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Preovulatory estradiol and the establishment and maintenance of pregnancy in suckled beef cows[1]

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ABSTRACT: In postpartum beef cows, GnRH-induced ovulation of small dominant follicles decreased pregnancy rates and increased late embryonic/fetal mortality. In Exp. 1, single ovulation reciprocal embryo transfer (ET) was used to examine the relationship between preovulatory serum concentrations of estradiol at GnRH-induced ovulation in donor and recipient cows and establishment and maintenance of pregnancy. Suckled beef cows (n = 1,164) were administered GnRH (GnRH1, 100 μg) on d –9 (GnRH1), $PGF_{2\alpha}$ on d –2, and GnRH2 (GnRH2, 100 μg) on d 0 (CO-Synch protocol) either with (donors; n = 810) or without (recipients; n = 354) AI. Single embryos (n = 394) or oocytes (n = 45) were recovered from the donor cows (d 7; ET) and all live embryos were transferred into recipients. Serum concentration of estradiol at GnRH2 was positively correlated with follicle size at GnRH2 (r = 0.45, P < 0.01) and progesterone at ET (r =0.34, P < 0.01). Donor cows with greater estradiol at GnRH2 were more likely to yield an embryo than an unfertilized oocyte (P < 0.01). Donor and recipient cows were retrospectively divided into 4 groups [low estradiol (<8.4 pg/mL) or high estradiol (≥8.4 pg/mL)] based on serum concentration of estradiol at GnRH2. Pregnancy rate at d 27 for low-low (n = 78), low-high (n = 80), high-low (n = 91), and high-high (n = 101) groups (donor-recipient, respectively) was 45, 65, 43, and 61% respectively (P < 0.02). Because recipient cows with greater estradiol concentration at GnRH2 had greater pregnancy rates in Exp. 1, the objective of Exp. 2 was to evaluate the effect of estradiol supplementation on pregnancy rate. Ovulation was synchronized in suckled beef cows (n = 600) using the CO-Synch protocol with the insertion of a controlled internal drug release (CIDR; intravaginal progesterone supplement) from d –9 until d –2. Approximately one-half of the cows (n = 297) received an injection of estradiol cypionate (ECP; 0.5 mg intramuscularly) 24 h before AI. Compared with the no treatment (Control) cows, ECP treatment increased (P < 0.01) pregnancy rates of cows induced to ovulate smaller dominant follicles (<12.2 mm). In conclusion, GnRH-induced ovulation of small dominant follicles was associated with reduced serum estradiol, fertilization rate (donor cows), and pregnancy establishment (recipient cows). Furthermore, ECP supplementation during the preovulatory period increased pregnancy rates in cows induced to ovulate smaller dominant follicles.

Key words: beef cattle, embryo transfer, estradiol, estradiol cypionate, follicle diameter, pregnancy

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INTRODUCTION

The microenvironment of a preovulatory follicle is unique relative to other follicles and a distinguishing characteristic is the relatively high secretion of estradiol. Estradiol coordinates a number of physiological processes that are essential for the establishment of pregnancy in cattle, including expression of

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estrus (Asdell et al., 1945), sperm transport within the uterus and oviduct (Hawk and Cooper, 1975), preparation of follicular cells for luteinization and secretion of progesterone (McNatty and Sawers, 1975; Welsh et al., 1983), induction of the preovulatory gonadotropin surge (Kesner et al., 1981), and induction of endometrial estrogen and progesterone receptors (Ing and Tornesi, 1997; Xiao and Goff, 1999).

Perry et al. (2005) reported that GnRH-induced ovulation of smaller follicles in beef cows was associated with decreased circulating concentrations of estradiol at time of AI, decreased pregnancy rates, and increased late embryonic/fetal mortality. We hypothesized that cows with reduced serum concentrations of estradiol at GnRH-induced ovulation would have decreased fertilization rates and/or pregnancy rates. Furthermore, we hypothesized that estradiol supplementation before GnRH-induced ovulation of smaller dominant follicles would increase pregnancy rate compared with cows induced to ovulate larger follicles. To distinguish between the effects of serum estradiol at insemination on fertilization rate and pregnancy rate, a reciprocal embryo transfer approach was used. The objectives were to examine the relationship between preovulatory concentrations of estradiol in serum from donor and recipient cows and the establishment and maintenance of pregnancy (Exp. 1) and to determine the effect of estradiol supplementation before GnRH-induced ovulation on pregnancy rates in suckled beef cows induced to ovulate small or large dominant follicles (Exp. 2).

MATERIALS AND METHODS

All protocols and procedures were approved by Fort Keogh Livestock and Range Research Laboratory (**LARRL**) Animal Care and Use Committee (Institutional Animal Care and Use Committee approval number 101106-3) and University of Missouri Animal Care and Use Committee (approval number 4016).

Experiment 1

These data were collected as part of a larger study that was reported by Atkins et al. (2013). Experimental design, treatments, and some of the methodologies were originally described by Atkins et al. (2013) and modified for inclusion in this publication.

Animal Handling. Multiparous suckled beef cows were used in a reciprocal embryo transfer study to differentiate between effects of ovulatory follicle size on oocyte competence and uterine environment. This trial was conducted over 3 yr at LARRL in Miles City, MT. Ovulation was synchronized in cows (1,164 single ovulations) with the CO-Synch protocol: administration of GnRH [**GnRH1**, 100 μg; intramuscularly (**i.m.**)] on d –9 followed by $PGF_{2\alpha}$; 25 mg i.m.; dinoprost) on d –2 and GnRH (**GnRH2**; 100 μg i.m.) on d 0 with (donor cows; $n = 810$) or without (recipient cows; $n = 354$) fixed-time AI. On d 0, donor cows were inseminated by 1 of 3 AI technicians with semen from a single sire. Single embryo flushes were performed on donor cows on d 7 after GnRH2 and all live embryos were transferred into recipients on the same day. Body weights and BCS were collected (scale of 1 to 9 in which1 = emaciated and 9 = obese; Whitman, 1975) at GnRH1. The experiment was conducted by synchronizing groups of approximately 100 cows and submitting each group to the experimental design outlined in Fig. 1. The number of groups in yr 1, 2, and 3 were 9, 11, and 5, respectively.

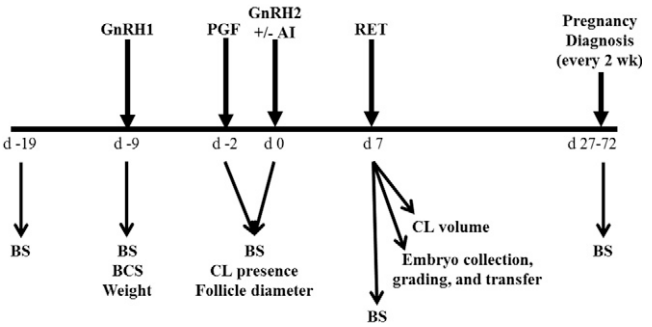

Figure 1. Design of Exp. 1 in which cows (1,164 single ovulations) were synchronized with the CO-Synch protocol: administration of GnRH (GnRH1, 100 μg) on d –9 followed by $PGF_{2\alpha}$ (PGF) on d –2 and GnRH (GnRH2, 100 μg) on d 0 with (donor cows; $n = 810$) or without (recipient cows; $n = 354$) fixed-time AI. Embryo collection, grading, and transfer occurred on d 7 (d 0 = GnRH2). Pregnancy status was determined on d 27 and continued every other week until d 72. BS = blood sample; CL = corpus luteum.

