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Research Article

Effects of preovulatory estradiol on uterine environment and conceptus survival from fertilization to maternal recognition of pregnancy†

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Abstract

Preovulatory estradiol is known to impact embryo quality and survival. The objective of this study was to determine the effects of preovulatory estradiol on the uterine environment and conceptus survival through maternal recognition of pregnancy. Beef cows/heifers were Aled following induced ovulation. Cows were grouped into high and low preovulatory estradiol. Conceptuses were collected on day 16 nonsurgically (Rep 1; n = 20), or following slaughter (Rep 2; n = 29). Blood was collected to determine plasma glucose concentrations, and uterine luminal fluid (ULF) was analyzed for protein, glucose, and interferon tau (IFNT) concentrations. Total cellular RNA was extracted from caruncular (CAR) and intercaruncular (INCAR) endometrial tissue. There was no effect of preovulatory estradiol on conceptus recovery rate ($P = 0.38$) or on apoptosis rate in the trophectoderm ($P = 0.64$). Cows in which a conceptus was recovered had greater concentrations of protein in the ULF ($P = 0.04$). Animals with elevated preovulatory estradiol had greater endometrial abundance of SLC2A1 ($P = 0.05$) and SLC5A1 ($P = 0.04$) in both INCAR and CAR tissue. Presence of a conceptus also tended to increase ($P = 0.10$) abundance of SLC5A1 in INCAR. In CAR tissue, cows with a conceptus had decreased SLC2A4 abundance ($P = 0.05$). In summary, conceptus recovery rates, apoptosis in the trophectoderm, IFNT, glucose, and protein concentration in ULF did not differ between cows that did or did not have an increase in preovulatory estradiol concentrations. Thus, there is no indication of increased conceptus survival to day 16 of pregnancy based on estradiol concentrations.

Summary Sentence

Conceptus survival to d 16 was similar among cows with and without elevated preovulatory estradiol; however, glucose transporter expression, and glucose and protein concentration in uterine fluids were influenced by estradiol and conceptus presence.

Key words: apoptosis, conceptus, maternal recognition of pregnancy, preovulatory estradiol, trophectoderm.

Introduction

Initiation of estrus occurs due to increased circulating concentrations of estradiol at a time when progesterone is not present [1]. In the absence of progesterone, estradiol acts on the hypothalamus to induce behavioral estrus and a Luteinizing Hormone (LH) surge resulting in ovulation [2]. Preovulatory estradiol impacts follicular growth, oocyte maturation, sperm transport, uterine environment, and embryo survival [3]. Cows that exhibited standing estrus within 24 h of fixed-time artificial insemination (AI) have greater circulating preovulatory estradiol concentrations [4] and greater pregnancy success compared to cows that do not [4], and cows that exhibited estrus have increased embryo survival to day 30 of gestation [5]. Ovariectomized cows that were treated with estradiol prior to embryo transfer were more likely to maintain a pregnancy to day 29 than ovariectomized cows that were not treated with estradiol prior to embryo transfer [6]. Because there were no differences in interferon-specific gene expression between the treatments during the window of maternal recognition of pregnancy (day 17–21), the authors hypothesized that early embryonic mortality occurred between maternal recognition of pregnancy and ultrasonographic confirmation of pregnancy at day 29 in the cows that were not treated with estradiol.

There are reasons, however, to believe that estradiol may improve the uterine environment prior to recognition of pregnancy and, thereby, improve early embryonic development and pregnancy success. Cows that exhibited estrus before an induced ovulation yielded longer conceptuses on day 19 [7]. This could indicate an improved uterine environment contributing to enhanced embryonic development by the time of recognition of pregnancy that could contribute to the improved pregnancy success observed by Madsen et al. [6]. Following embryo transfer on day 4, ewes that were given a small dose (25 μg) of estradiol had decreased uterine weight, total protein content, and pregnancy success compared to ewes given a larger dose (35 μg) [8]. This indicates that estradiol may contribute to histotroph production by the uterus.

The uterine histotroph is secreted by the uterine glands and is composed of a mixture of enzymes, growth factors, cytokines, lymphokines, hormones, amino acids, proteins, and glucose. It is important for conceptus growth and development, especially during the time when the embryo is undergoing morphological changes from spherical to filamentous [9]. Placing uterine gland knockout (UGKO) ewes with fertile rams resulted in no pregnancies on day 25 after intromission [10].

Of the uterine gland secretions, glucose in particular regulates trophoblast proliferation and function [11]. It is a major energy source used by the conceptus for growth and development, and is delivered into the uterus via glucose transporters [12, 13]. Transport of glucose across the plasma membrane is mediated by facilitative and/or sodium-dependent transporters. Previous studies have focused on select glucose transporters (SLC2A1, SLC2A3, SLC2A4, and SLC5A1) when examining the uterus. SLC2A1 is a facilitative transporter that is ubiquitous in all organs, and is responsible for basal level glucose uptake in most cells. Expression of this transporter appears to be regulated by both progesterone and interferon tau (IFNT) in the glandular and superficial glandular epithelium [14]. Additionally, estradiol treatment has been known to affect glucose utilization in the rat uterus, SLC2A1 mRNA and protein increased 3- to 4-fold within 4–8 h after estradiol administration [15]. SLC2A3 is a high affinity transporter that is primarily expressed in tissues with a high demand for glucose, such as a growing and developing embryo [16]. Mice lacking the SLC2A3 gene had restricted fetal growth and failed pregnancies [17]. SLC2A4 is the most studied transporter due to its role in whole body glucose homeostasis and type II diabetes mellitus [18]. SLC5A1 is a sodium-dependent glucose transporter that may function to transport sodium, urea, and water as a uniporter [19]. Expression of this transporter appears to be regulated by progesterone [14]. It has been suggested in pregnant ewes that facilitative transporters (SLC2A1, SLC2A3, SLC2A5) may transport glucose from the plasma across the basal and lateral membranes into endometrial epithelial cells, while sodium-dependent transporters (SLC5A1) may facilitate glucose transport from endometrial epithelial cells across the apical membrane into the uterine luminal fluid (ULF) between days 10 and 16 of gestation [14]. Both transporter types most likely synergistically work together to optimize glucose transport into the uterine lumen where it can be utilized for conceptus growth and development [14]. Therefore, the objective of this current study was to determine the impact of preovulatory estradiol prior to an induced ovulation on uterine environment and conceptus survival from fertilization to maternal recognition of pregnancy. Specifically, we hypothesized that beef cows that had greater circulating concentrations of estradiol at estrus would have a more suitable uterine environment for conceptus survival to day 16.

