

The role of preovulatory estradiol in fertility

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INTRODUCTION

The most efficient and cost-effective method for genetic improvement of economically important traits in the beef industry is artificial insemination (AI). Estrous synchronization and AI remain the most important and widely applicable reproductive biotechnology available for cattle (Seidel, 1995). However, the time and labor required to detect spontaneous estrus in beef cattle has been a deterrent to the widespread utilization of AI (Britt, 1987). Therefore, the development of procedures that facilitate high pregnancy rates at fixed-time insemination could expedite genetic progress.

At the initiation of estrus, gonadotropin -releasing hormone (GnRH) is secreted from the hypothalamus at a sufficient amplitude and frequency to stimulate a surge of luteinizing hormone (LH) to be released from the anterior pituitary to cause ovulation (Karsch et al., 1997). However, even when estrus does not occur a GnRH agonist is capable of causing ovulation of dominant bovine follicles (Ryan et al., 1998), and the luteal tissue that forms is capable of undergoing prostaglandin (PG)-induced luteal regression on days 6 or 7 (GnRH-PG protocol, Twagiramungu et al., 1995). The addition of a second injection of GnRH 48 hours after PG has been utilized to induce ovulation and eliminate the need for estrus detection. The second GnRH injection in this method resulted in induced ovulation of a wide range of follicle sizes (Perry et al., 2005).

A recent study involving the reciprocal embryo transfer of embryos to and from cows induced to ovulate either a large or small follicle with GnRH revealed some interesting results about the factors affecting fertility (Atkins et al., 2010, submitted). While ovulatory follicle size and serum concentrations of estradiol were highly correlated ($r = 0.49$; $P < 0.0001$), both concentrations of estradiol and follicle size had independent positive effects on fertilization success. Furthermore, a positive relationship between estrus expression at time of insemination and pregnancy success has been established in both beef and dairy cattle (Vasconcelos et al., 2001; Perry et al., 2005; Lopes et al., 2007; Perry et al., 2007). Among beef cows and heifers, analysis of more than 10,000 animals from 22 different studies, utilizing one of the top five recommended fixed-time AI protocols, indicated that $57.1\% \pm 3.5\%$ of animals exhibited standing estrus prior to fixed-time AI, and those animals that exhibited estrus averaged 27% greater pregnancy success compared to animals that did not (Richardson et al., 2016). Among high producing dairy cows, current rates of estrus detection are not sufficient for efficient reproductive management (Nebel and McGilliard, 1993; Senger, 1994; Dransfield et al., 1998; Lopez et al., 2004; Peralta et al., 2005).

In addition to the use of supplemental GnRH, several studies have investigated the role of supplemental estrogen on fertility (Cerri et al., 2004; Souza et al., 2005; Brusveen et al., 2009). Estradiol has a pivotal role on the initiation of estrus and the ovulatory cascade (Allrich, 1994). Preovulatory estradiol concentrations also play a critical role in sperm transport, regulation of the uterine environment, and embryo survival. Recent data from our laboratory has reported that some follicles that are ovulated in response to a GnRH injection are incapable of producing sufficient estradiol to initiate standing estrus while others are not.

ESTRADIOL PRODUCTION

A capillary network present in the thecal layer is responsible for supplying cholesterol for steroid production. Cholesterol is delivered to the theca interna cells where it is converted into androstenedione. Androstenedione is then transported to the granulosa cells where it is converted into estradiol by the aromatase enzyme (two cell – two gonadotropin concept, Fortune and Quirk, 1988). Thus, increased estradiol production by preovulatory follicles depends on the enhanced ability of the theca interna cells to produce androstenedione and the enhanced ability of granulosa cells to convert androstenedione to estradiol (Ginther et al., 1996). Estradiol production can also be increased by production of pregnenolone by granulosa cells, which can be converted to androstenedione by the theca interna cells, which in turn can be converted to estradiol by the granulosa cells (Fortune, 1986).

