Indian Journal of Animal Sciences **90** (4): 557–559, April 2020/Article

Effect of cervical insemination with frozen semen on fertility of Indian goat breed

R RANJAN*, A K GOEL, S D KHARCHE, R PRIYADHARSINI, N RAMACHANDRAN, M K SINGH, R KUMAR, M S DIGE, S BHUSHAN, U B CHOUDHARY, S KUMAR, S K JINDAL and M S CHAUHAN

ICAR-Central Institute for Research on Goats, Makhdoom, Farah, Mathura, Uttar Pradesh 281 122 India

Received: 26 March 2019; Accepted: 22 August 2019

ABSTRACT

Artificial Insemination (AI) has great potential to multiply superior quality of goat with faster rate in spite of lower conception rate. This technique will spread elite genetic material throughout a population and is also important for breed conservation and has paved the way for other reproductive biotechnologies. Ejaculates from bucks aged between 2–4 years old from Jamunapari, Barbari, Sirohi and Jakhrana were collected using artificial vagina, twice a week. Immediately after collection, the volume, colour, consistency, and mass motility of ejaculate were assessed and were extended with Tris -Egg yolk- Fructose diluent having 10% (v/v) egg yolk and glycerol 6% (v/v). Sperm concentrations were adjusted to 1×10^8/ml and diluted semen was equilibrated at 5°C for 4 h before being frozen. The post thaw motility in Jamunapari and Barbari was significantly higher then Jakhrana and Sirohi. In two major breeding seasons (May–June and October–November), 181 goats of different breeds (Barbari, Jakhrana, Jamunapari and Sirohi) including 35 goats in villages were intra-cervical inseminated with frozen semen in natural estrous condition. The kidding percentage in Barbari, Jamunapari, Jakhrana and Sirohi were 53.12±2.40%, 34.61±1.96%, 26.53±2.12% and 28.57±2.32% respectively. Overall, a success rate of 37.57% was recorded on the basis of actual kidding rate irrespective of goat breed maintained at this institute under semi-intensive management system. The best post thaw quality of buck semen and comparative deeper cervical insemination in Barbari and Jamunapri breeds results in higher conception and kidding percent compared to other breeds (Sirohi and Jakhrana).

Keywords: Artificial Insemination, Cryopreservation, Goat, Kidding per cent, Post thaw motility

The descript goat population (33%) is very less compared to non-descript and non-productive goats (67%). So, to increase the productivity per goat, we need to improve the breed quality scientifically. The sufficient elite germplasm of male buck is not available throughout the country to cover breeding programme by natural mating. We need 1.5–2 million bucks to cover 71 million breedable does for natural breeding as compared to only 50,000 bucks needed for Frozen Semen AI Technology. AI is the only solution to improve the quality and productivity per goat. The adoption index score possessed by Artificial Insemination was 42.50% in India (Gunaseelan *et al.* 2018). The pioneering work in goat semen freezing and AI in India carried out at Indian Veterinary Research Institute, Izatnagar and Veterinary College, Mathura (Roy *et al.* 1957) revealed around 50% conception after first insemination with washed spermatozoa diluted in EYC diluents. Chauhan and Anand (1990) had developed goat semen freezing protocol using egg yolk tris as a diluents and which was subsequently adopted and implemented by this Institute. Bhattacharyya *et al* (2012) reported 71.43% pregnancy rate with kidding rate of 1.27 by pellet semen. Goat has been playing multiple role in livelihood of the rural people by providing income, employment, nutrition, supporting crop production and risk aversion in case of crop failure. Goat farming has huge opportunity in rural development as goat has potential for export of products, capital storage, household income, employment and nutritional security. The greatest problem still existing with the cryopreservation of goat spermatozoa is that even with the best preservation techniques to-date available, post-thawing survival is restricted to approximately 50% of the sperm population. The frozen semen AI could successfully be used for preservation, conservation and propagation in different breeds of goat. Though the attempts of AI in goats have been undergoing on experimental basis at various organized farms and research institutes of India, still serious and coordinated efforts are lacking for taking up the AI in goats on large scale like AI in cattle and buffaloes. In this study, optimisation of goat semen freezing protocol was done to get optimum post thaw quality of frozen semen and subsequent conception by cervical AI method.

