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Intrauterine Insemination with Frozen-Thawed Semen in Creole Goats, Synchronized in Estrous During the Nonbreeding Season

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Abstract: In order to evaluate the fertility rate in Creole goats synchronized in estrous during the nonbreeding season and inseminated into the uterus with frozen-thawed semen or mating by fertile male 24 h after estrus detection, a study was carried out in Southern of Mexico, at 18° 15¨NL. Ninety multiparous (from 1 to 4 parity) non cycling Creole goats an five males (four Nubian and one Boer bucks) were utilized. Goats were synchronized in estrous by means of intravaginal sponges containing 40 mg of FGA during 11 d, plus an intramuscular injection of eCG (200 IU) at moment that sponges were withdrawal. Only 73 females showed heat manifestations. Twelve hours after estrous detection, 37 does receiving natural mating, while rest 36 goats were inseminated into the uterus by laparoscopy with frozen- thawed semen obtained from Boer male (100 million of spermatozoids), five month later fertility rate was registered. Chi-square test was utilized. No difference was found ($p>0.05$) for fertility rate between artificial insemination (33.3%) and natural mating (51.3%). It concluded that fertility rate registered inseminated into the uterus with thawed-frozen semen is similar that fertility registered in Creole goats receiving natural mating, synchronized in estrous during non reproductive season.

Key words: Intrauterine insemination, frozen-thawed semen, creole goats, nonbreeding season

INTRODUCTION

The Artificial Insemination (AI) in goats using thawed-frozen semen allows massive use of bucks that are genetically superiors. Although, unlike cattle, cervical insemination in sheep using frozen-thawed semen has not progressed due to low conception rates (Evans and Maxwell, 1990). The principal cause of reduced fertility appears to be an alteration in the capacity to transport the spermatozoids from the cervix to the site of the ova fertilization in the oviduct (Evans and Maxwell, 1990; Gillan and Maxwell, 1998). Intrauterine insemination using laparoscopy make it possible to deposit the semen thawed-frozen directly into the uterus near the oviduct, shortly before ovulation, which would produce fertility rates comparables to those obtained by natural mating. The other hand, Creole goat from Southern of Mexico show an a seasonal anoestrous during spring station (Mellado, 1997; Martínez *et al*., 2005) for this reason it is necessary utilized sponges containing Fluorogestone Acetate (FGA) for estrous synchronization (Martínez-Rojero *et al*., 2006). The objective of the present study, was to evaluate the fertility rate in Creole goats inseminated into the uterus with thawed-frozen semen or mating by fertile male 24 h after estrus detection.

MATERIALS AND METHODS

The present study was undertaken in Guerrero State that which located in Southern of Mexico, at 18°15´ 52´´ N latitude and 99°38´52´´ W longitude with an AW (W)(i´)g climate, that is hot and dry (García, 1988). Four fertile Nubian bucks, one Boer male and 90 Creole aged 2-5 years age were used, in corporal condition of 2.5-3.0, on a scale of 0-5 (Russel *et al*., 1969) during the anoestrus season (April of 2006). Goats are no cycling at the start of the study, they are multiparous females (from 1 to 4 parity) and had kidded five to six months prior to the study.

Bucks were kept under intensive conditions inside small roofed yards. They were fed stubble corn, chopped sorghum, water *ad libitum* and commercial concentrate feed (12% PC and 3500 kcal kg^{-1}) in a ratio of its body

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weight. Does were maintained under a extensive conditions, feeding exclusively on grasses, shrubs and native grass in the grasslands of the region. The goats were internally treated for parasites with levamisol chlorohydrate and externally treated with organophosphorated products, as well as being immunized against pasteurellosis and digestive problems with mixed bacterine.

For estrous synchronization, during 11 d the goats received an intravaginal sponge with 40 mg of Fluorogestone Acetate (FGA) plus an intramuscular injection of 200 IU of equine Corionic Gonadotropin (eCG) at removing the sponges. Once withdraw the sponges, estrous was detected in the experimental flock every 12 h with males fitted with an apron. Of the 90 goats considered initially, three expelled the sponge and 14 others did non present estrus, thus only 73 were used. Of those that presented estrus, 37 were served 24 h later by natural mating by 4 Nubian males which had proven to be fertile in previous mating. Another group of 36 does was inseminated into the uterus by laparoscopy with thawed-frozen semen obtained from Boer male.