Materials and methods

Animals

All procedures were approved by the South Dakota State University Institutional Animal Care and Use Committee.

Treatments

Reproductive cycles of Angus crossed beef cows/heifers (Rep 1: n = 30, Rep 2: n = 40) at the South Dakota State University Beef Breeding Unit were synchronized with a CO-Synch protocol [GnRH administered (100 μg as 2 mL of Factrel i.m.; Pfizer Animal Health, Madison, NJ) on day −7, followed by PGF2 alpha (PG; 25 mg as 5 mL of Lutalyse i.m.; Pfizer Animal Health) on day −2, and on day 0, cows were administered GnRH (100 μg as 2 mL of Factrel i.m.; Pfizer Animal Health) and artificially inseminated]. Estrus was monitored visually from day 0 through day 3 with the aid of Estro-Tect patches (Western Point, Inc., Apple Valley, MN). Cows were grouped into either high E2 (Rep 1: n = 15 Rep 2: n = 12) and low E2 (Rep 1: n = 5 Rep 2: n = 17) based on preovulatory estradiol concentrations and expression of estrus (day −2 to day 0). The threshold estradiol concentration that distinguished the two groups was 4.9 pg/mL. Previous research used a similar cutoff when evaluating changes in ovarian function associated with concentrations of estradiol before a GnRH-induced ovulation in beef cows [20].

Table 1. Genes, primer sequences, and product size for genes amplified during RTPCR.

Gene	Primer	Primer Sequence	Product Size
SLC2A1	Forward	5′ -TAACCGCAACGAGGAGAACC-3′	227
	Reverse	5′ -AGAAAACAGCGTTGATGCCG-3′	
SLC2A4	Forward	5′ -AGTTCCTAAGACAAGATGCCG-3′	103
	Reverse	5′ -AGAATACGCCAAGGACCAAG-3′	
SLC5A1	Forward	5′ -TCACCGCCCTTTACACAATC-3′	132
	Reverse	5′ -CACCATACCCTCCCACTTC-3′	
SLC2A5	Forward	5′ -CCATTCATCCAAGTGGGCCT-3′	203
	Reverse	5′ -GTCGACGGTGGAAACTCCTT-3′	
GAPDH	Forward	5′ -GATTGTCAGCAATGCCTCCT-3′	94
	Reverse	5′ -GGTCATAAGTCCCTCCACGA-3′	

Ultrasonography and detection of estrus

Follicular dynamics were assessed by transrectal ultrasonography using an Aloka 500V ultrasound with a 7.5-MHz linear probe (Aloka, Wallingford, CT) on day −9, day 0, and day 3 to characterize follicular development and ovulation. All follicles on each ovary >8 mm in diameter were recorded, and only cows that ovulated following the GnRH injection at fixed-time AI were utilized in the study (Rep 1: n = 20, Rep 2: n = 29). Ovulation was defined as the disappearance of a previously recorded large follicle, and confirmed by changes in circulating concentrations of progesterone. Only animals that ovulated were then used for the subsequent analyses discussed below.

Blood sampling and radioimmunoassay

Blood samples were collected by venipuncture of the jugular vein into 10 mL Vacutainer tubes (Fisher Scientific, Pittsburgh, PA). For the first replicate, blood was collected on day −2, day −1, day 0, then every other day through day 16. For the second replicate, blood was collected on day −2, day −1, day 0, then every other day through day 15. Blood was centrifuged at 1200 × g for 30 min at 4°C, and plasma was collected and stored at −20°C. Plasma collected on days 0, 5, 8, 10, 12, and 16 were later analyzed for glucose concentration using the Glucose Liquicolor assay (Boerne, TX). Radioimmunoassays were also performed on plasma samples to determine circulating progesterone concentrations [21]. Intra- and interassay CVs were 4.9% and 7.5% and 6.0% and 13.2% for replicate 1 and 2, respectively, and assay sensitivity was 0.4 ng/mL. Plasma concentrations of estradiol were determined within replicate by a single assay [22]. Intraassay CVs were 5.03% and 4.76%, for replicate 1 and 2, respectively. Assay sensitivity was 0.5 pg/mL.

Conceptus recovery

In replicate 1, uteri were flushed nonsurgically using a modified Foley catheter. The catheter was inserted into the vagina through the cervix, and into the uterus. A syringe was used to inflate the balloon; cows were flushed with 100 mL of flush media to maintain a constant volume. The uteri were massaged, and fluid drained through a filter above a conical tube. Flush media was assessed under a microscope at ×10 to determine whether a conceptus was present or not. If no conceptus was recovered, more flush media was added and this additional media was collected separately. The trophectoderm (high E2: n = 6, low E2: n = 3) was separated from the embryo proper, and was then stored at −80°C until BrdU staining.

In replicate 2, reproductive tracts were collected from the abattoir immediately following slaughter on day 16, and kept on ice. An incision was made at the anterior end of the uterine horn contralateral to the corpus luteum, a plastic tube was placed in the uterine tip and sutured to prevent any fluid loss while the other horn was clamped off. The uterine horns were flushed with 30 mL of flush media, and then massaged for equal fluid distribution in the uterus. The uterine flush was then collected in a 50 mL conical tube, and examined under a microscope at ×10 to determine if a conceptus was present. The trophectoderm (high E2: n = 6, low E2: n = 3) was separated from the embryo proper, and was then stored in OCT at −80°C until BrdU staining.

