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Breeding soundness evaluation of stall-fed bucks using ultrasonography

M SINGLA[1], A L SAINI[2], AJEET KUMAR[3], S KASWAN[4] and P S BRAR[5]

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab 141 004 India

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Breeding soundness evaluation (BSE) facilitates elimination of sub-fertile bucks from breeding programmes. It is therefore, necessary to develop fertility markers for early BSE and to improve productivity and genetic gain. Amongst various techniques, ultrasonographic evaluation of reproductive organs i.e. testes is an emerging technique for on-farm situations for differentiating potential males (Gnemmi and Lefebvre 2009) and can be correlated with semen quality (Barth *et al.* 2008). Furthermore, very little literature is available regarding ultrasonic BSE of indigenous bucks. Therefore, the present study was designed to assess the breeding soundness of bucks using ultrasonography and to compare the information with semen quality under stall-fed conditions.

Bucks (23: 20 Beetal and 3 cross (75% Beetal and 25% Boer inheritance)), sexually mature (mean age and weight 935.09±86.51 days and 55.10±2.27 kg, respectively) were distributed into 3 groups on the basis of ultrasonic examination of testicles. Bucks with mild (7) and extensive (5) fibrotic testicular lesions in one or both the testes were enrolled under T_1 and T_2 groups against T_0 with normal (11) homogenous pattern of echo texture with no visible lesions. All the animals were fed according to standard feeding schedule along with *ad lib*. green fodder. Live body weights (LBW) of all the bucks were recorded in the morning, before offering any feed and water. Ultrasonographic examinations of the testes were done with a B-mode ultrasound scanner connected to 5.0 MHz linear array transducer (Fig. 1a,b). Ultrasonographic probe of 5 MHz was placed halfway between the hook bone and the pin bone to measure rump fat thickness (RFT, Fig. 1c) in mm (Khushpreet *et al.* 2015). Scrotal circumference (cm) was measured with a looped measuring tape taken transversely at the greatest diameter of the scrotum. Testes were also examined by visual assessment and palpation for testicular tone.

Three semen samples (per buck) were collected at an interval of 2 days from 6, 6 and 5 donor bucks in T_0, T_1 and T_2 groups, respectively using artificial vagina (AV) connected to graduated collection tube. Immediately after collection and recording of volume, semen was graded descriptively for colour with unaided eye. Gross motility, sperm motility and percent live sperms were estimated as Bujarbaruah and Kumaresan (2013). Sperm concentration

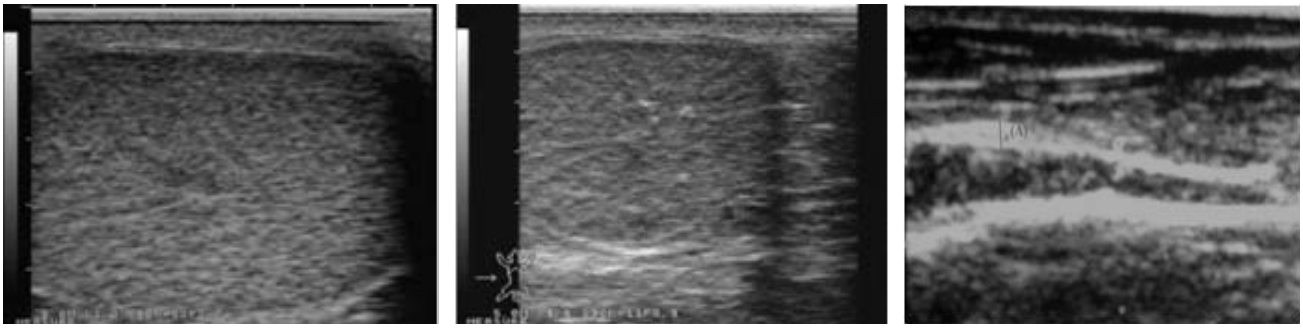

Fig 1. Ultrasonographic evaluation showing normal echo-texture (a), fibrosis (b) in testicular parenchyma and rump fat thickness (c).

Present address: [1]Assistant Animal Scientist (mandeep.bank @gmail.com), [2]Professor and Head (sainial@yahoo.co. in), [4]Assistant Professor (deepu02vet@ gmail.com), Department of Livestock Production Management. [3]Assistant Professor (ajeetvet@yahoo.com), [5]Professor and Head (parkashbrar @gmail.com), Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science.

