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Expression of estrus modifies the gene expression profile in reproductive tissues on Day 19 of gestation in beef cows

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ABSTRACT

The aim of this study was to test the effect of expression of estrus at artificial insemination (AI) on endometrium, conceptus, and CL gene expression of beef cows. Thirty-six multiparous nonlactating Nelore cows were enrolled on an estradiol- and progesterone (P4)-based timed AI protocol (AI = Day 0) and then slaughtered for the endometrium, CL, and conceptus collection on Day 19. The animals were retrospectively grouped on the basis of cows that (1) showed signs of estrus near AI (n = 19; estrus) and (2) did not show any signs of estrus (n = 17; nonestrus). Body condition score, blood sampling, and ultrasound examination were performed on Days 0, 7, and 18 of the experiment followed by messenger RNA extraction and quantitative reverse transcription polymerase chain reaction analysis of 58 target genes. Data were checked for normality and analyzed by ANOVA for repeated measures using proc GLM, MIXED, and UNIVARIATE of SAS. Only pregnant cows were included in the analyses (n = 12; nonestrus, n = 11). Estrous expression had no correlation with parameters such as body condition score, preovulatory follicle and CL diameter, P4 concentration in plasma on Days 7 and 18 after AI, and interferon-tau concentration in the uterine flushing ($P > 0.15$); however, a significant increase was observed in conceptus size from cows that expressed estrus ($P = 0.02$; 38.3 ± 2.8 vs. 28.2 ± 2.9 mm). The majority of transcripts affected by estrous expression in the endometrium belong to the immune system and adhesion molecule family (*MX1*, *MX2*, *MYL12A*, *MMP19*, *CXCL10*, *IGLL1*, and *SLPI*; $P \leq 0.05$), as well as those related with prostaglandin synthesis (*OTR* and *COX-2*; $P \leq 0.05$). Genes related to apoptosis, P4 synthesis, and prostaglandin receptor were downregulated (*CYP11A*, *BAX*, and *FPr*; $P < 0.05$) in the CL tissue of cows that expressed estrus. In addition, four genes were identified as differentially expressed in the 19-day-old conceptus from cows that expressed estrus (*ISG15*, *PLAU*, *BMP15*, and *EEF1A1*; $P < 0.05$). There was also a significant effect of Day 7 concentration of P4 mainly affecting the immune system, adhesion molecules, and wnt signaling pathway of the endometrium (*IGLL1*, *MX2*, *SLPI*, *TRD*, *APC*, *WNT2*, *GLYCAM1*, and *MYL12A*; $P < 0.05$). A significant interaction between estrous expression and P4 concentration on Day 7 was more pronounced in immune system genes (*MX1*, *MX2*, *TRD*, *SLPI*, and *IGLL1*; $P < 0.05$). This study reported that estrous expression at the time of AI favorably altered the gene expression profile in reproductive tissues during the preimplantation phase toward a more receptive state to the elongating conceptus. These effects seem to be more evident in the endometrium during the time of dynamic remodeling for embryo implantation.

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1. Introduction

Early and late embryonic loss occurs mainly in the first 6 weeks of gestation and is responsible for major losses in the beef and dairy industry. A great proportion of these embryonic losses occur between Days 8 and 21 after fertilization [1,2]. The effect of changes in steroid hormone concentrations is critical as they affect the ability of the endometrium to receive and maintain the conceptus. Previous studies have reported the correlation between the concentration of estradiol (E2) in plasma and the ovulation, increased pregnancy/artificial insemination (AI), and decreased pregnancy loss in beef and dairy cattle [3,4].

Estradiol initiates crucial modifications in the endometrium environment such as increased epithelial cell height and ciliation in the fimbria [5] and ampulla [6]. Indeed, E2 concentrations during the proestrus period are positively correlated with the diameter of the preovulatory follicle, subsequent CL diameter, concentration of progesterone (P4) during diestrus [7], and conception rates in dairy cows [8,9]. Pereira et al. [10] also reported that a shorter proestrus duration decreased conception rates even when embryo transfer technology was used. Furthermore, an increase in pregnancy maintenance from Days 7 to 27 after AI was observed when serum E2 concentration on Day 0 and P4 concentration on Day 7 were greater in recipient cows [11].

Comparing transcriptome of the receptive and non-receptive endometrium has led to identifying signaling pathways involved in embryonic growth and development [12]. Before implantation, during the receptivity phase of the endometrium, specific genes related to the immune system, adhesion molecules, and developmental genes are extensively regulated [12,13]. Some of these genes are activated once the conceptus starts secreting interferon-tau (IFNT), but the timing of this activation varies considerably.

