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Original article

Reproductive features and use of an anti-inflammatory drug in estrus-induced dairy goats artificially inseminated in a standing position with cervix immobilization

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ABSTRACT

This study evaluated reproductive features and role of Flunixin-Meglumine at timed artificial insemination (AI), using a new technique of standing position with cervix immobilization. In Experiment 1, 10 goats ($n = 5$ nulliparous [Null] and 5 pluriparous [Plu]) were evaluated after estrus induction by recorded reproductive parameters to define the ideal time for AI. In Experiment 2, goats were artificially inseminated 51–54 h after sponge removal with frozen–thawed semen. At AI, 1 mL saline (CONTROL; 18 Null and 14 Plu) or 50 mg Flunixin-Meglumine (FLUNIXIN; 15 Null and 18 Plu) was administered i.m. Location of semen deposition was recorded for both groups. In Experiment 1, all sexual behavior and ovulatory parameters were similar between Null and Plu for estrus response and ovulation (100%), interval from sponge removal to ovulation (~64.2 h), largest ovulatory follicle diameter (~6.6 mm), and number of ovulations (~2.0). In Experiment 2, pregnancy rate was superior ($P < 0.01$) for CONTROL (62.5%; 10 Null and 10 Plu) than FLUNIXIN (30.3%; 3 Null and 7 Plu) goats. Regardless of the treatment, intrauterine AI was more frequent ($P < 0.01$) in Plu (100.0%; 32/32) than in Null (69.7%; 23/33) goats. Moreover, AI was more time-consuming ($P < 0.01$) in Null (44 ± 37 s; 4–139 s) than in Plu (21 ± 19 s, 4–78 s) goats. Therefore, administration of Flunixin-Meglumine at the time of AI adversely affected pregnancy rate. High rates of intrauterine cervical penetration were obtained, achieving good pregnancy rates in goats not receiving Flunixin-Meglumine.

1. Introduction

In Brazil, goats are raised by both families and businesses, and goat production is an important part of society and agriculture [1]. Alpine, Anglo Nubian, Saanen, and Toggenburg are the main breeds explored for milk production, especially in the tropical area of southeast to northeast regions of the country. The Capragene, a genetic improvement program based on artificial insemination and progeny testing has been developed in Brazil [2]. To reach the goal, associated reproductive techniques needed to be developed. Focus was concentrated on control of the estrus cycle in dairy goats under tropical conditions, including protocols for estrus synchronization and induction, and a new artificial insemination (AI) technique was developed [3].

AI represents a safe and efficient form of reproduction and genetic improvement. Since the first report of AI in Brazil in the 1940s, there have been many examples of successful AI in goats by using a traditional bipedal position; however, unsatisfactory results have also been reported; the main cause of these results was intrauterine semen deposition efficiency [4,5]. In fact,

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intrauterine semen deposition and vaginal evaluation at the moment of AI are key factors affecting the establishment of pregnancy in goats [6]. Another important factor is the time required to perform AI; procedures that require more time for handling the animal are more difficult for technicians as well as for the animals and helpers. For more than a decade, Embrapa team has been focused on developing an alternative AI technique called the "Embrapa AI technique," which has not yet been described in detail in the literature. In the traditional technique, the goat is upside down in anterior bi-pedal position, and the Duckbill speculum is inserted to locate the cervix [7]. Conversely, in the Embrapa technique, the goat stays in a standing position, the Collins speculum is used, and the cervix is immobilized with Allis forceps.

Especially when using vaginal devices for estrus synchronization, inflammatory processes can occur, and vaginal mucus was recognized to be deleterious to spermatozoa [8]. This negative effect could be increased if these contents are carried into the cervix and uterus, which can be facilitated by the traditional bipedal technique. Moreover, it is noteworthy that the traditional technique is much more tiring and uncomfortable for the goat and for the helper holding it. Although the goat does not express any discomfort, the use of an anti-inflammatory could overcome any possible degree of pain from the need to clamp the cervix when using the Embrapa technique. Thus, the administration of anti-inflammatory agents during AI could provide a better rate of comfort and animal welfare to the females. Flunixin-Meglumine is a nonsteroidal anti-inflammatory drug commonly used for analgesic purposes. However, it is essential to detect any beneficial or harmful effect of such drug on ovulation, AI quality, and subsequent fertility of female goats.

Therefore, the present study aims (1) to assess the sexual behavior and ovulatory parameters in nulliparous and pluriparous Saanen goats after receiving a short-term estrus induction treatment to determine the ideal moment for AI for each category; (2) to describe an alternative AI technique in goats in a standing position with cervical immobilization; and (3) to evaluate the role of administering Flunixin-Meglumine at the moment of timed AI on pregnancy rate in goats.

