Original Research Article   https://doi.org/10.20546/ijcmas.2019.802.138

Laparoscopic Artificial Insemination with Different Liquid Semen Concentration in Nari Suwarna Ewes

**G.N. Rudresh, G. Sudha, K.H. Sandeepa, K.M. Hareesh, H.S. Shwetha*,
S. Mahendra, S. Vishwanath, M.A. Kshama and L. Ranganath**

Department of Veterinary Gynecology and Obstetrics, Veterinary College, Bangalore, India

*Corresponding author

A B S T R A C T

The study was conducted in eighteen NARI Suwarna ewes with the objectives to establish an effective liquid semen sperm concentration for laparoscopic AI and to study the conception rates with natural and laparoscopic AI method in ewes. The ewes in estrus were detected using vasectomized ram and external signs of estrus and were randomly allotted to three equal sized Groups of six each (n=6) namely, natural mating (Group I), laparoscopic AI with 20 million sperm dose (Group II) and laparoscopic AI with 40 million sperm dose (Group III). Thick creamy white semen samples with good mass activity, initial motility more than 70% and average sperm concentration around 5000 million was collected using an artificial vagina (AV). Freshly collected semen sample was diluted with Sodium citrate- egg yolk extender at 37∘C to achieve the concentration of 20 million spermatozoa/ dose for Group II and 40 million spermatozoa/ dose for Group III (Volume-0.25ml) for Laparoscopic AI. The laparoscopic AI was performed between 12-24 hrs after onset of estrus. Conception rates recorded for Group I, II and III were 33.33%, 16.66% and 66.66% respectively. Over all conception rate with laparoscopic AI was 41.66%. Laparoscopic AI with use of 40 million sperm/dose yields significantly better conception rates than 20 million sperm/ dose. It is concluded that laparoscopic AI can be effectively employed with the low sperm dose of 40 million when stress factors are taken care of.

Introduction

Reproduction in sheep is seasonal which limits its prolificacy. Artificial insemination (AI) can serve as a powerful tool to the sheep owners for making rapid genetic progress of their flock (Maxwell and Hewitt, 1986). Laparoscope is used in artificial insemination in ewes by direct manipulation of semen into the uterine horn as a means of genetic improvement (Dally, 2008). The laparoscopic intrauterine technique of insemination in ewes (Killen and Caffery, 1982) is now well-known in well-organized sheep farms. Laparoscopic insemination improves the flock health and management, reduces environmental effects and promotes estrus synchronization that promotes that shortens the lambing interval (Gourley and Riese, 1990). High fertilization rates of 89% (Mckelvey *et al.,* 1985), 55%

with 100 million fresh spermatozoa (Maxwell and Hewitt, 1986) and 64% with 600 fresh spermatozoa have been reported. The present study was conducted in Nari Suwarna ewes with the objectives to establish an effective liquid semen sperm concentration for laparoscopic AI in ewes and to study the conception rates with natural and laparoscopic AI method.

Materials and Methods

The study was conducted between February and May 2016 the summer months. Bangalore recorded higher atmospheric temperature and humidity during the study period than the previous years. During the study period highest environmental temperature of 39.2 ∘C and 89 per cent humidity was recorded, whereas the highest environmental temperature recorded during previous two years was 35 ∘C and approximately 4 ∘C rise in the temperature had been observed during the study period (© 1999-2016 weather online Ltd). Eighteen apparently healthy NARI Suwarna ewes, aged two to four years were selected for the study. Two NARI suwarna Rams aged between 2-3 years were used for semen collection and natural mating. AI was performed on naturally detected estrus.

The ewes in estrus were detected by vasectomized rams and also by external signs of heat like edematous vulva, switching of tail and hyperemic vaginal mucus membrane. On heat detection, ewes were randomly allotted to three equal sized Groups of six each (n=6) namely, Group 1: Natural mating, Group 2, ewes were inseminated by laparoscopic AI method with a dose of 20 million sperms and Group 3, ewes were inseminated by laparoscopic AI method with a dose 40 million sperms.

Semen was collected from rams by artificial vagina method on the day of laparoscopic insemination. Semen collected was evaluated for color, volume and concentration. Thick creamy white semen samples with good mass activity, initial motility more than 70% and average sperm concentration around 5000 million were used for laparoscopic artificial insemination method. Freshly collected semen sample was diluted with Sodium citrate- egg yolk extender at 37∘C to achieve the concentration of 20 million/ dose for Group II and 40 million/dose for Group III (Volume-0.25ml). Semen was deposited uterine by laparoscopy 12 hours after oestrus detection.

