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The Time of Artificial Insemination with Cooled Semen does not Affect the Pregnancy Rate in Anestrous Goats

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ABSTRACT

Background: The time of artificial insemination (AI) concerning ovulation is one of the main factors that affect the pregnancy rate. This study aimed to compare the time of AI with cooled semen in anestrous goats treated with progesterone (P4) plus hCG-based protocol and the effect of management condition on pregnancy rate.

Methods: Forty-nine crossbred (local × dairy goats) and thirty-nine Alpine anestrous multiparous goats managed under extensive and intensive production systems respectively, were used (June, 25°N). Goats were treated with 20 mg of P4 followed by 100 IU of hCG 24 h later. On the day of hCG administration (Day 0), goats in each management system were allocated randomly to each of the two treatment groups. Goats in the first group received a fixed-time AI (FTAI) at 48 and 60 h after hCG whereas goats of the second group received a FTAI at 60 and 72 h with cooled semen. Goats were examined by transrectal ultrasonography (7.5 MHz) 45 days after AI to determine the pregnancy rate.

Result: There was no significant difference in pregnant goats inseminated at 48 and 60 h (46.8%, 22/47) or 60 and 72 h (46.3%, 19/41) after hCG administration (P>0.05). There was a tendency for a higher pregnancy rate in intensive compared to extensive management conditions (P=0.09) and no interaction was detected between the time of insemination and the management condition system (P>0.05).

Key words: Breeding, Gonadotropin, Seasonality, Sperm, Ovulation.

INTRODUCTION

Artificial insemination (AI) has been widely used to disseminate the most valuable genetics to improve the production of milk, hair and meat in goats (Sharma and Sood, 2019; Gonzalez-Marin *et al.* 2021). In this specie, AI is usually undertaken using fresh or cooled semen due to the low fertility rates achieved with cryopreserved semen (Mocé *et al.* 2020). For liquid semen preservation, skimmed milk-based extenders are used (Susilowati *et al.* 2019) and after collection, semen is cooled for 1.5 to 4 h until it reaches 4-5°C (Sadeghi *et al.* 2020) to reduce the metabolism of sperm and the production of detrimental byproducts, such as reactive oxygen species, extending the sperm fertility for at least 12-24 h (Roof *et al.* 2012; Wiebke *et al.* 2021). This facilitated the storage and transport of semen for long distances allowing the dissemination of genetics from superior males between a group of herds located within a relatively small area (Baldassarre and Karatzas 2004).

AI may be performed at a fixed time without the necessity of estrus detection obtaining high pregnancy rates and making practical the wide adoption of AI mainly in extensive management conditions (Menchaca and Rubianes 2004). To establish a suitable FTAI program, knowledge of the time, rate and synchrony of ovulation after treatment is important since the time of AI can differ according to treatment, method of AI and the type of semen used (Cesh *et al.* 2012). FTAI protocols in small ruminants are based basically on progesterone releasing intravaginal devices and eCG obtaining a high pregnancy rate after AI (Gonzalez-Bulnes *et al.* 2020). On the other hand, the use of an injectable progesterone (P4) plus human chorionic gonadotropin (hCG)-based protocol has been successful for FTAI in anestrous multiparous and nulliparous goats using fresh semen with acceptable pregnancy rates in nulliparous and multiparous anestrous goats under intensive management conditions (Alvarado-Espino *et al.* 2019b). This protocol is less expensive and easier to administer than intravaginal P4 devices protocols (Alvarado-Espino *et al.* 2019b). However, the use of this protocol for FTAI with cooled semen has not been evaluated yet.

The present study hypothesized that the time of AI and management conditions could affect the pregnancy rate in goats treated with injectable P4 plus hCG-based protocol during the non-breeding season. The objectives of the present study were to evaluate the intervals for AI with cooled

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semen, after hCG administration and the management conditions on pregnancy rate in anestrous goats.