Estrous Detection. Cows were visually inspected for behavioral estrus once daily from GnRH1 to $PGF_{2\alpha}$ and twice daily from $PGF_{2\alpha}$ until GnRH2 in all groups and continued after GnRH2 in a subset of the groups (2 groups in yr 1 and all groups in yr 2 and 3). Estrotect patches (Western Point Inc., Apple Valley, MN) were applied at GnRH1 to aid in estrous detection. Cows that displayed estrus before GnRH2 or 2 to 7 d after GnRH2 were excluded from the study.

Ovarian Ultrasonography. Ovarian structures were monitored and recorded via transrectal ultrasonography using a 7.5 MHz linear array transducer (Aloka 500V; Corometrics Inc., Wallingford, CT) on d –2 ($PGF_{2\alpha}$), d 0 (GnRH2), and d 7 [embryo transfer (**ET**)]. The location and diameter of the largest follicle on each ovary was recorded as well as number of corpora lutea (**CL**). Follicle and CL diameters were calculated as the average diameter at the widest point and perpendicular to the widest measurement. The follicle of greatest diameter at GnRH2 was referred to as the dominant follicle. Dominant follicles <12.5 mm were considered small and dominant follicles ≥12.5 mm considered large based on

the previously reported range of follicle sizes ovulating to GnRH2 (Lamb et al., 2001; Perry et al., 2005). On d 7, donors and recipients that responded to GnRH2 (as determined by the presence of a CL on the same side as the dominant follicle at GnRH2 and did not express estrus in the previous 5 d) were included in the study.

Ovulation to the Initial Injection of Gonadotropin Releasing Hormone (GnRH1). Cows were classified as ovulating to GnRH1 by using estrous cyclicity status [based on blood samples collected on d –19 and –9 (at GnRH1); see below] and number of CL present at $PGF_{2\alpha}$. Cows with no CL or cycling cows with only 1 CL at the time of $PGF_{2\alpha}$ were considered not to have ovulated in response to GnRH1. Cows that were anestrous at the start of the experiment with 1 CL and estrous cycling cows with 2 CL were considered to have ovulated in response to GnRH1. A portion of cows were unable to be classified due to inconsistencies in the number of CL or cycling cows that had low serum progesterone concentration at GnRH1 and a CL at $PGF_{2\alpha}$ and/or GnRH2 (n = 217). This population of cows would likely include cows that were in proestrus during GnRH1 yet had low serum concentrations of progesterone at that blood sampling. From our data, we would not be able to determine if they responded to GnRH1 or ovulated on their own a couple of days later.

Embryo Recovery. On d 7 after AI, all donors were subjected to transcervical uterine catheterization. The side of ovulation was confirmed by the presence of 1 CL, a catheter was placed in the uterine horn ipsilateral to the CL, and the uterine horn was flushed with a ViGro Complete Flush Solution (Bioniche Animal Health, Athens, GA) and filtered with MiniFlush Filter System (MiniTube of America, Inc., Verona, WI). Upon completion of the flush, the filter was rinsed with flush solution and embryo visualized under dissecting microscope.

Embryo Handling. Each embryo was washed 3 times with holding media (Biolife Holding Media; AgTech Inc. Manhattan, KS) and incubated at 26°C until transfer into recipients. Embryos were assigned a quality grade [scale of 1 to 4 in which 1 = excellent to good (85% of the cellular mass intact and healthy appearance), 2 = fair (50 to 85% of the cellular mass intact and healthy appearance and no abnormalities in embryo shape), 3 = poor (over 50% of the cellular mass is extruded or degenerating or gross abnormalities in the structure of the embryo), and 4 = degenerate or dead] and a developmental stage (scale of 1 to 7 in which 1 = unfertilized oocyte, 2 = 2 to 12 cell embryo, 3 = early morula, 4 = morula, 5 = compact morula, 6 = blastocyst, and 7 = expanded blastocyst) according to International Embryo Transfer Society guidelines. All live embryos (grades 1, 2, and 3) were loaded into 0.25 mL straws and prepared for transfer into recipients the same day.

Embryo Transfer. Embryos were transferred into the uterine horn ipsilateral to the CL. Embryos collected from cows that ovulated a small follicle were transferred into cows that ovulated a large (n = 111) or small (n = 71) follicle. Similarly, embryos collected from cows that ovulated a large follicle were transferred into cows that ovulated a large (n = 50) or small (n = 122) follicle. Data from this study are presented elsewhere (Atkins et al., 2013). However, results of the aforementioned study were used here to retrospectively divide donor and recipient data into those with high and low serum estradiol concentration at GnRH2 (see below). Pregnancy diagnosis was performed by transrectal ultrasonography beginning on d 27 after GnRH2 and every other week until d 72. Viability of the embryo was confirmed at each exam by visualization of an embryonic heartbeat by 2 technicians.

Experiment 2

Animals and Treatments. Multiparous suckled beef cows (n = 600) were used to determine the effect of estradiol supplementation on pregnancy establishment. Replicates for this experiment were conducted at LARRL in yr 1 (n = 263) and 2 (n = 265) and at University of Missouri Beef Research and Teaching Farm in yr 3 (n = 72). In a subset of cows (n = 30; yr 3), serum concentrations of estradiol in periovulatory blood samples and concentrations of progesterone during the subsequent luteal phase were evaluated. The herd used in yr 1 and 2 included composite (one-half Red Angus, one-fourth Charolais, and one-fourth Tarentaise) cows and animals from this herd were assigned to treatment [estradiol cypionate (**ECP**)] or no treatment (**Control**) based on cow age and days postpartum. Crossbred beef cows were used in yr 3 and were assigned to treatment based on age, BW, BCS, days postpartum, and cyclicity status.

Suckled beef cows were synchronized with the CO-Synch + controlled internal drug release (**CIDR**) protocol, which included administration of 100 μg GnRH (GnRH1; i.m.) and CIDR (Eazi-breed CIDR; Pfizer Animal Health, Kalamazoo, MI) insertion on d –9 followed by $PGF_{2\alpha}$ injection (25 mg i.m.; dinoprost) and CIDR removal on d –2 and a second injection of GnRH (GnRH2) concurrent with AI on d 0. Cows were blocked by AI sire and randomly assigned to receive an injection of ECP [0.5 mg i.m (n = 297)] or sham injection (n = 303) on d –1 (24 h before GnRH2). Cows were inseminated by 1 of 7 AI technicians with semen from 1 of 18 sires in yr 1 and 2 and by 1 of 2 AI technicians with semen from 1 of 3 sires in yr 3.

