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Effect of Different Egg Yolk Concentration on Chilled Barbari Buck Semen During Short Term Storage

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ABSTRACT

The experiment was designed to evaluate the optimal concentration of egg yolk in extender for diluting Barbari buck semen during short-term storage at 4ºC. A total of four Barbari bucks were used as semen donors during the experiment. The observed mean (±SE) valuesrecorded for percent live spermatozoa ranged from 63.5 ± 1.08 to 75.33 ± 0.99, percent progressive motile spermatozoa ranged from 54.17 ± 1.56 to 69.00 ± 0.82, percentage of HOST positive spermatozoa ranged from 60.83 ± 1.62 to 71.33 ± 1.54 while the acrosomal integrity ranged from 58.67 ± 1.563 to 71.33 ± 0.92 during the experiment. Three different patterns to evaluate capacitation like changes were observed under fluorescence. The observed mean (±SE) values of pattern F ranged from 45.67 ± 1.54 to 62.33 ± 1.14, pattern B ranged from 22.00 ± 0.73 to 28.83 ± 1.58 while pattern AR represent capacitated spermatozoa ranged from 15.67 ± 0.71 to 25.00 ± 0.62 during the experiment. A significantly higher value (p<0.01) of different seminal attributes was recorded in samples diluted with 15% egg yolk followed by 10%, 20%, 5% and 2.5%. The results recorded during the experiment indicates that 15% egg yolk in the semen extender is best suited for semen dilution during chilling process in Barbaribuck.

Keywords: Barbari buck, Egg yolk, Short-term storage, Semen

Semen dilution is an integral component of cryopreservation protocol. It not only provides an opportunity to prepare multiple doses from single ejaculate but contains ingredients that provide energy, maintains osmotic balance, reduces oxidative stress, protects spermatozoa from cold shock, cryoinjuries and prevent microbial growth during short term or long term semen storage (Curry and Watson, 1994). Concentration of different ingredients has been standardized in extender that is utilized for semen dilution in different farm species. Egg yolk is an important ingredient of Tris based semen extender (Dorji *et al.*, 2014). It acts as non-penetrating cryoprotectant that provides protection through reduced mobilization of proteins, cholesterol and fatty acids from plasma membrane of spermatozoa during chilling and freezing process.

Chilling of diluted semen provides an efficient and successful means of short-term storage. During the process, spermatozoa are subjected to low temperature that adversely these reproductive cells, manifested through depression in viability, altered structural integrity, depressed motility and low conception rates (Batellier *et al.*, 2001; Watson, 2000). So, to minimize these losses, egg yolk is being incorporated in semen extender. With an exception in goat, the concentration of egg yolk has been standardized and is generally used @ 20% in semen extender. The process is complex in goats, due to persistence of lethal interaction between the seminal plasma and egg yolk (Leboeuf *et al.*, 2000). The reason for the interactive loss may be attributed to glycoprotein-60 (BUSgp60) in bulbourethral secretion that has a triacylglycerol hydrolase activity, which decreases sperm motility and movement quality by disruption of cell membrane (Pellicer- Rubio and Combarnous, 1998). Phospholipase A2 activity of egg yolk coagulating enzyme (EYCE) catalyse the hydrolysis of egg yolk phosphatidycholine (PC) into fatty acids and lysophophatidycholine (LPC). LPC has toxic

effect on buck spermatozoa by acting like a detergent on biomembrane, resulting in loss of motility, membrane integrity and consequently low fertility rate (Upreti *et al.*, 1999).

So, different protocols that include spermatozoa washing (Islam *et al.*, 2006) or semen dilution with low concentration of egg yolk (Bispo *et al.*, 2011) have been advocated. Further, different concentration of egg yolk in extender has been recommend by researchers (Gunjan *et al.*, 2014; Ranjan *et al.*, 2015) for various goat breeds giving a impression of breed specific variation in respect to egg yolk concentration. Hence, no standard concentration of egg yolk has been established till date. So, taking into account these facts, experiment was designed to evaluate the best possible concentration of egg yolk in extender for diluting Barbari buck semen used for short-term storage at 4ºC.

