Effects of Proportion of Egg Yolk and Preservation Time on Chilled Semen from Indigenous Rams

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***Abstract* -The study was set out to determine the effects of different percentages of egg yolk on quality of chilled semen in Indigenous Rams. Different percentages of egg yolk were used to preserve semen from indigenous rams in Tris based extender at 4°C. Nine 2 to 3 years old rams, weighing 21.5±1.2 kg, body condition score 3.9±0.1 with scrotal circumference of 22.4 ±0.4 cm were selected for collection of semen once a week using artificial vagina. Each ejaculate was divided into four portions, and extended with Tris based diluents containing 5, 10, 15, and 20% egg yolk and kept chilled at 4 to 5°C for up to 48h. Motility, viability, functional integrity and morphology were evaluated before and 24h and 48h of preservation. The results showed significantly ($p<0.05$) better motility, viability and functional integrity with 10% egg yolk compared to other concentrations of egg yolk during preservation. However, the proportion of egg yolk did not affect spermatozoa quality before preservation and normal morphology in any time during preservation. Time of preservation decreased ($p<0.01$) the rate of motility, viability, functional integrity, and normal morphology of spermatozoa. Positive correlation coefficient observed between spermatozoa motility, viability, and functional integrity. Functional integrity of spermatozoa positively correlated to morphologically normal spermatozoa. It is concluded that 10% egg yolk in Ttris based diluents may be best for chilled Indigenous ram semen.**

Key words: Chilled semen, Egg yolk, Indigenous Ram, preservation

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INTRODUCTION

Semen preservation enables its widespread use in artificial insemination (AI), conservation of endangered species and international exchange of germplasm [1]. AI allows the rapid dissemination of genetic material from a small number of superior sires to a large number of females [2]. However, freezing of semen decrease motility, compromise morphological integrity, increase embryonic loss and ultimately reduce fertility. In rams like other species, these damaging effects are less pronounced in diluted and chilled semen than in frozen thawed semen [3] [4]. Semen can be diluted and chilled as an alternative to freezing when insemination is performed within short time after collection [5].

The semen extender provides an energy source and a buffer of inorganic or organic salts [6]. Egg yolk is widely used as a cryoprotective component for spermatozoa preservation in bulls, rams, and goats [7]. Phospholipids, cholesterol and low density lipoprotein are the components present in the egg yolk that protect the integrity of the spermatozoa membrane [8] and prevent cold shock during cooling and freezing, with improved viability of spermatozoa [9]. Recently scientists have been using egg yolk from different avian species and Su *et al*. [10] have reported no difference in its cryoprotective action for ram spermatozoa when obtained from chicken, goose or duck Concentration of egg yolk in diluents may depends on the composition of diluents and also on the methods applied for preservation of semen. There is a huge variation in the proportion of egg yolk used in the extenders for semen preservation. Some report low levels of egg yolk quite satisfactory whereas others do not [11]. Evans and Setchell [12] stated that 20% egg yolk is standard.

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Considering the controversy in the literature regarding the effective proportion of egg yolk in semen extenders the present study was designed to investigate the effects of different proportions of egg yolk in the semen extender and preservation time on quality of chilled semen from indigenous rams under the local conditions in Bangladesh.

MATERIALS AND METHODS

Experimental animals and management

Nine rams were small sized nondescript indigenous types of prolific sheep in Bangladesh are probably originated from southeastern subtropical humid region's mutton type of sheep selected for this study. Rams were 2 to 3 years old, 21.5±3.5Kg body weight, and with scrotal circumferences of 22.4 ±1.2 cm, kept under semi intensive conditions at the departmental animal shed, Bangladesh Agricultural University, Mymensingh, Bangladesh. They were dewormed regularly and vaccinated against tetanus and rabies. Feeding consisted of natural grazing supplemented with concentrates that consisted of wheat bran (50%), crushed maize (25%), soy bean meal (20%), fish meal (1%), dicalcium phosphate powder (2%), vitamin mineral premix (0.5%), and salt (1.5%) 300g/ head/ day. Drinking water was supplied *ad libitum.*