Tissue collection, RNA extraction, and RT-PCR

In replicate 2, the ipsilateral uterine horn was cut anterior to the bifurcation, and then cut open to expose intercaruncular and caruncular tissue, which were collected and snap-frozen for later analysis of glucose transporter transcript abundance. Caruncular and intercaruncular endometrial tissue samples were homogenized prior to RNA isolation. Total cellular RNA was extracted using the Qiagen RNeasy Plus Mini Kit (Austin, TX) following the manufacturer's instructions. Pure RNA was dissolved in nuclease-free water, and a spectrophotometer (NanoDrop Technologies, Wilmington, DE) was used to determine RNA concentration for each sample. The RNA samples were then stored at −80°C. The RNA was diluted to 70 ng/μl and RT-PCR was performed in duplicate using iScript One-Step RT-PCR Kit with SYBR Green (BioRad) and Stratagene MX 3005P QPCR machine. Expression of SLC2A1, SLC2A4, SLC2A5, and SLC5A1 was measured using primers described in Table 1, with GAPDH used as an endogenous reference gene. All of the primers were diluted to a concentration of 10 μM. Each plate contained negative controls to assure no background contamination. The PCR program was 10 min at 50°C and 1 min at 95°C for inactivation of reverse transcriptase. Transcription was then followed by 15 s at 95°C for melting, 30 s at 60°C for annealing, and elongation for 40 cycles. All CVs were <21%. Amplicons were electrophoresed on 2% agarose gels to determine product size and were verified for identity by sequencing (Iowa State Genomics Core). Primers were previously published for SLC2A1, SLC2A5 [23], SLC2A4, and SLC5A1 [24].

Uterine luminal fluid analyses

Uterine luminal fluid underwent a 1:100 volumetric dilution. The glucose Liquicolor Kit (Boerne, TX) was used to determine glucose concentrations according to the manufacturer's instructions. Total protein concentration in the ULF was determined using the Micro BCA Protein Assay Kit (Rockford, IL) according to the manufacturer's directions. Interferon tau concentrations were determined for all ULF samples in Thomas Spencer's laboratory at the University of Missouri using a semiquantitative western blot method [25].

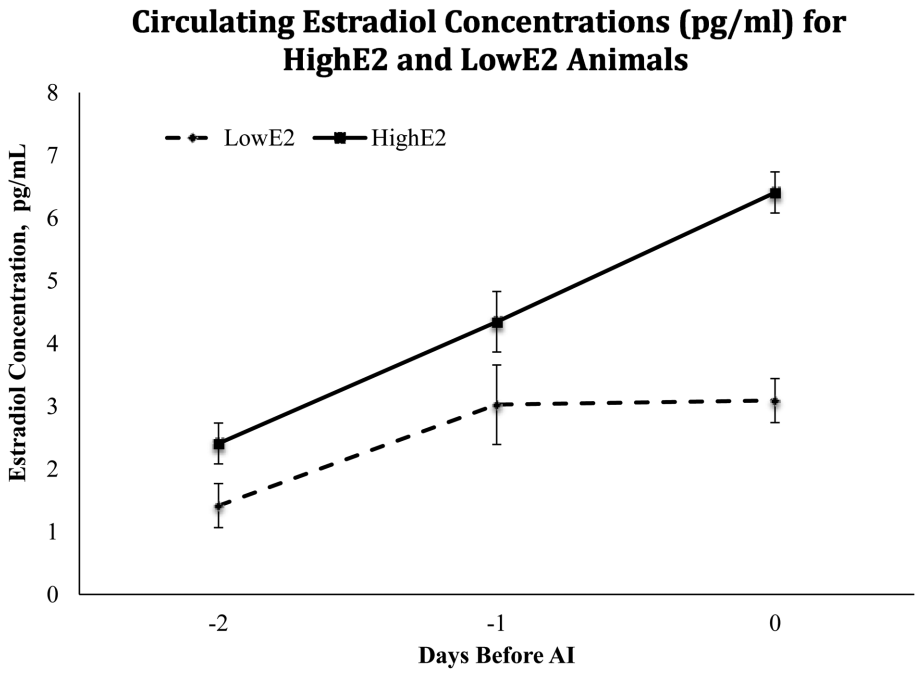

Figure 1. Circulating estradiol concentrations (pg/mL) for high E2 and low E2 animals. There was an effect of treatment, time, and treatment by time ($P < 0.01$) on plasma estradiol concentrations between high E2 and low E2 animals. There was no effect of replicate ($P = 0.70$).

Fluorescence BrdU staining of the trophectoderm

Trophectoderm was frozen in a 15 × 15 × 5 mm block (Fischer Healthcare, Houston, TX) with OCT prior to cryosectioning. Sections (7 μm) of trophectoderm were cut at −20°C using a Leica CM1850 UV cryostat and mounted onto warmed microscope slides (Thermo Scientific, Kalamazoo, MI). Tissue was stained using the Abcam In Situ BrdU-Red DNA Fragmentation (TUNEL) Assay Kit (Cambridge, UK) following the manufacturer's instructions. Instead of using the optional counter stain in the procedure, 50 μl of diluted DAPI (Thermo Scientific, Rockford, IL) was used for 1 min to stain normal trophoblastic cells. A Nikon M379E microscope was used to detect fluorescence. Three to five separate sections per conceptus were analyzed. Normal cells (blue) and apoptotic cells (red) were counted for each section to determine the percentage of apoptotic cells.

Statistical analysis

Circulating concentrations of progesterone, estradiol, and glucose were analyzed by analysis of variance for repeated measures using the MIXED procedure in SAS [26]. All covariance structures were modeled in the initial analysis. The indicated best fit covariance structure (Toeplitz) was used for the final analysis. The model included the independent variables of treatment, time, and the treatment × time interaction. The effects of treatment on circulating concentrations of progesterone, estradiol, and glucose were analyzed using animal within treatment as the error term, and effects of time and treatment by time were analyzed using treatment within pen as the error term.

The MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) was used for statistical analyses of uterine protein, glucose, and IFNT concentrations. The statistical model included treatment (high E2 or low E2), pregnancy status (conceptus or no conceptus), time, and all interactions. Replicate was included as a covariate. Relative abundance of day 16 glucose transporters was determined using the 2-VVCT method [27]. Transcript abundance was corrected for transcript abundance of the internal reference gene GAPDH, and the analyzed using the MIXED procedure of SAS. The statistical model included treatment (high E2 or low E2), pregnancy status (conceptus or no conceptus), time, and all interactions. Differences were considered to be significant when $P \leq 0.05$ and a tendency when $P > 0.05$ but $P \leq 0.10$. Conceptus recovery rate and percentage of apoptosis in the trophectoderm were analyzed using the GLIMMIX procedure of SAS, and included replicate in the statistical model.

RESULTS

Hormone profiles

Plasma estradiol concentrations during the preovulatory period were different between high E2 and low E2 animals ($P < 0.01$; Figure 1); however, plasma estradiol concentrations did not differ between animals that did and did not have a conceptus recovered on day 16 after insemination ($P = 0.51$; Figure 2). There was no difference in plasma progesterone concentrations between high E2 and low E2 animals ($P = 0.88$; Figure 3), nor was there a difference among animals that did and did not have a conceptus recovered on day 16 after insemination ($P = 0.58$; Figure 4).

Conceptus survival to day 16

There was no difference in conceptus recovery rates between high E2 and low E2 animals ($P = 0.38$; 43% vs 30%), nor was there a difference in recovery rates between replicates ($P = 0.56$; 45% vs 39%). Interferon tau concentrations in the ULF did not differ ($P = 0.70$) between high E2 and low E2 animals (1939,981 ± 286,251 and 1768,286 ± 336,481 volume intensity, respectively), nor was there a difference between animals that did and did not have a conceptus recovered ($P = 0.65$; 1953,655 ± 347,977 and 1754,612 ± 264,583 volume intensity, respectively). There was also no effect of replicate ($P = 0.22$; 31.6 ± 4.3% vs 25.8 ± 4.6%), estradiol ($P = 0.64$;

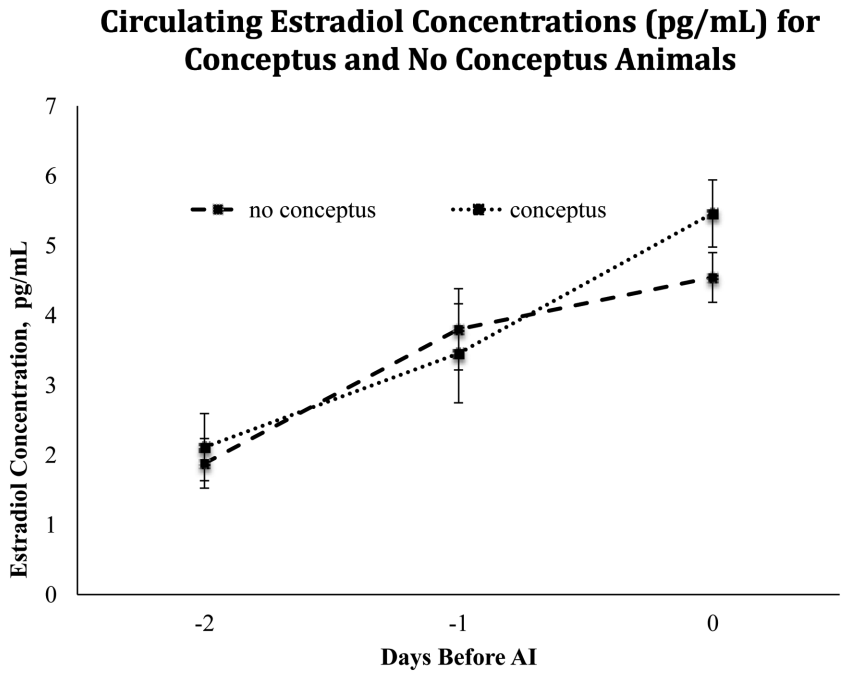

Figure 2. Circulating estradiol concentrations (pg/mL) for animals that did and did not have a conceptus recovered. There was no difference in estradiol concentrations between the conceptus and no conceptus animals ($P = 0.51$), while there was an effect of time ($P < 0.01$). There was no effect of replicate ($P = 0.17$) or time by conceptus interaction ($P = 0.46$).

