1 ± 1.6 pmols of pyruvate was oxidized per blastocyst in each hour in CON, LONO, HINO, NOLO, and NOHI treatments, respectively. There were no differences when metabolism data was normalized for the number of cells in each blastocyst (Table 5). Among treatments, 38.8 ± 16.3 fmols, 56.0 ± 15.1 fmols, 79.1 ± 16.5 fmols, 56.5 ± 16.0 fmols, 38.1 ± 17.0 fmols of glucose was metabolized per cell in each blastocyst, per hour in CON, LONO, HINO, NOLO, and NOHI treatments, respectively.

Orthogonal contrasts indicated the total quantities of pyruvate oxidized per blastocyst for Culture-1 (n = 88) compared to Culture-2 (n = 77) culture with

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progesterone tended to be different ($P = 0.089$; Table 6). Embryos exposed to

progesterone in Culture-1 oxidized 18.90 ± 2.75 pmols/blastocyst whereas 11.47 ± 2.91

pmols/blastocyst was oxidized in the Culture-2 group. Pyruvate oxidized per blastocyst

per hour tended to be different ($P = 0.089$; Table 6) for Culture-1 and Culture-2 with

6.30 ± 0.92 pmols and 3.82 ± 0.97 pmols oxidized each h per blastocyst, respectively.

Pyruvate oxidized by each cell in a blastocyst per h also tended to be different ($P =$

0.091) with 72.49 ± 9.02 fmols in the Culture-1 group and 48.66 ± 9.76 fmols in the

Culture-2 group (Table 6). Overall, embryos exposed to progesterone during Culture-1

had increased oxidation of pyruvate as compared to embryos exposed to progesterone in

Culture-2. Even though oxidation of pyruvate was greater in the Culture-1 group, we

do not believe this is abnormally high due to metabolic stress because the quantity of

glucose metabolized in this same group of embryos was not elevated compared to the

CON or the Culture-2 groups. Embryos in the Culture-1 group may not be meeting

their energy requirements using the glycolysis pathway and are relying more on

pyruvate as compared to embryos in Culture-2 or could indicate the beginnings of

metabolic stress and a change in glucose metabolism had not occurred yet (Khurana and

Niemann, 2000).

Number of cells per blastocyst

The number of cells per blastocysts were similar ($P = 0.16$) among treatment

groups. Among treatments (mean $\pm$ SEM), 102 ± 6 cells, 90 ± 5 cells, 96 ± 6 cells, $87 \pm$

6 cells, and 83 ± 7 cells in the CON, LONO, HINO, NOLO, and NOHI treatments,

respectively. Orthogonal contrasts indicated a difference in the number of cells per

blastocyst when comparing the CON group with P4 ($P = 0.039$). Blastocysts in the

CON group had 101.8 ± 5.55 cells per blastocyst and in the P4 group there were 89.2 ± 2.74 cells per blastocyst (mean ± SEM). These results may indicate that the addition of progesterone in the culture of in vitro produced embryos decreases development at a slow pace; a pace that is not sufficient to indicate differences at d 7 post-fertilization, when using the other measurements employed in this study.

In conclusion, treatment of in vitro produced bovine embryos with either 1 ng/mL or 100 ng/mL of progesterone during culture-1 or culture-2 did not impact the cleavage of presumptive zygotes nor the development of embryos to either the morula, the blastocyst, or the hatched blastocyst stages of development. The administration of 1 ng/mL of progesterone during culture-2 increased the quantity of glucose metabolized by embryos per cell. Furthermore, embryos exposed to 1 ng/mL progesterone metabolized greater quantities of glucose per cell than embryos exposed to 100 ng/mL. Concentration of progesterone did not impact pyruvate oxidation. Embryos exposed to progesterone during culture-1 oxidized greater quantities of pyruvate than embryos exposed to progesterone in culture-2. Treatment with progesterone at either concentration and at either interval decreased the numbers of cells in each blastocyst. These results indicate that administration of progesterone at 1 ng/mL or 100 ng/mL during the culture of in vitro produced embryos may have slight affects on embryo characteristics; however, there appears to be no advantage of supplementing progesterone to in vitro produced embryos to enhance cleavage rates, development to blastocysts, and metabolic characteristics.

LITERATURE CITED

Ball, G. D., M. L. Leibfried, R. W. Lenz, R. L. Ax, B. D. Bavister, and N. L. First. 1983. Factors affecting successful in vitro fertilization of bovine follicular oocytes. Biol. Reprod. 28:717-725.

Brenner, R. M. and I. A. Maslar. 1988. The primate oviduct and endometrium. In: Physiology of Reproduction. K. Knobil, J.D. Neill, L.L. Ewing, C.L. Markert, G.S. Greenwald, and D.W. Pfaff, eds. Raven Press, Ltd., New York, pp. 303-329.

Buhi, W. C., I. M. Alvarez, and A. J. Kouba. 1997. Oviductal regulation of fertilization and early embryonic development. J. Reprod. Fertil. 52:285-300.

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 number in artificially inseminated cattle. J. Anim. Sci. 70:484-491.

Donnay, I. and H. J. Leese. 1999. Embryo metabolism during the expansion of the bovine blastocyst. Mol. Reprod. Dev. 53:171-178.

Gandi, A. P., M. Lane, D. K. Gardner, and R. L. Krisher. 2000. A single medium supports development of bovine embryos throughout maturation, fertilization and culture. Hum. Reprod. 15:395-401.

Garrett, J. E., R. D. Geisert, M. T. Zavy, and G. L. Morgan. 1988. Evidence for maternal regulation of early conceptus growth and development in beef cattle. J. Reprod. Fertil. 84:437-446.

Geisert, R. D., G. L. Morgan, E. C. Short, and M. T. Zavy. 1992. Endocrine events associated with endometrial function and conceptus development in cattle. Reprod. Fertil. Dev. 4:301-305.

Javed, M.H. and R. W. Wright Jr. 1991. Determination of pentose phosphate and Embden-Meyerhof pathway activities in bovine embryos. Theriogenology 35:1029-1037.

Khurana, N. K. and H. Niemann. 2000. Energy metabolism in preimplantation bovine embryos derived in vitro or in vivo. Biol. Reprod. 62:847-856.

Lane, M. and D. K. Gardner. 1996. Prospective selection of viable mouse embryos prior to transfer using metabolic rate. Hum. Reprod. 11:1975-1978.

Leese, H. J. 1992. Metabolism of the preimplantation mammalian embryo. In: Milligan SR (ed.), Oxford Reviews of Reproductive Biology. Oxford: Oxford University Press 13:35-72.

Mann, G. R. and G. E. Lamming. 1999. The influence of progesterone during early pregnancy in cattle. Reprod. Domest. 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. Reproduction 121:175-180.

McNeill, R. E., J. M. Sreenan, M. G. Diskin, M. T. Cairns, R. Fitzpatrick, T. J. Smith, and D. G. Morris. 2006b. Effect of systemic progesterone concentration on the expression of progesterone-responsive genes in the bovine endometrium during the early luteal phase. Reprod. Fertl. Devl. 18:573-583.

Murray, M. K. 1992. The biosynthesis, immunocytochemical localization of an estrogen-dependent glycoprotein and associated morphological alterations in the sheep ampulla oviduct. Biol. Reprod. 47:889-902.

Ocon, O. M. and P. J. Hansen. 2003. Disruption of bovine oocytes and preimplatation embryos by urea and acidic pH. J. Dairy Sci. 86:1194-1200.

Parrish, J. J., J. L. Susko-Parrish, and N. L. First. 1985. Effect of heparin and chondroitin sulfate on the acrosome reaction and fertility of bovine sperm *in vitro*. Theriogenology 24:537-549.

Parrish, J. J., J. Susko-Parrish, M. A. Winer, and N. L. First. 1988. Capacitation of bovine sperm by heparin. Biol. Reprod. 38:1171-1180.

Rieger, D., B. Grisart, E. Semple, A. Van Langendonckt, K. J. Betteridge, and F. Dessy. 1995. Comparison of the effects of oviductal cell co-culture and oviductal cell-conditioned medium on the development and metabolic activity of cattle embryos. J. Reprod. Fertil. 105:91-98.

Rieger, D. and P. Guay. 1988. Measurements of the metabolism of energy substrates in individual bovine blastocysts. J. Reprod. Fertil. 83:585-591.

Rieger, D., N. M. Loskutoff, and K. J. Betteridge. 1992. Developmentally related changes in the uptake and metabolism of glucose, glutamine and pyruvate by cattle embryos produced in vitro. Reprod. Fertil. Dev. 4:547-557.

Rivera, R. M. and P. J. Hansen. 2001. Development of cultured bovine embryos after exposure to high temperatures in the physiological range. Reproduction 121:107-115.

