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Comparative efficacy of different concentrations of egg yolk for cryopreservation of goat semen

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ABSTRACT

Gaddi goats are important livestock species of Himachal Pradesh, India. The sensitivity to cryopreservation varies among different species as also between animals of same species. Ejaculates (180) from 11 adult Gaddi bucks aged between 1.1 to 4.5 years (2.16±0.36 years), weighing 31–57 kg (39.1±2.82 kg) were collected using artificial vagina and selected on basis of standard quality parameters. The ejaculates were extended in Tris citrate egg yolk extender containing 6% Glycerol with varying concentrations of egg yolk (EY; 5, 10, 15 and 20%) to maintain a concentration of 150 × 10^6 sperms/straws. Filled and sealed straws were equilibrated at 5°C for 4 h followed by vapour freezing of straws for 7 min at 4 cm above the liquid nitrogen and finally plunged into liquid nitrogen. The representative straws from each ejaculates were thawed at 37°C for 30 sec, 24 h post incubation to compare the progressive motility, viability, morphological abnormalities and HOST reactive sperms in between different EY concentrations along with per cent change due to the processing. The data was analyzed using package R version 3.4.3. Post thaw progressive motility (35.18±0.87) and viability (45.26±1.32) was higher with least per cent change due to processing (52.03 and 40.12) in 10% EY than other EY concentrations. Absolute average values of morphological abnormalities, were least in 10% EY (7.93±0.28) than 20, 15 and 5% EY (11.42±0.67, 10.84±0.53 and 8.39±0.35), respectively. The absolute average values of HOST did not differ between 15, 10 and 5% (59.96±1.93, 52.48±1.43 and 59.07±2.18) EY, all of which were higher than 20% (42.57±4.20) EY concentrations. In conclusion, extender containing 10% EY was best with respect to progressive motility and viability for Gaddi goat semen cryopreservation.

Keywords: Egg yolk, Extender, Gaddi goats, Semen cryopreservation

Small ruminants are important livestock species of India. A progressively declining cattle population in Himachal Pradesh accentuates their rearing under small household system. Gaddi breed of goats are the mainstay of a large portion of nomadic and hilly tribes of Himachal Pradesh (Dogra and Thakur 2010). Sperm preservation protocols differ between animal species, due to their inherent abilities to accommodate variations in semen extenders used in the cooling and freezing processes (Barbas and Mascarenhas 2009). The difference between species regarding the sensitivity of their sperm to cooling is largely attributed to the compositional variation of the sperm plasma membranes (Bailey *et al.* 2000). There may then also be considerable differences between breeds and between individual males of the same species, regarding the 'freezability' of their semen (Hiemstra *et al.* 2005). In general, the spermatozoa of small ruminants are extremely sensitive to cryopreservation compared to other species (Purdy 2006, Gangwar *et al.* 2016).

To mitigate the effects of cryopreservation, the components of extender have been subjected to hit and trial method culminating into most viable options. Egg yolk (EY) is an important constituent of semen extender providing cryoprotection to the sperm cells and has been used in varying concentrations in goats (Purdy 2006, Priyadarshini *et al.* 2011, Beltran *et al.* 2013, Ranjan *et al.* 2015, Singh *et al.* 2016, Anand *et al.* 2017, Tabarez *et al.* 2017). Due to variability in reports regarding best EY concentration in extender, a varying number of ejaculates in present study were utilized to establish most promising EY concentration (5, 10, 15 and 20%) for Gaddi goat semen cryopreservation.

MATERIALS AND METHODS

The study was conducted on apparently healthy Gaddi bucks (11) aged 2.16±0.36 years (1.1–4.5 years), weighing 39.1±2.82 kg (31–57 kg). These bucks were selected on basis of breeding history, breeding soundness evaluation and testicular diameters. All the bucks were maintained under identical conditions and were screened for diseases, Brucellosis (RBPT, OIE guidelines 2008), Chlamydiosis (AGPT, Chahota *et al.* 2015) to eliminate the possible transmission of infection.

Study was conducted at University Livestock Farm of CSK Himachal Pradesh Krishi Vishvavidyalya, Palampur

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from September-December 2016, January and March 2017 and September-December 2017, respectively. Average light**:** dark hours (h) and temperature (°C) during the period of study comprised the average of the values considered on the day of semen collection and the preceding day. The average light: dark hours (h) and temperature (°C) ranged from 7.07±0.41: 16.93±0.41 to 12.29±0.13: 11.71±0.13 and 9.88±0.51°C to 21.94±0.18°C, respectively.

The Gaddi bucks were subjected to grazing for 5 h and remained under confinement in a shed where they were fed as per the standards of Indian Council of Agricultural Research (ICAR 2013). All males had round the clock access to the clean drinking water. Bucks were supplemented extra concentrate ration @ 3–3.5% of body weight containing CP 11.59% and TDN 77%, respectively.

