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CORRELATION BETWEEN HYPO-OSMOTIC SWELLING TEST AND VARIOUS CONVENTIONAL SEMEN EVALUATION PARAMETERS IN FRESH NILI-RAVI BUFFALO AND SAHIWAL COW BULL SEMEN

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ABSTRACT

The present study was conducted to determine the correlation of hypo-osmotic swelling test with conventional semen evaluation parameters of fresh semen collected from two Nili-Ravi buffalo and two Sahiwal cow bulls. A total of 10 pooled samples (each comprising two consecutive ejaculates) from each bull were collected. Each semen sample was divided into two parts. One part was used for the evaluation of semen by conventional method, while the other part was subjected to hypo-osmotic swelling test by using 150 mOsm/L sodium citrate fructose solution. The mean sperm positive to HOS test was 85.25% both in Nili-Ravi buffalo and Sahiwal cow bull semen. Statistical analysis of the data revealed a significant ($P<0.05$) positive correlation between progressive motility, morphologically normal spermatozoa, sperm viability and percentage of HOS test positive spermatozoa for both species. It was inferred that HOS test could be a valuable method for routine evaluation of semen for artificial insemination.

Key words: Hypo-osmotic swelling, Nili-Ravi buffalo bull, Sahiwal cow bull, semen.

INTRODUCTION

An adequate evaluation of semen for breeding purposes has always been of great significance. Semen analysis is a valuable diagnostic tool to assess the fertility status of the male. However, the prediction of potential fertility of a male on the basis of a single assay is not reliable. Conventional parameters used for evaluation of semen have limited application because they only help to assess the structural integrity of the cell (Neild *et al.,* 1999). Each sperm cell consists of multiple sub cellular compartments with different functions, all of which must be intact for successful fertilization (Amann and Graham, 1993).

In recent years, more attention has been given to evaluating sperm membrane integrity as it is of fundamental importance in the fertilization process. Jeyendran *et al*. (1984) developed a hypo-osmotic swelling test (HOS) to evaluate sperm membrane function of human spermatozoa. Since its development, HOS test has been used for evaluation of sperm membrane integrity in bovines (Rota *et al*., 2000), equine (Neild *et al*., 2000), canine (Rodriguez-Gil *et al*., 1994), porcine (Perez-Llano *et al*., 2001) and rainbow trout (Cabrita *et al.,* 1999). The present study was conducted to find out the correlation, if any, between HOS test positive sperms and conventional semen quality parameters in Nili-Ravi buffalo and Sahiwal cow bull semen.

MATERIALS AND METHODS

Experimental animals

A total of four adult bulls i.e. two each of Nili-Ravi buffalo (B4 and B6) and Sahiwal cow (S2 and S3) kept at the Semen Production Unit, Department of Animal Reproduction, University of Agriculture, Faisalabad, Pakistan were used in this study. These bulls had clinically normal reproductive tract and were donating semen of acceptable quality for artificial insemination.

Collection and evaluation of semen

Semen from all the experimental bulls was collected with the help of an artificial vagina. From each experimental bull, semen was collected twice a week and a total of 10 pooled samples were collected from each bull. Immediately after collection, each ejaculate was shifted to 37^0C and subjected to microscopic evaluation i.e. estimation of progressive motility, viable spermatozoa and morphologically normal sperm (Salisbury and Van Demark, 1978). To access the functional status of sperm membrane, the spermatozoa were subjected to hypo-osmotic swelling test (Jeyendran *et al*., 1984).

Hypo-osmotic swelling test

The hypo-osmotic solution (150 mosm/L) was prepared by dissolving 7.35g sodium citrate and 13.5g fructose in 1000 ml of distilled water. The solution was stored at 4^0C till used. A 1.0 ml of hypo-osmotic solution was mixed with 0.1 ml of undiluted semen and incubated at 37 ^{0}C for 1 hour. A drop of diluted semen was placed on a clean sterilized dry glass slide and covered with a cover slip. A total of 200 spermatozoa were counted in different fields at 400X under phase contrast microscope and percentage of spermatozoa positive to HOS test (having coiled tails) was determined.