Estradiol production is further increased due to an increase in luteinizing hormone (LH) pulse frequency (Fortune et al., 2001). Pituitary gonadotropes produce and secrete LH, and the response of gonadotropes to GnRH is directly correlated with the number of GnRH receptors on the cell surface (Wise et al., 1984). Expression of the GnRH receptor can be regulated by estradiol, progesterone, and GnRH itself (Gregg et al., 1991; Wu et al., 1994; Looper et al., 2003). Increased sensitivity of gonadotropes to GnRH and increased expression of GnRH receptors occurs prior to the increase in concentrations of estradiol (Turzillo et al., 1994). Therefore, the decrease in progesterone before proestrus may be important in initiating the increase in LH secretion that leads to the increase in estradiol production. As progesterone decreases to basal concentrations at luteolysis, GnRH pulse frequency increased (Chabbert-Buffeta et al., 2000) and that increase in GnRH stimulated greater expression of GnRH receptors (Nett et al., 2002). Therefore, the ability of some dominant follicles to produce sufficient concentrations of estradiol to induce estrus may be a result of increased pituitary sensitivity to GnRH following a decrease in progesterone.

THE ROLE OF ESTRADIOL IN REGULATING FERTILITY

Estrus

Initiation of estrus occurs due to increased circulating concentrations of estradiol at a time when progesterone is not present (Allrich, 1994). Estrus refers to the period of time when a female is sexually receptive. Standing estrus refers to the visual sign that a female will stand to be

mounted by a male or another female. Among cattle, concentrations of estradiol peak approximately 36 hours before ovulation (Chenault et al., 1975), and this increased preovulatory concentrations of estradiol have been correlated with increased pregnancy success (Perry et al., 2005). The ability of estradiol to synchronize estrus and ovulation and its impact on overall fertility has been the focus of many investigations.

Pre-ovulatory follicular environment

The endocrine microenvironment of a preovulatory follicle has been correlated with oocyte quality and its ability to undergo germinal vesicle breakdown (McNatty et al., 1979). This microenvironment is unique relative to surrounding nonovulatory follicles and is important in the preparation of follicular cells for luteinization and secretion of progesterone (McNatty et al., 1975). Follicles containing oocytes that were more capable of being fertilized and developing to the blastocyst stage embryos contained less progesterone (Hazeleger et al., 1995), three to eight fold greater aromatase activity, greater amounts of the α subunit of inhibin (Driancourt et al., 1998), and greater concentrations of estradiol (Mermillod et al., 1999). In addition, the ability of human oocytes to develop into embryos increased when they were collected from follicles having greater follicular fluid concentrations of estradiol compared to oocytes collected from follicles that had lower concentrations of estradiol (Teissier et al., 2000). Follicular secretion of estradiol may also affect the establishment and maintenance of pregnancy through altering sperm transport and the uterine environment.

Sperm Transport

Sperm transport through the female reproductive tract is affected by estradiol and is optimized at estrus or when females are under the influence of estrogen (Hawk, 1983). Ovariectomized ewes (Allison and Robinson, 1972) and rabbits (Noyes et al., 1959) required estradiol for appropriate sperm transport through the reproductive tract. The dependence of sperm transport on the effects of estradiol is likely associated with changes in uterine pH as considerable evidence exists that pH regulates sperm motility and preserves viability (Wong et al., 1981). In cattle, there is a transient decline in uterine pH from 7.0 to 6.7 around the initiation of standing estrus (Elrod and Butler, 1993; Perry and Perry, 2008a). The importance of this decrease in uterine pH at the initiation of standing estrus to increase fertility has been reported in lactating beef cows (Bolzenius et al., 2016). Cows that initiated standing estrus had decreased uterine pH, 6.78, and increased pregnancy success, 52%, compared to cows that did not initiate standing estrus but were induced to ovulate, 6.96 and 38%, respectively.

