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Influence of Temperature on Motility and Viability of Bovine Spermatozoa during Cold Storage

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Abstract- The success of insemination programme depends on the appropriate management of semen collection, storage and use. According to some reports, the highest numbers of successful AIs were done in Central Province (CP) and the lowest were recorded in Eastern Province and Northern Province. So far there are no studies related to semen quality of bull which belongs to AI centre at Thirunelvely. The semen of Jersey was collected by means of artificial vagina and stored at 4-8°C. General examination was evaluated to assess volume, colour, opacity and pH. Fresh semen (0 h) and stored semen (24 h to 72 h) were compared by progressive individual motility and sperm count. Sperm count was evaluated by hemocytometer. Viability was determined by using 1% eosin. Repeated measures analysis of variance (ANOVA) with Dunnett's post test was performed using prism 5.04 to compare the viability of semen from 0 h to 72 h during storage. Viability decreased significantly (ANOVA $P<0.05$) in a time dependent manner from 0 h to 72 h during storage at 4-8°C. The percentage of viable sperms and sperm velocity gradually decreased when compared to control 0 h (93.17± 1.322%, 23.33 ± 1.419 μm/s), with 24 h (85.50 ± 0.8892 %, 13.33± 0.7698 μm/s), 48 h (79.22 ± 0.7731, 1.778 ± 0.2383 μm/s) and 72 h (69.17 ± 1.360 %, 0.817 ± 0.271μm/s). Our preliminary studies suggested that the fresh semen is better than the stored semen for higher successful rate of insemination in NP of Sri Lanka.

Index Terms- bull semen, hemocytometer, sperm count, sperm velocity, Viability

I. INTRODUCTION

Artificial insemination (AI) technique has been used to raise the genetic potential of cattle by using genetically superior bulls (Wishwanath, 2003). The success of an AI programme depends on the appropriate management of semen collection, storage and use (Etches, 1996). Preservation of semen using suitable extenders is an important ingredient in the success of AI in cattle reproduction. This is because it increases the volume of semen, thus permitting more insemination per ejaculate. The viability and fertilizing ability of ejaculated semen are mainly judged by progressive motility (Etches, 1996) and morphologically normal spermatozoa (Eva-Marie *et al.*, 1989). According to Abeygunawardena *et al.*, (2001) the highest numbers of successful AIs were done in Central Province (CP) and the lowest were recorded in Eastern Province and NP. The coverage in terms of the proportion of breedable cattle was highest in CP followed by Western Province (WP) where highest calving rate was recorded (Abeygunawardena *et al.*, 2001). More than four decades AI center at Thirunelvely has been giving AI service to many regions of Northern Province (NP). In this AI center, fresh semen is diluted with semen extenders to prepare insemination doses which is stored in the refrigerator (4-8°C) and supplied to other AI stations without assessing the viability before insemination. So far there are no studies related to semen quality of bull which belongs to AI centre at Thirunelvely. Thus the present study was undertaken to examine the viability of diluted semen during storage in the refrigerator at 4-8°C.

II. MATERIAL AND METHODS

Collection and storage of semen

The semen of Jersey was collected by means of artificial vagina once a week since Oct 2010 from AI center at Thirunelvely. To compare the viability of fresh and stored semen, adequate amount of semen was aliquoted into equal volume in the small vials. Vials were stored in the refrigerator at 4-8°C. Each vial was used for further evaluation per day.

Pre-extension evaluation of semen

General examination including volume, colour, opacity and pH was evaluated before dilution with 2.9% W/V sodium citrate buffer solution for initial assessment (quality) of the fresh semen. Volume of a fresh ejaculate was measured with the aid of graduated collection tube while pH was determined by pH paper (sigma). The colour and viscosity of the semen were evaluated by visual appraisal. Mass motility was assessed by placing a drop of semen on a slide, covered with a cover slip and examined under a light microscope to observe dark waves.

Comparison of viability of fresh and stored semen

Fresh semen (0 h) was evaluated immediately after collection from AI centre. Semen with 24 h of refrigeration, 48 h of refrigeration, and 72 h of refrigeration were also evaluated by microscopic examination particularly sperm concentration and sperm velocity. Sperm concentration was evaluated by using hemocytometer (improved NEUBAUER) which was loaded with a 1:100 dilution of semen. Semen sample was mixed well before loading into the chamber of the hemocytometer. The semen was immobilized by addition of formaldehyde to prevent movement of sperm cells. Sperm cells were counted in a "Zigzag" pattern in the middle big square of the hemocytometer. Semen was stained with 1% eosin and loaded again to assess the viability. Three readings for each sample were taken to calculate the average value of sperm concentration before and after staining. Sperm

velocity was also calculated for each sample by using the hemocytometer and stopwatch for assessing the progressive individual motility. The number of small boxes on which sperm moved was counted. Sperm morphology was also assessed by the preparation of the sperm smear with 1% eosin and 0.75% Giemsa stain to evaluate the viability and acrosome integrity respectively. The whole experiments were repeated at least more than 18 times.

