Trans-scrotal Ultrasonography and Breeding Soundness Evaluation of Bulls in a Herd of Dairy and Beef Cattle with Poor Reproductive Performance

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ABSTRACT

The present study was undertaken to determine the fertility soundness of unselected bulls used for breeding based on a standard breeding soundness evaluation technique (BSE) and trans-scrotal ultrasonography (TSU). A total of 8 bulls, with the mean age of 5½ years (ranged from 3½ - 8 years) and the mean weight of 651.5kg (ranged from 480-840 kg) were evaluated. Three bulls were Friesian Sahiwal, 2 Brangus and the rest were Brahman-KK (Kedah Kelantan) cross, Simmental-KK cross and KK breed (one from each). Out of the total bulls examined, 3 (37.5%) were found to be unfit to be used for breeding due to their physical unsoundness and/or poor semen quality. The use of TSU has revealed the presence of testicular lesions in 3 bulls. The finding of TSU (suggestive of testicular degeneration) in one bull was reflected by a poor semen quality of the BSE results. Despite the presence of lesions of idiopathic unilateral hydrocele and bilateral fibrotic foci lesion in the other two bulls, the BSE findings for semen quality were not compromised. In conclusion, there was enough evidence to support our hypothesis that the observed drop in the reproductive performance of the herd was partly the result of using bulls with poor breeding soundness for fertility.

Keywords: Breeding soundness evaluation, bulls, poor reproductive performance, trans-scrotal ultrasonography

INTRODUCTION

Although artificial technologies for cattle breeding are rapidly improving and have progressively displaced natural service, as the preferred method of breeding in the dairy industries of most developed countries of the world, natural breeding is still the most common procedure used in beef cattle operations throughout the world. In U.S, for instance, more than 90% of beef cows are bred by natural service and each year, the use of bulls in natural mating programmes accounts for over 95% of pregnancies (Kreplin, 1992; Perry, 2008). Where natural breeding is employed, reproductive statistics on the farm is greatly influenced by the fertility and handling of the bulls. To impregnate a cow in a natural mating situation, the bull must produce semen of satisfactory quality and be able to successfully mount and deposit the semen in the reproductive system of the cow. Failure to meet either criterion will result in poor reproductive performance (Kreplin, 1992).

Studies done on the prevalence of bulls unfit for use in breeding programmes showed that
approximately 1 in every 5 bulls had inadequate semen quality, physical soundness, or both (Barth & Waldner, 2002). Furthermore, some survey studies have indicated infertility as the most important reason considered when culling a bull (Parkinson, 2004). Moreover, unlike a cow which is responsible for half of the genetic makeup of one calf per year, the bull which is responsible for half of the genetic makeup of a number of calves per year depends on the bull to cow service ratio. This makes determining the potential fertility of the bull much more important than determining the fertility of any individual cow. Hence, the role of the bull in the fertility of both beef and dairy cows needs to be understood, particularly in terms of the overall constraints to cattle fertility (Kreplin, 1992; Chenoweth, 1999; Parkinson, 2004).

Breeding soundness evaluation (BSE) of bulls has been extensively used for evaluation of male fertility prior to the breeding period over the past 50 years (Hoflack et al., 2008). BSE is a useful tool in identifying bulls with reduced fertility or physical problems which lower their ability to sire calves. Thus, eliminating the bulls with physical problems or reduced fertility from the breeding herd improves the overall reproductive efficiency of the herd (Bagley & Chapman, 2005).

According to the Society for Theriogenology (SFT), bull BSE comprises general and reproductive physical examination, scrotal circumference indexed for age, semen motility and sperm morphology examinations (Alexander, 2008). Additional parameters, such as trans-scrotal ultrasonography, have been reported recently to enhance the routine BSE in bulls (Chapwanya et al., 2008). Trans-scrotal scanning is a convenient, non-invasive technique which allows an examination of both palpable and non-palpable testicular lesions in goats and rams (Ahmad & Noakes, 1995), making its use ideal in farm situations (Chapwanya et al., 2008). However, the application and interpretation of its findings in determining fertility status of the bulls in relation to the routine BSE technique still require further research.

