Research article

DOI: 10.48165/ijar.2024.45.02.6

The Indian Journal of Animal Reproduction

The official journal of the Indian Society
for Study of Animal Reproduction

ACS Publisher
www.acspublisher.com

Year 2024, Volume-45, Issue-2 (December)

ISSN 0970-2997 ***(Print)*** *ISSN 2583-7583* ***(Online)***

Effect of Quail Egg Yolk in Tris Based Extender on Cryopreservation of Sahiwal Bull Spermatozoa

Garima Singh, Atul Saxena*, Neha Chaudhary, Arun Kumar, Vikas Sachan, Anuj Kumar and Jitendra Kumar Agrawal

Department of Veterinary Gynaecology and Obstetrics
College of Veterinary Science and Animal Husbandry, U.P. Pandit Deendayal Upadhyay Pashu Chikitsa Vigyan Vishwavidhyala evam Go-Anusandhan Sansthan, Mathura, Uttar-Pradesh

ABSTRACT

The present study was designed to determine the effect of different concentrations of quail egg yolk (5%, 10%, 15% and 20%) in tris based semen extender on progressive motility of Sahiwal bull spermatozoa at pre-freeze as well as post-thaw stage. The progressive motility significantly (P<0.01) improved in 10% and 15% quail egg yolk concentration at pre-freeze stage whereas it increases significantly in 10% quail egg yolk concentration at post-thaw stage. The findings of the present study suggested that quail egg yolk improves post-thaw sperm progressive motility at 10% concentration.

***Key words*:** Bull semen, Cryopreservation, Sahiwal, Quail egg yolk

How to cite: Singh, G., Saxena, A., Chaudhary, N., Kumar, A., Sachan, V., Kumar, A., & Agrawal, J. K. (2024). Effect of quail egg yolk in Tris based extender on cryopreservation of Sahiwal bull spermatozoa.
The Indian Journal of Animal Reproduction, 45(2), 36–38. 10.48165/ijar.2024.45.02.6

INTRODUCTION

Artificial insemination with frozen semen has been proved to be the best tool worldwide for mass genetic improvement through dissemination of superior germplasm. This objective can be achieved only if the frozen semen used in AI programme conforms to certain prescribed quality standards. Due to the importance of cryobiology in reproductive technologies, new protocols are being developed and cryo-protective agents tested for enhanced cryo-survival of sperm. Cryopreservation causes significant changes in the morphological and functional attributes of spermatozoa, which ultimately lead to poor fertility of cryopreserved semen as compared to fresh semen. Egg yolk is a normal component of semen extenders which protects the sperm cell against cold shock and the cell membrane during freezing and thawing. The protective mechanisms are determined by the phospholipids (lec-

*Corresponding author.
E-mail address: dratulsaxena@rediffmail.com (Atul Saxena)
Received 02-02-2024; Accepted 24-07-2024

ithin) and low-density lipoproteins (Situmorang, 1985; Simpson *et al.,* 1986; Sansone *et al.,* 2000; Medeiros *et al.,* 2002; Moussa *et al.,* 2002; Amirat *et al.,* 2004; Purdy, 2006). Traditionally, commercial hen egg yolk has been used in extenders or freezing media because of its easy availability but it has high amount of unsaturated fatty acids. Quail egg yolk (QEY) also contains significantly more phosphatidylcholine, less phosphatidylethanolamine, less polyunsaturated fatty acids and more saturated fatty acids in comparison to chicken egg yolk (Trimeche *et al.*, 1997). Quinn et al (1980) noticed that phospholipids form a protective film at the surface of spermatozoa membranes after disruption of LDL (Low density lipoproteins). The low saturated fatty acids to polyunsaturated fatty acid ratio tended to render sperm membrane less susceptible to cold shock because saturated fatty acids crystallize in more regular form compared to polyunsaturated fatty acids. Graham and Foote (1987) suggested that phosphotidylserine alone or in combination with phosphatidylcholine is most effective phospholipid to protect spermatozoa. Inclusion of quail egg yolk in the semen extender has been reported to improve post-thaw quality of stallion sperm (Webb *et al.,* 2011). Seshoka et al (2016) reported that quail egg yolk extender provided sufficient cryopreservation to Nguni bull semen. Akhter et al (2017) reported that QEY at 5% and Turkey egg yolk (TEY) at 10% offers advantages over 20% Chicken egg yolk (CEY) in terms of *in-vitro* post-thaw semen quality and in vivo fertility of Buffalo semen. Saieed et al (2018) also reported that individual sperm motility in ram significantly increases (P≤ 0.05) after addition of Quail egg yolk. Based on the above facts the study has been designed to cryopreserve the Sahiwal bull spermatozoa in different concentrations of quail egg yolk *viz*. 5%. 10%, 15%, 20% in tris based semen.