Sartori, R., R. Sartor-Bergfelt, S. A. Mertens, J. N. Guenther, J. J. Parrish, 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.

Schumacher, A. and B. Fischer. 1988. Influence of visible light and room temperature on cell proliferation in preimplantation rabbit embryos. J. Reprod. Fert. 84:197-204.

Telford, N. A., A. J. Watson, and G. A. Schultz. 1990. Transition from maternal to embryonic control in early mammalian development: a comparison of several species. Mol. Reprod. Dev. 26:90-100.

Thompson, J. G. 1996. Defining the requirements for bovine embryo culture. Theriogenology 45:27-40.

Thompson, J. G., R. G. Partridge, F. D. Houghton, C. I. Cox, and H. J. Leese. 1996. Oxygen uptake and carbohydrate metabolism by in vitro derived bovine embryos. J. Reprod. Fertil. 106:299-306.

Tiffin, G., D. Rieger, K. J. Betteridge, B. R. Yadav, and W. A. King. 1991. Glucose and glutamine metabolism in pre-attachment cattle embryos in relation to sex and stage of development. J. Reprod. Fertil. 93:125-132.

Verhage, H. G., P. A. Mavrogianis, M. L. Boice, W. Li, and A. T. Fazleabas. 1990. Oviductal epithelium of the baboon: Hormonal control and the immuno-gold localization of oviduct-specific glycoproteins. Am. J. Anat. 187:81-90.

Zheng, P., S. Wei, B. D. Baviser, J. Yang, C. Ding, and W. Ji. 2003. 17β-Estradiol and progesterone improve in-vitro cytoplasmic maturation of oocytes from unstimulated prepubertal and adult rhesus monkeys. Human Reprod. 18:2137-2144.

Table 1. Composition of synthetic oviductal fluid (SOF) medium used to fertilize oocytes, wash spermatozoa and culture bovine zygotes and embryos in vitro[1]

Reagent (mmol/L)	Fertilization	Sperm Wash	Culture-1	Culture-2
Sodium pyruvate	0.33	1.0	0.33	0.33
Alanine/Glutamine	-	-	1.0	1.0
Glucose	-	-	1.5	3.0
Sodium lactate	-	18.3	-	-
HEPES	-	12.5	-	-
Bovine serum albumin[2]	6 mg/mL	3 mg/mL	8 mg/mL	8 mg/mL
EDTA	-	-	0.1	-
Taurine	-	-	0.1	-
NEAA[3]	2×	-	1×	1×
EAA[4]	-	-	1×	2×
Vitamins	-	-	-	1×
PSA[5]	-	10 µL/mL	-	-

[1] Supplements added to a base SOF medium consisting of 99.70 mmol/L NaCl, 7.16 mmol/L KCl, 1.19 mmol/L KH_2PO_4, 0.49 $MgCl_2 \cdot 6H_2O$, 3.30 mmol/L lactic acid (L) free acid (MP Biomedicals, LLC), 25.07 mmol/L $NaHCO_3$, 1.71 $CaCl_2 \cdot 2H_2O$.

[2] Bovine serum albumin used in fertilization was Fatty Acid Free (Sigma A7511), used in sperm wash was Fraction V (Sigma A3311), and used in culture-1 and culture-2 was crystallized (Cat. No. 810012; MP Biomedicals, LLC).

[3] Non-essential amino acids (MP Biomedicals, LLC).

[4] Essential amino acids (MP Biomedicals, LLC).

[5] Penicillin/streptomycin/amphotericin (PSA; Invitrogen Corp., Grand Island, NY).

Table 2. Percentage of oocytes that were fertilized and cleaved and percentage of cleaved embryos that developed to morula or greater, blastocysts or greater, or hatched blastocysts by replicate.

			Percentage of cleaved embryos that developed to		
Replicate	No. droplets	Percentage cleaved (no.)[1]	$\geq$ Morula	$\geq$ Blastocyst	Hatched blastocyst
		---------------------------------- Mean $\pm$ sd ----------------------------------			
1	28	$78.1^{cb} \pm 14.3$ (132)	$47.4^c \pm 19.5$	$29.9^e \pm 17.1$	$4.5^{de} \pm 8.9$
2	28	$81.9^c \pm 13.3$ (94)	$33.9^b \pm 16.7$	$28.5^e \pm 14.7$	$8.4^{ef} \pm 8.6$
3	30	$49.2^a \pm 11.8$ (118)	$39.3^b \pm 26.0$	$28.2^e \pm 22.3$	$9.8^f \pm 13.5$
4	28	$74.7^b \pm 14.3$ (57)	$20.4^a \pm 17.0$	$18.6^d \pm 15.4$	$4.2^d \pm 6.3$

[1] no. = Number of oocytes that cleaved.

[abc] Within a column, means without a common superscript letter differ ($P \leq 0.05$).

[def] Within a column, means without a common superscript letter differ ($P \leq 0.10$).

Table 3. Percentage of oocytes that were fertilized and cleaved and percentage of cleaved embryos that developed to morula or greater, blastocysts or greater, or hatched blastocysts by treatment.

| Treatment | No. droplet | Percentage cleaved (no.)[1] | Percentage of cleaved embryos that developed to | | |
			$\geq$ Morula	$\geq$ Blastocyst	Hatched blastocyst
			------------------------------------- Mean $\pm$ sd ----------------------------		
CON[2]	23	67.8 $\pm$ 18.8 (156)	30.5 $\pm$ 22.2	26.8 $\pm$ 20.5	7.2 $\pm$ 9.0
LONO[3]	21	74.1 $\pm$ 17.4 (156)	41.8 $\pm$ 15.5	33.0 $\pm$ 16.7	8.1 $\pm$ 9.4
HINO[4]	24	70.1 $\pm$ 19.3 (168)	30.1 $\pm$ 22.6	20.9 $\pm$ 17.6	4.0 $\pm$ 6.7
NOLO[5]	23	72.7 $\pm$ 19.5 (167)	37.0 $\pm$ 24.2	26.6 $\pm$ 20.1	8.7 $\pm$ 13.5
NOHI[6]	23	69.6 $\pm$ 19.1 (157)	38.1 $\pm$ 24.9	25.2 $\pm$ 13.8	5.9 $\pm$ 10.2

[1] no. = Number of oocytes that cleaved.

[2] Included droplets exposed to no progesterone (CON).

[3] Included droplets exposed to 1 ng/mL progesterone (LO) during culture-1 and no progesterone during culture-2.

[4] Included droplets exposed to 100 ng/mL progesterone (HI) during culture-1 and no progesterone during culture-2.

[5] Included droplets exposed to no progesterone during culture-1 and 1 ng/mL progesterone (LO) during culture-2.

[6] Included droplets exposed to no progesterone during culture-1 and 100 ng/mL progesterone (HI) during culture-2.

Table 4. Orthogonal contrasts of percentage of oocytes that were fertilized and cleaved and percentage of cleaved embryos that developed to morula or greater, blastocysts or greater, or hatched blastocysts by treatment factor.

Factor	No. droplet	Percentage cleaved	Percentage of cleaved embryos that developed to		
			$\geq$ Morula	$\geq$ Blastocyst	Hatched blastocyst
		-------------------------------- Mean $\pm$ sd -------------------------------			
CON vs. P4[1]:					
CON	23	67.8 $\pm$ 18.8	30.5 $\pm$ 22.2	26.8 $\pm$ 20.5	7.2 $\pm$ 9.0
P4	91	71.3 $\pm$ 18.7	36.5 $\pm$ 22.3	26.2 $\pm$ 17.6	9.6 $\pm$ 10.1
HIP4 vs. LOP4[2]:					
HIP4	47	69.9 $\pm$ 19.0	34.0 $\pm$ 23.8	23.0 $\pm$ 15.8	5.0 $\pm$ 8.5
LOP4	44	72.9 $\pm$ 18.3	39.3 $\pm$ 20.4	29.7 $\pm$ 18.6	8.4 $\pm$ 11.6
Culture-1 vs. Culture-2[3]:					
Culture-1	45	72.0 $\pm$ 18.3	35.5 $\pm$ 20.3	26.6 $\pm$ 18.1	5.9 $\pm$ 8.2
Culture-2	46	70.7 $\pm$ 19.1	37.5 $\pm$ 24.3	25.9 $\pm$ 17.1	7.3 $\pm$ 11.9

[1] CON included droplets exposed to no progesterone treatment (CON) compared to P4 which included all droplets that received progesterone (1 ng/mL or 100 ng/mL; NOLO, NOHI, LONO, and HINO)

[2] HIP4 included droplets exposed to high concentrations of progesterone (100 ng/mL; NOHI and HINO) compared to LOP4 which included droplets that were exposed to low concentrations of progesterone (1 ng/mL; NOLO and LONO) .

