Research article

DOI: 10.48165/ijar.2024.45.01.6

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-1 (June)

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

Effect of Supplementation of Different Levels of Egg Yolk on Cryopreservation of Pantja Buck Semen in Tris Dilutor

Rashmi, Sunil Kumar*, Harihar Prasad Gupta and Shiv Prasad[2]

Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Sciences, GBPUAT, Pantnagar-263145, Uttarakhand, India

ABSTRACT

Present study was conducted to determine the optimum concentration of egg yolk in extender for diluting Pantja buck semen and its effect on post-thaw semen quality. Tris-citric acid-fructose-egg yolk-glycerol (TCF-EY) extender containing 4 different levels (5, 10, 15 and 20%) of egg-yolk was used for cryopreservation. Thirty-two (32) ejaculates were collected from four (04) bucks (eight ejaculates from each buck). Semen ejaculates after collection were pooled (4 ejaculates = 1 pooled ejaculate) on daily basis and this pooled ejaculate was divided and diluted into four groups as D1[Neat semen + tris-fructose-citric acid-egg yolk (@5%)-glycerol], D2[Neat semen + tris-fructose-citric acid-egg yolk (@10%)-glycerol], D3[Neat semen + tris-fructose-citric acid-egg yolk (@15%)-glycerol] and D4[Neat semen + tris-fructose-citric acid-egg yolk (@20%)-glycerol], and evaluated for physico-morphological parameters (sperm motility, live spermatozoa count, abnormal spermatozoa percent, plasma membrane integrity and acrosomal integrity) at post-dilution, post-equilibration and post-thaw stages. Biochemical parameters {Malondialdehyde (MDA), Glutamic Oxaloacetic Transaminase (GOT), Glutamic Pyruvate Transaminase (GPT) and Glutathione Peroxidase (GSH-Px)}were also estimated before dilution and at post-thaw stages of semen freezing. Significant difference ($P< 0.05$) was observed for all the physico-morphological and biochemical parameters at various stage of semen freezing in various diluters. All seminal parameters except total sperm abnormality showed higher values in D2 (10% EY) group at post-dilution, post-equilibration and post-thaw stages compared to other groups. Likewise, significantly lower values for MDA, GOT, GPT and higher value for GSH-Px were observed in D2 group at post-thaw stage of semen freezing. It was therefore, concluded that 10% egg yolk level (V/V) supplementation in Tris-citric acid-fructose-egg yolk -glycerol extender provides comparatively better post-thaw semen quality of Pantja buck semen.

Key words: Semen, cryopreservation, Pantja buck, egg yolk, tris citrate extender

How to cite: Rashmi, Sunil Kumar, Harihar Prasad Gupta, & Shiv Prasad. (2024). Effect of Supplementation of Different Levels of Egg Yolk on Cryopreservation of Pantja Buck Semen in Tris Dilutor. *The Indian Journal of Animal Reproduction, 45*(1), 25–28. 10.48165/ijar.2024.45.01.6

*Corresponding author.

E-mail address: sunilvgo16@gmail.com(Sunil Kumar)

Received 10-02-2024; Accepted 07-03-2024

INTRODUCTION

Goat can be raised in a wide range of climatic conditions and may be fed with a variety of plants. Goat farming is a source of income as well as source of protein for about 35 million households in India (Yadav *et al*., 2018). Majority of goat farming done in India comes under extensive system with uncontrolled natural mating. Finding a buck with a high level of genetic vigor is a major challenge for this agricultural operation. The success of artificial insemination (AI) is completely dependent on the successful cryopreservation of the semen. As far as freezing of buck spermatozoa is concerned, tris-based extenders including egg yolk has been widely used. The low-density lipoproteins (LDL) in egg yolk can help to protect and preserve spermatozoa by forming a protective film on spermatozoa surface or by simply replacing for phospholipids in the sperm membrane that have been lost or damaged during freezing process (Manjunath,2012). Dhara (2020) reported potentially hazardous interaction between the seminal plasma and egg yolk. Egg yolk coagulating enzyme (EYCE) is found in buck seminal plasma, which is responsible for sperm damage (Ustuner *et al*., 2009; Sharma *et al,* 2018). This enzyme is responsible for catalyzing the breakdown of egg yolk lecithin into lysolecithin, which is harmful to spermatozoa and hence it must be avoided. Therefore, present study was undertaken to study the effect of different levels of egg yolk on physico-morphological and biochemical characteristics of Pantja buck semen during freezing-thawing process.

