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Serum profile of cytokines interferon gamma and interleukin-10 in ewes subjected to artificial insemination by cervical retraction

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ABSTRACT

This study evaluated the influence of artificial insemination (AI) by cervical retraction (CRI) on serum levels of interferon gamma (IFNγ) and interleukin-10 (IL-10) in ewes. Synchronized pluriparous Santa Inês ewes were subjected to natural mating (NM, n = 8) and AI, which was performed for a fixed time (55 ± 1 hour) by CRI (n = 8) or laparoscopy (n = 8). Ewes were classified as pregnant, with return to estrus (RE) or with embryonic loss (EL). Blood samples were collected on Day 0, Day 3, Day 5, Day 12, and Day 17 (Day 0 = AI/NM) for progesterone dosage and cytokines were quantified from Day 0 to Day 12. Progesterone levels were constant, except for a decrease in ewes with RE at Day 17 ($P < 0.05$). Regardless of the reproductive method used, there was no difference in the IFNγ and IL-10 levels at any time, with averages of 642.1, 713.2, and 741.2 pg/mL for IFNγ and 667.1, 616.8, and 721.1 pg/mL for IL-10 when using CRI, laproscopy, and NM, respectively. Regarding the physiological status, ewes with EL had lower serum levels of IFNγ and IL-10 than pregnant ewes and ewes with RE, regardless of the reproductive method used, with averages of 769.1, 714.9, and 555.7 pg/mL for IFNγ and 713.8, 699.3, and 578.7 pg/mL for IL-10 in pregnant ewes, ewes with RE and EL, respectively ($P < 0.01$). In conclusion, AI by CRI in Santa Inês ewes does not alter the profile of serum cytokines IFNγ and IL-10 and does not induce an inflammatory reaction that can compromise pregnancy.

1. Introduction

Intrauterine artificial insemination (AI) in sheep with the use of frozen semen still faces obstacles. Despite laparoscopy giving the best fertility results, it is not widely used because of its high costs and need for anesthetic procedures and specialized personnel [1]. To circumvent these limitations, transcervical AI technique was developed, which enables the intrauterine deposition of semen through the cervical canal [2].

However, transcervical AI has yet to become a widely used technique, owing mostly to the sinuousness of the sheep cervix and its variable fertility success rate [3,4]. Several alternatives have been created to improve the performance of transcervical AI, including the use of cervix-dilating drugs [5–7] and development of tools adapted to the cervical anatomy [8–10].

Some studies suggest that cervical handling may cause tissue damage, thereby altering the uterine environment by proinflammatory cells, which could subsequently result in loss of fertility or embryonic death [11–13]. The immune system plays a decisive role in reproduction, because it protects the organism against external pathogens and

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modulates the process that avoids rejection of a "semi-allograft" conceptus [14].

Cytokines are a heterogeneous and pleiotropic group of hydrosoluble extracellular polypeptides or glycoproteins with molecular weights ranging from 5 to 100 kDa. They are chemical signals that trigger biochemical cascades, resulting in the production of other cytokines, and ultimately regulating the inflammatory response [15].

T-helper 1 (Th1) cytokines are considered as proinflammatory and T-helper 2 (Th2) cytokines as anti-inflammatory. In the reproductive system, both contribute to the communication among cells, and they are not only secreted by embryos but also by peripheral blood lymphocytes, macrophages, endometrial cells, and cells from the uterine tubes [16].

Interferon gamma (IFNγ) is one of the main cytokines secreted by Th1 cells. Activated monocytes secrete proinflammatory interleukins (ILs) IL-12, IL-15, and IL-18, which enhance the cytotoxic activity of natural killer (NK) cells and CD4 + CD8 + T cells. Consequently, paracrine and systemic IFNγ secretion activate cytolytic and cytotoxic immunity [17,18]. On the other hand, IL-10 is an important Th2 cytokine secreted by several immune and nonimmune cells, which induces the production of antibodies through B cells and regulates the release of Th1 cytokines. Thereby, playing immunosuppressant and tolerogenic roles [19,20].

Numerous studies indicate that some extent of systemic or uterine inflammation is necessary for the normal process of embryo implantation, which demands a Th1/Th2 balance that can successfully control the maternal immune system response against conceptus tissues [21,22]. Tissue or systemic alterations that markedly disturb that Th1/Th2 balance may cause reproductive disorders such as infertility, embryonic loss (EL), and abortion [23,24].