*Corresponding author e-mail: dr_raviranjan@yahoo.co.in

MATERIALS AND METHODS

Animals and their management: The adult bucks of different breeds (Jamunapari, Barbari, Sirohi and Jakhrana) of 2–4 years old (40) were selected for the study. The bucks were kept under semi-intensive system of management at

Table 1. Post thaw quality of frozen semen and kidding per cent in different goat breeds (Mean±SE)

Breed	Progressive motility %	Live %	Acrosome intact %	Hypoosmotic swelling %	Kidding %
Barbari	54.40^{a}±2.84	65.35^{a}±2.94	72.51^{a}±3.24	68.72^{a}±2.81	53.12^{a}±2.40
Jamunapari	52.80^{a}±3.54	61.21^{b}±3.26	68.45^{b}±2.46	65.65^{b}±2.56	34.61^{b}±1.96
Jakhrana	48.80^{b}±3.12	54.39^{c}±3.12	64.42^{c}±2.54	61.23^{c}±3.12	28.57^{c}±2.32
Sirohi	46.20^{b}±2.32	58.28^{bc}±2.86	65.42^{c}±2.86	63.22^{bc}±2.38	26.53^{c}±2.12

*Different superscript differ significantly within a column.

this institute. All the animals were handled as per Ethical rule. Institute Animal Ethical Committee (IAEC) had approved the work and all procedure was followed as per IAEC rule.

Semen collection, evaluation and dilution: Ejaculates from bucks aged between 2–4 years old from Jamunapari, Barbari, Sirohi and Jakhrana were collected using artificial vagina, twice a week at this Institute. Immediately after collection, the volume, colour, consistency, and mass motility of ejaculate were assessed and were extended with Tris-Egg yolk-Fructose diluent (Tris, 3.604 g; Citric acid, 1.902 g; Fructose, 1 g; Streptomycin, 100 mg; Penicillin, 100000 IU; Triple distilled water, 100 ml; *p*H, 6.75–6.8), having 10% (v/v) egg yolk and glycerol 6% (v/v). Samples having mass motility >4 and progressive motility >70% were taken for this study as these quality of semen sample qualify for freezing process. Sperm concentrations were adjusted to 1×10^{8}/ml and diluted semen was equilibrated at 5°C for 4 h before being frozen (Ranjan *et al.* 2009a, 2009b, 2014, 2015, 2017).

Sperm staining and evaluation: Post thaw semen (10 µl) was placed on a clean grease free warm slide (38°C) with cover slip and observed under 200 × magnification of phase contrast microscope for assessing the progressive motility. The average values of two independent experts were considered for calculating the progressive motility. For calculating the live and dead sperm count, a method of Hancock (1951) using Eosin-Nigrosine stain was followed. Abnormal sperms were counted with the same staining technique. Giemsa stain was used to assess the acrosomal integrity of frozen thawed buck spermatozoa as per modified by Ranjan *et al.* (2014). Hypoosmotic swelling test was carried out as described by Ranjan *et al.* (2009).

Artificial insemination in estrous goat: Intra-cervical AI was used to get maximum benefits. For Intra cervical AI, the oestrous goat lifted from back for clear visualization of genitalia. A lubricated glass vaginal speculum was inserted through vagina for visualization of cervical opening under sunlight. Then frozen thawed semen straw was inserted through vaginal speculum which passed through cervical opening and semen was deposited there and waited for 2-3 min. In two major breeding seasons (May-June and October-November), 181 goats of different breeds (Barbari, Jakhrana, Jamunapari and Sirohi) including 35 goats in villages were inseminated with frozen semen in natural estrous condition. The insemination was carried out 12 h after detection of estrous and repeated after 12 h of first insemination.

Trans rectal ultrasonography of goats: Trans rectal ultrasonography was carried out using 5/7 MHz transducer in inseminated does with frozen semen at or after 28 days post mating.

Statistical analysis: Data were analyzed by two-way analysis of SPSS package 16. The factorial model included the effect of percent post thawed motility as independent variables and percent of goat kidded as dependent variables.

