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Cervical or Intrauterine Artificial Insemination in Pelibuey Ewes, with Chilled Semen

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Abstract: In order to evaluate the effect of the site of cooled semen deposition (intrauterine or cervical) on subsequent fertility in Pelibuey ewes, a study was carried out during the breeding season in the dry tropic region from the Southern of Mexico, at 18°15´N and 99°38´W. A total of 40 cycling Pelibuey ewes from 2-4 years of age were used with corporal condition from 2-3, on a scale of 0-5. To synchronize estrus, intravaginal sponges were applied to the females, containing 20 mg of Flourogestone Acetate (FGA) for 11 days, plus 200 IU of equine Corionic Gonadotropine (eCG) administered intramuscularly when, the sponges were removed. Once removed, estrous was detected every 12 h with males fitted with an apron. Of the 40 ewes considered initially, five expelled the sponge and three others did no present estrus, thus 32 were used. Of those that presented estrus, 16 received cervical insemination using cooled semen 12 later and 16 others too were inseminated 12 h later with cooled semen deposited into the uterus by laparoscopy. The semen was collected by artificial vagina from a Dorper ram 12 h before the start of inseminations with an artificial vagina and an evaluation was made of its volume (mL), mass motility (percentage) and spermatic concentration (millions of cells mL^{-1}). For the preparation of the diluents, the following were used: Triladyl (20%), distilled water (60%) and egg yolk (20%). The diluted semen was manually placed into straws with a capacity of 0.25 mL, at 30°C, which were sealed with powdered polyvinyl alcohol. The sealed straws were placed in a plastic container with a water at 30°C, which was introduced into a cooler with ice cubes, where, it was maintained at 5°C, until the insemination took place, within the 12-16 h following its collection. The fertility rate of 75% obtained by intrauterine insemination was higher ($p<0.05$) than that registered for vaginal insemination (50.0%).

Key words: Pelibuey ewes, insemination, fertility, intrauterine, Mexico

INTRODUCTION

As with other domestic species, Artificial Insemination (AI) in ewes is an alternative for the maximum utilization of sires generally of high genetic value. The semen of the improved male can be used for AI in fresh, cooled or frozen form. In its fresh form maintained at 30°C (diluted or undiluted), the semen should be used immediately after it is collected, as the motility and viability of the spermatozoids under these conditions is quickly reduced, due to the increase in the concentration of lactic acid in the ejaculate (Vivanco, 1998). The above does not give the opportunity to transport the fresh semen to the used in farms that are distant, either from the breeding centers or from the farms where the improved males are found. However, the viability of the semen preserved in refrigeration can be maintained 48 h or more (Evans and Maxwell, 1990), which allows more flexibility for its use in AI programs for nearby sheep farms, which wish to use genetically superior males for the local Creole herds.

The simplest technique for AI in small ruminants consists of depositing the semen at the entrance of or within the cervix, or in passing the insemination pistol through the cervix, depositing the semen directly into the uterus (Evans and Maxwell, 1990), which is sometimes