Despite the potential effect of the bulls’ fertility on the overall reproductive efficiency of the cattle herd, their role in the breeding programmes is often underestimated and the use of standard BSE for bulls before use for breeding has been overlooked. This allows poor performing bulls to be used for breeding and hence leads to reduction in an overall reproductive performance of the herd. The farm at Universiti Putra Malaysia, where this study was conducted, follows a similar extensive management system, whereby the bulls of unknown fertility status have been used without prior detail BSE. A drop in the overall reproductive performance of the cattle in the farm was observed with a number of beef and dairy cows with prolonged post-partum period and heifers with delayed age at first calving (Yimer et al., 2010). Our previous study (Yimer et al., 2010) has shown a considerable number of females having normal ovarian activity but failed to conceive. Thus, it was hypothesized that these animals are unable to become pregnant due to bulls’ fertility-related factors. As a result, this study was designed to investigate the soundness of breeding bulls for fertility in a dairy and beef cattle herd.

MATERIALS AND METHODS

Animals and Management

A total of 8 bulls with the average age of 5½ yrs (ranged from 3½ - 8 years), and with a mean body weight of 651.5 kg were used for natural mating on the farm in this study. Three of the bulls were Friesian Sahiwal, which were used to serve dairy cows of the same breed. Meanwhile, the remaining 5 bulls consisted of one KK, a Simmental-KK cross, a Brahman-KK cross, and 2 Brangus bulls. The 5 bulls were used to serve the beef females of KK cross, Brangus and Bradford crossbreeds.

The bulls were mixed with the females for breeding at a service ratio of 1 to 30. A bull will remained with the females for breeding for about 2 to 3 weeks before it was replaced by another. During the resting period, the bulls were kept in stalls with shades. Feeding was mainly
based on palm kernel cake (PKC), daily supply of 5 kg/animal, in combination with mineral supplement. Fresh grass supplement and water were provided ad libitum. As part of the herd’s health management, all the bulls were vaccinated against foot and mouth disease and haemorrhagic septicaemia. These bulls were also diagnosed to be negative for diseases, such as vibriosis and brucellosis.

**Breeding Soundness Evaluation**

**Medical history and physical examination**
A standard BSE technique, developed by the Society for Theriogenology SFT (Hopkin & Spitzer, 1997) was used in this study. Prior to semen collection, the identification of each bull, as well as the medical and breeding histories, were recorded. The general and detail physical clinical examinations were performed on each bull, following the procedure described by Alexander (2008). Moreover, information related to the evidence of pain during mounting was also recorded. General clinical examination was made by giving attention to the condition of teeth and jaws, eyes, thorax, abdomen, feet, and the legs. A detail physical examination of the reproductive organs was performed to evaluate the scrotum for abnormalities and its circumference, testicles (consistency and asymmetry), penis and prepuce for any defects, and all accessory sex glands palpated per rectum for abnormalities.

**Semen collection**
A total of 32 semen collections (4 from each bull on average) were made at 2 to 3 weeks interval. Semen samples which were contaminated with urine were not used for the evaluation. As the bulls were not trained for artificial vagina, electroejaculation (EE) was used to collect semen for evaluation. In some bulls, rectal massaging at the area of ampulla was applied to get semen when they failed to respond to EE. An electroejaculator (The P-T electronics, model 304, Oregon, USA), fixed to a probe measuring 3 cm in diameter with 3 longitudinal electrodes, was used to stimulate ejaculation. After placing the probe in the rectum with electrodes facing down, a voltage range (0-15 volt, depending on the response of the animal) was used with gradual increase to the desired stimulus, holding for 2-3 seconds and then decreased to zero over a 2-3 second span. After 2-3 seconds in the off position, the cycle was repeated. Prior to semen collection, hair around the prepuce was removed and the area of the prepuce was cleaned thoroughly with water and dried using clean paper towels to minimize contamination. Immediately after the semen was obtained using clean graduated test tube attached to a rubber cone, records were taken for colour and volume; the semen was then kept in warm water (37°C) and transported to the laboratory (Faculty of Veterinary Medicine, Universiti Putra Malaysia) for further analysis within 20-30 minutes.