RESULTS AND DISCUSSION

The post thaw motility in Barbari, Jamunapari, Jakhrana and Sirohi were 54.40±2.84%, 52.80±3.54%, 48.80±3.12% and 46.20±2.32% respectively. The post thaw motility irrespective of breed was 50.55%. The corresponding values of live percent were 65.35±2.94, 61.21±3.26, 54.39±3.12 and 58.28±2.86 respectively. The corresponding values of acrosome integrity percent were 72.5±3.24, 68.50±2.46, 64.42±2.86 and 65.42±2.86 respectively. The corresponding values of hypoosmotic swelled sperm percent were 68.72±2.81, 65.65±2.56, 61.23±3.12 and 63.22±2.38 respectively. AI by using these frozen semen straws having above mentioned post thaw motility was carried out in two major breeding seasons (May-June and October-November). Goats of different breeds (181; Barbari, Jakhrana, Jamunapari and Sirohi) including 35 goats in villages were intra cervical inseminated with frozen semen in natural estrous condition. A total of 68 goat conceived by using frozen semen AI technology and total 121 kids (65 female and 56 male) were born through this technology. The kidding percentage in Barbari, Jamunapari, Jakhrana and Sirohi are given in Table 1 and Table 2. Overall, a success rate of 37.57% was recorded on the basis of actual kidding rate irrespective of goat breed maintained at this Institute under semi-intensive management system.

It is possible to maintain good fertility in goats after AI with semen stored for 24 h in TEMPOL (Mara *et al.* 2007). Commercially available soy-based extender (Bioxcell®) was found superior to an egg yolk-based extender (Irvine

Table 2. Kidding per cent by AI with frozen semen

Breed	Does inseminated	Does that kidded	Kidding %
Barbari	64	34	53.12
Jamunapari	26	9	34.61
Sirohi	42	12	28.57
Jakhrana	49	13	26.53
Overall	181	68	37.57

TYB) in preserving motility of cryopreserved goat sperm using a two-step method (Roof *et al*. 2011). Jimenez-Rabadan *et al.* (2012) studied effect of different extender on buck semen cryopreservation using Tris-based extender with moderate success. In recent years the main focus was on the various additives in the dilutor in order to obtain the higher post thaw motility and fertility rates. Padilha *et al.* (2012) reported supplementation of Tris extender with IGF-I improved subjective sperm motility and structural integrity of the plasma membrane without a significant effect on pregnancy rates of ewes with frozen thawed semen. Due to the complex cervical anatomy in goats, it becomes very difficult to pass the AI gun throughout the cervix. Therefore, the conception rate highly correlated with depth of penetration. Although the laparoscopic AI involving deposition of frozen-thawed semen directly in to the uterus generally results 60–70% fertility (Salamon and Maxwell 1995), the conception rate of cryo-preserved semen following trans-cervical AI (TCAI) is still very low (16 – 40%; Kharche *et al.* 2013, Kumar and Naqvi 2014). The Embrapa AI technique resulted in satisfactory rates of cervical transposing and intrauterine AI, achieving reasonable pregnancy rates in goats (Jeferson *et al.* 2017). Inhibition of angiotensin-converting enzyme in goats under protocol of fixed-time artificial insemination improves pregnancy rates, parturition, twinning and proved to be a good alternative for increasing the efficiency of such a biotechnique (Fernandes *et al.* 2018). The premature capacitation as a consequence of freezing and thawing curtails the lifespan of spermatozoa having very shorter time to achieve fertilization compared to the fresh sperm. Therefore, further research efforts are required to develop better freezing protocols and diluents that minimize the ultra-structural and biochemical alterations in spermatozoa resulting from the freezing/thawing process, particularly if intended for TCAI; because spermatozoa have to survive longer to traverse through cervical mucosa before reaching the site of fertilization.

It can be concluded that the best post thaw quality of buck semen and deeper intra cervical insemination in Barbari and Jamunapri breeds results in higher conception and kidding percent compared to other breeds (Sirohi and Jakhrana).

ACKNOWLEDGEMENT

Authors are thankful to Director, ICAR-CIRG, Makhdoom, Farah, Mathura, Uttar Pradesh, India for providing necessary facilities to conduct this experiment.

REFERENCES

Bhattacharyya H K, Ganai N A and Khan H M. 2012. Fertility of local goats of Kashmir using frozen semen of Boer bucks. *Wudpecker Journal of Agricultural Research* **1**: 346–48.

Chauhan M S and Anand S R. 1990. Effect of egg yolk lipids on the freezing of goat semen. *Theriogenology* **34**(5): 1003–13.