[3] Culture-1 were droplets that received progesterone (1 ng/mL or 100 ng/mL) during culture-1 compared to culture-2 where droplets received progesterone (1 ng/mL or 100 ng/mL) during culture-2.

Table 5. Embryo metabolism of glucose and pyruvate after in vitro culture by treatments.

Treatment	No.	Glycolysis[1]/embryo	Glycolysis[2]/ h/embryo	Glycolysis[3]/cell	Pyruvate oxidation[4] / embryo	Pyruvate oxidation[5]/ h/embryo	Pyruvate oxidation[6]/cell
				------- Mean ± SEM -------			
CON[7]	38	17.89 ± 2.42	5.96 ± 0.81	61.30[a] ± 7.68	12.78 ± 4.57	4.26 ± 1.52	38.82 ± 16.30
LONO[8]	50	15.45 ± 2.15	5.15 ± 0.72	59.67[a] ± 6.72	13.40 ± 4.17	4.47 ± 1.39	55.98 ± 15.09
HINO[9]	35	19.37 ± 2.47	6.46 ± 0.82	66.39[a] ± 7.96	22.28 ± 4.62	7.43 ± 1.54	79.14 ± 16.51
NOLO[10]	39	20.65 ± 2.35	6.88 ± 0.78	84.83[b] ± 7.55	13.82 ± 4.45	4.61 ± 1.48	56.49 ± 15.99
NOHI[11]	32	13.99 ± 2.49	4.67 ± 0.78	52.06[a] ± 8.32	9.23 ± 4.66	3.08 ± 1.55	38.11 ± 16.98

[1] [5-^{3}H]Glucose metabolized in pmols/embryo in 3 h.

[2] [5-^{3}H]Glucose metabolized in pmols/embryo in 1 h.

[3] [5-^{3}H]Glucose metabolized in fmols/cell in 1 h.

[4] [2-^{14}C]Pyruvate oxidized in pmols/embryo in 3 h.

[5] [2-^{14}C]Pyruvate oxidized in pmols/embryo in 1 h.

[6] [2-^{14}C]Pyruvate oxidized in fmols/cell in 1 h.

[7] Included droplets exposed to no progesterone (CON).

[8] Included droplets exposed to 1 ng/mL progesterone (LO) during culture-1 and no progesterone during culture-2.

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[9] Included droplets exposed to 100 ng/mL progesterone (HI) during culture-1 and no progesterone during culture-2.

[10] Included droplets exposed to no progesterone during culture-1 and 1 ng/mL progesterone (LO) during culture-2.

[11] Included droplets exposed to no progesterone during culture-1 and 100 ng/mL progesterone (HI) during culture-2.

[a,b] Within a column, means without a common superscript letter differ ($P \leq 0.05$).

Table 6. Orthogonal contrasts of embryo metabolism of glucose and pyruvate after in vitro culture by treatment factors.

Factor	No.	Glycolysis[1]/embryo	Glycolysis[2]/ h/embryo	Glycolysis[3]/cell	Pyruvate oxidation[4] / embryo	Pyruvate oxidation[5]/ h/embryo	Pyruvate oxidation[6]/cell
		--- Mean ± SEM ---					
CON vs. P4[7]:							
CON	39	17.56 ± 2.30	5.85 ± 0.77	61.07 ± 7.65	13.28 ± 3.83	4.43 ± 1.28	43.51 ± 12.30
P4	165	17.28 ± 1.12	5.76 ± 0.37	65.91 ± 3.77	15.13 ± 1.86	5.04 ± 0.62	60.86 ± 6.07
HIP4 vs. LOP4[8]:							
HIP4	73	16.59 ± 1.67	5.53 ± 0.56	59.09† ± 5.74	16.38 ± 2.98	5.46 ± 0.99	63.01 ± 10.00
LOP4	92	18.06 ± 1.50	6.02 ± 0.50	72.33† ± 5.02	13.99 ± 2.67	4.66 ± 0.89	58.15 ± 8.74
Culture-1 vs. Culture-2[9]:							
Culture-1	88	17.45 ± 1.54	5.82 ± 0.55	63.12 ± 5.17	18.90† ± 2.75	6.30† ± 0.92	72.49† ± 9.02
Culture-2	77	17.20 ± 1.63	5.73 ± 0.55	68.31 ± 5.60	11.47† ± 2.91	3.82† ± 0.97	48.66† ± 9.76

[1] [5-^{3}H]Glucose metabolized in pmols/embryo in 3 h.

[2] [5-^{3}H]Glucose metabolized in pmols/embryo in 1 h.

[3] [5-^{3}H]Glucose metabolized in fmols/cell in 1 h.

[4] [2-^{14}C]Pyruvate oxidized in pmols/embryo in 3 h.

[5] [2-^{14}C]Pyruvate oxidized in pmols/embryo in 1 h.

[6] [2-^{14}C]Pyruvate oxidized in fmols/cell in 1 h.

[7] CON included droplets exposed to no progesterone treatment (CON) compared to P4 which included all droplets that received progesterone (1 ng/mL or 100 ng/mL; NOLO, NOHI, LONO, and HINO)

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[8] HIP4 included droplets exposed to high concentrations of progesterone (100 ng/mL; NOHI and HINO) compared to LOP4 which included droplets that were exposed to low concentrations of progesterone (1 ng/mL; NOLO and LONO) .

[9] Culture-1 were droplets that received progesterone (1 ng/mL or 100 ng/mL) during culture-1 compared to culture-2 where droplets received progesterone (1 ng/mL or 100 ng/mL) during culture-2.

[†] Indicates treatment factor tended to affect means ($P \leq 0.10$).

THESIS LITERATURE CITED

Abecia, J. A., F. Forcada, and J. M. Lozano. 1999. A preliminary report on the effect of dietary energy on prostaglandin $F_{2\alpha}$ production in vitro, interferon-tau synthesis by the conceptus, endometrial progesterone concentration on days 9 and 15 of pregnancy and associated rates of embryo wastage in ewes. Theriogenology 52:1203-1219.

Adler, H. C. 1959. Genital vibriosis in the bovine. An experimental study on the influence on early embryonic mortality. Acta. Vet. Scand. 1:1-11.

Ahmad, N., E. C. Townsend, R. A. Dailey, and E. K. Inskeep. 1997. Relationships of hormonal patterns and fertility occurrence of two or three waves of ovarian follicles, before and after breeding, in beef cows and heifers. Anim. Reprod. Sci. 49:13-28.

Al-Katanami, Y. M., F. F. Paula-Lopes, and P. J. Hansen. 2002. Effect of season and exposure to heat stress on oocyte competence in Holstein cows. J. Dairy Sci. 85:390-396.

Attardi, B. and H. K. Happe. 1986. Modulation of the estradiol-induced luteinizing hormone surge by progesterone or antiestrogens: effects on pituitary gonadotropin-releasing hormone receptors. Endocrinology 119:274-283.

Ayalon, N. 1978. A review of embryonic mortality in cattle. J. Reprod. Fertil. 54:483-493.

Ball, G. D., M. L. Leibfried, R. W. Lenz, R. L. Ax, B. D. Bavister, and N. L. First. 1983. Factors affecting successful in vitro fertilization of bovine follicular oocytes. Biol. Reprod. 28:717-725.

Bavister, B. D. 1995. Culture of preimplantation embryos: facts and artifacts. Hum.

Reprod. Update 1(2):91-148.

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

Black, W. G., J. Simon, S. H. McNutt, and L. E. Cassida. 1953. Investigations on the physiological basis for the differential response of estrous and pseudopregnant rabbit uteri to induced infection. Am. J. Vet. Res. 11:318-323.

Bottari, S. P., A. Vokaer, E. Kaivez, J. P. Lescrainer, and G. P. Vauquelin. 1983. Differential regulation of alpha-adrenergic receptor subclasses by gonadal steroids in human myometrium. J. Clin. Endocrinol. Metab. 57:937-941.

Boué, J., A. Boué, and P. Lazar. 1975. Retrospective and prospective epidemiological studies of 1500 karyotyped spontaneous human abortions. Teratology 12:11-26.

Brenner, R. M. and I. A. Maslar. 1988. The primate oviduct and endometrium. Pages 303-329 in Physiology of Reproduction. K. Knobil, J. D. Neill, L. L. Ewing, C. L. Markert, G. S. Greenwald, and D. W. Pfaff, eds. Raven Press, Ltd., New York.

Brenner, R. M., J. A. Resko, and N. B. West. 1974. Cyclic changes in oviductal morphology and residual cytoplasmic estradiol binding capacity induced by sequential estradiol-progesterone treatment of spayed Rhesus monkeys. Endocrinology 95:1094-1104.

Buhi, W. C., I. M. Alvarez, and A. J. Kouba. 1997. Oviductal regulation of fertilization and early embryonic development. J. Reprod. Fertil. 52:285-300.

