RESEARCH ARTICLE

Assessment of Body Weight and Scrotal Circumference as Determinants of Semen Production Traits in Kankrej Bulls Using a Mixed Model Approach

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Abstract

The present study was conducted to evaluate the influence of body weight (BW) and scrotal circumference (SC) at the time of semen collection on semen production traits in Kankrej bulls. A total of 1307 ejaculates collected from 26 Kankrej bulls maintained at the Dama Semen Production Unit, Gujarat, were analyzed. The semen production traits studied included semen volume, sperm concentration, and number of semen doses per ejaculate. Data were analyzed using a linear mixed model with Restricted Maximum Likelihood (REML) method, considering BW and SC as fixed effects and bull as a random effect. The overall least squares means for semen volume, sperm concentration, and number of semen doses per ejaculate were estimated as 5.90 ± 0.30 mL, 1302.83 ± 48.66 million/mL, and 335.36 ± 20.58, respectively. BW at semen collection had a highly significant ($p<0.01$) effect on semen volume and sperm concentration, and a significant ($p<0.05$) effect on the number of semen doses per ejaculate. Semen volume and number of doses increased with increasing BW, with bulls weighing ≥700 kg producing superior performance. In contrast, sperm concentration decreased with increasing BW, likely due to dilution effects associated with increased seminal plasma. Scrotal circumference significantly ($p<0.01$) influenced semen volume and sperm concentration but had no significant effect on the number of semen doses per ejaculate. Bulls with larger SC generally exhibited improved semen volume and sperm concentration, although the relationship was not strictly linear. The findings indicate that BW is a more consistent predictor of semen production efficiency, whereas SC is more closely associated with semen quality traits. Incorporating these parameters in selection and management strategies may enhance reproductive efficiency in Kankrej breeding bulls.

Key words: Body weight, Kankrej bulls, Scrotal circumference, Semen traits.

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Introduction

Reproductive efficiency of breeding bulls plays a crucial role in the success of artificial insemination (AI) programs and genetic improvement in cattle and buffaloes. Semen production traits such as semen volume, sperm concentration, motility, morphology, and number of semen doses per ejaculate are important indicators of a bull's fertility and economic value. Evaluation of these traits is essential for selection of superior breeding bulls and for improving the efficiency of semen production systems. Several studies published have emphasized the multifactorial nature of semen production and quality traits. Physico-morphological characteristics and oxidative stress markers have been reported to significantly influence semen quality and fertility potential in Gir bulls (Prajapati *et al.,* 2022). Likewise, management practices such as nutritional supplementation and improved housing conditions have been shown to enhance semen quality traits like motility, viability, and morphology in crossbred bulls (Ali *et al.,* 2021). Comparative studies across breeds have further indicated that semen

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characteristics vary depending on genetic makeup and environmental conditions (Chaudhary *et al.,* 2017).

In addition to these factors, early reproductive traits such as body weight (BW) and scrotal circumference (SC) at first semen collection are considered important indicators of future reproductive performance. In this context, Dangar *et al.* (2021) reported that pubertal age, BW and SC at first semen collection in Kankrej bulls were not significantly influenced by season or year of birth, suggesting the adaptability of this breed under varying environmental conditions. Further, Dangar *et al.* (2022) observed that non-genetic factors had limited influence on BW and SC at the first semen collection as well as on semen production traits, indicating that these parameters may be relatively stable and could serve as reliable selection criteria. Studies on semen preservation and fertility have also demonstrated that physico-morphological traits are closely associated with semen freezability and reproductive performance in buffalo bulls (Vijyeta *et al.,* 2024). Advances in reproductive technologies, such as intracytoplasmic motile sperm selection, have further improved the assessment of semen quality and selection of superior spermatozoa in Kankrej bulls (Chaudhary *et al.,* 2025). Moreover, molecular studies have revealed that expression of sperm-specific genes related to energy metabolism pathways plays a significant role in determining fertility status in bulls (Ramya *et al.,* 2024).

Despite these advancements, semen production traits are influenced by a complex interaction of genetic, physiological, environmental, and management factors, and their relative importance may vary across breeds. Indigenous cattle breeds like Kankrej, known for their adaptability and genetic potential, require more focused investigation to understand the influence of physical traits such as BW and SC at the time of semen collection on semen production performance. Therefore, the present study was undertaken to evaluate the effect of BW and SC of Kankrej bulls at semen collection on semen production traits, with the hypothesis that the findings will be useful in formulating effective selection and management strategies for improving semen production efficiency in breeding bulls.

Materials and Methods

The DSPU, Dama is located in Gujarat state, India, in the outskirts of Deesa city (24.2585° N, 72.1907° E) and at an altitude of 144.58 m above sea level. The region experiences three different seasons, *viz.,* winter (November to February), summer (March to June), and monsoon (July to October) (https://www.accuweather.com).

