Haryana Vet. (Dec., 2017) **56 (2),** 154-157

Research Article

CAPACITATION STATUS AND MEMBRANE INTEGRITY OF BUCK SPERMATOZOA STORED AT 4^0C IN DUCK EGG YOLK BASED SEMEN EXTENDER

MUKUL ANAND[1*], S. YADAV[1], J. KUMAR[1] and S. VASWANI[2]

[1]Department of Veterinary Physiology, [2]Department of Animal Nutrition, College of Veterinary Sciences and Animal Husbandry, Uttar Pradesh Pandit Deen Dayal Upadhayaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan, Mathura-281001, Uttar Pradesh, India

ABSTRACT

The experiment was designed to evaluate the storage ability of duck egg yolk in buck semen during short-term storage at 4°C. Four healthy adult Barbari bucks of 2-3 years of age, weighing 25-35 kg reared at Departmental Goat Farm were used as semen donor. Semen was collected twice a week using artificial vagina. A total of 32 ejaculates (8 from each buck) were collected during the study. The collected semen from four bucks after initial evaluation was pooled. Pooled sample was divided into five aliquot and diluted with Tris based extender containing variable duck egg yolk level *viz.* 2.5% (L-I), 5% (L-II), 10% (L-III), 15% (L-IV) and 20% (L-V) to reach final concentration of 200 million spermatozoa per ml and stored at 4^0C for 48 h and later evaluated for sperm characters. Sperm plasma membrane was evaluated through sperm viability, progressive motility, hypo–osmotic swelling test, acrosomal integrity test and capacitation like changes in sperm head. Significantly ($p<0.01$) higher value of sperm viability, motility, hypo osmotic swelling test (HOST), sperm with intact acrosome and those exhibiting pattern F (uncapacitated sperm) was observed in L-IV with 15% duck egg yolk. Thus, it is concluded that 15% duck egg yolk has a better capacity to maintain sperm morphology and membrane integrity and this level can be preferred for short term semen storage in goat.

Key words: Duck egg yolk, semen, sperm characters, short-term storage

Egg yolk is the most widely used non-penetrating cryoprotectant in semen extender. The cryoprotective capacity of egg yolk may be attributed to low-density lipoproteins (LDL; Bergeron *et al.,* 2004). It is considered as the most active constituent responsible for sperm protection against cold shock and cryoinjuries during freeze thawing process. Apart from LDL, cholesterol and protein also contribute to cryoprotection incurred by egg yolk. Any variation in the chemical composition of egg yolk constituents may elicit a differential response to cryoprotective capacity of extender. The seminal proteins in buck semen react with egg yolk to form compounds lethal to sperm (Leboeuf *et al.,* 2000). Egg yolk from different avian species vary their chemical composition, hence can elicit differential response to cryoprocessing. Use of varied egg yolk level within a species have been reported to elicit different response to cryoprocessing and has been well studied in goat (Anand *et al.,* 2016).

Among the different avian species, duck egg has easy availability next to chicken egg. The duck egg yolk has also been reported to have better cryoprotective capacity in buffalo bull spermatozoa (Andrabi *et al.,* 2008; Waheed *et al.*, 2012). Therefore, the experiment was designed to evaluate the cryoprotective effect of duck egg yolk on membrane integrity and capacitation like changes in buck sperm during short term storage at 4^0C.

*Corresponding author: drmukulanandvet@gmail.com

MATERIALS AND METHODS

Four healthy adult Barbari bucks of 2-3 years of age, weighing 25-35 kg reared at University goat farm were used as semen donor. Semen was collected twice a week using artificial vagina (length=20cm and diameter=4.5cm). Immediately after collection, semen was transferred to analytical laboratory in thermoflask maintained at 37^0C. The collected semen from four bucks after initial evaluation for mass motility, live percent and sperm abnormality was pooled. Pooled sample was divided into five aliquot and diluted with Tris based extender containing variable duck egg yolk level *viz.* 2.5% (L-I), 5% (L-II), 10% (L-III), 15% (L-IV) and 20% (L-V) to reach final concentration of 200 million spermatozoa per ml, the samples were stored at 4^OC for 48 h and later evaluated for sperm characters. Percent live spermatozoa were evaluated using eosin-nigrosin staining technique (Hancock, 1952). The progressive motility of spermatozoa was estimated immediate after dilution using a 10X objective of phase contrast microscope