2. Materials and methods

2.1. Ethics and experimental conditions

This study was approved by the Animal Care Committee of Fluminense Federal University (protocol number 116/11), and it was conducted according to the principles of the Brazilian Society of Laboratory Animal Science. The study was conducted during the anestrous season in Coronel Pacheco, Minas Gerais, in the southeast region of Brazil. The research unit is located at 435 m altitude and S 21°35′ and W 43°15′. The area receives an average annual precipitation of 1581 mm^3. The average annual temperature at site is 21 °C. Animals were housed with intensive management, receiving triturated Napier grass (*Pennisetum purpureum*) and a protein concentrate according to maintenance demand. Mineralized salt and water were offered ad libitum. A total of 84 Saanen goats were used, 43 nulliparous (Null) and 41 pluriparous (Plu) lactating goats at middle to end of lactation. Goats were allocated in two experiments.

2.2. Experiment 1

Ten goats (five Null and five Plu) were subjected to an estrus induction treatment and evaluated regarding their sexual behavior and ovarian parameters by ultrasonography to determine the optimal time to perform AI for both categories. Goats received an intravaginal sponge containing 60 mg medroxyprogesterone acetate (MAP; Progespon®, Syntex S.A., Industria Bioquímica y Farmacéutica, Buenos Aires, Argentina) that was maintained for 6 d. An administration of 30 μg d-cloprostenol (Prolise®, ARSA S.R. L., Buenos Aires, Argentina) i.m. was performed at sponge insertion (day 0) plus 200 IU equine chorionic gonadotrophin (eCG; Novormon® 5000, Syntex S.A., Industria Bioquímica y Farmacéutica, Buenos Aires, Argentina) i.m. 24 h before sponge removal (day 5). All hormonal procedures were done between 6:00 and 7:00 am. Goats were subjected to a teaser male after sponge removal, twice a day (7:00 am and 6:00 pm), for 4 d until they showed no more estrus signs. Females were considered to be in estrus when allowed to be mounted.

2.3. Experiment 2

The same estrus induction protocol was applied in Experiment 2. A total of 65 goats (33 Null and 32 Plu) were assigned according to parity, body weight, and condition score (BCS; scale 1–5) to two treatment groups: FLUNIXIN (51.8 ± 8.9 kg and 3.1 ± 0.6 of BCS) and CONTROL (52.0 ± 8.7 kg and 3.1 ± 0.5 of BCS). Goats were artificially inseminated (AI) 51–54 h after sponge removal with frozen–thawed 0.25 mL straws (100×10^6 spermatozoa). At the moment of AI, goats received either Flunixin-Meglumine (1 mL, 50 mg; Banamine®, Schering-Plough, Cotia, Brazil, $n = 33$, 15 Null and 18 Plu) or saline (1 mL, control group, $n = 32$, 18 Null and 14 Plu) intramuscularly.

2.4. Ultrasonography

Transrectal ovarian ultrasonography was performed in all goats of Experiment 1 by the same operator. Ultrasound assessments were performed every 8 h from sponge removal until ovulation in all goats. Ovulation was confirmed in a subsequent evaluation 8 h after ovulation detection. The examinations were conducted with a B-mode transrectal ultrasonographic scanner (KX 2000G Vet®, Kaixin, Xuzhou, China) with 7.5 MHz transducer fitted to a plastic rod that allowed the transrectal manipulation of the probe. Does were maintained in a standing position, fecal pellets were removed manually (with a finger), and 20 mL of carboxymethyl cellulose gel was placed into the rectum with a syringe. Ovaries were located as previously described [9], and the number, diameter, and position of ovarian follicles ≥3 mm in size were recorded. The day of ovulation was defined as the day when the largest follicle, previously identified, was no longer detected. The preovulatory follicle diameter was considered the last measurement obtained before ovulation. Approximately 60 d after AI (Experiment 2), the same equipment was used to check pregnancy.

2.5. Artificial insemination technique

The Embrapa technique of transcervical AI through cervical immobilization was applied. Goats were contained in the milking room in a standing position, allowing the inseminator to stand. Another person maintained elevation of the goat's tail while the inseminator cleaned the external genitalia of the animal with a dry paper towel (Fig. 1A). At the end of this stage, the inseminator stimulated the clitoris, massaging the bottom of the vulva with the paper towel avoiding direct contact with the animal.

Collin-type specula are used in sizes 0–3. In the case of nulliparous goats, speculum 0 was first inserted and gently opened to provide vaginal dilation, facilitating the introduction of speculum 1. In most cases, speculum 1 was the appropriate choice for young goats. For pluriparous ones, speculum 2 was the appropriate choice. The speculum was lubricated by applying non-spermicidal gel (Fig. 1B) before insertion through the vulva and