Evaluation of semen production traits were carried out on Kankrej bulls of Dama Semen Production Unit (DSPU), Dama (A Grade semen station) managed by Banaskantha District Cooperative Milk Producers' Union Limited (Banas Dairy), Palanpur, Gujarat (India). Information on breed characteristics is available on the national portal of the NBAGR website (National Bureau of Animal Genetic Resources, 2021).

Data on semen production traits of 26 Kankrej cattle bulls born during year 2006 to 2015*, viz.,* bull number, date of birth, date of semen collection, ejaculation number, number of semen doses (per ejaculate), semen volume (mL), sperm concentration (million/mL), monthly body weight (kg) and scrotal circumference (cm) of bulls were collected. Semen production traits considered to study the effects of various non-genetic factors were semen volume, sperm concentration and number of semen doses per ejaculate for Kankrej cattle. Non-genetic factors affecting these traits were BW of bull at semen collection and SC of bull at semen collection. Collected data were used to estimate other traits like BW of bull at semen collection and SC of bull at semen collection.

The bulls were maintained under proper housing, feeding, management and health care. The young bulls were trained for semen collection using artificial vagina. The semen was collected twice a week from individual bull and ejaculates were obtained with an interval of 15 - 30 min. The nutrition requirement was standardized, so bulls were fed *ad-libitum* chaffed green and dry fodder mixture as per seasonal availability, concentrate mixture as per requirement based on BW with area specific mineral mixture.

The semen volume (mL) of the each ejaculate recorded using graduated collection tube to the nearest 0.1 mL by semen stations was utilized for the study. The semen station is using photometer (IMV, France) for estimation of sperm concentration per mL and per ejaculate (x 10^6/mL). To study the effect of BW and SC of bull on semen quality traits at particular semen collection, the bulls were grouped according to BW with interval of 100 kg, and SC with interval of 2 cm according to Society of Theriogenology (Table 1) with sizable number of animal in each group. BW and SC were measured routinely on monthly basis at the semen station. Semen collected in particular month was compared with BW and SC of bulls in that particular month.

Table 1: Grouping of Kankrej bulls as per their body weight and scrotal circumference at the time of semen collection

Code for BW of bull		Code for SC of bull	
BW (kg)	**Code for bulls**	**SC (cm)**	**Code for bulls**
<500	BWSC1	< 24	SCSC1
500-599	BWSC2	24-25	SCSC2
600-699	BWSC3	26-27	SCSC3
≥ 700	BWSC4	28-29	SCSC4
		30-31	SCSC5
		32-33	SCSC6
		34-35	SCSC7
		36-37	SCSC8
		38-39	SCSC9
		40-41	SCSC10
		≥ 42	SCSC11

Statistical Analysis

The effects of non-genetic factors were studied by multivariate analysis under mixed model and restricted maximum likelihood (REML) method considering all of them as fixed effect and bull as random effect. The data were analysed using SAS software version 9.3 and PROC MIXED as base command SAS (2011).

The differences between the least squares means for subclasses under a particular effect were tested by Scheffe test (Scheffe, 1959) to check the significance. The high heterogeneous variances between the subclasses, unequal group size, pairwise and unpairwise comparison lead to use of Scheffe test as other tests find differences between the least squares subclass means. The F-ratio used in the calculation is unique in that the mean square (MS) for only two groups being compared is used in the numerator and the MS for all respective comparison is used in the denominator. This means that each pairwise comparison has to have the same significance as the variance for all comparisons when using Scheffe test.

$$TS: Fs = \frac{(\bar{x}_i - \bar{x}_j)^2}{S_w^2\left(\frac{1}{n_i} + \frac{1}{n_j}\right)}$$

Where, TS = Test of significance, Fs = F for Scheffe, X_i = Mean of i^{th} group, X_j = Mean of j^{th} group, S_w = Within group variance, n_i = Total number of observation of i^{th} group, n_j =Total number of observation of j^{th} group.

Present mixed model is designed to estimate least squares means of semen production traits listed above for fixed effects of non-genetic factors, *i.e.* BW and SC of bull at semen collection.

$Y_{rijx} = \mu + R_r + S_i + T_j + e_{rijx}$

Where, Y_{rijx} = Semen production trait of x^{th} individual observation belonging to, r^{th} bull random effect, i^{th} body weight of bull at semen collection, j^{th} SC of bull at semen collection. μ = Overall mean, $R_r = r^{th}$ bull random effect (r = 1 to 26), S_i = Effect of i^{th} BW of bull at semen collection (i = 1 to 4), T_j = Effect of j^{th} scrotal circumference of bull at semen collection (j =1 to 11), e_{rijx} = Residual random error, NID (0 and σ^2_e).

