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Hypo osmotic swelling test as screening for evaluation of semen of bull

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ABSTRACT

The hypo osmotic swelling test is a simple laboratory test which is used for the assessment of functional integrity of sperm membrane. When the sperm are put in hypo osmotic solution, water moves within cell membrane and swelling of membrane will take place. If the membrane is already damaged then there will be no swelling of membrane. The percentage of swollen spermatozoa is the measure of intact membrane. The semen of bull is evaluated on the basis of conventional parameters like motility, morphology and livability. To evaluate the semen on the basis of functional integrity, Fresh semen collected from 3 bulls was subjected to the hypo osmotic swelling (HOS) test in order to determine if the results could be correlated with conventional semen evaluation parameters. A total of 20 pooled samples from each bull were collected. Each semen sample was separated into two parts. One fraction was used for the evaluation of semen by usual method, while the other part was subjected to hypo-osmotic swelling test by using 150 mOsm/L sodium citrate and fructose solutions. The mean sperm positive to HOS test was 47% in Sahiwal cow bull semen, 40% was in Frisian semen and 27% in crossbred semen. On the statistical analysis of the data there was a significant ($P < 0.05$) positive correlation between progressive motility, morphologically normal spermatozoa, sperm viability and percentage of HOS test positive spermatozoa for three bulls. It was proved that HOS test could be a screening test for routine evaluation of semen which is used for artificial insemination.

Keywords: Hypo-osmotic swelling; Bull and Semen.

1. Introduction

The appropriate evaluations of the semen for artificial insemination and in embryo transfer technology are of significant importance in livestock industry. Although a wide variety of diagnostic tools are used to investigate the quality of bull sperm, it is still difficult to determine to which extent subfertile sperm contributes to the inability achieving the conception. If one simple test can be used to conclude that a semen sample is adequate for potential fertility, routine semen quality screening could be done in a much easier way. The analysis of semen provides the actual information about the fertility of an individual [1]. The semen is evaluated conventionally on the basis of motility, morphology and livability. The conventional semen evaluation methods limit the fertilizing potential of male animals [2]. The process of fertilization comprises the many steps which involve the many physiological and chemical events. The measurement of motility of spermatozoa was known as dependable parameter of good quality of semen [3]. The evaluation of membrane integrity and motility predict the good fertility rate of an individual [4]. Due to high importance of plasma membrane in the process of fertilization, the evaluation of plasma membrane integrity has got the significant attention in the semen evaluation of male animal. For the evaluation of membrane integrity of sperm, the eosine and nigrosine staining is used [5] but it measures simply structural integrity of plasma membrane of spermatozoa and there is no help for the functional active of membrane [6]. The staining of spermatozoa is based on the principal that membrane of dead spermatozoa allows to inter the stain in the cytoplasm but live spermatozoa do not permit it and remain colorless [7]. The process of fertilization which comprises the certain physiological events (union of sperm to egg, reaction of acrosome and capacitation) require the active plasma membrane of sperm and it is not possible for the occurrence of fertilization with inactive plasma membrane [8].

The Hypo-osmotic swelling test which was developed by [8] in human and later on this test was used in the evaluation of semen in dog [9], boars [10] and bulls [11].

This HOS test is based on the principal of semipermeability of integral cell membrane of spermatozoa. This ability helps the sperm to swell under the hypo osmotic situations. Under the exposure of hypo osmotic conditions, swelling of the sperm tail indicates the movement of water across the membranes to get the equilibrium [12]. The objective of this study was to find the effectiveness of HOST in cow bull and its correlation with conventional semen evaluation parameters. The aim of present study was to simplify the semen evaluation and instead of evaluation with conventional parameters only HOS can be used as screening test for semen evaluation.

2. Materials and Methods

2.1. Experimental animals

The semen of three cow bulls; Sahiwal, Friesian and cross bred (Sahiwal X Friesian) was used. The bulls were kept at Semen Production Unit, Department of Theriogenology, University of Agriculture, Faisalabad. These bulls had normal reproductive genitalia with good quality of semen for artificial insemination. The animals were kept in individual pens and provided plenty of green fodder and fresh water.

2.2. Collection and evaluation of semen

Semen was collected with the help of artificial vagina early in the morning. Before collection of semen, the bulls were given bath to remove dung from prepuce of animal. The semen from each experimental animal was collected two times in a week and twenty semen samples were collected from each bull. The collected semen was shifted immediately to water bath at 37 °C and evaluated for livability, assessment of motile spermatozoa and morphology [13]. The hypo osmotic swelling test was used for the functional integrity of spermatozoa [8].