FernandesV P, Silva M N N, Costa A S, Kunkel D, Júnior A S, Feitosa L C S, Muratori M C S and Costa A P R. 2018. CE inhibition in goats under fixed-time artificial insemination protocol increases the pregnancy rate and twin births. *Reproduction in Domestic Animal* **53**: 1006–08.

Gunaseelan M, Singh B P, Verma M R and Pachaiyappan K. 2018. Adoption level of improved goat farming technologies by commercial Goat Farmers in Tamil Nadu, India. *International Journal of Current Microbiology and Applied Science* **7**(05): 3293–3300.

Hancock J L. 1951. A staining technique for the study of temperature shock in semen. *Nature* (*London*) **167**: 323.

Jeferson F F, Gilmar P A, Joanna M G S, Maria E F O, Viviane L B, Felipe Zandonadi B and Olivardo F. 2017. Reproductive features and use of an anti-inflammatory drug in estrus-induced dairy goat sartigicially inseminated in a standing position with cervix immobilization. *Reproductive Biology* **17**: 268–73.

Jimenez-Rabadana P, Ramona M, Garcia-Alvarezh O, Maroto-Moralesh A, Olmob E, Perez-Guzmana M D, Bisballo A, Fernadez-Santoosb M R, Gardeb J J and Silerb A J. 2012. Effect of semen collection method (artificial vagina vs electro ejaculation), extender and centrifugation on post-thaw semen quality of Blanca-celtibercia buck ejaculate. *Animal Reproduction Science* **132**: 88–95.

Kharche S D, Jindal S K, Priyadhrashini R, Kumar Satish, Goel A K, Ramachandran N and Rout P K. 2013. Fertility following frozen semen artificial insemination in Jamunapari goats. *Indian Journal of Animal Sciences* **83**(10): 1071–73.

Kumar D and Naqvi S M K. 2014. Effect of time and depth of insemination on fertility of Bharat Merino sheep inseminated trans-cervical with frozen-thawed semen. *Journal of Animal Science and Technology* **56**: 8.

Mara L, Dattena M, Pilichi S, Sanna D, Branca A and Cappai P. 2007. Effect of different diluents on goat semen fertility. *Animal Reproduction Science* **102**(1–2): 152–7.

Padilha R T, Magalhaes-Padilha D M, Cavalecante M M, Almeida A P, Haan K T, Gastal M O, Nunes J F, Rodrigues A P R, Figueiredo J R and Oliveria M A L. 2012. Effect of insulin-like growth factor-I on some quality trials and fertility of cryopreserved ovine semen. *Theriogenology* **78**: 907–13.

Ranjan R, Goel A K, Kharche S D, Ramachandran N, Gangwar C and Jindal S K. 2014. Comparison between normal and dual staining technique for evaluating acrosome status and viability in frozen thawed buck spermatozoa. *Indian Journal of Small Ruminants* **20**(2): 50–53.

Ranjan R, Goel A K, Ramachandran N, Kharche S D and Jindal S K. 2015. Effect of egg yolk levels and equilibration periods on freezability of Jamunapari buck semen. *Indian Journal of Small Ruminants* **21**(1): 32–36.

Ranjan R, Priyadharsini R, Goel A K, Singh B, Kumar S, Kharche S D and Jindal S K. 2017. Effect of membrane stabilizer on the freezability of buck semen. *Indian Journal of Animal Sciences* **87**(4): 435–36.

Ranjan R, Ramachandran N, Jindal S K and Sinha N K. 2009b. Hypoosmotic swelling test in frozen thawed goat spermatozoa. *Indian Journal of Animal Science* **79**(10): 1022–23.

Ranjan R, Ramachandran N, Jindal S K and Sinha N K. 2009a. Hypoosmotic swelling test in frozen thawed goat spermatozoa. *Indian Journal of Animal Sciences* **79**: 1022–23.

Roof D J, Bowley S, Price L L and Matsas D J. 2011. Comparison of two commercial extenders for cryopreservation of goat semen without sperm washing. *Theriogenology* **77**: 412–20.

Roy A. 1957. Egg-yolk coagulating enzyme in the semen and Cowper's gland of the goat. *Nature London* **179**: 318.

Salamon S and Maxwell W M C. 1995. Frozen storage of ram semen II. Causes of low fertility after cervical insemination and methods of improvement. *Animal Reproduction Science* **38**: 1–36.