with thermostatically controlled stage. To analyze the functional integrity of plasma membrane, hypo-osmotic swelling test was performed following the technique described by Jeyendran *et al.* (1984). Acrosome integrity was judged by Giemsa staining technique (Watson, 1975). Capacitation status and acrosome reaction were assessed using chlortetracycline (CTC) staining (Collin *et al*., 2000) with a little modification. Semen sample was washed in phosphate buffered saline (PBS), pH 7.4 and centrifuged (1500 rpm, 15 min). The CTC stock solution containing 750 µM CTC-HCl (Sigma), 130 mM NaCl, 5 mM L-cysteine, 20 mM Tris acid (pH 7.8) was prepared daily in amber colored bottle and stored at 4^0 C until required. Ten microliters of sperm suspension was mixed with 15µL of CTC solution on a slide at room temperature. Then, 0.3µL of 12.5% glutaraldehyde in 2.5M Tris base was added as a fixative. Samples (in duplicate) were covered with coverslips and stored in the dark at 4°C. A total of 200 sperm per slide were observed within 24 h using a Nikon Eclipse TE 2000-S microscope with phase contrast and epifluorescence optics under blue-violet illumination (excitation at 400–440 nm and emission at 470 nm). Three different patterns exhibited by sperm viz. Pattern F (uncapacitated sperm), Pattern B (capacitated, acrosome intact sperm) and Pattern AR (acrosome-reacted sperm) were evaluated during the experiment. Statistical analyses were performed using Statistical Package for Social Science (SPSS® Version 20.0 for Windows®, SPSS Inc., Chicago, USA). The means were compared using one way Analysis of Variance, Duncan's multiple range tests and presented as mean ± standard error (SE).

RESULTS AND DISCUSSION

The response of sperm membrane to different concentration of duck egg yolk during short-term storage has been presented in Table 1.. A significantly ($p<0.01$) higher value of sperm viability, motility, HOST responsive sperms and those with intact acrosome was observed in L-IV as compared to others. Based upon the capacitation test, the sperm exhibiting the pattern F was significantly ($p<0.01$) higher in L-IV indicative of its better capacity to prevent cryocapacitation.

Chilling of semen provides an efficient and successful means of short-term storage, it has yet some adverse effects on the spermatozoa (Watson, 2000; Batellier *et al.*, 2001). Induction of premature acrosomal reaction, altered mitochondrial function, reduction of motility and failure of chromatin decondensation are manifestation during sperm storage at low temperature (Chaverio *et al*., 2006; Wongtawan *et al.*, 2006). Plasma membrane that maintains both biochemical and structural integrity of the sperm is most liable to fluctuation in temperature (Cabrita *et al*., 1999). Temperature fluctuations and cell dehydration induce changes in lateral phase separation of lipids and reordering of membrane components (Drobnis *et al.,* 1993). It stimulates loss of poly-unsaturated fatty acids and cholesterol from sperm membrane. This alters the permeability of the sperm surface to water, ions and cryoprotectants (Hagiwara *et al*., 2009; Oldenhof *et al*., 2010). Sperm undergoes alterations especially in the head region, similar to those occurring during capacitation (Bailey *et al.,* 2000). These capacitation-like alterations could

Table. 1

Physical attribute of semen diluted with extender containing different level duck egg yolk after equilibration

