



Testicular biometry and semen quality is not altered by the process of fine needle aspiration in crossbred bulls

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ABSTRACT

Testicular fine needle aspiration cytology (FNAC) is widely being used in humans to evaluate the functional testicular mechanism, but its application is very limited in animals especially breeding bulls. This preliminary study was conducted to assess the effect of testicular fine needle aspiration (FNA) technique on testicular biometry and seminal characteristics in Karan Fries crossbred bulls. Eight bulls within the age range of 4–6 years were utilized for study. Before and after fine needle aspiration, testicular parameters were measured and seminal quality was assessed as per standard protocols. There was no significant difference observed in scrotal circumference and testicular length before and after the treatment. The testicular width was 6.59 ± 0.32 and 7.08 ± 0.27 during pre- and post- aspiration, while the corresponding values for testicular mass were 355.77 ± 39.20 and 427 ± 28.75 , respectively. There was no significant changes observed in ejaculate volume, mass activity and individual motility in bulls during pre-FNA and post-FNA period. Similarly, the percentage of live spermatozoa, membrane intact spermatozoa and acrosome intact spermatozoa did not differ significantly between pre-FNA and post-FNA period. These results indicated that testicular fine needle aspiration technique can be used as a routine diagnostic method to detect sub-fertility and infertility in crossbred bulls without affecting their reproductive health.

Key words: Crossbred bull, Testicular fine needle aspiration, Testicular parameters, Seminal characteristics

In India, infertility and sub-fertility are the major reasons for disposal of large number of breeding bulls from semen stations. More than 50% crossbred young bulls, which are progenies of elite dams and proven sires, inducted for semen collection are straightaway rejected because of poor semen quality and low cryosurvivability of spermatozoa (Mandal and Tayagi 2004, Mukhopadhyaya *et al.* 2010). In Karan Fries crossbred (Holstein-Friesian $\times$ Tharparkar) bulls it was reported that the ejaculate rejection rate (owing to poor initial semen qualities) ranged from 26 to 74% (Vijetha 2011). There are also reports citing increased sperm abnormalities (even up to 70%) in crossbred bulls (Khatte 2005, Chauhan 2007).

Almost half of the reserved stock (for future breeding) is disposed due to poor quality semen production. This clearly indicated that the present method used for selection of the

future breeding bulls could not predict the future semen production ability and fertility. The use of sub-fertile bulls in dairy production system may lead to lower calving percentage, extended calving interval, reduced genetic progress, expense of carrying empty cows, wastage of dairy bulls and finally loss to the dairy industry in terms of labour, money and time. Thus, precise determination of fertility of a bull is very important to avoid the loss associated with maintaining unproductive bulls. Several methods were tried to appraise the grounds for sub-fertility and infertility bull but the results were not satisfactory. Testicular fine needle aspiration cytology (FNAC) is a technique that is routinely used to diagnose infertility in human (Jha and Sayami 2009, Agarwal *et al.* 2004, Arora *et al.* 2000). Although FNA procedure is proven to be a less painful, simple and minimally invasive procedure in human, the technique is not widely practiced in animals. Only few reports are available on use of FNA to diagnose sub-fertility in bulls (Chapwanya *et al.* 2008), horse (Leme and Papa 2010) and dog (Dahlbom *et al.* 1997, Johnston *et al.* 2000, De Souza *et al.* 2004). Moreover, the effect of FNA procedure on the testicular characteristics and semen quality has not been studied in detail especially in bulls. Before using the technique as a routine diagnostic

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tool, we should make sure that the procedure do not have any harmful effect on the testicular function and the quality of semen produced, and there is lack of reports on this aspect. Hence an investigation was conducted to study the effect of testicular fine needle aspiration (FNA) technique on testicular biometry and seminal characteristics in Karan Fries crossbred bulls.

MATERIALS AND METHODS

Experimental animals and their management: The present investigation was conducted on Karan Fries (Tharparkar × Holstein-Friesian (HF) crosses between 50 to 75% exotic inheritance) breeding bulls maintained at Artificial Breeding Research Complex, Karnal, Haryana, India. Randomly 8 bulls were selected from 4–6 years of age. Bulls were kept in individual pens (30¢×10¢) under loose housing system and fed with 2.5 kg concentrate ration containing 21% CP and 70% TDN. Seasonal green fodder such as maize, cowpea, berseem, jowar etc., depending on their availability, along with mixture of maize and oat silage was fed *ad lib* to the animals. The bulls have free access to clean drinking water throughout the day. Vaccination, de-worming, regular check-up for communicable diseases and other herd-health programmes were followed as per the farm schedule, to protect the animals from diseases.