Semen was collected in December 2005 with artificial vagina from Boer buck of proven fertility. Two ejaculated were pooled and the semen was observed under a microscope and evaluated for ejaculated volume, sperm concentration, motility, morphology and abnormalities. Semen was frozen with the method described by Valencia (1997) and stored in liquid nitrogen for at least four months before use. A extender Triladyl (commercially available product; 20%) in egg yolk (20%) plus bidestilled water (60%) was utilized. Briefly, the semen was diluted to obtain a concentration of about 100×10^6 sperm mL^{-1} in the extender at 30°C. Diluted semen was then loaded into 0.25 mL PVC straws using an manual filled system and cooled to 5°C in 2 h. The straws were then dried and transferred to precooled rack in liquid nitrogen vapor at 4.0 cm above the level of the liquid. After 7 min the frozen straws were transferred to liquid nitrogen thermo for storage (Valencia, 1997). The straws were thawed by plunging them directly into a water bath at 37°C for 20 sec. The straws analyzed after thawing showed a mean of 50% viable spermatozoa.

For artificial insemination laparoscopy was utilized, for which goats were dieted for 24 h before being subjected to intrauterine insemination with frozen semen (straws of 0.25 mL, with 100 million spermatozoids). For pre-anesthetic tranquilizing xilacine hydrochloride at 2% (0.1 mL 10 kg^{-1} live weight) was applied by intramuscular injection. Ketamine chloridrate (0.2 mL 10 kg^{-1} live weight) was applied intravenously as anesthetic, 10 min after administering xilacine hydrochloride (Mejía, 1997). Once anesthetized, the ewes were placed in dorsal position on a surgery table, leaning back at the angle of 45°, the animal´s head downwards, with the purpose to cause the viscera to move towards the diaphragm, in order to avoid harm at the introducing the Verres needle and the trocar-cannula in the wall of the abdomen and the same time to uncover the uterus from the major mesentery (Ramírez-Molina *et al*., 2005).Two incisions were made, parallel to the middle line of the abdomen, at 4 cm distance from it and approximately 8 cm from the edge of the udder. First, by the right incision the Verres needle was introduced to insufflate air into the abdominal cavity, with the purpose to distend and permit good visibility of the uterus through the endoscope lens. Afterwards, by the incision on the right a trocar-cannula was inserted, through which the endoscope lens was introduced, while by the incision of the left the trocar-cannula got embedded, where the doses of aspics were introduced with the insemination pistol, containing the straw with frozen-thawed semen to deposit within the uterus (half dose in each uterine horn) (Maxwell and Butler, 1986).

Fertility rate (goats kidding/goat served or inseminated) was determined five mounts after service, when the kidding or non kidding occurrence in the experimental flock it was registered. The percentage of does kidding recorded between groups evaluated, was compared by using the Chi-square test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Only 73 (81.1%) of the 90 Creole goats treated with FGA plus eCG showed induced estrous within 72 h after the end of the treatment; whereas the proportion of does exhibiting estrual cycle at least twice, was 0%.

No difference was found ($p>0.05$) for fertility rate obtained between goats inseminated with thawed-frozen semen (33.3%) and goats served by natural mating (51.3%), as it is showed into Table 1.

At the present study, only 81.1% of the Creole goats treated with hormones showed estrous. This result is in agreement with the observations of Schoenian (2000) who found that no all females respond to the hormonal manipulations. Moreover, goats exhibiting a second estrous were no found in this study. Similarly, Estrada and Gutiérrez (1997) in anestric does induced to cycle by

Table 1: Fertility rate in creole goats inseminated into the uterus with thawed-frozen semen or served by natural mating

Treatment	Served goats	Kidding goats	Fertility rate (%)
Artificial insemination	36	12	33.3
Natural mating	37	19	51.3

Differences between treatments are not statistically significant ($p>0.05$)

means of fluorogestone acetate (45 mg) plus eCG (250 IU), too registered that neither of female treated with hormones showed a second estrous. Ortega *et al.* (1998) utilizing intravaginal sponges containing fluorogestone acetate (40 mg) during the non breeding season, observed that only the 44% of non pregnancy does repeated estrous.