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 (Steel and Torrie, 1980). The significance of correlation was checked by applying "T test".

RESULTS

The data on hypo-osmotic swelling test positive sperm and its correlation with other semen evaluation parameters in Nili-Ravi buffalo and Sahiwal cow bulls are presented in Tables 1 and 2, respectively. The mean sperm positive to HOS test was 85.25% both in buffalo and Sahiwal cow bull semen. In buffalo bull semen samples, HOS test showed significant ($P<0.05$) positive correlation with progressive motility ($r=0.649$), sperm viability ($r=0.880$) and morphologically normal spermatozoa ($r=0.611$). In Sahiwal cow bull semen samples, the HOS test also showed a positive significant ($P<0.05$) correlation with progressive motility ($r=0.612$), sperm viability ($r=0.902$) and morphologically normal spermatozoa ($r=0.661$).

DISCUSSION

In the present study, 85.25 percent of the Nili-Ravi buffalo and Sahiwal cow bull spermatozoa were positive to HOS test. The high number of positive sperms in both the species shows the higher concentration of biochemically active sperm cells with intact functional membrane. Carreras *et al*. (1990) indicated that proportion of positive spermatozoa to HOS test was lower in oligozoospermia. The percentage of positive sperms to HOS test varies with bull (Prasad *et al*., 1999), season (Kale *et al*., 2000), mass activity, progressive motility, sperm count, total sperm with intact acrosome (Prasad *et al.,* 1999) and individual fertility level (Jeyendran *et a*l., 1984).

The results of the present investigation showed highly significant correlation between HOS test score and progressive motility (%), sperm viability (%) and morphologically normal spermatozoa (%) both in Nili-Ravi buffalo and Sahiwal cow bull semen. This was in agreement with previous work on human (Jeyendran *et al*., 1984), equine (Mantovani *et al.*, 2002) and fresh goat spermatozoa (Fonseca *et al*., 2005) semen samples. Under the conditions of the present study, it was inferred that HOS test could be a valuable and practical tool to know the functional capacity of fresh Nili-Ravi buffalo and Sahiwal cow bull spermatozoa. It could be added in the routine analysis of semen samples for artificial insemination. However, the results of the present study are not supported by field fertility trials, so further studies are suggested to evaluate this parameter under field conditions.

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Table 1: Correlation between hypo osmotic swelling test and routine semen evaluation parameters in Nili-Ravi buffalo bull semen samples

Buffalo bull	No. of samples	Hypo osmotic positive sperm (%)	Percent motile	Percent live	Percent normal sperm
B4	10	84.6	81.5 (r=0.644)*	83.9 (r=0.881)*	83.9 (r=0.683)*
B6	10	85.9	80.0 (r=0.654)*	84.7 (r=0.880)*	84.0 (r=0.540)*
Mean	20	85.25	80.75 (r=0.649)*	84.3 (r=0.880)*	83.95 (r=0.611)x*

* Significance correlation ($P<0.05$) with HOS positive sperms.

Table 2: Correlation between hypo osmotic swelling test and routine semen evaluation parameters in Sahiwal cow bull semen samples

Sahiwal cow bull	No. of samples	Hypo osmotic positive sperm (%)	Percent motile	Percent live	Percent normal sperm
S2	10	84.9	81.0 (r=0.664)*	83.8 (r=0.91)*	86.9 (r=0.542)*
S3	10	86.6	80.0 (r=0.561)*	85.9 (r=0.895)*	85.6 (r=0.781)*
Mean	20	85.25	80.5 (r=0.612)*	84.85 (r=0.902)*	86.25 (r=0.661)*

* Significance correlation ($P<0.05$) with HOS positive sperms.

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