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impossible (especially in young animals), given the size and structure of the cervix (Vivanco, 1998; Mareco, 2004). Therefore, cervical insemination offers a faster and practical option; however, when frozen semen is used, fertility obtained could to be lower (Salamon and Lightfoot, 1967; Maxwell *et al*., 1984). As the main cause of reduced fertility of the frozen semen appears to be an alteration in the capacity to transport the spermatozoids form the cervix to the place of fertilization in the oviduct (Evans and Maxwell, 1990). It is has been observed in sheep that the cooling and thawing of the ram semen induces the premature capacitation of the spermatozoids, reducing their fertilizing capacity (Gillan and Maxwell, 1998). By reason of lower pregnancy rates obtained for cervical insemination, when frozen-thawed semen is used, the intrauterine insemination performed by laparoscopy, is the most popular method for AI in ewes (Salamon and Maxwell, 1995a, b; Gil, 2001); because this technique makes it possible to deposit the semen directly into the uterus near the oviduct, shortly before ovulation (Evans and Maxwell, 1990), resulting in acceptable conception rates. Even so, due to surgical requirements and its high cost, the laparoscopic technique of intrauterine insemination is no suitable for routine use in many commercial programs. To overcome this problem, an alternative is the use of liquid semen stored between 3-5°C; the cooled semen has resulted in higher pregnancy rates when it is deposited into the cervix, compared to the poor fertility achieved with frozen-thawed semen (Maxwell and Salamon, 1993; Salamon and Maxwell, 2000). However, no studies in hair tropical ewes were found aimed at evaluating the fertility rate obtained with intrauterine or cervical insemination in sheeps, using cooled semen. The objective of the present study was to evaluate the effect of the site of cooled semen deposition, intrauterine or cervical, on subsequent fertility in Pelibuey ewes.

MATERIALS AND METHODS

The study was carried out during the breeding season (October 2008) in the dry tropic region from the Southern of Mexico (Iguala, Guerrero City), at 18°15´N and 99°38´W. The climate of the region is Awo (w)(i´)g: warm sub-humid, with rains in summer and with no defined winter season (Garcia, 1988).

For this study, 40 cycling Pelibuey ewes from 2-4 years of age were used with corporal condition scores from 2-3, on a scale of 0-5 (Russel *et al*., 1969). The experimental flock was managed in a semi-confined system; during the morning ewes grazed on African Star pastures (*Cynodonn nlemfuencis*), by the evening and night they were kept in roofed pen; were they received water *ad libitum*, besides a commercial concentrate with 12% of crude protein and 3500 kcal kg^{-1}. The ewes were internally treated for parasites with levamisol chlorohydrate, as well as being immunized every 6 months against pasteurellosis and digestive problems with mixed bacterine.

To synchronize estrus, intravaginal sponges were applied to the females, containing 20 mg of Flourogestone Acetate (FGA) for 11 days, plus 200 IU of equine Corionic Gonadotropine (eCG) administered intramusculary when the sponges were removed. Once removed, estrous was detected every 12 h with males fitted with an apron. Of the 40 ewes considered initially, five expelled the sponge and three others did no present estrus, thus 32 were used. Of those that presented estrus, 16 received os-cervical insemination using cooled semen 12 later and 16 others too were inseminated 12 h later with cooled semen deposited into the uterus by laparoscopy.

The semen was collected with an artificial vagina from a Dorper ram of the UNAM sheep farm, located to 120 km from Iguala City, 12 h before the start of inseminations and an evaluation was made of its volume (mL), mass motility (percentage) and spermatic concentration (millions of cells mL^{-1}). For the preparation of the diluents, the following were used: Triladyl (20%), distilled water (60%) and egg yolk (20%). The diluent was added to the ejaculate at 30°C in a single step, using 100 million spermatozoids per dose. The diluted semen was manually placed into straws with a capacity of 0.25 mL, at 30°C, which were sealed with powdered polyvinyl alcohol (Evans and Maxwell, 1990). The sealed straws were placed in a plastic container with a water at 30°C, which was introduced into a cooler with ice cubes, where it was maintained at 5°C until the insemination took place, within the 12-16 h following its collection.

For intrauterine insemination, laparoscopy was utilized, for which 16 ewes from this group were dieted for 24 h before being subjected to intrauterine insemination with cooled semen. For pre-anesthetic tranquilizing xilacine hydrochloride at 2% (0.1 mL 10 kg^{-1} live weight) was applied by intramuscular injection. Ketamine (0.2 mL 10 kg^{-1} live weight) was applied intravenously as anesthetic, 10 min after administering xilacine hydrochloride (Mejia, 1997). Once anesthetized, the ewes were placed in dorsal position on a surgery table, leaning back at an 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 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.