Considering the inconsistency in fertility rates of transcervical AI, compared with those of laparoscopy [3,13], we hypothesized that cervical manipulation can lead to inflammatory reaction capable of raising serum Th1/Th2 ratio, thereby threatening the viability of the embryo. Thus, this study aimed to evaluate the influence of AI by cervical retraction on serum IFNγ and IL-10 levels in Santa Inês ewes.

2. Materials and methods

2.1. Animals and treatments

The experimental procedures performed in this study were approved by the Ethics Committee in the Use of Animals of the Southwest Bahia State University, under protocol no. 68/2014.

The study was conducted in the sheep farming station of the Santa Cruz State University, Ilhéus, Bahia, (14°47′20″S, 39°02′56″W). Twenty-four Santa Inês pluriparous ewes from commercial herds (4–8 years, weighing 52.20 ± 4.60 kg, with a body condition score 3.4 ± 0.4, scale 0–5 [25]) were used. One ram and four teaser males were also used. Animals grazed in pastures of *Brachiaria humidicola* from 8 AM to 4 PM and were supplemented with *Pennisetum purpureum* and corn/soy-based concentrates (15% crude protein) at a rate of 400 g/animal/day. Vitamin-mineral premix (Guabiphos Ovinos AE; Guabi, Brazil) and water were available ad libitum. Hormonal protocol consisted of intravaginal sponges containing 60 mg of medroxyprogesterone acetate (Progespon; Zoetis, Brazil), which were kept in place for 12 days. After sponge withdrawal, 200 IU i.m. of eCG (Folligon, MSD Saúde Animal, Brazil) was administered. Ewes were bred using transcervical AI by cervical retraction (CRI, n = 8), AI by laparoscopy (LAI, n = 8), or natural mating (NM, n = 8). AI was conducted 55 ± 1 hour after eCG administration. In the NM group, ewes were mated in the subsequent natural estrus to the hormonal protocol.

For the CRI, the ewes were kept standing in a cradle (standing position). After the vulvar region was sanitized, a 15-cm vaginal speculum with a light source was used to visualize the cervical os, and the cervix was retracted out to the vulvar opening. Fixation was achieved using two 25-cm Allis tweezers. Next, a 12-cm metal applicator containing a mandrel (Aplicador Expansor Ovino; Alta Genetics, Brazil) was used to cross the cervical rings as much as possible; then, a 0.25-mL straw was introduced, containing the commercially available frozen-thawed semen.

The LAI procedure was adapted from Evans and Maxwell [26]. The ewes were previously submitted to a 24-hour fasting period. Afterward, their abdominal regions were shaved and disinfected, and the ewes were placed in a special cradle in dorsal recumbency at an angle of 60°, with their hind feet raised. Local anesthesia was performed by injecting lidocaine hydrocloride 2% (Anestésico L; Pearson, Brazil) at two abdominal points near the uterine horns. After making two small incisions, two trocars were inserted: one to introduce the laparoscope (Karl Storz, Germany), and the other one for multiple uses: pumping air, using a handler to locate the uterus, or introducing the insemination pipette with a puncturing end. Once the uterine horns were located, the insemination was performed with the frozen-thawed semen, using half of the dose (0.25 mL) for each uterine horn.

2.2. Progesterone dosage

To establish serum progesterone (P_4) concentration, blood samples were collected from the ewes on Day 0, Day 3 Day 5, Day 12, and Day 17. Day 0 corresponds to the day when the AI or NM was performed. The samples were collected from the jugular veins using needles and vacuum blood collection tubes with no anticoagulant (BD Vacutainer; England). The samples were centrifuged at 1500 × *g* for 10 minutes and the obtained serum was stored in a freezer at −20 C. Hormone dosing was performed with duplicate samples through a radioimmunoassay in the Animal Reproduction Laboratory of the Federal Fluminense University, Rio de Janeiro. In order to quantify P_4, a progesterone radioimmunoassay kit (IM1188, Beckman Coulter; Immunotech, Czech Republic) was used, with a sensitivity of 0.05 ng/mL and with intraassay and interassay coefficients of variation of 6.5% and 7.2%, respectively.

2.3. Reproductive rates and physiological status

Non-return to estrus (RE) was determined with the aid of teasers up to 21 days after AI or NM. The pregnancy diagnosis was conducted through ultrasonography