All the experimental procedures and animal experimentation methods were approved by the Institutional Ethical Committee.

Testicular fine needle aspiration cytology (FNAC): FNAC was carried out under epidural anaesthesia (2% lignocaine hydrochloride @ 7–10 ml) after proper restraining. Once desensitization of the posterior part of animals was achieved, the posterior parts of testes were held tightly with hand and antiseptic solution was applied over the scrotum. A 22 gauge needle attached to 5 ml syringe was inserted into the testis through scrotum at right angle to the testis. When plunger was pulled back, the needle was moved little forward and backward within the testis 2 to 3 times for approximately 4 sec for dislodging of cells and easy suction into the needle. After aspiration, animals were treated with an antibiotic @ 6.6 mg/kg body weight subcutaneously and anti-inflammatory drug @ 2–3 mg/kg body weight intramuscularly.

Testicular parameters assessment: Testicular parameters, viz. scrotal circumference (SC), testicular width, length, volume and mass were measured 1 week before and after testicular FNA as method described by society for Theriogenology (Ball *et al.* 1983). SC was measured with metal scrotal tape at widest point of circumference. The tape was manually tightened with slight pressure on the scrotum and the measurement was recorded and other testicular parameters like total width of testis, length of individual right and left testis were measured using digital callipers. Average values of length of both right and left testis for each bull were taken as length of testis for that particular bull. Testicular

length (TL) and width (TW) were used to calculate testicular mass (TM) and volume (TV), considering the testicle as a prolate spheroid. The following mathematical formulas were used as reported by Bailey *et al.* (1996).

Semen quality assessment: Semen samples were collected at 15 days interval for 2 months before FNA and at day 7, 15, 30, 60, 75 and 90 days after FNA from all 8 bulls, 2 ejaculates on the same day with 15 min rest between successive ejaculates. Semen was evaluated for mass activity, individual motility, viability, sperm concentration and membrane integrity of spermatozoa. The mass motility was measured in 0 - 5 scale on the basis of swirls and eddies activity. The percentage of motile spermatozoa were measured by mixing 100 µl of undiluted semen into pre-warmed 2 ml eppendorf tubes containing 900 µl of Tris buffer. A thin drop of diluted semen was placed on a pre-warmed glass slide (37 °C) and allowed to spread uniformly under the cover slip (18 × 18 mm) and examined under 200 × phase contrast microscope provided with a warm stage.

The sperm concentration was estimated using the haemocytometer (Salisbury *et al.* 1985) and expressed as million spermatozoa per ml. Sperm viability was determined by Eosin-Nigrosin stain (Campbell *et al.* 1953). Total or partly stained spermatozoa were considered as dead. The membrane integrity of spermatozoa was evaluated using hypo osmotic swelling test method as described by Jeyandran *et al.* (1984). For sperm viability, acrosome integrity and membrane integrity at least 200 sperm per smear were counted using tally counter. All the semen evaluation was performed by same person throughout the study. Data obtained were analyzed statistically by Sigma Plot 11® software package. Paired t-Test was used for analysis of testicular and semen quality parameters before and after FNA.

RESULTS AND DISCUSSION

Effect of FNA on testicular parameters: The effect of FNA procedure was studied on testicular biometry parameters viz. scrotal circumference, testicular length, width, volume and mass in all the eight bulls. Results of various testicular parameters recorded before and after the FNA technique are depicted in Table 1 and Fig. 1. No significant difference was observed in scrotal circumference and testicular length

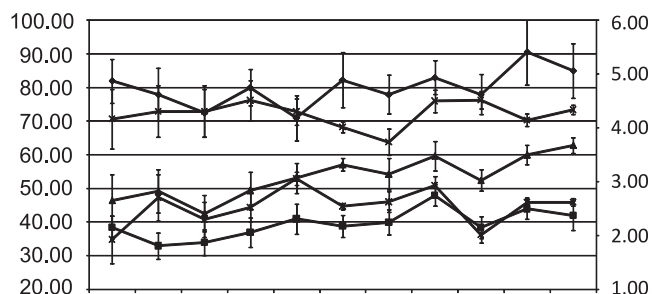


Fig. 1. Pre- and post- FNA ejaculate quality in crossbred bulls.