MATERIALS AND METHODS

Experimental procedure

The study was designed to evaluate the optimal concentration of egg yolk in semen extender for diluting Barbari buck semen for short-term storage at 4ºC. Four Barbari bucks aged between 2-3 years of age weighing between 30-35 kg were used as semen donor during the experiment. Semen was collected from each buck twice a week using artificial vaginal. A total of 24 ejaculates (6 from each buck) were collected during the experiment. Immediately after collection the semen samples were evaluated and the samples with more that 80% live spermatozoa were selected for further experimentation. After initial evaluation, collected samples were pooled to remove individual variation. The pooled samples were divided in five equal parts and diluted with Tris extender

Table 1: Physical attributes of semen diluted with extender containing different levels of egg yolk

Parameter / % Egg Yolk	Live Percent (%)	Progressive motility (%)	HOST (%)	Acrosomal integrity (%)	Pattern F (%)	Pattern B (%)	Pattern AR (%)
2.5% egg yolk	$63.50^{D} \pm 1.09$	$54.17^{C} \pm 1.56$	$60.83^{B} \pm 1.62$	$58.67^{C} \pm 1.56$	$45.67^{D} \pm 1.54$	$28.83^{A} \pm 1.58$	$25.50^{A} \pm 0.62$
5% egg yolk	$69.33^{B} \pm 1.65$	$63.00^{B} \pm 1.65$	$64.50^{B} \pm 1.77$	$63.83^{B} \pm 1.89$	$50.67^{C} \pm 0.88$	$28.17^{A} \pm 0.87$	$21.17^{B} \pm 0.65$
10% egg yolk	$74.50^{A} \pm 1.57$	$66.00^{AB} \pm 1.57$	$71.17^{A} \pm 0.75$	$68.33^{AB} \pm 1.76$	$58.17^{B} \pm 1.01$	$23.83^{B} \pm 0.83$	$18.00^{C} \pm 0.82$
15% egg yolk	$75.33^{A} \pm 0.99$	$69.00^{A} \pm 0.81$	$71.33^{A} \pm 1.54$	$71.33^{A} \pm 0.92$	$62.33^{A} \pm 1.15$	$22.00^{B} \pm 0.73$	$15.67^{D} \pm 0.71$
20% egg yolk	$73.50^{A} \pm 0.89$	$63.83^{B} \pm 2.06$	$64.00^{B} \pm 1.15$	$64.33^{B} \pm 1.08$	$52.05^{C} \pm 0.56$	$27.83^{A} \pm 0.31$	$19.67^{BC} \pm 0.80$

Mean with capital letter (A,B,C) show difference at (p<0.01) between rows.

containing glycerol (6%) to reach the final concentration of 200 × 10^6 per ml while the egg yolk concentration was varying in each group *viz.* 2.5%, 5%, 10%, 15% and 20%. The diluted semen samples were refrigerated at 4ºC for four hours. The stored semen samples were subjected to thawing. The semen was evaluated for per cent live spermatozoa (Hancock, 1952), progressive motility and Hypo osmotic swelling test (HOST) (Jeyendran *et al.,* 1984), per cent intact acrosome (Watson, 1975) and capacitation like changes (Collin *et al.,* 2000).

Statistical analyses

Statistical analyses were performed using Statistical Package for Social Science (SPSS® Version 20.0 for Windows®, SPSS Inc., Chicago, USA). The values recorded during the experiment were analyzed statistically using one way ANOVA at the significance level of $p< 0.01$ and presented as mean ± standard error (SE).

RESULTS

The observed mean (±SE) values of different seminal attributes evaluated during the study are presented in Table 1. The observed mean (±SE) value of per cent live spermatozoa ranged from 63.5±1.08 to 75.33±0.99. A non significant difference was observed in the per cent live spermatozoa for 15%, 10% and 20% egg yolk with higher values observed with 15% egg yolk while a significantly ($p< 0.01$) lower values were observed with 5% and 2.5% egg yolk, respectively. The observed mean (±SE) value of per cent progressive motile spermatozoa ranged from 54.17±1.56 to 69.00±0.82 with significantly ($p< 0.01$) higher values observed in extender diluted with 15% egg yolk and lowest with 2.5% egg yolk. Unlike per cent live spermatozoa, the values of progressive motile spermatozoa were significantly ($p< 0.01$) lower in sample diluted with 20% egg yolk in extender as compared to samples with 15% egg yolk. The observed mean (±SE) value of percentage of HOST positive spermatozoa ranged from 60.83±1.62 to 71.33 ±1.54 while the acrosomal integrity ranged from 58.67±1.563to 71.33±0.92. A significantly ($p< 0.01$) higher value was observed in the semen samples diluted with 15% and 10% egg yolk while lower values were observed in samples with 2.5% egg yolk. The acrosomal status in respect to capacitation like changes induced during the chilling was studied using CTC dye. Three different patterns were observed under fluorescence. The observed mean (±SE) value of pattern F exhibited by spermatozoa at different egg yolk concentration in extender ranged from 45.67±1.54 to 62.33±1.14. The pattern F indicative of incapacitated or intact acrosome, was significantly ($p< 0.01$) higher in sample with 15% egg yolk compared to other groups. Pattern B indicating capacitated spermatozoa ranged from 22.00±0.73 to 28.83±1.58 while pattern AR represent capacitated spermatozoa ranged from 15.67±0.71 to 25.00±0.62. Significantly ($p< 0.01$) higher values of Pattern B and AR were observed in the sample diluted with 2.5% egg yolk while lower value was observed in sample diluted with 15% egg yolk.

