Reproductive behaviour and semen characteristics of Binjharpuri bulls

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ANIMAL REPRODUCTION, GYNAECOLOGY AND OBSTETRICS

By

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CERTIFICATE-I

This is to certify that the thesis entitled **"Reproductive behaviour and semen characteristics of Binjharpuri bulls"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Veterinary Science (Animal Reproduction, Gynaecology and Obstetrics)** to the Odisha University of Agriculture and Technology is a faithful record of bonafide and original research work carried out by **Susanta Kumar Singh**, **Adm. No. 18192C08** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received by him from various sources during the course of investigation has been duly acknowledged.

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CERTIFICATE-II

This is to certify that the thesis entitled **"Reproductive behaviour and semen characteristics of Binjharpuri bulls"** submitted by **Susanta Kumar Singh**, **Adm. No. 18192C08** to the Odisha University of Agriculture and Technology, Bhubaneswar in partial fulfilment of the requirements for the degree of **Master of Veterinary Science (Animal Reproduction, Gynaecology and Obstetrics)** has been approved/ disapproved by the students' advisory committee and the external examiner.

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Place : Bhubaneswar

Date : **(Susanta Kumar Singh)**

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LIST OF ABBREVIATIONS

%	:	Percent
&	:	And
@	:	At the rate
^{0}C	:	Degree centigrade
A.I.	:	Artificial Insemination
ATP	:	Adenosine triphosphate
AV	:	Artificial vagina
avg	:	Average
CBJ	:	Cross bred Jersey
FSAI	:	Frozen semen artificial Insemination
HF	:	Holstein Friesian
HLB	:	High libido bulls
HOST	:	Hypo-Osmotic Swelling Test
i.e.	:	That is
JY	:	Jersey
LLB	:	Low libido bulls
M.S.P.	:	Minimum Standard Protocol
ml	:	Milliliter
mm	:	Millimeter
mOsm	:	Milliosmole
NRR	:	Non return rate
PBS	:	Phosphate buffered saline
ROS	:	Reactive Oxygen Species
SI	:	Service behaviour index
vs	:	Versus
ZP	:	Zona pellucida

ABSTRACT

The present investigation was carried out in the Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry (OUAT), Bhubaneswar in collaboration with Frozen Semen Bank, Cuttack under Fisheries & Animal Resources Development Department, Govt. of Odisha. A total of 192 ejaculates were collected from healthy six Binjharpuri bulls by artificial vagina method. These ejaculates were evaluated, processed and cryopreserved in liquid nitrogen (LN_2) with Tris-Egg yolk-Citrate-glycerol extenders. Routine behavioural parameters like Approach to test site, Eagerness to approach stimulus, Approach to dummy, Sniffing and licking, Flehman's reaction, Libido, Protrusion of penis, Mounting, Copulatory thrust and ejaculation, Reaction time & seminal parameters such as volume, pre-freeze motility, mass activity, livability, sperm concentration and sperm abnormality were recorded whereas, post-thaw motility evaluation was done after 24 hours of cryopreservation of collected semen samples. The service behaviour index was calculated as the mean of 10 parameters related to sexual behaviour. Statistical analysis of the data obtained during the present course of investigation was carried out as per one way analysis of variance of the ANOVA procedure and using the software IBM SPSS 25.0. The routine semen parameter evaluation of all the six Binjharpuri bulls yielded no significant difference among them so far as volume (ml) and sperm concentration (millions per ml), mass activity, pre-freeze motility, post thaw motility, livability and sperm abnormality were concerned.

The following are the overall mean of different parameters of Binjharpuri breeding bulls. This is the first study of this kind, which might be helpful to evaluate the breeding bulls in future.

Service Behaviour Index (1-5)	:	2.82±0.03
Semen Volume (ml)	:	3.76±0.09
Semen Concentration (million/ ml)	:	888.71±36.05
Mass Activity (0-4)	:	2.74±0.07
Livability (%)	:	75.21±0.47
Pre-freeze motility (%)	:	77.66±1.67
Post-thaw Motility (%)	:	44.04±1.37
Sperm Abnormality (%)	:	8.76±0.14

INTRODUCTION

Binjharpuri commonly called as ‘Deshi’, a breed of cattle, is mostly found in Jajpur, Kendrapara and Bhadrak districts of Odisha. Their population is about 110553 (2016, NDDB). It is named after the local area "Binjharpur" of Jajpur district in Odisha. They are maintained for milk production, draught as well as manure production. Animals of this breed are chiefly white in colour and occasionally grey, black and some brown. Their milk yield ranges from 915-1350 kg per lactation with milk fat per cent ranging from 4.3-4.4. Govt. of Odisha has decided to induct the breed to the Frozen Semen Bank, Cuttack for semen collection for artificial insemination. Binjharpuri breed is very much adaptable to our local environment. Binjharpuri cow has already been registered as 33rd cattle breed in India. Potentiality of Binjharpuri cattle in terms of milk production under semi-intensive management, disease resistance, fertility and draftability of bullocks, pushed it to be placed under programme for conservation of indigenous germplasm. The demand for elite indigenous animals is getting very high in India over the recent years owing to their milk quality, adaptability to harsh environment as well as their survivability on available poor quality type of feeds and fodders. The Odisha breeding policy 2015 puts thrust on conservation and improvement of native germplasm in the state. Moreover, Binjharpuri breed will be used for genetic up gradation programme as Haryana will be slowly eliminated by replacing with Tharparkar and Binjharpuri. Under national project on bovine breeding, procurement of Binjharpuri bulls for AI in cattle was planned. As per the Minimum Standard Protocols of Govt of India, bull that is to be used at sperm station, need to undergo a series of screening process. A detailed study has already been made about the fertility and production status of Binjharpuri cows (Dash and Sethi, 2008). However, no such effort has been made to have an extensive study about the male fertility traits of a Binjharpuri bull, which is absolutely needed for selection of bull calves for adopting selective breeding.

The indigenous breeds of cow are an integral part of traditional agriculture. However they are progressively diluted due to the interventions of crossbreeding programmes and modern mechanization of agriculture in India. Indigenous cattle contribute approximately 50 per cent of total milk production in India and are also able to withstand extreme weather condition (FAO, 2012). The demand for frozen

semen from the elite indigenous bulls is increasing throughout the country due to their milk productivity, adaptability, heat tolerance, disease resistance and survivability on available poor feeds and fodders. Breeding soundness evaluation (BSE) of the bulls is very important to achieve the target of producing quality frozen semen with better fertility. For this, the relationship between the sexual behaviour and the quality of semen of the breeding bulls should be studied. Sexual behaviour traits reflect the reproductive efficiency of the breeding bulls and are of paramount importance in a breeding program. Libido characteristic of bulls is a heritable trait. Aggressive and efficient breeder bulls are more likely to have offspring those also good breeders. Sexual behaviour is a very complex phenomenon, controlled by the endocrine constitution of an animal, influenced by the social environment, sensory capacities and sexual stimuli (Naskar and Nagpaul, 2005). Joshi and Kharche (1992) reported that the intensity of sexual expression not only reflects the sex drive (libido) but also the seminal attributes of bull. Also, sexual behaviour stimulation could increase spermatozoa production (Sholikah *et al.,* 2018).

Among different advancements in reproductive biotechnologies, Artificial Insemination has made a profound contribution in genetic improvement of the dairy animals. By this technique, a single ejaculate from the male is used to impregnate multiple number of females. Cryopreservation of semen further adds a beneficial role in adopting this technology. These techniques have been used commercially in dairy industry. Semen freezing process is a time consuming, laborious and costly venture, requiring lots of inputs. A large number of sperms are rendered incapable to fertilize the ovum following freezing and thawing. Besides, rejection of frozen semen straws for poor post thaw quality and freezability results in wastage of all efforts. The freezability of semen varies between species, breeds and among individual males within the same breed. Genetic diversity presents a natural barrier to the spread of diseases. This is of course, the opposite of the current trend towards uniformity and genetic monocultures in much of the livestock sector, with respect especially to poultry and pigs, but also dairy cattle.

Promoting robust local breeds is the antidote to this risk and the measure we need to take to lessen risks. Apart from being genetically less homogeneous, these animal genetic resources have been selected over many generations for what scientists

call ‘fitness traits’ and are less likely to succumb to diseases. Such animals have the added advantage of being able to cope with fluctuating feed supplies, and withstand some lack of feed by down-grading their metabolic rate. These populations need to be preferred, taken very seriously at the highest level by policy makers with lion’s share of investment for protecting the requirement of future generations. A lot of individual variability exists in these populations and superiority in each economic trait needs to be explored to enhance productivity. Sustainable use of these animals will lead to conservation. That is why we have to intensify conservation of local breeds like Binjharpuri in its native tract, so that our goal can be achieved. In this competitive growing market, rearing or conserving a native breed, is really a difficult task. There must be such intensive support systems for conservators or else soon they will perish.

Looking to the above need a novel attempt has been made with the following objectives.

- To study the sexual behavior in Binjharpuri breeding bulls.
- To study the spermiogram after collection, processing and evaluation of neat and frozen thawed semen samples of Binjharpuri breeding bulls.

REVIEW OF LITERATURE

The breeding soundness evaluation (BSE) of bulls has been a very cost effective procedure in veterinary field providing benefits like risk reduction and improvement in the intensive usage of bulls, enhanced herd fertility and farmer's economy. When performed appropriately, semen evaluation is an important component of the breeding soundness evaluation. Proficient physical/ reproductive examination, including measurement of scrotal circumference and evaluation of semen contribute greatly to the fertility and economics of the individual herds, adding the importance of understanding those factors affecting cow fertility. In spite of so many advantages, there exist challenges in achieving full acceptance of Bull Breeding Soundness Evaluation (BBSE), particularly by the dairy industry in the developing countries.

In dairy sector, to improve the performance of the offspring, insertion of superior quality breeding bulls at semen collection centres is need of the hour. Good quality semen production not only reduces the post-thaw rejection of semen straws, but also improves conception rate of the herds following insemination. Therefore, study of service behaviour of breeding jersey bulls has been focussed along with the estimation of their concurrent relationships with the quality and fertility of the semen.

2.1 Service behaviour of the bull

Bishop *et al*. (1954) reported no relationship found between the palpable characteristics of the genital organs or the libido of the bulls with their fertility.

As analyzed by Fraser (1960) in a group of 102 bulls, 50% of the bulls had a reaction time of approximately 2 minutes, while 12% of them did not react-even up to one hour. Therefore he suggested that bulls may be selected on the basis of their reaction time.

Kerruish (1955) observed a significant improvement in sexual behaviour and a rise in conception rate by 8.7 % when ten bulls were placed for five months on regimes of intensive sexual stimulation prior to semen collection.

Crombach *et al*. (1956) used identical twin bulls and showed that if one of the twin bulls was allowed a false mount following restraint for 5 min, the number of active sperms in the bull was doubled as compared to that of the non-restrained twin bull.

As reported by Hale and Almquist (1960), the recovery from sexual satiation to a particular stimulus varied from individual to individual and those who quickly recovered showed a short reaction time.

Watson (1964) gave opinion that sexual desire or libido in the male domestic animals is largely inherited but could be modified widely by the environmental influence.

Tomar *et al.* (1966) reported that in the Haryana bulls, reaction time varied significantly between months.

Roberts (1971) reported a positive correlation of optimum sexual desire with different seminal attributes and fertility of all domestic ruminants. Irrespective of delay involved in allowing bulls to mount without ejaculation resulted in significant improvement in different semen characters being greater after two mounts than that after one mount (Signoret, 1962; Almquist, 1973).

Blockey (1978) recorded a higher first cycle pregnancy rates in heifers, mated with bulls having higher serving capacity, compared to those mated with lower serving capacity bulls.

Chenoweth *et al*. (1979) found that out of three methods of assessing sex drive in yearling beef bulls like service capacity score, libido score and reaction time to the first service, the libido scoring pattern was the most credible. Whereas, the LH and testosterone values were not significantly correlated or were poorly correlated with sex drive measurements.

Smith *et al.* (1981) observed that in bulls the libido assessment provides greater prediction of bull fertility than did semen assessment alone.

Chenoweth (1981) reported that proper assessment of factors such as libido and mating ability before breeding can greatly reduce the possibility of poor

reproductive performance from single sire. He also opined that in eliciting male sexual response ,visual cues are more effective compared to olfactory cues.

Blockey (1981) opined that allowing the bulls to watch their counter-parts engaged in copulatory act enhanced their sexual performance.

Chenoweth (1983) recorded that characteristics like motility and survival rate of spermatozoa as well as conception rate are influenced positively by the precoital stimulation.

Bikner *et al*. (1983) found that the conception rate for the bulls with high libido was 51.4% whereas that of bulls with low libido was 30.6% ($P<0.01$).

Crichton and Lishman (1985) opined that it was sexual experience rather than the age that determined the mating activity of the bulls.

Bhosrekar *et al.* (1988) reported no significant difference between breeds for different components of the service behaviour. However, they showed seasonal variation significantly for reaction time. Use of bulls with greater libido has been shown to benefit pregnancy rates, time of conception, length of calving seasons, homogenity of calves at weaning and more efficient use of personnel (Blockey *et al*., 1978, 1989; Godfrey and Lunstra, 1989).

Libido is a response to endogenous or exogenous stimuli mediated through a variety of physiological mechanism, previous sexual exposure and motivation (Bryant, 1989). Pathak *et al*. (1990) evaluating the libido and comparing with semen characters in young (3-5 years) and old (9-12 years) crossbred bulls observed that libido was long standing in young bulls but old bulls had a greater ($P<0.01$) semen volume and lower ($P<0.01$) sperm concentration than young bulls. The overall average reaction time was 289 ± 31.20 Sec.

Amann (1990) opined that absence of adequate sexual preparation prior to semen collection reduces the libido and courtship behaviour with concurrent deficiency in vigour of the ejaculatory reflex Whereas, sexual preparation will increase the number of sperm ejaculated. Anzar *et al*. (1993) found that sexual behaviour had no relationship with fertility rate of buffalo bulls ($r = 0.42$, $P>0.05$) and sexual behaviour index can be used for selection of buffalo breeding bulls.