Cartmill, J. A., S. Z. El-Zarkouny, B. A. Hensley, G. C. Lamb, and J. S. Stevenson. 2001. Stage of cycle, incidence, and timing of ovulation, and pregnancy rates in dairy cattle after three timed breeding protocols. J. Dairy Sci. 84:1051-1059.

Casida, L.E. 1961. Present status of the repeat-breeder cow problem. J. Dairy Sci. 44:2323-2329.

Cerri, R. L. A., K. N. Galvão, S. O.Juchem, R. C. Chebel, and J. E. P Santos. 2003. Timed AI (TAI) with estradiol cypionate (ECP) or insemination at detected estrus in lactating dairy cows. J. Dairy Sci. 86(Suppl. 1):181. (Abstr.)

Chebel, R. C., J. E. Santos, R. L. Cerri, K. N. Galvao, S. O. Juchem, and W. W. Thatcher. 2003. Effect of resynchronization with GnRH on day 21 after artificial insemination on pregnancy rate and pregnancy loss in lactating dairy cows. Theriogenology 60(8):1389-1399.

Chenault, J. R., J. F. Boucher, K. J. Dame, J. A. Meyer, and S. L. Wood-Follis. 2003. Intravaginal progesterone insert to synchronize return to estrus of previously inseminated dairy cows. J. Dairy Sci. 86:2039-2049.

Chenault, J. R., J. F. McAllister, and C. W. Kasson. 1990. Synchronization of estrus with melengestrol acetate and prostaglandin $F_{2\alpha}$ in beef and dairy heifers. J. Anim. Sci. 68:296-303.

Chew, B. P., R. E. Erb, J. F. Fessler, C. J. Callahan, and P. V. Malven. 1979. Effects of ovariectomy and of prematurely induced parturition on progesterone, estrogens, and calving traits. J. Dairy Sci. 62:557-566.

Cicinelli, E., N. Einer-Jensen, M. Cignarelli, L. Mangiacotti, D. Luisi, and S. Schonauer. 2004. Preferential transfer of endogenous ovarian steroid hormones to the uterus during both the follicular and luteal phases. Hum. Reprod. 19(9):2001-2004.

Clarke, C.L. 1990. Cell-specific regulation of progesterone receptor in the female

reproductive system. Mol. Cell. Endocrinol. 70:C29-C33.

Colazo, M. G., J. P. Kastelic, R. C. Mainar-Jaime, Q. A. Gavaga, P. R. Whittaker, J. A. Small, M. F. Martinez, R. E. Wilde, D. M. Veira, and R. J. Mapletoft. 2006. Resynchronization of previously timed-inseminated beef heifers with progestins. Theriogenology 65:557-572.

Committee on Bovine Reproductive Nomenclature, 1972. Recommendations for standardizing bovine reproductive terms. Cornell Vet. 62:216-237.

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.

Darwash, A. O. and G. E. Lamming. 1998. The importance of milk progesterone concentrations during early pregnancy in the cow. J. Anim. Breed. 2:41-43.

Dawson, F. L. M. 1954. Progesterone in functional infertility of cattle. Vet. Rec. 66:324-326.

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 number in artificially inseminated cattle. J. Anim. Sci. 70:484-491.

De Vries, A. 2006. Economic value of pregnancy in dairy cattle. J. Dairy Sci. 89:3876-3885.

Diskin, M. G., D. A. Kenny, L. D. Dunne, and J. M. Sreenan. 2002. Systemic progesterone pre- and post-AI and embryo survival in heifers. Page 27 in Irish Agricultural Research Forum. M.D. Diskin, ed. Tullamore, Co. Offaly, Ireland.

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Donnay, I. and H. J. Leese. 1999. Embryo metabolism during the expansion of the bovine blastocyst. Mol. Reprod. Dev. 53:171-178.

Dransfield, M. B., R. L. Nebel, R. E. Pearson, and L. D. Warnick. 1998. Timing of insemination for dairy cows identified in estrus by a radiotelemetric estrus detection system. J. Dairy Sci. 81:1874-1882.

Drost, M., J. E. Ambrose, M-J Thatcher, C. K. Cantrell, K. E. Wolsdorf, J. F. Hansen, and W. W. Thatcher. 1999. Conception rates after artificial insemination or embryo transfer in lactating dairy cows during summer in Florida. Theriogenology 52:1161-1176.

Ealy, A. D., M. Drost, and P. J. Hansen. 1993. Developmental changes in embryonic resistance to adverse effects of maternal heat stress in cows. J. Dairy Sci. 76:2899-2905.

Eicker, S. and J. Fetrow. 2003. New tools for deciding when to replace used dairy cows. Pages 33-46 in Proc. Kentucky Dairy Conf., Cave City, KY. Univ. Kentucky, Lexington.

El-Zarkouny, S. Z. and J. S. Stevenson. 2004. Resynchronization of estrus with progesterone or progesterone plus estrogen in lactating dairy cows of unknown pregnancy status. J. Dairy Sci. 87:3306-3321.

Eppig, J. J., K. Wigglesworth, and M. J. O'Brien. 1992. Comparison of embryonic developmental competence of mouse oocytes grown with and without serum. Mol. Reprod. Dev. 32:33-40.

Erickson, R. P. 1977. Differentiation and other alloantigens of spermatozoa. Pages 85-94 in Immunology of Gametes. M. Edidin and M. H. Johnson, eds. Oxford University Press, London.

Erickson, R. P., S. E. Lewis, and M. Butley. 1981. Is haploid gene expression possible for sperm antigens? J. Reprod. Immunol. 3:195-219.

Estergreen, V. L., O. L. Frost, W. R. Gomes, R. E. Erb, and J. F. Bullard. 1967. Effect of ovariectomy on pregnancy maintenance and parturition in dairy cow. J. Dairy Sci. 50: 1293-1295.

Evans, R. W. and W. W. Leavitt. 1980. Progesterone inhibition of uterine nuclear estrogen receptor: dependence on RNA and protein synthesis. Proc. Natl. Acad. Sci. USA 77:5856-5860.

Eyestone, W. H. and N. L. First. 1989. Co-culture of early cattle embryos to the blastocyst stage with oviductal tissue or in conditioned medium. J. Reprod. Fertil. 85:715-720.

Farin, G. 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.

Favero, R. J., D. B. Faulkner, and D. J. Kesler. 1993. Norgestomet implants synchronize estrus and enhance fertility in beef heifers subsequent to timed artificial insemination. J. Anim. Sci. 71:2594-2600.

Fricke, P. M. 2002. Scanning the future-Ultrasonography as a reproductive management tool for dairy cattle. J. Dairy Sci. 85:1918-1926.

Funston, R. N., R. J. Lipsey, T. W. Geary, and A. J. Roberts. 2005. Effect of

administration of human chorionic gonadotropin after artificial insemination on concentrations of progesterone and conception rates in beef heifers. J. Anim. Sci. 83:1403-1405.

Gandi, A. P., M. Lane, D. K. Gardner, and R. L. Krisher. 2000. A single medium supports development of bovine embryos throughout maturation, fertilization and culture. Hum. Reprod. 15:395-401.

Garrett, J. E., R. D. Geisert, M. T. Zavy, and G. L. Morgan. 1988. Evidence for maternal regulation of early conceptus growth and development in beef cattle. J. Reprod. Fertil. 84:437-446.

Gayerie de Abreu, F., G. E. Lamming, and R. C. Shaw. 1984. A cytogenetic investigation of early stage bovine embryos; relation with embryos mortality. Proc. 10th Int. Congr. Anim. Reprod. and A.I., Urbana. 2:82.

Geisert, R. D., G. L. Morgan, E. C. Short, and M. T. Zavy. 1992. Endocrine events associated with endometrial function and conceptus development in cattle. Reprod. Fertil. Dev. 4:301-305.

Griffiths J. G., R. G. Gunn, and J. M. Doney. 1970. Fertility in Scottish Blackface ewes as influenced by climatic stress. J. Agric. Sci., Camb. 75:485.

Gröhn, Y. T. and P. J. Rajala-Schultz. 2000. Epidemiology of reproductive performance in dairy cows. Anim. Reprod. Sci. 60-61:605-614.

Hansel, W., P. W. Concannon, and J. H. Lukaszewska. 1973. Corpora lutea of the large domestic animals. Biol. Reprod. 8:222-245.

Hansel, W., L. E. Donaldson, W. C. Wagner, and M. A. Brunner. 1966. A comparison

of estrous cycle synchronization methods in beef cattle under feedlot conditions. J.

Anim. Sci. 25:497-503.

Hansel, W. and P. V. Malven. 1960. Estrus cycle regulation in beef cattle by orally

active progestational agents. J. Anim. Sci. 19:1324. (Abstr.)

Hansen, P. J., M. Drost, R. M. Rivera, F. F. Paula-Lopes, Y. M. Al-Katanani, C. E.

