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Changes in ovarian function associated with circulating concentrations of estradiol before a GnRH-induced ovulation in beef cows

E.L. Larimore [a], O.L. Amundson [a], G.A. Bridges [b,†], A.K. McNeel [c,‡], R.A. Cushman [c], G.A. Perry [a,*]

[a] Department of Animal Science, South Dakota State University, Brookings, SD, USA
[b] North Central Research and Outreach Center, University of Minnesota, Grand Rapids, MN, USA
[c] USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE, USA

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ABSTRACT

These studies were conducted to evaluate causes for differences in circulating concentrations of estradiol before a GnRH-induced ovulation. Beef cows were synchronized by an injection of GnRH on day −7 and an injection of prostaglandin F2α ($PGF_{2\alpha}$) on day 0. In experiment 1, blood samples were collected every 3 h from $PGF_{2\alpha}$ on day 0 to hour 33 after $PGF_{2\alpha}$ and at slaughter (hour 36 to 42; n = 10). Cows were assigned to treatment group based on circulating concentrations of estradiol (E2): HighE2 vs LowE2. At slaughter, follicular fluid (FF) and granulosa cells were collected from the dominant follicle. In experiment 2, blood samples (n = 30) were collected every 8 h from $PGF_{2\alpha}$ until the dominant follicle was aspirated via ultrasound-guided follicular aspiration to collect FF and granulosa cells (hour 38 to 46). In experiment 1, HighE2 had increased abundance of 3β-hydroxysteroid dehydrogenase, cytochrome P450 aromatase, and LHR ($P \leq 0.02$), and greater concentrations of estradiol and androstenedione ($P \leq 0.02$) in the FF. In experiment 2, HighE2 had increased abundance of CYP11A1, 3β-hydroxysteroid dehydrogenase, cytochrome P450 aromatase, and LHR ($P \leq 0.03$) vs either LowE2 or GnRHLowE2. There was a tendency ($P = 0.07$) for LH pulse frequency to be increased in both the GnRHLowE2 and HighE2 compared with LowE2. HighE2 cows experienced increas in circulating concentrations of estradiol compared with LowE2. In conclusion, animals with greater concentrations of circulating estradiol before fixed-time AI experienced an upregulation of the steroidogenic pathway during the preovulatory period.

Names are necessary to report factually on available data; however, either South Dakota State University or the USDA neither guarantees nor warrants the standard of the product, and the use of names by South Dakota State University or the USDA implies no approval of the product to the exclusion of others that may also be suitable.

* Corresponding author. Tel.: 605-688-5456; fax: 605-688-6170.
E-mail address: george.perry@sdstate.edu (G.A. Perry).
† Current Address: GAB: Elanco Animal Health, Greenfield, IN.
‡ Current Address: Zoetis Animal Genetics, Kalamazoo, MI.

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1. Introduction

Previous reports have indicated increased circulating concentrations of estradiol before GnRH-induced ovulation improved conception rates and pregnancy maintenance in beef cattle [1–3]. Estradiol may improve pregnancy success in cattle by improving oocyte maturation and competence [4], increasing sperm transport to the site of fertilization [5,6], advancing embryo development [6], and promoting a uterine environment more conducive to maintaining pregnancy [3].

Circulating concentrations of estradiol during the preovulatory period have been correlated to size of the ovulatory follicle [2]; however, some large dominant follicles (DFs) fail to produce elevated concentrations of estradiol, whereas some smaller follicles do produce elevated concentrations of estradiol [2,7]. Luteinizing hormone pulse frequency drives ovarian follicular development and estradiol production [8,9], and systemic infusion of LH during the midluteal phase increased messenger RNA (*mRNA*) abundance for P45017α and 3β-hydroxysteroid dehydrogenase (3β-HSD), subsequently increasing androstenedione and estradiol concentrations within the follicular fluid (FF) [10].

Administration of a single dose (5 μg) of GnRH stimulated an LH pulse similar to that of a natural pulse and stimulated an increase in estradiol consistent with estradiol increases during a natural GnRH pulse [11]. Therefore, the objectives of these projects were (1) to evaluate causes for differences in circulating concentrations of estradiol between cows with high and low circulating concentrations of estradiol before a GnRH-induced ovulation and their relationship to follicle populations and (2) to determine if a small dose (5 μg) of exogenous GnRH administration could alter LH secretion and subsequent follicular estradiol production during the preovulatory period in beef cows.