Experimental design

Semen was collected using an artificial vagina (AV) once a week. Semen diluents were prepared using reagent grade chemicals from Sigma Aldridge (Spain). Each ejaculate was examined for evaluation of volume, color, density, concentration and mass motility. Semen volume was estimated in a graduating tube just after collection. Color and density of semen were estimated in a graduating tube by eye estimation and tube slant, respectively. Spermatozoa concentration was measured using a Neubauer counting chamber. Mass motility was estimated by assessment of wave motion of fresh undiluted semen under microscope 10 × with 0 to 5 score. Then each ejaculate was divided into 4 portions and each portion was extended with one of the four experimental extenders to a final concentration of 300-400x10^6 spermatozoa/ml. All the experimental extenders were consisted of Tris 3.6g, Fructose 0.5g, citric acid 2.0g, penicillin 100,000 IU and streptomycin 100 mg with double-distilled water up to 100 ml) but differed in egg yolk concentrations and had either 5 or 10 or 15 or 20% egg yolk, respectively. After dilution, semen was stored at 4°C for 48h. Semen was evaluated at before (0h), 24h and 48h after storage for motility percentage, viability, functional integrity and morphology of spermatozoa. Preserved semen was warmed to 37°C before evaluation. Spermatozoa motility was evaluated subjectively using a phase contrast microscope (Gallenhamp, No. 82TT8, Cat No.M/6-200-H HZ 60, England) (400×). Diluted (5µL) semen was placed directly on a microscope slide and covered by a cover slip. For each sample, different microscopic fields were examined. The mean of the three successive evaluations was recorded as the final percentage motility. Spermatozoa viability was assessed by staining with eosin-nigrosin, Hypo osmotic swelling (HOST) test was used to detect the functional integrity of spermatozoa [13]. Spermatozoa morphology was assessed by microscopic examination after Sparmac® (Minitube, Box 152, Wellington, 7654, South Africa) staining [14].

Statistical analysis

The data were subjected to analysis of variance with respect to different percentages of egg yolk and preservation time using SPSS 17.0 computer program package (SPSS, Chicago, IL, USA). Repeated analysis of variance followed by Tukey comparison test was done to observe the effects of different concentrations of egg yolk and preservation time on semen quality of spermatozoa. Pearson's correlation coefficients were used to evaluate the correlations among the parameters. Significance was accepted at $p< 0.05$

RESULTS

Effects of different concentrations of egg yolk on the quality of chilled semen

The mean volume of the semen ejaculate from the rams was 1.4 ± 0.01 ml. The semen was creamy to milky white in color. Mean density was 3.0 ± 0.04, and mean mass activity 4.6 ± 0.05. Mean spermatozoa concentration was 4.6±0.14x10^9 spermatozoa per mL of ejaculate.

Motility

The % of spermatozoa motility at 24h and 48h was significantly ($p<0.05$) higher (84.0±0.4, 76.1±0.5) in samples preserved with diluents containing 10% egg yolk, than in those containing 5%, 15% or 20%. At the 0h of observation there was no difference of spermatozoa motility in respect of egg yolk concentration. Spermatozoa motility was reduced ($p<0.01$) with increased preservation time irrespective of egg yolk concentration (Fig.1).

Viability

Nonsignificant difference of % viable spermatozoa was observed at 0h, however, tendency to higher in diluents with 10% egg yolk than in others. Likewise spermatozoa motility, the proportion of viable spermatozoa was significantly higher ($p<0.05$) at 24h and 48h in semen with 10% egg yolk (88.2±0.3, 80.5±0.4) than in semen with 5%, 15% or 20%. The rate of viable spermatozoa

was reduced ($p<0.01$) with increased preservation time (0h, 24h 48h) in semen diluted with Tris based diluents containing (5,10, 15, and 20) percent egg yolk (Fig. 2).