Ejaculates (180) from 11 adult healthy Gaddi bucks were collected twice weekly by artificial vagina (AV) maintained at 42–43°C using induced estrus females as teaser. Ejaculates were primarily evaluated for colour, volume and concentration (Caprine Photometer, IMV 1409®) followed by microscopic examination for mass motility. Ejaculates were judged by classifying the semen sample as suitable or unsuitable according to standard criterion (Kharche *et al.* 2013, Rather *et al.* 2016). Absence of any gross abnormality in semen colour, mass motility of ≥3 and initial progressive motility of ≥70% were considered as main criterion for selection or rejection of semen sample. The post – thaw cryopreserved semen samples were evaluated for progressive motility (Hafez and Hafez 2000), viablility (Hannock 1951), morphological abnormalities (Bloom 1977) and HOST reactivity (Pant *et al.* 2002). Percent change in the aforesaid parameters between fresh and post-thaw semen was also calculated to determine the effect of cryopreservation. The seminal plasma was removed as described (Nuti 2007, Sharma *et al.* 2018). The semen pellet thus obtained after removal of Ringer's solution was extended with two equal fractions of tris citrate egg yolk extender, TEY (TRIS 1.21 g, citric acid 0.685 g, D-Fructose 0.5 g, benzyl penicillin 1000 IU/ml, streptomycin sulphate 1 mg/ml) along with variable concentrations of EY (20, 15, 10 and 5%) and 6% Glycerol. TEY extender was finally added at a gap of 2–3 min to yield a final concentration 150×10^6 spermatozoa per straw. *p*H of the buffer was adjusted to 6.7–6.9.

Extended semen was filled in 0.25 ml French mini straws (IMV Technologies, L'Aigle, Cedex, France), by aspiration using micropipette (Minitube, Germany) and subsequently sealed at free end with the help of polyvinyl alcohol (PVA) (IMV Technologies, L'Aigle, Cedex, France) powder. The straws were laid on a stainless steel rack and placed in cooling cabinet (Macro Scientific works Pvt. Ltd. India) for 4 h and thereafter exposed to liquid nitrogen vapours for 7 min. Thereafter, the straws were plunged into liquid nitrogen for storage. The inventory of semen storage was also maintained. Thawing of semen straws was done at 37°C for 30 sec (Evans and Maxwell 1987, Sariozkan *et al.* 2010). Proper ethical considerations related to animal handling and semen collections were observed and ensuring not to cause any injury during sampling. The data obtained were analysed using package R version 3.4.3. Paired sample t-test was used to see the significant difference between fresh and post thaw evaluation of quality seminal parameters with TEY extender containing variable concentrations of EY and 6% Glycerol. Results are presented as mean ±SEM and differences were considered significant when $P<0.05$.

RESULTS AND DISCUSSION

Perusal of the results for varing concentrations of EY (Table 1) revealed significantly higher ($P<0.05$) post thaw progressive motility with least per cent change due to processing (52.03) in TEY extender containing 10% EY than 20 and 15%, respectively. Priyadharsini *et al.* (2011) found better functional membrane integrity and intactness of acrosome, using 10 than 20% EY in Jakhrana goat semen. Ranjan *et al.* (2015) studied the effect of 2.5, 5, 7.5 and 10% egg yolk and concluded that 10% egg yolk showed higher post thaw motility and livability in Sirohi buck semen held at refrigeration temperature for 72 h.

Viablity was also better in 10% EY in terms of absolute values with least per cent change due to processing (40.12,

Table 1. Average (Mean±SEM) post thaw semen quality parameters extended with different per cent of egg-yolk in Gaddi goats

Seminal parameter (%)	Egg yolk (%)			
	20% (n=21) (% change due to processing)	15% (n=25) (% change due to processing)	10% (n=106) (% change due to processing)	5% (n=28) (% change due to processing)
Progressive motility	25.09 ± 2.07^{C} (65.03)	29.52 ± 1.64^{BC} (58.56)	35.18 ± 0.87^{A} (52.03)	32.14 ± 1.60^{AB} (56.85)
Viability	40.47 ± 4.18^{A} (47.24)	41.8 ± 3.04^{A} (44.08)	45.26 ± 1.32^{A} (40.12)	39.85 ± 2.15^{A} (47.01)
Morphological abnormalities	11.42 ± 0.67^{A} (22.13)	10.84 ± 0.53^{A} (8.83)	7.93 ± 0.28^{B} (20.15)	8.39 ± 0.35^{B} (24.29)
HOST reactive	42.57 ± 4.20^{B} (41.62)	59.96 ± 1.93^{A} (22.08)	52.48 ± 1.43^{A} (26.26)	59.07 ± 2.18^{A} (24.91)

$^{A-C}$Values with different superscripts within same row differs ($P<0.05$). n, number of ejaculates.