Importantly, work from our laboratory indicates that cattle which exhibited standing estrus and possessed elevated concentrations of estradiol prior to insemination had increased pregnancy success compared to cattle that did not exhibit standing estrus (Perry et al., 2005; Perry et al., 2007). This increased pregnancy success among cows that exhibited standing estrus is likely due to increased sperm transport to the site of fertilization (Larimore et al., 2015). Subsequent work

by our laboratory (Perry and Perry, 2008a, b) has focused on the effects of estradiol on uterine pH of cattle where supplementation of estradiol decreased uterine pH to a level similar to cows in standing estrus. Similar evidence can be found in the mouse, where Wang and others (Wang et al., 2003) reported Na⁺/H⁺ exchanger (NHE) isoforms 1, 2 and 4 were present in mouse endometrial epithelial during the luteal phase and regulated uterine pH by extruding H⁺ into the uterine lumen to increase endometrial secretion of HCO₃⁻ and uterine pH. Among cows that exhibited estrus, expression of mRNA for NHE1 was decreased at 48 hours compared to hour 0, mRNA for NHE2 was decreased at 12, 24, 36, 48 and 60 hours compared to hour 0, and mRNA for NHE4 was decreased at 36, 48 and 60 hours compared to hour 0. Among cows that did not exhibit estrus, expression of NHE1, NHE2 and NHE4 did not change over time (Bolzenius et al., 2016).

Following estrus, cattle exhibit a rapid increase in uterine pH from 6.7 (day 0) to 7.0 prior to the time of ovulation (Perry and Perry, 2008a, b), and the neutral pH of 7 is maintained through the luteal phase of the cycle (Elrod et al., 1993). As extracellular pH increased up to 7.5, sperm motility increased in demembrated bull (Goltz et al., 1988), membrane intact bull (Contri et al., 2013), and human (Giroux-Widemann et al., 1991) sperm. Increased sperm motility appeared necessary for sperm to penetrate the viscous oviductal mucus and the cumulus matrix (Suarez and Dai, 1992) as well as the zona pellucida for fertilization (Stauss et al., 1995). Furthermore, capacitation (a process necessary for sperm egg binding) is initiated through an increase in intracellular pH (Fraser et al., 1993). Following capacitation and initiation of hyperactive motility, sperm must undergo the acrosome reaction in order to fertilize an ovum (Yanagimachi and Bhattacharyya, 1988). Sperm did not undergo the acrosome reaction when an increase in intracellular pH was prevented (Parrish et al., 1989; Zeng et al., 1996). Estradiol induced a decrease in uterine pH at the initiation of standing estrus, which may initially decrease sperm motility, inhibit capacitation, and the acrosome reaction thereby increasing sperm longevity. The rapid rise in uterine pH prior to ovulation is necessary for increased sperm motility, and to initiate capacitation and the acrosome reaction in order to be able to fertilize the oocyte.

Fertilization

A recent review by Santos (2004) reported fertilization failure in lactating beef and dairy cows was as high as 45%. A recent study in beef cows, in which embryos were flushed from cows that had high or low concentrations of estradiol at time of fixed-time AI revealed that cows with greater concentrations of estradiol at the GnRH-induced ovulation were more likely to yield a fertilized embryo than an unfertilized oocyte (Jinks et al., 2013). Data from our laboratory has reported that animals that exhibited estrus prior to fixed-time AI had increased accessory sperm numbers and improved embryo quality compared to animals that did not exhibit estrus (Larimore et al., 2015). Although accessory sperm are not involved in fertilization, they represent sperm that were able to access the oviduct, undergo capacitation and the acrosome reaction, recognize and bind to the oocyte, and partially penetrate the zona pellucida (Dalton et al., 2006). The number of

accessory sperm trapped in the zona pellucida has been positively associated with fertility (Hunter and Wilmut, 1984; Hawk and Tanabe, 1986; DeJarnette et al., 1992; Nadir et al., 1993), and are thought to be an indirect measure of both sperm transport and the availability of healthy competent sperm competing for fertilization (DeJarnette et al., 1992). These studies indicate the important role preovulatory estradiol concentrations have on sperm transport and fertilization success.