Functional integrity

Similarly, the % functional integrity of spermatozoa was significantly ($p<0.05$) higher at 24h and 48h in semen preserved with 10% egg yolk (82.0±0.4, 73.8±.6) than in others (5%, 15%, 20%). There was nonsignificant difference in the functional integrity of spermatozoa at 0h. Functional integrity of spermatozoa was reduced ($p<0.01$) with increased preservation time irrespective of egg yolk concentration (Fig. 3).

Morphologically normal spermatozoa

There was no significant differences ($p>0.05$) were found in % spermatozoa with normal morphology in semen with diluents containing different percentages of egg yolk. However, the rate of normal morphology of spermatozoa decreased ($p<0.01$) over the time of preservation (0h, 24h, and 48h) (Fig. 4).

Correlation

The rate of percent spermatozoa motility at 0h were positively correlated with viability ($r=0.467$ ($p<0.01$), functional integrity ($r=0.807$ ($p<0.01$). Similarly spermatozoa motility at 24h positively correlated with viability ($r=0.337$ ($p<0.01$), and functional integrity ($r=0.335$ ($p<0.01$), respectively. In addition to these, motility of spermatozoa at 48h positively correlated to viability ($r=0.293$ ($p<0.01$). Functional integrity of spermatozoa 24h after preservation positively correlated with morphology of normal spermatozoa ($r=0.252$ ($p<0.01$).

DISCUSSION

Motility

Motility is the most common and important parameter for evaluation of spermatozoa quality and viability, although its relation to fertilization capacity of spermatozoa is often contradictory [15] [16] [17]. No information was available in relation to the effects of egg yolk on chilled semen from indigenous rams in Bangladesh. The present study showed better quality of chilled semen preserved with Tris based diluents containing 10% egg yolk than with other concentrations. Similar result was observed by Ahangari *et al*.[18] reported there was no benefit from increasing the concentration of egg yolk above 10% in Tris extender on motility of ram spermatozoa at 5°C. The mean motility was similar to that observed by other scientists [19] at 0h. They reported the mean motility of fresh Garole ram semen was 91.6 % and 85.5% in 0h and 24h, respectively. However, the rate of spermatozoa motility at 48h (73.2%) lower than our finding. The mean motility in semen preserved with 5%, 15%, and 20% egg yolk was similar. The minimal value of spermatozoa motility for the ram is 60% as reported [20]. Kasimanickam *et al*. [21] stated that breeding ram lambs should have more than 30% spermatozoa motility. The storage time (0h, 24h, and 48h) reduced ($p<0.01$) spermatozoa motility of indigenous ram semen preserved in Tris based diluents with irrespective of egg yolk concentration in this study. Gundogan *et al*. (22) observed the same effects of time of preservation on spermatozoa motility in ram. Beside this, the similar observation was reported by Kasimanickam *et al*. (21). Even though the spermatozoa motility was reduced ($p<0.01$) with increasing preservation time the rate of motile spermatozoa was above 70% up to 48h of storage time which is acceptable for AI.

Viability

Determination of % viable spermatozoa is important in assessing semen but preservation damages the spermatozoa membrane, resulting in loss of membrane permeability and cell death [23]. However, egg yolk consist of phospholipids, cholesterol and the low density lipoprotein are the protective components which prevent cold shock during cooling with improves viability of spermatozoa [9] [24]. Our results produced significant ($p<0.05$) differences in spermatozoa viability at 24h and 48h of preservation with 10% egg yolk compared to other percentages. The present result agreed by other scientists [18] reported that more than 10% of egg yolk in Tris based diluents had no effect on viability of ram spermatozoa at 5°C. Equal % of viable spermatozoa was obtained in semen preserved with 5%, 15%, and 20% egg yolk at both 24h and 48h. The positive effects of 5% egg yolk on viability of spermatozoa were next to 10% egg yolk. The present findings for all percentages of egg yolk were similar (Fig. 2) those of other researchers at 0h [25] [26] [27]. They found 90-93% viable spermatozoa in fresh ram semen. Viability of spermatozoa was sharply reduced ($p<0.01$) over the time of preservation in this study (Fig 2). The effects of storage time on viability of ram spermatozoa in our study was supported by Kasimanickam *et al*. [21] and Gundogan *et al*. [22]. They reported that the viability of ram semen reduced with increasing storage time.