Krininger 3rd, and C. C. Chase, Jr. 2001. Adverse impact of heat stress on embryo

production: causes and stratagies for mitigation. Theriogenology 55(1):91-103.

Hansen, P. J., P. Soto and R. P. Natzke. 2004. Mastitis and fertility in cattle – Possible

involvement of inflammation or immune activation in embryonic mortality. Am. J.

Reprod. Immunol. 51(4):294-301.

Hassold, T., N. J. Chen, J. Funkhouser, T. Jooss, B. Manuel, J. Matsura, A.

Matsuyarma, C. Wilson, J. A. Yamane, and P. A. Jacobs. 1980. A cytogenetic study

of 1000 spontaneous abortions. Annls Hum. Genet. 44:151-178.

Hare, W. C. D., E. L. Singh, K. J. Betteridge, M. D. Eaglesome, G. C. B. Randall, D.

Mitchell, R. J. Bilton, and A. O. Trounson. 1980. Chromosomal analysis of 159

bovine embryos collected 12-18 days after estrus. Can. J. Genet. Cytol. 22:615-626.

Henricks, D. M., D. R. Lamond, J. R. Hill, and J. F. Dickey. 1971. Plasma progesterone

concentrations before mating and in early pregnancy in the beef heifer. J. Anim. Sci.

33:450-454.

Herrick, J. B. 1953. Clinical observation of progesterone therapy in repeat breeding

heifers. Vet. Med. 48:498-490.

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.

Hugentobler, S. A., M. G. Diskin, H. J. Leese, P. G. Humpherson, T. Watson, J. M. Sreenan, and D. G. Morris. 2007a. Amino acids in oviduct and uterine fluid and blood plasma during the estrous cycle in the bovine. Mol. Reprod. Devl. 74:445-454.

Hugentobler, S. A., D. G. Morris, J. M. Sreenan, and M. G. Diskin. 2007b. Ion concentrations in oviduct and uterine fluid and blood serum during the estrous cycle in the bovine. Theriogenology 68:538-548.

Ing, N. H., H. Francis, J. J. McDonnell, J. F. Amann, and R. M. Roberts. 1989. Progesterone induction of the uterine milk proteins: Major secretory proteins of sheep endometrium. Biol. Reprod. 41:643-654.

Iwai, M., H. Kanzaki, M. Fujimoto, K. Kojima, H. Hatayama, T. Inoue, T. Higuchi, H. Nakayama, T. Mori, and J. Fujita. 1995. Regulation of sex steroid receptor gene expression by progesterone and testosterone in cultured human endometrial stromal cells. J. Clin. Endocrinol. Metab. 80:450-454.

Javed, M. H. and R. W. Wright Jr. 1991. Determination of pentose phosphate and Embden-Meyerhof pathway activities in bovine embryos. Theriogenology 35:1029-1037.

Johnson, K. R., R. H. Ross, and D. L. Fourt. 1958. Effect of progesterone administration on reproductive efficiency. J. Anim. Sci. 17:386-390.

Kasa-Vubu, J. Z., G. E. Dahl, N. P. Evans, L. A. Thrun, S. M. Moenter, V. Padmanabhan, and F. J. Karsch. 1992. Progesterone blocks the estradiol-induced

gonadotropin discharge in the ewe by inhibiting the surge of gonadotropin-releasing hormone. Endocrinology 131:208-212.

Kesler, D. J., H. Khazali, and R. J. Favero. 1990. Quantification of steroids via a polymer linked second antibody enzyme immunoassay system: Methods of linking anti-rabbit IgG to polystyrene. Pages 157-170 in Progress in Biomedical Polymers. C. G. Gebelein and R. L. Dunn, eds. Plenum Publishing Co., New York, NY.

Khurana, N. K. and H. Niemann. 2000. Energy metabolism in preimplantation bovine embryos derived in vitro or in vivo. Biol. Reprod. 62:847-856.

King, W. A. 1990. Chromosome abnormalities and pregnancy failure in domestic animals. Adv. Vet. Sci. Comp. Med. 34:229-250.

Kirby C. J., M. F. Smith, D. H. Keisler, and M. C. Lucy. 1997. Follicular function in lactating dairy cows with sustained-release bovine somatotropin. J. Dairy Sci. 80:273-285.

Kovats, S., E. K. Main, C. Librach, M. Stubblebine, S. J. Fisher, and R. DeMars. 1990. A class I antigen HLA-G, expressed in human trophoblasts. Science 248:220-223.

Krohn, P. L. 1954. The effect of steroid hormones on the survival of skin homografts in the rabbit. J. Endocrinol. 11:78-82.

Lamb, G. C., C. R. Dahlen, and M. Maddox. 2008. What is the economic impact of infertility in beef cattle? University of Florida, IFAS Florida Coop. Ext. Serv., Animal Science Dept., EDIS Publication AN208.

Lamb, G. C., J. E. Larson, T. W. Geary, J. S. Stevenson, S. K. Johnson, M. L. Day, R. P. Ansotegui, D. J. Kesler, J. M. DeJarnette, and D. G. Landblom. 2006. Synchronization of estrus and artificial insemination in replacement beef heifers

using gonadotropin-releasing hormone, prostaglandin F2{alpha}, and progesterone. J. Anim. Sci. 84:3000-3009.

Lamming, G. E., and A. O. Darwash. 1995. Effect of inter-luteal interval on subsequent luteal phase length and fertility in post partum dairy cows. Biol. Reprod. 52(Suppl.1):72.

Lamming, G. E., A. O. Darwash, and H. L Back. 1989. Corpus luteum function in dairy cows and embryo mortality. J. Reprod. Fertil. 37: 245-252.

Lane, M. and D. K. Gardner. 1996. Prospective selection of viable mouse embryos prior to transfer using metabolic rate. Hum. Reprod. 11:1975-1978.

Larson, S. F., W. R. Butler, and W. B. Currie. 1995. Progesterone supplementation increases pregnancy rates in lactating dairy cattle. J. Reprod. Fertil. Abst. Series 15:63. (Abstr.)

Larson, J. E., G. C. Lamb, J. S. Stevenson, S. K. Johnson, M. L. Day, T. W. Geary, D. J. Kesler, J. M. DeJarnette, F. N. Schrick, A. DiCostanzo, and J. D. Arseneau. 2006. Synchronization of estrus in suckled beef cows for detected estrus and artificial insemination and timed artificial insemination using gonadotropin-releasing hormone, prostaglandin $F_{2\alpha}$, and progesterone. J. Anim. Sci. 84:332-342.

Larson, J. E., K. N. Thielen, B. J. Funnel, J. S. Stevenson, D. J. Kesler, and G. C. Lamb. 2009. Influence of a controlled internal drug release after fixed-time artificial insemination on pregnancy rates and returns to estrus of nonpregnant cows. J. Anim. Sci. 87:914-921.

Lauderdale, J. W. 1972. Effects of $PGF_{2\alpha}$ on pregnancy and estrous cycle of cattle. J. Anim. Sci. 35:246. (Abstr.)

151

Leese, H. J. 1992. Metabolism of the preimplantation mammalian embryo. Pages 35-72 in Oxford Reviews of Reproductive Biology (13). S. R. Milligan ed. Oxford University Press, Oxford.

Liehr, R. A., G. B. Marion, and H. H. Olson. 1972. Effects of prostaglandin on cattle estrous cycle lengths. J. Anim. Sci. 35:247. (Abstr.)

López-Gatius, F. 2003. Is fertility declining in dairy cattle? A retrospective study in northeastern Spain. Theriogenology 60:89-99.

López-Gatius, F., P. Santolaria, J. Yániz, J. Rutllant, and M. López-Béjar. 2002. Factors affecting pregnancy loss from gestation day 38 to 90 in lactating dairy cows from a single herd. Anim. Reprod. Sci. 57:1251-1261.

Lord, E. M., G. F. Sensabaugh and D. P. Stites. 1977. Immunosuppressive activity of human seminal plasma. I. Inhibitory in vitro lymphocyte activation. J. Immunol. 118:1704-1711.

Louis, T. M., H. D. Hafs, and D. A. Morrow. 1972. Estrus and ovulation after uterine $PGF_{2\alpha}$ in cows. J. Anim. Sci. 35:247. (Abstr.)

Lucy, M. C. 2001. Reproductive loss in high-producing dairy cattle: where will it end? J. Dairy Sci. 84:1277-1293.

Lucy, M. C., W. J. Weber, L. H. Baumgard, B. S. Seguin, A. T. Koenigsfeld, L. B. Hansen, H. Chester-Jones, and B. A. Crooker. 1998. Reproductive endocrinology of lactating dairy cows selected for increased milk production. J. Anim. Sci. 76(Suppl. 1):296. (Abstr.)