In the disinfected area of the abdomen three incisions were made, a small one (5 cm) on the left of the udder, where the Verres needle was introduced to insufflate air into the abdominal cavity, with the purpose to distend it and permit good visibility of the uterus through the endoscope lens. Afterwards, two incisions of greater size were made, parallel to the middle line of the abdomen, at 4 cm distance from it and approximately, 8 cm from the front edge of the udder. By the incision on the right a trocar-cannula was inserted, through, which the endoscope lens was introduced, while by the incision on the left the trocar-cannula got embedded, where the doses of aspic were introduced with the insemination pistol, containing the pipette with cooled semen to deposit it in the uterine horn, ipsilateral to the corpus luteum.

For cervical insemination, ewes were restrained and the rear quarters of the animal were lifted, with the front legs remaining on the ground. A vaginal speculum, fabricate using a hard plastic cylinder, with an outer diameter of 2.5 cm and length 15 cm equipped with a light source, was used to examine the vaginal and locate the external cervical os and the chilled semen was deposited with the aid of an insemination gun.

Just before semen was deposited either into the uterus or at the cervical os, masal motility of spermatozoa (wave motion) was evaluated. A small drop of cooled semen was deposited an a glass slide and placed on a warmed stage (37-38°C) under microscope (magnification 10X; Evans and Maxwell, 1990).

The variable evaluated was the fertility rate (ewes lambing/ewes inseminated) and it was determined 5 mounts after service, when the lambing occurrence in the experimental flock it was registered. The percentage of ewes lambing recorded between groups evaluated, was compared by using the Z for proportions test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The fertility rate obtained in Pelibuey ewes using intrauterine insemination with cooled semen was 75% (12/16). The fertility resulting from os-cervical insemination (43.7%; 7/16) was lower ($p<0.05$) than with laparoscopic artificial insemination (Table 1).

The data demonstrate that fertility rate obtained for cervical insemination are not comparable at fertility resulting from intrauterine insemination and suggest that differences exist between both insemination types in the fertilizing capacity of chilled semen, which is attribute to the site of spermatozoids deposition, considering that the same cooled-stored spermatozoa was utilized either for laparoscopic or for exocervical insemination. At the present research, good motility has been achieved after cooled process of ram semen (70%), but it has not been reflected in improvement in lambing rate after os-cervical insemination. In addition, in critical studies fertility declined rapidly fertility when semen stored for >24 h was used for cervical insemination, but after intrauterine insemination some spermatozoa maintained their fertilizing capacity for up 10 days (Salamon *et al*., 1979). Stojanov *et al*. (1994) further showed that fertilizing capacity of ram spermatozoa *in vitro* is maintained for up to 14 days in a Tris-citrate diluents at 5°C.

Table 1: Fertility rate of pelibuey ewes that received intrauterine or vaginal insemination with cooled semen, 12 after presenting estrus

Insemination site	n	Ewes lambing	Fertility rate
Cervical	16	7	43.7%[a]
Intrauterine	16	12	75.0%[b]

[a,b]Different letters indicate significant differences ($p<0.05$) between insemination site

Frozen-thawed spermatozoa generally exhibit reduced fertility after cervical insemination, compared to that obtainable with fresh diluted semen or after intrauterine insemination by laparoscopy (Maxwell and Watson, 1996). No studied has been made for chilled semen in sheep, but Eppleston *et al*. (1994) demonstrated that one of important factors affecting the fertility of frozen-thawed ram semen inseminated into the cervix of sheep is the deep of insemination. The overall lambing rate achieved from deposition of frozen-thawed semen into the cervix or uterus using the transcervical technique was 22.7% even though, the mean post-thaw motility was 78.7%; however, no lambing rate was obtained with os-cervical insemination was utilized (Naqvi *et al*., 1998). Impaired sperm transport through the genital tract of the ewe, is the principal cause of low fertilization rates after cervical insemination with frozen semen (Salamon and Lightfoot, 1967).