Parameter	Live	Progressive	HOST	Acrosomal	Capacitation like changes		
Level	sperm (%)	motility (%)	(%)	integrity (%)	Pattern F(%)	Pattern B(%)	Pattern AR
Level -I (2.5% EY)	66.67^{C} ± 0.92	60.33^{C} ± 1.05	60.00^{C} ± 0.97	57.50^{C} ± 1.18	48.00^{B} ±1.06	28.33^{BC} ± 1.12	23.67^{A} ± 1.36
Level -II (5% EY)	67.17^{C} ± 0.70	61.50^{BC} ± 0.76	65.00^{B} ± 0.73	63.50^{B} ± 0.67	49.67^{B} ± 1.12	27.67^{C} ± 1.20	22.67^{AB} ± 0.71
Level -III (10% EY)	69.67^{BC} ± 0.76	64.00^{B} ± 1.03	67.50^{B} ± 0.85	66.17^{B} ± 0.87	51.50^{B} ± 1.34	28.33^{BC} ± 1.05	20.17^{B} ± 0.54
Level -IV (15% EY)	73.00^{A} ± 0.52	68.33^{A} ± 0.56	70.33^{A} ± 0.67	69.00^{A} ± 0.77	60.5^{A0} ± 1.09	22.83^{D} ± 0.79	16.67^{C} ± 1.05
Level -V (20% EY)	68.33^{C} ± 0.84	61.67^{BC} ± 0.88	66.00^{B} ± 1.10	64.33^{B} ± 1.05	47.83^{B} ± 1.62	31.50^{B} ± 1.18	20.67^{B} ± 0.61

Mean with different superscripts differ significantly within the column ($p<0.01$); EY=duck egg yolk

bring about a premature spontaneous acrosome reaction reducing sperm capacity to fertilize ova before reaching the ampulla of the oviduct. It is believed that the phospholipids, cholesterol and low-density lipoproteins in egg yolk provide protection to sperm against cold shock during its storage at low or ultra low temperature. Chemical composition of egg yolk varies in different avian species and can elicit differential response to cold shock resistance in diluted goat semen. Duck egg yolk has more monounsaturated fatty acid (Bathgate *et al*., 2006).

Surai *et al*. (1999) hypothesized that the main mechanism whereby egg yolk constituents especially LDL protect goat spermatozoa via the sequestration of GSP (Goat seminal plasma) proteins that bind to the sperm surface at ejaculation, triggering cholesterol and phospholipids efflux from the sperm membrane (Manjunath *et al.*, 2002; Bergeron *et al.,* 2004). The results recorded during the experiment indicates that duck egg yolk with variable LDL, phospholipid and cholesterol composition as compared to hen egg yolk exhibit differential responds to impart sperm protection at refrigerated temperature. The composition of egg yolk that vary between the species and concentration of egg yolk that regulates the level of cryoprotective constituents in extender influence the efficiency of semen extender to protect sperm against cold shock. The significantly ($p<0.01$) higher values recorded for different seminal attributes in L-IV with 15% duck egg yolk may be the result of better counter balance between egg yolk constituents and the different factors in diluted semen that affect the semen quality during its short term storage. In conclusion, 15% duck egg yolk has a better capacity to maintain sperm characters and membrane integrity and this can be preferred for short-term semen storage in goats.

REFERENCES

Anand, M., Baghel, G. and Yadav, S. (2016). Effect of egg yolk concentration and washing on sperm quality following cryopreservation in Barbari buck semen. *J. Appl. Anim. Res.***1**: 560-565.

Andrabi, S.M.H., Ansari, M.S., Ullah, N., Anwar, M., Mehmood, A. and Akhter, S. (2008). Duck egg yolk in extender improves the freezability of buffalo bull. *Anim. Repro. Sci.* **109**: 115-126.

Bailey, J.L., Bilodeau, J.F. and Cormier, N. (2000). Semen cryopreservation in domestic animals, a damaging and capacitating phenomenon. *J. Androl.* **21**: 1-7.

Batellier, F., Vidament, M., Fauquant, J., Duchamp, G., Arnaud, G., Yvon, J.M. and Magistrini, M. (2001). Advances in cooled semen technology. *Anim. Reprod. Sci.* **68**: 181-190.

Bathgate, R., Maxwell, W.M.C. and Evans, G. (2006). Studies on the effect of supplementing boar semen cryopreservation media with different avian egg yolk types on *in vitro* post-thaw sperm quality. *Reprod. Dom. Anim.* **41**: 68-73.

Bergeron, A., Crete, M.H., Brindle, Y. and Manjunath, P. (2004). Low-density lipoprotein fraction from hen's egg yolk decreases the binding of the major proteins of bovine seminal plasma to sperm and prevents lipid efflux from the sperm membrane. *Biol. Reprod.* **70**: 708-717.

