CORRELATION BETWEEN DIFFERENT POST-THAW QUALITY PARAMETERS OF MALABARI BUCK SEMEN

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Received: 17-09-2015, Accepted: 24-09-2015

ABSTRACT

As single evaluation technique has limited value in predicting the actual fertilizing potential, adequate evaluation techniques must be used for evaluating a particular semen processing protocol. In this study, freezing of buck semen was carried out using egg yolk based and soybean lecithin based extenders at two extension rates. The parameters included for post- thaw evaluation were progressive motility, viability, acrosomal integrity, and functional membrane integrity (Hypo Osmotic Sperm swelling Test). Pearson's correlation coefficient between the parameters reveals the reliability of them in the post thaw evaluation of Malabari buck semen with the freezing protocol, adopted.

Key Words: Malabari buck semen, Motility, viability, acrosomal integrity, HOST, Pearson's correlation coefficient

INTRODUCTION:

Assessment of the degree of cryodamage happened to the spermatozoa during their cryopreservation is crucial in predicting their fertilizing potential. Therefore, selection of reliable methods of semen evaluation is one of the most important factors in a breeding programme. If two parameters are significantly correlated, one of them can be used to predict trend of the other (Taylor & Francis, 2006). At the same time, evaluation of one of the parameters may be enough instead of doing all the qualitative tests so that the evaluation procedure will be simplified.

Hence, we studied the correlation between post-thaw motility, viability, acrosomal integrity and functional membrane integrity of Malabari buck spermatozoa in the freezing protocol using egg yolk based and soybean lecithin based extenders at two extension rates.

MATERIALS AND METHODS:

Forty eight semen samples collected following double ejaculate regime from two Malabari bucks, maintained at the Artificial Insemination centre, Dept. of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Mannuthy, Thrissur were utilised for the study. Sperm concentration was determined using haemocytometer after diluting the semen at 1:200 using hypertonic eosin- saline.

Semen samples with more than 80 per cent initial motility were divided into four groups. Samples of group I and II were extended with Tris egg yolk based extender and soyabean lecithin based extender (Andromed) respectively at the rate of 400

million spermatozoa per ml. Group III and IV were extended with Tris egg yolk based extender and soyabean lecithin based extender (Andromed) respectively at the rate of 800 million spermatozoa per ml. Group I and III were initially extended with non glycerolated fraction of the extender. Group II and IV were fully extended with Andromed. One hour after attaining 5ºC in the cold handling chamber, glycerolated fraction of the extender was added to the non glycerolated fraction in three steps at 10 minutes interval so that the fully extended samples of group I and III contained six per cent glycerol. French medium straws were used to fill the semen samples manually. After an equilibration period of 2 hours, the straws were undergone conventional freezing for 10 minutes and were plunged into liquid nitrogen.

Post thaw evaluation of semen from each group was done 24 h after freezing. Post-thaw quality parameters such as motility, viability, acrosomal integrity and functional membrane integrity were assessed after thawing the straws at 37°C for 60 seconds.

Motility was assessed at 400X objective of the light microscope. Sperm viability was assessed using eosin-nigrosin staining technique (Campbell *et al.,* 1953). Acrosomal integrity was assessed by Giemsa staining technique (Watson, 1975). Hypo osmotic sperm swelling test (HOST) was carried out to assess the functional membrane integrity of spermatozoa as per the method used for human spermatozoa described by Jeyendran *et al.* (1984). The data were statistically analysed using SPSS (Statistical Package for Social Studies) software. Correlation between the variables was assessed using Pearson correlation.

RESULTS AND DISCUSSION

Correlation between different parame ters is expressed in Fig1 to Fig.6. Here, progressive motility was found to be highly

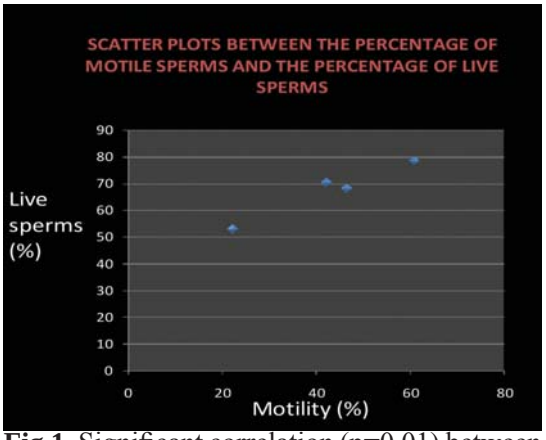

Fig.1. Significant correlation (p=0.01) between progressive motility and viability (R=0.848

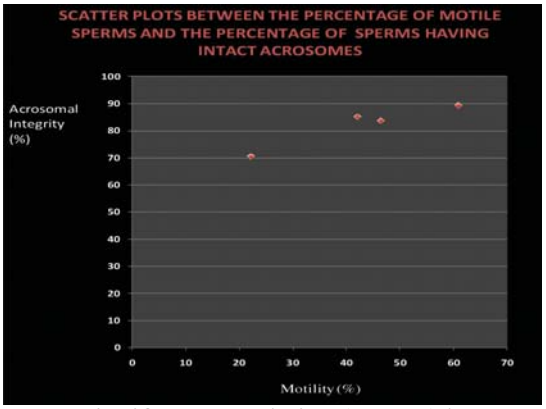

Fig.2. Significant correlation (p=0.01) between progressive motility and acrosomal integrity R=(0.760)

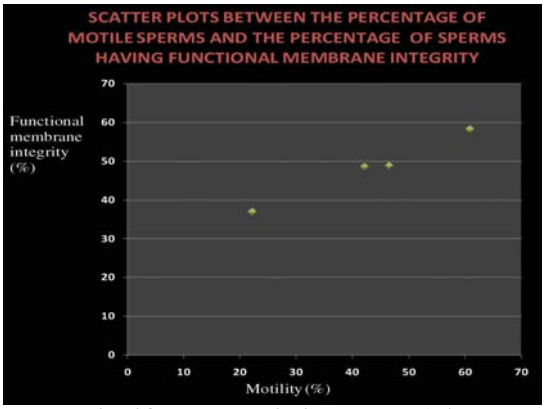

Fig.3. Significant correlation p=0.01) between progressive motility and functional membrane integrity (R=0.760)

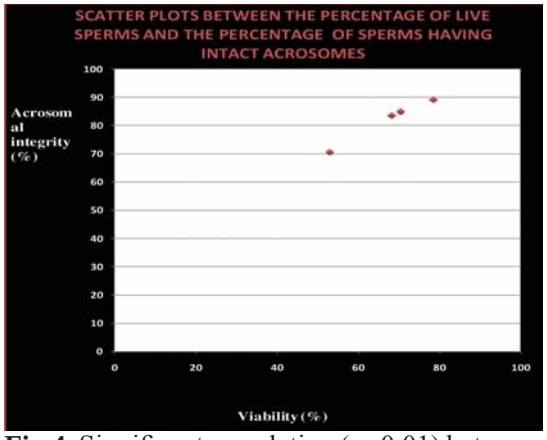

Fig.4. Significant correlation (p=0.01) between viability and acrosomal Integrity (R=0.790)

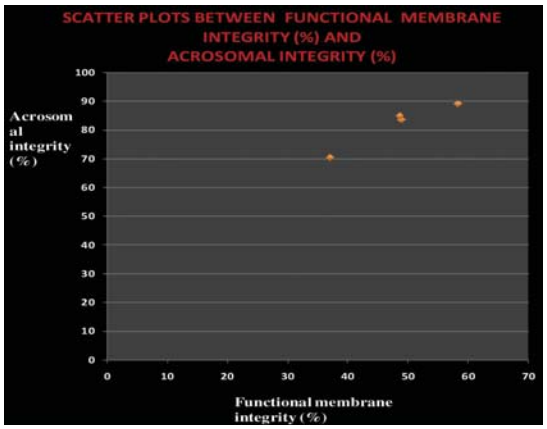

Fig.5. Significant correlation p=0.01) between viability and functional membrane Integrity (R=0.732)

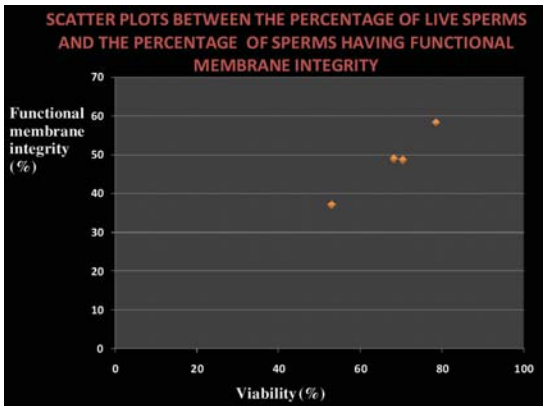

Fig.6. Significant correlation (p=0.01) between functional membrane integrity and acrosomal integrity (R=0.634).

correlated (p=0.01) with viability (R=0.848), acrosomal integrity (R=0.760) and functional membrane integrity (R=0.760). Percentage of viable spermatozoa was also highly correlated with (p=0.01) percentage of spermatozoa with intact acrosomes (R=0.790) and functional membrane integrity (R=0.732). Significant correlation (p=0.01) existed between functional membrane integrity and acrosomal integrity (R=0.634). Similar results were obtained previously by Jeyendran *et al*. (1984) in human semen, Mantovani *et al*. (2002) in equine semen Bohlooli S. *et al*. (2012) in ram semen.

SUMMARY

Results of the present study revealed the reliability of quality parameters such as motility, viability, acrosomal integrity and functional membrane integrity in the post-thaw evaluation of Malabari buck semen with the freezing protocol adopted using egg yolk based and soybean lecithin based extenders at two extension rates. However, research should be extended to find out the correlation of each of them to both *in vivo* and *in vitro* fertility rate of Malabari buck semen.

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