Estrous Detection. Visual estrous detection occurred once daily from GnRH1 to $PGF_{2\alpha}$ and twice daily

for 1 h from $PGF_{2\alpha}$ until GnRH2 in all groups and continued until 24 h after GnRH2 in a subset of the groups (2 groups in yr 1 and all groups in yr 2 and 3). Estrotect (Western Point Inc.) estrous detection patches were applied to all cows to aid in estrous detection.

Ultrasonography. Ovarian structures were monitored as described above. The location and diameter of the largest follicle on each ovary was recorded at the time of AI. The follicle of greatest diameter at GnRH2 was referred to as the dominant follicle. Additionally, in yr 2 and 3, growth of the dominant follicle was monitored by daily ultrasound from d –2 until d 0, and ovulation was confirmed on d 8. Pregnancy diagnosis occurred on d 27 to 29 after GnRH2 as described above.

Blood Collection and Hormone Quantification for Experiments 1 and 2

Blood was collected via coccygeal venipuncture into 10 mL Vacutainer tubes (Fisher Scientific, Pittsburgh, PA) and stored at 4°C for 24 h and centrifuged at 1,200 × *g* for 25 min at 4°C. Serum was harvested and stored at –20°C until RIA. In Exp. 1, blood was collected on d –19, –9 (GnRH1), –2 ($PGF_{2\alpha}$), 0 (GnRH2), d 7 (ET), and at each pregnancy exam. In yr 3 replication of Exp. 2, samples were collected from a subset of 30 cows for quantification of serum estradiol at –48, –36, –24, –20, –16, –12, 0, 12, 24, 36, 48, 60, and 96 h relative to AI. Samples were also collected for determination of serum progesterone on d –19, –9, –2, –1, 0, 2, 4, 6, 8, 10, 12, and 14 relative to AI.

Serum concentration of estradiol-17β was quantified in both experiments by RIA as described previously (Kirby et al., 1997). Intra- and interassay CV for estradiol assays were 3.5 and 14% for Exp. 1 and 2.9 and 14% for Exp. 2, respectively. Assay sensitivity, defined as the lowest standard in our assay that was more than 2 SD from tubes with no estradiol, was 0.5 pg/mL. Serum concentrations of estradiol in all samples fell within the standard curve or were below the sensitivity of our assay. Serum concentration of progesterone was quantified with a Coat-a-Count RIA kit (Diagnostic Products Corporation, Los Angeles, CA) as described previously (Bellow et al., 1991; Kirby et al., 1997). Cows were considered to have resumed estrous cycling if progesterone was >1.0 ng/mL in at least 1 sample collected before GnRH1. Intra- and interassay CV for progesterone assays were 1.8 and 13% in Exp. 1 and 1.9 and 12% in Exp. 2, respectively. Assay sensitivity, defined as the lowest standard in our assay that was more than 2 SD from tubes with no progesterone, was 0.08 ng/mL. Serum concentrations of progesterone in all samples fell within the standard curve or were below the sensitivity of our assay.

Data and Statistical Analysis

All reported means are the adjusted least squares means ± SEM and statistical significance was declared when $P < 0.05$.

Experiment 1. All variables that were hypothesized to affect serum estradiol concentration at GnRH2 were included in a general linear model procedure (PROC GLM; SAS Inst. Inc., Cary, NC) and multiple regression analysis was performed. Independent variables in the model included follicle size, serum progesterone at $PGF_{2\alpha}$, ovulatory response to GnRH1, cyclicity status, age, BW, BCS, and days postpartum. Nonsignificant variables were removed from the model, beginning with the variable with the largest *P*-value, until all variables had a *P*-value of ≤0.1. Correlations between serum estradiol and dominant follicle diameter at GnRH2 and serum concentrations of progesterone on d 7 were determined by multivariate ANOVA using the MANOVA/PRINTE statement in PROC GLM. Logistic regression (PROC GENMOD in SAS) was conducted to determine the relationship between serum concentration of estradiol at GnRH2 and binary variables including ovulation to GnRH1, expression of estrus within 24 h after GnRH2, recovery of embryo or unfertilized oocyte from donor cows, and pregnancy at d 27 or 72 in recipients and donors. Similarly, the relationship between expression of estrus within 24 h after GnRH2 and probability of pregnancy establishment on d 27 was determined using logistic regression.

From the data of Perry et al. (2005), it was determined that pregnancy rate decreased when serum estradiol concentration at GnRH2 was <8.4 pg/mL. Therefore, we retrospectively classified donors and recipients into low estradiol (<8.4 pg/mL) or high estradiol (≥8.4 pg/mL) groups based on serum concentration of estradiol at GnRH2 to investigate the relationship of donor and recipient serum concentrations of estradiol at GnRH2 on pregnancy outcome (Table 1). This classification by serum concentration of estradiol resulted in these donor and recipient groupings: 1) low into low (n = 78 transfers), 2) low into high (n = 80 transfers), 3) high into low (n = 91 transfers), and 4) high into high (n = 101 transfers). Effects of donor/recipient group on pregnancy establishment as measured on d 27 and maintenance to d 72 were assessed using logistic regression (as implemented in GENMOD).

Experiment 2. The effects of estradiol supplementation and estrus expression on serum concentration of estradiol and progesterone were analyzed by ANOVA with repeated measures (PROC MIXED in SAS). Factors in the model statement included ECP treatment, expression of estrus, time, and their interactions. Time points in the analysis of serum estradiol included –20, –16, –12, 0, 12, 24, 36, 48, 60, and 96 h relative to AI (h 0). Time

Table 1. Retrospective categorization of embryo transfer groups based on serum estradiol concentration at GnRH-induced ovulation (GnRH2) in embryo donor and recipient cows

No. of transfers	Embryo donor (estradiol pg/mL)	Embryo recipient (estradiol pg/mL)
78	Low (<8.4)	Low (<8.4)
80	Low (<8.4)	High (≥8.4)
91	High (≥8.4)	Low (<8.4)
101	High (≥8.4)	High (≥8.4)

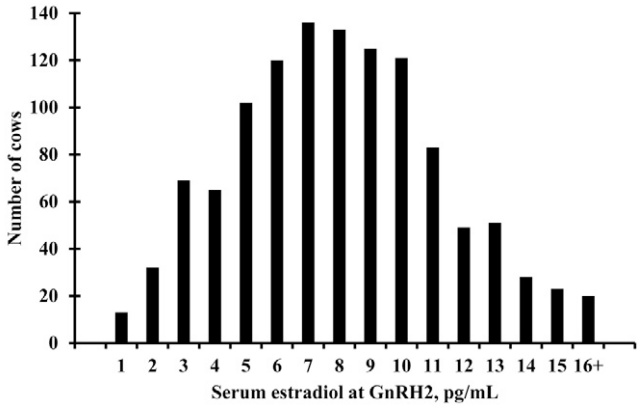

Figure 2. Distribution of serum estradiol concentration (pg/mL) at GnRH-induced ovulation (GnRH2, d 0) in donor and recipient cows (n = 1,135). Mean serum estradiol concentration at GnRH2 was 8.50 ± 0.10 pg/mL.

points in the analysis of serum progesterone included 0, 2, 4, 6, 8, 10, 12, and 14 d relative to AI (d 0). Serum samples collected before ECP administration at h –36 and –24 (for estradiol) and d –2 and –1 (for progesterone) were used as covariates in the model. The effect of ECP treatment was assessed in the model with animal within treatment as the error term. The effect of time and the ECP by time interaction effect was determined in the model with the residual as the error term.