**Semen analysis**
A conventional method was used to analyse sperm morphology using eosin nigrosin stain, which was dried in air and examined under light microscope, 1000x magnification (Goovaerts et al., 2006). A drop of net semen was mixed to 4 drops of eosin nigrosin stain for 30 seconds and a smear was prepared by taking 5μl of the mixed sample. A total of 200 spermatozoa was examined in each sample, and expressed as percentages of the normal spermatozoa and spermatozoa with abnormal morphology, such as head, mid-piece and tail abnormalities, and spermatozoa with a proximal or distal protoplasmic droplet.

In addition to the conventional method, Computer Assisted Semen Analysis (CASA) technique, which involves a computer aided semen analyser with animal motility software (IVOS 12.2, Hamilton Thorne Biosciences, USA), was used to objectively determine the concentration, general, and progressive motility of spermatozoa.

Depending on the density of the ejaculate, the sample was diluted in normal saline (1:100 or 1:50). From the diluted sample, a 10μl
 aliquot was loaded on a 2X-CEL, 20μm dual side sperm analysis chamber (Hamiltone Thorne Research, USA) and inserted into the computer for analysis at a stage temperature of about 37°C. A total of 8 fields (4 fields from each chamber) was randomly analyzed and their results were averaged and used to compare with the standards. Based on the guideline by SFT, minima of 30% progressive motility, 70% normal morphology and age-related scrotal circumference (minimum 34 cm for age >2 yrs) were used as standards. Bull set up number 4 of the CASA software with slight adjustment on some parameters, based on the preliminary trials for the exact identification of sperm cells using the play back facility, was used for the analysis. The parameters set during the analysis are presented in Table 1 below.

**Trans-scrotal ultrasonography**
A B-mode, real time portable ultrasound scanner with a 5 MHz linear array transducer (SSD 555, Aloka, Japan) was used for the trans-scrotal ultrasonography along the mid-sagittal and transverse plane of each testis. An ultrasound gel was applied to the face of the transducer to maintain a proper contact and to avoid interference of air between the surface of the transducer and the testis.

**Statistical analysis**
In this study, descriptive statistics was used to express the mean and percentage of each semen quality parameter, whereas motility and morphology were computed using SPSS version 16.

**RESULTS**

**Evaluations of Breeding Soundness**
A summary of the results from the BSE for each bull is given in Table 2. Out of the total stud bulls examined, 37.5% (3/8) of them failed to meet the standards for BSE and found to be unfit

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter function</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frame rate (Hz)</td>
<td>Image capture</td>
<td>60</td>
</tr>
<tr>
<td>Number of frame required</td>
<td>Image capture</td>
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<tr>
<td>Minimum contrast</td>
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<td>Straightness cut-off (% STR)</td>
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Trans-scrotal Ultrasonography and Breeding Soundness Evaluation of Bulls

for breeding, due to either physical unsoundness and/or poor semen quality. One bull (ID T025) failed BSE because of the left hind limb lameness, which has existed for a year before the evaluation. The lameness was pronounced during mounting. For this bull, however, other parameters related to semen quality and scrotal circumference were above the minimum standards set by the SFT. The mean values for general sperm motility, progressive motility, and spermatozoa with normal morphology were 63.6%, 41.5%, and 74.5%, respectively, with 43 cm scrotal circumference (Table 2).