($\times 10^6$/ ml) was recorded with Accucell photometer (IMV Technologies, L'Aigle, France). Semen pH was tested using digital FiveEasy^{TM}PluspH meter (Mettler-Toledo AG, 8603 Schwerzenback, Switzerland). All procedures involving animals were approved by the Institutional Animal Ethics Committee. The statistical significance of the mean

differences between groups was analyzed by ANOVA, with Duncan's multiple range tests using IBM SPSS 22.0 statistical software at P<0.05.

The mean age (days) of bucks in T_2 (1389.40±299.03) was statistically (P<0.05) higher than those in T_1 (830.14±62.77) and T_0 (795.36±68.29). The mean LBW (kg) was 6.70% and 19.88% more in T_1 and T_2 over T_0, respectively though difference was not statistically significant (Table 1). It indicates that with advancement of age, the fibrosis of testicular tissue also increased. Here it is also important to mention that appearance of fibrosis in any buck was not associated with any clinical symptoms resembling orchitis, pain, fever, lethargy, off-fed etc. Similarly, it was found in bulls that fibrotic lesions appeared as early as 5–6 months of age and continued to increase with ageing (Barth *et al.* 2008). The mean RFT (mm) was significantly (P<0.05) higher in bucks with extensive testicular fibrosis (3.42±0.30) over bucks under mild (2.51±0.27) and no fibrosis (2.05±0.12). This could be an important observation for selection and judging of bucks under field conditions of traditional farming where no authenticated documents of age and body weight are available. It is indicated that bucks with higher RFT tends to have higher fibrosis or poorer semen characteristics. In the same way, Khushpreet *et al.* (2015) reported significantly (P<0.05) higher RFT (mm) in poor libido buffalo bulls (7.29±0.93) over high libido buffalo bulls (5.29±0.62). Scrotal circumference (mean±SE, cm) was recorded nonsignificantly higher in T_0 (26.72±0.76) and T_1 (26.31±0.91) over T_2 (25.65±0.46). Likewise, nonsignificantly higher SC was reported in high libido buffalo bull over low libido buffalo bull, respectively (Khushpreet *et al.* 2015). On the contrary, Barth *et al.* (2008) found no association between SC or testis size and fibrosis.

Bucks with extensive fibrosis showed abundance of hyperechoic areas scattered in the testicular parenchyma, acoustic shadowing, showing testicular degenerations with mineralization and many anechoic areas in the testicular parenchyma. Out of total 46 right and left testes of 23 bucks scanned, extensive, mild and no fibrosis (normal) was found in 15.22, 19.57 and 65.22% of testicle's parenchyma tissue, respectively. Within T_0, T_1 and T_2, the per cent testes with variable degree of fibrosis were 0, 64.29 and 70.00, respectively. Fibrotic lesions were reported common in the testes of bulls (Barth *et al.* 2008). The causes of appearance of fibrotic lesions in testis parenchyma with advancement of age were unknown and speculative. Some of earlier workers had also failed to establish the cause of fibrosis in testicles and viewed that developmental changes of the testes might be involved in the etiology of testis fibrotic lesions (Barth *et al.* 2008). Thus, ultrasonography of external genitalia can be a powerful, applied and easy to operate non-invasive tool under field conditions for efficiently diagnosing lesions causing partial or complete loss of fertility in bucks. Similarly, it was demonstrated that ultrasound based evaluation of testicular echotexture can be used to demarcate the good and poor libido buffalo breeding bulls as variation in testicular echogenecity might affect the physiology leading to loss of libido (Singh *et al.* 2015).

Table 1. Phenotypic traits of bucks with homogenous echo-texture (T_0), mild (T_1) and extensive (T_2) hyperechoic areas in testicular parenchyma

Parameter	T_0	T_1	T_2
Number of bucks	11 (Normal)	7 (Mild)	5 (Extensive)
Number of testicles with fibrosis	0	9	7
Number of donor bucks	6	6	5
Age (days)	795.36± 68.29[a]	830.14± 62.77[a]	1389.40± 299.03[b]
LBW (kg)	51.80±3.21	55.27±2.49	62.10±6.55
Scrotal circumference (cm)	26.72± 0.76	26.31± 0.91	25.65± 0.46
Rump fat thickness (mm)	2.05± 0.12[a]	2.51± 0.27[a]	3.42± 0.30[b]

Means bearing different superscripts in a row differ significantly (P<0.05).