The relationship between expression of estrus within 24 h after AI and serum progesterone concentration from d –2 to 14 was analyzed by ANOVA with expression of estrus as the dependent variable and serum progesterone concentration as the independent variable. Orthogonal contrast comparison of means was used to assess specific treatment differences at each time point for those variables shown to have a significant treatment by time effect in the ANOVA. The effect of ECP treatment on dominant follicle growth rate from $PGF_{2\alpha}$ administration (d –2) to GnRH2 and on expression of estrus within 24 h after GnRH2 was analyzed by using analysis of variance (PROC MIXED in SAS).

To investigate the effect of estradiol supplementation on pregnancy rate the probability of pregnancy at d 28 was modeled by logistic regression using the GENMOD procedure. The model statement included treatment, follicle size at GnRH2, year, and their interactions. Contrast of pregnancy rate was used to assess specific treatment differences at each follicle size.

RESULTS

Experiment 1

Mean serum concentration of estradiol at GnRH2 for all donors and recipients was 8.50 ± 0.10 pg/mL (Fig. 2). Serum concentrations of estradiol at GnRH2 differed between donors and recipients (8.37 ± 0.12 compared with 8.81 ± 0.18 pg/mL, respectively; P = 0.04). These independent variables accounted for a significant amount of variation in estradiol concentration at GnRH2: ovulatory follicle size (P < 0.01), serum progesterone concentrations at $PGF_{2\alpha}$ (d –2; P = 0.01), ovulatory response to GnRH1 (P = 0.01), and whether or not cows were cycling before GnRH1 (P < 0.01). Serum estradiol increased in association with increased ovulatory follicle size, increased d –2 progesterone concentration, ovulation to GnRH1, and among anestrous cows. Serum concentrations of estradiol at GnRH2 were not associated with BCS, days postpartum, age, or BW.

Ovulatory follicle size in both donors and recipients was positively correlated with serum concentration of estradiol at GnRH2 (r = 0.45, P < 0.01; Fig. 3) and estradiol at GnRH2 was greater in serum from cows that ovulated in response to GnRH1 (8.8 ± 0.1 pg/mL) compared with cows that did not ovulate (7.9 ± 0.2 pg/mL; P < 0.01). Serum concentration of estradiol at GnRH2 was positively correlated with progesterone at ET (r = 0.34, P < 0.01; Fig. 4). Mean serum concentration of estradiol was greater (P < 0.01) in donor cows from which a fertilized embryo was recovered (8.8 ± 0.2 pg/mL) versus recovery of an unfertilized oocyte (6.7 ± 0.4 pg/mL). Increased serum concentration of estradiol of donor cows tended to improve embryo quality and viability (P < 0.10) but not embryo stage (P > 0.10). Donor and recipient cows detected in estrus within 24 h after GnRH2 had significantly greater concentrations of serum estradiol at GnRH2 compared with cows that did not express estrus (10.7 ± 0.2 vs. 7.9 ± 0.1 pg/mL, respectively; P < 0.01) and recipients detected in estrus within 24 h after GnRH2 had increased pregnancy rates at d 27 (61.2 ± 0.2 vs. 51.2 ± 0.1%; P < 0.01). Serum concentration of estradiol at GnRH2 was greater (P < 0.01) in recipient cows that were pregnant on d 27 compared with those that did not establish a pregnancy (9.5 ± 0.25 vs. 7.9 ± 0.28 pg/mL). However, there was no direct relationship between estradiol concentration at GnRH2 in donor cows and pregnancy establishment (P = 0.69). Pregnancy rate at d 27 for transfers between

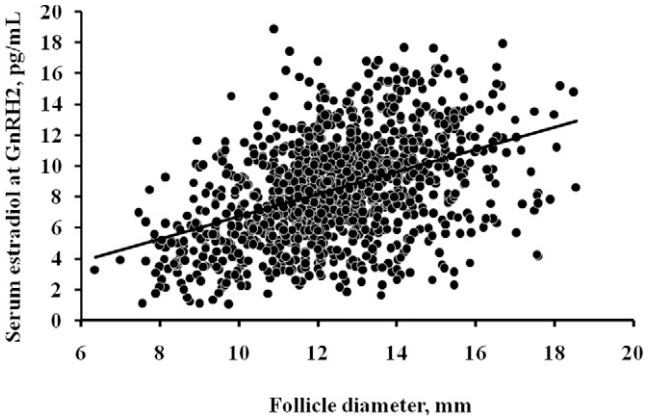

Figure 3. Scatter plot illustrating association (r =0.45, $P < 0.01$, n = 1,135) between dominant follicle diameter and serum estradiol concentration at GnRH-induced ovulation (GnRH2; d 0).

donor and recipient cows with low (<8.4 pg/mL) or high (≥8.4 pg/mL) serum estradiol concentration at GnRH2 were low into low (45%^{a}), low into high (65%^{b}), high into low (43%^{a}), and high into high (61%^{b}), respectively ($^{a,b}P < 0.02$; Fig. 5). Pregnancy maintenance from d 27 to 72 was similar (90%; $P = 0.32$) between donor/recipient cows in the preceding groups.

Alternatively, pregnancy rate was analyzed to circumvent categorizing animals based on a set concentration of estradiol. To do this, estradiol concentration at GnRH2 was included as a continuous variable in a logistical regression of pregnancy rate. Logistic regression of pregnancy rate on serum estradiol for donors and recipients was curvilinear. As recipient estradiol concentration increased, the likelihood of a transferred embryo resulting in a pregnancy at d 27 increased ($P < 0.01$); however, donor estradiol concentration was not predictive of pregnancy at d 27. A logistic regression of serum estradiol on pregnancy maintenance to d 72 was not predictive ($P > 0.10$) in donors or recipients.

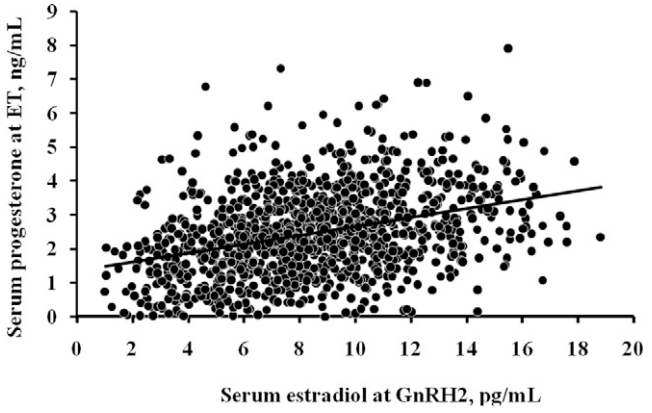

Figure 4. Scatter plot illustrating association (r = 0.34, $P < 0.01$, n = 1,134) between serum estradiol concentration at GnRH-induced ovulation (GnRH2, d 0) and serum progesterone concentration at embryo transfer (ET; d 7).