Ludwick, T. M. and E. R. Rader. 1968. Diagnosis of early pregnancy in cattle by ovarian analysis. J. Dairy Sci. 51:74-77.

Lynch, P. R., K. L. Macmillan, and V. K. Taufa. 1999. Treating cattle with

    progesterone as well as GnRH analogue affects oestrous cycle length and fertility.

    Anim. Reprod. Sci. 56:189-200.

Macmillan, K. L. and A. J. Peterson. 1993. A new intravaginal progesterone releasing

    device for cattle (CIDR-B) for oestrous synchronisation, increasing pregnancy rates

    and the treatment of post-partum anoestrus. Anim. Reprod. Sci. 33:1-25.

Macmillan, K. L., V. K. Taufa, A. M. Day, and V. M. Eagles. 1999. Some effects of

    post-oestrous hormonal therapies on conception rates and resubmission rates in

    lactating dairy cows. Pages 195-208 in Fertility in the High Producing Dairy Cow,

    Vol. 1. Occasional pub. No. 26. Br. Soc. Anim. Sci., Penicuik, Scotland.

MacMillan, K. L., V. K. Taufu, A. M. Day, and A. J. Peterson. 1991. Effects of

    supplemental progesterone on pregnancy rates in cattle. J. Reprod. Fertil.

    (Suppl.)43:304. (Abstr.)

Mann, G. E. 2008. Meta-analysis of progesterone supplementation during early

    pregnancy in cattle. J. Anim. Sci. (Suppl.)86:387. (Abstr.)

Mann, G. E. and G. E. Lamming. 1999. The influence of progesterone during early

    pregnancy in cattle. Reprod. Domest. 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. Reproduction 121:175-180.

Mann, G. R., G. E. Lamming and P. A. Fisher. 1998. Progesterone control of embryonic

    interferon tau production during early pregnancy in the cow. J. Reprod. Fertil.

    19:132. (Abstr.)

153

Mann, G. R., P. Merson, M. D. Fray, and G. E. Lamming. 2001. Conception rate following progesterone supplementation after second insemination in dairy cows. Vet. J. 162:161-162.

Marsh, J. M., R. W. Butcher, K. Savard, and E. W. Sutherland. 1966. The stimulatory effect of luteinizing hormone on adenosine 3',5'-monophosphate accumulation in corpus luteum slices. J. Biol. Chem. 241:5436-5440.

Maslar, I. A., P. Powers-Craddock, and R. Ansbacher. 1986. Decideral prolactin production by organ cultures of human endometrium: effects of continuous and intermittent progesterone treatment. Biol. Reprod. 34:741-750.

Maurer, H. R. 1992. Towards serum-free, chemically defined media for mammalian cell culture. Pages 15-46 in Animal Cell Culture: A Practical Approach, 2nd ed. R. I. Freshney, ed. Oxford University Press, Oxford.

McFeely, R. A. and E. Rajakoski. 1968. Chromosome studies on early embryos of the cow. Proc. 6th Int. Cong. Reprod. Anim. and A.I., Paris. 2: 905-907.

McNeill, R. E., M. G. Diskin, and J. M. Sreenan. 2002. Progesterone and early embryo survival in dairy cows. Page 23 in Irish Agricultural Research Forum. M.D. Diskin, ed. Tullamore, Co. Offaly, Ireland.

McNeill, R. E., D. G. Morris, M. G. Diskin, and J. M. Sreenan. 2006a. Associations between milk progesterone concentration on different days and with embryo survival during the early luteal phase in dairy cows. Theriogenology 65: 1435-1441.

McNeill, R. E., J. M. Sreenan, M. G. Diskin, M. T. Cairns, R. Fitzpatrick, T. J. Smith, and D. G. Morris. 2006b. Effect of systemic progesterone concentration on the

expression of progesterone-responsive genes in the bovine endometrium during the early luteal phase. Reprod. Fertil. Devl. 18:573-583.

Michael, S. D., I. I. Geschwind, G. E. Bradford, and G. H. Stabenfeldt. 1975. Pregnancy in mice selected for small little size: Reproductive hormone levels and effect of exogenous hormones. Biol. Reprod. 12:400-407.

Moriyama, I. and T. Sugawa. 1972. Progesterone facilitates implantation of xenogeneic cultured cells in hamster uterus. Nat. New. Biol. 236:150-152.

Munroe, J. P. 1971. Progesteroids as immunosuppressive agents. J. Reticuloendothel. Soc. 9:361-375.

Murray, M. K. 1992. The biosynthesis, immunocytochemical localization of an estrogen-dependent glycoprotein and associated morphological alterations in the sheep ampulla oviduct. Biol. Reprod. 47:889-902.

Nayak, R. K. and D. R. Zimmerman. 1971. Ultrastructural changes in porcine oviduct epithelium during the estrous cycle. J. Anim. Sci. 33:262.

Nebel, R. L., J. M. DeJarnette, D. A. Whitlock, C. E. Marshall, M. R. Mink, and R. Kasimanickam. 2007. Effect of human chorionic gonadotropin or gonadotropin releasing hormone injected 5 or 7 days after 72 h Co-Synch on first service pregnancy rates in lactating dairy cows. J. Dairy Sci. 90(Suppl. 1): 327. (Abstr.)

Ocon, O. M. and P. J. Hansen. 2003. Disruption of bovine oocytes and preimplatation embryos by urea and acidic pH. J. Dairy Sci. 86:1194-1200.

Padykula, H. A., L. G. Coles, W. C. Okulicz, S. I. Rapaport, J. A. McCracken, N. W. King, Jr., C. Longcope, and I. R. Kaiserman-Abramof. 1989. The basalis of the

primate endometrium: a bifunctional germinal compartment. Biol. Reprod. 40:681-690.

Padykula, H. A. and T. R. Tansey. 1979. The occurrence of uterine stromal and intraepithelial monocytes and heterophils during normal late pregnancy in the rat. Anat. Rec. 193(3):329-356.

Parr, R. A. 1992. Nutrition-progesterone interactions during early pregnancy in sheep. Reprod. Fertil. Dev. 4:297-300.

Parrish, J. J., J. L. Susko-Parrish, and N. L. First. 1985. Effect of heparin and chondroitin sulfate on the acrosome reaction and fertility of bovine sperm *in vitro*. Theriogenology 24:537-549.

Parrish, J. J., J. Susko-Parrish, M. A. Winer, and N. L. First. 1988. Capacitation of bovine sperm by heparin. Biol. Reprod. 38:1171-1180.

Pereira, R. M., C. C. Marques, M. C. Baptista, M. I. Vasques, and A. E. Horta. 2009. Embryos and culture cells: a model for studying the effect of progesterone. Anim. Reprod. Sci. 111(1):31-40.

Perry, R. C., L. R. Corah, R. C. Cochran, W. E. Beal, J. S. Stevenson, J. E. Minton, D. D. Simms, and J. R. Brethour. 1991. Influence of dietary energy on follicular development, serum gonadotropins, and first postpartum ovulation in suckled beef cows. J. Anim. Sci. 69:3762-3773.

Pope, W. F., R. R. Maurer, and F. Stormshak. 1982. Distribution of progesterone in the uterus, broad ligament, and uterine arteries of beef cows. Anat. Rec. 203:245-250.

Portaluppi, M. A. and J. S. Stevenson. 2005. Pregnancy rates in lactating dairy cows after presynchronization of estrous cycles and variations of the Ovsynch protocol. J.

Dairy. Sci. 88:914-921.

Pursley, J. R., M. R. Kosorok, and M. C. Wiltbank. 1997a. Reproductive management of lactating dairy cows using synchronization of ovulation. J. Dairy Sci. 80:301-306.

Pursley, J. R., R. W. Silcox, and M. C. Wiltbank. 1998. Effect of time of artificial insemination on pregnancy rates, calving rates, pregnancy loss, and gender ratio after synchronization of ovulation in lactating dairy cows. J. Dairy Sci. 81:2139-2144.

Pursley, J. R., M. C. Wiltbank, J. S. Stevenson, J. S. Ottobre, H. A. Garverick, and L. L. Anderson. 1997b. Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized estrus. J. Dairy Sci. 80:295-300.

Purvis, H. T. and J. C. Whittier. 1997. Use of short-term progestin treatment to resynchronize the second estrus following synchronized breeding in beef heifers. Theriogenology 48:423-434.

Rabiee, A. R., K. L. Macmillan, and F. Schwarzenberger. 2000. Effect of level of feed intake on plasma progesterone concentrations in desolrelin-implanted dairy cows treated with a CIDR device. J. Anim. Sci. 78(suppl. 1):221. (Abstr.)

Rieger, D., B. Grisart, E. Semple, A. Van Langendonckt, K. J. Betteridge, and F. Dessy. 1995. Comparison of the effects of oviductal cell co-culture and oviductal cell-conditioned medium on the development and metabolic activity of cattle embryos. J. Reprod. Fertil. 105:91-98.