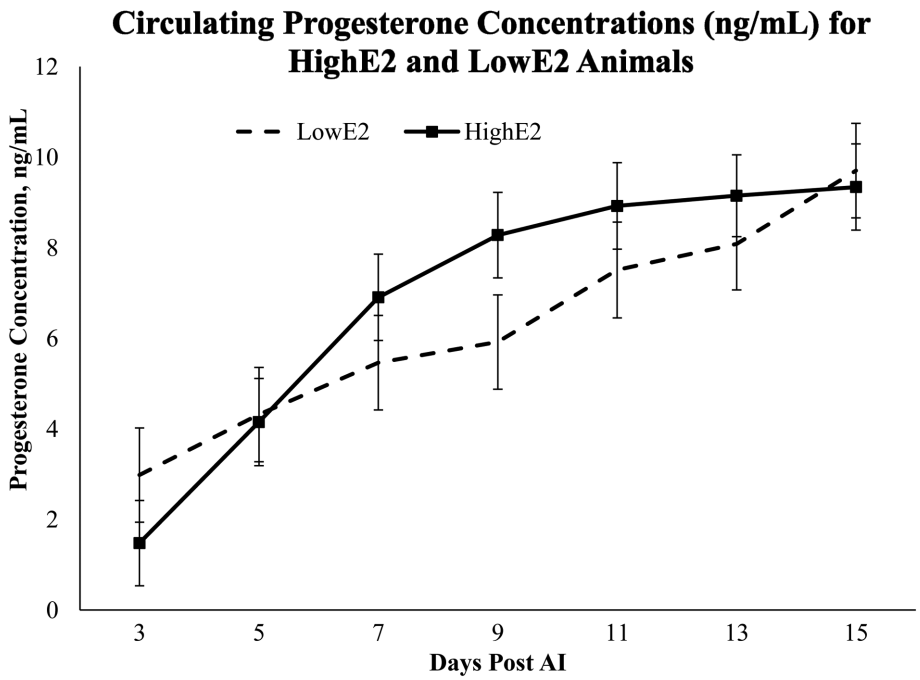

Figure 3. Circulating progesterone concentrations (ng/mL) among high E2 and low E2 animals. There was no difference in circulating progesterone concentrations among high E2 and low E2 animals ($P = 0.88$), while there was an effect of time ($P < 0.01$), treatment by time ($P = 0.05$), and replicate ($P < 0.01$).

29.8 ± 4.0% vs 27.6 ± 4.9% for estrus cows), or estradiol by replicate interaction ($P = 0.13$) on apoptosis rate in the trophectoderm.

Uterine flush media analysis

Total protein concentration in ULF was greater ($P = 0.04$) among animals that a conceptus was recovered from (2750 ± 599 and 1189 ± 456 mcg/mL, respectively). However, there was no difference ($P = 0.65$) in total protein concentration among high E2 and low E2 animals (2145 ± 493 and 1794 ± 580 mcg/mL, respectively). Glucose concentration in ULF did not differ among high E2 and low E2 animals ($P = 0.16$; 54.44 ± 1.33 and 51.46 ± 1.59 mg/dL, respectively); however, animals that had a conceptus tended to have decreased glucose concentration in

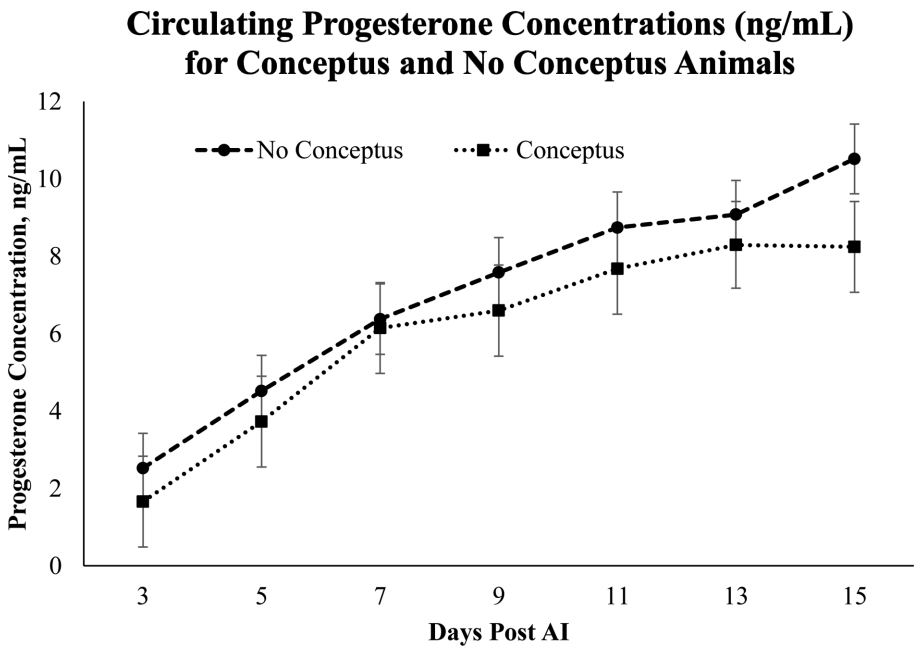

Figure 4. Circulating progesterone concentrations (ng/mL) among animals that did and did not have a conceptus recovered. There was no difference in progesterone concentrations between the conceptus and no conceptus animals ($P = 0.58$), while there was an effect on time ($P < 0.01$) and replicate ($P < 0.01$)

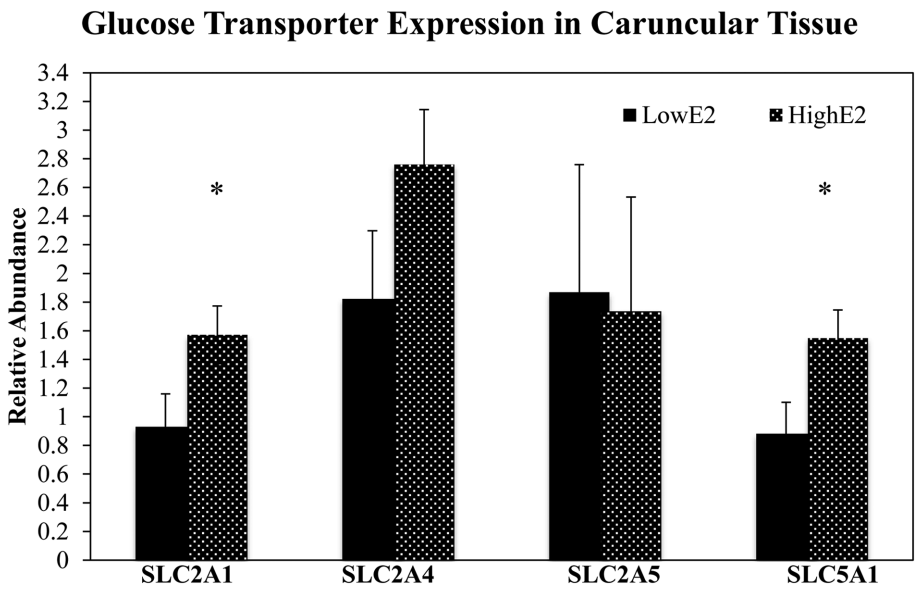

Figure 5. Glucose transporter expression (SLC2A1, SLC2A4, SLC2A5, SLC5A1) in caruncular endometrial tissue among high E2 and low E2 animals. High E2 animals had greater SLC2A1 and SLC5A1 mRNA abundance ($P < 0.05$).

their ULF ($P = 0.10$; 51.25 ± 1.62 and 54.65 ± 1.25 mg/dL, respectively).