Oviduct and uterine gene expression changes

Ovarian estradiol has been reported to play an important role in establishing the timing of uterine receptivity (Ozturk and Demir, 2010). More specifically, estradiol likely plays a direct role in regulating oviductal secreted glycoproteins (Buhi, 2002) and in the regulation of the biological clock in the uterus (Nakamura et al., 2005). Whether or not an animal is exposed to elevated concentrations of estradiol impacts gene expression changes within the endometrium (Bridges et al., 2012). Specifically, estrus expression at time of AI has been shown to influence endometrium and corpus luteum gene expression. Cows that showed estrus have been reported to have increased expression of genes related to the maternal immune system and adhesion molecules, and downregulation of genes associated with prostaglandin synthesis and CL maintenance (Davoodi et al., 2016).

The TGF- β superfamily is involved in changes in the endometrium, development of the placenta, and maintenance of the pregnancy (Jones et al., 2006). At estrus there is also up-regulation of several genes involved in the remodeling of the extracellular matrix (Bauersachs et al., 2005), and changes in expression of inhibin α subunit (a member of the TGF- β signaling pathway) in the bovine intercaruncular area suggests it is involved in coordinating endometrial remodeling (Ishiwata, 2003 #1582). Furthermore, a recent study in cattle examined the role of high and low preovulatory estradiol concentrations on regulation of progesterone and estradiol receptors later in the estrous cycle. The authors reported that progesterone receptors in the deep glandular epithelium as well as ER α receptor mRNA in the endometrium were up-regulated in cows with elevated preovulatory estradiol concentrations (Bridges et al., 2012).

Milk protein (SERPINA14) is expressed in the endometrium of ruminants during pregnancy and is likely involved in nutrition of the embryo/fetus, growth of the embryo/fetus, and suppression of the maternal immune system (Ing and Roberts, 1989). Expression of SERPINA14, as determined by Real-time RT-PCR, was greatest on the day of estrus and expression was also up-regulated after stimulation with estradiol. Furthermore, detection of SERPINA14 protein revealed increased protein concentrations on the day of estrus and was localized to the glandular epithelium (Ulbrich et al., 2009). This suggests a possible preparatory role for preovulatory estradiol in establishing a uterine environment conducive to pregnancy. Estradiol induced progesterone (Zelinski et al., 1980) and oxytocin (Lamming and Mann, 1995) endometrial receptors in ruminants. Expression of uterine oxytocin and steroid receptors (estradiol receptor α and nuclear progesterone receptor) changed throughout the estrous cycle (Robinson et al.,

2001), and they play a vital role in regulating the uterine environment. Therefore, estradiol may not only have a direct effect on regulating the uterine environment, but may also have an indirect role by regulating response to progesterone through regulation of the progesterone receptor.

Subsequent Progesterone effects

The endocrine microenvironment of a preovulatory follicle is also unique relative to surrounding nonovulatory follicles and is important for preparation of follicular cells for luteinization and secretion of progesterone (McNatty et al., 1975). McNatty et al. (1979) suggested that development of a normal CL depends upon a follicle meeting the following criteria: 1) an adequate number of granulosa cells, 2) an adequate number of LH receptors on the granulosa and thecal cells, and 3) granulosa cells capable of synthesizing adequate amounts of progesterone following luteinization. Within granulosa cells, estradiol is reported to cause: 1) increased cellular proliferation (Goldenberg et al., 1972; Parrott and Skinner, 1998; Dupont et al., 2000), 2) formation of gap junctions (Merk et al., 1972; Burghardt and Anderson, 1981), 3) increased stimulatory action of FSH on aromatase activity (Adashi and Hsueh, 1982; Zhuang et al., 1982; Reilly et al., 1996), 4) enhanced stimulation of progesterone synthesis following gonadotropin stimulation (Welsh et al., 1983; Fanjul et al., 1984), and 5) enhanced acquisition of LH receptors (Kessel et al., 1985; Farookhi and Desjardins, 1986; Wang and Greenwald, 1993). Furthermore, luteinized granulosa cells secreted increased amounts of progesterone when they were collected from follicles having increased concentrations of estradiol compared to granulosa from follicles that had lower concentrations of estradiol (McNatty et al., 1979).