Laparoscopic AI was performed under aseptic condition in ewes with 12 hour off feed and water. A specially designed mobile cradle tilted at an angle of 30∘ to the horizontal was used to restrain the ewe in a dorsal recumbent position with the head down.

A local anesthetic (0.5 ml xylocaine; 2% lignocaine hydrochloride) was injected at two sites approximately 5-8cm cranial to the udder and 3-6cm lateral to the ventral midline, avoiding prominent blood vessels. Using a 5.5 mm trocar and cannula, the laparoscope (Karl Storz GmbH and Co., Germany) was introduced into the abdomen to the left of the midline (Plate 5A) and the uterus was viewed through the telescopic lens (Plate 5B). A 5.5 mm trocar and cannula was inserted into the abdomen on the right side of the midline to enable the introduction of a specially designed laparoscopic insemination gun (IMV Company, India). Semen sample with sperm concentration 20 million/dose for Group II and 40 million/dose for Group III was deposited into the lumen of uterine horn (Plate.5C) by penetrating the uterine wall with the needle attached to the plastic sheath mounted on the insemination gun. Laparoscopic wound was cleaned with povidone iodine solution and povidone iodine ointment applied. Wound healed in 5days without any complications.

Pregnancy diagnosis was done on day 30 after natural mating and AI using an ultra sound scanner (Plate 6) (Aloka prosound α 6, Hitachi Aloka medical limited). The conception rate was then calculated as the number of animals that became pregnant in relation to the number of inseminations carried out and the same was expressed in per cent.

Results and Discussion

The comparison of conception rates between the Groups I, II and III by chi-square test showed there was a significant difference between the Groups (P>0.05).

In the present study, Group III which was inseminated laparoscopically with 40 million spermatozoa had a significantly better conception of 66.66 per cent in comparison to the conception of 16.66 per cent in Group II which was inseminated with 20 million spermatozoa (Table 1). Many studies have recorded similar conception rates (Smith *et al.,* 1995; Maxwell and Hewitt, 1986; Milovanovic *et al.,* 2013 and El-Badry *et al.,* 2014) and or higher conception rate with this technique (Ghalsasi and Nimbkar, 1996; Al-

wataar *et al.,* 2009 and Abdalbari *et al.,* 2012).

One notable feature of this study is that this study has used a lower dose of 40 million in Group III, whereas others have used 80 million sperms and obtained conception of 48 per cent (Windsor *et al.,* 1994), 100 million with conception rates of 55 per cent (Maxwell and Hewitt, 1986). Some have used 200 million spermatozoa with conception rates of 63 per cent (Smith *et al.,* 1995), with 600 million spermatozoa conception of 64 per cent has been achieved by Milovanovic *et al.,* (2013). Ghalsasi and Nimbkar (1996) have obtained pregnancy rates of 77 per cent and 72 per cent with fresh diluted semen (dilution ratio 1:2) and fresh undiluted semen.

The difference in conception rates in this study and others may be attributed to the higher dose of spermatozoa used by others which was either 100, 200, 600 million spermatozoa or use of fresh undiluted semen, fresh semen diluted at 1:2 ratio whereas our study has used a much smaller dose of 20 and 40 million spermatozoa in Group II and III respectively.

Table.1 Conception rates of ewes mated naturally (Group I) and inseminated by laparoscopic AI method (Group II and Group III)

Groups (n=6)	No. of ewes Pregnant
Group I (Natural mating)	2(33.33%)
Group II (Laparoscopic AI with 20million sperm dose)	1(16.66%)
Group III (Laparoscopic AI with 40million sperm dose)	4(66.66%)

Between Group II (inseminated with 20 million spermatozoa) and Group III (inseminated with 40 million spermatozoa),

there was significantly lesser conception rate in Group II as compared to Group III. The possible reason for lowered conception

between laparoscopic AI Group i.e. Group II may be lower dose of semen used and ram variation (Anel *et al.,* 2005) since both the Groups were inseminated with different ram semen. Also in the present study, the only fertility parameters assessed is sperm motility and fertility is not related to motility in rams (Colas, 1979) and it could have contributed to lower conception rate in Group II in relation to Group III.