Rieger, D. and P. Guay. 1988. Measurements of the metabolism of energy substrates in individual bovine blastocysts. J. Reprod. Fertil. 83:585-591.

Rieger, D., N. M. Loskutoff, and K. J. Betteridge. 1992. Developmentally related changes in the uptake and metabolism of glucose, glutamine and pyruvate by cattle embryos produced in vitro. Reprod. Fertil. Dev. 4:547-557.

Rivera, H., H. Lopez, and P. M. Fricke. 2005. Use of intravaginal progesterone-releasing inserts in a synchronization protocol before timed AI and for synchronizing return to estrus in Holstein heifers. J. Dairy Sci. 88:957-968.

Rivera, R. M. and P. J. Hansen. 2001. Development of cultured bovine embryos after exposure to high temperatures in the physiological range. Reproduction 121:107-115.

Robinson, N. A., K. E. Leslie and J. S. Walton. 1989. Effect of treatment with progesterone on pregnancy rate and plasma concentrations of progesterone in Holstein cows. J. Dairy Sci. 72:202-207.

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. Endocrinology 160:21-33.

Rodgers, J. C. 2008. An economic evaluation of estrous synchronization and timed artificial insemination in beef cows. Master's Thesis. University of Minnesota, Saint Paul.

Royal, M., G. E. Mann, and A. P. Flint. 2000. Strategies for reversing the trend towards subfertility in dairy cattle. Vet. J. 160:53-60.

Sangsritavong, S., D. K. Combs, R. Sartori, L. E. Amentano, and M. C. Wiltbank. 2002. High feed intake increases liver blood flow and metabolism of progesterone and estradiol-17β in dairy cattle. J. Dairy Sci. 85:2831-2842.

Sangsritavong, S., D. K. Combs, R. F. Sartori, and M. C. Wiltbank. 2000. Liver blood flow and steroid metabolism are increased by both acute feeding and hypertrophy of the digestive tract. J. Anim. Sci. 78(Suppl. 1):221. (Abstr.)

Santos, J. E. P., S. O. Juchem, R. L. A. Cerri, K. N. Galvão, R. C. Chebel, W. W. Thatcher, C. Dei, and C. Bilby. 2004a. Effect of bST and reproductive management on reproductive and lactational performance of Holstein dairy cows. J. Dairy Sci. 87:868-881.

Santos, J. E. P., W. W. Thatcher, R. C. Chebel, R. L. A. Cerri, and K. N. Galvão. 2004b. The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. Anim. Reprod. Sci. 82-83:513-535.

Santos, J. E. P., W. W. Thatcher, L. Pool, and M. W. Overton. 2001. Effect of human chorionic gonadotropin on luteal function and reproductive performance of high-producing lactating Holstein dairy cows. J. Anim. Sci. 79:2881-2894.

Sartori, R., A. Gümen, J. N. Guenther, A. H. Souza, and M. C. Wiltbank. 2003. Comparison of artificial insemination (AI) versus embryo transfer (ET) in lactating dairy cows. J. Dairy Sci. 86(Suppl. 1):238. (Abstr.)

Sartori, R., J. M. Haughian, R. D. Shaver, G. J. M. Rosa, and M. C. Wiltbank. 2004. Comparison of ovarian function and circulating steroids in estrous cycles of Holstein heifers and lactating cows. J. Dairy Sci. 87:905-920.

Sartori, R., G. J. M. Rosa, and M. C. Wiltbank. 2002. Ovarian structures and circulating steroids in heifers and lactating cows in summer and lactating and dry cows in winter. J. Dairy Sci. 85:2813-2822.

Sartori, R., R. Sartor-Bergfelt, S. A. Mertens, J. N. Guenther, J. J. Parrish, 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.

Schmitt, E. J., T. Diaz, M. drost, and W. W. Thatcher. 1996. Use of a gonadotropin-
releasing hormone agonist or human chorionic gonadotropin for timed insemination
in cattle. J. Anim. Sci. 74:1084-1091.

Schumacher, A. and B. Fischer. 1988. Influence of visible light and room temperature
on cell proliferation in preimplantation rabbit embryos. J. Reprod. Fert. 84:197-204.

Short, R. E., R. A. Bellows, R. B. Staigmiller, J. G. Berardinelli, and E. E. Custer. 1990.
Physiological mechanisms controlling anestrus and infertility in postpartum beef
cattle. J. Anim. Sci. 68:799-816.

Siiteri, P. K., F. Febres, L. E. Clemens, R. J. Chang, B. Gondos, and D. Stites. 1977.
Progesterone and the maintenance of pregnancy: Is progesterone nature's
immunosuppressant? Ann. N.Y. Acad. Sci. 286:384-397.

Siiteri, P. K. and D. P. Stites. 1982. Immunologic and endocrine interrelationships in
pregnancy. Biol. Reprod. 26:1-14.

Silke, V., M. G. Diskin, D. A. Kenny, M. P. Boland, P. Dillon, J. F. Mee, and J. M.
Sreenan. 2002. Extent, pattern, and factors associated with late embryonic losses in
dairy cows. Anim. Reprod. Sci. 71:1-12.

Simmons, R. L., A. L. Price, and A. J. Ozerkis. 1968. The immunologic problem of
pregnancy. V. The effect of estrogen and progesterone on allograft survival. Am. J.
Obstet. Gynecol. 100:908-911.

Skemesh, M., N. Ayalon, and H. R. Lindner. 1973. Early pregnancy diagnosis based

upon plasma progesterone levels in the cow and ewe. J. Anim. Sci. 36:726-729.

Smith, D. L., B. M. Stinefelt, K. P. Blemings, and M. E. Wilson. 2006. Diet-induced alterations in progesterone clearance appear to be mediated by insulin signaling in hepatocytes. J. Anim. Sci. 84:1102-1109.

Snijders, S. E., P. Dillon, D. O. O'Callaghan, and M. P. Boland. 2000. Effect of genetic merit, milk yield, body condition and lactation number on in vitro oocyte development in dairy cows. Theriogenology 53:981-989.

Spencer, T. E. and F. W. Bazer. 1995. Temporal and spatial alterations in uterine estrogen receptor expression and progesterone receptor gene expression during the estrous cycle and early pregnancy in the ewe. Biol. Reprod. 53:1527-1543.

Spencer, T. E., W. C. Becker, P. George, M. A. Mirando, T. F. Ogle, and F. W. Bazer. 1995a. Ovine interferon-τ inhibits estrogen receptor up-regulation and estrogen-induced luteolysis in cyclic ewes. Endocrinology 136:4932-4944.

Spencer, T. E., W. C. Becker, P. George, M. A. Mirando, T. F. Ogle, and F. W. Bazer. 1995b. Ovine interferon-τ regulates expression of endometrial receptors for estrogen and oxytocin but not progesterone. Biol. Reprod. 53:732-745.

Sreenan, J. M. and M. G. Diskin. 1983. Early embryonic mortality in the cow: its relationship with progesterone concentration. Vet. Rec. 112:517-521.

Starbuck, G. R., A. O. Darwash, and G. E. Lamming. 1999. The importance of progesterone during early pregnancy in the dairy cow. Cattle Practice 7:397-399.

Starbuck, G. R., A. O. Darwash, G. E. Mann, and G. R. Lamming. 2001. The detection and treatment of post-insemination progesterone insufficiency in dairy cows. Pages

447-450 in Fertility in the High Producing Dairy Cow. M.G. Diskin, ed. BSAS Occasional Publication No. 26(2).

Starbuck, G. R., C. G. Gutierrez, A. R. Peters, and G. E. Mann. 2006. Timing of follicular phase events and the postovulatory progesterone rise following synchronization of oestrus in cows. Vet. J. 172:103-108.

Sterry, R. A., M. L. Welle, and P. M. Fricke. 2006. Treatment with gonadoptropin-releasing-hormone after first timed artificial insemination improves fertility in noncycling lactating dairy cows. J. Dairy Sci. 89:4237-4245.

Stevenson, J. S. 2001. Reproductive management of dairy cows in high milk-producing herds. J. Dairy Sci. 84(E. Suppl.):E128-E143.

Stevenson, J. S., S. K. Johnson, M. A. Medina-Britos, A. M. Richardson-Adams, and G. C. Lamb. 2003. Resynchronization of estrus in cattle of unknown pregnancy status using estrogen, progesterone, or both. J. Anim. Sci. 81:1681-1692.

Stevenson, J. S. and M. O. Mee. 1991. Pregnancy rate of Holstein cows after post-insemination treatment with a progesterone-releasing intra-vaginal device. J. Dairy Sci. 74:3849-3856.

