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Original article

Evaluation of cervical mucus and reproductive efficiency of seasonally anovular dairy goats after short-term progestagen-based estrous induction protocols with different gonadotropins

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ABSTRACT

The use of three different gonadotropins was tested for estrous induction in dairy goats during the non-breeding season. All does received an injection of 30 μg of d-cloprostenol and intravaginal sponges containing 60 mg of medroxyprogesterone acetate (MAP) for 6 d plus 20 IU of porcine FSH (pFSH), 200 IU of eCG or 250 IU of hCG 24 h before sponge removal. In Experiment 1 (n = 24), ovarian ultrasound parameters were recorded and cervical mucus was evaluated daily for 5 d after sponge removal or until ovulation. In Experiment 2 (n = 80), reproductive efficiency of artificially inseminated or naturally mated does was assessed. The mean interval from sponge removal to ovulation (73.5 ± 23.7 h), number of ovulations (1.6 ± 0.7) and ovulatory follicle diameter (7.2 ± 0.8 mm) did not vary ($P > 0.05$) among the three groups. At ovulation, cervical mucus had crystalline-striated to striated (22.2%), striated to striated-caseous (72.2%) and striated-caseous to caseous (5.6%) appearance. The largest follicle diameter was greater ($P < 0.05$) in does with crystalline (6.7 ± 1.4 mm), crystalline-striated (7.2 ± 1.1 mm) or striated (7.3 ± 1.3 mm) mucus than in those with striated-caseous (5.3 ± 1.4 mm) or caseous (4.5 ± 1.1 mm) mucus. Percentage of animals exhibiting estrus (92.5%) and conception rate (60.8%) were similar ($P > 0.05$) among the three gonadotropins groups. Results of this study support the use of eCG (200 IU), hCG (250 IU) and pFSH (20 IU) for the estrous induction protocols in dairy goats during the non-breeding season. Cervical mucus evaluation can be used as an additional method to determine the optimal time for artificial insemination in goats.

1. Introduction

In southeastern Brazil, commercial dairy goat operations using specialized breeds constantly require controlled reproductive management in order to obtain sufficient amount of milk throughout the year. The most frequently used estrous induction protocols are based on the use of equine chorionic gonadotropin (eCG) and intravaginal progesterone- or progestagen-releasing devices [1]. During the non-breeding season, gonadotropin is usually injected one day before the end of progestagen treatment to stimulate ovarian follicular development [2,3]. eCG is a glycoprotein secreted by trophoblast cells in the mare during gestation. The dual (FSH- and LH-like) activity, long half-life and widespread availability make it a convenient hormone for estrous induction treatment in goats. However, eCG is the most heavily glycosylated glycoprotein hormone and, from the first treatment, it may induce the production of anti-eCG antibodies [4]. These antibodies may delay the preovulatory LH surge and ovulation leading to poor fertility of the eCG-treated females [5].

It is necessary to circumnavigate the effects of such an immunological reaction to the gonadotropic agent by using an alternative hormone in order to maintain the efficiency of estrous induction programs for artificial insemination (AI). However, there is a paucity of literature on the use of other gonadotropins for estrous induction in goats. Human chorionic gonadotropin (hCG) with a potent LH-like

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activity has been used in estrous and ovulation synchronization protocols in domestic animals [6]. This hormone has a long half-life of approximately 39 h [7] and, although there are only a few reports on its effects in goats, the results obtained were very encouraging: hCG stimulated follicular development and induced fertile estrus in the early postpartum period [8], it resulted in estrous behavior and ovulation in all treated, sexually mature goats in the breeding season [9] and in the 61.1% conception rate after natural mating [2].

Follicle-stimulating hormone (FSH) is a primary promoter of antral follicular growth and has a short half-life of about 10 h [10]. Although FSH is widely used in superovulatory treatments, there is no report of its use for estrous synchronization in goats. In sheep, when combined with progesterone pre-treatment, porcine FSH was effective in the synchronization of estrus and ovulation [11], and its administration was associated with high pregnancy rates [66% and 79% using long- (12-day) or short-term (5-day progesterone treatment) protocol, respectively] [12].

It is known that the macroscopic appearance of cervical mucus changes from crystalline to caseous from the onset to the end of behavioral estrus [13] when ovulation normally occurs [14]. However, there are presently no data on the relationship between changes in cervical mucus and antral follicular dynamics that would allow for the prediction of the ovulation time in goats. We hypothesized that evaluation of cervical mucus could assist in determining the optimal time for AI in goats. Therefore, the aims of the present study were to: i) evaluate the use of hCG and pFSH as substitutes for eCG in estrous synchronization protocols; and ii) characterize the temporal changes in cervical mucus during various estrous induction protocols and their associations with ovulatory dynamics in dairy goats during the non-breeding season.

2. Material and methods

2.1. Location and experimental conditions

This research project had been reviewed and approved by the Animal Care Committee of Fluminense Federal University (protocol 048/08), and was in compliance with the guidelines of the Brazilian Society of Laboratory Animal Science (SBCAL) that contain regulations on the use of experimental animals.

The present study was conducted from September to October and it utilized animals housed at two different locations. The first experiment (Experiment 1) was conducted in the rural area of Piau, MG, Brazil (latitude 21°35′S, longitude 43°15′W and altitude of 435 m). The second experiment (Experiment 2) was conducted both in Piau, MG, Brazil as well as in the rural area of Florestal, MG, Brazil (latitude 19°53′S, longitude 44°25′W and altitude of 776 m). In such conditions, the breeding season (period of shortening daylengths) is from March to June, the non-breeding season (period of lengthening daylengths) is from August to November, and the transitional period (period of relatively stable long daylengths) is from December to February [15].