Part of the explanation for the lower fertility of stored-chilled semen compared with fresh spermatozoa is that the proportion of progressively motile cell is reduced by process such as cooling, freezing and thawing (Maxwell and Watson, 1996). For this reason, another alternative explanation for the lower lambing rate obtained with cervical insemination, is that the process of cooling semen damages many sperm cells, which causes reduced fertility. It is has been observed in sheep that the cooling and thawing of the ram semen induces the premature capacitation of the spermatozoids, reducing their fertilizing capacity (Gillan and Maxwell, 1998). Therefore, semen conserves high fertilizing capacity only when deposited in the uterus next to the oviduct, close to the moment of ovulation. For this reason, probably the lower lambing rate resulted from os-cervical artificial insemination is likely associated to the fact that appears

to be substantial reduction in sperm motility a viability and short half-life of the spermatozoa after the process of cooling semen (Haresign, 1992). On the other hand, the period of time of sperm transportation through the female reproductive tract could be also considered as one factor affecting fertility rate in the sheep, when cooled semen is placed at the external cervical os (Maxwell and Salamon, 1993; Menchaca *et al*., 2005). By reason of this, deep uterine insemination has been shown to be advantageous in the sheep (Salamon and Maxwell, 1995b; Wulster-Radcliffe *et al*., 2004), especially when sperm quality is suboptimal (Sohnrey and Holtz, 2007). Endoscopy is a technique that overcome this problem; whereas, the advantage of laparoscopic insemination is that the semen is deposited closer to the site where, the fertilization occurrs.

Poor fertility generally is obtained after cervical insemination in ewes with semen that has been frozen and thawed (Eppleston *et al*., 1994; Maxwell and Watson, 1996). Even so, the study seem be suggested that middle fertility rate obtained after os-cervical insemination using chilled semen in Pelibuey ewes, can be considered as acceptable. Little information is available on os-cervical artificial insemination using cooled semen, but the fertility rate of 43.7% registered in this study, agrees with the conception rate of 42.7% obtained for Menchaca *et al*. (2005) in ewes inseminated vaginally (semen placed at the external cervical os) with chilled semen started at 5°C for 12 h, whereas Paulenz *et al*. (2003) reported a slightly higher lambing rate (51.5%) in ewes using semen stored at 5°C diluted in a commercial TRIS-based extender.

In comparison with os-cervical insemination, the present study shows that good fertility rate (75.0%) can be obtained using chilled ram semen stored for 12 h at 5°C that is deposited into the uterus by laparoscopic artificial insemination. There is a few information on aspects of cooled preservation of ram semen such as processing and storage damage and fertility of ram spermatozoa, but it is generally assumed that the process reduce the motility and is associated with a loss of fertilizing capacity. In contrast, there are a wealth studies on frozen storage and freezing injury of ram semen has been made. An problem with frozen-thawed semen is the reduced viability of spermatozoa in the female reproductive tract, characterized by longevity often half that of fresh spermatozoa (Salamon and Maxwell, 1995a) and impaired ability to penetrate the cervix (Lopyrin and Loginova, 1958). Evidence suggest that while many spermatozoa remain motile after storage, the membrane of the motile cells are destabilized to the point where they may not survive further ageing in the female tract after cervical insemination. These membrane changes are similar to the capacitation and acrosome reaction of spermatozoa. Thus, stored spermatozoa may require less capacitation time in the female tract and may readily fertilize oocytes if placed in their immediate vicinity tubal or even intrauterine insemination (Maxwell and Watson, 1996). For this reason, fertility is generally lower after cervical insemination with frozen-thawed semen.

CONCLUSION

It concluded that middle fertility rate of 43.7% resulting from os-cervical insemination in Pelibuey ewes with chilled ram semen stored for 12 h at 5°C can be considered as acceptable, but it is no possible to obtain similar fertility rate (75.0%) as with laparoscopic intrauterine insemination.

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