SCIENCE LETTERS 2013 | Volume 1 | Issue 1 | Pages 9-12

Conventional semen processing technique and breeding soundness of various cattle breed bulls used in sub-tropical conditions

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Abstract

Protocol was developed to evaluate the best performing breed in the local conditions of tribal areas which are slightly different from other parts of the country; moreover the conventional method was used to evaluate its effect on the semen quality during processing. Four different breeds (Holstein Friesian, Jersey, Sahiwal, and Holstein-Friesian Sahiwal Cross) in four different time intervals (pre-dilution, post-dilution, post-equilibration and post-freezing) were evaluated to see their effect on the semen quality such as individual motility, sperm membrane integrity, sperm morphology and live percentage. The breed effected individual motility of semen significantly ($p<0.05$), while the effect on volume, sperm abnormalities and membrane integrity was highly significant ($p<0.01$). Highest individual motility observed for FSC was 74.0%, as compare to Jersey, Holstein Friesian and Sahiwal, i.e. 72.9%, 70.95% and 68.9 %, respectively. However, the primary abnormalities observed were highest in FSC (15.39%) and least in Jersey (11.70%). Membrane integrity damage was found in FSC, Sahiwal, Jersey and HF as 24.06%, 22.66%, 20.60% and 18.90% respectively. The effect of different time intervals on the sperm motility, live percentage and secondary abnormities was highly significant ($p<0.01$). The individual sperm motility during different time interval i. e. pre-dilution, post-dilution, post-equilibration and post-freezing was (93.34%, 80.31%, 79.30% and 49.92%) respectively. The live sperm percentage was highest at pre-dilution (92.72%) and least observed at post freezing (53.60%), showing 42.19 percent change. Secondary abnormalities observed were 8.19%, 18.23%, 21.35% and 29.96% respectively, during different time intervals. The overall results suggest that jersey breed performance was good in hilly area of the region and should be continued to breed the local breed *Achai*. Moreover highest sperm abnormalities and other damage to sperm cell were occurred post-freezing by using conventional way of freezing.

Key words: Cattle breeds, conventional method, semen quality, sub-tropical region.

Received May 25, 2013; **Revised** August 22, 2013; **Accepted** September 04, 2013

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Introduction

Artificial Insemination has been introduced in Khyber Pakhtunkhwa/Federally Administered Tribal Areas, since last four decades and has found popularity in cattle then buffalo [1]. The conception rate is lower in artificial insemination as compare to natural service, which is dependent upon the estrus detection, nutrition and environmental stress [2] and semen quality. Semen is mostly processed and cryopreserved by conventional (manual liquid nitrogen tank) method. This process is less expensive and coveniant as compare to computer controlled freezing technique but due human errors most of semen quality is damaged which leads to low conception rate in cows.

Environmental stress mostly affect exotic (*Bos tauras*) cattle breed as compare to the local breeds (*Bos indicus*), the local breeds are more adapted to environmental stressors [3]. In recent era of dairy revolution in a country due to poor genetics and low production performance of local breeds, the exotic breeds are preferred and imported. But their production and reproductive performance is compromised as compare to their native countries (temperate zone), i.e. mainly affected by heat stress and endemic diseases in the region. Comparatively cattle from *Bos indicus* are better able to regulate body temperature in response to heat stress than cattle from most of *Bos taurus* breeds from European origin [4, 5] but their production and reproductive performance is very low due to poor genetics. Therefore, In order to evaluate the reproductive performance of various cattle breed bulls locally and check sperm cell damage in different steps of conventional method for cryopreservation the current study was conducted.

Material and Methods

Equal no of bulls (two from each breed) were selected from four different breeds of cattle (Holstein Friesian (HF), Jersey (Jr), Sahiwal (Sw) and Holstein-Friesian-Sahiwal Crossbred (FSC)). Daily two semen ejaculates were collected for one month, twice a week on Monday and Thursday

early morning before dawn. Total 128 semen ejaculates were collected for analysis of different parameters.

Data analysis

The initial Data were recorded by using MS-Excel sheet and then statistically analyzed by computer software program SPSS version 16. The mean of multiple variables was ranked by Duncan Multiple Range (DMR) test [6].

Sample collection

Semen was collected in artificial vagina (42 ^{0}C @ 45-55mm hg pressure). The collection tube was then shifted to hot water bath with temperature of 35 ^{0}C. Two ejaculates were collected with 10-15 min of intervals. Then the sample were observed for any foreign body, color, volume, mass motility, individual motility, abnormal sperm cell count, Hypo-osmotic swelling test and live to dead ratio [7].

