EFFECT OF DIFFERENT GLYCEROL LEVELS IN EGG YOLK CITRATE EXTENDER ON FREEZABILITY OF RAM SEMEN

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ABSTRACT

An experiment was carried out to evaluate the optimum glycerol level in egg yolk citrate diluent on freezability of ram semen. The ejaculates were obtained from six crossbred rams (Malpura X Bharat Merino). The semen was diluted with the diluent containing different levels of glycerol (4, 6, 8 and 10 %) and was frozen in liquid nitrogen at -196°C. The post-thaw motility and live sperm per cent were higher at 6 per cent glycerol level as compared to 8 per cent glycerol level. The values of post-thaw dead and abnormal sperm per cent were significantly lower at 6 per cent glycerol level as compared to 8 per cent glycerol level.

KEY WORDS: Ram semen, Glycerol level, Extender egg yolk citrate.

INTRODUCTION

Genetic improvement in sheep production can be brought about by selection within native breed, which apparently is a slow and time consuming process. Accordingly, cross- breeding as a national policy has been adopted as the main tool for improving sheep and wool production in India. Hence, the programmes under this policy will require the availability of a large number of exotic rams, which is not cost effective. Secondly, imported rams will take more time for adoptation to the climate of Indian subcontinent and require a very special care with respect to general and nutritional management. Therefore, for genetic improvement widespread use of artificial insemination is the only option. The ram semen is generally preserved at -196°C in liquid nitrogen. Sudden cooling of sperm during freezing may cause cold shock, which may ultimately leads to reduction in fertility. Glycerol is highly water soluble, hygroscopic, and bacteriostatic in action and can easily permeate into live cell due to their cryoprotectant activity. It was used successfully by Smith and Polge (1950) in the cattle and buffalo semen as a freezing buffer. Therefore, present study was undertaken to evaluate the influence of different glycerol levels in egg yolk citrate diluents on freezability of ram semen.

MATERIALS AND METHODS

Six crossbred (Malpura × Bharat merino) rams with an average age of two and half years and with an average body weight of 35 kg were randomly selected from Sheep Research Center of Post Graduate Institute of Veterinary and Animal Sciences, Akola. All the rams were kept under similar managemental conditions prior to start of the experiment. Semen was collected from all the rams twice in a week, early in the morning with the help of artificial vagina. Immediately after collection semen was evaluated qualitatively and quantitatively for various semen attributes, viz. ejaculate volume, colour, consistency, mass activity, live, dead and abnormal sperm count. It was then diluted with egg yolk citrate (EYC) diluent with four different glycerol levels, i.e. 4, 6, 8 and 10 per cent. The EYC diluent was prepared by dissolving 2.8 g Sodium citrate, 0.64 g Glucose, 0.3 g Sulphonilamide, 1 lakh IU Benzyl Penicilin and 50,000 IU of Streptomicin in 90 ml of triple distilled water to which 10 ml Egg yolk was added. The semen samples were diluted 1:5 with diluents having

different levels of glycerol and filled in plastic German mini straws of 0.25 ml capacity with the help of glass syringe and a long spinal needle. The straws were sealed by fixing the metal balls at both the ends. Filled straws were gradually cooled from 30°C to 5°C in 30 to 60 minutes in refrigerator. The straws were kept at 5°C for 8 hr in refrigerator to achieve equilibration. Thereafter, freezing of straws was done by keeping straws on the metal net of freezing chamber with the help of forceps. This freezing was done for 10 minutes duration and then straws were transferred directly in to vapors of liquid nitrogen (-196°C). The effects of various levels of glycerol on above parameters were studied for 5, 10, 15, 20, 25 and 30 days after freezing. Post-thaw average microscopic attributes of semen samples (in %) at five days intervals for one month was carried out at all the glycerol levels. The percentages of live, dead and abnormal sperm are estimated by staining technique using eosin-nigrosin stain (Tomar, 1996). The data was statistically analyzed as per standard method.

RESULTS AND DISCUSSION

The average results of macroscopic examination and microscopic examination of fresh semen samples are depicted in Table 1.

Table 1: Average result of macro and microscopic examination of fresh semen sample.

Sr. No.	Macro/microscopic Examination of fresh semen	Average Results
Macroscopic Examination		
1	Volume (ml)	0.96±0.90
2	Colour	Milky white
Microscopic Examination		
1	Mass activity	++++
2	Initial sperm motility (%)	80.50±2.23
3	Live sperm count (%)	77.66±3.43
4	Dead sperm count (%)	22.33±3.43
5	Abnormal sperm count (%)	12.83±1.60

The present findings with respect to average semen volume are in accordance with those reported by Carvalho *et al*. (2002) and Hassanin *et al*. (2013). However, Moghaddam and Pourseif (2014), Recai Kulaksiz *et al*. (2012) and Al-Samarrae (2009) reported comparatively lower semen volume than the present study in different breeds of ram. The difference in seminal volume reported in different studies might be attributed to different factors, viz. breed, age, health and genetic make-up, nutritional status, season/ climatic temperature and individual variation apart from semen collection techniques as reported in buffalo bulls by Chaudhari *et al.* (2014).

Colour of semen in present study is in agreement with that reported by Londhe *et al.* (2005) and Bhojne (1993). However, it is in contrast to the findings of Oyeyemi *et al.* (2009) in different breeds of sheep.

The average mass activity corroborated with Lyer (1994). Whereas, Oyeyeme *et al.* (2009) reported the mass activity +2 in African dwarf rams. The average sperm motility in present study was in accordance with that reported by Londhe *et al*. (2005) and Oyeyemi *et al*. (2009) and differed from that reported by Carvalho *et al*. (2002), Reza Asadpour (2012) and Hegedusova *et al*. (2012) in different breeds of ram.

The average live sperm count and dead sperm count found in present study are in agreement with Moghaddam and Pourseif (2014), Londhe *et al.* (2005) and Folch and Colas (1982). However, Olfati

et al. (2013) reported lower and Bhojne (1993) and Oyeyemi *et al*. (2009) reported higher live sperm count than the present finding. The average abnormal sperm count in present study is in agreement with that reported by Folch and Colas (1982). However, the present finding are not in agreement with the values reported by Olfati *et al*. (2013), Al-Samarrae *et al.* (2012) and Hegedusova *et al*. (2012) for different breeds of sheep.

The mean ± SE of post-thaw sperm motility, live sperm, dead sperm and abnormal sperm of semen samples (%) diluted in egg yolk citrate (EYC) with different glycerol levels studied at five day interval over a period of one month after freezing are depicted in Table 2.

The average post-thaw motility in 6 per cent glycerol level was significantly higher as compared to the value found with 8 per cent glycerol level. These findings corroborated with the results of Londhe *et al*. (2005). On the other hand, Ferdean and Bragaru (1964) reported the best sperm motility at 7 per cent glycerol level. However, addition of glycerol above the optimum level increases the detoriation of acrosome in the frozen thawed semen. In present study, post-thaw live sperm per cent in EYC diluent at 6 per cent glycerol level was significantly higher followed by 8 per cent and 10 per cent glycerol levels. In the post- thaw semen percentage of live sperms declined sharply as the period of freezing storage increased irrespective of the glycerol level as well as diluent.

Table 2: Post-thaw average microscopic parameters of semen samples (in %) at five days intervals for one month after freezing in EYC with different levels of glycerol.

Glycerol levels (%)	Days post-freezing							Mean ± SE
	0	5	10	15	20	25	30	
Sperm motility								
4	27.16	25.50	23.83	22.17	20.50	18.83	17.17	22.14^{d} ±1.28
6	42.17	40.50	38.83	37.17	35.50	36.83	32.17	37.17^{a}±1.31
8	33.83	32.17	30.50	28.83	27.17	25.50	23.83	28.83^{b}±1.36
10	32.17	30.50	28.83	27.17	25.50	23.83	22.16	27.17^{c}±1.45
Live sperms								
4	32.00	31.50	31.33	30.83	30.50	29.83	28.67	30.66^{d}±0.42
6	56.33	56.17	56.00	54.50	53.83	53.50	52.67	54.71^{a}±0.55
8	45.33	45.17	44.50	44.33	43.50	43.33	42.83	44.14^{b}±0.35
10	44.33	43.67	43.50	43.17	41.50	40.67	39.67	42.35^{c}±0.60
Dead sperms								
4	68.00	68.50	68.67	69.17	69.50	70.17	71.30	69.33^{a}±0.42
6	43.67	43.83	44.00	45.50	46.17	46.50	47.33	45.28^{d}±0.55
8	54.67	54.83	55.50	55.67	56.50	56.67	57.17	55.85^{c}±0.35
10	55.67	56.33	56.33	56.83	58.50	59.33	60.33	57.64^{b}±0.66
Abnormal sperms								
4	15.67	15.83	16.00	16.17	16.23	16.67	17.00	16.23^{b}±0.17
6	14.67	14.83	15.00	15.17	15.50	15.83	16.33	15.33^{d}±0.22
8	16.67	16.33	16.50	16.67	17.00	17.17	17.50	16.76^{a}±0.18
10	15.50	15.67	15.83	16.17	16.33	16.50	16.83	16.11^{c}±0.17

Note: Mean ± SE values for each parameter in last column with dissimilar superscripts indicates significant difference at 5% as well as 1% levels.

In EYC diluent, live sperm at 6 per cent glycerol level was in agreement with the findings of Bane *et al*. (2005) and Sinha *et al.* (1992), while Jones and Martin (1964) and Srivastav *et al*. (1989)

reported the lower live sperm than the present finding. Londhe *et al*. (2005) reported higher live sperm at 4 per cent glycerol level compared to the present finding. The mean ± SE of post-thaw dead sperm at various glycerol levels clearly indicated that 6 per cent glycerol was found to be the best, where dead sperm per cent was found to be the lowest.

The mean ± SE of post-thaw abnormal sperm was maximum at 8 per cent glycerol level, while it was lowest at 6 per cent glycerol level. The value obtained in the present experiment was comparable with findings of Londhe *et al*. (2005), who reported lower sperm abnormality at 6 per cent glycerol level in EYC while Bhojane (1993) observed less abnormal sperm in EYC at 4 per cent glycerol level and hence was not in accordance with the present result.

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