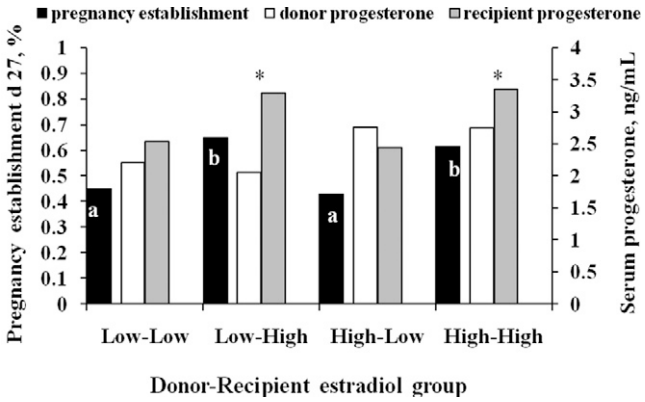

Figure 5. Relationship between donor-recipient serum estradiol concentration at GnRH-induced ovulation (GnRH2, d 0) and pregnancy establishment at d 27 (d 20 after embryo transfer; black bar), and corresponding mean serum progesterone concentrations at embryo transfer for donors (white bar) and recipients (grey bar), respectively. Pregnancy rate at d 27 for low-low (n = 78), low-high (n = 80), high-low (n = 91), and high-high (n =101) groups was 45^{a}, 65^{b}, 43^{a}, and 61^{b}%, respectively. Bars with different letters differ ($^{a,b}P < 0.02$). Asterisks (*) denote differences in mean serum progesterone concentration within donor-recipient group ($P < 0.01$).

Experiment 2

Proportion of cows exhibiting standing estrus from $PGF_{2\alpha}$ until GnRH2/AI was similar for yr 1 and 2 (18.5 and 22.0%; $P = 0.26$) respectively. Proportion of cows that exhibited estrus before GnRH2 was not different between ECP treatment groups (20.5 vs. 20.1% in ECP and Control, respectively; $P = 0.88$). Cows displaying estrus before GnRH2 were excluded from further analysis. Among cows that were subjected to intensive blood sampling in yr 3, no cows were detected in estrus before GnRH2. However, 5 of 16 and 8 of 14 of these cows in the ECP and control treatments, respectively, displayed estrus within 24 h after GnRH2 ($P = 0.10$). Cows displaying estrus had greater serum concentration of progesterone on d 12 and 14 after GnRH2 compared with cows that did not exhibit estrus ($P < 0.01$; Fig. 6).

Serum concentration of estradiol was monitored from d –2 until 96 h after ECP administration in a subset of cows in yr 3. No difference in serum estradiol between ECP and control cows was detected during this period ($P = 0.65$). However, it was determined that the estradiol-17β antibody used in the RIA did not cross-react with ECP at the dosage administered in this experiment (data not shown). Additionally, no effect of treatment with ECP on serum concentration of progesterone in the next luteal phase was detected ($P = 0.65$). A proportion of animals displayed estrus within 24 h of GnRH2/AI; however, serum concentration of estradiol and progesterone were not affected ($P > 0.10$) by the interaction between estrus and treatment throughout the experiment.

Dominant follicle growth rate from $PGF_{2\alpha}$ until GnRH2 was similar (yr 2 = 0.9 ± 0.1 mm/d compared

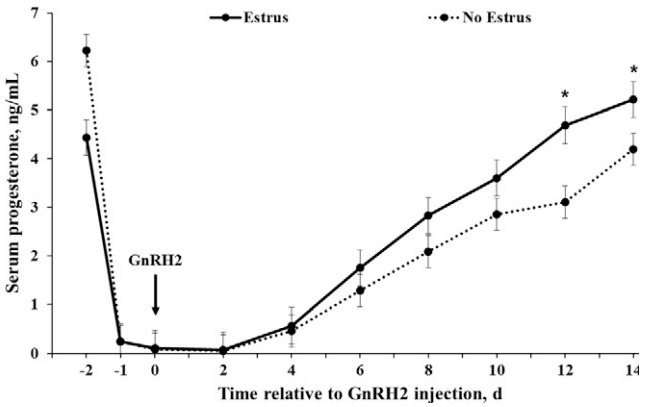

Figure 6. Mean serum progesterone concentration in cows that exhibited estrus (Estrus; $n = 13$) or did not exhibit estrus (No Estrus; $n = 17$) within 24 h after GnRH-induced ovulation (GnRH2, d 0). Prostaglandin F2α was administered d –2, estradiol cypionate on d –1, and GnRH2/AI on d 0). Asterisks (*) denote difference in mean serum progesterone within day ($P < 0.01$).

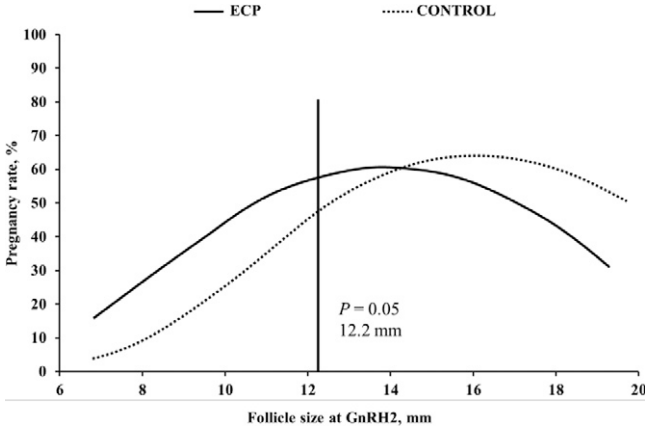

Figure 7. Logistic regression of follicle size on pregnancy rate at d 28 after AI for estradiol cypionate (ECP)-treated ($n = 297$) and no treatment (Control) cows ($n = 303$). Pregnancy rate of ECP treated cows was greater than Control cows induced to ovulate follicles <12.2 mm in diameter ($P < 0.05$).

with 0.9 ± 0.1; yr 3 = 0.7 ± 0.1 compared with 0.7 ± 0.8 mm/d) for ECP and Control groups, respectively ($P > 0.10$). Likewise, ovulatory follicle size was unaffected ($P > 0.10$) by ECP treatment in all replicates.

The effect of ECP treatment on pregnancy rate was analyzed by logistic regression with follicle size at GnRH2 as a continuous variable. There was a significant effect of year on pregnancy rate ($P < 0.01$), but interactions between year and treatment and/or follicle size did not affect pregnancy rate. Logistic regression of pregnancy rate on follicle size was curvilinear and significantly different ($P < 0.01$) for ECP and Control treatments. Compared with the Control, ECP treatment increased ($P < 0.05$) pregnancy rate of cows induced to ovulate a dominant follicle less than 12.2 mm in diameter (Fig. 7).