Individual motility

The initial motility of semen sample was done by taking a 3-4 mm diameter drop of diluted (physiological saline, before freezing) semen (by the use of a micropipette and a tip) onto a warm slide (maintained on a slide warmer at 37^o C) and a cover slip (18 mm square or round) was put on the drop.
The slide was placed on the biotherm (warm stage at 37^{o}C) of the phase contrast microscope and the motility (movement of individual sperms) at a magnification of 100 times (10X eyepiece and 10X objective lens) was examined. Motility was expressed as percentage i.e. Very good (A), 80-100% motile; Good (B) 60-79% motile; Fair (C) 40-59% motile; Poor (D) 40% motile.

Sperm morphology

A 100 µl volume of the semen sample was added to 500 µl buffered formal citrate solution (2.9 g sodium citrate, 1 ml 37% formaldehyde in 100 ml distilled water).A drop of a solution was placed on a clean glass slide, covered with a cover slip and examined under phase contrast microscope under oil emersion lens (1000 X) with at least 100 cells were examined [8].

Sperm morphological abnormalities were classified as primary and secondary abnormalities [9]. Primary abnormalities were (abnormal head and midpiece, abaxial attachment of midpiece, tightly coiled tails) secondary abnormality (separated heads, distal droplets, bent tail).

Sperm membrane integrity

The HOS test was used to evaluate the membrane as either biochemically active or not. When active spermatozoa were exposed to hypo-osmotic stress they showed swelling because of influx of water and increased in volume to establish equilibrium between the intracellular and extracellular fluid environment. The tail region attached near the head showed the swelling because of loose attachment of membrane in the region. 0.25 ml of semen was incubated in 1.0 ml of normal saline solution (0.9% NaCl) for 5 minutes & numbers of spermatozoa with curled tails were calculated [8]. The number in normal saline was deducted from number in HOS solution / distilled water solution. The resultant figure was taken in as the sperm tail curling as an effect of HOS Test. That was denoted, as ‘Reactive sperm’ & the spermatozoa did not show any effect was ‘non-reactive sperms’ & expressed in percentage.

Live to dead ratio

One drop of semen along with one drop of Eosin-Nigrosin stain was placed and mixed on clean slide, mixed with clean slide or stick. A thin smear was made; air dried and examined under microscope 400X: 10X eye piece and 40X objective lens [7]. The stain was composed of sodium citrate dehydrate 3 g, distal water 100 ml, eosin 1 g, Nigrosin 5 g. The Nigrosin-stain produced dark back ground on which sperm cells stand out as lightly colored objects. Normal live sperm appear as white and dead sperm appeared pinkish in color by taking Eosin stain because of loosing of membrane integrity.

Results and Discussion

Breed effect

The research data reveals that the Jersey was the best breed. The individual motility was high and less primary abnormalities were observed (Table 1;

Fig 1), which were significantly ($p<0.01$) different among all the breeds [10]. The sperm viability was

Table 1: Breed effect on the individual motility, sperm primary abnormalities and membrane integrity reactive sperm cells of different bulls.

Breed	Individual Motility (%)	Primary Abnormalities	Host (%)
Friesian	70.9a,b	12.23c	20.60c
Jersey	72.9a	11.70d	18.90c
Sahiwal	68.9b	14.33b	22.66b
FSC	74.5a	15.39a	24.06a
P (value)	<0.05	<0.01	<0.01

Different English letters mean that values in columns differ significantly at p <0.01; FSC; Holstein Friesian Sahiwal crossbred.

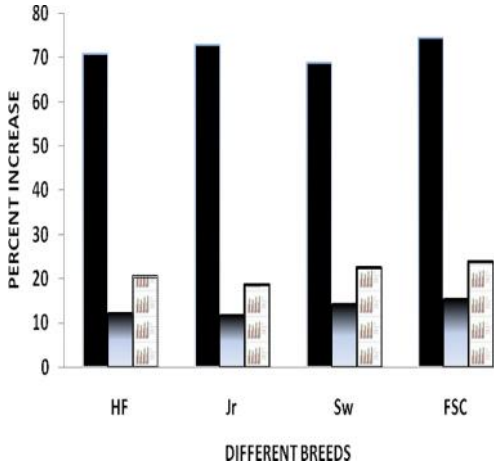

Fig. 1: The effect of different breeds (HF: Holstein Friesian; Jr: Jersey; Sw: Sahiwal; FSC: Fresian Sahiwal crossbred) on the individual motility (%, solid bars, $p<0.05$), primary abnormality (%, Gradient bars, $p<0.01$) and sperm membrane intigrity (%, Stack bars, $p<0.01$) in bovine semen (n= 832).

also higher than motility in all breeds [11]. The study area is arid in nature with relatively higher altitude as compared to Peshawar valley. The fodder availability in the region is low and the temperature is relatively low. These environmental conditions are more favorable for lighter breeds like Jersey. Also the breeding policy of Government of Pakistan recommends light breed i.e. Jersey semen for up gradation of cattle breeds in hilly areas, because the exotic (*Bos tauras*) breed performance is better than local breeds (*Bos indicus*) [12].