Stevenson, J. S., M. A. Portaluppi, D. E. Tenhouse, A. Lloyd, D. R. Eborn, S. Kacuba, and J. M. DeJarnette. 2007. Interventions after artificial insemination: conception rates, pregnancy survival, and ovarian responses to gonadotropin-releasing hormone, human chorionic gonadotropin, and progesterone. J. Dairy Sci. 90:331-340.

Stevenson, J. S., S. M. Tiffany, and E. K. Inskeep. 2008. Maintenance of pregnancy in dairy cattle after treatment with human chorionic gonadotropin or gonadotropin-

releasing hormone. J. Dairy Sci. 91:3092-3101.

Stites, D. P. and P. K. Siiteri. 1983. Steroids as immunosuppressants in pregnancy. Immunol. Rev. 75:117-138.

Strinden, S. T. and S. S. Shapiro. 1983. Progesterone-altered secretory proteins from cultured human endometrium. Endocrinology 112:862-870.

Stronge, A. J. H., J. M. Sreenan, M. G. Diskin, J. F. Mee, D. A. Kenny, and D. G. Morris. 2005. Post-insemination milk progesterone concentration and embryo survival in dairy cows. Theriogenology 64:1212-1224.

Szenci, O., J. F. Beckers, P. Humblot, J. Sulon, G. Sasser, M. A. M. Taverne, J. Varga, R. Baltusen, and G. Schekk. 1998. Comparison of ultrasonography, bovine pregnancy-specific protein B, and bovine pregnancy-associated glycoprotein 1 tests for pregnancy detection in dairy cows. Theriogenology 50(1):77-88.

Telford, N. A., A. J. Watson, and G. A. Schultz. 1990. Transition from maternal to embryonic control in early mammalian development: a comparison of several species. Mol. Reprod. Dev. 26:90-100.

Thatcher, W. W. 1974. Effects of season, climate, and temperature on reproduction and lacation. J. Dairy Sci. 57:360-368.

Thatcher, W. W., F. W. Bazer, D. C. Sharp, and R. M. Roberts. 1986. Interrelationships between uterus and conceptus to maintain corpus luteum function in early pregnancy: sheep, cattle, pig and horses. J. Anim. Sci. 62(Suppl. 2):25-46.

Thatcher, W. W., A. Guzeloglu, R. Mattos, M. Binelli, T. R. Hansen, and J. K. Pru. 2001. Uterine-conceptus interactions and reproductive failure in cattle. Theriogenology 56:1435-1450.

Thompson, J. G. 1996. Defining the requirements for bovine embryo culture. Theriogenology 45:27-40.

Thompson, J. G., R. G. Partridge, F. D. Houghton, C. I. Cox, and H. J. Leese. 1996. Oxygen uptake and carbohydrate metabolism by in vitro derived bovine embryo**s**. J. Reprod. Fertil. 106:299-306.

Thurmond, M. C. and J. P. Picanso. 1990. A surveillance system for bovine abortion. Prev. Vet. Med. 9:41-53.

Tiffin, G., D. Rieger, K. J. Betteridge, B. R. Yadav, W. A. King. 1991. Glucose and glutamine metabolism in pre-attachment cattle embryos in relation to sex and stage of development. J. Reprod. Fertil. 93:125-132.

Townson, D. H., P. C. W. Tsang, W. R. Butler, M. Frajblat. L. C. Griel Jr., C. J. Johnson, R. A. Milvae, and J. L. Pate. 2002. Relationship of fertility to ovarian follicular waves before breeding in dairy cows. J. Anim. Sci. 80:1053-1058.

Van Cleeff, J., M. Drost, and W. W. Thatcher. 1991. Effects of post-insemination progesterone supplementation on fertility and subsequent estrous responses of dairy heifers. Theriogenology 36:795-807.

Van Cleeff, J., K. L. Macmillan, M. Drost, and W. W. Thatcher. 1996. Effects of administering progesterone at selected intervals after insemination of synchronized heifers on pregnancy rates and resynchronization of returns to estrus. Theriogenology 4:1117-1130.

Verhage, H. G., P. A. Mavrogianis, M. L. Boice, W. Li, and A. T. Fazleabas. 1990. Oviductal epithelium of the baboon: Hormonal control and the immuno-gold localization of oviduct-specific glycoproteins. Am. J. Anat. 187:81-90.

Villarroel, A., A. Martino, R. H. BonDurant, F. Deletang, and W. M. Sischo. 2004. Effect of post-insemination supplementation with PRID on pregnancy in repeat-breeder Holstein cows. Theriogenology 61:1513-1520.

Walker, R. S., P. D. Burns, T. E. Engle, G. E. Sides, D. D. Zelesky. 2005. Effects of human chorionic gonadotrophin administration on artificial insemination pregnancy rates in beef heifers. Prof. Anim. Sci. 21:361-364.

Walton, J. S., G. W. Halbert, N. A. Robinson, and K. E. Leslie. 1990. Effects of progesterone and human chorionic gonadotrophin administration five days post-insemination on plasma and milk concentrations of progesterone and pregnancy rates of normal and repeat breeder dairy cows. Can. J. Vet. Sci. 54:305-308.

Wathes, D. C., G. E. Mann, J. H. Payne, P. R. Riley, K. R. Stevenson, and G. E. Lamming. 1996. Regulation of oxytocin, oestradiol, and progesterone receptor concentrations in different uterine regions by oestradiol, progesterone and oxytocin in ovariectomised ewes. Endocrinology 151:375-393.

Wathes, D. C., V. J. Taylor, Z. Cheng, and G. E. Mann. 2003. Follicle growth, corpus luteum function and their effect on embryo development in postpartum dairy cows. Reproduction Suppl. 61:219-237.

Watnick, A. S. and R. A. Russo. 1968. Survival of skin homografts in uteri of pregnant and progesterone-estrogen treated rats. Proc. Soc. Exp. Biol. Med. 128:1-4.

Weems, C. W., C. N. Lee, Y. S. Weems, and D. L. Vincent. 1988. Distribution of progesterone to the uterus and associated vasculature of cattle. Endocrinol. Jpn. 35:625-630.

West, N. B., M. C. McClellan, M. D. Sternfeld, and R. M. Brenner. 1987. Immunocytochemistry versus binding assays of the estrogen receptor in the reproductive tract of spayed and hormone-treated macaques. Endocrinology 121:1789-1800.

Whisnant, C. S., L. A. Pagels, and M. G. Daves. 2001. Case study: Effectiveness of a commercial early conception factor test for use in cattle. Prof. Anim. Sci. 17:51-53.

Whitman, R. H. 1975. Weight changes, body condition and beef-cow reproduction. Ph.D. Dissertation. Colorado State University, Fort Collins.

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, J. N., H. W. Hawk, H. E. Kidder, W. G. Black, L. C. Ulberg, and L. E. Casida. 1956. Effect of progesterone therapy on embryo survival in cows of lowered fertility. J. Dairy Sci. 39:456-461.

Xu, Z. Z., L. J. Burton, and K. L Macmillan. 1997. Reproductive performance of lactating dairy cows following estrus synchronization regimens with PGF2alpha and progesterone. Theriogenology 47(3):687-701.

Xu, K. P., B. K. Yadav, R. W. Rorie, L. Plante, K. J. Betteridge, and W. A. King. 1992. Development and viability of bovine embryos derived from oocytes matured and fertilized in vitro and co-cultured with bovine oviduct epithelial cells. J. Reprod. Fert. 94:33-43.

Yie, S. M, R. Xiao, and C. L. Librach. 2006. Progesterone regulates HLA-G gene expression through a novel progesterone response element. Human Reprod. 21:2538-2544.

Zeron, Y., A. Ocheretny, O. Kedar, A. Borochov, D. Sklan, and A. Arav. 2001. Seasonal changes in bovine fertility: relation to developmental competence of oocytes, membrane properties and fatty acid composition of follicles. Reproduction 121:447-454.

Zheng, P., S. Wei, B. D. Baviser, J. Yang, C. Ding, and W. Ji. 2003. 17β-Estradiol and progesterone improve in-vitro cytoplasmic maturation of oocytes from unstimulated prepubertal and adult rhesus monkeys. Human Reprod. 18:2137-2144.

Zimbelman, R. G. 1963. Determination of the minimal effective dose of 6a-methyl-17α-acetoxyprogesterone for control of the estrual cycle in cattle. J. Anim. Sci. 22:1051-1058.

Zimbelman, R. G. and L. W. Smith. 1966. Control of ovulation in cattle with melengestrol acetate. I. Effect of dosage and route of administration. J. Reprod. Fertil. 11:185-191.

Zoli, A. P., L. A. Guilbault, P. Dhlahaut, W. B. Ortiz, and J. F. Beckers. 1992. Radioimmunoassay of a bovine pregnancy-associated glycoprotein in serum: Its application for pregnancy diagnosis. Biol. Reprod. 46:83-92.