Prajapati (1995) observed that temperament of the bulls was less affected phenotypically and usually governed by the genetic makeup of an animal. It was also marked that various service behaviour indices like temperament, libido, erection, protrusion and intensity of thrust improved significantly in bulls undergoing regular exercise. Narasimha Rao *et al*. (1996) found that flehman's reflex was exhibited more frequently ($P<0.01$) by Ongole than Murrah, Jersey x Ongole cross and jersey bulls in descending sequence. They also found the desire for copulation was very strong ($P<0.01$) for Jersey bulls followed by Murrah and Jersey x Ongole cross. The frequency of weak desire was significantly higher ($P<0.01$) among Jersey x Ongole cross bulls compared to pure Jersey bulls. Similarly, the copulatory thrust score was higher for young bulls and was very good to excellent for Jersey bulls followed by others.

Chenoweth (1997) opined that bull sex drive or libido is a measurable trait, which is mostly genetically inherited. It also represents an important aspect of bull reproductive performance, with positive effects on herd pregnancy rates. Purohit *et al*. (1998) found libido score was significantly positively correlated ($P<0.5$) with semen volume in Surti buffaloes and all other correlations between sexual behaviour and semen quality parameters were not significant. Silva Mena *et al*. (2000) explored a positive relationship between bull sex drive and reproductive performance in BOS indicus bulls in Mexico. Chandler *et al*. (2003) stated that a shorter reaction time yielded better quality semen, being significant only for volume and individual motility. Further they suggested that stronger intensity of thrust produced better quality semen compared to visual quantification of libido scoring in breeding bulls.

Mandal and Tyagi (2004) found significant ($P<0.01$) difference in reaction time among adult and young Sahiwal bulls. Seasonal variation showed significant ($P<0.05$) effect on reaction time of young and was non significant in adult bulls. They observed significant ($P<0.05$) influence of season on penile erection of both young and adult Sahiwal bulls. Further, significant ($P<0.01$) difference in intensity of thrust between young and adult bulls was also noticed. The values for different pre-copulatory service behaviour in Sahiwal bulls was also reported such as chin resting on back of dummy ($11.40 \pm 3.37\%$),

frequency of flehman's reaction (25.95 ± 9.37%), smelling the hind part of dummy (15.43 ± 3.69%), dribbling from prepuce (100 ± 0.0%), licking of genetalia (1.23 ± 0.67%) and raising of tail (7.26 ± 4.66%).

Parkinson (2004) found that the potential fertility of bulls can be evaluated by assessment of mating ability and physical examination. Both methods are useful for screening out low fertility bulls. Togun *et al*. (2006) opined that the efficiency of sperm production, libido and quality of sperm tend to remain uniform throughout the reproductive life but may be significantly altered by aging, nutrition, environment, health status, drugs and chemicals.

Galina *et al*. (2007) observed that screening and selecting bulls for desirable reproductive traits and high libido is known to improve reproductive performance of the herd. Mandal *et al*. (2008) observed that flehman's reaction was not significantly related with good sexual behaviour as only 36% of bulls showed flehman's reflex during courtship. Mandal *et al*. (2008) found that young bulls producing freezable quality semen had higher libido score and greater intensity of thrust as compared to that producing non-freezable quality semen. Elrabie *et al*. (2008) found that libido score, temperament score and semen volume (ml) were significantly ($P<0.01$) higher for group of bulls having rubber mattress collection floor, where as concrete floor showed significantly ($P<0.01$) higher values of erection score, protrusion score and intensity of thrust than brick clay and rubber mattress floors.

Evelin *et al.* (2009) observed that behavioural alterations and slipping are less frequent in bulls kept on rubber mats than in bulls on slatted floors. Bulls in straw bedded pens showed hardly any behavioural alterations and no slipping. This indicates that rubber mats improve the situation of fattening bulls in an animal welfare point of view, but do not reach the softness of straw bedding. Bulls which have been moved from straw into slatted floor pens at an average live weight of 450 kg very quickly developed the same behavioural alterations as bulls housed in slatted floor pens during the whole fattening period. However, the extended rearing period on straw can be regarded as an improvement of the bulls' situation. Therefore, this alternative is to prefer to the conventional slatted floor system.

Mishra *et al.* (2011) stated that service behaviour index analysis might be a simple, economic and dependable method for screening of breeding bulls which has shown a linear relevancy with semen quality and conception rate that can suitably be employed in frozen semen stations.

Singh *et al.* (2015) opined that the correlation of various sexual behavior and semen quality parameters reflected the importance of sexual behavior in predicting the quality of semen and their future utility. Islam *et al.* (2018) opined that libido, semen quality and fertility are linked together and taken into consideration during breeding bull selection for the higher fertility in AI program. Chikhaliya *et al*. (2018) opined that semen characteristics, freezability and semen production efficiency of Gir bulls are comparable to other breeds of cattle.

2.2 Semen analysis

Improving the quality of frozen semen, minimizing the post thaw discard rate and increasing conception rate have been tried by several workers by screening different semen samples through routine semen evaluation, post-thaw semen evaluation.

2.3 Routine semen evaluation

2.3.1 Volume

Increase or decrease in volume of semen is not usually correlated with fertility or sterility in a male unless ejaculation fails to occur (Roberts, 1971). The ejaculated volume significantly increased with age (Abdel-Raouf, 1965; Colchen-Bourlaoud, 1973; Rao and Rao, 1979) and the same was increased linearly with age along with motile spermatozoa and sperm concentration, which was correlated with total sperm count (Almquist and Cunningham, 1967; Colchen-Bourlaoud and Thibier, 1973). A correlation between total number of sperm and semen volume was also reported by Nishiyama *et al*. (1968).

The overall mean seminal volume(ml), mass activity (0-4), individual motility (%), live sperm (%), sperm concentration (million/ml), normal sperm (%) and conception rate (%) are 2.42 ± 0.22, 2.77 ± 0.09, 57.48 ± 1.83, 86.51

± 0.81, 1316 ± 44, 87.30 ± 1.64 and 35.53 ± 2.16 respectively in Crossbred Jersey bulls (Bedi *et al*., 1984). The average volume for 62.5% JY bulls was significantly higher than other genetic groups except 75% HF bulls (Sagdeo *et al*., 1990). The pooled over breed groups mean volume was 4.11 ± 0.02ml, in monsoon, 3.62 ± 0.03ml in summer and 3.18 ± 0.02 ml in winter season. Individual breed wise mean also indicate that maximum volume is in monsoon followed by summer with minimum volume in for all breeds in winter (Sadgeo *et al*., 1991).

The mean ejaculated volume (double thrust) recorded in Gir bulls (7.03 ± 0.44 ml) did not differ significantly from that in Jaffarabady bulls (6.36 ± 0.33ml) (Raina and Dhami, 2004). It has been reported that the average volume(ml), pH ,mass activity, percent motility, concentration (million /ml), live sperm %, sperm head, mid piece, tail and total abnormalities (%) of neat semen were 1.82 ± 0.16, 6.51± 0.46, 3.24 ± 0.14, 72.92 ± 2.70, 1335.42 ± 58.36, 85.01 ± 2.21, 1.35 ± 0.21, 1.78 ± 0.28 3.10 ± 0.55 and 6.44 ± 0.16 respectively. The acrosome reaction was 48.08 ± 1.39 % indicates its fitness (Pandey and Gupta, 2004).

The effect of season on volume, sperm motility and percent live spermatozoa is reported to be non significant to half bred and triple cross Gir bulls (Bhoite *et al*., 2008). Volume of Friesian Gir cross bred (5.278 ± 0.056) was significantl higher than JY Friesian cross bred (IFJG) (4.473 ± 0.083) and Brown Swiss Fresians Gir cross bred (IBFG) in triple cross breeds (Bhoite *et al*., 2008).

Volume (ml), colour, concentration ($x10^6$/ml), mass activity, motility (%), normal acrosome, mid-piece, tail (%) and normal head (%) were 4.5 ± 0.4, 3.5 ± 0.3, 1410.0 ± 70.0, 3.8 ± 0.3, 77.5 ± 1.5, 93.5 ± 0.5 and 93.5 ± 1.0 respectively in fresh semen of HF Crossbred bull (Munsi *et al*., 2007).

Ejaculate volume (ml), sperm motility (%), concentration ($X10^6$/ml), total abnormality (%), total viability, HOST and pH were 5.03 ± 0.2, 83.5 ± 2.4, 1480.0 ± 70.0, 5.40 ± 0.6, 82.30 ±2.8, 83.80 ± 3.3 and 6.59± 0.0 respectively in fresh semen of bull (Uysal *et al*., 2007). Ejaculate volume (ml), Mass activity

(0–5 scale), Initial motility (%), Sperm concentration (million/ml) and Sperm concentration per ejaculate (million) were 3.81±0.03, 2.36±0.02, 56.18±0.001, 792.36±6.09, 3175.38±33.18 respectively in Sahiwal Bulls (Bhakat *et al*., 2011). Semen volume (ml), Semen pH, Sperm concentration ($x10^9$), Total sperm count ($x10^9$), Sperm motility of fresh semen (%), Total motile sperm count ($x10^9$), Sperm motilit before-freezing (%), Sperm motility post-thawing (%) were 6.46 ± 0.13, 6.3 ± 0.01, 1.29 ± 0.02, 8.35 ± 0.24, 70.4 ± 0.15, 5.88 ± 0.17, 55.7 ± 0.21, 40.9 ± 0.21 respectively in Ongole bull (Isnaini *et al.*, 2019).

2.3.2 Mass activity

Motility below 50% was often associated with low conception rates and poor fertility (Blom, 1950). The initial motility was more closely related with semen characteristics and fertility (Bishop *et al*., 1954 and Erb *et al*., 1956). Mass activity is evaluated as a routine test carried out to assess semen quality done immediately after collection. Wave motion is the outcome of oriented sperm cell movement, which come into phases and it can be observed only in high density specimens such as ram or bull semen, but hardly in human, rabbit or other animals, whose semen is less dense (Mann and Lutwak-Mann, 1981).

Gross motility of spermatozoa reflects the combined effect of sperm cell concentration and the viability of sperm cells (ZemJanis, 1970). Mass activity is most subjective test and can vary from individual to individual (Bhosrekar, 1990). Average mass activity of Pure JY, 75% JY, 62.5% JY,50% JY, 75%HF and 62.5% HF was 3.04 ± 0.03, 2.07 ± 0.12, 2.30 ± 0.15, 1.93 ± 0.06, 1.77 ± 0.19 and 2.27 ±0.08 respectively and 50% and 75% JY bulls have showed similar values. 62.5% JY and HF bulls are also similar in this respect but were slightly superior to 75% HF bulls (Sagdeo *et al*., 1990). The mean mass activity score and percent individual sperm motility were significantly ($p<0.05$) higher in Gir bulls (3.33 ± 0.11 and 7.50 ± 0.89) than Jaffarabady buffalo semen (2.80 ±0.06 and 66.75 ±1.04) (Raina and Dhami, 2004).

The mass activity of sperms is considered as viability characteristic and is determined through microscope by low magnification. The mean numerical value for mass activity of the sperm collected in June, 2013 was 2.49, 3.83 and 2.16 respectively (Kiani *et al*., 2014). The mean mass activity of semen (0-5 scale)

estimated in the Hallikar cattle (considered as one of the premier draught breed of India) was 4.32 ± 0.01 with range of 0.50 to 4.50 (Prem Kumar *et al*., 2020).

2.3.3 Individual motility

Bovine semen should contain a minimum of 40 to 50% motile sperm, as low motility was associated with infertility (Blom, 1950). While, Branton *et al.* (1951) opined a minimum initial sperm motility of 70% required for efficient breeding policy. Although the motility of fertile bulls was higher than infertile bulls, the decrease in motility did not parallel to the decrease in fertility (Rollinson, 1951).

Blom (1950) and Bishop *et al.* (1954) reported that, the initial motility has a positive correlation with sperm concentration and fertility. Again Lasley (1951) found a highly significant correlation among percentage of live motile and progressively motile spermatozoa in fresh semen.

Abdel–Raouf (1965), Almquist and Cunnigham (1967) found increase in percentage of motile spermatozoa with advancement of age. However, the effect of season on initial motility was demonstrated by Tripathy and Prabhu (1968),who could not found significant effect of season. However, Dojcseva *et al.* (1979) reported significant decrease in motile sperm.

The average motility of pure bred and cross bred bull was 82.84 ± 0.77 and 75.26 ± 2.57% respectively. Live sperm and sperm concentration millions/ml were 84.37±0.81, 73.63±2.5%, 1003.92±50.79 and 1176.31±16.13 (Barik *et al*., 1987). The initial motility of Pure JY, 75% JY, 62.5% JY, 62.5% HF and 75% HF are 72.14±0.65%, 47.50±4.62%, 52.07±1.77%, 46.69±2.23% and 30.24 ± 2.24%, respectively (Sagdeo *et al*., 1990). Mean value for initial and pre-freeze motility were significantly ($p< 0.05$) higher in cattle (81.67± 1.28 % and 72.87 ± 1.47 %) than in buffalo (76.94±1.81 % and 67.76±2.03%) semen. Post thaw motility did not vary between them (45.23 ±1.71 % Vs 44.49 ±1.99 %) (Dhami *et al*., 1991).

Similarly, the initial motility in 75% cross bred bull was 67.77% (Chauhan *et al*., 1983). However, good initial motility alone is not an accurate indicator of fertility in bulls (Swanson and Herman, 1994). The velocity of sperm is

an indirect measure of the force of movement. Penetration of cumulous oophorus does not depend upon the degree of progressiveness of movement but it does require reasonable velocity. A small reduction in velocity may affect sperm penetration of zona pellucida (Olds – Clarke, 1996).

Baumber *et al*. (2000) had reported that sperm motility may be a more sensitive indicator of oxidative stress and is therefore inhibited before any measurable increase in lipid peroxidation and the sperm motility may be affected by ROS through a mechanism of action separate to that of lipid peroxidation. In somatic cell systems, it has been proposed that H_2O_2 causes perturbations in important biochemical functions, including increased formation of oxidized intracellular sulfa-hydryls, rapid decrease in ATP levels and a consequent depression of glycolytic flux.