Table 1. Effect of fine needle aspiration (FNA) procedures on testicular characteristics (mean±SE) in bulls (n=8)

Parameters	Pre- FNA	Post- FNA
SC (cm)	38.07±0.81	38.50±0.70
TL (cm)	14.60±0.24	15.31±0.31
TW (cm)	6.59 ^a ±0.32	7.08 ^b ±0.27
TV (cm ³)	338.25 ^a ±37.09	404.28 ^b ±27.21
TM (g)	355.77 ^a ±39.20	427.2 ^b ±28.75

SC, Scrotal circumference; TL, testicular length; TW, testicular width; TV, testicular volume; TM, testicular mass; mean with different superscripts within a row differ significantly (TW, P=0.008; TM, P=0.021; TV, P=0.021).

between pre-FNA and post-FNA. But, the testicular width, testicular volume and testicular mass showed a significant difference between pre-FNA and post-FNA with comparatively higher values in post-FNA. The mean±SE testicular width was 6.59±0.32 during pre-FNA assessment while it was 7.08±0.27 during post-FNA assessment, while the corresponding values for testicular mass was 355.77±39.20 and 427±28.75, respectively.

To find out why there was an increase in testicular width after FNA, we looked into the raw data of individual bulls and found that there was not much change in the testicular width of 7 bulls after FNA but 1 bull showed much increase in testicular width (from 6.75 cm during pre-FNA to 7.7 cm during post-FNA measurement). This might be the reason for overall significant increase in testicular width leading to significant increase in testicular volume and mass. In spite of an increase in testicular parameters, the particular bull mounted normally and ejaculated semen with characteristics normal to that bull on the very next day of FNA. Further we have monitored the rectal temperature of the bulls daily up to a week after FNA and observed no fever in any of the bulls. In bulls, no literature is available on this aspect to compare the findings of the present study. But, similar reports are available on dogs and stallion. No clinical changes were observed in dogs during and after FNA procedure (De Souza *et al.* 2004) and no side effects of this procedure on libido and semen quality even after repeated FNA in dogs (James *et al.* 1979, Dahlbom *et al.* 1997). In line with our observation, Leme and Papa (2010) observed a small hematoma after FNA in stallion, which subsided after 48 h and there were no difference in seminal volume, sperm concentration, total sperm count, sperm motility and sperm morphology before and after testicular FNA.

Effect of FNA on semen quality parameters: The effects of FNA procedure on seminal parameters were studied (Table 2). There was no significant change observed in ejaculate volume (4.63±0.33 vs 4.73±0.20), mass activity (2.32±0.33 vs 2.42±0.22), and individual motility (53.15±4.29 vs 58.60±3.19) in bulls during pre-FNA and

Table 2. Effect of FNA procedures on semen quality (mean±SE) in bulls (n=8)

Parameters	Pre-FNA	Post- FNA
Volume (ml)	4.63±0.33	4.73±0.20
Mass activity (0–5 scale)	2.32±0.33	2.42±0.22
Individual motility (%)	53.15±4.29	58.60±3.19
Live spermatozoa (%)	77.4±2.19	71.16±3.33
Sperm concentration (10 ⁶ /ml)	761.40±52.62	768.72±33.98
Membrane intact spermatozoa (%)	42.64±3.32	42.06±2.24

post-FNA period. Similarly, the percentage of live spermatozoa and membrane intact spermatozoa did not differ significantly between pre-FNA and post-FNA period. Our results clearly indicated that the FNA procedure does not affect the ejaculate quality in crossbred bulls. As indicated earlier, FNA technique did not had adverse effect on libido and seminal quality in dog and horse (Dahlbom *et al.* 1997, Leme and Papa 2010). No literature was available regarding effect of FNA technique on seminal quality in bull to compare our results.

Since early 20th century, testicular cytogram has evolved as a means for the evaluation of spermatogenic function in infertile men. Testicular functions can be appraised by determining various cell indices of spermatogenic cells, Sertoli cells and Leydig cells. In humans, the testicular cytogram is being evaluated through many methods, like open method, split needle biopsy, needle punch biopsy and fine needle aspiration cytology (FNAC). Compared to other techniques, testicular FNAC is considered as simple, quick and less invasive method to evaluate spermatogenesis (Rajwanshi *et al.* 1991, Foresta and Varott 1992). Representative samples obtained using testicular FNAC is routinely used to study the details of testicular cytogram and to identify the fertility status of a male in humans (Meng *et al.* 2001). But in animals very limited reports are available about these techniques, especially the use of FNAC for studying testicular cytogram (Chapwanya *et al.* 2008, Bagley and Chapman 2005) and reports were available about the effect of FNA on testicular parameters and semen quality.