The second bull (ID T036) showed unsatisfactory semen quality for the percentage of normal sperm morphology (66.9%), which was less than the minimum standard (70%). The semen from this bull contained 63.8% general sperm motility and 43.7% progressive motility. This bull had the smallest scrotal circumference (35cm) compared with the other bulls, but this was slightly above the minimum standard set by SFT (Table 2).

Right testicular degeneration and poor semen quality were the findings in the third bull (ID 3770, Brangus breed). The right testicle appeared to be atrophied or degenerated as its size was about 1/3 from the left testicle. The semen quality analysis showed all the parameters far below the minimum standards; 63.4% mean sperm cells with normal morphology, progressive motility (19.8%) and asymmetrical testicles. The mean value for sperm concentration (425 X10^6/ml) from this bull was also the lowest compared with the other bulls examined (Table 2). Meanwhile, the medical history of the bull indicated the presence of inflammatory swelling of the right testicle previously, which later recovered following antibiotic treatment.

**Findings of Trans-scrotal Ultrasonography**

The use of trans-scrotal ultrasonography has revealed the presence of lesions in the testes of three bulls. One of them (ID 3770) had right testicular atrophy that failed to meet the minimum standards for BSE. The ultrasonographic examination of the right testicle of this bull (Fig. 1a) showed the presence of non-homogeneous, hyperechoic fibrotic testicular parenchyma with the size reduced to about 1.6cm (as measured from the mediastinum to the border of the testis) compared with the normal left testicle which had a medium to

<table>
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<tr>
<th>ID</th>
<th>Breed</th>
<th>Age (yrs)</th>
<th>SC (cm)</th>
<th>Physical soundness</th>
<th>Average semen quality parameters from CASA/ conventional method (morphology)</th>
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<td></td>
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<td></td>
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<td>Concentration (M/ml)</td>
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<td>43</td>
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<td>35</td>
<td>Sound</td>
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<td>1042</td>
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<td>693</td>
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<tr>
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<td>3549</td>
<td>KK</td>
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<td>36</td>
<td>Sound</td>
<td>1250</td>
</tr>
<tr>
<td>3568</td>
<td>Brah.KKx</td>
<td>5</td>
<td>43</td>
<td>Sound</td>
<td>827</td>
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</tbody>
</table>

NA: not applicable; KK: Kedah-Kelantan; Fr: Friesian; Brah: Brahman; Simm: Simmental; SC: Scrotal Circumference; *parameters below the minimum standard
Minimum standards: 30% progressive motility; 70% normal morphology (< 30% abnormal morphology); 34cm SC for age ≥ 2yrs
coarse echogenicity (Fig. 1b). A unilateral anechoic fluid-filled space (hydrocoele) between the parietal and visceral layers of the vaginal tunic which measured about 1.6 cm in the right testicle of bull 3568 was the other lesion detected with ultrasonography (Figs. 2b and 2c). However, ultrasonogram of the left testicle of the same bull (Fig. 2a) and also testes of the other bulls examined did not show such anechoic space. The third lesion, which involved both testicles, was noted in bull with ID 1545. The ultrasonogram of the testes showed the presence of hyperechoic foci scattered throughout the testicular parenchyma with acoustic shadowing, consistent with fibrosis and possible mineralization (Fig. 3b).

Despite the presence of lesions mentioned in the bulls, ID 3568 and 1545, semen quality parameters, such as motility and morphology, were not affected. Moreover, ultrasonography of the testes of a bull (ID T036) that failed BSE because of the high percentage of abnormal sperm morphology (Fig. 4) and other bulls did not show any abnormal lesion.