Glucose transporter transcript abundance

In caruncular endometria, high E2 animals had greater abundance of SLC2A1 ($P = 0.05$) and SLC5A1 ($P = 0.04$) mRNA (Figure 5), but there was no difference in SLC2A1 and SLC5A1 mRNA abundance between conceptus and no conceptus animals ($P > 0.13$; Figure 6). Animals from which a conceptus was recovered had decreased SLC2A4 mRNA abundance ($P = 0.04$; Figure 6), while there was no difference in SLC2A4 transcript abundance between high E2 and low E2 animals ($P = 0.15$; Figure 5). There was no difference in SLC2A5 mRNA abundance between high E2 and low E2 animals ($P = 0.91$; Figure 4), nor between conceptus and no conceptus animals ($P = 0.58$; Figure 6) in caruncular tissue.

In intercaruncular tissue, there was no difference in SLC2A4 and SLC2A5 mRNA abundance between high E2 and low E2 animals, nor between conceptus and no conceptus animals ($P > 0.20$; Figures 7 and 8). The presence of a conceptus tended to increase ($P = 0.10$)

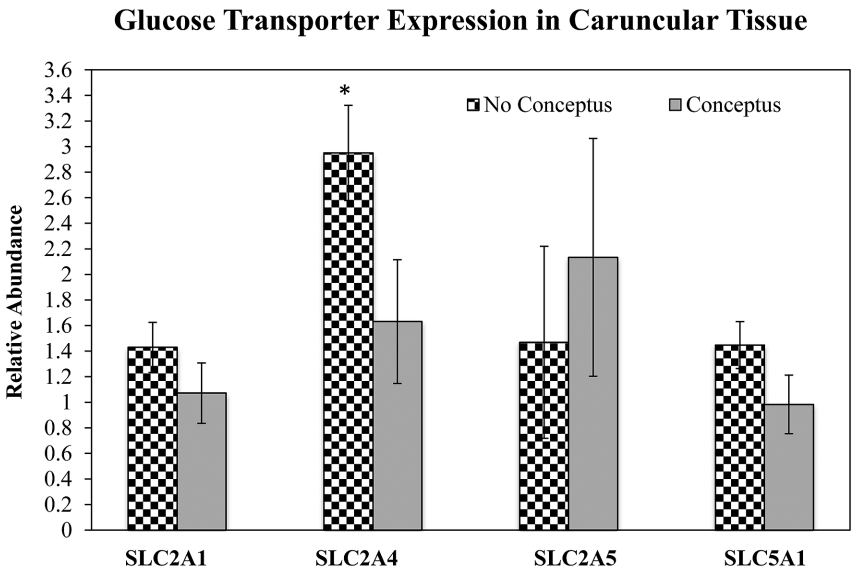

Figure 6. Glucose transporter expression (SLC2A1, SLC2A4, SLC2A5, SLC5A1) in caruncular endometrial tissue among animals that did and did not have a conceptus recovered. Animals that had a conceptus recovered had decreased SLC2A4 abundance ($P < 0.05$).

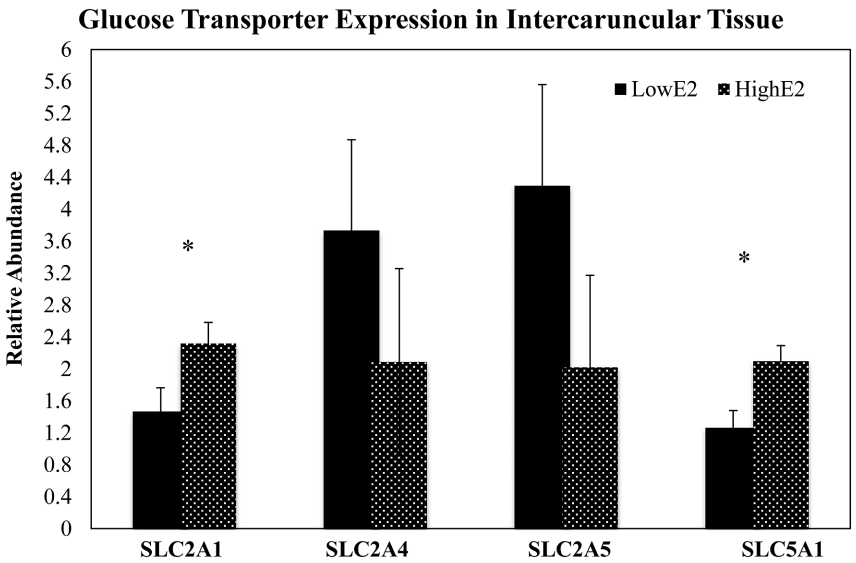

Figure 7. Glucose transporter expression (SLC2A1, SLC2A4, SLC2A5, SLC5A1) in intercaruncular endometrial tissue among high E2 and low E2 animals. High E2 animals had greater SLC2A1 and SLC5A1 mRNA abundance ($P < 0.05$).

abundance of SLC5A1 mRNA in intercaruncular tissue (Figure 8), while high E2 animals had increased SLC5A1 mRNA abundance ($P < 0.01$; Figure 7). There was no difference in SLC2A1 abundance between conceptus and no conceptus animals ($P = 0.17$; Figure 8), while high E2 animals had greater SLC2A1 abundance in intercaruncular tissue ($P < 0.05$; Figure 7).