Functional integrity of spermatozoa

Intact functional integrity of spermatozoa membrane is essential for fertilization. Recently the hypo-osmotic swelling test (HOST) has been used to evaluate the functional integrity of the spermatozoa membrane. Spermatozoa with normal membrane function exposed to hypo-osmotic conditions show swelling of the tail because of water influx [28] [29]. Integration of HOST into

the spermatozoa selection procedure may provide a valuable tool for selection of functional spermatozoa. Our results showed that functional integrity of spermatozoa membrane was higher ($p<0.05$) at 24h and 48h of preservation in semen preserved with 10% egg yolk compared to other percentages. Similar result was reported in frozen semen in ram [30]. The semen extended with 5% egg yolk produced the second highest HOST positive spermatozoa at 24h and 48h of storage time followed by 20% and 15%. There were different results regarding HOST test. One of the previous studies conducted by Azizunnesa *et al.* [31] showed that the rate of spermatozoa functional integrity (on 0h, 24h, and 48h) was similar to this present result. However, Juyena [32] reported 76% HOST positive spermatozoa in fresh semen in Padovana rams which was lower than the present result. The present result showed decreased functional integrity of spermatozoa with progressing time (Fig. 3). This result for spermatozoa functional integrity was similar to those reported by Gundogan *et al*. [22]. Kasimanickam *et al*. [21] also reported the quality of spermatozoa was decreased when preservation time increased.

Morphology of spermatozoa

Breeding ram should have more than 70% morphologically normal spermatozoa [21]. The present findings showed nonsignificant differences ($p>0.05$) among different concentration of egg yolk in morphology of spermatozoa. Our results agreed with others [18] who reported nonsignificant difference ($p>0.05$) in morphology of spermatozoa preserved with different percentages of egg yolk. The mean values of their result regarding normal morphology were similar to our results with 10%, 15% and 20% egg yolk. The proportion of abnormal spermatozoa may vary with different factors. Variation in proportion of abnormal spermatozoa was observed by other scientists [27] in different breeds of ram. They reported 12.5 ± 0.3%, 16.9 ± 0.5% and 15.0 ± 0.3% morphologically abnormal spermatozoa in Suffolk, Walachian and Sumava sheep, respectively, in fresh semen. Good quality spermatozoa was observed [33] in 2-3-year-old rams and after that quality declined gradually [32]. In general, our results showed good quality semen from Indigenous rams of 2-3 years age. The mean percentage of normal spermatozoa was dramatically reduced over the time of preservation (Fig. 4). Our study was similar to Gundogan *et al*. [20] regarding the effects of preservation on normal spermatozoa morphology rate was gradually decreased during storage period.

Correlation

Regarding the correlation coefficient, our results were similar to Gundogan *et al*. [20].They reported that the positive correlation coefficient between motility, viability and functional integrity of spermatozoa. Therefore, it is likely that the motile spermatozoa included viable and functionally integrated. This results also supported by Juyena [30]. However, contradictory relationship was reported between functional integrity and other vital stain results [33]. Our report showed that positive relationship between functional integrated spermatozoa and morphologically normal spermatozoa. It would be said that morphologically normal spermatozoa were able to get influx of water and swollen tail.

Some report shown that low level of egg yolk (1.5–3.75%) produced quite satisfactory semen quality while others do not [11]. Another report showed that 20% egg yolk is standard for ram semen [12]. However, studied above, these results represent that 10% egg yolk containing Tris based diluents supports better to spermatozoa quality regarding motility, viability, and functional integrity after preserved at 4-5^{o}C than other concentration of egg yolk. The better quality semen with 10% egg yolk could be due to the proper ratio of phospholipid, cholesterol and the low-density lipoprotein in the diluents are the protective components which protect the spermatozoa membrane and prevent cold shock during cooling with improves quality of spermatozoa [9] [24]. Although, the time of preservation decreased ($p<0.01$) the quality of spermatozoa in each concentration of egg yolk, however, the rate of quality regarding motility, viability, functional integrity and normal morphology were above 70% up to 48h of preservation which was acceptable for breeding ram (Kasimanickam *et al*. [21].