The FSC breed had higher individual motility and live percentage, as compare to Sw and HF (Fig. 1) showing the hybrid vigor. The reason for the better performance of FSC is that it has developed genes from the HF and resistant genes from Sw to local environment leading to its higher individual motility and live percentage. The higher abnormalities were also recorded for the same breed showing it genetic disturbance and imbalance.

HF bull's semen showed lesser abnormalities, highest volume than FSC and Sw. However, the live percentage was high in FSC and high concentration was recorded for Jr (Table 1). The HF performance is very good in plan areas but in the study area there is fodder shortage and its large body size leading to relatively low performance. The Sw cattle are local breed of Pakistan from plan areas (Punjab) but its performance is compromised in the relatively higher altitude i.e. tribal areas of Pakistan. Some other factors including lacking of genes for higher production and delayed age at maturity lead to poor productivity and reproductive performance as compare to exotic breeds (*Bos tauras*) [3].

Table 2: Effect of time interval on individual motility, live percent and secondary abnormalities of sperm cells in the semen, fresh and after dilution.

Time interval	Individual motility (%)	Live (%)	Secondary Abnormality
Pre dilution	93.34^{a}	92.72^{a}	8.19^{d}
Post dilution	80.31^{b}	79.10^{b}	18.23^{c}
Post equilibration	79.30^{b}	78.12^{b}	21.35^{b}
Post freezing	49.92^{c}	53.60^{c}	29.96^{a}
P (value)	<0.01	<0.01	<0.01
Percent change	46.52	42.19	-265.81

Different English letters mean that values in columns differ significantly at p <0.01.

Time interval effect (conventional method)

There is gradual decline in each parameter because of more exposure to environmental influences. Secondary abnormalities increased significantly ($p<0.01$) after each time interval and highest secondary abnormalities observed were post-freezing (29.96%) (Table 2), supporting the statement that more exposure to external stressors lead to increase in sperm abnormalities [13, 14].

Findings of the research data revealed that membrane integrity, live percent and individual motility decreased after each time interval (Table 2) (Fig 2), suggesting that membrane injury resulted from phase events in the lipid bilayer [15, 16], there was almost 50% decrease in individual motility pre-dilution to post-freezing. Highest decrease in

individual motility was documented at the time interval (post-equilibration to post freezing).

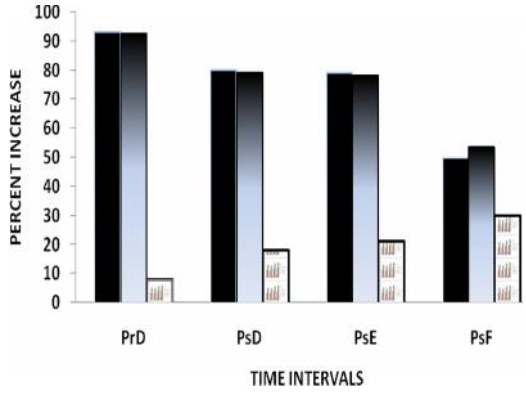

Fig. 2: The effect of different Time intervals (PrD: Pre-dilution; PsD: Post-dilution; PsE: Post-equilibration; PsF: Post-freezing) on the individual motility (%, solid bars, $p<0.01$), live sperm cells (%, Gradient bars, $p<0.01$) and secondary abnormalities (%, Stack bars, $p<0.01$) in bovine semen (n= 832).

The membrane integrity was less damaged post-dilution and more damaged post-freezing (Fig 2). The largest decrease in motility occurred at post-dilution cooling on 5°C [17]. The individual motility and live percentage was almost same in post-dilution to post-equilibration and then there was a drastic change in both post-freezing (Table 2). Secondary abnormalities increased after each time interval in a constant manner (Figure 4.3). The sudden decrease in individual motility, live percentage and increase in abnormalities, post freezing showed that there was a cryogenic shock to sperm cells as the temperature decreased from +5°C to 196°C. Sperm abnormalities and loss of membrane integrity showing that the membrane elements may have been altered by temperature stress. In the sperm cell membrane Integral membrane proteins are clustered by lipid phase separation, which undergo structural modulation to carry out their function, i.e. ion channel proteins due to cryoshock leads to membrane permeability or injuries; leading to sperm defects [18].

Conclusions

From the above discussion it was concluded that the Jersey is the best breed regarding semen quality parameters and short body size which is well adapted to the hilly area and service of local breed *Achai*. Also with the advancement in the processing stage from collection to freezing; resulted in decrease of sperm motility, integrity and increased secondary abnormalities.

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