Cabrita, E., Alvarez, R., Anel, E. and Herraez, M.P. (1999). The hypo-osmotic swelling test performed with coulter counter, a method to assay functional integrity of sperm membrane in rainbow trout. *Anim. Reprod. Sci.* **55**: 279-287.

Chaverio, A., Machado, L., Frijters, A., Engel, B. and Woelders, H. (2006). Improvement of parameters of freezing protocol for bull sperm using two osmotic supports. *Theriogenology* **65**: 1875-1890.

Collin, S., Sirard, M., Dufour, M. and Bailey, J. (2000) Sperm calcium levels and chlortetracycline fluorescence patterns are related to the in vivo fertility of cryopreserved bovine semen. *J. Androl.* **21**: 938-943.

Drobnis, E.Z., Crowe, L.M., Berger, T., Anchordoguy, T.J., Overstreet, J.W. and Crowe, J.H. (1993). Cold shock damage is due to lipid phase transitions in cell membranes, a demonstration using sperm as a model. *J. Exp. Zool.* **265**: 432-437.

Hagiwara, M., Choi, J.H., Devireddy, R.V., Roberts, K.P., Wolkers, W.F., Makhlouf, A. and Bischof, J.C. (2009) Cellular biophysics during freezing of rat and mouse sperm predicts post-thaw motility. *Biol. Reprod.* **81**: 700-706.

Hancock, J.L. (1952). The morphology of bull spermatozoa. *J. Expt. Biol.* **29**: 445-453.

Jeyendran, R.S., Van, H.H., Perez-Pelaez, M., Crabo, B.G. and Zanevald, L.J.D. (1984). Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. *J. Repro. Fert.* **70**: 219-228.

Leboeuf, B., Restall, B. and Salomon, S. (2000). Production and storage of goat semen for artificial insemination. *Anim. Reprod. Sci.* **62**: 113-141.

Manjunath, P., Nauc, V., Bergeron, A. and Menard, M. (2002). Major proteins of bovine seminal plasma bind to the low-density lipoprotein fraction of hen's egg yolk. *Biol. Reprod.* **67**: 1250-1258.

Oldenhof, H., Friedel, K., Sieme, H., Glasmacher, B. and Wolkers, W.F. (2010). Membrane permeability parameters for freezing of stallion sperm as determined by Fourier transform infrared spectroscopy. *Cryobiol.* **61**: 115-122.

Surai, P.F., Speake, B,K., Noble, R.C. and Mezes, M. (1999). Species-specific differences in the fatty acid profiles of the

lipids of the yolk and of the liver of the chick. *J. Sci. Food Agri.* **79**: 733-736.

Waheed S., Ahmed N., Najib-ur-Rahman, Jamil-ur-Rahman H., Tounis M. and Iqbal S. (2012). Evaluation of duck egg yolk for the cryopreservation of Nili-Ravi buffalo bull semen. *Anim. Reprod. Sci.* **131:** 95-99.

Watson, P.F. (1975) Use of geimsa stain to detect changes in acrosomes of frozen ram spermatozoa. *Vet. Res.* **97**: 12-15.

Watson, P.F. (2000). The causes of reduced fertility with cryopreserved semen. *Anim. Reprod. Sci.* **61**: 481-492.

Wongtawan, T., Saravia, F., Wallgren, M., Caballero, A. and Rodriguez-Martinez, H. (2006). Fertility after deep intra-uterine artificial insemination of concentrated low-volume boarsemen doses. *Theriogenol.* **65**: 773-787.

CONTRIBUTORS MAY NOTE

- Research/Clinical articles are invited for next issue from the Scientists/Veterinarians engaged in Veterinary profession.
- Please follow strictly the format of 'The Haryana Veterinarian' for manuscript writing/ submission.
- Please send a D.D. (payable at Hisar) of Rs. 700/- in favour of Dean, College of Veterinary Sciences, as processing fee alongwith each article.
- After revision, please return the revised manuscript and rebuttal at the earliest.
- Please mention your article reference number in all correspondence for a quick response.
- We solicit your co-operation.
- All correspondance should be addressed to The Editor, Haryana Veterinarian, Department of Veterinary Public Health and Epidemiology, College of Veterinary Sciences, LUVAS, Hisar-125 004.

Editors