The results of this study have shown beneficial effects of 10% egg yolk in the Tris based semen extender on motility, viability and functional integrity of spermatozoa up to 48h of preservation. The spermatozoa morphology was not affected by different concentrations of egg yolk. A positive correlation coefficient was presented in motility, viability, and functional integrity of spermatozoa. Functional integrity of spermatozoa positively correlated to morphologically normal spermatozoa. The pregnancy rate obtains by AI using semen diluted with Tris based diluents containing 10% egg yolk requires to investigate.

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REFERENCES

[1] M. Yoshida. (2000). Conservation of sperms: current status and new trends. *Anim. Reprod. Sci.* 60(61), pp. 349-35.

[2] R. Vishwanath and P. Shannon. (1997). Do sperm cells age? A review of the physiological changes in sperm during storage at ambient temperature. *Reprod. Fertil. Dev*. 9, pp. 321-331.

[3] P.S. Fiser and R.W. Fairful. (1984).The effect of glycerol concentration and cooling velocity on cryosurvival of ram spermatozoa frozen in straws. *Cryobiology*. 21, pp. 542-551,

[4] E.G. Aisen, V.H. Medina and A. Venturino. (2002). Cryopreservation and post-thawed fertility of ram semen frozen in different trehalose concentrations. *Theriogenology*. 57, pp. 1801-1808.

[5] A. Menchaca, A. Pinezak and D. Queirolo. (2005). Storage of ram semen at 5ºC: effects of preservation period and timed artificial insemination on pregnancy rate in ewes. *Anim. Reprod*. 2, pp. 195–198.

[6] G.W. Salisbury, N.L. Van Demark and J.R. Lodge, Physiology of reproduction and A1. 2nd Ed. W.H. Freeman and Co. San Francisco, USA. 1978, pp. 199, 250.

[7] M. Moussa, V. Matinet, A. Trimeche, D. Tainturier and M. Anton. (2002). Low density lipoproteins extracted from hen egg yolk by an easy method: cryoprotective effect on frozen-thawed bull semen. *Theriogenology*. 57, pp. 1695–706.

[8] C. Aurich. (2005). .Factors affecting the plasma membrane function of cooled-stored stallion spermatozoa. *Anim. Reprod. Sci*. 89, pp. 65-75.

[9] S. Salamon and W.M.C. Maxwell. (2000). Storage of ram semen. *Anim. Reprod. Sci*. 62, pp. 77–111,

[10] L. Su, X. Li, X. Quan, S. Yang, Y. Li, X. He and X. Tang. (2008). A comparision of the protective action of added egg yolks from five avian species to the cryopreservation of bull sperm. *Anim. Reprod. Sci*.104, pp. 212–219.

[11] E.F. Graham, M.K.L. Schmehl, B.K. Evensen and D.S. Nelson. (1978). Viability Assays for Frozen Semen. *Cryobiology*. 15(2), pp. 242-244.

[12] R.W. Evans and B.P. Setchell. (1978). .Association of exogenous phospholipids with spermatozoa. *J. Reprod. Fertil*. 53(2), pp. 357-362.

[13] R.G. Saacke and J.M. White, Semen quality tests and their relationship to fertility. Proceedings of the 4th technical conference on Artificial Insemination and reproduction, N A A B. 1972, pp. 22-27.

[14] E.F. Grahman, M.K.L. Schmehl and D.S. Nelson, Problem with laboratory assays. Proceedings of the 8th technical conference on Artificial Insemination and reproduction, N A A B. 1980, pp. 1-8.