Table 1). Similarly, Ferreiral *et al.* (2014) also observed better viability with 10% EY than lower EY concentration in Sannen goat semen cryopreserved at –196°C. Alternatively, Anand *et al.* (2017) reported better cryoprotection in Barbari buck spermatozoa with 20% than 3% EY. Contrarily, various workers have used even much lower EY concentration of 2.5% yielding good seminal parameters (Singh *et al.* 2016).

In terms of absolute average values of morphological abnormalities (Table 1), 10% EY was superior to 20 and 5% EY. Whereas, it was inferior to 15% EY, the corresponding average values being 7.93±0.28, 11.42±0.67, 8.39±0.35 and 10.84±0.53, respectively. However, in terms of per cent change due to processing, 15% EY followed by 10% can be considered the better amongst the lot as indicated by a minimal change of 8.83% against other concentrations of EY.

The absolute average values of HOST did not differ between 15, 10 and 5% EY concentration, all of which were significantly higher than 20% (42.57±4.20) EY concentrations (Table 1).

Freezing effects with regard to post thaw motility in bucks have been observed by various workers (37.8–51.4%, Singh *et al.* 2016; 36–40%, Narwade *et al.* 2017; 55.4–62.6%, Priyadarshini *et al.* 2011) using different extenders. Besides being a source of nutrition and alteration of viscosity of the medium in which sperms are suspended, EY is most common non-penetrating cryoprotectant used (Tuli and Holtz 1994, Purdy 2006, Beltran *et al.* 2013, Gangwar *et al.* 2016). Egg yolk protects sperm plasma membrane against cold shock, and interacts with sperm plasma membrane (Watson 1995). The action of egg yolk may be attributed to phospholipids, cholesterol (Moce *et al.* 2010) and low-density lipoprotein (LDL) content (Bergeron and Manjunath 2006), which afford successful protection to the sperm plasma membrane against cold shock and cryoinjuries (Moussa *et al.* 2002). Graham *et al.* (1980) suggested that phospholipids from egg yolk could merge with the sperm membrane, replacing some of the sperm phospholipids and, thereby, decreasing their phase-transition temperature. The addition of egg yolk to the extenders during the freezing step conferred significantly higher post-thaw motility and acrosomal integrity (Dorado *et al.* 2007).

In conclusion, extender containing 10% EY was best with respect to progressive motility and viability for Gaddi goat semen cryopreservation.

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REFERENCES

Anand M, Baghel G and Yadav S. 2017. Effect of egg yolk concentration and washing on sperm quality following cryopreservation in Barbari buck semen. *Journal of Applied Animal Research* **45**(1): 560–65.

Bailey J L, Bilaodeau J F and Cormier N. 2000. Semen cryopreservation in domestic animals: a damaging capacitating phenomenon. *Journal of Andrololgy* **20**: 1–7.

Barbas J P and Mascarenhas R D. 2009. Cryopreservation of domestic animal sperm cells. *Cell Tissue Bank* **10**: 49–62.

Beltran M A G, Eufrocina P A, Edwin C A, Emilo M C, Flocerfida P A and Libertado C C. 2013. Optimized extenders for cryopreservation of buck semen for artificial insemination. *Philippines Journal of Veterinary and Animal Sciences* **39**(1): 1–10.

Bergeron A and Manjunath P. 2006. New insights towards understanding the mechanisms of sperm protection by egg yolk and milk. *Molecular Reproduction and Development* **73**: 1338–1344.

Bloom E. 1977. Sperm morphology with reference to bull infertility. Some papers contributed to the 1st All India Symposium on Animal Reproduction, Punjab Agricultural University, Ludhiana, India, pp. 66–8.

Chahota R, Gupta S, Bhardwaj B, Malik P, Verma S and Sharma M. 2015. Seroprevalence studies on animal Chlamydiosis amongst ruminants in five states of India. *Veterinary World* **8**(1): 72–75.

Dogra P K and Thakur Y P. 2010. Livestock biodiversity of Himachal Pradesh with special reference to pashmina goats. Proceeding of National Training on "Production, processing and utilization of pashmina fibre" 1–7 Dec Palampur, HP, India. pp. 1–12.

Dorado J, Rodríguez I and Hidalgo M. 2007. Cryopreservation of goat spermatozoa: comparison of two freezing extenders based on post-thaw sperm quality and fertility rates after artificial insemination. *Theriogenology* **68**: 168–177.

Evans G and Maxwell W M C. 1987. Frozen storage of semen. Salamon's Artificial Insemination of Sheep and Goats. Butterworths, Wellington, pp. 122–141.