Thus based on findings of present study, it can be concluded that the FNA procedure is safe and can be employed in crossbred bulls to study the testicular cytogram to evaluate sub-fertility and infertility in crossbred bulls without affecting their testicular and seminal characteristics.

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REFERENCES

- Agarwal M S, Gupta A, Chaturvedi K and Lavania P. 2004. Assessment of the utility of testicular FNAC in infertile males with special reference to differential counts. *Indian Journal of Urology* **20**(2): 148–53.
- Arora V K, Singh N, Bhatia A, Rashmi R G, Jain B P and Agrwal N. 2000. Testicular fine needle aspiration cytology for the diagnosis azoospermia and oligospermia. *Acta Cytologica* **44**(3): 349–56.
- Bagley C V and Chapman C K. 2005. Breeding Soundness Evaluation of bulls. *Animal Health, Utah state University* **1**: 1–2.
- Bailey T L, Monke D, Hudson R S, Wolfe D F, Carson R L and Riddell M G. 1996. Testicular shape and its relationship to sperm production in mature Holstein bulls. *Theriogenology* **46**: 881–87.
- Ball L, Ott R S, Mortimer R G and Simons J C. 1983. Manual for breeding soundness examination of bulls. *Journal for Society of Theriogenology* **12**: 1–65.
- Campbell R C, Dott H M and Glover T D. 1956. Nigrosin-eosin as a stain for differentiating live and dead spermatozoa. *Journal of Agricultural Science* **48**: 1–8.
- Chauhan I. 2007. 'Influence of genetic and non-genetic factors in semen production in Karan Fries bulls.' M.V.Sc Thesis, NDRI (Deemed University), Karnal, Haryana, India
- De Souza F F, Leme D P, Uechi E, Trinca L A and Lopes M D. 2004. Evaluation of fine needle aspiration cytology and serum testosterone levels in dogs. *Brazilian Journal of Veterinary Research and Animal Science* **41**: 98–105.
- Foresta C and Varott A. 1992. Assessment of testicular cytology by fine needle aspiration as a diagnostic parameter in the evaluation of the oligospermic subject. *Fertility and Sterility* **58**: 1028–33.
- James R, Heywood W R and Fowler D J. 1979. Serial percutaneous testicular biopsy in the Beagle dog. *Journal of Small Animal Practice* **20**: 219–28.
- Jeyendran R S, Vander Ven H H, Perez Pelaez M, Crabo B G and Zaneveld L J D. 1984. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. *Journal of Reproduction and Fertility* **70**: 219–28.
- Jha R and Shayami G. 2009. Testicular fine needle aspiration in evaluation of male infertility. *Journal of Nepal Medicine Association* **48**(173): 78–84.
- Johnston S D, Root Kustritz M V and Olson P N S. 2000. Clinical approach to infertility in the male dog. *Canine and feline Theriogenology*. pp. 370–95. W B Saunders, Philadelphia.
- Khate K. 2005. 'Studies on multistage selection of dairy bulls.' M.V.Sc. Thesis, National Dairy Research Institute, Deemed University, Karnal, Haryana, India.
- Leme D P and Papa F O. 2010. How to perform and interpret testicular fine needle aspiration in stallion. *Journal of Equine Veterinary Science* **30**(10): 590–96.
- Mandal D K and Tyagi S. 2004. Pre-copulatory behavior of Sahiwal bulls during semen collection and effects of age and season on their sexual performance. *Indian Journal of Dairy Science* **57**(5): 334–38.
- Meng M V, Cha I, Ljung B M and Turek P J. 2001. Testicular fine needle aspiration in infertile men : Correlation of cytologic pattern with biopsy Histology. *American Journal of Surgery and Pathology* **25**(1): 71–79.
- Mukhopadhyay C S, Gupta, A K, Yadav B R, Khate K, Raina, V S, Mohanty T K and Dubey P P. 2010. Subfertility in males: an important cause of bull disposal in bovines. *Asian - Australasian Journal of Animal Sciences* **23**: 450–55.
- Rajwanshi A, Radhika S, Vaidyanathan S. 1991. Fine needle aspiration cytology in azoospermic males. *Diagn Cytopathology* **7**: 3–5.
- Salisbury G W, Van Denmark N L and Lodge JR. 1985. *Physiology of Reproduction and Artificial Insemination of Cattle*. 2nd edn. CBS Publication and Distributors, New Delhi, India.
- Vijetha B T. 2011. 'Pedigree analysis of semen quality and its relationship with fertility in Karan Fries crossbred bulls.' M.V.Sc Thesis, NDRI (Deemed University), Karnal, Haryana, India.