[15] R.S. Jeyendran, H.H. Van-Der-Ven, M. Perez-Pelaez and L.J.D. Zaneveld. (1984). Nonbeneficial effects of glycerol on the oocyte penetrating capacity of cryopreserved and incubated human spermatozoa. *Cryobiology*. 22, pp. 434-437.

[16] S. Schafer and Holzman. (2000).The use of transmigration and Spermac stain to evaluate epididymal cat spermatozoa. *Anim. Reprod. Sci*. 59, pp. 201-211.

[17] J.F. Fonseca, C.A.A. Torres, V.V. Maffili, A.M. Borges, A.D.F. Santos, M.T. Rodrigues and R.F.M. Oliveira. (2005). The hypo-osmotic swelling test in fresh goat spermatozoa. *Anim. Reprod*. 2(2), pp. 139-144.

[18] Y.J. Ahangari, M.A. Hamedani and S.R. Hassani.(2010).Effect of various concentrations of egg yolk on Dallagh Ram semen characteristics at 5ºC. Proc. *Aust. Soc. Anim. Prod*. 28, p. 88.

[19] A. Joshi, S. Bag, S.M.K. Naqvi, R.C. Sharma, P.S. Rawat and J.P. Mittal. (2001). Effect of Short term and long term preservation on motion haracteristics of Garole Ram Spermatozoa: A prolific Microsheep breed of Indoia. *Asian Aus. J. Anim. Sci.* 14(11), pp. 1527-1533.

[20] D.L. Garner and E.S.E. Hafez, Spermatozoa and seminal plasma. In: Reproduction in farm animals. (Ed) E.S.A. Hafez. Philadelphia, Lea and Febriger, 1982, pp. 12-7.

[21] R. Kasimanickam, V. Kasimanickam, K.D. Pelzer and J.J. Dascanio. (2007). Effect of breed and sperm concentration on the changes in structural, functional and motility parameters of ram-lamb spermatozoa during storage at 4 ◦C. *Anim. Reprod. Sci.* 101, pp. 60–73.

[22] M. Gundogan, F. Avdatek and D.,Yen. (2011). Effect of extenders on motility, morphology and osmotic resistance parameters of ram sperm during liquid storage. *Rev. Méd. Vét*. 162(11), pp. 546-551.

[23] N.L. Cross and S.E. Hanks. (1991). Effects of cryopreservation on human sperm acrosomes. *Hum. Reprod*. 6, pp. 1279–1283.

[24] J. Beran, L. Stádník, F. Bezdíček, L. Louda, J. Čítek and J. Ducháček. (2012). Effect of sire and extender on spermatozoa motility and share of live or dead sperm in bulls' fresh ejaculate and in AI doses after thawing. *Archiv Tierzucht.* 55 (3), pp. 207-218.

[25] M. Gundogun. (2009). Short Term preservation of ram semen with different extenders, Afyon Kocatepe Üniversitesi Veteriner Fakültesi Dölerme ve Sun'i Tohumlama Anabilim Dalı. *afkas Univ vet fak derg*. 15(3), pp. 429-435.

[26] V.H. Guerrero, L.W. Huanta, T.F. Raymundo, O.S. Huerta and R.D. Daphne. (2009).Uso de dilutores hipertónicos en la criopreservación de semen ovino. *Rev. Investig. Vet. Peru*. 20(1), pp. 41-46.

[27] P.J.E. Hernández, R.F. Fernández, S.J.L. Rodríguez, R.E. Juárez, M.Y.G. (2012). Soto and R.A.D. García, Effect of cryopreservation of sheep semen related to its viability and acrosomal status. *Rev. Salud Anim*. 34(2), pp. 78-83.

[28] S. Avery, V.N. Bolton and B.A. Mason. (1990). Int. J. Androl.13, pp. 93–99.

[29] D.Y. Liu and H.W.G. Baker. (1992). *Fertil. Steril.* 8, pp. 465–483.