It has been proposed that sperm immobilization was due to a decreased phosphorylation of axonemal proteins required for sperm movement and also reported that ROS inhibited one or more enzymes of oxidative phosphorylation, glycolysis, or both, thus limiting ATP generation by the sperm cell (Baumber *et al*., 2000).

Sperm motility is correlated with sperm abnormalities and membrane integrity. Live sperm percentage is the only parameter correlated with fertility. Motility and live sperm percentage could be used as a predicative measure for fertility in semen analysis of buffalo (Mahmoud *et al*., 2013).

The individual motility of Kankrej bull semen ranged between 80 to 92 per cent with the mean values of 86.15 + 0.30 per cent. Individual motility of spermatozoa in present study was at par with reports of HF bulls, Sahiwal and Jersey bulls (Patel *et al*., 2013).

Pure breed bull showed significantly high values of individual motility than cross bred bull. We can define individual motility percentage as, spermatozoa with any movement in microscopic field called sperm individual motility (Khan *et al.,* 2017).

2.3.4 Sperm concentration

Lagerlof (1934) reported that, the sperm concentration in fertile bulls varied from $30X10^6$ to $200X10^6$ per mm^3. Haq (1949) found that, spermatozoan density remained within normal limits in many bulls with testicular degeneration. However, ininfertile or sterile bulls, there was a rapid decline in sperm concentration among first, second and third ejaculates indicting a poor reserve with reduced sperm production (Rollinson, 1951). Branton *et al*. (1951) reported that sperm concentration of at least 900 million per mm^3 is required for efficient breeding. A proportionate increase in sperm concentration with advancement of age was reported by Colchen – Bourlaud and Thibier (1973) along with Rao and Rao (1978). On contrary, Schluter (1975) observed no significant effect of age on sperm concentration.

Nishiyama *et al*. (1968) found a correlation between sperm concentration and environmental temperature. Similarly, Porwel *et al*. (1977) reported highly significant effect of season, while Dojcseva *et al*. (1979) described a decrease in sperm concentration during winter season. Sperm concentration per ml is one of the most important traits of semen quality for acceptance in frozen semen production. The average sperm concentration in Gir and Jaffarabady bulls semen was 2608 ± 53 and 1384 ± 40 millions, respectively (Raina and Dhami, 2004).

Haugan *et al.* (2007) found that the natural range of sperm concentration at collection within some of the individuals was associated with reproductive performance. However, the most favorable sperm concentration at semen collection differed among bulls and no primary effect (when adjusted for bull) on calving rate was found. Further, the inherent fertility potential of individual sires was not mirrored by the average sperm concentration in their ejaculates.

An insignificant correlation was observed between the sperm concentration in the ejaculate and the morphological traits, dimensions and shapes of bull spermatozoa. The less concentrated ejaculates contained spermatozoa with a slightly larger head circumference and a more elongate head shape in comparison with the spermatozoa in the more concentrated ejaculates. The highest frequency of morphologically malformed spermatozoa, both in the case of primary and secondary

alterations, was observed in ejaculates with a sperm concentration of no more than 1000 x 103/ mm3 (Kondracki *et al*., 2012). Morrell *et al*. (2018) found that the mean concentration (±SD) was $88\pm20\times10^6$ /ml and $55 \pm 19\times10^6$ /ml for beef and dairy bull semen, respectively ($P < 0.001$).

2.3.5 Live spermatozoa count

A Staining Mixtures for Differentiating Live and Dead Spermatozoa have been proposed since the original report of this technique by Lasley *et al.* (1942). Since the background stain (opal blue) used in their method was not available during the war, mixtures using fast green were reported satisfactory by Mayer *et al*.(1947).

A different staining method for differentiating live and dead bull spermatozoa was proposed by Blom (1950) using eosin as the sperm staining substance and nigrosin as the background stain. This mixture gave a very uniform preparation with stained (dead) cells clearly distinguishable from the live or unstained cells. The nigrosin provides a smoother background stain than fast green, opal blue, or aniline blue. It seems to obscure the sperm less and mixes with seminal plasma or diluter more uniformly. For this reason it can be used as easily with egg-yolk diluted-semen as with semen not mixed with egg yolk.

The proper staining of dead sperm was found by Mayer *et al*. (1951) depended upon careful control of pH, osmotic pressure, stain concentration and ionizable salts. Bishop *et al*. (1954) described that variation in the metabolic activity of the semen cause variation in concentration of live spermatozoa and incidence of dead spermatozoa. The proportions of live and dead spermatozoa are varying between ejaculates and bulls. Moreover, semen with 30% of dead spermatozoa reduced fertility in cattle (Bonnadonna and Kann, 1955). Colchen–Bourlaoud and Thibier (1973) observed that percentage of dead spermatozoa decreased with increase in age.

Mohanty (1981) observed that increase in dead spermatozoa with advancement of age was attributed to impairment of epididymal function.

The proportion of dead cells was 18% in the fresh semen and increased to 46% after freezing-thawing (Chen *et al*., 1989). The mean percentages of live and

dead sperms found in fresh semen of Gir bulls were 71.85±1.49 and 22.50±1.40, respectively and the corresponding values in Jaffarabady buffalo bulls were 77.90 ± 2.08 and 22.30 ± 1.57. The mean live sperm percentage differed significantly between them (Raina and Dhami, 2004). Overall mean value of semen volume (ml), concentration ($X10^6$/ml), mass activity (0-5 scale), initial motility (%), live spermatozoa (%), tail abnormality (%) and mid piece abnormality(%), abnormal tail (%) and total abnormal sperm(%) were 3.15±0.13, 1335.25±51.00, 2.66±0.007, 52.7±1.31, 70.40±3.91, 4.50±1.37, 6.80±1.82, 7.10±1.26 and 18.40±3.03, respectively in Sahiwal bulls (Mandal *et al*., 2005).

Mathur *et al*. (1991) who recorded the abnormality in semen of crossbred bull is 12.16%. Singh *et al*. (1997) recorded overall sperm abnormality is 2.03% to 3.90% in crossbred semen. In adult bulls of Sahiwal breed, the semen volume, mass activity, initial motility and post thaw motility were 125.37, 10.63, 28.77 and 12.86 % higher as compared to young bulls. Initial and post thawvmotility were highest during winter season (Mandal *et al*., 2005). Live spermatozoa (%) observed in IBFG (78.58 ± 0.288) was significantly higher than IFG (76.59 ± 0.14) and IJFG (73.84 ± 0.29) genetic groups. The live spermatozoa was highest in CB bulls having 50% Brown Swiss inheritance following Friesian and Jersey crosses (Bhoite *et al*., 2008)

The association of sperm volume with semen motility and live spermatozoa was positive and significantly ($p<0.01$) in IFG (0.258 and 0.297) and IFJG (0.261 and 0.239) groups and negative and significant ($p< 0.01$) in IJFG (-0.157 and –0.134) and IBFG correlation between semen volume and sperm motility in JY X Sahiwal (Bhoite *et al*., 2008). Mondal *et al*. (2010) found that the percentage of live sperm decreased ($P < 0.01$) from 86 ± 6 in fresh to 65 ± 6% in frozen–thawed semen. Patel *et al*. (2013) found that the individual sperm motility, live sperm count and acrosomal integrity were significantly decreased, where as abnormal sperm count was significantly increased as freezing process progressed.

Gangwar *et al*. (2018) stated that Dead spermatozoa could be differentiated by their ability to be stained by Eosin dye. The live spermatozoa, which are alive at the time of staining, remain colourless since they were impermeable to the Eosin

stain. Sabes-Alsina *et al*. (2019) stated that Husbandry conditions for bulls used for semen collection should be adapted to allow the animals' physiological responses for temperature regulation to operate fully so that the effects of increased temperature and humidity can be mitigated. Extreme of temperature and humidity should be avoided.

2.3.6 Sperm abnormality

Chenoweth (2005) stated that Genetic sperm defects are specific sperm defects, which have been shown to have a genetic mode of transmission. Such genetic linkage, either direct or indirect, has been associated with a number of sperm defects in different species, with this number increasing with improved diagnostic capabilities.

Enciso *et al*. (2011) observed in his study a clear relationship between morphologically abnormal bull sperm and poor DNA quality. In particular, major sperm abnormalities, that potentially might have a genetic origin or be the result of an abortive apoptotic mechanism, appear to be closely associated with the presence of a highly damaged DNA molecule.

Beletti1 *et al*. (2017) studied that variations in the abnormalities in chromatin condensation influenced the head morphology in different ways. The finding that spermatozoa with chromatin abnormalities did not necessarily show morphological alterations demonstrates the need for the morphological analysis of sperm to be followed by an evaluation of chromatin structure.

Taylor *et al.* (2020) studied that Taking into account the impact of age, season, region, and breed there were differences between breeds in both percent morphologically normal sperm and in some individual categories of sperm abnormality ($P<0.001$). Independent of breed, season and region, proximal droplets were significantly increased in bulls less than 20 months of age. This is the first study to comprehensively collect data from this wide geographical area and compare sperm morphology profiles among the *Bos indicus* and *Bos taurus* breeds

2.4 Post thaw semen evaluation

Cryopreserved bovine spermatozoa, when used for artificial insemination, generally produced lower conception rates than fresh spermatozoa for which post thaw evaluation is one of the important factor in semen processing laboratories as motility, reasonable velocity, acrosome integrity, membrane stability contributed to fertility. Moustafa and Meszaros (1980) observed that semen collected from 69 AI bulls having good semen quality with good freezing capacity, good semen quality with poor freezing capacity had 50.0, 21.5 and 12% post thaw motility respectively.

Wood *et al.* (1986) demonstrated through multiple regression analysis that a combination of sperm motility after dilution in saline, motility after thawing and the proportion of coiled tails and proximal protoplasmic droplets provide the best prediction of fertility with a significant correlation. Belorkar *et al*. (1990) found that semen samples with 50 % post thaw motility or more were mostly from bulls with larger ejaculates, higher sperm concentration and lower percentage of abnormal and dead spermatozoa.

The ability to predict post thaw sperm quality and fertility from a routine sperm function assay would be beneficial, considering the extended period of progeny testing (Garner *et al*., 1994). Nair (1997) observed the mean post thaw motility and post thaw livability in half bred and three bred cross bulls to be 23.65 ± 2.28, 22.54 ± 2.84, 39.75 ± 3.0 and 40.42 ± 3.38% respectively.

Brahmakshtri *et al*. (1999) established highly significant correlation ($P<0.01$) between post thaw motility and viability. The reduced fertility of frozen thawed spermatozoa is thought to be not only due to a low sperm survival post thaw rate but also due to an impaired function of the surviving spermatozoa (Watson, 2000). Muino *et al.* (2008) indicated that, in terms of total post-thaw sperm motility and integrity of plasma and acrosomal membranes, there was no difference between the three thawing rates compared.

Kumar *et al*. (2018) indicated that deoxygenation of the semen extender with N_2 gassing improves post-thaw semen quality by reducing oxidative stress and increasing the plasma membrane integrity, mitochondrial membrane potential and the DNA integrity of the spermatozoa.

2.4.1 Post thaw motility

The mean post thaw motility was 47.14 ± 0.92 % , 28.33 ± 5.26%, 44.16 ± 2.99%, 42.65 ±1.54%, 23.64 ± 5.32 and 28.33 ± 2.35% for Pure JY, 75% JY, 62.5% JY, 50 % JY and 75% HF and 62.5% HF bulls, respectively (Sagdeo *et al*., 1980) 62.5 % Brown Swiss: 44.79%, 62.5 % JYCB: 44.79% (Mathew *et al*., 1982) 50% JY CB bulls: 43.33+ 1.86% (Tuli *et al*., 1988).

The overall post thaw motility and total abnormality in the thawed semen was 42.56 ± 1.29 and 14.26 ± 0.04 % respectively. Fertility rate of bulls showed negative correlation ($p<0.05$) with sperm abnormality percent ($p=0.93$) (Mandal *et al*., 2007).

Leite *et al*. (2010) concluded that Equilibration time is necessary for preservation of motility and integrity of sperm membranes. Equilibration for 4 h resulted in the greatest preservation of total and progressive motility, as well as the integrity of plasma and acrosomal membranes during cryopreservation.

Das *et al.* (2013) registered a new breed in Odisha. Binjharpuri is medium sized, horned, strong, dual type docile cattle with good posture. Bulls look very strong, vigorous with well-developed hump, penis, naval flap and dewlap. The hump, neck and some regions of the face and back are black in colour irrespective of the coat colour of the males. Bulls have majestic gait. Long tail is an important feature of this breed of cattle, which almost touches the ground. It is tapering towards tip with voluminous switch. The ears are short and dewlap is thin, small and soft.

Fakhrildin *et al.* (2014) indicated that the supplementation of honey (10%) to cryoprotectant solution results in enhancement of sperm quality post-thawing.

Anzar *et al.* (2019) concluded that bull semen can be frozen successfully without egg yolk by treating sperm with CC complex alone. It appears that CC (cholesterol-cyclodextrin) complex protected bull sperm during the initial (supra-zero) cooling phase. Glycerol, regardless of its addition at room or refrigerated temperature, is still required to protect bull sperm during the deep (sub-zero) freezing phase. Electrophoresis data revealed that protein profiles of plasma membrane of fresh sperm and frozen sperm TG (without egg yolk) are similar. In contrast, egg yolk proteins bind and modify sperm plasma membrane. Both, semen frozen with and without egg yolk has similar fertilization potential *in vitro*.

MATERIALS AND METHODS

The present study was undertaken in the Department of Animal Reproduction Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar, in collaboration with Frozen Semen Bank, Cuttack, under Fisheries and Animal Resources Development Department, Govt. of Odisha during the period of January 2020 to April 2020. The experiment was envisaged to study the reproductive behaviour and semen characteristics of breeding Binjharpuri bulls.