Ferreiral V S, Mello M R B, da Fonseca C E M, Dias A C F, Cardoso J M, Silva R B and Martins W P. 2014. Effect of seminal plasma and egg yolk concentration on freezability of goat semen. *Revista Brasileira de Zootecnia* **43**: 513–18.

Gangwar C, Kharche S D, Kumar S and Jindal S K. 2016. Cryopreservation of goat semen: status and prospects. *Indian Journal of Small Ruminants* **22**: 1–10.

Graham J K, Schmehl K L and Nelson D S. 1980. Problems with laboratory assays. Proc. *8th Tech Conference AI and Reproduction* Milwaukee, Wisconsin. NAAB, 1–8.

Hafez B and Hafez E S E. 2000. *Reproduction in Farm Animals*. 7th ed. Lippincott Williams and Wilkens, New York. pp. 509.

Hannock J L. 1951. A staining technique for study of temperature shock in semen. *Nature* **167**: 323–24.

Hiemstra S J, Van der Linde T and Woelders H. 2005. The potential of cryopreservation and reproductive technologies for animal genetic resources conservation strategies. The role of biotechnology. Villa Giuliana, Turin, Italy. FAO. Rome, Italy.

ICAR. 2013. *Nutrient Requirements of Sheep, Goats and Rabbits*. 3rd ed. ICAR, New Delhi.

Moce E, Blanch E, Tomas C and Graham J K. 2010. Use of cholesterol in sperm cryopreservation: present moment and future prospects. *Reproduction in Domestic Animal* **45**: 57–66.

Moussa M, Martinet V, Trimeche A, Tainturier D and Anton M. 2002. Low density lipoproteins extracted from hen egg yolk by an easy method: cryoprotective effect on frozen–thawed bull semen. *Theriogenology* **57**: 1695–1706.

Narwade B M, Mohanty T K, Bhakat M and Rahim A. 2017. Goat semen cryopreservation using egg yolk and soya based extenders containing trehalose. *Indian Journal of Animal Sciences* **87**(7): 851–55.

Nuti I. 2007. Techniques of artificial insemination in goats. (Eds.) Youngquist R S and Threlfall W R (*Current Therapy in Large Animal Theriogenology*. 2nd ed. Sounders-Elsevier, St. Louis, MO. pp. 529–534.

Kharche S D, Jindal S K, Priyadarshini R, Kumar S, 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.

OIE. Quality Standards and Guidelines for Veterinary Laboratories, 2nd ed., 2008, OIE, Paris.

Pant H C, Barot L R, Kasiraj R, Misra A K and Prabhakar J H. 2002. Acrosomal integrity of buffalo spermatozoa and its relation to fertility. *Indian Journal of Animal Reproduction* **23**: 110–112.

Priyadharsini R, Jindal S K, Sharma D, Ramachandran N, Karche S D and Goel A K. 2011. Effect of different egg yolk level on the cryopreservation capability of Jakhrana goat semen. *Journal of Animal Science Advances* **1**(1): 28–37.

Purdy P H. 2006. A review on goat sperm cryopreservation. *Small Ruminant Research* **63**: 215–225.

R Core Team. 2013. R: *A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.

Ranjan R, Goel A K, Ramchandran 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 Ruminant* **21**(1): 32–36.

Rather H A, Islam R, Malik A A and Lone F A. 2016. Addition of antioxidants improves quality of ram spermatozoa during preservation at 40°C. *Small Ruminant Research* **141**: 24–28.

Sariözkan S, Bucak M N, Tuncer P B, Taºdemir U, Kinet H and Ulutaº P A. 2010. Effects of different extenders and centrifugation/washing on post thaw microscopic-oxidative stress parameters and fertilizing ability of Angora buck sperm. *Theriogenology* **73**: 316–23.

Singh L G, Ray K and Sarkar B. 2016. Effect of different levels of egg yolk on cryopreservation of Black Bengal buck semen in tris egg yolk citrate fructose glycerol extender. *Iranian Journal of Applied Animal Science* **6**: 101–06.

Sharma A, Sood P and Dogra P. 2018. Seminal plasma removal improves cryopreserved semen quality in Gaddi bucks. *Indian Journal of Animal Reproduction* **39**(2): 25–28.

Tabarez A, Garcia W and Palomo M J. 2017. Effect of the type of egg yolk, removal of seminal plasma and donor age on buck sperm cryopreservation. *Small Ruminant Research* **149**: 91–98.

Tuli R K and Holtz W. 1994. Effect of glycerolization procedure and removal of seminal plasma on post-thaw survival and GOT-release from Boer goat spermatozoa. *Theriogenology* **42:** 547–555.

Watson P F. 1995. Recent developments and concepts in the cryopreservation of spermatozoa and the assessment of their post-thawing function. *Reproduction Fertility and Development* **7**: 871–891.