Plasma glucose concentration

There was an effect of replicate on mean plasma glucose concentration ($P < 0.02$; 71.91 ± 1.26 and 67.89 ± 1.09 mg/dL, respectively); however; there was no effect of time ($P = 0.80$). High E2 animals had decreased plasma glucose concentrations compared to low E2 animals ($P = 0.02$; 67.99 ± 1.02 and 71.81 ± 1.28 mg/dL, respectively), and animals that had a conceptus recovered had increased glucose concentrations compared to no conceptus animals ($P = 0.02$; 71.76 ± 1.29 and 68.04 ± 0.98 mg/dL, respectively). There was an estradiol by conceptus interaction ($P = 0.04$). The low E2 cows with a conceptus had a greater mean plasma glucose concentration compared to the other three groups ($P < 0.006$). There was no correlation between uterine and plasma glucose concentrations in either replicate on day 10, day 12, and day 16 ($P > 0.10$).

DISCUSSION

Previous research reported that ovariectomized cows that were exposed to estradiol prior to progesterone treatment were more likely to maintain pregnancy to day 29, and it was hypothesized that conceptus survival to maternal recognition of pregnancy was similar

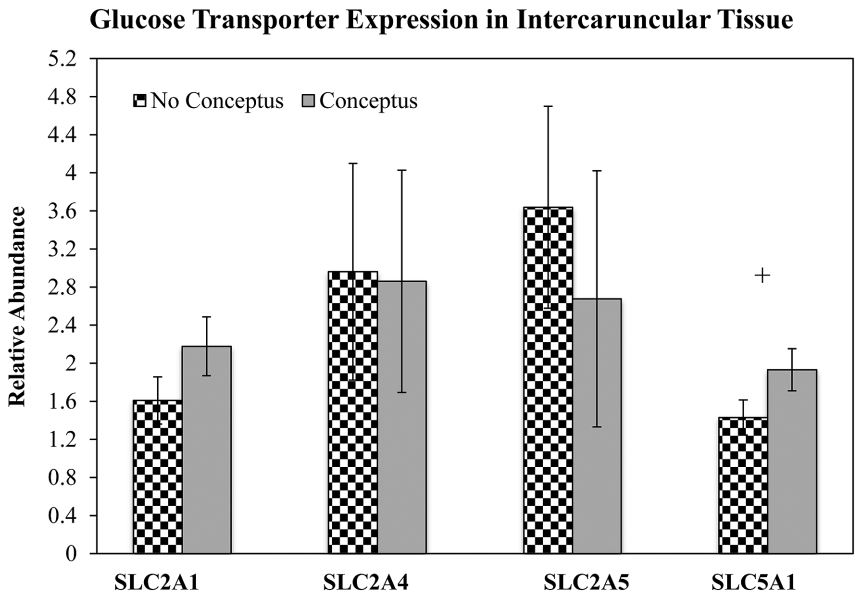

Figure 8. Glucose transporter expression (SLC2A1, SLC2A4, SLC2A5, SLC5A1) in intercaruncular endometrial tissue among animals that did and did not have a conceptus recovered. Presence of a conceptus tended to decrease abundance of SLC5A1 mRNA ($P < 0.10$).

between cows that were and were not exposed to estradiol [6]. The results of the present study clearly demonstrate that conceptus survival to maternal recognition of pregnancy is not influenced by circulating preovulatory estradiol concentrations. It has been well established that the initiation of estrus occurs due to increased circulating concentrations of estradiol at a time when progesterone is not present [1], and as demonstrated in the current study and previously [28], during a fixed-time AI protocol cows that exhibited estrus had greater preovulatory estradiol concentrations compared to cows that did not exhibit estrus. In the current study, however, the percentage of conceptuses recovered on day 16 after insemination did not differ between cows with high E2 and cows with lowE2.

In cattle, maternal recognition of pregnancy occurs around day 16 after estrus [29]. The conceptus must produce and secrete IFNT, which acts on the uterus and ovary to ensure the maintenance of a functional corpus luteum so progesterone production and pregnancy can be maintained [30]. Intrauterine IFNT concentrations were increased from day 14 to day 18 among pregnant cows, and were positively correlated with embryo size [31]. In the current study, there was no difference in conceptus recovery rates or in concentrations of IFNT in the ULF between high E2 and low E2 animals. Furthermore, apoptosis is critical for normal differentiation and development of the embryo; however, the importance of apoptosis past the hatched blastocyst stage and the impact of increased preovulatory estradiol concentrations have not been well characterized. In the current study, there was no difference in the rate of apoptosis of the trophectoderm between high E2 and low E2 animals. Taken together, these data strongly indicate that there is no difference in early embryonic development prior to recognition of pregnancy between high E2 and low E2 animals.

The corpus luteum is the main source of progesterone, and it is essential for the maintenance of pregnancy [32]. Adequate progesterone secretion is necessary for stimulating endometrial secretions, embryo growth and development, and maintenance of pregnancy by altering endometrial gene expression [33]. The postovulatory rise of progesterone is associated with an increase in pregnancy success [34]. The current study found no differences in circulating progesterone concentrations between high E2 and low E2 animals, and there was also no difference in animals that did and did not have a conceptus recovered.