3.1 Source of animals

Six good breedable Binjharpuri bulls (BH1, BH4, BH5, BH6, BH8 and BH9) belonging to Frozen Semen Bank, Cuttack under Department of Fisheries and Animal Resources Development, Government of Odisha, were selected for this study. The semen from these bulls was routinely used for artificial insemination following collection and ultra low temperature freezing. The bulls under investigation were within the age group of 4 to 6 years and maintained under optimum nutritional and managemental practices as per the minimum standard protocol (MSP) fixed for maintenance of breeding bulls at sperm stations.

3.2 Collection of semen

The semen was collected at homosexual mount by artificial vagina. The bulls were given exercise in the morning as a part of sexual preparation (Fig.1).

Fig. 1: Bulls on exerciser

They were allowed at least one to two false mounts before collection of the ejaculate. The sheath was lightly grasped and deviated into the artificial vagina. Immediately after collection, the collecting tube was detached from the AV, taken to the laboratory and placed in water bath maintained at 35^{o}C until evaluation and processing. A total of 192 ejaculates @ two from each bull per week were collected for assessment and preservation. During collection of semen, each bull was observed for their service behaviour and was assessed with a scoring system (Appendix -1). The samples collected from various bulls were subjected to routine semen analysis pertaining to physical and microscopic attributes. Physical characteristics evaluated was volume. The parameters studied under routine microscopic examination were mass activity, individual motility, livability, sperm concentration, sperm abnormality and post thaw motility.

The semen was then processed with tris-egg yolk-glycerol extender with a four hour equilibration time and static vapour freezing. Packaging of extended glycerinated semen dose with twenty million spermatozoa was accomplished in 0.25ml polyvinyl French mini straws prior to equilibration.

3.3 Service behaviour

Mating or service behaviour is that behaviour exhibited immediately before, during and after service (Chenoweth, 1981). Anderson (1945) differentiated sexual behaviour in the bull into two components, i.e. libido or sex drive and mating ability or ability to copulate. Libido has also been defined as the willingness and eagerness to mount and complete service of a female, where as mating ability is the ability to complete service (Hultans, 1959). In the present study the components of service behaviour were observed during semen collection and assessed as per procedure laid down by Chenoweth (1980), Narashima Rao *et al*. (1996) and Mohanty (1999) with some minor modifications (Mishra, 2011) in the indices of scoring system. The scoring system undertaken during the present investigation were as follows:

3.3.1 Approach to the test site

Approach of a breeding bull to the collection site develops by virtue of its sexual experience. It can be classified and scored as:

Keen	5 points
Less keen	3 points
Lethargic	2 points
Not interested	1 points

3.3.2 Eagerness to approach stimulus

Sexual stimulation of bulls is the presentation of a stimulus situation adequate to elicit mounting and ejaculation (Amann and Almquist, 1976), where as sexual preparation is defined as prolongation of the period of stimulation beyond that which is sufficient for mounting and ejaculation (Hale and Almquist, 1960).

The promptness with which a breeding bull approaches a sexual stimulus is an important index of service behaviour of that bull. A suitable stimulus need not even be a female (Amann and Almquist, 1976), confirming that an inverted "U" shape is the most stimulatory visual configuration to provoke mounting behaviour in bulls (Hafez and Boissou, 1975). Basing on these criteria the eagerness of the bull can be classified and scored as:

Very eager	5 points
Eager	3 points
Slow	2 points
Indifferent	1 points

3.3.3 Approach to dummy

In Frozen semen stations a bull is used as a dummy to elicit sexual response from a breeding bull. As discussed earlier immobility of an inverted “U” shaped structure is the most sensuous stimulus for a bull. Hence, approach to a dummy can be taken as an important index of sexual behaviour of a bull which can be assessed and scored as:

Very eager	5 points
Eager	3 points
Slow	2 points
Indifferent	1 points

3.3.4 Sniffing and licking

Some breeding bulls display their courtship behaviour by sniffing and licking the genitalia of the dummy and this can be taken as criteria while assessing their sexual behaviour. Sniffing of perineal region elicits tactile stimulus and is essential for sex arousal (Fig.2).

Fig. 2: Licking and sniffing of dummy by a breeding bull

It can be assessed and scored as:

Conspicuous sniffing	5 points
Moderate sniffing	3 points
Poor sniffing	2 points
Rare sniffing	1 points

3.3.5 Flehman's reaction

With mounting evidence it becomes clear that olfaction is fundamental in the stimulation of reproductive responses. Substances eliminated in urine are specific for reflex, since it is almost invariably induced after the subject has odour tested freshly voided urine particularly from the female. It is known that urine carries breakdown products of hormones including reproductive hormones and it is reasonable assumption that oestrous cycle phasing may be recognizable to male animal by the odour testing of urine. Odorous substances, eliminated by the animal have a specific stimulatory effect on the animal of opposite sex, are termed pheromones its role in reproductive behaviour is considerable (Bhosrekar, 1990).

Flehman's reaction is defined as a flexing of bull's nostrils and retraction of upper lip following investigation of a female's perineal, flank or leg

region (Seneger, 1999). It is meant to detect the pheromones present in the urine by help of the vomeronasal organ (Fig.3).

Fig. 3: Flehman's Reaction

It is one of the important courtship behaviour exhibited by a breeding bull and the scoring is done as follows:

Conspicuous Flehman's reaction	5 points
Moderate Flehman's reaction	3 points
Poor Flehman's reaction	2 points
Rare Flehman's reaction	1 points

3.3.6 Libido

It is the desire or eagerness of a breeding bull for mating and is considered as the most important component of service behaviour of a breeding bull. In the present study it was assessed as per the procedures laid down by Chenoweth (1980), Narashima Rao *et al*. (1996) and Mohanty (1999) with slight modifications (Mishra, 2011). Libido in breeding bulls has been classified and scored as follows:

Very strong or uncontrolled desire with intensive holding and seeking	5 points
Strong libido i.e., eager mounting with good seeking and holding	3 points
Moderate libido i.e., mounting with repeated hesitation	2 points
Weak libido i.e., little interest in mounting despite licking and sniffing	1 point

3.3.7 Protrusion of penis

Penile protrusion (Fig.4) is an indication of the degree of sex arousal of a breeding bull and has been classified and scored as follows:

Strong	5 points
Moderate	3 points
Weak	2 points
None	1 points

Fig. 4: Strong penile protrusion

3.3.8 Mounting

Sexual desire culminates in mounting. In this the pubic region of the bull comes in apposition with the dummy. The legs of the bull remains at the pin bone region of dummy and its chin rests on its back giving an intense holding and seeking.

Fig. 5: Ideal mounting

Thus, a good mount results in a good intromission and subsequent ejaculation. So mounting is assessed and scored as follows

Energetic	5 points
Steady	3 points
Slow	2 points
Poor	1 point

3.3.9 Copulatory thrust and ejaculation

After a successful mounting the penis enters into the vagina resulting in intromission and finally culminating in ejaculation after a violent copulatory thrust. During this process i.e., at the time of ejaculation the hind limbs of the bull gets lifted from the ground along with arching of back, giving an impression of good ejaculation and completion of service.

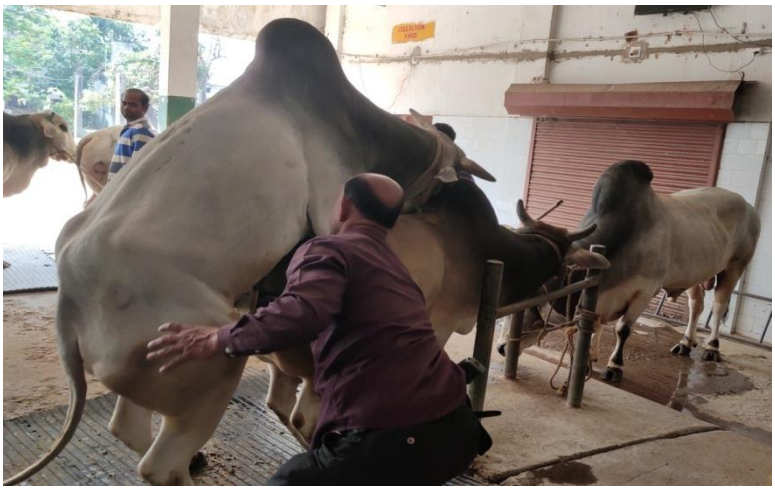

Fig. 6: Copulatory thrust

The copulatory thrust is classified and scored as follows

Too vigorous	5 points
Vigorous	3 points
Moderate	2 points
Weak	1 point

3.3.10 Reaction time

The time elapsing between introduction of a bull into the test area and the first mount is defined as reaction time (Singh and Pangawkar, 1989) or in other

words it is the time taken from introduction of the bull to the dummy and its first mount. It is measured in seconds with the help of a stop watch.

Thus reaction time scoring was undertaken as follows

Very good (between 0 to 10 seconds)	5 points
Good (between 10 to 20 seconds)	3 points
Average (between 20 to 30 seconds)	2 points
Poor (more than 30 seconds)	1 point

3.4 Service behavior index

Taking into account the above parameters of service behaviour an average value for scoring the service behaviour index was devised.

The semen quality of all bulls was ascertained basing on seminal traits.

3.5 Routine semen analysis

3.5.1 Volume

Volume of the ejaculate was recorded in millilitre directly from the graduated tube attached to the artificial vagina and volumes of less than 2 ml were discarded.

Fig. 7: Collected semen volume

3.5.2 Mass activity

Soon after collection of semen, a small thick drop of undiluted semen was taken on a pre-heated clean glass slide and examined on a thermostatic stage

at 37^0C under low power phase contrast optics. Mass activity was graded with a scale "O" to ++++ and score (0-5) (Bhosrekar, 1990).

Very intense eddies and waves were observed with whirls	5 points
Strong eddies and waves were observed	3 points
The waves and eddies were present but they were mild and the force with vigour was not observed	2 points
The waves or eddies were absent and the movement of individual spermatozoa was seen	1 point
The waves or eddies were absent and spermatozoa were immotile	0 point

3.5.3 Pre-freeze motility

This was done to estimate total percentage of motile spermatozoa in the ejaculate with special reference to the type of motility. Neat semen was diluted with 2.9 per cent sodium citrate solution. A small drop of diluted semen was placed on a pre-heated slide, covered with a cover slip and examined under phase contrast optics with thermostatic stage at 37^0C. Motile spermatozoa were expressed as percentage (Zemjanis, 1970).

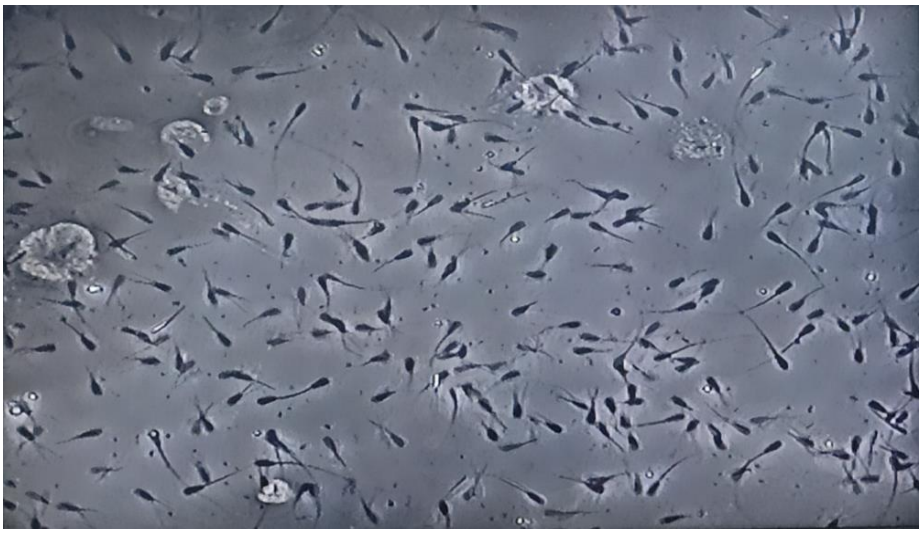

Fig. 8: Individual motility of semen

3.5.4 Sperm concentration

The sperm concentration was measured using bovine photometer Accucell (IMV make). After warming up the equipment, 6.0 ml sodium citrate solution

(2.9%) was taken in a cuvette and the equipment was set to 100% transmittance. To this 0.1 ml neat semen was added and gently mixed. The cuvette was placed inside the previously standardized bovine photometer and reading (% transmission / optical density) was recorded.

- Distilled water of 1.6 ml containing 2.96 % sodium citrate was taken in the cuvette.
- The cuvette was inserted into the photo electric meter.
- The refractive index is adjusted to 100% by using knob.
- The optical density of the semen is recorded and using the ready reckoner chart, sperm concentration was assessed and recorded.

3.5.5 Livability

Live and dead count of spermatozoa of the ejaculate was performed by counting 100 spermatozoa in a smear stained with Eosin–Nigrosin Stain. Eosinophilic spermatozoa were considered dead. Live spermatozoa were expressed as per cent. Eosin-Nigrosin stain was prepared well in advance, filtered and preserved in amber coloured reagent bottle for one month before use (Saxena, 2000).

Eosin-Nigrosin stain

Eosin –y (water soluble)	1.67 gm
Nigrosin (water soluble)	10.00 gm
Glass distilled water	100 ml.