MATERIALS AND METHODS

Study was conductedat the Goat Unit, Department of Livestock Production Management, C.V.A.Sc., G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand to determine the optimal concentration of egg yolk in extender for diluting Pantja buck semen and its effect on post thaw semen quality. Four sexually mature Pantja bucks with 2.5 to 4 years of age were used and a total of thirty-two ejaculates were collected from selected bucks (eight ejaculates from each buck). Semen ejaculates after collection were pooled on day basis and the pooled ejaculate was divided and diluted into four groups as D1[Neat semen + tris-fructose-citric acid-egg yolk (@5%)-glycerol], D2[Neat semen + tris-fructose-citric acid-egg yolk (@10%)-glycerol], D3[Neat semen + tris-fructose-citric acid-egg yolk (@15%)-glycerol] and D4[Neat semen + tris-fructose-citric acid-egg yolk (@20%)-glycerol], and evaluated for physico-morphological parameters viz; sperm motility (Tomar, 1997), live spermatozoa count (Mamuad *et al.,* 2004), abnormal spermatozoa per cent (Roberts, 1971), plasma membrane integrity (Zubair *et al*., 2013) and acrosomal integrity (Pant *et al.,* 2002) at post-dilution, post-equilibration and post-thaw stages. Biochemical parameters viz; Malondialdehyde, Glutamic Oxaloacetic Transaminase, Glutamic Pyruvate Transaminase (AUTOSPAN® Liquid Gold AST, ALT kits) and Glutathione Peroxidase were estimated before dilution and at post-thaw stages of semen freezing as per standard techniques.

RESULTS AND DISCUSSION

Effect of varying concentration of egg yolk supplementation in tris dilutor on physico-morphological seminal attributes and biochemical parameters of Pantja buck semen at different stages of semen freezing has been shown in table 1 and 2, respectively.

Observations in the present study showed a significant reduction in the mean values of sperm progressive motility, live sperm percentage, plasma membrane integrity, acrosome integrity and an significant increase in the mean values of abnormal spermatozoa at each freezing stage viz; post-dilution, post-equilibration and post-thawing (Table1). These significant changes in seminal attributes may be due to cryo-injuries arouse at the time of deep freezing. Group D3 and D4 showed comparativelya higher reductionin the values of sperm progressive motility, live sperm percentage, sperm plasma membrane integrity and acrosome integrity but an increased level of sperm morphological abnormalities compared to group D1 and D2. This change might be due to higher levels of egg yolk coagulating enzyme. Group D1 and D2 showed an increased level of sperm motility, live sperm percentage, plasma membrane integrity and acrosome integrity and reduced level of morphological abnormalities compared to group D3 and D4. This improvement in semen quality may be because of addition of optimum levelof egg yolk viz; 5 and 10%, which may protect the spermatozoa from freezing related damages. At post-equilibration and post-thawing stage of semen freezing, D2 group showed best seminal attributes compare to other groups. This may be due to optimum concentration of egg yolk (10%) used, which provides significant protection from cryo-injury to spermatozoa. Our findings strongly corroborate with the findings of Priyadharsini *et al.*(2011) in Jakhrana buck; Yimer *et al.* (2014) in Red Kalahari buck; Sen *et al.* (2015) in Norduz buck and Ranjan *et al.* (2015) in Jamunapari buck. On the other hand, Bispo *et al.* (2011) concluded that lower level of egg yolk (2.5%) is better than higher level (20%) for semen cryopreservation in Alpino and Saanen buck.

Table 1: Effect of egg yolk supplementation in tris dilutor on physico-morphological seminal attributes of Pantja buck at different stages of semen freezing