The overall conception rate with laparoscopic AI irrespective of semen volume used was 41.66 per cent which was significantly higher than naturally mated Group (33.33%) (Table 1). Stellflug *et al.,* (1993) have recorded 29 per cent conception with laparoscopic AI and complete absence of conception by natural mating in Targhee ewes. Ghalsasi and Nimbkar (1996), showed a significantly better conception rates in natural mating (83%) than in laparoscopic AI (75%).

The low conception rate that was recorded in laparoscopic AI method with 20 million sperm dose and natural mating may be attributed to higher environmental temperature (39.2 ◦C) and humidity (89%) (© 1999-2016 weather online Ltd) recorded during the study period as compared to previous years, handling stress during preparation of surgical site and the laparoscopic AI procedure, inherent variation in the ewe and ram, season, breed, experience of the inseminator, flock management and early embryonic mortality.

References

Abdalbari, A. Alfaris., Tahir, A. Fahid., Baqer Jafer and Hassan Al-Dahabi., 2012. Laparoscopic intrauterine artificial insemination and ultrasonic pregnancy diagnosis in Arabi ewes. Journal of Evolutionary Biology Research, 4(1): 1-12

Al-Wataar, B.D., Taha, M.B. and Al-Badrany, M.S., 2009. Co-partion of Laparoscopic intrauterine insemination in synchronized Awassi ewes. J. Iraqi Vet. Sci., 22 (1): 53-57

Anel, L., Kaabi, M., Abroug, B., Alvarez, E.M., Anel, E., Boixo, J.C., De La Fuente, L.F. and De Paz, P., 2005. Factors influencing the success of vaginal and laparoscopic artificial insemination in churra ewes: a field assay. Theriogenology, 63: 1235–1247

Colas, G., 1979. Fertility in ewe after artificial insemination with fresh and frozen semen at the induced oestrus and influence of the photoperiod on the semen quality of the ram semen. Live. Prod. Sci., 6: 153-166

Dally, R.M., 2008. Laparoscopic artificial insemination a means to improve genetics. Animal science department, University of California-Hopland field station. (net.www.topram.com)

El-Badry, D.A., Aml Z. Leil and Mona H. Shaker., 2014. Studies on laparoscopic intrauterine insemination of barki ewes (using different insemination doses) as compared with cervical insemination. Assiut Vet. Med. J., 60: 172-178

Ghalasasi, P.M and Nimbkar, C., 1996. Evaluation of laparoscopic intrauterine insemination in ewes, Small Ruminant Research, 23: 69-73

Gourley, D.O and Riese, R.L., 1990. Laparoscopic artificial insemination in sheep. Vet. Clinics of N. America: Food Animal Practice, 6: 615-633.

Killeen, I.D. and Caffery, G.J., 1982. Uterine insemination of ewes with the aid of a laparoscope. Australian Veterinary Journal, 46: 327-333

Maxwell, W.M.C. and Hewitt, L.J., 1986. A comparison of vaginal, cervical and intrauterine insemination of sheep. Journal of agricultural science, Cambridge, 106: 191-193

Mckelvey, W.A.C., Robinson, J.J and Aitken, R.P., 1985. The evaluation of a laparoscopic insemination technique in ewes. Theriogenology, 24: 519-535

Milovanović, A., Maksimović, N., Barna, T., Lazarević, M and Delić, N., 2013. Laparoscopic insemination of sheep in Republic of Serbia. Biotechnology in Animal Husbandry, 29 (3): 449-456

Smith, J.F., Parr, J., Beaumont, S., Oliver, J.E. and Upreti, G.C, 1995. Comparison of cervical, transcervical and laparoscopic insemination of ewes with chilled stored and frozen ram semen. Proc. N. Soc. Anim. Prod., 55: 248-250

Stellflug, J.N., Rodriguez, F. and Fitzgerald., 1993. Influence of estrus induction with artificial insemination or natural mating on reproductive performance of fall-barn ewe lambs during out-of-season breeding. Sheep research journal, 9(3): 115-117

Windsor, D., Szell, A. and Buschbeck, C., 1994. Transcervical artificial insemination of Australian Merino ewes with frozen-thawed semen. Theriogenology, 42: 147-57

How to cite this article: