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Correlation between conventional sperm assay parameters in cryopreserved Ram Semen

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ABSTRACT

This study investigated the correlation of cryopreserved sperm membrane integrity with viability and motility in ram semen diluted in five different extenders and storage in LN (Liquid Nitrogen). Semen was collected with an artificial vagina from five adult rams. The ejaculates were pooled and diluted in five different extenders: Milk, Sodium citrate, Tris, Lactose and Sucrose. The diluted semen was frozen in vapor of LN (Liquid Nitrogen) and stored in LN.Afterthawed, sperm viability, progressive motility and membrane integrity was evaluated. Statistical analysis of the data revealed a significant ($p<0.01$) positive correlation between viability, progressive motility and membrane integrity in cryopreserved ram semen. Therefore, these results have indicated that there are statisticallysignificant correlations between evaluated parameters. It was concluded that semen characteristics could be evaluated by each of this parameters instead of evaluation all of them, accompanied with other assays for use in fertilization in vivo and in vitro.

Keywords: Correlation, Sperm Parameters, Simplifying, Extenders.

INTRODUCTION

A suitable evaluation of semen for breeding and in vitro fertilization purposes has always been of great importance. Evaluation of sperm quality usually is linked with the desire for predicting fertility in a clinical setting or to enable maximum number of offspring from a valuable sire [13, 16]. The traditional evaluation of the quality of ejaculate has been mainly based on routine semen analyses (motility, morphology and acrosomal integrity) which have a limited capacity for the prediction of the potential fertility of an ejaculate [6].The hypo-osmotic swelling test (HOST) was developed to evaluate the functional integrity of the sperm membrane [6]. Live spermatozoa with normal membrane function show swelling of the tail due to water influx when exposed to

hypo-osmotic conditions. The clinical predictive value of this 'functional' test, however, is still being debated [18, 19].The relationship between the fertility and sperm motility [20, 21] or morphology [20] is not consistent. Although these laboratory tests can be used to rapidly evaluate a semen sample, they do not incorporate information on sub-cellular physical damage that can occur during cryopreservation. The aim is to simplification sperm evaluation then, the correlation between these characteristics could be evaluated and instead of evaluation all of them, one of them could be evaluated and evaluation process could be simplified.

In terms of prediction, if two variables were correlated perfectly, then knowing the value of one score permits a perfect prediction of the score on the second variable. Generally, whenever two variables are significantly correlated, the researcher may use the score on one variable to predict the score on the second [4]. Thus, with calculation of correlation between this routine sperm analysis parameters, would not be necessary to assay of other related parameters and instead of them, other tests could be used to evaluate the functional characteristics of sperm like Zona-free hamster ova test, and cervical mucus penetration test [3].

For this reason, this study investigates the correlation between sperm viability, progressive motility and membrane integrity of post-thaw spermatozoa. To avoid of likely effects of different extender on these parameters, semen diluted in five different extenders.

MATERIALS AND METHODS

All chemicals used in this study were provided from Merck (Germany), the exceptions are otherwise indicated.

2.1. Animals and Semen Collection

Five 3-5 years old sexually mature and healthy rams was selected for semen collection. Semen was collected 3 times in a week during the breeding season using an artificial vagina. The ejaculates were placed in a thermos (35 ºC) immediately after collection, and semen quality was assessed and pooled. The pooled semen had macroscopic good visual mass activity, sperm concentration 2.8×10^9/ml, progressive sperm motility ≥ 75%, and normal sperm morphology ≥ 90%. Thus, the pooled ejaculate divided into five equal parts and diluted with Milk, Sodium Citrate, Tris, Lactose and Sucrose extenders to a final concentration of approximately 400×10^6 spermatozoa per ml. The extended semen was then cooled to 5ºC during nearly 1h by placing in a refrigerator. The cooled semen was then filled into 0.5 ml plastic straws and prepared to cryopreservation, which done by N2 vapor for 8 minute and stored in liquid nitrogen. Cryopreserved semen was thawed in a thermostatic bath at 37 ºC and then evaluated [10, 17].

2.2. Semen Extender:

The composition of the citrate diluent was: 2.37 g sodium citrate (2H O2), 0.50 g glucose, 20 ml egg yolk, 5% glycerol, 100,000 IU penicillin, 100 mg streptomycin and glass distilled water to 100 ml. Then pH adjusted to 6.8-6.6. The Tris-based medium made by mixing 3.63 g Tris, 0.50 g fructose, 1.99 g citric acid, 20 ml egg yolk, 5% glycerol and glass-distilled water to 100 ml [10]. Pasteurized and homogenized skim milk heated for 10 minute at 90ºC used in milk-base extender [9, 2]. Milk extender composed from 75% skim milk, 20% egg yolk and 5% glycerol. Preparation of lactose extender was performed by adding 10 g lactose, 0.5 g glucose, 20 ml egg

yolk and 5 ml glycerol to 75ml distillated water (6.6≥ pH ≤6.8). For preparation sucrose extender, 10 g sucrose, 0.5 g glucose 20 ml egg yolk and 5 ml glycerol added to 75ml distillated water then pH adjusted by NaOH 10% to 6.6-6.8. To control microbial growth 1000 IU penicillin and 1 mg streptomycin added to each milliliter of each extender.

2.3. Semen Evaluation

Sperm motility estimated by phase contrast microscope. To evaluate this parameter, one drop of thawed sperm was placed on a warm (37ºC) stage and spermatozoa with progressive motility counted used 20X objective. Sperm viability was assessed by Eosin Y-Nigrosin staining. A cell with an intact cell membrane does not take up the stain Eosin Y, while a dead takes up the red stain. Nigrosin was used as a background stain to provide contrast for the unstained (white) live cells. For more guarantee, 200 spermatozoa assessed under oil immersion with a high-resolution 100 X objective with correct adjustment of the bright field optics. Spermatozoa that were white (unstained) classified as "live" and those that show any pink or red coloration classified as "dead" [1]. To detection of cytoplasmic membrane integrity, one drop of each treatment added to 8 drop of hypoosmotic solution (13.5 g fructose, 7.35 g Tri citrate sodium, Eosin Y 0.5% w/v) and after incubation (for 45 minute at 38 ºC) one drop of mixture place on microscope lam and covered with cover slip. Sperm with swelling tail was evaluated as sperm with membrane integrity [11].

2.4. Statistical Analysis

The conformity of variables with the normal distribution was examined using Shapiro-Wilk W-test. Data was reported as mean±SD. The Correlation between two parameters was evaluated using Pearson correlation. Statistical significance was considered as $P<0.01$. Calculating of correlation and regression was done with PASW 18 and Minitab 15 Statistic software package.

RESULTS AND DISCUSSION

The results of the present study are summarized in Table 1.The mean percentage of viability, progressive motility and membrane integrity were 21.53±9.06%, 11.54±6.91% and 5.23±3.91%, respectively.

Table1. Mean percentage of evaluated sperm parameters for the different extenders

Parameters / Extenders	Viability %	Progressive Motility%	Membrane Integrity%
Tris	31.52±1.26	19.21±0.82	8.37±0.89
Milk	20.09±2.00	9.23±1.05	7.71±0.60
Sucrose	13.50±1.14	5.689±1.05	0.87±0.42
Lactose	12.28±1.02	5.00±0.67	1.02±0.39
Citrate	30.26±1.68	18.60±0.82	8.15±1.30
Mean	21.53±9.06	11.54±6.91	5.23±3.91

Correlation among viability, progressive motility and membrane integrity are illustrated in figure 1-3.