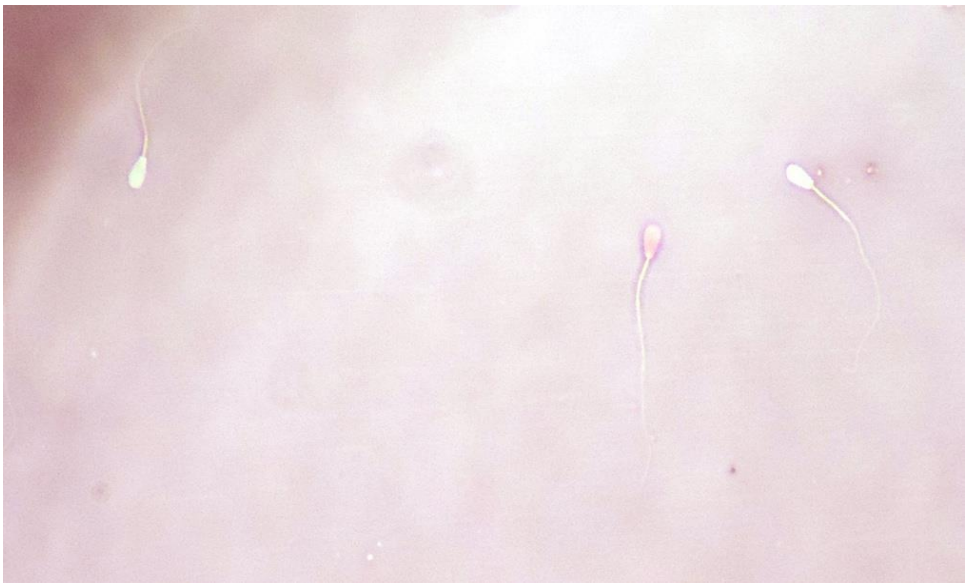

Fig. 9: Live (white) and dead (pink) spermatozoa in Eosin- Nigrosin staining

A relatively small drop of semen was placed on a pre-heated slide (37^0C) and 5 to 10 times larger drop of warm stain (37^0C) was placed next to semen and was gently mixed for 10 to 15 seconds. Then a thin smear was drawn, air dried and examined under high power objective with phase contrast optics.

The disruption of spermatozoan cell membrane as well as acrosomal cap facilitates stain penetration, thus differentiating live and motile spermatozoa from dead and immotile ones.

3.6 Post thaw semen evaluation

3.6.1 Post thaw motility

The post thaw motility was assessed as per method described by Amelar *et al*. (1980). After thawing, the frozen semen straw in 35^oc water for 30 second, the lab end is cut and straw is emptied in a 2 ml test tube. Test tube was kept in water bath for 5 minutes. A small drop of the semen was placed over a clean, pre heated, grease free slide, covered with a cover slip (18 mm) and was examined under high power magnification with thermostatically controlled phase contrast microscope. Care was taken to avoid air bubbles in the smear. Generally a post thaw motility of 40 % is considered as a minimum acceptable motility for frozen semen.

3.7 Statistical analysis

Statistical analysis of the data obtained during the present course of investigation was carried out to find out the descriptive values as per one way analysis of variance the ANOVA procedure and using the software IBM SPSS 25.0.

RESULTS

4.1 Service behavior index

The various parameters included under service behaviour index of individual Binjharpuri breeding bull such as approach to test site, eagerness to approach stimulus, approach to dummy, sniffing, Flehman's reaction, reaction time, protrusion of penis, mounting on dummy, copulatory thrust and libido are depicted in table 1 to 10 and fig. 11 to 17. The service behaviour Index (SI) has been computed as the mean figure of all the above scores and is presented in table 11, where rating of each trait and SI, varied between 1 and 5, according to the scoring method adopted. Accordingly approach to test site has got the overall mean value of 3.01±0.09, while the other parameters have recorded the values as under: eagerness to approach stimulus (2.99±0.09), approach to dummy (3.46±0.08), sniffing and licking (2.54±0.04), Flehman's reaction (2.59±0.04), libido (2.59±0.04), protrusion of penis (2.94±0.04), mounting on dummy (3.50±0.08), copulatory thrust (2.60±0.04) and reaction time (2.00±0.06). The overall mean of SI is recorded to be 2.82±0.03 (table 11) during the present study.

Variation in these traits have been observed among bulls with respect to Approach to test site, Protrusion of penis, Mounting, Copulatory Thrust and Ejaculation & Reaction time.

Table 1: Mean Score (1-5) of Approach to test site of Binjharpuri Breeding Bulls

Bull No.	N	Approach to test site
BH1	32	2.71^{b}±0.20
BH4	32	2.93^{ab}±0.20
BH5	32	2.75^{b}±0.22
BH6	32	3.43^{a}±0.22
BH8	32	3.15^{ab}±0.21
BH9	32	3.06^{ab}±0.23
Overall mean	**192**	**3.01±0.08**

*Mean with different superscripts differ significantly (P<0.05)

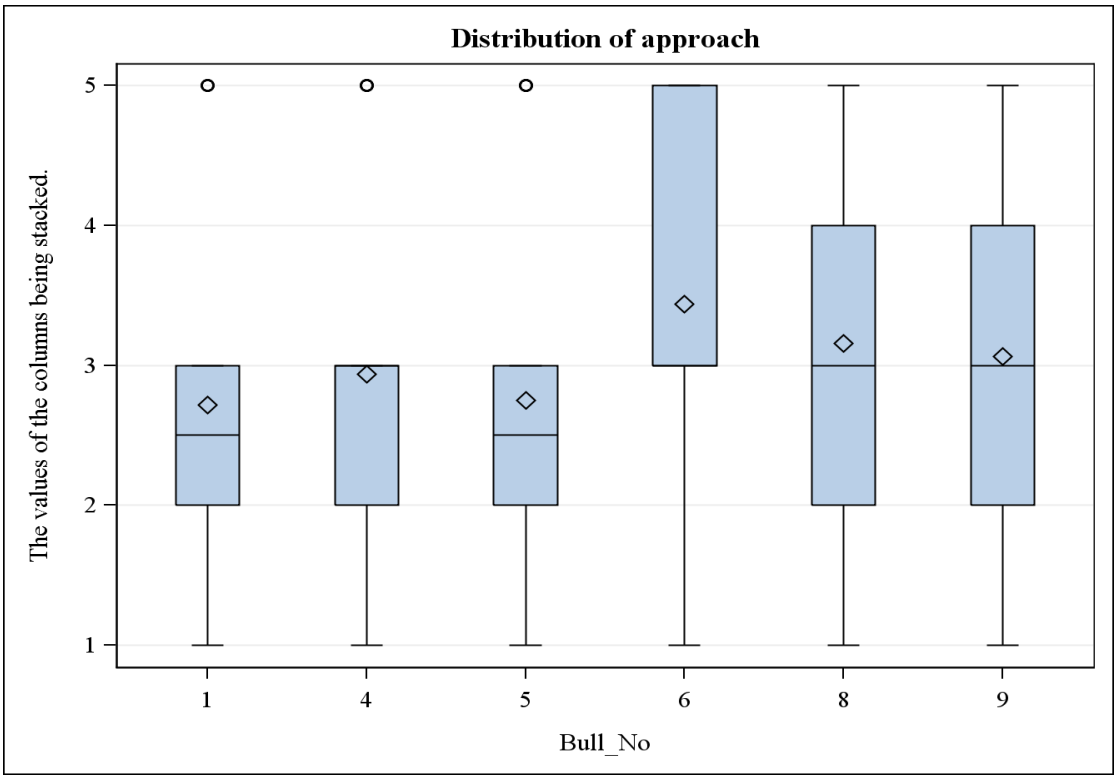

Fig. 10: Mean distribution of approach among different bulls

Table 2: Mean Score (1-5) of Eagerness to approach stimulus of Binjharpuri breeding bulls

Bull No.	N	Eagerness to approach stimulus
BH1	32	2.78±0.21
BH4	32	2.90±0.18
BH5	32	2.81±0.20
BH6	32	3.31±0.22
BH8	32	3.09±0.22
BH9	32	3.03±0.23
Overall mean	**192**	**2.99±0.09**

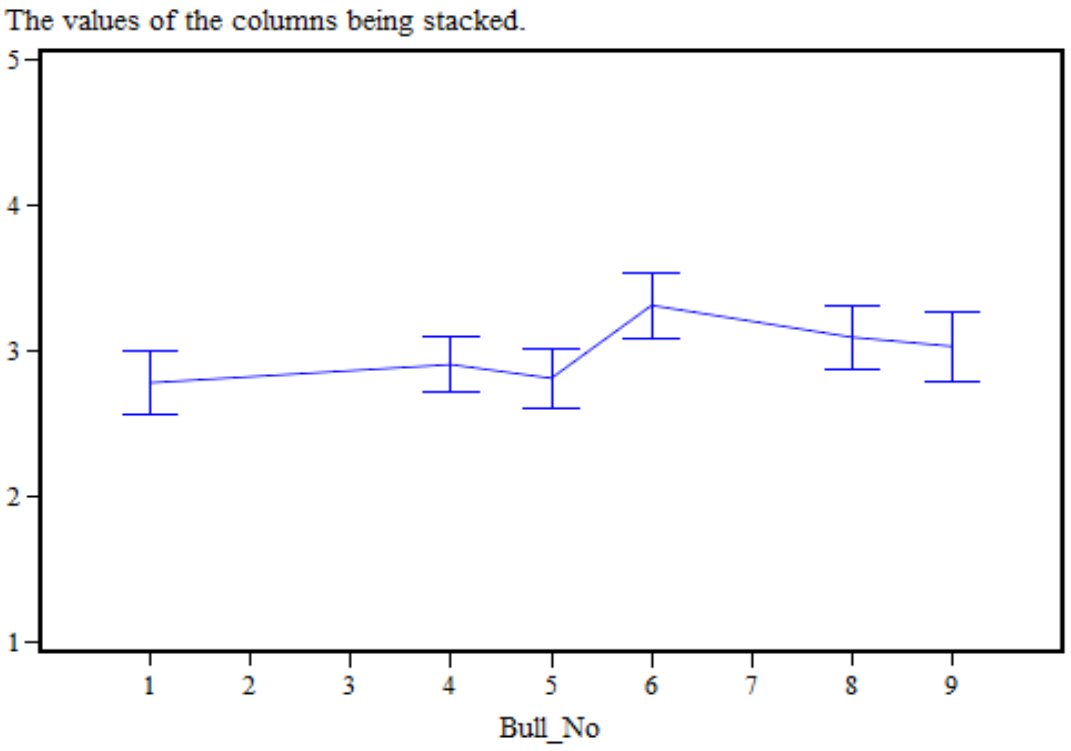

Fig. 11: Mean Distribution of Eagerness to approach stimulus of different bulls

Table 3: Mean Score (1-5) of Approach to dummy of Binjharpuri breeding bulls

Bull No.	N	Approach to dummy
BH1	32	3.18±0 .20
BH4	32	3.46±0 .21
BH5	32	3.78±0 .18
BH6	32	3.56±0 .21
BH8	32	3.43±0 .22
BH9	32	3.34±0 .18
Overall mean	**192**	**3.46±0.08**

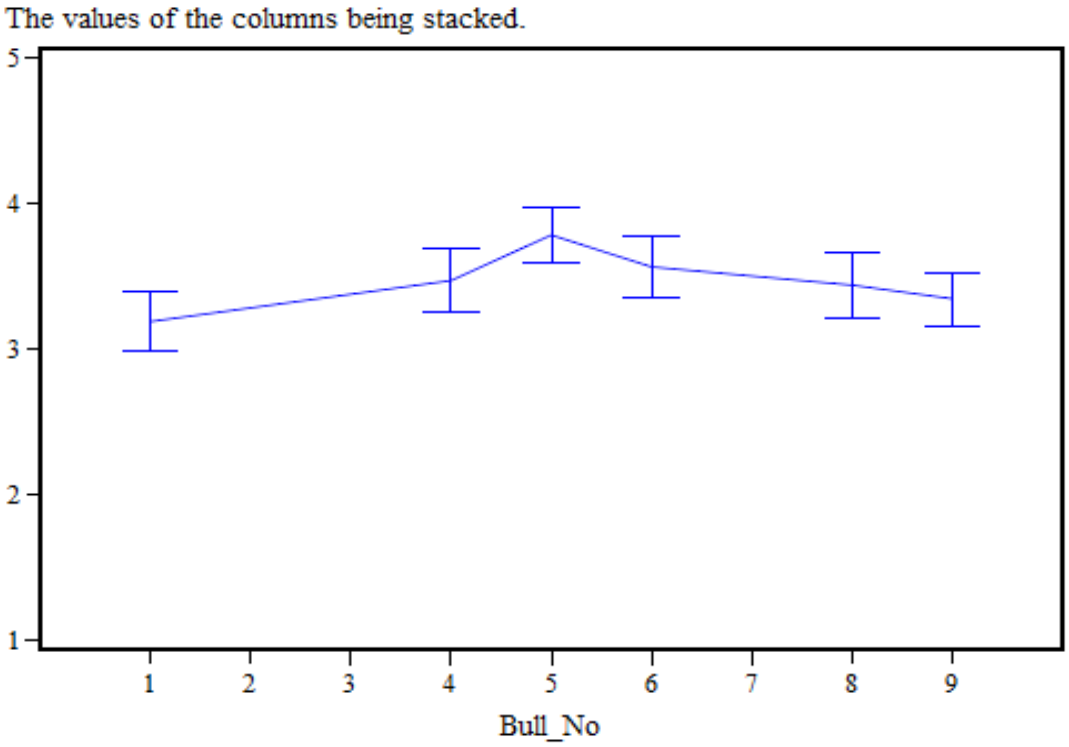

Fig. 12: Mean Distribution of Approach to dummy of different bulls

Table 4: Mean Score (1-5) of Sniffing and licking of Binjharpuri breeding bulls

Bull No.	N	Sniffing and licking
BH1	32	2.53±0 .10
BH4	32	2.59±0 .08
BH5	32	2.53±0 .08
BH6	32	2.46±0 .10
BH8	32	2.50±0 .08
BH9	32	2.62±0 .08
Overall mean	**192**	**2.54±0.04**