Results and Discussion

The least squares means (LSMs) estimates for semen production traits, namely semen volume, sperm concentration, and number of semen doses per ejaculate are presented in Table 2, while the results of Type-III tests of fixed effects are summarized in Table 3.

Semen Volume

The overall LSM for semen volume recorded was 5.90 ± 0.30 mL. Both BW and SC of bulls at semen collection exerted a highly significant (p≤0.01) influence on semen volume. A clear positive association was observed between BW and semen volume, wherein semen volume per ejaculate increased progressively with increasing BW. Bulls weighing ≥700 kg produced the highest semen volume (6.64 ± 0.35 mL), whereas those in the 500-599 kg BW group exhibited the lowest semen volume (5.57 ± 0.30 mL). This trend may be attributed to enhanced physiological maturity, increased testicular mass, and greater secretory activity of accessory sex glands in heavier bulls, leading to increased seminal plasma output. The present findings are in agreement with those of Asad *et al.* (2004) and Gopinathan *et al.* (2018) indicating that BW and age are positively associated with semen volume due to improved reproductive organ development and endocrine function (Bhakat *et al.,* 2014). However, Asad *et al.* (2004) documented a contrasting trend, wherein bulls within the 400-600 kg BW range exhibited higher semen volume compared to those in the 600-700 kg group. Such discrepancies may arise due to differences in breed, nutritional status, management practices, environmental influences, and sample size.

BW at semen collection also had a highly significant (p≤0.01) effect on semen volume. Bulls with larger SC (40-41 cm) produced higher semen volume (6.71 ± 0.40 mL), whereas those with smaller SC (24-25 cm) exhibited lower semen volume (5.19 ± 0.40 mL). Despite this, the relationship did not follow a consistent linear pattern across all classes, suggesting that while SC reflects testicular development, semen volume is additionally influenced by accessory gland function and other physiological and environmental factors. These findings are consistent with earlier reports by Sudheer (2000), Sarder (2005), Gopinathan *et al.* (2018), and further corroborated by studies demonstrating a positive association between SC and semen production capacity (Brito *et al.,* 2004). The non-linear trend observed in the present study underscores the multifactorial nature of semen volume.

Sperm Concentration

The overall least squares mean (LSM) for sperm concentration was 1302.83 ± 48.66 million per mL. Both BW and SC at semen collection exhibited a highly significant (p≤0.01) effect on sperm concentration. Interestingly, sperm concentration demonstrated an inverse association with BW. Bulls weighing less than 500 kg produced the highest sperm concentration (1667.71 ± 138.23 million per mL), whereas bulls in higher weight categories (500 kg and above) showed comparatively lower but statistically similar values. This inverse relationship may be attributed to a dilution effect, wherein increased seminal plasma volume in heavier bulls leads to reduced sperm concentration despite potentially higher total sperm output. These findings are in accordance with those of Asad *et al.* (2004) and Gopinathan *et al.* (2018), and further supported by reports indicating that semen volume and sperm concentration often exhibits an inverse relationship due to dilutional effects (Bhakat *et al.,* 2014). However, Asad *et al.* (2004) observed lower sperm

concentration in bulls weighing 600-700 kg and higher concentration in those weighing 700-900 kg, indicating variability across studies.

Scrotal circumference also significantly influenced sperm concentration. The highest sperm concentration (1692.85 ± 110.58 million per mL) was recorded in bulls with SC of 26-27 cm, whereas the lowest (988.15 ± 116.19 million per mL) was observed in bulls with SC ≤24 cm. However, except for these extreme categories, differences among other SC groups were non-significant. This suggests that optimal testicular size contributes to efficient spermatogenesis, while excessively small SC may indicate underdeveloped testes and reduced sperm production. These observations are in agreement with the findings of Sarder (2005) and Gopinathan *et al.* (2018), and are further supported by Brito *et al.* (2004), who reported a strong association between SC and sperm production traits. In contrast, Sudheer (2000) reported a non-significant effect, indicating possible variation due to breed and management conditions.

Number of Semen Doses per Ejaculate

The overall LSM for the number of semen doses per ejaculate was 335.36 ± 20.58. BW at semen collection had a significant (p≤0.05) effect on this trait, whereas SC did not exert a significant influence. Higher numbers of semen doses per ejaculate were obtained from bulls weighing less than 500 kg and those weighing ≥600 kg, with values ranging from 333.41 ± 56.80 to 368.50 ± 27.04. In contrast, bulls in the 500-599 kg weight category produced the lowest number of doses (296.05 ± 20.89). This pattern may be explained by the combined influence of semen volume and sperm concentration, as semen dose production is a function of both these parameters. These findings are in agreement with Gopinathan *et al.* (2018) and further supported by reports suggesting that semen dose yield is primarily governed by total sperm output rather than individual semen quality parameters alone (Bhakat *et al.,* 2014).