Figure 1.The distribution of membrane integrity with progressive motility

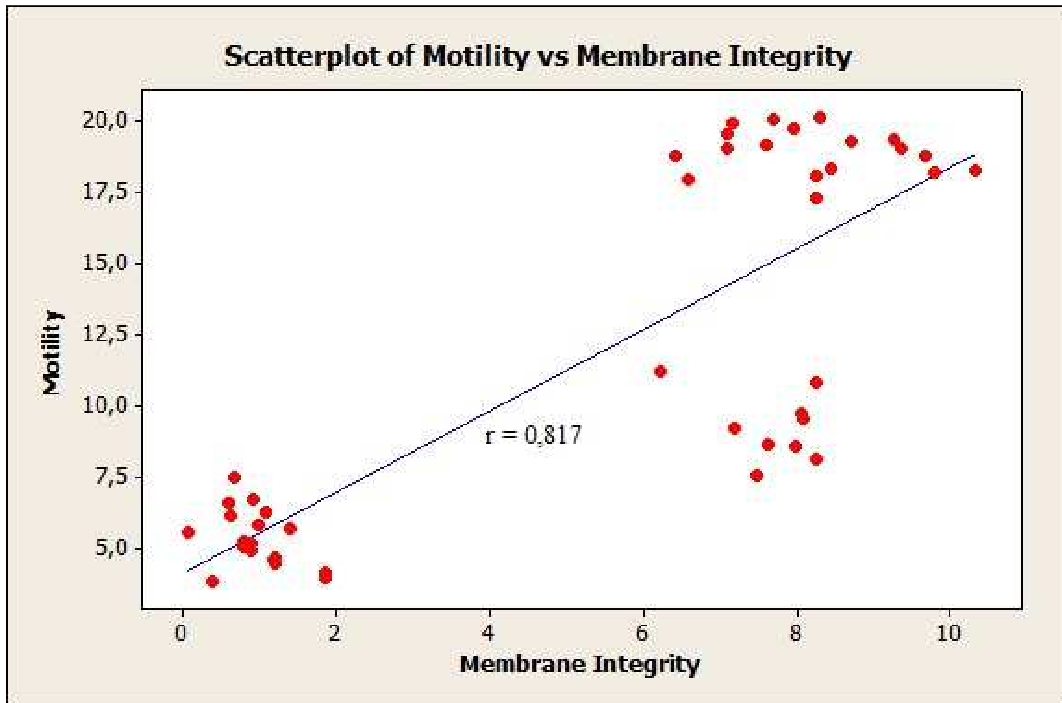


Figure 2.The distribution of membrane integrity with sperm viability

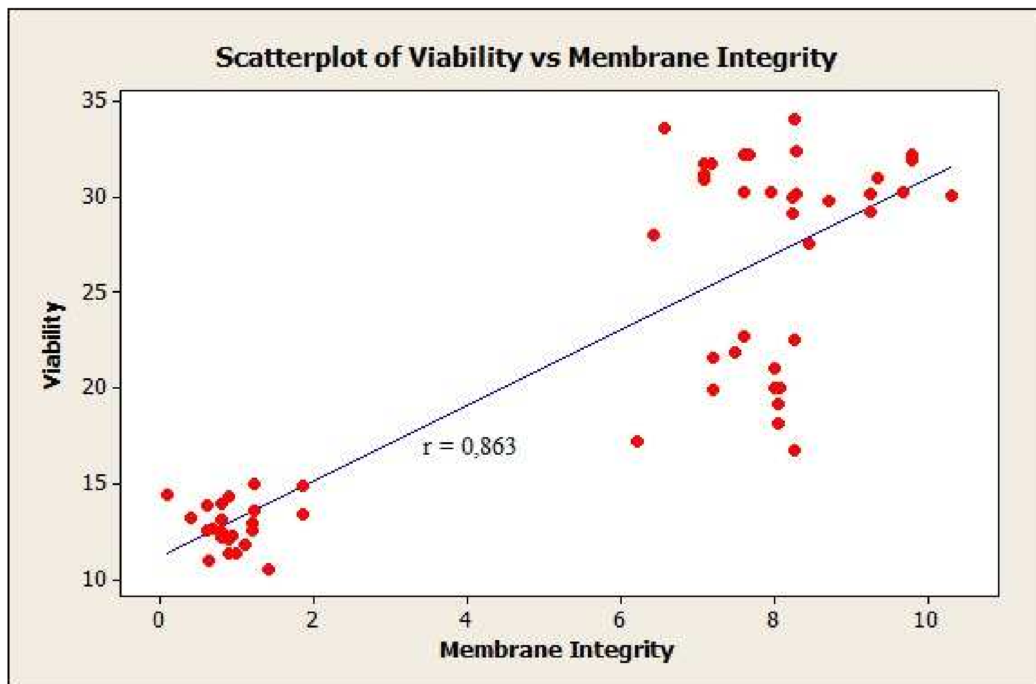
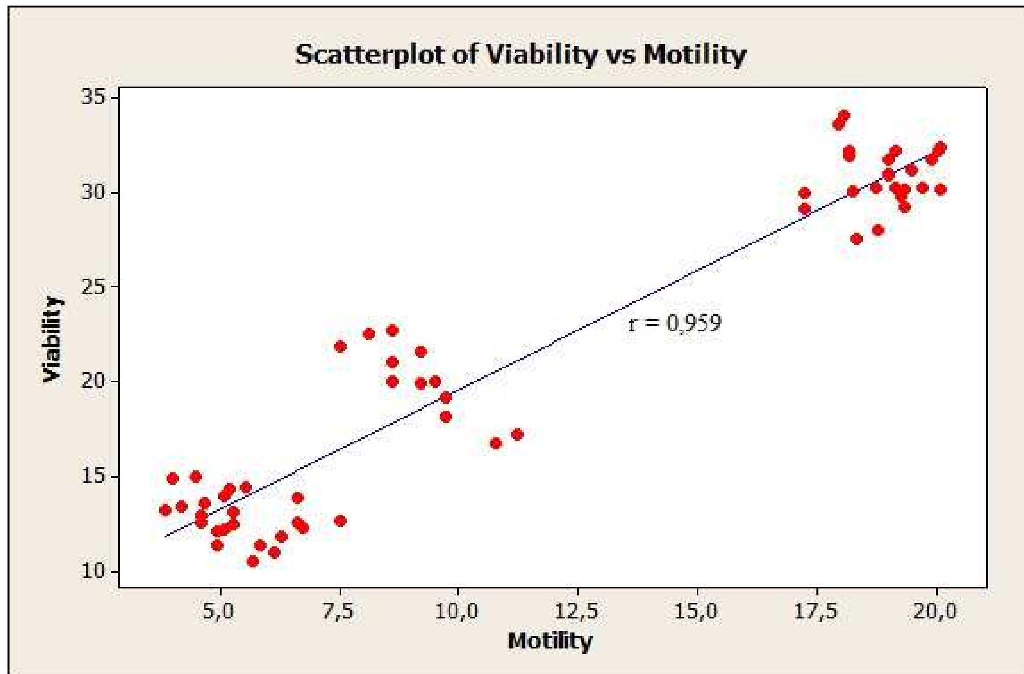


Figure 3.The distribution of progressive motility with viable spermatozoa



Data of this experiment revealed statistically significant correlation between sperm membrane integrity with viability and motility. This was in agreement with previous work on human [6], equine [14] and fresh goat spermatozoa [12, 15]. Zekariya has calculated correlation between Saanen buck's spermatozoa motility and HOST. He found significant correlation (r=0.523, P<0.001) between percentage of swollen spermatozoa obtained with the HOS and the motility[12].It is not interesting because spermatozoon motility partly depends on transports of compounds across membrane of spermatozoa [6]. Therefore, Plasma membrane damage due to death or anisosmoticconditions causes a rapid leakage of intracellular adenosine triphosphate (ATP), which is required to maintain sperm motility [7,8]. The ATP content was highly correlated with progressive motility of spermatozoa of fresh and cryopreserved bull semen [5].

CONCLUSION

In conclusion, our results indicate very strong correlation between the evaluated sperm parameters which have been cryopreservedand thawed in different extenders. Therefore,we canevaluateone of them and if necessary calculate other related parameters in order to facilitate this process.However further studies performed with data fromdifferent breeds and farms,will be needed to calculate of similar correlations between sperm assay parameters.

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