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Effect of membrane stabilizer on the freezability of buck semen

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Goats play a vital role in the economy of the poor and marginal farmers of rural India and very little effort has been taken for genetic improvement at farm level. A cryopreservation protocol developed for one species may not be ideal for the sperm of other species due to their difference in morphology and certain biochemical constituents. The greatest problem still existing with the cryopreservation of goat spermatozoa is that even with the best preservation techniques to-date available, post-thaw survival is restricted to approximately 50% of the sperm population (Ranjan *et al*. 2014, 2015). Increasing evidence suggests that certain substances that reduce calcium ionization (e.g. chlorpromazine) are expected to exert beneficial effect on semen cryopreservation by protecting sperm membrane from freeze thawing (Verma 1999). Chlorpromazine is a well-known membrane stabilizing agent and tranquilizer, and one of the aminopropyl compounds which belong to the phenothiazine derivatives. The effect of chlorpromazine on the motility of spermatozoa in farm animals had been reported by several workers (Paudel *et al*. 2010, Hong *et al*. 1982, Verma *et al*. 1999). Some of them indicated that the addition of chlorpromazine increased the sperm survival in the egg yolk extenders (Foote and Gray 1960). Jamunapari is one of the important milch breed of goats in India. The frozen semen AI could successfully be used for preservation, conservation and propagation in this breed (Kharche *et al*. 2013) and other breeds (Bhattacharyya *et al*. 2012). The freeze-thawing may cause membrane disruption, so, the addition of membrane stabilizing agent may be helpful in membrane protection. Therefore, the objective of the present experiment was to determine the effects of membrane stabilizing agent chlorpromazine hydrochloride (CPH) in goat semen dilutor on post thaw quality of Jamunapari buck semen.

Ejaculates (30) from Jamunapari bucks aged between 2–4 years old, were collected using artificial vagina, twice a week at Central Institute for Research on Goats, Makhdoom, Farah, Mathura, U.P. Immediately after collection, the volume, colour, consistency and mass motility of ejaculate were assessed. Semen 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 I.U., Triple distilled water-100 ml, pH-6.75–6.8) at the rate of 1:10, 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. Semen was divided into equal volumes, and diluted with dilutor having different levels (0 µM-control, 100 µM, 200 µM, 300 µM, 400 µM) of chlorpromazine hydrochloride. Sperm concentrations were adjusted to 1×10^8/ml and diluted semen was equilibrated at 5°C for 4 h before being frozen. Progressive motility was assessed in diluted semen (10 µl) and live and dead sperm count was done using Eosin-Nigrosine stain and abnormal sperms were counted with the same staining technique as per Ranjan *et al*. (2009). Giemsa stain was used to assess the acrosomal integrity of frozen thawed buck spermatozoa and Hypo-osmotic swelling test was done as per Ranjan *et al*. (2009). Data were analysed by SPSS data analysis software package (SPSS, Chicago, IL, USA). The factorial model included the effect of CPH concentration (0 µM-control, 100 µM, 200 µM, 300 µM, 400 µM) as independent variables and percent post thawed motility, live sperm count, acrosome intact sperm and hypo osmotic swelled sperm as dependent variables.

The effects of CPH on the membrane protective action in frozen semen were evaluated and the percentages of motile spermatozoa, live and dead spermatozoa, hypo osmotic swelling test (HOST) positive spermatozoa and acrosome integrity for each CPH concentration were observed. The effect of the different concentration of CPH in diluents on post thaw sperm quality is summarized in Table 1. The results showed that the progressive motility, live sperm count, acrosomal integrity and HOST positive spermatozoa (Mean±SE) were not significantly ($P<0.05$) different with 100 µM and 200 µM CPH concentration as compared to control group. The results showed that the

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Table 1. Effect of different concentration of Chlorpromazine hydrochloride in semen dilutor on post thaw quality of buck semen

Post thaw parameters	Concentration of chlorpromazine hydrochloride in semen dilutor				
	0µM-Control	100 µM	200 µM	300 µM	400 µM
Motility %	42.33± 1.88[a]	42.00± 1.45[a]	38.00± 1.44[a]	33.33± 0.93[b]	20.66± 1.53[c]
Live %	49.84± 1.91[a]	49.34± 1.58[a]	48.27± 1.8[a]	32.58± 1.37[b]	28.87± 1.65[b]
Acrosome %	48.23± 1.32[a]	47.66± 1.19[a]	47.03± 1.54[a]	29.57± 1.65[b]	27.82± 1.74[b]
HOS %	47.23± 1.11[a]	45.77± 1.92[a]	47.06± 1.81[a]	35.34± 2.15[b]	12.55± 0.85[c]

#Different superscripts (a, b, c) differed significantly within row (P<0.05).

progressive motility, live sperm count, acrosomal integrity and HOST positive spermatozoa (Mean±SE) were highest in control followed by decreasing concentration of CPH. The data also revealed that the post thaw quality was superior in control group than with 300 µM and 400 µM concentration of CPH used in present study and CPH showed negative effect in higher concentration when used at the concentration more than 200 µM. None of the levels of CPH tested improved sperm survival, and the highest levels of drug (300 µM and 400 µM) were found to be spermicidal (P<0.05).

CPH did not show any special beneficial effect on the viability and protective effect of goat spermatozoa in the egg yolk extender. Chlorpromazine alone did not improve the post-thaw semen quality but when combined with either ascorbic acid or catalase, improvement in semen quality was noticed (Paudel *et al*. 2010). The *in vitro* effects of chlorpromazine on human sperm motility were investigated. Chlorpromazine inhibited human sperm motility and the concentration which decreased sperm motility to 50% of control was 0.22 mM. They supported the hypothesis that chlorpromazine acts on the cellular membrane but consider the inhibition of sperm motility an unlikely cause of decreased fertility in chlorpromazine treated patients (Hong *et al*. 1982).

Consequently, better post-thaw recovery of crossbred bull semen can be achieved in TRIS dilutor after supplementing chlorpromazine as an additive in the diluent (Verma *et al*. 1999). The modified extender containing chlorpromazine but no sulfanilamide may have fertility advantage (Foote and Grey 1960).

The overall results suggested that the addition of CPH, a major cryoprotective agent during the cryopreservation did not showed any significant effect on post thaw quality of goat spermatozoa. None of the levels of CPH tested improved sperm survival, and the highest levels of drug (300 µM and 400 µM) were found to be spermicidal (P<0.05).

SUMMARY

Chlorpromazine hydrochloride (CPH) acts as a membrane stabilizer during semen freezing. The ejaculates from Jamunapari bucks (2–4 years old) maintained at this institute under semi intensive management system were utilized to find out the freezability of buck semen at different levels of CPH by conventional method of freezing. The ejaculates were collected twice a week using artificial vagina and were extended to maintain sperm concentration approximately 100 million per dose (0.25 ml) with Tris-Citric acid- Fructose (TCF) diluent. The post thaw motility, live sperm count, abnormalities, acrosomal integrity and hypo-osmotic swelling positive spermatozoa did not differ significantly at different levels of CPH. There was no significant effect of CPH inclusion in goat semen dilutor on post thaw qualities. None of the levels of CPH tested improved sperm survival, and the highest level of drug (400 µM) was found to be spermicidal.

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