2.3. Hypo-osmotic swelling test

The functional integrity of sperm was evaluated by using the HOS test. To get the maximum reactive spermatozoa for osmolarity, different osmolar solutions (50, 100, 150, 200, 250 and 300 mOsm/L) with sodium citrate and fructose were used in this study. By using these solutions, most reactive spermatozoa for swelling with optimal osmolarity could be assessed. The mixture of semen and HOS solutions together were incubated at temperature of 35 °C and 37 °C for time of 15 minutes intervals. This process continued for 120 minutes. The objective of this process was to determine the suitable time and temperature for maximum results of HOS test. To get the consistent results, this experiment was performed three times. On the basis of preliminary results, the hypo osmolar solution of 150 mOsm/L and incubation period of 37 °C for 30 minutes was chosen for these studies. The HOS test was conducted according to the method of [8]. Briefly the 1ml of 150 mOsm/L solution of was mixed with 50 ul of semen and incubated for the temperature of 37 °C for 60 minute.

One drop of the incubated semen and hypo osmolar solution was placed on the glass slide and covered with coverslip. This slide was observed at 400X under the microscope magnification and 200 sperms were counted. Spermatozoa with swollen tail were counted as percentage of HOS positive.

2.4. Statistical analysis

The values collected on % age of sperms positive to hypo-osmotic swelling test and progressive motility (%), viable spermatozoa (%) and morphologically normal sperm (%) were subjected to Pearson linear correlation method [14]. The significance of correlation was checked by applying "T test".

3. Results

Evaluating the semen, we focused on the semen parameters; motility, morphology, Livability and then hypo osmotic swelling test for assessment of membrane functional integrity of spermatozoa. The mean percentage of motility, Morphology, Livability and HOS coiling of Friesian bull was 62.5, 57.5, 65 and 40 respectively. There was a significant correlation between HOS coiling, motility, normal spermatozoa and livability as shown in Figure 1, 2 and 3.

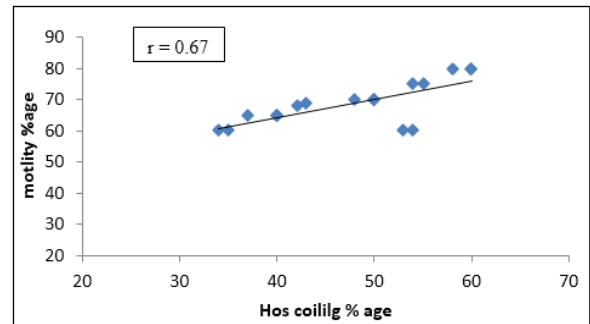


Fig 1: Significant correlation between HOS coiling and motility of sperms in fresh semen of Friesian bull

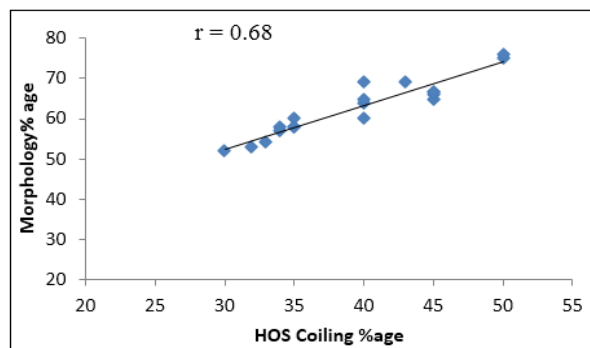


Fig 2: Significant correlation between HOS coiling and normal morphology of spermatozoa in fresh semen of Friesian bull

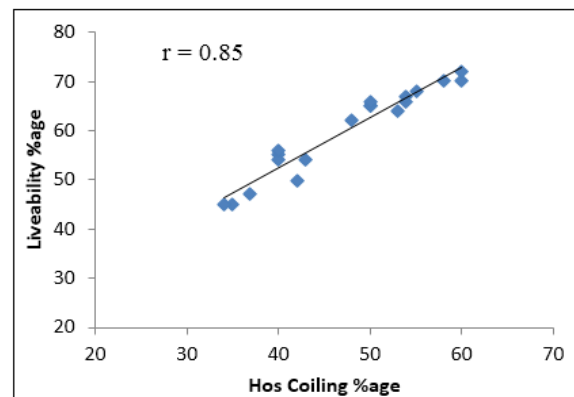


Fig 3: Significant correlation between HOS coiling and Livability of spermatozoa in fresh semen of Friesian bull

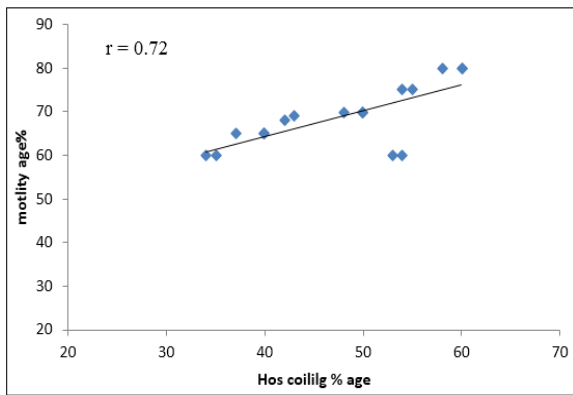


Fig 4: Significant correlation between HOS coiling % age and motility % age of spermatozoa in fresh semen of Sahiwal bull