BW at semen collection did not significantly influence the number of semen doses per ejaculate in the present study. This suggests that although SC is associated with semen quality traits such as sperm concentration, its direct influence on semen dose output is limited. However, Sarder (2005) and Gopinathan *et al.* (2018) reported a significant effect of BW on this trait, indicating that its influence may vary depending on genetic and environmental factors.

Table 2: Least-squares means of semen production traits for BW and BW (SC) of bull at semen collection in Kankrej bulls

Factors	n	Semen volume (mL)	Sperm concentration (millions per mL)	Number of semen doses per ejaculate
Overall	1307	5.9 ± 0.3	1302.83 ± 48.66	335.36 ± 20.58
BW at semen collection		**	**	*
BWSC1 (<500 kg)	16	4.99 ± 0.66^{ab}	1667.71 ± 138.23^{a}	333.41 ± 56.80^{ab}
BWSC2 (500-599 kg)	257	5.57 ± 0.30^{b}	1192.70 ± 49.56^{b}	296.05 ± 20.89^{b}
BWSC3 (600-699 kg)	498	6.39 ± 0.29^{a}	1169.41 ± 46.04^{b}	343.48 ± 19.50^{a}
BWSC4 (≥700 kg)	536	6.64 ± 0.35^{a}	1181.50 ± 65.07^{b}	368.50 ± 27.040^{ab}
SC at semen collection		**	**	NS
SCSC1 (<24 cm)	16	6.37 ± 0.52^{ab}	988.15 ± 116.19^{b}	296.68 ± 47.30
SCSC2 (24-25 cm)	9	6.38 ± 0.63^{ab}	1354.82 ± 145.05^{ab}	368.49 ± 58.95
SCSC3 (26-27 cm)	32	4.66 ± 0.61^{ab}	1692.85 ± 110.58^{a}	385.12 ± 46.48
SCSC4 (28-29 cm)	88	5.19 ± 0.40^{b}	1312.03 ± 75.80^{ab}	324.41 ± 31.53
SCSC5 (30-31 cm)	177	5.82 ± 0.38^{ab}	1283.85 ± 69.91^{ab}	336.12 ± 29.22
SCSC6 (32-33 cm)	232	5.45 ± 0.37^{b}	1405.19 ± 67.06^{ab}	339.14 ± 28.02
SCSC7 (34-35 cm)	49	5.79 ± 0.42^{ab}	1262.08 ± 83.74^{ab}	326.38 ± 34.53
SCSC8 (36-37 cm)	123	5.85 ± 0.39^{b}	1227.48 ± 71.16^{ab}	295.12 ± 29.72
SCSC9 (38-39 cm)	140	6.12 ± 0.41^{ab}	1301.80 ± 77.72^{ab}	327.73 ± 32.41
SCSC10 (40-41 cm)	398	6.71 ± 0.40^{a}	1286.30 ± 76.24^{ab}	363.23 ± 31.74
SCSC11 (≥42 cm)	43	6.53 ± 0.46^{ab}	1216.55 ± 93.99^{ab}	326.54 ± 38.69

**Highly significant (p<0.01), *Significant (p<0.05) and NS-Non-significant, n = Number of observations.

Means with at least one common superscript within classes do not differ significantly (p>0.05)

Table 3: Type-3 tests of BW and BW (SC) of bull at semen collection affecting semen production traits in Kankrej bulls

Source of variation	df	Semen production traits					
		Semen volume		Sperm concentration		Number of semen doses per ejaculate	
		F Value	P Value	F Value	P Value	F Value	P Value
BW at semen collection	3	9.26	<.0001	4.03	0.0072	3.49	0.0153
SC at semen collection	10	4.19	<.0001	4.28	<.0001	1.59	0.105
Error	1294	MSS		MSS		MSS	
		2.2542		142903		23187	

MSS = Mean sum of squares, df = Degree of freedom

Conclusions

The overall least squares means for semen volume, sperm concentration, and number of semen doses per ejaculate indicated satisfactory semen production performance of Kankrej bulls under the prevailing management conditions. BW at the time of semen collection emerged as a significant determinant of all semen production traits. An increase in BW was associated with a significant increase in semen volume and number of semen doses per ejaculate, whereas sperm concentration exhibited a declining trend. Scrotal circumference at semen collection significantly influenced semen volume and sperm concentration but did not have a significant effect on the number of semen doses per ejaculate. Larger SC was generally associated with improved semen volume and sperm concentration, although the relationship was not strictly linear. This non-linear association indicates that, while SC is a reliable indicator of testicular development and spermatogenic capacity, semen production is governed by multiple interacting physiological and environmental factors. Therefore, incorporation of BW along with BW in selection and management strategies may enhance semen production and improve the efficiency of artificial insemination programs in Kankrej bulls. The study highlights the importance of integrating physiological traits into breeding and management decisions to achieve optimal reproductive performance in indigenous cattle breeds.

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