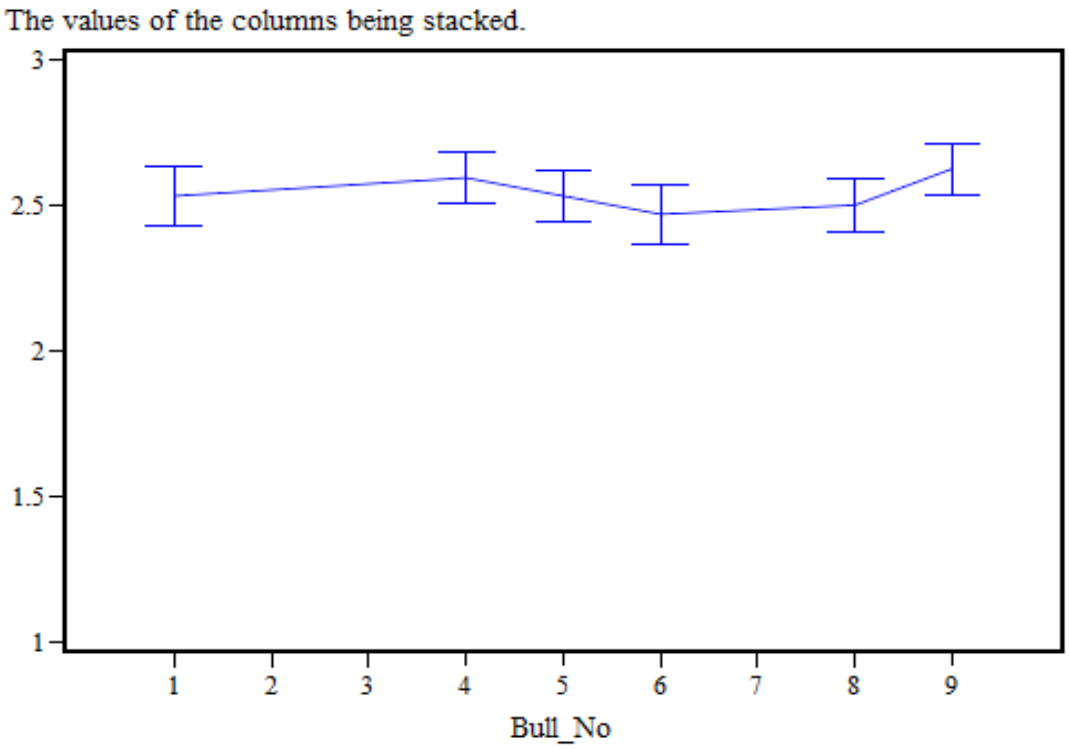

Fig. 13: Mean Distribution of of Sniffing and licking different bulls

Table 5: Mean Score (1-5) of Flehman's reaction of Binjharpuri breeding bulls

Bull No.	N	Flehman's reaction
BH1	32	2.59±0 .08
BH4	32	2.62±0 .08
BH5	32	2.68±0 .08
BH6	32	2.53±0 .08
BH8	32	2.53±0 .08
BH9	32	2.56±0 .08
Overall mean	**192**	**2.59±0.04**

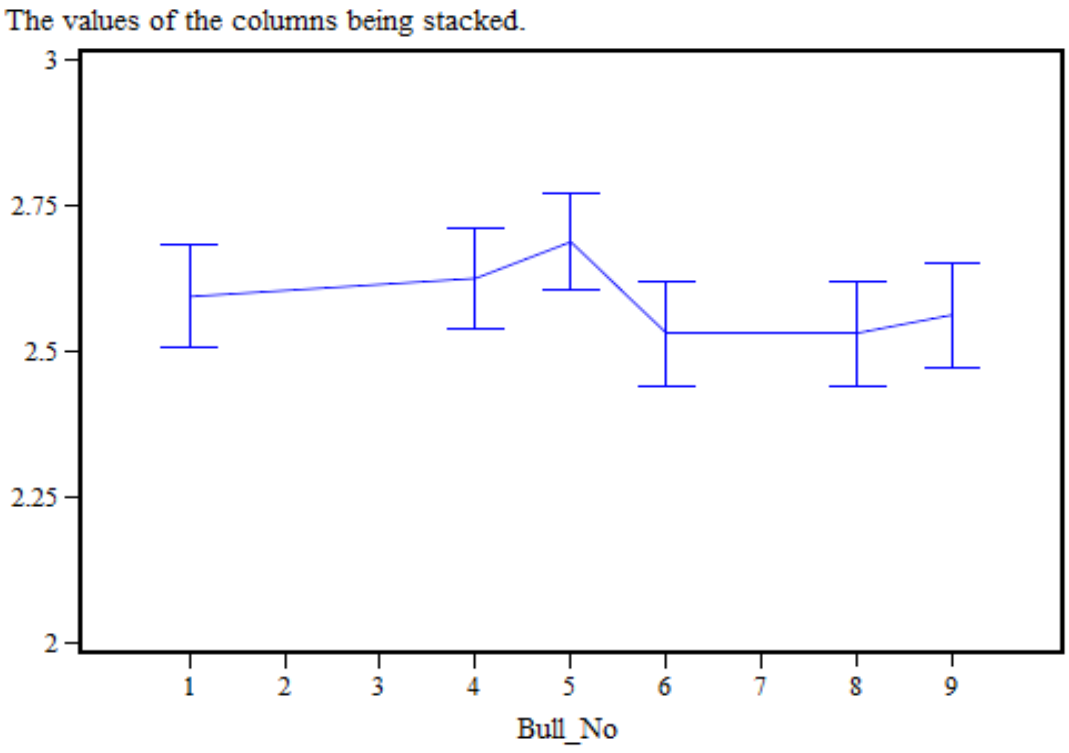

Fig. 14: Mean Distribution of Flehman's reaction of different bulls

Table 6: Mean Score (1-5) of Libido of Binjharpuri breeding bulls

Bull No.	N	Libido
BH1	32	2.59±0 .08
BH4	32	2.62±0 .08
BH5	32	2.68±0 08
BH6	32	2.53±0 .08
BH8	32	2.53±0 .08
BH9	32	2.56±0 .08
Overall mean	**192**	**2.59±0.04**

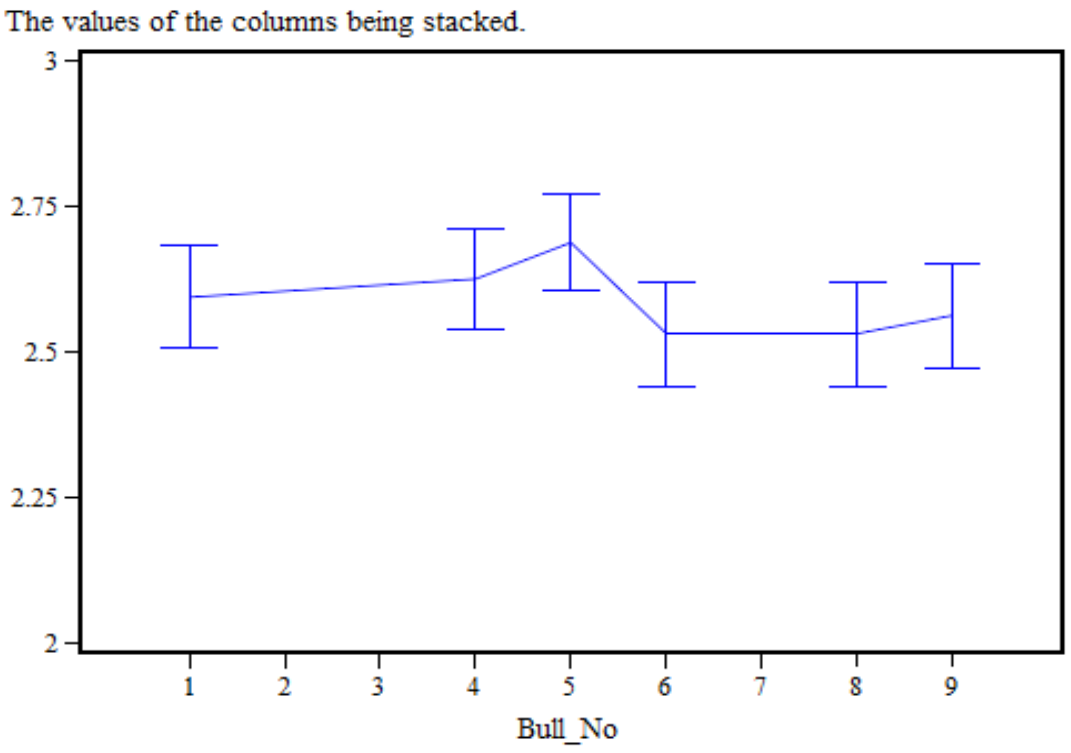

Fig.15: Mean Distribution of Libido of different bulls

Table 7: Mean Score (1-5) of Protrusion of penis of Binjharpuri breeding bulls

Bull No.	N	Protrusion of penis
BH1	32	2.87^{ab}±0 .05
BH4	32	2.75^{b}±0 .07
BH5	32	3.00^{ab}±0 .15
BH6	32	3.12^{a}±0 .14
BH8	32	3.00^{ab}±0 .00
BH9	32	2.87^{ab}±0 .05
Overall mean	**192**	**2.94±0.04**

*Mean with different superscripts differ significantly (P<0.05)

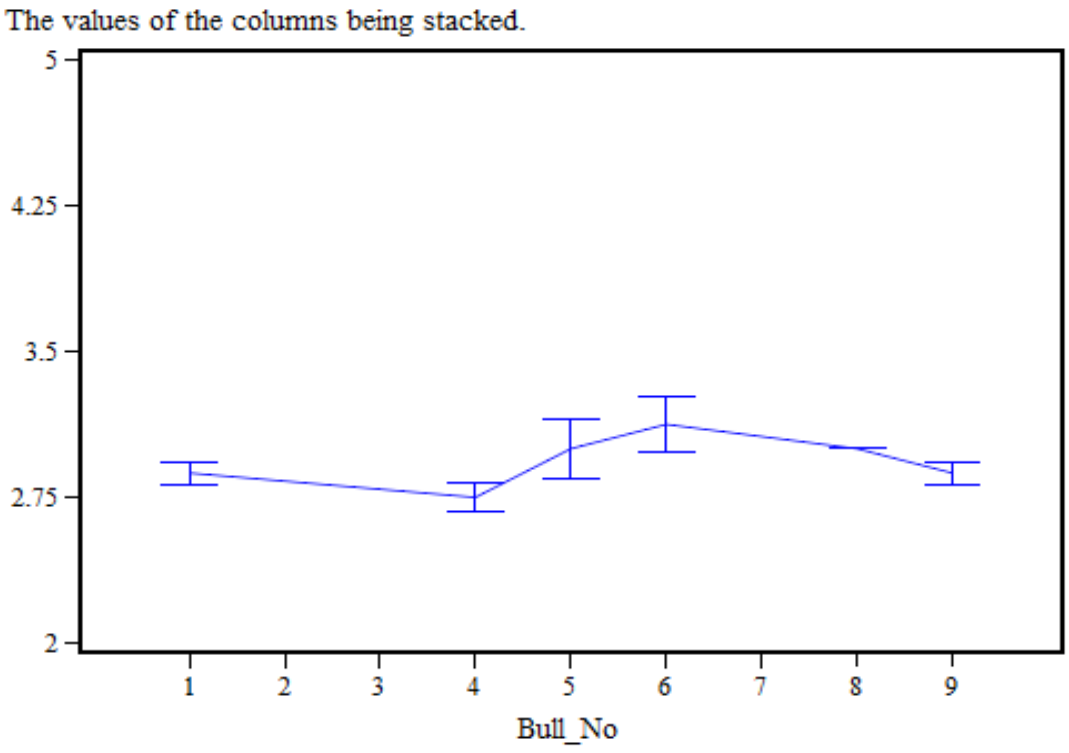

Fig. 16: Mean Distribution of Protrusion of penis of different bulls

Table 8: Mean Score (1-5) of Mounting of Binjharpuri breeding bulls

Bull No.	N	Mounting
BH1	32	4.31^{a}±0.17
BH4	32	4.37^{b}±0 .16
BH5	32	4.31^{b}±0.17
BH6	32	2.68^{b}±0.08
BH8	32	2.65^{a}±0.08
BH9	32	2.65^{c}±0.08
Overall mean	**192**	**3.50±0.08**

*Mean with different superscripts differ significantly (P<0.05)

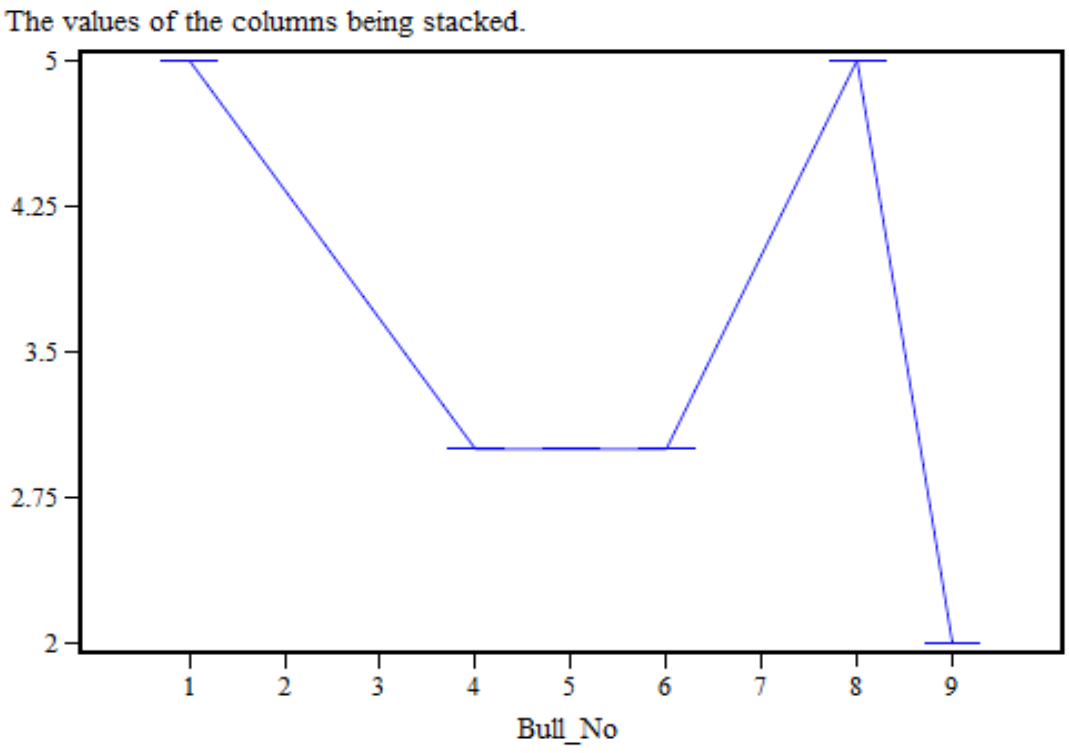

Fig. 17: Mean Distribution of Mounting of different bulls

Table 9: Mean Score (1-5) of Copulatory Thrust and Ejaculation of Binjharpuri breeding bulls

Bull No.	N	Copulatory thrust and ejaculation
BH1	32	2.75^{a}±0.07
BH4	32	2.75^{c}±0.07
BH5	32	2.75^{c}±0.07
BH6	32	2.50^{c}±0.08
BH8	32	2.43^{a}±0.08
BH9	32	2.43^{b}±0.08
Overall mean	**192**	**2.60±0.04**

*Mean with different superscripts differ significantly (P<0.05)

Table 10: Mean Score (1-5) of Reaction time of Binjharpuri breeding bulls

Bull No.	N	Reaction time
BH1	32	2.06^{ab}±0.11
BH4	32	2.03^{ab}±0.13
BH5	32	2.15^{b}±0.12
BH6	32	1.84^{ab}±0.15
BH8	32	2.06^{a}±0.15
BH9	32	1.84^{ab}±0.14
Overall mean	**192**	**2.00±0.06**

*Mean with different superscripts differ significantly (P<0.05)

Table 11: Mean Score (1-5) of Service Behaviour Index of Binjharpuri breeding bulls

Bull No.	N	Service Behaviour Index
BH1	32	2.91±0.05
BH4	32	2.74±0.05
BH5	32	2.72±0.06
BH6	32	2.82±0.06
BH8	32	3.06±0.06
BH9	32	2.66±0.06
Overall mean	**192**	**2.82±0.03**

4.2 Routine semen analysis

4.2.1 Volume, concentration and mass activity

In the present investigation the overall mean values with standard error for semen volume (ml), concentration (millions/ ml) and mass activity (0-5 scale) were recorded to be 3.76±0.09 (table 12, fig. 18), 888.71±36.05 (table 13) and 2.74±0.07 (table 14, fig. 19) respectively.