Immunologically, the embryo is an allograft for the dam and more specifically for the uterine tissue. Therefore, a complex modulation of immune cells and its signals are necessary to allow the maintenance of the conceptus. The uterus is an immunologically privileged site [14], and E2 has shown to play an important role by upregulating *SERPINA14* messenger RNA (mRNA) synthesis during estrus [15]. On the basis of studies performed in sheep [16], this serpin family member has immunomodulatory roles which include (1) blocking T cell proliferative responses [17], (2) impairing natural killer cell activity [18], and (3) decreasing antibody production [19]. A second group of genes critical for the survival of the early embryo are related to cell adhesion. Proper attachment and invasion of the embryo in the endometrium depend on adhesion-related molecules. In ruminants, the fetal tissue invades [20] the endometrium and establishes a synepitheliochorial type of placentation [21]. Apposition, adhesion, and invasion performed by the conceptus are controlled by the endometrium [22]. Studies have found upregulation of some adhesion molecules such as *SPP1* and *GLYCAM1* during the implantation phase in ruminants [23,24]. The canonical wnt signaling pathway, which is regulated by sexual steroids including E2 [25], is critical for morphogenesis and development of the preimplantated conceptus [26,27]. The wnt regulatory role in embryonic development is still unknown as previous studies have shown that the wnt activation improves [28], reduces [29], or has no effect [30] on the proportion of embryos that can develop to the blastocyst stage.

The function of the CL and consequent P4 synthesis in the preimplantation phase is key for proper embryo elongation and IFNT synthesis. However, it is unclear whether estrous expression could further modify the transcriptome of the CL. It is reasonable to believe that a fully mature preovulatory follicle could improve the chances for a more developed CL.

The objective of this study is to test the effects of behavioral expression of estrus before AI on gene expression of target transcripts in the endometrium, CL, and conceptus on Day 19 of gestation. We hypothesized that expression of estrus is associated with a complete maturation and function of the preovulatory mechanisms, therefore improving the transcriptome profile in reproductive tissues during the preimplantation phase.

2. Materials and methods

2.1. Animals and housing

Thirty-six nonlactating multiparous Nelore cows (body condition score [BCS] = 5.5 ± 0.1) [31] were assigned to an estrous synchronization plus timed AI protocol [32] (Fig. 1). All animals were cycling and with absence of any clinical disorder. The animals were between 48 and 72 months of age. The animals were not lactating at the time of study, and previous parturition occurred 300 to 360 days before enrollment. The cows were enrolled onto a synchronization protocol that was carried out as follows: 2-mg injection of estradiol benzoate (Estrogin; Farmavet, São Paulo, SP, Brazil) and a second-use (previously used for 9 days) intravaginal P4-releasing device (CIDR, originally

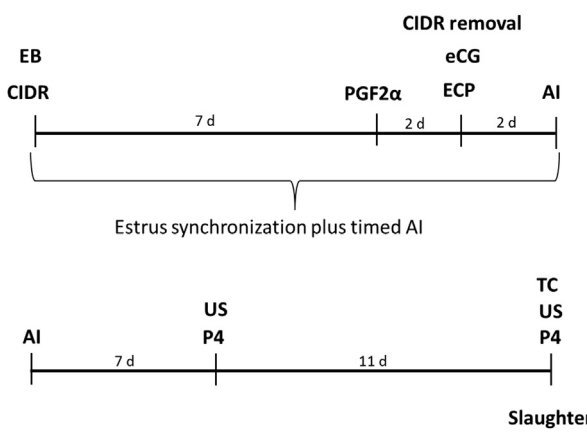

Fig. 1. Diagram of study. Cows received a 2 mg injection of estradiol benzoate (EB, Estrogin; Farmavet, São Paulo, SP, Brazil) and a second-use intravaginal progesterone-releasing device (CIDR, originally containing 1.9 g of progesterone; Zoetis, São Paulo, Brazil) on study Day −11, a 12.5-mg injection of PGF2α (Lutalyse; Zoetis, São Paulo, Brazil) on Day −4, CIDR removal in addition to 0.6 mg of estradiol cypionate (ECP; Zoetis, São Paulo, Brazil) and 300 IU of eCG (Novormon, Schering-Plough Co., São Paulo, Brazil) on Day −2, and timed artificial insemination (AI) on Day 0. P4, blood collection for progesterone analysis, TC, tissue collection; US, ultrasonographic examination of ovaries.