DISCUSSION

Lethal interaction between the egg yolk and seminal plasma (Roy, 1957; Leboeuf *et al.,* 2000) together with protective action of egg yolk (Anand *et al.,* 2014) in semen extender are two major factors that determines the semen quality after dilution in goats. The increased egg yolk concentration elevates the extent of lethal interaction (Anand *et al.,* 2016), but simultaneously enhances the protection against cold shock and cryoinjuries during semen storage (Drobins *et al.,* 1993). The trend observed for per cent live spermatozoa during the experiment indicates that the croprotective effect of egg yolk dominates over the lethal interactive losses during cold storage resulting in higher values in sample diluted with 15%, 10% and 20% egg yolk as compared to 5% and 2.5%. Further the higher values observed in 15% egg yolk may be the result of a higher level of cold shock resistance accompanied with lower interactive losses at higher dilution of 200 million spermatozoa per ml. Semen dilution might have reduced the concentration of reactive proteins decreasing the intensity of lethal interaction with egg yolk. Supplemented with protective action of egg yolk, the diminished interactive losses established a counter balance between the two forces in sample with 15% egg yolk giving better results. Motility a characteristic unique to spermatozoa is influenced by egg yolk concentration in extender. Egg yolk is thought to increase the viscosity of medium affecting the movements of spermatozoa (Vishwanath and Shannon, 2000). It has been reported that several components in egg yolk affect the plasma membrane integrity and interferes the energy production

system of spermatozoa thus affecting the progressive motion (Kampshmidt *et al.,* 1953). Unlike live per cent, a significant higher value of progressive motile spermatozoa in sample diluted with 15% compared to 20% egg yolk is indicative of detrimental effect of egg yolk on motility at concentration higher than 15%. The lower values in 2.5% and 5% may be effect of poor protection against cold shock during chilling process. HOS test is performed to evaluate the physical and chemical health of spermatozoa. The spermatozoa with intact membrane respond to hypo osmotic solution while the acrosomal status is evaluated through Geimsa that has been related with fertilizing ability of spermatozoa. Reactive oxygen species are one of the main causes for oxidative stress that leads to destruction of plasma membrane integrity (Bucak *et al.,* 2008). The dead and abnormal spermatozoa are the main sourceof ROS production that intern affect the plasma membrane and integrity of acrosome resulting in poor response (Aziz *et al.,* 2004). The results recorded during the study can be well correlated with the per cent live spermatozoa. The lower values in 2.5% and 5% egg yolk may be the result of higher ROS level in seminal plasma generated from dead and stressed spermatozoa affecting membrane. Further, the poor protection at low concentration egg yolk making membrane more susceptible to oxidative stress might have intensified the effect, resulting in poor values.

Chilling induce capacitation-like changes and reduced ability of spermatozoa to bind to the cells of the reproductive tract and may contribute to the reduced fertility of cryopreserved spermatozoa. Capacitation including membrane destabilization (Harrison, 1996), an influx of calcium ions (Yanagimachi, 1994), and protein phosphorylation (Visconti *et al.,* 1998). Cryocapacitation is a similar process (capacitation-like) but displaying a different pattern and number of tyrosine phosphorylated proteins (Green and Watson, 2001). The values recorded during the study can be the result of high rate of O_2- and H_2O_2 generation or in the intracellular concentration of free calcium ions (Ca2+) (Ball, 1999) leading to oxidation of thiols in sperm proteins resulting membrane destabilization making spermatozoa susceptible to capacitation. Higher values of pattern F in the group diluted with 15% egg yolk is indicative of lower level of ROS generation accompanied with better protection against cold shock protecting the acrosonal intrigity and preventing the capacitation like changes. So, it can be concluded that 15% egg yolk in the semen extender to be utilized for semen dilution during chilling process is most suitable in Barbari goat. Further studies may be conducted to study the effect on motility pattern and *in vivo* fertility trials for better conception rates.

REFERENCES

Anand, M., Yadav, S. and Shukla, P. 2014. Cryoprotectant in semen extender: From egg yolk to low-density lipoprotein (LDL). *Livestock. Res. Int.,* **2:** 48-53.