A total of 104 dairy goats (15 Alpine, 38 Saanen and 51 Toggenburg), of eight months to five years old, were used in this study. Goats were kept in an intensive management system, and grouped according to physiological status and milk production in pens housing 10 to 12 goats each (2.5 to 3 m^2 per animal). Goats were fed corn silage and *Pennisetum purpureum K. Schum* as forage, and balanced concentrate supplement (soy bean, corn and mineral nucleus based mixture). Non-lactating multiparous and nulliparous goats received daily rations of 0.5 kg of concentrate/goat (homemade mixture with 16% crude protein and 68% total digestible nutrients - (TDN) and 4.0 kg of chopped elephant grass/goat. Lactating goats received daily rations 1.5 kg of concentrate/goat (homemade mixture with 22% crude protein and 69% TDN) until the attainment of daily milk yields of 3 kg/goat plus 0.33 kg of concentrate for each additional kg of milk produced and 4.0 kg/goat of corn silage. Mineralized salt licks (Salminas Goats®, Nutriplan, Juiz de Fora, MG, Brazil) and drinking water were available *ad libitum*.

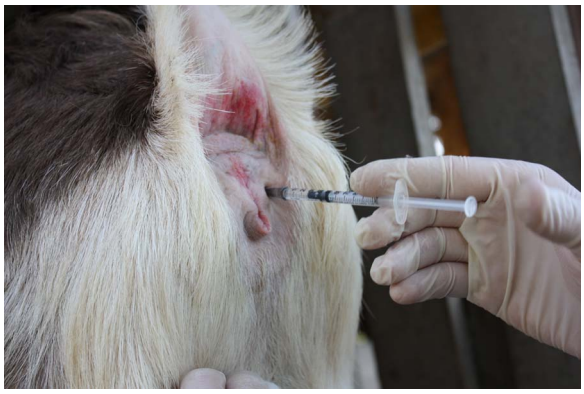

Fig. 1. Cloprostenol administration by latero-vulvar route. A 1 mL syringe coupled to a 0.7 mm × 25 mm needle was used for each animal.

2.2. Experimental procedures

Estrous synchronization was performed as described previously by our group [2,16]. All goats received intravaginal sponges containing 60 mg of medroxyprogesterone acetate (MAP; Progespon®, Syntex S.A., Buenos Aires, Argentina) for 6 d as well as 30 μg of d-cloprostenol (Veteglan®, Laboratórios Callier S.A., Barcelone, Spain) administered by latero-vulvar route (Fig. 1) and a dose of gonadotropin (20 IU of porcine FSH (pFSH), 200 IU of eCG or 250 IU of hCG, i.m.) administered 24 h before sponge removal (Day 5).

In Experiment 1, nine nulliparous and 15 multiparous Toggenburg goats (nine lactating and six non-lactating) were allocated according to parity and lactation status, body weight (BW) and body condition score (BCS, range 1 to 5), into three subsets: (i) pFSH (n = 8, BW: 47.8 ± 15.0 kg, BCS: 3.1 ± 0.5) receiving 20 IU of pFSH (Pluset®, Hertape-Calier, São Paulo, Brazil) i.m.; (ii) hCG (n = 8, BW: 47.9 ± 13.9 kg, BCS: 3.2 ± 0.5) treated with 250 IU of hCG (Vetecor®, Hertape-Calier, São Paulo, Brazil) i.m.; and (iii) eCG (n = 8, BW: 48.0 ± 13.5 kg, BCS: 3.1 ± 0.3) treated with 200 IU of eCG (Novormon 5000®, Schering Plough Animal Health, São Paulo, Brazil) i.m. as described earlier.

After MAP sponge removal, estrous behavior was monitored twice a day (06:00 and 17:00 h) with hand-controlled bucks to avoid penile penetration and the does were considered to be in estrus when they stood to be mounted. Cervical mucus appearance was evaluated twice daily for 5 d, starting 12 h after sponge removal. A sterilized Collin vaginal speculum (lighted) was used to classify cervical mucus types using the 1–5 scale as follows: crystalline – 1 (mucus completely translucent); crystalline/striated – 2 (mucus with some opacity but devoid of striation); striated – 3 (evident striation within crystalline areas); striated/caseous – 4 (striation coalescing and no visible translucent areas); and caseous – 5 (mucus appearing as a caseous mass with evident flocculation). Different types of cervical mucus observed in the cervical os and vulva of goats are shown in Figs. 2 and 3, respectively. Note that the diameter of the cervical os opening expands as the cervical mucus changes from crystalline to striated/caseous (Fig. 2).

Transrectal ovarian ultrasonography was performed by the same, experienced operator every 12 h from 24 h after sponge removal until ovulation. All examinations were conducted with a B-mode ultrasonographic scanner connected to a 6.5-MHz transrectal transducer (Pie Medical Aquila Vet®, Campinas, Brazil). The transducer was fitted with a plastic rod that allowed external transrectal manipulation of the probe. All does were examined in a standing position; fecal pellets were removed and 20 mL of carboxymethylcellulose gel was injected into the rectum with a syringe prior to scanning. Ovaries were located as previously described [17], and the number, diameter, and position of