MATERIALS AND METHODS

Experimental animals and their feeding schedule and management: Four healthy breeding Sahiwal bulls of the age group between 4-7years and weighing between 450-500 kg body weight, reared at Semen Biology Lab, Department of Veterinary Gynaecology and Obstetrics, C.V.Sc & A.H., DUVASU, Mathura were utilized for the present study. The experimental bulls were kept in individual pens in sheds made up of brick and cement with concrete floor and asbestos roof. The bulls were being fed with balanced ration comprising of green fodder, straw and concentrate which was available at farm. The bulls were vaccinated against important contagious and infectious diseases on regular basis along with regular deworming.

Semen collection: All glasswares used during the course of study were sterilized. Semen was collected by using Artificial Vagina (AV) method. A total 06 ejaculates from each bull were collected (Total ejaculates from all bulls 06×4= 24). False mounting of each bull was done at least once to improve the ejaculated semen quality.

Evaluation of ejaculated semen sample: The ejaculated semen sample was initially evaluated for following parameters such as color, consistency, volume, pH and mass motility. The samples having creamy white color, thick consistency, sufficient volume, 6.6-6.8 pH and +3 mass motility were further extended in quail egg yolk based semen extender containing 5%, 10%, 15% and 20% quail egg yolk concentration.

Progressive motility: The progressive motility of spermatozoa was examined under 40X magnification of Leica DM-1000 microscope where glass slides were kept on thermostatic stage maintained at 37˚C. The slide was then examined for observing vigorously motile spermatozoa exhibiting progressive path.

$$\text{Progressive motility (\%)} = \frac{\text{No. of progressively motile sperms}}{\text{Total No. of sperms counted}}$$

Semen extension: Semen was diluted in different concentrations of QEYTG diluent upto 100×10^6 spermatozoa/ml of semen sample followed by filling of straws in French mini straws. The equilibration was done in equilibration chamber at 4˚C temperature for 4 hours. Straws were taken from each group (groups containing 5%, 10%, 15% and 20% quail egg yolk) at this time for evaluation of progressive motility.

Freezing of semen straws was done in programmable biological freezer as per the following freezing rates:

5 °C / min upto a temperature from 4 to -10 °C
40 °C / min upto a temperature from -10 to -100 °C
20 °C / min upto a temperature of -100 to -140 °C

Post Thaw evaluation of frozen semen

The frozen semen was thawed between 24-72 hours after freezing in a thawing unit at 37˚C temperature for 45 seconds. The thawed semen samples were subjected to assess progressive motility.

Statistical analysis: Data was analyzed by using one way ANOVA statistical method using SPSS16 software. Statistical significance was set at 0.05 probability level. If the effect was found significant, comparison was done by Duncan Multiple Range Test. Results are expressed as Mean ±Standard Error of Mean.

Table 1: Percent progressive motility of spermatozoa in different concentration of quail egg yolk based tris extender at pre-freeze and post-thaw stages of Sahiwal bull semen.

Stages	Progressive motility (%)				'F' Value
	Egg yolk 5%	Egg yolk 10%	Egg yolk 15%	Egg yolk 20%	
Pre-freeze	4.79 ± 1.00[a] (5-15)	63.96 ± 2.57[c] (40-80)	56.25 ± 3.60[c] (25-80)	47.7 ± 3.45[b] (15-70)	82.59**
Post-thaw	1.25 ± 0.44[a] (0-5)	46.04 ± 2.04[d] (30-65)	29.79 ± 2.85[c] (5-55)	10.00 ± 1.56[b] (0-30)	103.99**