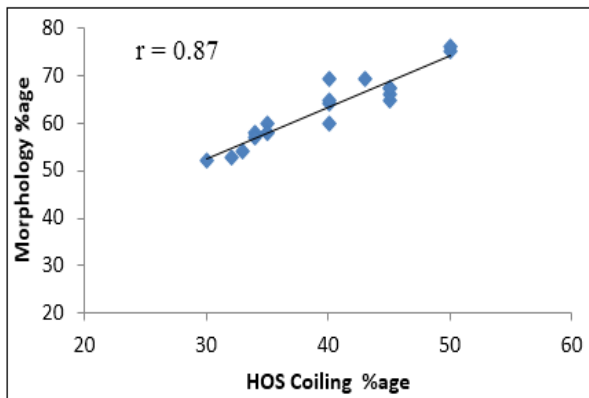


Fig 5: Significant correlation between HOS coiling % age and normal morphology % age of spermatozoa in fresh semen of Sahiwal bull

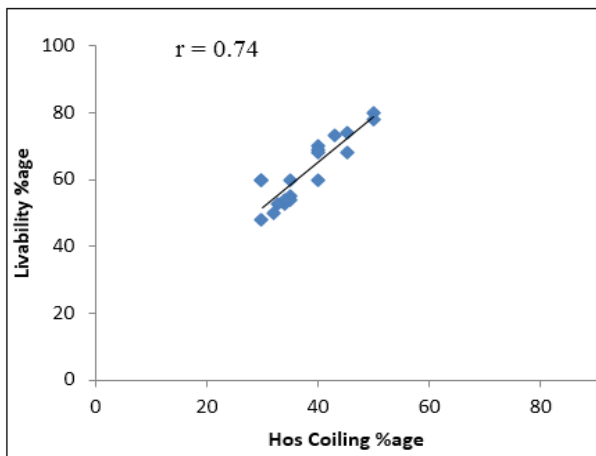


Fig 6: Significant correlation between HOS coiling % age and livability % age of spermatozoa in fresh semen of Sahiwal bull

The mean values of semen evaluation parameters including HOS coiling, motility, morphology and livability of Sahiwal bull was 47%, 60%, 56% and 70% respectively. The correlation between HOS coiling, motility, normal spermatozoa and livability was significant as in Figure 4, 5 and 6. The mean value of the HOS coiling was 27% in crossbred bull. Whereas the motility, normal morphology and livability were 50%, 40% and 55.8% respectively and these last three values were significantly correlated with HOS swelling as in Figure 7, 8 and 9.

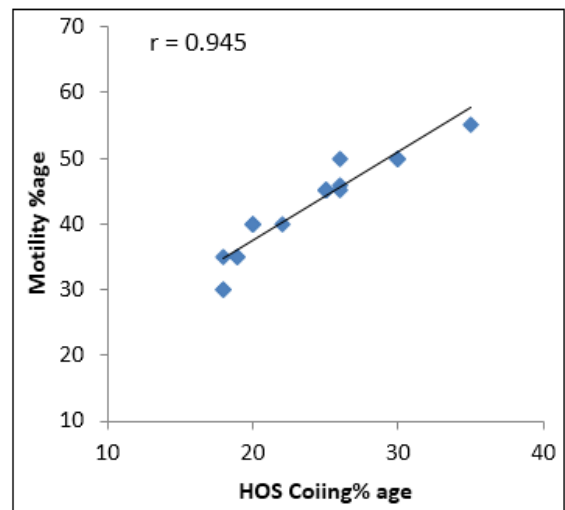


Fig 7: Significant correlation between HOS coiling % age and motility % age of spermatozoa in fresh semen of Crossbred bull

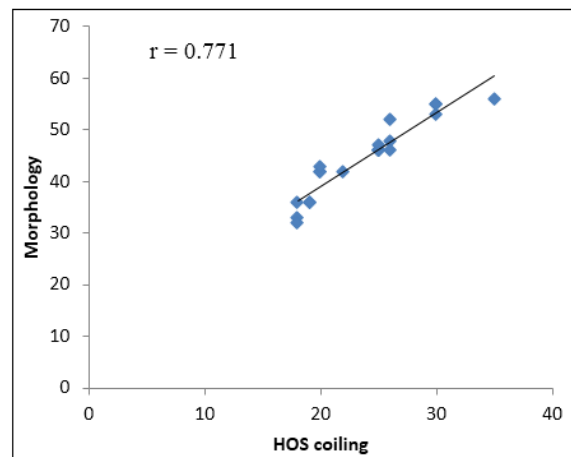


Fig 8: Significant correlation between HOS coiling % age and normal morphology % age of spermatozoa in fresh semen of Crossbred bull