Statistical analysis

Statistical analysis was performed by using prism 5.04 to compare the viability of bull semen during storage conditions. Mean ± SEM was used to describe data. Repeated measures analysis of variance (ANOVA) with Dunnett's post test for comparison of treatment groups with a control (0 h) was used to analyze the viability of semen and sperm velocity during storage.

III. RESULTS

During general examination creamy white colour of the semen was observed in the Jersey bull. Volume varied from 4 to 11 ml per ejaculate, while pH of semen fluctuated between the ranges of 6.5 to 7.0. Opacity of semen was high due to the high concentration of sperms. Sperm abnormalities were less than 8% in almost all semen samples, but some samples from Jersey bull contained high percentage (>15%) of abnormal sperms including double headed sperm, detached head, crooked tail **(**Figure 1A) and coiled midpiece. After overnight from the Giemsa staining almost all sperms (97%) possessed intact acrosome **(**Figure 1B), but some sperms lost their acrosome (Figure 1C).

Before the eosin staining average sperm concentration did not vary greatly (2.14-2.27× 10^9 sperms/ml). However number of dead sperms (Figure 1D, E) increased gradually from day 0 to day 3 during storage conditions. Fresh semen of Jersey showed dense of dark waves but this wave pattern and mass motility decreased from 24 h to 72 h of storage gradually due to the reduction in number of live sperms (Figure 1F). Sperm velocity and progressive individual motility were high in fresh semen compared to stored semen (Table 1) Progressive individual motility was greater than 70% in fresh semen.

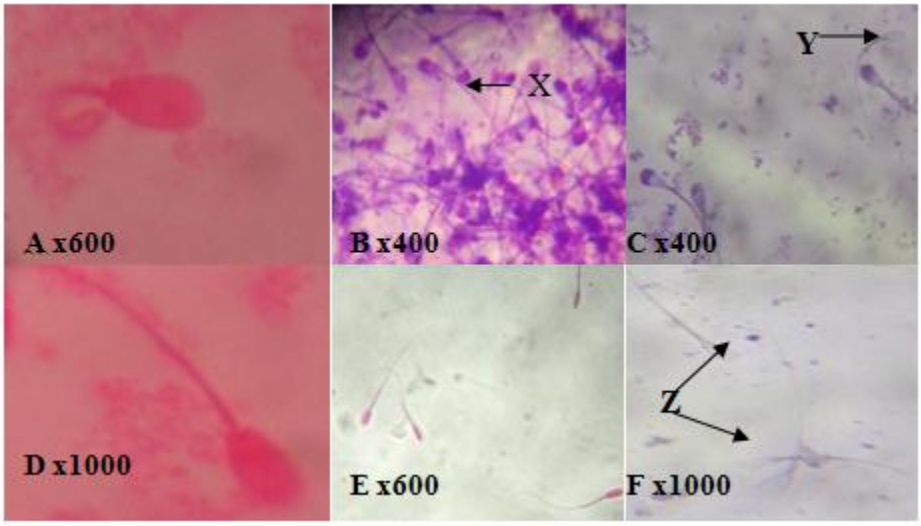

Figure 1: Assessment of the acrosome integrity (Giemsa staining) and sperm membrane (eosin staining) under the light microscope. A: Sperm with crooked tail. B: Sperms with intact acrosome (X) C: acrosome intact sperms and acrosome reacted sperm (Y) D, E: dead sperms F: live sperms (Z)