2. Materials and methods

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

2.1. Experiment 1

2.1.1. Experimental design

Beef cows (n = 32) were synchronized by administration of GnRH (100 μg as 2 mL of Factrel, intramuscularly; Pfizer Animal Health, Madison, NJ, USA) on day −7 and administration of prostaglandin F2α ($PGF_{2\alpha}$) (PG; 25 mg as 5 mL of Lutalyse intramuscularly; Zoetis, Florham Park, NJ, USA) on day 0. Estrus was monitored every 3 h from PG on day 0/hour 0 to hour 33 and at slaughter (hour 36 to 42) with the aid of EstroTect (Western Point, Inc, Apple Valley, MN, USA) estrus detection aids. Follicular dynamics and ovulatory response were assessed by transrectal ultrasonography using an Aloka 500V ultrasound with a 7.5-MHz linear probe (Aloka, Wallingford, CT, USA) on day −7, −4, and 0. Twenty cows initiated a new follicular wave (62.5%) after the initial GnRH injection, and only cows that had initiated a new follicular wave by day −4 were slaughtered on day 2 (hour 36 to 42) for ovary collection (n = 10). One cow was determined to have a follicular cyst at time of slaughter and was removed from the data set. Animals were classified as either HighE2 (peak estradiol ≥6.0 pg/mL; n = 4) or LowE2 (peak estradiol ≤4.5 pg/mL; n = 5) according to Jinks et al, [2]; thus 9 cows were used for data analysis.

2.1.2. Tissue collection

Immediately after ovary collection, all visible surface follicles were counted and classified as small (<5 mm), medium (5–10 mm), or large (>10 mm). Follicular fluid was aspirated from DF and from all small follicles (<5 mm; pooled within size group and animal). Granulosa cells were separated from FF by centrifugation, placed in RNase Free tubes (USA Scientific), and snap frozen in liquid nitrogen. Samples were stored at −80°C until total RNA was extracted. A representative piece of ovarian cortex which did not contain the corpus luteum or a large DF was fixed in 4% paraformaldehyde for subsequent histology [12,13].

2.1.3. Blood sampling and RIA

Blood samples were collected by venipuncture of the jugular vein into 10-mL Vacutainer tubes (Fisher Scientific, Pittsburgh, PA, USA) every 3 h from PG on day 0 to hour 33 and at slaughter. Blood was allowed to coagulate at room temperature for 1 h and centrifuged at 1,200 × *g* for 30 min at 4°C. Serum was harvested and stored at −20°C until radioimmunoassays was performed. Radioimmunoassays (RIA) was performed on all serum and FF samples to measure estradiol concentrations using the methods described by Perry and Perry [14]. Inter- and intra-assay CV were 4.4% and 6.9%, respectively and assay sensitivity was 0.4 pg/mL. All serum and DF FF samples were analyzed in a single assay for concentrations of progesterone using the methods described by Engel et al [15], and intra-assay CV was 4.62% and assay sensitivity was 0.4 ng/mL.

Concentrations of androstenedione were analyzed in all serum and DF FF samples by RIA. All samples were run in duplicate (100 μL). Androstenedione standards (0.25, 0.5, 1, 2.5, 5, 7.5, 10, and 20 pg per tube) were incubated with 100 μL of androstenedione antisera (MP biomedical; 1:280,000 vol/vol dilution; 45% binding) at 37°C for 1 h. After incubation, 100 μL of [^{125}I] androstenedione (MP Biomedical; 2,000 μCi/μg; adjusted to 5,000–6,000 cpm) was added to each tube. Tubes were incubated at 4°C for 20 h. Bound and free androstenedione was separated by addition of 0.5-mL dextran-coated charcoal solution (10 min incubation) followed by centrifugation at 3,000 × *g* for 10 min. Supernatants were counted in a gamma counter for 5 min per tube. Cross-reactivities of the antibody as reported by MP Biomedical are 100% for androstenedione, 4.4% for dehydroepiandrosterone sulfate, 3.5% for dehydroepiandrosterone, 1.79% for estrone, 0.64% for testosterone, 0.07% for progesterone, 0.02% for estradiol-17β, and <0.01% for aldosterone, cholesterol, corticosterone, cortisol, dihydrotestosterone, desoxycorticosterone, 11-desoxycortisol, estriol, 17α-hydroxyprogesterone, pregnenolone, pregnenolone sulfate, and 17α-hydroxypregnenolone. Increasing volumes of bovine serum (200, 300, 400, and 500, μL) produced a displacement curve that was parallel ($P = 0.87$) to the standard curve (slope = 1.77 ± 0.10 for standard curve; slope = 1.80 ± 0.1.2 for bovine serum). Addition of known amounts of androstenedione (5, 50, and