For embryo survival to occur the maternal uterine environment needs to provide sufficient nutrients to the developing embryo, these nutrients are provided in what is known as the uterine histotroph. The uterine histotroph is composed of a mixture of enzymes, growth factors, cytokines, lymphokines, hormones, amino acids, proteins, and glucose [11]. Estradiol has been reported to induce endometrial receptors and expression of uterine proteins [35], as well as induce the expression of many genes involved in uterine extracellular matrix remodeling that are necessary for embryo growth and a successful pregnancy [36]. In addition, IFNT has also been reported to influence uterine gene expression [37]. When pregnant and cyclic ewes were compared, 30 genes were upregulated and 9 were downregulated during pregnancy. Many of the upregulated genes were associated with antiviral responses, while the downregulated genes were associated with regression of the corpus luteum. Davoodi et al. [7] reported that pathways associated with maternal immune system suppression, attachment between the endometrium and conceptus, and corpus luteum (CL) maintenance were favorably expressed in cows that exhibited estrus near the time of AI. Miller and Moore [8] conducted a study administering small and large doses of estradiol; ovariectomized ewes given a small dose of estradiol had decreased uterine weight, total protein content, and pregnancy success prior to reaching maternal recognition of pregnancy compared to ewes given a larger dose. However, preovulatory estradiol concentrations did not cause differences in ULF protein concentration in the current study on day 16 of pregnancy, while animals that had a conceptus recovered had greater protein concentrations in the ULF compared to animals from which no conceptus was recovered. The difference between the current study and the study by Miller and Moore is likely in the timing of uterine collection. They examined the uterus prior to maternal recognition of pregnancy, and in the current study uteri were flushed/collected after maternal recognition had occurred.

Thus, the stimulation of uterine genes by IFNT may have masked a difference in uterine protein between high E2 and low E2 animals.

Glucose is a major fuel source used by the conceptus, and is transported into the uterus via glucose transporters [12, 13]. Glucose can then be used by the conceptus to make glycogen, nucleic acids, proteins, and lipids during the peri-implantation period [11]. In sheep, total glucose content in uterine flushes has been reported to increase sixfold between days 10 and 15 of gestation, during this time period the embryo is undergoing morphological changes from spherical to filamentous [9]. In the current study, glucose concentrations in the ULF did not differ between high E2 and low E2 animals. However, cows from which a conceptus was recovered had decreased glucose concentration in the ULF, and increased mean glucose concentration in their plasma. This may serve as a possible mechanism to avoid excess glucose, which has been reported to negatively impact stem cell differentiation in mice [38] and to negatively impact implantation in humans [39]. There was no correlation between uterine and plasma glucose concentration so therefore the differences in glucose concentration are most likely due to changes in specific glucose transporters.

Transport of glucose across the plasma membrane is mediated by facilitative and/or sodium-dependent transporters. Glucose transporters are found in various tissues throughout the body [40]. Previous studies have focused on select glucose transporters (SLC2A1, SLC2A3, SLC2A4, and SLC5A1) when examining the uterus. The expression of these select transporters differed between cyclic and pregnant ruminants [14]. In the present study, in caruncular endometria, high E2 animals had greater abundance of SLC2A1 and SLC5A1 mRNA, but there was no difference in SLC2A4 and SLC2A5 abundance between high E2 and low E2 animals. In intercaruncular tissue, there was no difference in SLC2A4 and SLC2A5 mRNA abundance between high E2 and low E2 animals, while high E2 animals had increased SLC2A1 and SLC5A1 mRNA abundance. In caruncular endometria, there was no difference in SLC2A1, SLC2A5, and SLC5A1 mRNA abundance between conceptus and no conceptus animals. However, animals from which a conceptus was recovered had decreased SLC2A4 mRNA abundance. In intercaruncular tissue, there was no difference in SLC2A1, SLC2A4, and SLC2A5 mRNA abundance, but presence of a conceptus tended to increase abundance of SLC5A1 mRNA. A recent study reported that SLC2A1 mRNA was increased in pregnant ewes starting at day 10 compared to cyclic ewes [14]. Furthermore, it was reported that expression of SLC2A1 appeared to be regulated by both progesterone and IFNT. According to Gao et al., [14] SLC2A4 mRNA abundance also increased in pregnant ewes between day 10 and 18 of pregnancy, and treatment of ovariectomized ewes with progesterone and IFNT increased SLC2A4 mRNA levels 1.9-fold. Pregnant ewes had an increase in endometrial expression of SLC5A1 mRNA between day 10 and 12 of the cycle, and expression remained elevated through day 16 [14]. However, in the present study there was no difference in circulating concentrations of progesterone or concentrations of IFNT in the ULF. Furthermore, the reduction in SLC2A4 may indicate the ability of the conceptus to partially regulate glucose in the uterine environment. It was reported that there was no difference in SLC2A1, SLC2A4, SLC2A5, and SLC5A1 transcript abundance or in uterine concentrations of glucose on day 10 between large follicle (LF)-large CL (LCL) cows versus small follicle (SF)-small CL (SCL) cows. However, large follicle cows had greater plasma estradiol concentrations on day −2, day −1, and day 0 compared to SF-SCL cows [41]. The endocrine environment along with the conceptus itself helps to induce uterine changes that are necessary for the growth and development of the conceptus. Estradiol, progesterone, and IFNT most likely play a role in modulating endometrial levels of glucose transporter transcripts. Differential expression of some of these glucose transporters in caruncular and intercaruncular endometrium suggests that they may have a tissue/cell specific role in glucose metabolism during pregnancy. Both the facilitative and sodium-dependent transporters likely function synergistically in the different tissue types to avoid excess glucose transport from the plasma into the uterine lumen.

In summary, there were no differences between high E2 and low E2 animals in conceptus survival to day 16 based on recovery rates, apoptosis in the trophectoderm of conceptuses, and IFNT concentrations. However, glucose transporter expression in the endometrium, and also glucose and protein concentration in ULF were influenced by preovulatory estradiol concentrations and conceptus presence. From a physiology perspective, the most important differences are the differences in the uterine environment (glucose and protein concentrations) and the lack of difference in conceptus survival. It remains possible that the differences in glucose and protein concentrations could still lead to conceptus loss sometime after day 16. It is this difference in glucose and protein concentrations that actually from a physiological perspective suggests and supports the mRNA results that there are differences in transporter abundance causing these physiological differences. This study provides the discovery work to suggest that increased pregnancy success among animals that exhibit estrus prior to fixed-time AI (high E2) is not a result of increased conceptus survival to maternal recognition of pregnancy and must be regulated by differences is conceptus survival after day 16. Further research is necessary to determine the timing and mechanism by which embryonic loss occurs in cattle.

Acknowledgment

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