RESULTS AND DISCUSSION

The progressive motility of the spermatozoa contributes as one of the major factor in assessment of fertility and quality of bull. The results of least square mean and standard error among different concentration of quail egg yolk is presented in Table 1. The overall least square mean of progressive motility at 5%, 10%, 15% and 20% quail egg yolk concentration at pre-freeze stage is 4.79 ± 1.00, 63.96 ± 2.57, 56.25 ± 3.60 and 47.7 ± 3.45 respectively and at post-thaw stage is 1.25 ± 0.44, 46.04 ± 2.04, 29.79 ± 2.85 and 10.00 ± 1.56 respectively. Similar results at 10% QEY concentration were reported by Saieed et al. (2018) in awassi Ram spermatozoa. However, Akhter et al. (2017) and Seshoka et al (2016) reported improved post-thaw progressive motility of bull spermatozoa in 5% QEY concentration. Rauch (2013) found that post-thaw progressive motility improved when beef bull semen was extended in 20% clarified QEY extender as compared to 20% CEY. Statistical analysis revealed that progressive motility at pre-freeze stage was significantly higher (P<0.01) for 10% and 15% QEY concentration and at post-thaw stage it was significantly higher (P<0.01) for 10% QEY concentration.

CONCLUSIONS

Quail egg yolk concentration of 10% in tris based extender provides better cryopreservation as compared to 5%, 15% and 20%. It may be further concluded that 5% quail egg yolk concentration does not provide sufficient cryoprotection whereas 20% concentration have detrimental effect on Sahiwal bull spermatozoa in terms of progressive motility.

REFERENCES

Akhter, S., Rakha, B.A., Ansari, M.S., Husna, A.U., Iqbal, S. and Khalid, M. (2017). Evaluation of quail and turkey egg yolk for cryopreservation of Nili-Ravi buffalo bull semen. *Theriogenology*, **87**:259-265.

Amirat, L., Tainturier, D., Jeanneau, L., Thorin, C., Gerard, O., Courtens, J.L. and Anton, M. (2004). Bull semen in vitro fertility after cryopreservation using egg yolk LDL: a comparison with Optidyl®, a commercial egg yolk extender. *Theriogenology*, 61(5): 895–907.

Medeiros, C.M., Forell, F., Oliveira, A.T. and Rodrigues, J.L. (2002). Current status of sperm cryopreservation: why isn't better. *Theriogenology*, **57**(1): 327-344.

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**(6): 1695–1706.

Purdy, P. (2006). A review on goat sperm cryopreservation. *Small Rumin. Res.*, **63**(3): 215–225.

Rauch, A. (2013). Cryopreservation of bovine semen in egg yolk based extenders. Doctoral thesis, University of Saskatchewan, Canada.

Saieed, A.Y., Mahdi, Z.A. and Ibrahim, A. (2018). Effect of addition from egg yolk of different avian to tris extender on freezing semen traits of awassi rams. *Iraqi J. Agric. Sci.*, **49**(4): 663- 669.

Sansone, G., Nastri, M.J.F. and Fabbrocini, A. (2000).Storage of buffalo (Bubalusbubalis) semen. *Anim. Reprod. Sci.*, **62**(1-3): 55–76.

Seshoka, M., Mphaphathi, M.L., Mafolo, K. and Nkadimeng, M. (2016). 38 quail egg yolk in citrate extender is suitable for cryopreservation of Nguni bull semen. *Reprod., Fertil. Dev.*, **28**(2): 149.

Simpson, A.M., Swan, M.A. and White, I.G. (1986).Action of phosphatidylcholine in protecting ram sperm from cold shock.*Gamete Res.*, **15**: 43-56.

Situmorang, P.Z. (1985). Deep-freezing of bull and ram semen and assessment of deep-frozen semen relating cytological characteristics of spermatozoa to their fertilizing capacity. PhD. Thesis, University of Sydney, Australia.

Trimeche, A., Anton, M., Renard, P., Gandemer, G. and Tainturier, D. (1997). Quail egg yolk: a novel cryoprotectant for the freeze preservation of Poitou jackass sperm. *Cryobiology*, **34**(4): 385–93.

Webb, G.W., Burris, C.L., Harmon, S.E. and Baker, R.H. (2011). Effects of egg yolk source on the cryopreservation of stallion spermatozoa. *J. Equine Vet. Sci.*, **31**(4): 166–73.