MATERIALS AND METHODS

This study was conducted in northern Mexico during the nonbreeding season (June 2020, at 25°N). Forty-nine crossbred (local × dairy goats) and thirty-nine dairy (Alpine) anestrous multiparous goats managed under extensive and intensive production systems, respectively, were used. All females were in anestrus at the onset of the experiment which was determined by the absence of corpus luteum (CL) detected by two transrectal ultrasound examinations (Aloka SSD 500, Tokyo, Japan; transducer 7.5 MHz) performed 8 days apart with the second one just before starting the experiment. Goats in extensive management conditions had a body condition score (BCS) of 2.8±0.3 (mean ± SD; scale 1-5, Atasever *et al.* 2015) and bodyweight of 45.2±7.2 kg. During the study, goats grazed herded by the owner in a landscape of shrubs such as creosote bush (*Larrea tridentata*), mesquite (*Prosopis glandulosa*), *Acacia farnesiana*, grasses (*i.e., Bouteloua gracilis* and *B. curtipendula*) and occasionally on crop residues such as corn (*Zea mais*) and sorghum for approximately 8 h daily. At night, the goats were maintained housed in pens and received mineral salt and water *ad libitum*. Goats were hand milked every day in the morning and received supplemental concentrate (150 g/animal) that was offered once daily during milking. In intensive management conditions, females had a BCS of 3.0±0.3, were fed with alfalfa hay *ad libitum* and received 250 g of concentrate per animal during machine milking in the mornings.

All animals were in good health status and all the procedures were in strict accordance with international and national accepted guidelines for ethical use, care and welfare of animals in research (FASS 2010), with institutional approval reference number UAAAN/UL-2807.

Goats in both management conditions were treated with an injectable P4 plus hCG-based protocol as previously described by Rodríguez-Martínez *et al.* (2018). Briefly, goats were injected with 20 mg of P4 in oil adjuvant (0.4 mL/goat, Progesterona, Zoetis, Mexico), plus 100 IU of hCG (0.1 mL/goat, Chorulon, Intervet, Mexico) 24 h later, administered by the intramuscular route and applied in the morning. On the day of hCG administration (Day 0), all goats were assigned randomly to each of two treatment groups and blocked by their body condition score as part of the randomization procedure to minimize confounding of this variable between treatments (Fig 1). In one group, goats received a FTAI at 48 and 60 h after hCG injection whereas goats in the second group received a FTAI at 60 and 72 h with cooled semen without estrus detection in the groups.

For AI, semen was collected from two Alpine and one Toggenburg young bucks of proven fertility housed at the Universidad Autónoma Agraria Antonio Narro-Unidad Laguna. Semen was collected between 6 to 16 h before AI using an artificial vagina. Raw semen was placed into a 15-mL tube and each ejaculate was assessed immediately after collection for volume (mL), sperm concentration determined with a SDM 1 (Minitube ®, Alemania), mass motility on a 6-point scale (0 = no motion; 5 = numerous rapid waves; David *et al.* 2015) and viability (%). Microscopic assessments were conducted by the same technician and only those ejaculates that had a volume ≥0.5 mL, mass motility ≥3 (scale 0-5) and concentration ≥2500×10^6 cells per mL were used. After evaluation, the semen was maintained in a water bath at 30 °C for 5-10 minutes until dilution. Semen was extended in commercial ultra-high temperature bovine skim milk in one step to get a final concentration of 800×10^6 per mL without seminal plasma removal (Andrabi *et al.* 2016). Then, ejaculates were transferred in a water bath (30°C) to the refrigerator and cooled slowly at 0.25°C/min until reached a temperature of 4-5°C.