Table 12: Mean Volume of Binjharpuri breeding bulls

Bull No.	N	Volume (ml)
BH1	32	3.50±0 .19
BH4	32	3.78±0 .16
BH5	32	3.53±0 .28
BH6	32	4.10±0 .22
BH8	32	3.89±0 .20
BH9	32	3.73±0 .23
Overall mean	**192**	**3.76±0.09**

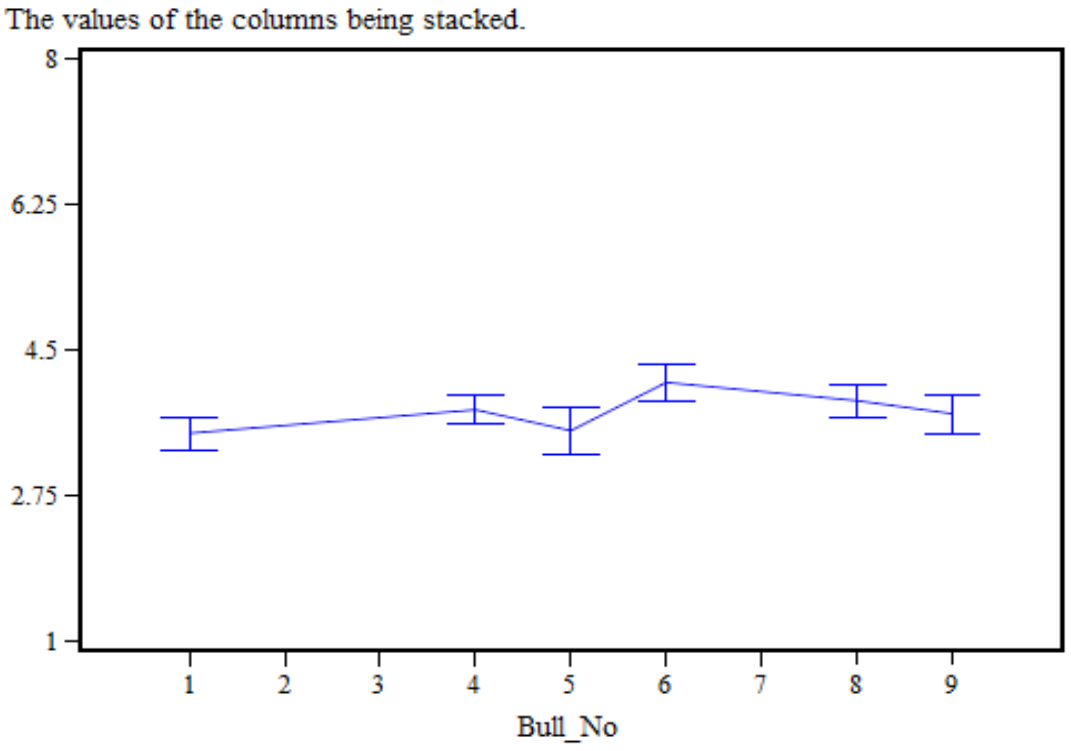

Fig.18: Mean Distribution of Semen volume of different bulls

Table 13: Mean Mass Activity Score and Sperm Concentration of Binjharpuri breeding bulls

Bull No.	N	Mass Activity (0-5)	Sperm concentration (Million/ ml)
BH1	32	2.59±0.20	826.28± 93.53
BH4	32	2.65±0.18	832.65± 84.05
BH5	32	3.00±0.18	1074.75± 100.20
BH6	32	2.62±0.17	848.78± 80.27
BH8	32	2.68±0.15	869.90± 81.93
BH9	32	2.87±0.14	879.87± 87.25
Overall mean	**192**	**2.74±0.07**	**888.71±36.05**

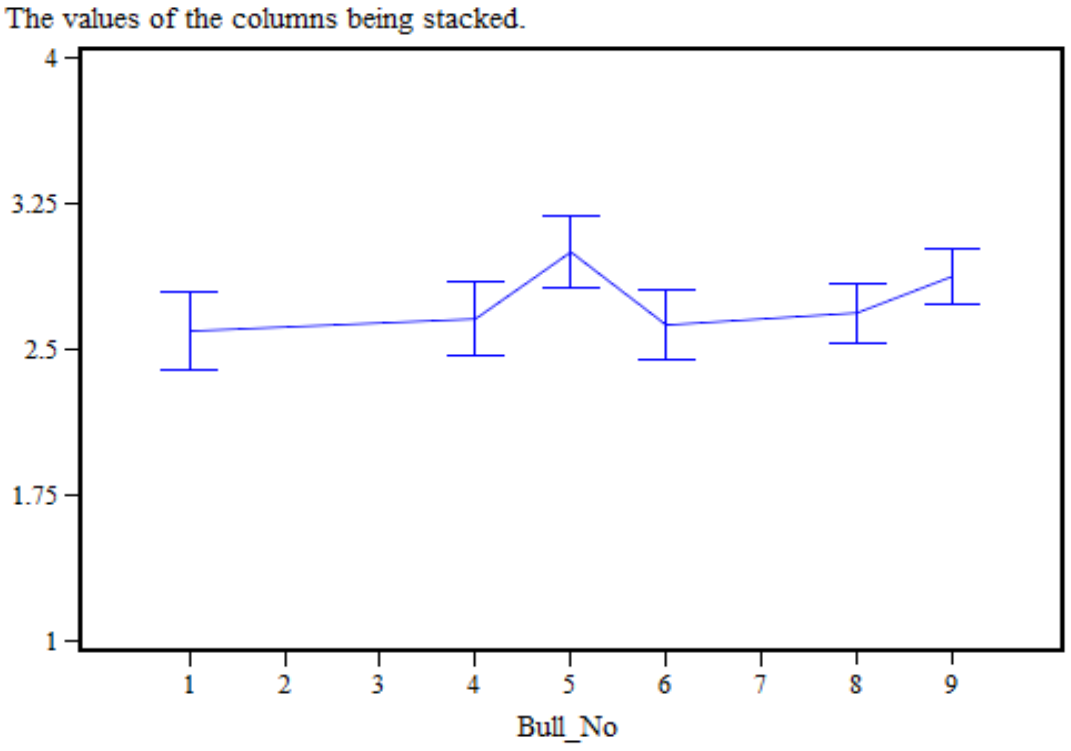

Fig. 19: Mean distribution of sperm concentration of different bulls

4.2.2 Mass activity and sperm concentration

During the present study, the overall mean values with standard error for Mass activity (o-5) and sperm concentration (million/ml) are recorded to be 2.74±0.07 and 888.71±36.05 (table 13, fig. 19) respectively.

4.2.3 Motility, livability and sperm abnormality

In the present investigation the overall mean values with standard error for pre-freeze motility, Post thaw motility, Livability, Sperm abnormality are recorded to be 77.66±1.67 (table 14), 44.04±1.37 (table 14) and 75.21±0.47 (table 15, fig. 20) and 8.76±0.14 (table 15, fig. 20) respectively.

Table 14: Mean Pre-freeze and Post Thaw Motility of Binjharpuri breeding bulls

Bull No.	N	Pre-freeze motility (%)	Post thaw motility (%)
BH1	32	78.75± 4.13	41.87± 3.43
BH4	32	74.06± 4.16	48.43± 3.39
BH5	32	79.68± 3.94	42.18± 3.26
BH6	32	75.00± 4.16	48.43± 3.42
BH8	32	80.31± 4.10	43.43± 3.31
BH9	32	78.12± 4.22	41.87± 3.43
Overall mean	**192**	**77.66±1.67**	**44.04±1.37**

Table 15: Mean Livability and Sperm Abnormality of Binjharpuri breeding bulls

Bull No.	N	Livability (%)	Sperm abnormality (%)
BH1	32	75.62± 1.26	9.25±0.42
BH4	32	75.62±1.34	8.59±0.27
BH5	32	75.62±1.09	8.75±0.37
BH6	32	75.56±1.15	8.62±0.26
BH8	32	74.37±1.18	8.40±0.30
BH9	32	73.43±0.85	8.90±0.33
Overall mean	**192**	**75.21±0.47**	**8.76±0.14**

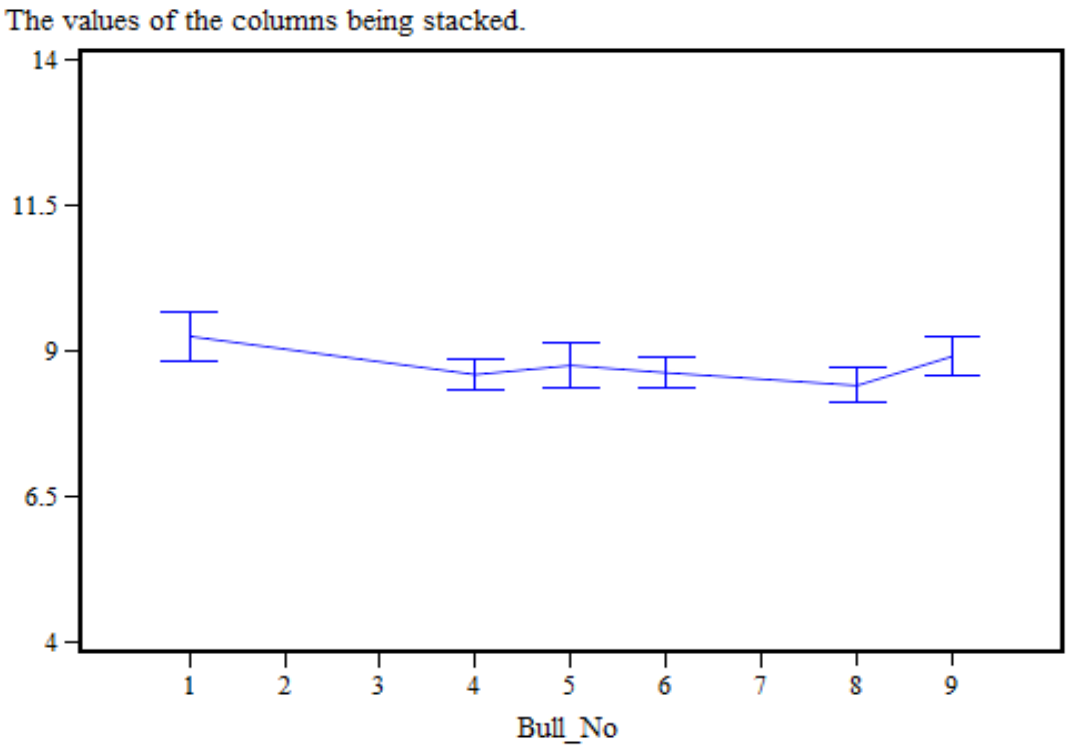

Fig. 20: Mean Distribution of Sperm Abnormality of different bulls

DISCUSSION

5.1 Service behaviour

The service behaviour of breeding bulls used for artificial insemination is not only important for maximizing semen production but also advantageous to herd fertility which has probable beneficial effects on the fertility of subsequent female progeny. The interest in bull sexual behaviour dates back to the first literature published on bull libido in 1941 and after various observations and field trials with the semen collected from the selected bulls, a widely accepted nomenclature regarding breeding soundness examination of bulls has been put into practice. In an organized bull station, different bulls are being utilized as per necessity of the existing agro-climatic region though very little information on sexual behaviour of bulls is available. Despite routine screening of bulls for libido and mating ability, it is evident that a number of substandard bulls have also been considered as satisfactory potential breeders. However, James (1950), Olsen and Petersen (1951), Bane (1954) and Blockey *et al.* (1978) were of opinion that both libido and mating ability are important in bulls and there is ample evidence to substantiate that the two traits are influenced by genetic factors.

There is also an interesting possibility that bull libido and female fertility are linked genetically in a similar fashion to scrotal circumference in bulls and age at puberty in females (Brinks *et al*., 1978). This has been substantiated by Kilgour (1980) that female progeny of high libido rams have significantly higher cycling and lambing rates than female progeny of rams of low libido. So it is probable that genetic linkages occur between other fertility components in both male and female cattle.

