



## SHORT COMMUNICATION

### Association of Scrotal Circumference with Sperm Production and Semen Quality in Sahiwal Bulls

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#### ABSTRACT

The present study was aimed to investigate the association of scrotal circumference (SC) with sperm production and semen quality in Sahiwal bulls. Mature semen donor bulls (n=76), 3 to 5 years old, were divided into three groups according to SC: GI, GII, and GIII with 28-30, 31-35, and >35 cm, respectively. Semen was collected using artificial vagina once a week for five successive weeks. Positive correlations ( $P<0.05$ ) were observed between SC and mean ejaculate volume ( $r=0.2$ ) and percentage of live sperm ( $r=0.12$ ). Mean volume per ejaculate differed amongst the GI, GII, and GIII ( $4.3\pm 0.1$ ,  $5.0\pm 0.1$ ,  $5.7\pm 0.1$ ;  $P<0.05$ ). Sperm concentration and percentage of live sperm were greater ( $P<0.05$ ) in GII and GIII compared to GI. Scrotal circumference was not significantly correlated with sperm motility, membrane integrity, normal acrosome or morphology; no significant differences among groups were observed for these endpoints. In conclusion, greater SC in Sahiwal bulls was associated with greater production of live sperm.

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#### INTRODUCTION

Scrotal circumference (SC) measurement is an important feature of breeding soundness examination of bulls. This has long been used as an indicator for the estimation of the sperm producing ability and fertility of bulls. The semen volume, sperm concentration, motility and morphology are attributes having the positive association with the scrotal circumference of bulls (Chacon *et al.*, 1999). Although, the standards for breeding soundness examination of bulls "set by Society for Theriogenology in 1993" have been used in various dairy and beef breeds for many years, to categorize the bulls based on their breeding potential (Sylla *et al.*, 2007). However, these standards have great variation in their use at field level for AI sires selection. Because of the fact that every breed has its minimum acceptable SC size which is potential indicator of semen production ability. Therefore, it is imperative to study the association of SC with semen characteristics and variability that exists within the same breed in order to classify the bulls according to their breeding potential.

Sahiwal is well adaptive breed of tropical and subtropical region that is now being raised in different

parts of the world for enhancement of production through crossbreeding. The physical reproductive parameters of this breed have not been well studied. Relationship of age with semen quality in Sahiwal bulls has already been documented (Ahmad *et al.*, 2011; Bhakat *et al.*, 2011), however, relationship of SC with seminal attributes has not yet been reported. Therefore, this study was designed to evaluate the association of SC with sperm production and semen quality in Sahiwal bulls.

#### MATERIALS AND METHODS

Mature semen donor bulls (n=76), 3 to 5 years-old, resident at a semen processing center were used. Bulls were divided into three groups according to SC: GI, GII, and GIII with 28-30, 31-35, and >35 cm, respectively. The body weight of all bulls was measured before the start of study. The study was conducted during the spring season (February to March, 2009). Bulls were housed in optimal conditions and received 20 to 30 kg green fodder and 2 to 3 kg concentrate/day.

Semen was collected through artificial vagina once a week (two ejaculates per collection) for five consecutive weeks. Immediately after collection, the volume of each

ejaculate was recorded. Sperm motility was evaluated subjectively, whereas, sperm concentration ( $\times 10^6/\text{ml}$ ) was determined through hemocytometer. Live sperm and morphologic abnormalities were assessed by using eosin-nigrosin staining procedure as described previously (Ahmad *et al.*, 2011). One hundred sperm were counted for live/dead and morphologic abnormalities under bright field microscope at  $400\times$  and  $1000\times$  respectively.

Percentages of plasma membrane integrity and normal acrosome were assessed by using hypo-osmotic swelling assay and normal apical ridge (NAR) assay respectively, as reported earlier (Ahmad *et al.*, 2011). One hundred sperm were counted separately for their membrane integrity ( $400\times$ ) and normal acrosome ( $1000\times$ ) with phase contrast microscope.