Parameters	Stage of freezing	D1 (5%)	D2 (10%)	D3 (15%)	D4 (20%)
Sperm Motility (%)	PD	77.25±0.89^{aB}	82.75±0.41^{aA}	69.28±1.63^{aC}	58.37±1.14^{aD}
	PE	71.02±0.2^{bB}	76.6±0.41^{bA}	64.97±1.09^{bC}	54.38±1.86^{bD}
	PT	46.8±1.01^{cB}	53.03±0.33^{cA}	37.53±0.73^{cC}	23.13±0.37^{cD}
Live Spermatozoa (%)	PD	73.98±0.78^{aB}	82.47±0.5^{aA}	68.72±0.71^{aC}	56.96±1.03^{aD}
	PE	69.24±1.06^{bB}	75.03±0.51^{bA}	63.21±1.36^{bC}	51.28±2.25^{bD}
	PT	46.38±0.82^{cB}	52.86±0.32^{cA}	37.22±0.65^{cC}	23.76±0.63^{cD}
Sperm abnormality (%)	PD	8.38±0.1^{cC}	7.81±0.04^{cD}	9.56±0.1^{cB}	10.23±0.14^{cA}
	PE	9.22±0.1^{bC}	8.58±0.06^{bD}	10.27±0.09^{bB}	10.86±0.07^{bA}
	PT	11.16±0.05^{aC}	10.46±0.09^{aD}	11.68±0.06^{aB}	12.08±0.05^{aA}
Plasma membrane integrity (%)	PD	72.6±1.57^{aB}	82.18±0.41^{aA}	65.38±0.99^{aC}	52.82±1.44^{aD}
	PE	67.29±0.67^{bB}	73.43±0.37^{bA}	57.96±0.67^{bC}	49.05±0.91^{bD}
	PT	45.64±1.00^{cB}	52.13±0.36^{cA}	35.97±0.86^{cC}	22.26±0.35^{cD}
Acrosomal integrity (%)	PD	69.54±0.45^{aB}	83.13±0.77^{aA}	63.88±1.13^{aC}	52.32±0.43^{aD}
	PE	66.84±0.9^{bB}	77.33±0.76^{bA}	58.84±0.98^{bC}	47.59±1.55^{bD}
	PT	45.15±1.09^{cB}	51.43±0.37^{cA}	35.82±0.74^{cC}	22.01±0.39^{cD}

*__PD__-Post dilution; __PE__-Post Equilibration and __PT__- Post thaw

**Mean value with at least one common superscript do not differ significantly (p<0.05).

***Mean bearing different lower superscripts in a column differ significantly (p<0.05).

****Mean bearing different upper superscripts in a row differ significantly (p<0.05).

Significantly, higher level of malondialdehyde (MDA) was observed in D4 group followed by D3 then D1 and D2 (Table 2). Neat semen showed the lowest value (2.22±0.16 nmol/ml) of MDA assay. MDA is the end product of lipid peroxidation (LPO) and is considered an indirect indicator of lipid peroxidation resulting in to plasma membrane damage due to structural changes in lipid matrix during cryopreservation. (Hsieh *et al.,* 2006; Ducha, 2018). In several studies MDA has been negatively correlated with the spermatozoa motility (Hsieh *et al*., 2006). Group D4 showed highest level of MDA,which might be due to adverse reaction of buck seminal plasma with higher egg yolk level in extender. Present study showed that 10% egg yolk reduces the MDA production effectively indicating a decline in lipid peroxidation and subsequently better post-thawed semen quality.

The lowest Glutamic Oxaloacetate Transaminase (GOT) and Glutamic Pyruvate activity (GPT) was observed in neat semen (127.36±0.05U/L and 16.99±0.34U/L, respectively), whereas it was highest in D4 group (166.62±0.66U/L and 41.28±0.46U/L, respectively). Significant (p<0.05) difference was observed in the mean values of activity of these enzymes between all the groups. GOT and GPT activity in semen is a measure of sperm cell damage and membrane integrity. Thus, high concentrations of GOT and GPT in D4 group were indicative of sperm membrane damage and leakage of enzyme from spermatozoa (Rastegarnia *et al.,* 2010). On the other hands, mean values of glutathione peroxidase (GPX) was found the lowest in D4 group, while it was the highest in neat semen (Table2).

Table 2: Effect of varying concentration of egg yolk in tris dilutor on biochemical parameters in Pantja buck semen

S. No	Parameter	Neat semen	Diluters (Post-thaw values)			
			D1	D2	D3	D4
1	MDA (nmol/ml)	2.22±0.05^{e}	5.55±0.06^{c}	4.39±0.11^{d}	5.88±0.02^{b}	6.36±0.04^{a}
2	GOT(U/L)	127.36±0.05^{e}	156.45±0.55^{c}	141.25±0.72^{d}	160.13±0.18^{b}	166.62±0.66^{a}
3	GPT(U/L)	16.99±0.34^{e}	32.42±0.23^{c}	23.14±0.81^{d}	37.79±0.34^{b}	41.28±0.46^{a}
4	GPx(U/ml)	10.94±0.19^{a}	9.29±0.03^{b}	9.76±0.03^{a}	8.62±0.11^{c}	8.12±0.10^{d}

*Mean value with at least one common superscript do not differ significantly (p<0.05).

**Mean bearing different upper superscripts in a row differ significantly (p<0.05).

CONCLUSIONS

From the present study, it may be concluded that 10% egg yolk level (V/V) supplementation in Tris-citric acid-fructose-egg yolk glycerol extender provides comparatively better post-thaw semen quality of Pantja buck semen.