Anand, M. and Yadav, S. 2016. Assessment of motion and kinematic characteristics of frozen-thawed Sirohi goat semen using computer-assisted semen analysis, *Vet. World.,* **9**: 203-206.

Aziz, N., Saleh, R.A., Sharma, R.K., Lewis-Jones, I., Esfandiari, N., Thomas Jr, A.J. and Agarwal, A. 2004. Novel association between sperm reactive oxygen species production, sperm morphological defects, and the sperm deformity index. *Fertil. Steril.,* **81:** 349-354.

Ball, B.A. 1999. Reactive oxygene species generation by equine spermatozoa. *Biol. Reprod.,* **60:** 136-137.

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.

Bispo, C.A.S., Pugleisi, G., Galvao, P., Rodrigues, M.T., Ker, P.G., Filgueiras, B. and Carvalho, G.R. 2011. Effect of low and high egg yolk conc. In the semen extender for goat semen cryopreservation. *Small Rumin. Res.*,**100:** 54-58.

Bucak, M.N., Atessahin, A. and Yuce, A. 2008. Effect of antioxidant and oxidative stress parameters on ram semen after the freeze-thawing process. *Small Rumin. Res.*,**75:** 128-134.

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.

Curry, M.R. and Watson, P.F. 1994. Osmotic effects on ram and human sperm membranes in relation to thawing injury. *Cryobiology,* **31:** 39-46.

Dorji, P., Pattarajinda, V. and Vongprolub, T. 2014. Cryopreservation of Mithunans Siri bull. *Bangl. J. Vet. Med.,* **12:** 147-153.

Drobnis, E.Z., Crowe, L.M., Berger, T., Anchordoguy, T.J., Overstreert, 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.

Green, C.E. and Watson, P.F. 2001. Comparison of the capacitation-like state of cooled boar spermatozoa with true capacitation. *Reproduction.*, **122:** 889-898.

Gunjan, B., Anand, M. and Yadav, S. 2014. Effect of different egg yolk conc. On sperm membrane integrity in Barbari buck subjected to cryopreservation. *Vet. Pract.*, **15:** 264-266.

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

Harrison, R. 1996. Capacitation mechanisms and the role of capacitation as seen in eutherian mammals. *Reprod. Fertil. Dev.*, **8:** 581–594.

Islam, R., Ahmed, K. and Deka, B.C. 2006. Effect of holding and washing on the quality of goat semen. *Small Rumin. Res.*, **66:** 51-57.

Jeyendran, R.S., Vander-Ven, H.H., Perez-Pelaez, M., Crabo, B.G. and Zanevld, L.J.D. 1984. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characters. *J. Repord. Fertil.*,**70:** 219-228.

Kampshmidt, R.F., Mayer, D.T. and Herman, H.A. 1953. Lipid and lipoprotein constituents of egg yolk in the resistance and storage of bull spermatozoa. *J. Dairy. Sci*., **36**: 733-742.

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

Pellicer-Rubio, M.T. and Combarnous, Y. 1998. Deterioration of goat spermatozoa in skimmed milk-based extenders as a result of oleic acid released by the bulbourethral lipase BUSgp60. *J. Reprod. Fertil.*, **112:** 95-105.

Ranjan, R., Goel, A.K., Ramachandean, N., Kharche, S.D. and Jindal, S.K. 2015. Effect of egg yolk and equilibration period on freezability of jamunapari buck semen. *Ind. J. Small Rumin. Res.*, **21:** 32-36.

Roy, A. 1957. Egg yolk-coagulation enzyme in the semen and Cowper's gland of the goat. *Nature.*, **179:** 318-319.

Upreti, G.C., Hall, E.L., Koppens, D., Oliver, J.E. and Vishwanath, R. 1999. Studies on the measurement of phospholipase A2 (PL A2) and PL A2 inhibitor activities in ram semen. *Anim. Repord. Sci.*, **56:** 107-121.

Visconti, P.E., Galantino-Homer, H., Moore, G.D., Bailey, J.L., Ning, X.P., Fornes, M. and Kopf, G.S. 1998. The molecular basis of sperm capacitation. *J. Androl.*, **19:** 242-248.

Vishwanath, R. and Shannon, P. 2000. Storage of bovine semen in liquid and frozen state. *Anim. Repord. Sci.*, **62:** 23-53.

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. Repro. Sci.*, **61:** 481-492.

Yanagimachi, R. 1994. Mammalian fertilization. In: Knobil, E., Neill, J.D. (Eds.), The Physiology of Reproduction. Raven Press, New York. 289-317.