DISCUSSION

A major aim of the current study was to determine the association of preovulatory estradiol at time of induction of ovulation (GnRH2) in donor cows with subsequent fertilization rate, stage of embryo development, and embryo quality on d 7 after AI. In an effort to determine whether concentrations of estradiol at GnRH2 affect oocyte competence and/or the maternal environment, a reciprocal embryo transfer approach was used in Exp. 1. Donor cows with greater circulating concentrations of estradiol at induction of ovulation were more likely to yield an embryo than an unfertilized oocyte. Consequently, preovulatory follicular environment, as reflected by estradiol secretion, was shown to affect oocyte competence. Whether this effect on oocyte competence is mediated directly on the oocyte or indirectly remains to be determined. Although competence of bovine oocytes increased as follicular diameter increased (Arlotto et al., 1996), direct effects of estradiol on the maturing oocyte are equivocal. Inclusion of estradiol during in vitro maturation resulted in either a detrimental effect (Beker-van Woudenberg et al., 2004), no effect (Beker-van Woudenberg et al., 2006), or a positive effect (Tesarik and Mendoza, 1995; Kim et al., 2005) on the nuclear maturation of oocytes of various species. Evidence for in vivo effects of estradiol on oocyte nuclear maturation and ability to be fertilized was demonstrated after administration of an aromatase inhibitor. Treatment of rhesus monkeys with an aromatase inhibitor decreased circulating concentrations of estradiol by 63%, disrupted progression of meiosis in oocytes to metaphase II, and decreased in vitro fertilization rate of oocytes that reached metaphase II (Zelinski-Wooten et al., 1993). In mice, treatment with an aromatase inhibitor reduced estradiol production and in vitro fertilization rate but did not affect nuclear maturation (Hu et al., 2002).

Although the positive relationship between donor cow serum estradiol at GnRH2 and fertilization, embryo survival (to d 7), and embryo quality suggest that follicular environment improves oocyte competence, embryos and unfertilized oocytes were not collected until d 7; therefore, an early effect of the maternal environment cannot be eliminated. Increased estradiol may increase fertilization success via the maternal environment through more efficient transport of sperm and ova through the female reproductive tract to the site of fertilization (Crisman et al., 1980; Hawk, 1983). Estradiol effects on sperm may be due to a decrease in uterine pH (7.0 to 6.7; Perry and Perry, 2008a,b), which increased viable lifespan of sperm (Jones and Bavister, 2000), or by modulating timing of the acrosome reaction via estradiol binding to a nongenomic estrogen receptor (**ER**) present on sperm plasma membrane (Baldi et al., 2000). Others have reported ER transcript presence in the oo-

cyte of mice and pigs, but the maternal ER mRNA was degraded early during the maternal to zygotic transition (Wu et al., 1992; Ying et al., 2000). To our knowledge there are no reports of estradiol receptor expression in bovine embryos from the early cleavage to blastocyst stage. Therefore, potential contributions of estradiol to early embryonic development would likely be through its effects on oviductal and uterine environments.

Although the physiological maturity of an ovulatory follicle may affect fertilization rate, data from Exp. 1 and 2 provide strong support for a positive effect of estradiol on the preparation of the maternal environment for the establishment of pregnancy. In Exp. 1, increased serum estradiol at GnRH2 in recipient but not donor cows was predictive of pregnancy. Additionally, recipients that were detected in estrus within 24 h after GnRH2 had greater circulating concentrations of estradiol and pregnancy rate at d 27 than recipients not detected in estrus after GnRH2. Therefore, although cows that expressed estrus within 24 h after GnRH2 likely had a more mature follicle/oocyte at GnRH2, the fact that this variable improved pregnancy establishment only among recipients indicates that the increased serum concentration of estradiol was more important than follicle/oocyte maturity level using this model. In Exp. 2, ECP supplementation increased pregnancy rate in cows with follicles <12.2 mm in diameter at GnRH2. This positive effect of ECP on pregnancy in cows induced to ovulate smaller follicles is likely independent of an effect on the oocyte and/or cumulus cells because it is unlikely that an injection of 0.5 mg ECP on d –1 could directly alter intrafollicular concentrations of estradiol. However, ECP administration during proestrus might indirectly increase follicular synthesis of estradiol through increased LH stimulation (Stumpf et al., 1989).

In the present study, there was a positive correlation between ovulatory follicle size and serum estradiol concentration at GnRH2 in animals not exhibiting standing estrus and these data are similar to previous reports in beef heifers (Perry et al., 2007; Atkins et al., 2008) and postpartum beef cows (Perry et al., 2005; Atkins et al., 2010a,b). It is not clear whether ovulatory follicle size affects follicular estradiol secretion or vice versa, but strong correlations between size of the dominant follicle and intrafollicular estradiol concentrations during the preovulatory period have been reported previously (Ireland and Roche, 1982; Kruip and Dieleman, 1985). Although larger follicles have more theca (i.e., synthesize more androgen substrate) and granulosa (convert androgen to estradiol) cells, estradiol was also reported to increase granulosa cell mitosis (Goldenberg et al., 1972) and increase aromatase activity (Zhuang et al., 1982). In spite of the positive relationship between follicle size and estradiol, follicle size was not always predictive of serum estradiol. For example, 39% of recipient cows with small follicles were classified as having increased serum concentrations of estradiol (≥8.4 pg/mL) at GnRH2. The discrepancy between ovulatory follicle size and serum estradiol at GnRH2 may reflect differences in physiological maturity of the follicle.

Moore (1985) reported that a transient rise in estradiol was necessary for establishment of pregnancy in ovariectomized ewes that received embryos and that the uterine environment was affected by the sequential exposure of ewes to a period of progesterone priming followed by a transient increase in estradiol and a subsequent sustained increase in progesterone. Ewes that did not receive estradiol to simulate preovulatory concentrations of estradiol had decreased total protein in the uterine lumen, decreased progesterone and estrogen receptors in the endometrium, and reduced uterine weight at embryo transfer (Miller et al., 1977). Bridges et al. (2010) studied the effects of a long versus short proestrus period in beef cattle. Follicles induced to ovulate after a short proestrus (1.2 d) had shorter exposure to preovulatory estradiol and reduced pregnancy rates compared with those having a longer proestrus (2.2 d). The mechanism by which preovulatory estradiol facilitates the establishment of pregnancy is unknown; however, estradiol is known to induce endometrial progesterone receptors (Zelinski et al., 1982; Ing and Tornesi, 1997) and to induce the expression of various oviductal and uterine proteins (Bartol et al., 1981; Buhi, 2002).

In Exp. 1, cows that had greater serum estradiol concentrations at GnRH2 also had greater concentrations of progesterone on d 7. Because of this associated response, the effects of estradiol and progesterone on pregnancy establishment could only be separated by use of reciprocal embryo transfer. Preovulatory estradiol is known to induce uterine progesterone receptors, which could enhance the effects of progesterone on the endometrium. However, Atkins et al. (2013) reported that estradiol at GnRH2 affected d 27 pregnancy rate of recipient cows independent of progesterone on d 7. Furthermore, donor estradiol was not predictive of pregnancy at d 27 suggesting that the primary beneficial effect of increased estradiol on pregnancy was mediated through the maternal environment of the recipient cow after ET. Serum estradiol concentration at GnRH2 was not predictive of pregnancy maintenance to d 72 in donor or recipient cows, suggesting that the effects of estradiol on pregnancy occur before d 30.