The relationship between pregnancy success and circulating concentrations of progesterone during early pregnancy in cattle is equivocal, as luteal secretion of progesterone is required for the survival of the embryo/fetus (McDonald et al., 1952). Several studies have reported elevated concentrations of progesterone in pregnant cows compared to nonpregnant cows beginning as early as day 4 (Butler et al., 1996) or day 6 (Henricks et al., 1971; Erb et al., 1976) after insemination. Furthermore, cows that had an earlier rise in progesterone had embryos that were more advanced developmentally, produced more interferon τ (INF- τ) and were capable of inhibiting the PGF_{2 α} release on day 16 after breeding (Kerbler et al., 1997; Mann et al., 1998; Mann et al., 1999; Mann and Lamming, 2001). Similarly, cows supplemented with progesterone during early gestation had advanced endometrial expression of several genes associated with uterine secretions and conceptus development (Forde et al., 2009; Forde et al., 2011). It is suspected that progesterone induces changes in endometrial gene expression that leads to changes in uterine histotroph composition (Spencer et al., 2008). Bartol et al. (1981) determined that protein accumulation within the uterine lumen is related to length of progesterone stimulation. However, direct supplementation of progesterone following insemination has produced mixed results. Some studies have reported a 10 to 60% increase in pregnancy rates following progesterone supplementation (Johnson et al., 1958; Robinson et al., 1989; Macmillan and Peterson, 1993), but in other studies using sheep (Diskin and Niswender, 1989; Nephew et

al., 1994) and cattle (Walton et al., 1990; Van Cleef et al., 1991) progesterone supplementation had no benefit on pregnancy success. A consistent characteristic among animals in which progesterone supplementation increased pregnancy rate was that the animals appeared to have fertility problems, since control animals had unusually low pregnancy rates.

Work by Atkins et al. (2013) reported that estradiol concentrations at GnRH-induced ovulation affected day 27 pregnancy rate of recipient cows independent of progesterone on day 7. Furthermore, work from our laboratory has reported when ovulatory follicle size was controlled there was no difference in day 10 CL weight, circulating concentrations of progesterone, or expression of luteal steroidogenic enzymes between cows that exhibited standing estrus and cows that did not (Fields et al., 2012). Therefore, improved pregnancy success among cattle with elevated preovulatory concentrations of estradiol are likely independent of the impact of progesterone on the uterine environment during the subsequent estrous cycle.

Uterine environment.

The uterine environment plays a critical role in early embryo development, recognition of pregnancy, elongation, and attachment. During the estrous cycle changes occur in the endometrium regarding its composition and differentiation status. These changes are mainly regulated by estradiol, progesterone, and oxytocin (Spencer and Bazer, 2004). The timing of changes in the uterine environment is of critical importance to embryo survival. In cattle, synchrony between the embryo and the uterus must be ± 24 hours (Hasler, 2001). Ovarian estradiol plays an important role in establishing the timing of uterine receptivity (Ozturk and Demir, 2010).