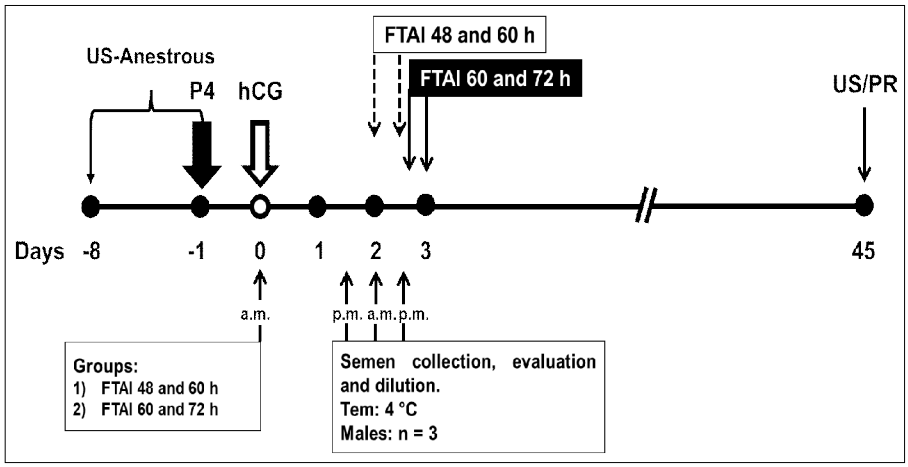

Fig 1: Treatment schedules for multiparous anestrous goats under extensive and intensive management conditions FTAI with cooled semen (4-5°C) at 48 and 60 h or 60 and 72 h after human chorionic gonadotropin (hCG). US= Ultrasound. FTAI= Fixed-Time artificial Insemination. PR= Pregnancy rate.

Semen of each male was transported in a Styrofoam box under refrigerated conditions to the farms. At the time of AI, semen was loaded in 0.25 ml French straws containing 200×10^6 spermatozoa and was equally distributed for insemination to each group. Cervical insemination was performed with the aid of a tubular vaginoscope equipped with a light source and an ovine-caprine AI catheter (IMV, France). The goats were lifted by their hind limbs and semen was deposited in the external cervical *os*. To avoid bias, all inseminations were performed by the same technician.

To determine the pregnancy rate, goats were examined by transrectal ultrasonography (7.5 MHz, Aloka SSD 500, Tokyo, Japan) 45 days after AI. Goats were confirmed pregnant by the presence of at least one fetus and extra-embryonic membranes (Rickard *et al.* 2017).

Statistical analyses were performed using SAS software (SAS Institute Inc., Cary, NC). The pregnancy rate was analyzed by logistic regression using GENMOD with the link logit of SAS. The effect of time of AI, management conditions (extensive and intensive) and the interaction between timed AI and management conditions were determined. A significant difference between treatment groups was considered when $P\leq0.05$.

RESULTS AND DISCUSSION

The results are summarized in Table 1. There were no significant differences in the proportion of pregnant goats at day 45 after FTAI with cooled semen at 48 and 60 h or 60 and 72 h after hCG administration (P>0.05) and there was no interaction between FTAI and management condition. However, there is a tendency for a higher pregnancy rate in goats under intensive production systems (P=0.09).

The present study showed that the time of FTAI using buck semen stored at 5°C does not affect pregnancy rates in anestrous goats treated with an injectable P4 plus hCG-based protocol. These results were in line with previous studies that reported a similar pregnancy rate (52.2%) after FTAI performed at 48 and 60 h in goats treated with intravaginal sponges and eCG (Lehloenya *et al.* 2005) or after single FTAI using the same protocol (Alvarado-Espino *et al.* 2019a) and was higher than reported for sheep (Menchaca *et al.* 2005). In intensive management conditions, a tendency (P=0.09) for higher pregnancy rates was observed with no interaction between FTAI and management conditions on pregnancy rates (P>0.05).

Table 1: Pregnancy rates obtained in anestrous goats inseminated at different times with cooled semen under extensive and intensive management conditions and treated with an injectable progesterone plus hCG-based protocol.

Treatments	Pregnancy rate (%)
Time of AI	
48 + 60 h	46.8% (22/47)[a]
60 + 72 h	46.3% (19/41)[a]
Management systems	
Intensive	56.4% (22/39)[a]
Extensive	38.7% (19/49)[b]
Interaction TAI*system	P= NS

Different superscript P<0.10.

NS= Non-significant.