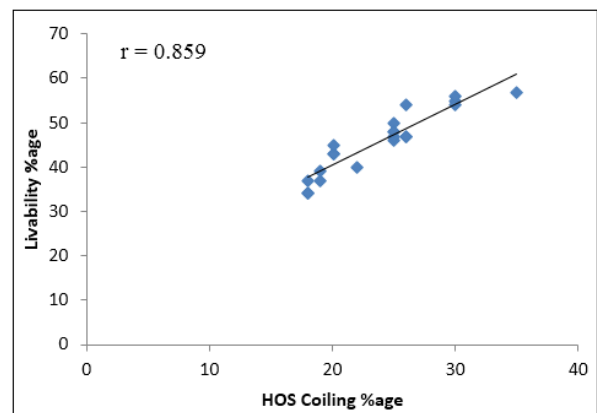


Fig 9: Significant correlation between HOS coiling % age and livability % age of spermatozoa in fresh semen of Crossbred bull.

4. Discussion

The most widely used parameters to do an initial screening on semen are sperm count, morphology and motility. The progress of new simple tests of functional integrity for screening of semen could helpful in the diagnosis the quality of semen for artificial

insemination. The basic objective of research in semen evaluation is to find out the physiological properties of sperm which could help in the infertility [15]. The conventional parameters of semen evaluation have limited function for functional integrity of plasma membrane of spermatozoa [16]. The two important properties of viability of sperm are structural and functional integrity [17]. Many scientists are convinced on the importance of functional integrity of sperm plasma membrane for the estimation of fertility of semen of an individual [4]. The spermatozoa obtain a hard capsule during the process of spermatogenesis which gives them a proper shape and in the maturation of spermatozoa in epididymis this capsule is layered by lipoprotein. This capsule has the feature of livability of spermatozoa. We generally use the eosin and nigrosine staining for the evaluation of fresh semen and this staining can also be used for extended semen to find the live sperm. Similarly, abnormal shapes of sperms can be determined through this staining [7]. The use of HOS test in semen evaluation is much applicable than eosin and nigrosine staining. The better fertilizing capacity of sperm plasma membrane can be estimated through functional activity by HOS test [1]. This test has the ability to sign whether the plasma membrane of sperm is biochemically active or dead.

On exposing the sperm to hypo osmolar solution, the liquid moves into the sperm cell via the cell membrane. The sperm cell with functionally active membrane swells from the tail region to achieve the balance for concentrations of solutes for extra and intracellular space [19]. Due to swelling of the membrane, curling of tail takes place [8]. The osmolarity of the hypoosmolar solution must have the capacity for creation of best result without the death of sperm. Different solutions which have different osmolarities of osmotic pressure of semen in dogs [20], stallions and bucks [16]. In the present studies, the best number of swellings in three bulls was observed in 150 mOsm/ L with sodium citrate and fructose and similar kind of findings were observed by [8].

The findings of the present investigation showed highly significant correlation between HOS coiling and motility (%), sperm livability (%) and morphologically normal spermatozoa in Frisian, Sahiwal and crossbred bulls. High correlation was found between HOS reactive and staining ($r = 0.65$) [21] in rainbow trout and these results are in close line with the present study.

The study conducted by [22], the evidence of significant correlation was reported between HOS test and common semen assessment parameters such as motility of sperm and concentration in human. The similar kinds of results were reported by [23, 24].

Results of present research were in close to previous findings which were $r = 0.98$ given by [20]. Strong correlation in dog was reported by [9] between HOS and supravital staining and motility $r = 0.94$ and $r = 0.82$ respectively and these findings were similar to present study. Some scientist [25] reported the strong correlation between fraction of structural integrity of sperm membrane and HOS positive sperms ($r = 0.81$) in bull to see the effectiveness of supravital staining and HOS test. The results of present studies were in accord with these findings.

The findings of present study were also similar with preceding reports on human [8], equine [26] and fresh spermatozoa goat [27] semen samples where significant correlations were reported between HOS test and motile, normal morphology and livability of spermatozoa.

It is not likely to be easy and inexpensive test that obviously differentiate between productive and non-productive semen. There is real need for easy and simple test which can help in the screening of semen.

5. Conclusion

HOS test does not provide us all the information which we need about the evaluation of semen but it has the ability to tell that semen is potential for fertility or not. The results of our study showed that the HOS coiling significantly correlated with motility, normal morphology and livability and HOS test is suitable for the early screening, of semen.

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