CLOCK genes regulate biological oscillations and the transcription of other genes in a tissue specific manner in response to changes in nutrient status, day length, and possibly other environmental influences (Albrecht, 2006). Decreased litter size, lower frequency of successful matings, and increased cycle length have been reported in *Clock* ^{Δ 19} (mice in which CLOCK is not functional) female mice (Chappell et al., 2003). When *Clock* ^{Δ 19} female mice were treated hormonally to induce ovulation and mated with fertile males, *Clock* ^{Δ 19} females produced fewer embryos than control females (Kennaway, 2005). It is possible from these results that defects in circadian clock gene expression at the level of the uterus may disrupt the synchrony between the uterine environment and the developing embryo. He and co-workers (2007) reported that Period 1 mRNA (part of the positive feedback loop of CLOCK) was expressed in the uterus of diestrus rats and that both estradiol and progesterone stimulated uterine Period 1 mRNA expression in the uterus of ovariectomized rats. Furthermore, Nakamura and co-workers (2005) reported that estradiol altered the normal circadian rhythms in the uterus. Therefore, the circadian clock genes may influence fertility at the level of the uterus.

The uterine histotroph is composed of nutrients, growth factors, proteins, glucose, immunosuppressive agents, enzymes, and ions. It is secreted by the endometrium, and is necessary for early conceptus growth/survival (Geisert et al., 1992; Gray et al., 2001). Any changes can greatly influence early embryonic viability. In particular, glucose is a major fuel source used by the conceptus for growth and development. Work from our laboratory found that animals that showed estrus during a fixed-time AI program had greater glucose in the uterine luminal fluid compared to no estrus animals (unpublished). A subsequent study determined that animals that exhibit estrus had increased mRNA expression of specific glucose transporters in the endometrium. In both caruncular and intercaruncular endometrial tissue, both SLC2A1 and SLC5A1 mRNA abundance was increased in animals that exhibited estrus ($P \leq 0.05$). This may serve as a possible mechanism to deliver glucose into the uterus where it can be utilized by the developing conceptus.

When an injection of estrogen, corresponding with initiation of estrus, was omitted in ovariectomized hormone-supplemented ewes, embryo survival following embryo transfer (Miller and Moore, 1976), uterine weight, uterine protein, RNA to DNA ratio, and the rate of protein synthesis were decreased (Miller et al., 1977). Elevated concentrations of estradiol did not impact protein to DNA, RNA to DNA ratios or in-vitro rate of protein synthesis in intercaruncular and caruncular endometrium on the day of estrus, but increased all measures from day 2 to 4 after estrus (Miller and Moore, 1983). Furthermore, cows that exhibit standing estrus around the time of fixed-time insemination had increased preovulatory concentrations of estradiol (Perry et al., 2005; Perry and Perry, 2008a, b), decreased uterine pH (Perry and Perry, 2008a, b), and increased pregnancy success (Perry et al., 2005) compared to cows that did not exhibit standing estrus. Consequently, preovulatory concentrations of estradiol may play a major role in the uterine environment and the establishment and maintenance of pregnancy.

Early embryo development and survival

Production of a glycoprotein, known as oviductal glycoprotein (OGP) also occurs during estrus by the non-ciliated epithelial cells of the oviduct from the late follicular phase through early embryo development (Buhi, 2002). When cyclic ewes were ovariectomized production of OGP was abolished, but administration of estradiol benzoate restored OGP production (Sutton et al., 1986). Oviductal glycoprotein has been reported to be present on the head, midpiece (King and Killian, 1994), and tail (Abe et al., 1995) of bovine sperm in oviductal fluid, and OGP may be instrumental in fertilization success and early embryo development. *In vitro* matured oocytes cultured in media containing varying concentrations of OGP resulted in increased cleavage rates and blastocyst formation following IVF in a dose-dependent manner (Hill et al., 1996).