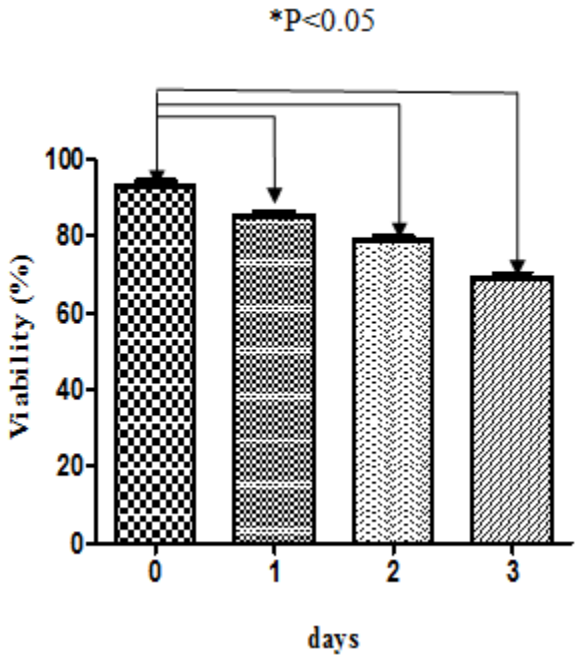

Figure 2: Viability of bull semen during first 3 days of storage

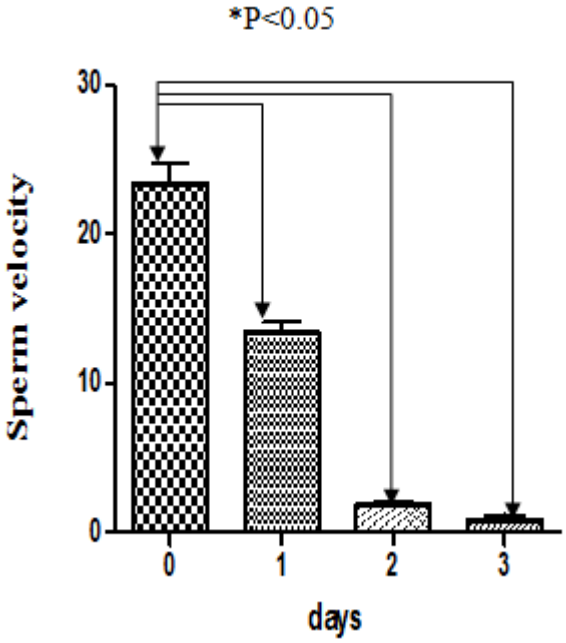

Figure 3: Sperm velocity (µm/s) during first 3 days of storage

Table 1. Comparison of viability and sperm velocity of fresh and stored semen samples

Sperm parameters	0 h	24 h	48 h	72 h
Viability (%)	93.17± 1.322	85.50 ± 0.8892	79.22 ± 0.7731	69.17 ± 1.360
Sperm velocity (µm/s)	23.33 ± 1.419	13.33± 0.7698	1.778 ± 0.2383	0.817 ± 0.271

The highest motility was obtained during the first day of storage (24 h), thereafter, the motility declined drastically from day 2 to day 3. Progressive individual motility was 60% during the first day of storage whereas 48 h and 72 h samples showed less than 40% of progressive motility. After 72 hrs of refrigeration, sample showed very less progressive motility. After 3 days of storage, sperms showed very less motility. Percentage of viability (Figure 2) and sperm velocity (Figure 3) significantly decreased (ANOVA $P< 0.05$) in a time dependent manner from 0 h to 72 h during storage.

IV. DISCUSSION AND CONCLUSION

In the present study, there were significant differences between viability of fresh semen and stored semen due to some other reasons such as gradual degradation of nutrients, sperm damages and acrosome reaction during freezing and thawing procedures, formation of toxic compounds and metabolic byproducts etc. We evaluated the acrosome status, because percentage of live sperm with intact acrosome is essential for successful fertilization with oocyst. When eosin staining smears are evaluated, non stained sperm cells were considered as alive, whereas, partially and totally stained cells were considered as dead, since their plasma membrane was altered and allowed stain penetration (Felipe-Pérez *et al*., 2008).

Udeh and Oghenesode, (2011) concluded that diluted liquid goat semen stored in refrigerator condition should not be kept beyond 24 h before insemination in order to obtain a good conception rate, because live sperms must be greater than 70% for artificial insemination. The storage of diluted semen at reduced temperature helps to extend the sperm life by slowing their metabolism as well as by inhibiting bacterial growth. Bacteria grow by utilizing the nutrients in semen extenders, thus competing with spermatozoa for these limited resources, and release metabolic byproducts, thus creating an environment that is not conducive to maintaining viable spermatozoa. The drastic decline in sperm motility and viable sperms could be attributed to gradual depletion of nutrients such as potassium, sodium and plasma protein (Udeh and Oghenesode, 2011).

Approximately 50% of viable sperm are detrimentally affected after exposure to either fresh or frozen storage procedures. Cell damage during cooling or freezing procedures can be caused by ionic compounds present in the storage medium. Egg yolk contains lipoproteins and lecithin that stabilize the plasma membrane of sperm (Bencharif *et al.*, 2008) and prevent hypermotility and capacitation (Amirat *et al.*, 2004), but it is also a potential source of bacterial contamination. Buffer solution doesn't alter the physiology of the sperms. The addition of sodium citrate buffer helps to control the pH of the medium and regulation of the osmotic pressure (Nilani *et al*., 2012). The increased concentration of buffer solution in the extender reduces the deleterious effects of the great amount of hydrogenic ions was produced from the metabolic activity of spermatozoa. Adeyemo *et al.*, (2007) who reported that the sodium citrate performed excellently well in maintaining the viability of stored fish semen. Sodium ions are also common compounds in sperm preservation media, and possibly have adverse effects on sperm.

Replacement of sodium ions with choline chloride in medium has been shown to improve viability of sperm cells subjected to cooling or freezing procedures. Toner *et al.*, (1993) suggested that choline chloride is an organic compound similar to sodium that possesses protective properties against cold exposure, but apparently cannot cross cell membranes. Aboagle and Terada, (2003) stated that refrigerator temperature helps to stop metabolic process of stored liquid semen which resulted in the utilization of nutrients such as fructose by the sperm cells. Udeh and Oghenesode, (2011) also recommended that the refrigerator temperature should be used as a storage medium.

In conclusion, our preliminary studies suggested that fresh semen is better than stored semen for higher successful rate of insemination in NP of Sri Lanka. As for successful conception, viability must be more than 70 %. In our studies sperm viability is more than 70 % for 0 h, 24 h and 48 h of stored semen at low temperature (4-8°C). Therefore the reason for weakness of AI performance in NP could be some other reasons such as failure of wise use of estrus detection methods or else.

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