Granulosa cells differentiate into large luteal cells (reviewed in Smith et al., 1994), which have greater basal secretion of progesterone (Hansel et al., 1987), and secrete approximately 80% of the luteal progesterone in sheep (Niswender et. al., 1985). Therefore, follicles with presumably more granulosa cells may differentiate into

CL that have more large luteal cells and subsequently secrete increased amounts of progesterone. Alternatively, increased progesterone secretion by the subsequent CL could be due to a positive effect of estradiol on the preparation of granulosa cells within the ovulatory follicle for luteinization. McNatty (1979) reported that human granulosa cells collected from follicles with increased follicular fluid concentrations of estradiol produced more progesterone after luteinization. Furthermore, luteal insufficiency occurred after premature induction of ovulation in ewes and may have been associated with inadequate preparation of follicular cells for luteinization and secretion of progesterone (Murdoch and van Kirk, 1998). Although ECP administration had no effect on postovulatory serum progesterone in Exp. 2, the increased percentage of control cows exhibiting estrus after GnRH2 may have affected our ability to detect a difference due to ECP.

Administration of GnRH1 is intended to induce ovulation and synchronize a follicular wave that will culminate in ovulation to GnRH2. In Exp. 1, cows that ovulated in response to GnRH1 had greater serum estradiol concentration at GnRH2. Cycling cows that ovulated after GnRH1 had larger follicles (11.4 vs. 9.5 mm) and tended ($P = 0.07$) to have greater serum estradiol (4.5 vs. 3.3 pg/mL) at GnRH2 than cows that did not ovulate after GnRH1 (Atkins et al., 2010a). Likewise, anestrous cows that ovulated after GnRH1 had larger follicles (12.3 vs. 11.0 mm) and greater serum estradiol (3.4 vs. 2.3 pg/mL) at GnRH2 (Atkins et al., 2010b). Therefore, synchronization of a follicular wave in response to GnRH1 likely increases estradiol secretion at GnRH2.

In summary, serum concentration of estradiol at GnRH2 was positively correlated with ovulatory follicle size and cows ovulating to GnRH1 had larger follicles and greater serum concentrations of estradiol at GnRH2 than cows not ovulating after GnRH1. Embryo donor cows with greater circulating concentrations of estradiol were more likely to yield an embryo than an unfertilized oocyte and recipient cows with greater estradiol at GnRH2 had increased pregnancy establishment. For cows that were pregnant on d 27, serum concentrations of estradiol at GnRH2 were not predictive of pregnancy maintenance to d 72. Injection of ECP 24 h before GnRH2/AI increased pregnancy rate in cows that ovulated smaller follicles (<12.2 mm) after GnRH2 and this effect was likely due to effects of estradiol on maternal environment.

LITERATURE CITED

Arlotto, T., J. L. Schwarts, N. L. First, and M. L. Leibfried-Rutledge. 1996. Aspects of follicle and oocyte stage that affect in vitro maturation and development of bovine oocytes. Theriogenology 45:943–956.

Atkins, J. A., D. C. Busch, J. F. Bader, D. H. Keisler, D. J. Patterson, M. C. Lucy, and M. F. Smith. 2008. Gonadotropin-releasing hormone-induced ovulation and luteinizing hormone release in beef heifers: Effect of day of the cycle. J. Anim. Sci. 86:83–93.

Atkins, J. A., M. F. Smith, M. D. MacNeil, E. M. Jinks, F. M. Abreu, L. J. Alexander, and T. W. Geary. 2013. Pregnancy establishment and maintenance in cattle. J. Anim. Sci. 91:722–733.

Atkins, J. A., M. F. Smith, K. J. Wells, and T. W. Geary. 2010a. Factors affecting preovulatory follicle diameter and ovulation rate after gonadotropin-releasing hormone in postpartum beef cows. Part I: Cycling cows. J. Anim. Sci. 88:2300–2310.

Atkins, J. A., M. F. Smith, K. J. Wells, and T. W. Geary. 2010b. Factors affecting preovulatory follicle diameter and ovulation rate after gonadotropin-releasing hormone in postpartum beef cows. Part II: Anestrous cows. J. Anim. Sci. 88:2311–2320.

Asdell, J. A., J. de Alba, and J. S. Roberts. 1945. The levels of ovarian hormones required to induce heat and other reactions in the ovariectomized cow. J. Anim. Sci. 4:277–284.

Baldi, E., M. Luconi, M. Muratori, and G. Forti. 2000. A novel functional estrogen receptor on human sperm membrane interferes with progesterone effects. Mol. Cell. Endocrinol. 161:31–35.

Bartol, F. F, W. W. Thatcher, G. S. Lewis, E. L. Bliss, M. Drost, and F. W. Bazer.1981. Effect of estradiol-17beta on PGF and total protein content in bovine uterine flushings and peripheral plasma concentration of 13, 14-dihydro-15-keto-PGF(2alpha). Theriogenology 15:345–358.

Beker-van Woudenberg, A. R., H. T. A. van Tol, B. A. J. Roelen, B. Colenbrander, and M. M. Bevers. 2004. Estradiol and its membrane-impermeable conjugate (estradiol-bovine serum albumin) during in vitro maturation of bovine oocytes: Effects on nuclear and cytoplasmic maturation, cytoskeleton, and embryo quality. Biol. Reprod. 70:1465–1474.

Beker-van Woudenberg, A. R., E. C. Zeinstra, B. A. J. Roelen, B. Colenbrander, and M. M. Bevers. 2006. Developmental competence of bovine oocytes after specific inhibition of MPF kinase activity: Effect of estradiol supplementation and follicle size. Anim. Reprod. Sci. 92:231–240.

Bellow, R. A., R. B. Staigmiller, J. M. Wilson, D. A. Phelps, and A. Darling. 1991. Use of bovine FSH for superovulation and embryo production in beef heifers. Theriogenology 35:1069–1082.

Bridges, G. A., M. L. Mussard, C. R. Burke, and M. L. Day. 2010. Influence of the length of proestrus on fertility and endocrine function in female cattle. Anim. Reprod. Sci. 117:208–215.

Buhi, W. C. 2002. Characterization and biological roles of oviduct-specific, oestrogen-dependent glycoprotein. Reproduction 123:355–362.

Crisman, R. O., L. E. McDonald, and F. N. Thompson. 1980. Effects of progesterone or estradiol on uterine tubal transport of ova in the cow. Theriogenology 13:141–154.

Goldenberg, R. L., J. L. Vaitukaitis, and G. T. Ross. 1972. Estrogen and follicle stimulating hormone interactions on follicle growth in rats. Endocrinology 90:1492–1498.

Hansel, W., H. W. Alila, J. P. Dowd, and X. Yang. 1987. Control of steroidogenesis in small and large bovine luteal cells. Aust. J. Biol. Sci. 40:331–347.