Although there was no significant difference in fertility rate registered in this work between the groups, there was still a numerical difference of almost 18%, (artificial insemination, 31.3% vs natural mating, 55.1%); which could be due to the low number of repetitions for treatment utilized in this work. However, this result suggest too that fertility was not affected for the type of service used.

In the present research, the fertility rate was low for both evaluated groups. The causes of this poor fertility are unknown, but it may be related to different factors. Estrous synchronization methods there may be reductions in fertility and increased reproductive losses (Schoenian, 2000). It has been suggested that other factors, such as variation in the estrous presentation, abnormal luteal corpus and embryo mortality could affect too the fertility rate (Cameron and Batt, 1991; Niswender and Nett, 1994; Thatcher *et al.*, 1994). The hormonal treatments using progestagens associated with synchronization of estrus, seem to provoke a fertility reduced attributed to the fact that no all females respond to the hormonal stimulation, as well as to alterations in spermatozoa viability and motility (Hawk and Conley, 1971; Haresign and Read, 1986). It is documented that inseminations or natural mating made in the first estrous cycle after synchronization with progestagens generally had a lower fertility, due to an adverse effect of the treatment on the spermatic transport into the reproductive tract of the female (McDonald *et al.*, 1998). It is also known that low conception rates registered for artificial insemination too appears to be substantial reduction in sperm motility and viability after freezing (Lucidi *et al.*, 2001). Finally, conception rate obtained by natural mating is low during Spring and Summer due to lower sperm motility, than in Autumn and Winter (Tuli and Holtz, 1995).

There is a few information in the revised literature related to intrauterine artificial insemination in goats using frozen semen. Average fertility obtained in our study (33.3%) is near than in the work of Amaro *et al.* (1997) who reported a fertility of 29.2% in Creole goats from Mexico, induced in estrous 60 d after kidding and inseminated using frozen-thawed semen. In contrast, Dickson *et al.* (2001) registered a fertility rate of 59,5% in Alpine and Saanen goats inseminated into the uterus with frozen semen; while Ritar *et al.* (1987) obtained fertility rate of 71.2% in Cashmer goats; in both experiments fertility rates are higher than that 33.3% registered in the present study. Karatzas *et al.* (1997) and Goonewardene *et al.* (1997) too obtained during the nonbreeding season slightly higher fertility rates (44.9 and 41.0%, respectively) than in our work. Lowinger *et al.* (2001) found a variable fertility (from 0.0-40.0%) in goats of different flocks from Argentina, inseminated by laparoscopy within the uterus with thawed-frozen semen.

The fertility rate obtained in this experiment (51.3%) by natural mating is within the intervals reported of the Creole goats from Southern of Mexico, that fluctuate between 39 y 96% (Mellado, 1997) and is similar that of 58.1% found by Martínez *et al.* (2005) in Creole goats from this same region from Mexico. In the arid zones of northern of Mexico, Mellado *et al.* (1991) found a fertility rate of 54% in goats mated during the first trimester of the year. Fertility increased to 85% when mating occurred during the last trimester of the year. In those studies, does were served in natural estrous, commonly during the breeding season.

It is important to consider that in controlled mating after estrous synchronization programs using progestagens, the number of services per female was restricted to one. It therefore, seems likely that in our study with this type of breeding system, fertility is lower that when free mating is allowed in continuous traditional breeding, commonly taking place in commercial herds. In the Southern from Mexico, Aguilar *et al.* (1998) obtained a fertility rate of 38.8% in Croele goats treated with intravaginal sponges containing fluorogestone acetate and served by natural mating; while Ortega *at al.* (1998) registered a fertility of 31.25% in Creole goats synchronized during the non breeding season. Both fertility rates are lower that the 51.3% of does kidding recorded in that research.

CONCLUSION

It concluded that fertility rate registered for insemination into the uterus with frozen-thawed semen in Creole goats synchronized in estrous during the nonbreeding season, is low and similar that fertility registered in does receiving natural mating.

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