ACKNOWLEDGEMENTS

The authors would like to thank Director Extension Research, G.B.P.U.A.T., Pantnagar, Uttarakhand (India) and Dean College of Veterinary Sciences, Pantnagar for providing all facilities to conduct the entire research work and also acknowledged the support of AICRP on Uttarakhand Goat Unit (Code-189).

CONFLICT OF INTEREST

The authors have no conflict of interest.

REFERENCES

Bispo, C.A.S., Pugliesi, G., Galvao, P., Rodrigues, M.T., Ker Filgueiras, P.G.B. and Carvalho, G.R. (2012). Effect of low and high egg yolk concentrations in the semen extender for goat semen cryopreservation. *Small Rum. Res.*, **100**:54-58.

Dhara, S. (2020). Studies on cryopreservation of Pantja buck semen. *M.V.Sc. thesis submitted to G.B.U.A.T., Pantnagar, Uttarakhand, India.*

Ducha, N. (2018). The test about blood serum capabilities in maintaining the quality of bull spermatozoa during storage in cep diluent at refrigerator temperature. *IOP Conf. Series: Earth and Environ Sci.,* **130**: 012043.

Hsieh, Yao-Yuan, Chang, Chi-Chen and Lin, Chich- Sheng. (2006). Seminal malondialdehyde concentration but not glutathione peroxidase activity is negatively correlated with seminal concentration and motility. *Int. J. Biol. Sci.*, **2**(1): 23–29.

Mamuad, F.V., Venturina, E.V. and Saito, H. (2004). Collection, processing and handling buffalo semen. Water Buffaloes and Beef cattle improvement project (WBBCIP). A joint-JICA assisted project of Philippine carabao center and the Bureau of Animal Industry, Philippines.

Manjunath, P. 2012. New insights into the understanding of the mechanism of sperm protection by extender components. *Anim Reprod*., **9**:809-15.

Pant, H.C., Barot, L.R., Kasiraj, R., Misra, A.K., Prabhakar, J.H. (2002). Acrosomal integrity of buffalo spermatozoa and its relation to fertility. *Indian J. Anim. Reprod.*, **23:** 110-112.

Priyadharsini, R., Jindal, S. K., Sharma, D., Ramachandran, N., Kharche, S. D. and Goel, A. K. (2011). Effect of different egg yolk level on the cryopreservation capability of Jakhrana goat semen. *J. Anim. Sci. Adv.,***1**(1): 28–37.

Ranjan, R., Goel, A.K., Ramachandran, N., Kharche, S.D. and Jindal, S.K. (2015). Effect of egg yolk levels and equilibration periods on freezability of Jamunapari buck semen. *Indian. J. Small Rum.,* **21**: 32-36.

Rastegarnia, A., Eviaz, S. Y. and Shafepour, V. (2010). Studies on seminal plasma enzymes (GOT, GPT and LDH) profile and its relationship with quality of buffalo frozen semen. *J. Vet. Clin. Res.,***1**: 189–199.

Roberts, S.J. (1971). Veterinary Obstetrics and Genital Diseases. 2nd Edn. Scientific Book Agency, Calcutta.

Sen, C. C., Tekin, K. and Akcay E., (2015). Effect of Egg Yolk and Removal of Seminal Fluid on Semen Cryopreservation in Norduz Goat. *Harran Üniv. Vet. Fak. Derg.,***4** (2) 64-67.

Sharma, A., Sood, P. and Dogra, P. (2018). Seminal plasma removal improves cryopreserved semen quality in Gaddi bucks. *Indian J. Anim. Reprod.* 39 **(2)**: 25-28.

Tomar, N.S. (1997). Artificial insemination and reproduction of cattle and buffaloes (4th Edn.), Saroj Prokashan, Allahabad.

Ustuner, B., Gunay, U. and Nur, Z. (2009). Effect of seminal plasma, egg yolk, and season on the freezability of saanen buck semen. *Bull Vet. Inst. Pulawy*., **53**: 369-374.

Yadav S., Yadav, B., Anand, M. and Swain, D.K. (2018). Artificial Insemination in Goats- Prospect and Challenges. In *XXVII Annual conference of society of Anim. Physiology of India*: 21-25.

Yimer,N., Noraisyah, A.H., Rosnina, Y. and Hafizal, A.M. (2014). Comparison of Cryopreservative Effect of Different Levels of Omega-3 Egg-Yolk in Citrate Extender on the Quality of Goat Spermatozoa. *Pak. Vet. J.,***34** (3):347-350

Zubair, M., Lodhi, L.A., Ahmad, E. and Ghulam, M. (2013). Hypo osmotic swelling test as screening for evaluation of semen of bull. *J. Entomol. Zool. Stud*., **1**: 124-128.