Hawk, H. W. 1983. Sperm survival and transport in the female reproductive tract. J. Dairy Sci. 66:2645–2660.

Hawk, H. W., and B. S. Cooper. 1975. Improvement of sperm transport by the administration of estradiol to estrous ewes. J. Anim. Sci. 41:1400–1406.

Hu, Y., R. Cortvrindt, and J. Smitz. 2002. Effects of aromatase inhibition on in vitro follicle and oocyte development analyzed by early preantral mouse follicle culture. Mol. Reprod. Dev. 61:549–559.

Ing, N. H., and M. B. Tornesi. 1997. Estradiol up-regulates estrogen receptor and progesterone receptor gene expression in specific ovine uterine cells. Biol. Reprod. 56:1205–1215.

Ireland, J. J., and J. F. Roche. 1982. Development of antral follicles in cattle after prostaglandin-induced luteolysis: Changes in serum hormones, steroids in follicular fluid, and gonadotropin receptors. Endocrinology 111:2077–2086.

Jones, J. M., and B. D. Bavister. 2000. Acidification of intracellular pH in bovine spermatozoa suppresses motility and extends viable life. J. Androl. 21:616–624.

Kesner, J. S., E. M. Convey, and C. R. Anderson. 1981. Evidence that estradiol induces the preovulatory LH surge in cattle by increasing pituitary sensitivity to LHRH and then increasing LHRH release. Endocrinology 108:1386–1391.

Kim, M. K., Y. H. Fibrianto, H. J. Oh, G. Jang, H. J. Kim, K. S. Lee, S. K. Kang, B. C. Lee, and W. S. Hwang. 2005. Effects of estradiol-17β and progesterone supplementation on in vitro nuclear maturation of canine oocytes. Theriogenology 63:1342–1353.

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

Kruip, Th. A. M., and S. J. Dieleman. 1985. Steroid hormone concentrations in the fluid of bovine follicles relative to size, quality and stage of the oestrus cycle. Theriogenology 24:395–408.

Lamb, G. C., J. S. Stevenson, D. J. Kesler, H. A. Garverick, D. R. Brown, and B. E. Salfen. 2001. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin F2α for ovulation control in postpartum suckled beef cows. J. Anim. Sci. 79:2253–2259.

McNatty, K. P. 1979. Follicular determinants of corpus luteum function in the human ovary. Adv. Exp. Med. Biol. 112:465–481.

McNatty, K. P., and R. S. Sawers. 1975. Relationship between the endocrine environment within the graafian follicle and the subsequent rate of progesterone secretion by human granulosa cells in vitro. J. Endocrinol. 66:391–400.

Miller, B. G., N. W. Moore, L. Murphy, and G. M. Stone. 1977. Early pregnancy in the ewe. Effects of oestradiol and progesterone on uterine metabolism and on embryo survival. Aust. J. Biol. Sci. 30:379–388.

Moore, N. W. 1985. The use of embryo transfer and steroid hormone replacement therapy in the study of prenatal mortality. Theriogenology 23:121–128.

Murdoch, W. J., and E. A. van Kirk. 1998. Luteal dysfunction in ewes induced to ovulate early in the follicular phase. Endocrinology 139:3480–3484.

Niswender, G. D., R. H. Schwall, T. A. Fitz, C. E. Farin, and H. R. Sawyer. 1985. Regulation of luteal function in domestic ruminants: New concepts. Recent Prog. Horm. Res. 41:101–151.

Perry, G. A., and B. L. Perry. 2008a. Effect of preovulatory concentrations of estradiol and initiation of standing estrus on uterine pH in beef cows. Dom. Anim. Endocrinol. 34:333–338.

Perry, G. A., and B. L. Perry. 2008b. Effects of standing estrus and supplemental estradiol on changes in uterine pH during a fixed-time artificial insemination protocol. J. Anim. Sci. 86:2928–2935.

Perry, G. A., M. F. Smith, M. C. Lucy, J. A. Green, T. E. Parks, M. D. MacNeil, A. J. Roberts, and T. W. Geary. 2005. Relationship between follicle size at insemination and pregnancy success. Proc. Nat. Acad. Sci. 102:5268–5273.

Perry, G. A., M. F. Smith, A. J. Roberts, M. D. MacNeil, and T. W. Geary. 2007. Relationship between size of the ovulatory follicle and pregnancy success in beef heifers. J. Anim. Sci. 85:684–689.

Smith, M. F., E. W. McIntush, and G. W. Smith. 1994. Mechanisms associated with corpus luteum development. J. Anim. Sci. 72:1857–1872.

Stumpf, T. T., M. L. Day, M. W. Wolfe, A. C. Clutter, J. A. Stotts, P. L. Wolfe, R. J. Kittok, and J. E. Kinder. 1989. Effect of estradiol on secretion of luteinizing hormone during the follicular phase of the bovine estrous cycle. Biol. Reprod. 41:91–97.

Tesarik, J., and C. Mendoza. 1995. Non-genomic effects of 17 beta-estradiol on maturing human oocytes: Relationship to oocyte developmental potential. J. Clin. Endocr. Metab. 80:1438–1443.

Welsh, T. H., L. Zhuang, and A. J. Hsueh. 1983. Estrogen augmentation of gonadotropin-stimulated progestin biosynthesis in cultured rat granulose cells. Endocrinology 112:1916–1924.

Whitman, R. H. 1975. Weight changes, body condition and beef cow reproduction. PhD Diss. Colorado State Univ., Fort Collins, CO.

Wu, T. C. J., L. Wang, and Y.-J. Y. Wan. 1992. Expression of estrogen receptor gene in mouse oocyte and during embryogenesis. Mol. Reprod. Dev. 33:407–412.

Xiao, C. W., and A. K. Goff. 1999. Hormonal regulation of oestrogen and progesterone receptors in cultured bovine endometrial cells. J. Reprod. Fert. 115:101–109.

Ying, C., W. Hsu, W. Hong, W. T. K. Cheng, and Y. C. Yang. 2000. Estrogen receptor is expressed in pig embryos during preimplantation development. Mol. Reprod. Dev. 55:83–88.

Zelinski, M. B., P. Noel, D. W. Weber, and F. Stormshak. 1982. Characterization of cytoplasmic progesterone receptors in the bovine endometrium during proestrus and diestrus. J. Anim. Sci. 55:376–383.

Zelinski-Wooten, M. B., D. L. Hess, W. L. Baughman, T. A. Molskness, D. P. Wole, and R. L. Stouffer. 1993. Administration of an aromatase inhibitor during the late follicular phase of gonadotropin treated cycles in rhesus monkeys: Effects on follicle development, oocyte maturation, and subsequent luteal function. J. Clin. Endo. Metab. 76:988–995.

Zhuang, L.-Z., E. Y. Adashi, and A. J. W. Hsueh. 1982. Direct enhancement of gonadotropin-stimulated ovarian estrogen biosynthesis by estrogen and clomiphene citrate. Endocrinology 110:2219–2221.