When an injection of estrogen, corresponding with initiation of estrus, was omitted in ovariectomized hormone-supplemented ewes, embryo survival following embryo transfer was decreased (Miller and Moore, 1976). In a recent study from our laboratory ovariectomized cows

received exogenous hormones to mimic the luteal phase and luteolysis and then received either estradiol cypionate (ECP), estradiol benzoate (EB) or no treatment (CON) according to Latin Square assignment to mimic a preovulatory period. Ovulation was simulated with an injection of gonadotropin releasing hormone (GnRH, 100 µg; day 0), and embryos were transferred on day 7. Pregnancy was maintained by exogenous progesterone supplementation. Expression of interferon stimulated genes (ISG) was used as a marker for pregnancy on day 17, 19, 21, and 28 and pregnancy-specific protein B (PSPB) was used as a marker for pregnancy on day 22-28. Transrectal ultrasonography was used on day 29 to diagnose pregnancy. Cows that received preovulatory estradiol exposure had greater embryonic survival and pregnancy establishment compared to control animals (4%, 29%, and 21% for CON, EB, and ECP, respectively; (Madsen et al., 2015). Additionally, preovulatory estradiol has been reported to have a positive impact on conceptus development. Cows that exhibit estrus have been shown to have a longer conceptus length compared to no estrus cows on day 19 of gestation (Davoodi et al., 2016).

USE OF ESTRADIOL TO SYNCHRONIZE ESTRUS AND OVULATION

The ability of estradiol to synchronize standing estrus and ovulation has been the focus of many investigations. Using estradiol following PGF_{2α} administration increased expression of estrus (Cerri et al., 2004; Souza et al., 2005; Brusveen et al., 2009), reduced the interval to onset of estrus (Nancarrow and Radford, 1975; Ryan et al., 1995; Evans et al., 2003), induced a surge of LH (Short et al., 1973; Short et al., 1979), and reduced the interval from PGF_{2α} to ovulation (Ryan et al., 1995; Evans et al., 2003). However, the effect of supplemental estradiol on fertility during a synchronization protocol is not clear. When estradiol was administered at follicular wave emergence, fertility was reduced, but when administered when a dominant follicle was present on the ovary, it had no influence on fertility (Lane et al., 2001). Estradiol administered during follicular wave emergence reduced the maximum diameter of the ovulatory follicle, but estradiol administered when a dominant follicle was present did not affect maximum ovulatory follicle size (Evans et al., 2003). When estradiol was administered pre-breeding in sheep bred by natural mating or artificial insemination, there was no effect on pregnancy success (Inskeep et al., 1979). However, in the preceding studies all animals were inseminated following detection in standing estrus, and when cows were detected in standing estrus no differences were detected in circulating concentrations of estradiol (Perry et al., 2005). Administering estradiol before artificial insemination following a fixed-time insemination protocol improved fertility (Ahmadzadeh et al., 2003) when small follicles were induced to ovulate (Jinks et al., 2013). Time of estradiol administration and type of estradiol used can also influence fertility. Administering 1 mg of Estradiol Cypionate (ECP) did not induce an LH surge until 42 ± 2 hours and ovulation until 61 ± 3 hours after administration (Stevenson et al., 2004). However, administering 0.5 mg of estradiol benzoate induced an LH surge and ovulation 30 ± 3.1 and 72 ± 0.0 hours, respectively, after administration (Evans et al., 2003). Administration of estradiol-17β 8 hours (Souza et al., 2007) or 16 hours (Brusveen et al., 2009) before insemination did not improve pregnancy success and even tended to have a negative effect on fertility, but when

concentrations of estradiol were elevated for 1.9 days before insemination (follicle size held constant) pregnancy success was increased (Bridges et al., 2010). Therefore, the addition of estradiol to a synchronization protocol must consider dose, type of estrogen, and timing of administration in maximizing the benefits.

CONCLUSION

These studies indicate not only an importance in increasing preovulatory concentrations of estradiol but also the timing of the increase in estradiol and that increasing circulating concentrations of estradiol through supplementation may not be sufficient to increase fertility. Increased production of estradiol by the ovulatory follicle may be required to enhance fertility through the regulation of sperm transport, fertilization, oviductal secretions, uterine environment, and embryo survival.

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