**Statistical analysis:** The data were analyzed using Statistical Package software (SPSS version 17; SPSS Inc., Chicago, IL). One-way ANOVA was applied to compare the semen parameters in GI, GII and GIII. Tukey's test was applied to find the significant differences ( $P<0.05$ ) among the groups. The means and SEM were calculated. Association of SC with semen parameters was studied using Pearson correlation procedure.

## RESULTS AND DISCUSSION

Comparison of semen parameters among SC groups (GI, GII and GIII) in Sahiwal bulls is presented in Table 1. SC is positively correlated ( $P<0.05$ ) with mean ejaculate volume ( $r=0.2$ ) and percentage of live sperm ( $r=0.12$ ). Mean volume per ejaculate was different among GI, GII, and GIII significantly ( $P<0.05$ ). Sperm concentration and percentage of live sperm were greater ( $P<0.05$ ) in GII and GIII when compared to GI. Scrotal circumference was not significantly correlated with sperm motility, membrane integrity, normal acrosome or morphology; no significant differences among groups were observed for these endpoints.

**Table 1:** Comparison of body weight and semen parameters among scrotal circumference groups (Mean $\pm$ SE) in Sahiwal bulls

Parameters	Scrotal Circumference		
	28-30cm (n=12)	31-35cm (n=38)	>35cm (n=26)
Body weight (kg)	12.5 $\pm$ 18.5 <sup>a</sup>	504.1 $\pm$ 11.6 <sup>b</sup>	512.2 $\pm$ 8.9 <sup>c</sup>
Volume per ejaculate (ml)	4.3 $\pm$ 0.1 <sup>a</sup>	5.0 $\pm$ 0.1 <sup>b</sup>	5.7 $\pm$ 0.1 <sup>c</sup>
Motility (%)	67.7 $\pm$ 1.0 <sup>ab</sup>	67.7 $\pm$ 0.4 <sup>b</sup>	69.7 $\pm$ 0.5 <sup>a</sup>
Concentration ( $\times 10^6/\text{ml}$ )	1028.7 $\pm$ 50 <sup>a</sup>	1235.2 $\pm$ 49 <sup>b</sup>	1245.7 $\pm$ 55 <sup>b</sup>
Live sperm (%)	60.7 $\pm$ 1.5 <sup>a</sup>	66.3 $\pm$ 1.2 <sup>b</sup>	67.7 $\pm$ 1.5 <sup>b</sup>
PMI (%)	50.0 $\pm$ 2.3	52.1 $\pm$ 1.3	52.5 $\pm$ 1.5
NAR (%)	72.5 $\pm$ 1.7	73.1 $\pm$ 1.0	74.6 $\pm$ 1.1
Morphological abnormalities (%)	18.4 $\pm$ 1.4	19.0 $\pm$ 0.8	19.1 $\pm$ 0.8

Within the same row, the values with different superscripts are different,  $P<0.05$ . PMI=Plasma membrane Integrity, NAR=Normal Apical Ridge.

The present study is the first report which illustrates that greater SC in Sahiwal bulls is associated with more production of live sperm. This information can be used for the selection of Sahiwal/zebu bulls in general and in the many cross breeding programs where the Sahiwal is involved. SC is positively correlated with mean ejaculate volume and live sperm percentage. These results are in

agreement with earlier work in dairy and crossbred cattle (Devkota *et al.*, 2008; Latif *et al.*, 2009).

The association of SC with semen production and quality has also been reported in other species (Pant *et al.*, 2003; Okere *et al.*, 2011). These studies concluded that SC is a good marker of sperm output and the male with larger SC produce large numbers of live spermatozoa. Thus, sperm production is primarily estimated by the scrotal size; a figure which is most simply evaluated by measurement of SC in the live bull. However, age, breed, season and managemental practices do contribute the variation in the sperm production ability of bulls (Ha *et al.*, 2012).

The relationships of SC with plasma membrane integrity, and normal apical ridge and sperm defects were not positively associated in present study; however, variability in these parameters might be related to subfertility or infertility in bulls (Kastelic and Thundathil, 2008).

**Conclusion:** Greater scrotal circumference in Sahiwal bulls was associated with greater production of live sperm. However, determination of relationship of different scrotal circumference sizes and seminal attributes in a longitudinal study is required for better information to conduct the breeding soundness evaluation for artificial insemination sires.

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