Bucks with no and mild testicular tissue fibrosis ejaculated same mean volume of semen, which was nonsignificantly higher than bucks with extensive fibrosis of testis (Table 2). The semen donated by all the bucks was creamy white indicating no attributing effect of testes fibrosis on semen colour. Interestingly, sperm concentration (× 10^6/ml) and total sperms (millions) ejaculated per ejaculate (mean±SE) were nonsignificantly higher in bucks with mild fibrosis over bucks with homogenous echo texture of testicular parenchyma, respectively. Whereas, bucks with abundant hyperechoic areas in testis parenchyma (T_2) yielded statistically least (P<0.05) total number of sperms per ejaculate (Table 2). From the findings, it is concluded that increase of fibrosis lead to significant (P<0.05) decline in gross motility, sperm motility and live sperm percentage. Confirming to the effect of testis lesions on semen quality, Ahmad and Noakes (1995) in an ultrasonograhic study on induced testicular lesions in goats found that development of hyperechoic areas resulted in decrease in semen volume, mass and individual motility, sperm concentration and total sperm per ejaculate, and an increase in the percentages of dead and abnormal spermatozoa. However, many researchers failed to demonstrate any association between semen quality and the extent of testis fibrosis indicating that the presence of relatively large amounts of scar tissue within the testis parenchyma did not prevent the remaining unaffected parenchyma from producing normal sperms. They had also considered areas of increased echogenicity (testicular fibrosis) common especially in young bulls and are not associated with decreased semen quality (Barth *et al.* 2008). Higher (P<0.05) mean pH was recorded in the semen of bucks with extensive fibrosis of parenchyma (Table 2). The semen pH seemed to be the least variable parameter to evaluate BSE. It was also documented that semen pH remained within normal limits in bucks suffering from acute, subacute and chronic orchitis (Njenga *et al.* 1999).

Table 2. Semen characteristics of bucks with homogenous echo-texture (T_0), mild (T_1) and extensive (T_2) hyperechoic areas in testicular parenchyma

Parameter	T_0	T_1	T_2	Normal range*
Volume per ejaculate (ml)	1.44± 0.06	1.44± 0.06	1.26 ± 0.06	0.5 to 2
Concentration ($\times 10^6$/ml)	2297.00± 93.16	2418.22± 72.07	2121.33± 136.13	1500 to 4000
Total sperm per ejaculate ($\times 10^6$)	3344.63 ± 222.87[a]	3541.52 ± 239.57[a]	2603.91 ± 154.39[b]	1250 to 5000
Gross motility	4.50 ± 0.12[a]	3.83 ± 0.09[b]	2.80 ± 0.11[c]	4 to 5
Sperm motility (%)	85.00 ± 1.21[a]	76.67 ± 1.81[b]	56.00 ± 2.14[c]	> 80.00
Live sperm (%)	85.77 ± 1.13[a]	81.14 ± 1.50[b]	69.01 ± 2.03[c]	> 80.00
Semen pH	6.41 ± 0.04[a]	6.53 ± 0.06[a]	6.99 ± 0.04[b]	6.2 to 6.7

Means bearing different superscripts in a row differ significantly (P<0.05). *Bujarbaruah and Kumaresan (2013).

The usefulness of ultrasonography along with semen evaluation in identifying normal testicular parenchyma for breeding soundness assessment had been substantiated in bucks under stall-fed conditions. The bucks with normal or mild fibrosis produced better quality semen over bucks with extensive fibrosis. Thus, the safe, non-invasive and reliable ultrasonography technique can be utilized for reproductive evaluation and selection of breeding bucks than other qualitative traits alone.

SUMMARY

Bucks (20 Beetal and 3 cross (75% Beetal and 25% Boer inheritance), sexually mature (mean age and weight 935.09±86.51 days and 55.10±2.27 kg, respectively), were distributed into 3 groups on the basis of testicular fibrotic lesions examined using ultrasonography. Bucks with mild and extensive fibrotic lesions in one or both the testes were enrolled under T_1 and T_2 groups against T_0 i.e. normal (11) homogenous pattern. Increase of fibrosis lead to significant decline in gross motility, sperm motility and live sperm percentage. It can be concluded that ultrasonographic techniques can be adopted as indicator of reproductive performance of breeding bucks for selection under stall-fed conditions.

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