The interval from AI to ovulation is one of the major factors that affect the pregnancy rate in FTAI protocols (Stelletta *et al.* 2017). To get acceptable pregnancy rates, AI must be performed before or around the expected time of ovulation to allow sufficient time for sperm transport and capacitation relative to ovulation (Romano *et al.* 2016; Lauber *et al.* 2020). In our study, the pregnancy rate was similar between those goats with FTAI performed at 48 and 60 h and 60 and 72 h by cervical insemination. In goats treated with an injectable P4 plus hCG-based protocol, the onset of estrus occurs between 48 and 60 h after hCG administration and ovulated 24-36 h later (Alvarado-Espino *et al.* 2019a; 2019b). According to this, it is probably that in both FTAI schedules the AI occurred around the expected ovulation time allowing that AI could be performed in the morning-afternoon or the afternoon-morning after hCG administration without affecting fertility.

The results of pregnancy rates after single or double insemination are contradictory. Previous studies have reported an increase in fertility after double insemination with fresh or frozen-thawed semen (Karatzas *et al.* 1997; Pellicer-Rubio *et al.* 2016). In contrast, other studies have reported that fertility after one insemination with 200 million frozen-thawed spermatozoa was similar to that obtained after two inseminations with 200 million spermatozoa in each one in hormonally synchronized dairy goats (Corteel *et al.* 1988), or after an equal number of spermatozoa in goats inseminated after natural estrus (Nordstoga *et al.* 2010). It is probable that the advantages of double AI depended on the timing of insemination concerning ovulation, the tightest synchrony of goats after treatment, or related to lower estrus detection rates (Roca *et al.* 1997; Nordstoga *et al.* 2010). Since in the present study goats were inseminated twice, further studies are required to determine if one simple AI with cooled semen could induce similar pregnancy rates.

In goats, semen can be stored at 5°C for 12 or 24 h without affecting its fertility, facilitating the transportation and exchange of genetic material between flocks (Gororo *et al.* 2019). In addition, the cooling process causes less cell damage and increases sperm survival, increasing fertilization and therefore pregnancy (Borges-Silva *et al.* 2015; Gororo *et al.* 2019). In the present study, the time between semen collection and the time of insemination was 12 h, sufficient time to transport semen from the collection center to farms. So, the use of FTAI and using cooled semen could facilitate the adoption of AI at farm level because the semen collection time and timing of insemination could be planned in conjunction with ovulation (Borges-Silva *et al.* 2015).

A tendency for decreasing pregnancy rate per AI in goats from extensive compared to intensive management

conditions has been observed, independently of the time of FTAI. These results are in line with those previously reported in goats in which there was imposing the same estrus synchrony treatment (Carrillo *et al.* 2019; Alvarado-Espino *et al.* 2019a) or after treatment with intravaginal sponges and eCG inseminated with fresh or cooled extended semen under extensive or semi-extensive management conditions (Mehmood *et al.* 2011; Arrebola *et al.* 2012; Muayad *et al.* 2019). Under extensive management conditions like those observed in northern Mexico, low reproductive rates are common (Mellado *et al.* 2020). In this kind of marginal goat production system goats are prone to get a low plane of nutrition that may negatively impact their fertility (Meza-Herrera *et al.* 2017; Alvarado-Espino *et al.* 2019b; Mellado *et al.* 2020). Although breed could be a factor that influences fertility rate, a previous study shows that the treatment used here induces a similar estrous response and ovarian activity in crossbreed and dairy goats (Alvarado-Espino *et al.*2019a). In addition, health and animal handling, could be also contributed to the low fertility observed in these production systems (Nordstoga *et al.* 2010; Arrebola *et al.* 2012; Salinas-Gonzalez *et al.* 2016).

CONCLUSION

In conclusion, the results of the current study showed that the time of FTAI with cooled semen did not affect the pregnancy rate in anestrous goats submitted to an injectable P4 plus hCG-based protocol. The use of cooled semen and FTAI could facilitate producers' application and adoption of AI in their herds since no estrus detections are required and semen can be transported long distances, improving the reproductive performance and genetic selection of goats in developing countries. More studies are needed to increase the pregnancy rate after FTAI in goats under extensive management conditions.

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Conflict of interest: None.

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