Basing on all these aspects the present investigation was undertaken to evaluate the breeding Binjharpuri bulls used at sperm stations with respect to their service behaviour index and were graded basing on their performances related to different sexual behaviours. The patterns of male sexual behaviour as per Hafez and Bouisou (1975), encompassing sequential behavioural elements like approach to test site, eagerness to approach stimulus, approach to dummy, sniffing, Flehman's reaction, reaction time, protrusion of penis, mounting on dummy and libido were studied.

The effect of service behaviour has been summarized by Anderson (1945) where he differentiated sexual behaviour into two components such as libido (sex drive) and ability to copulate (mating ability). Bull libido was extensively measured by Hultnas (1959) and was further substantiated by Hale and Almquist (1960) that accuracy of assessment could be

influenced by presence or absence of prior stimulation. In the present context, thirty two ejaculates from each bull were collected and various relevant sexual parameters were effectively monitored and judiciously evaluated during semen collection by artificial vagina. The study was conducted for four months with a view to eliminate the effect of heat, relative humidity and other extraneous factors in order to rule out the adverse psychological buildup of the bulls due to routine semen collection.

Narasimha Rao *et al*. (1996) made a descriptive study on the sexual behaviour by considering various traits in Jersey and cross bred Jersey bulls. The parameters considered in the present study were comparable to the aforesaid workers. However, the units of assessment of sexual behaviour traits were different. They have individualized each trait but the reported reaction time observed by them was higher from present study.

The sex drive rating for Binjharpuri bulls were comparable to that of NarasimhaRao *et al*. (1996), where they observed a stronger sex drive and a better copulatory thrust for Jersey bulls compared to others.

Mandal and Tyagi (2004) observed copulatory behaviour and relevant sexual performance in Sahiwal bulls. They have assessed various sexual behaviour indices on the basis of libido scoring. They have studied parameters like reaction time, erection score and protrusion score, intensity of thrust and dismount time in various seasons, both for adult and young bulls. Although many of the parameters considered in the present study were similar in many respects to the aforesaid worker, the scoring pattern bears a little deviation.

The scoring indices in the present study were formulated as 1, 2, 3 and 5 for poor, average, good and excellent, respectively as per the degree of intensity of each individual sexual trait. Further, this scoring index was uniform for all the parameters to rule out error of judgment.

Although the present scoring system could not be validated due to paucity of literature but similar scoring pattern have been recorded in other domestic animals like horse (Dixit *et al*., 1999) and yak (Kataktalware *et al*., 2008).

In India rearing of Binjharpuri breed is limited and reports regarding their sexual behaviour are not available, this being a newly registered breed of dairy cattle of Odisha. Moreover, the sexual behaviour scoring pattern undertaken with bull performance was a new concept to screen good and poor performer. It is not possible to justify or corroborate this new concept of scoring of service behaviour due to lack of relevant literature. The present scoring

pattern has also been followed by Mishra (2011) while studying on poor and good freezable Jersey bulls in Odisha.

This type of analysis of service behaviour could be a useful tool for basic and comprehensive assessment of service behaviour of breeding bulls in frozen semen stations.

The objective and selection of highly fertile bulls not only relied upon phenotypic characteristics but also necessitates examination of seminal attributes which are imperative to differentiate between high and low fertile bulls. Routine semen analysis of bull semen pertaining to volume, mass activity, sperm concentration, live sperm percentage and sperm morphology are useful undertakings for assessment of semen quality and subsequent extension. The routine semen analysis comprising six Binjharpuri bulls were carried out by taking thirty two ejaculates from each bull and are presented in table 1 to 11 and fig. 11 to 17.

5.2 Semen volume

No doubt semen volume is an important productive parameter for a breeding bull, but the quality has been of paramount importance as far as production of frozen semen is concerned. However volume is highly variable and should be within a range. For frozen semen purpose, the minimum acceptable limit is one ml. (MSP, 2005). The overall mean volume of Binjharpuri bull was recorded to be 3.76±0.09 which ranged between 3.5 to 4.1 ml (table 12, fig.18). This finds the support of most of the workers (Bhoite *et al*., 2008 and Bhakat *et al*., 2011) experimenting on indian breeds of cattle. However higher semen volume in Haryana breed and lower volume in different buffalo breeds have also been recorded (Bhosrekar, 1990).

Ejaculate volume (ml), Sperm concentration (million/ml) and Sperm concentration per ejaculate (million) were 3.81±0.03, 2.36±0.02, , 792.36±6.09 respectively in Sahiwal Bulls (Bhakat *et al*.,2011).However, higher semen volume have been reported by Bhat and Chauhan(1982), Singh and Pangawkar (1990) and Moharatha (2001).

5.3 Mass activity and sperm concentration

Mass motility is the sum total effect of individual motility of spermatozoa in undiluted semen. The index of motility is represented by the intensity of their collective motion exhibiting waves, eddies and swirls of different degrees. It is a subjective measurement and can give a tentative assessment of sperm concentration and their individual motility. The mass motility of spermatozoa immediately after collection is commonly used as a measure of fertilizing ability of sperm (Roberts, 1971). Blom (1950) indicated that bovine

semen with low initial motility were associated with infertility. However, the initial motility estimation could only detect the gross differences in semen quality but it has a limited value in detecting fertility differences (Maule, 1962). Mass motility represented by immotile weakened rotatory movements with no waves or eddies are considered to be associated with poor conception rate (Swanson and Herman, 1944). The present findings partially corroborates the finding of Belorkar *et al*. (1990), Gupta *et al*. (1990) and Maharatha (2001) in cross bred and pure bred Jersey bulls. Conversely higher values for mass motility have also been reported in pure bred Jersey bulls by NarasimhaRao *et al*. (1996), Johar *et al*. (2006) and Pandey *et al*. (2010). The variation in mass motility may be possible depending upon the interval between collection and examination. Further, variation in mass motility could occur in ejaculates with poor sperm concentration and viability. In the present study the high libido bulls showed significantly higher mass activity values compared to bulls having lower service behaviour indices. As the selection process is based on various sexual behaviour, which have related with genetic, endocrine and sexual orientation that are reflected in their seminal attributes.

The mean values for mass activity of Binjharpuri bulls was found to be 2.74±0.07. The mass activity of sperms is considered as viability characteristic and is determined through microscope by low magnification. The mean numerical value for mass activity of the sperm collected in June, 2013 was 2.49, 3.83 and 2.16 respectively (Kiani *et al*., 2014). The mean mass activity of semen (0-5 scale) estimated in the Hallikar cattle (considered as one of the premier draught breed of India) was 4.32 ± 0.01 with range of 0.50 to 4.50 (Prem Kumar *et al*., 2020).

The sperm concentration recorded in the present study for aforesaid bulls were at par with observations made by Johar *et al*. (2006), whereas higher values of sperm concentration were reported by NarasimhaRao *et al*. (1996), Maharatha (2001) and Pandey *et al*. (2010) in pure bred Jersey bulls along with Belorkar *et al*. (1988), Rao *et al*. (1996) and Mohanty (1999) in cross bred bulls. However it is in consonance with the findings of many authors (Prem Kumar *et al*., 2020; Kiani *et al*., 2014) experimenting in different Indian breeds of cattle. The variation in volume and sperm concentration may be due to genotype, management, nutrition, climatic effect, age, type of bio-stimulation and method of collection.

5.4 Sperm motility

One can define individual motility percentage as, spermatozoa with any movement in microscopic field called sperm individual motility (Khan *et al.*, 2017).The mean values for

pre-freeze motility and post thaw motility of semen of breeding bulls has been presented in table 14.

The individual motility of Kankrej bull semen ranged between 80 to 92 per cent with the mean values of 86.15 + 0.30 per cent. Individual motility of spermatozoa in present study was at par with reports of HF bulls, Sahiwal and Jersey bulls (Patel *et al*., 2013). Pure breed bulls show significantly higher values of individual motility than cross bred bulls.

The findings are in accordance with the reports of Robbins *et al*. (1976) and Singh *et al*. (1997) in pure bred Jersey and that of Bonia *et al*. (1980), Tuli *et al*. (1985) and Mohanty (1999) in cross bred bulls. On the other hand higher values for individual motility have been reported by Sagdeo *et al*. (1990), NarasimhaRao *et al*. (1996), Johar *et al*. (2006) and Pandey *et al*. (2010) in other Indian breeds.

Assessment of pre-freeze motility is a valuable index to distinguish between fertile and infertile sperms (Roberts, 1971). Although, motility may not be required for transportation of spermatozoa in lower tubular genital tract but it is essential for distributing sperm cells in the infundibulum randomly in which fertilizable ovum is found, thus ensuring successful ovum spermatozoa encounter (Salisbury *et al.,* 1985).

Available evidence indicates that motility facilitates sperm transport through cervix and it is essential in oviduct for fertilization (Asdell and Salisbury, 1941). The importance of actively motile sperm cells in the ejaculate and the fertility has been stressed in Guernsey bulls used in artificial insemination. It has been reported that gradual decrease or increase in motility is positively correlated with fertility (Roberts, 1971).

In the present study the values of pre-freeze motility and post thaw motility for Binjharpuri bulls were comparatively lower than the findings of various workers studied in other zebu cattle. Significant decrease in post thaw motility per cent is obvious as the freezing process drastically reduces the number of sperms. The appreciable loss of spermatozoa during freezing may be due to exposure to very low temp (-196° C) and their inherent ability to withstand cold shock. Besides that some genetic factors and individual idiosyncrasy might have contributed towards this loss during freezing.

Similar reports of decline in motility in post thaw samples have also been reported by many workers (Sagdeo *et al*., 1990; Singh *et al*., 1997; Singh and Pant, 1999; and Moharatha, 2001).

Incidentally during service behaviour index studies, the motility and viability percentages of 192 ejaculates were clubbed for all the bulls, without omitting any semen sample with lower values for the aforesaid seminal traits. For this reason the mean values for individual and post thaw motility were relatively lower compared to contemporary workers. However, ejaculates having more than 70 per cent initial motility and 50 per cent post thaw motility were only considered for acceptance of frozen semen straws for artificial insemination.

The testicular functions and secretions from accessory sex glands have an overriding influence on motility, which is an inherent character of spermatozoa. Two contractile proteins i.e. spermosins and flactins have been isolated from sperm tail and the energy source for tail movement is from ATP which is maintained by glycolytic respiration. As such the ejaculates showing low motility value might have variable aforesaid components meant for spermatozoa propagation. All these factors might have contributed for exhibiting relatively low motility in bulls manifesting low sexual libido.

5.5 Livability and abnormality

Gangwar *et al*. (2018) stated that Dead spermatozoa could be differentiated by their ability to be stained by eosin dye. The live spermatozoa, which are alive at the time of staining, remain colourless since they were impermeable to the Eosin stain.

Sabes-Alsina *et al*. (2019) stated that Husbandry conditions for bulls used for semen collection should be adapted to allow the animals' physiological responses for temperature regulation to operate fully so that the effects of increased temperature and humidity can be mitigated. Extreme of temperature and humidity should be avoided. During the present study similar strategy was adopted to ascertain the live sperm % and the abnormal sperm %. The present finding of overall mean live % of 75.21±0.47 (table 15) and sperm abnormality of 8.76±0.14 % (table 15 and fig. 20) finds the support of minimum standard protocol fixed for sperm stations by Govt. of India which describes the minimum permissible limit of livability of neat semen as 70% with maximum of 15% abnormal sperm. Since the present study satisfied both the criteria, much of the literatures were not reviewed to support our findings. Moreover the low livability % might be attributed to very young bulls being put to use.

Finally, it can be postulated that this being the first report of semen parameters of Binjharpuri bulls, further controlled studies can be taken up in future to establish the findings.

SUMMARY AND CONCLUSION

The present investigation was carried out in the Department of Animal Reproduction Gynaecology and Obstetrics & Frozen Semen Bank, Cuttack with an objective to evaluate the sexual behaviour of breeding Binjharpuri bulls during collection of semen and its influence on semen quality. To achieve this objective, the service behaviour of breeding Binjharpuri bulls were assessed with a scoring system and the quality of the corresponding ejaculates were evaluated by routine semen analysis.

Six healthy and matured Binjharpuri bulls were included in the present investigation. Depending on the intensity of their sexual behaviour manifested during semen collection, each bull was ascribed with an index, termed as service behavior index (SI) basing on which the bulls were evaluated. However the SI was computed from 10 different qualitative parameters. Approach to test site has got the overall mean value of 3.01±0.09, while the other parameters have recorded the values as eagerness to approach stimulus (2.99±0.09), approach to dummy (3.46±0.08), sniffing and licking (2.54±0.04), Flehman's reaction (2.59±0.04), libido (2.59±0.04), protrusion of penis (2.94±0.04), mounting on dummy (3.50±0.08), copulatory thrust (2.60±0.04) and reaction time (2.00±0.06). The overall mean of SI is recorded to be 2.82±0.03 (table 11) during the present study.

A total of 192 ejaculates were collected with equal number of samples (32) from each Bull. The quality of ejaculates were evaluated by routine semen analysis like volume, concentration, mass activity, live sperm count, individual motility and post thaw motility.

The overall mean semen volume (ml), concentration (millions/ ml) and mass activity (0-5 scale) were recorded to be 3.76±0.09, 888.71±36.05 and 2.74±0.07 respectively. The overall mean values with standard error for pre-freeze motility, Post thaw motility, livability, sperm abnormality are recorded to be 77.66±1.67, 44.04±1.37 and 75.21±0.47 and 8.76±0.14 respectively.

CONCLUSION

The following are the overall mean service behaviour index and seminal parameters of Binjharpuri breeding bulls.

Service Behaviour Index (1-5)	:	2.82±0.03
Semen Volume (ml)	:	3.76±0.09
Semen Concentration (million/ ml)	:	888.71±36.05
Mass Activity (0-5)	:	2.74±0.07
Livability (%)	:	75.21±0.47
Pre-freeze motility (%)	:	77.66±1.67
Post-thaw Motility (%)	:	44.04±1.37
Sperm Abnormality (%)	:	8.76±0.14

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