[30] J. Gil, M. Rodiguez-Irazoqui, N. Lundeheim, L. Soderquist and H. Rodriguez- Martinez. (2003). Fertility of ram semen frozen in bioexcell and used for cervical artificial insemination. *Theriogenology*. 59, pp. 1157–70.

[31] Azizunnesa, B.F. Zohara, F.Y. Bari and M.G.S. Alam. (2013). Effects of Concentrate Supplementation on Reproductive Perfoermances and Semen Quality of Indigenous Rams in Bangladesh. *J. Emb. Trans.* 28(4), pp. 325-335.

[32] N.S. Juyena, Protein profiles and biochemical characteristics of semen: Influence on frozen-thawed spermatozoa quality in rams (*Ovis Aries*) and Alpacas (*Vicugna Aacos*). PhD Thesis, Faculty of Veterinary Medicine, Department of Clinics Veterinary Science. University of Paduva, Italy, 2011, pp. 123-173.

[33] I. David, L. Bodin, G. Lagriffoul, E. Manfredi and C. Robert-Granie. (2007). Character process model for semen volume in AI rams: evaluation of correlation structures for long and short term environmental effects. *Genet. Sel. Evol*. 39, pp. 55-57.

[34] V. Tufarelli, G.M. Lacalandra, G. Aiudi, F. Binetti and V. Laudadio. (2011). Influence of feeding level on live body weight and semen characteristics of Sardinian Rams reared under intensive conditions. *Trop. Anim. Health and Prod.* 43, pp. 339–345.

[35] D. Neild, G. Chaves, M. Flores, N. Mora, M. Beconi and A. Aguero. (1999). Hypo osmotic test in equine spermatozoa. *Theriogenology*. 51, pp. 721-727.

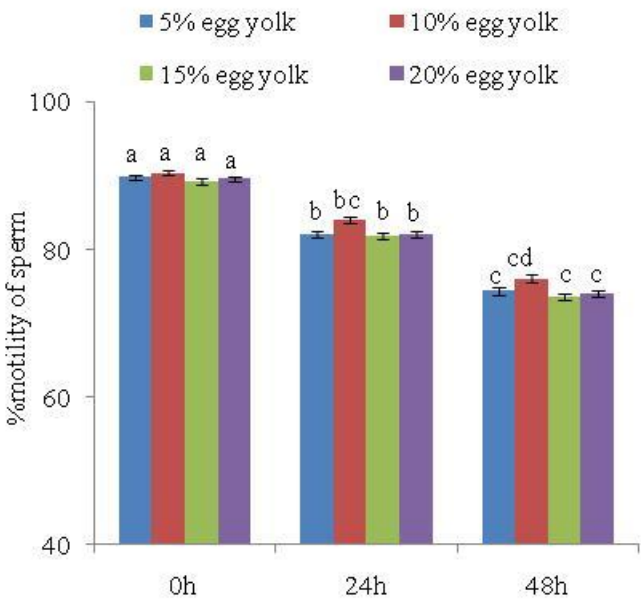

Fig. 1. Effect of different % of egg yolk on motility of indigenous ram chilled semen at 4-5°C for 48h. The values are mean ±SEM. Different lowercase letters indicate significant difference ($p<0.05$) between extenders and within preservation time

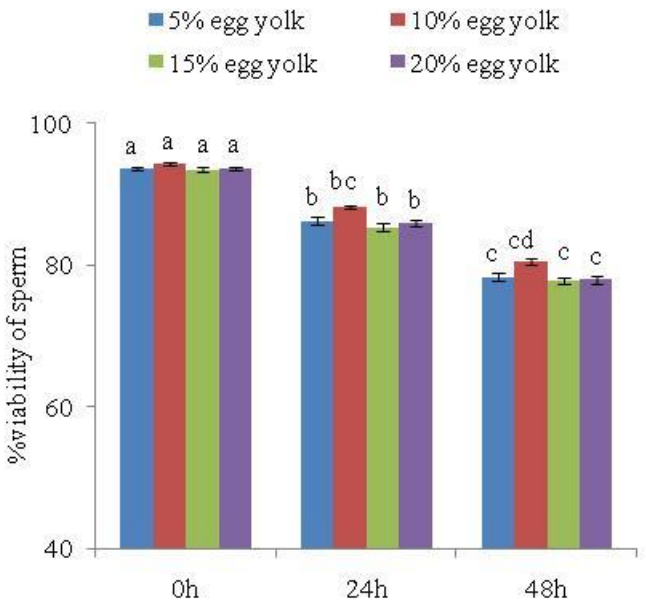

Fig. 2. Effect of different % of egg yolk on viability of indigenous ram chilled semen at 4-5°C for 48h. The values are mean ±SEM. Different lowercase letters indicate significant difference ($p<0.05$) between extenders and within preservation time

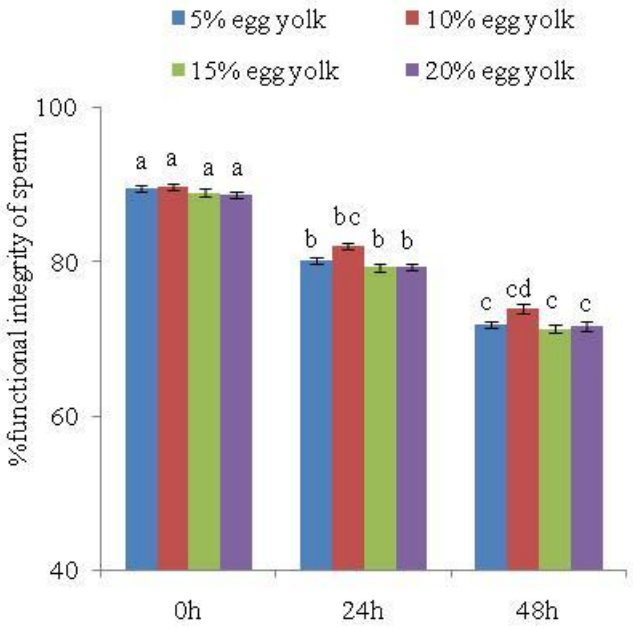

Fig. 3. Effect of different % of egg yolk on functional integrity of indigenous ram chilled semen at 4-5°C for 48h. The values are mean ±SEM. Different lowercase letters indicate significant difference ($p<0.05$) between extenders and within preservation time

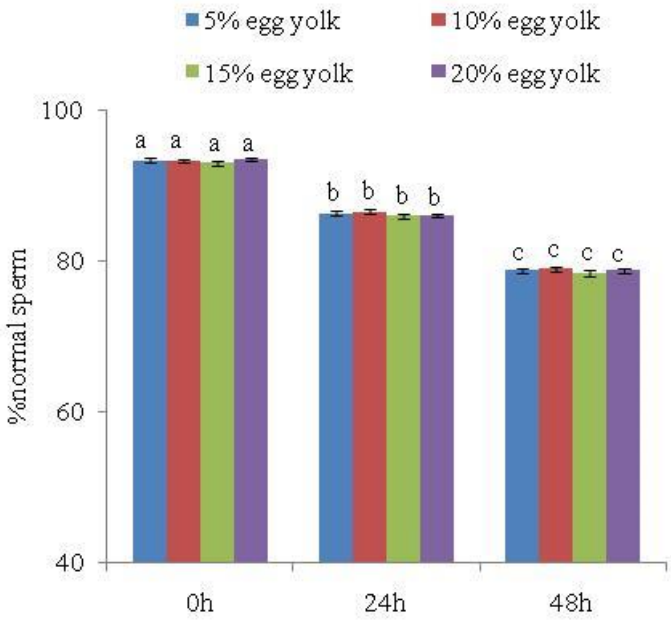

Fig. 4. Effect of different % of egg yolk on normality of indigenous ram chilled semen at 4-5°C for 48h. The values are mean ±SEM. Different lowercase letters indicate significant difference ($p<0.05$) between extenders and within preservation time

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