**DISCUSSION**

Kastelic & Thundathil (2008) showed that 20 – 40% of bulls had reduced fertility in an unselected population for fertility soundness. Moreover, a 33% prevalence of the bulls unfit for breeding had also been reported by Chacon *et al.* (1999) from extensively managed bulls in Costa Rica based on BSE. Eliminating such bulls with physical problems or reduced fertility from the breeding herd improved the overall reproductive efficiency of the herd (Bagley & Chapman, 2005). In the present study, 37.5% (3/8) of the stud bulls, which have been used for breeding in the farm, were found to be unfit for breeding due to either physical problems or inadequate semen quality, or both. The occurrence of subfertile bulls indicates their role in the reduction in the overall fertility of the herd. All these subfertile bulls were eventually culled from the breeding group.

During the mating act, the full weight of the bull is borne on the hind legs and feet, therefore, any unsoundness in this area drastically interferes with its breeding ability (Barth, 2007). A bull with physical problems of the legs, eyes, testicles, penis, or internal...
reproductive structures that interfere with its ability to impregnate females is unfit for breeding, as long as the problems are unlikely to improve with time (Bagley & Chapman, 2005). In the present study, two of the bulls examined showed physical problems related to the leg (hind limb lameness in bull T025) and the testis (testicular atrophy in bull 3770). According to the medical history, both problems had chronically existed for about a year prior to the evaluation, indicating that these problems were unlikely to recover with time. In addition to the

Fig. 2: Testicular ultrasonography of a Brahman-KK cross bull (ID. 3568); a) normal ultrasonogram of the left testicle scanned along the mid-saggital plane with no space between the testicular parenchyma (TP) and scrotal layer (SL), b) right testicular ultrasonogram with completely dark nonechoic (NE) fluid filled space (hydrocele) between the border of the testis and the scrotal layer (SL) measuring 1.6 cm, and c) shows the same right testis when scanned along the transverse plane with the hydrocele (NE) indicated by the arrow

Fig. 3: Testicular ultrasonography of a Friesian Sahiwal bull (ID. 1545). Ultrasonogram of both the left (a) and the right (b) testes with hyperechogenic foci (F) scattered throughout the parenchyma forming acoustic shadow beneath the foci as indicated by the biggest arrow in (b)
unilateral testicular atrophy observed in bull 3770, the quality of semen was demonstrated by low percentage of progressive sperm motility (20%) and normal sperm morphology (63.3%), which were both below the minimum standards set by SFT. The poor semen quality is likely due to the degeneration of the left testicle. Testicular degeneration is an acquired condition in which one or both testicles that were once normal undergo pathologic changes consequently, resulting in small testicular size and abnormal function (Hopkins, 2007). It has been reported that degenerative changes in the testicle are frequent causes of infertility in males, including bulls which commonly occur following inflammatory reaction to infection (Barham & Pennington, 2006). Testicular degeneration results in testicular dysfunction which is responsible for poor semen quality (Hoflack et al., 2008). The swelling associated with inflammation, coupled with an inelastic tunica albuginea, tends to cause pressure necrosis of the testicles. Thrombosis of blood vessels results in degeneration which causes the testicle to lose its ability to function normally. In addition, the increased temperature of the scrotal contents has been shown to result in testicular degeneration with a higher percentage of abnormal ejaculated sperm (Hopkins, 2007).

Several researchers have shown that there is a positive relationship between scrotal circumference, male reproductive traits and semen quality parameters, such as the number of normal sperm, sperm concentration, sperm motility, and total daily sperm production (Madrid et al., 1988; Geske et al., 1994; Chacon et al., 1999). Consequently, many reports have demonstrated that scrotal circumference is positively related to conception and/or pregnancy rates (Coulter & Kozub, 1989; McCosker et al., 1989; Makarechian & Farid, 1985; Larsen et al., 1990; Brito et al., 2002; Fitzpatrick et al., 2002; Holroyd et al., 2002). In addition, selecting bulls for large testes has been reported to improve the fertility of their female offspring by reducing the age at puberty and breeding (Barham & Pennington, 2006). One of the bulls evaluated in this study (ID T036) had high abnormal sperm morphology (33.1%), beyond the minimum standard (30%). No clinical abnormalities were apparent upon palpation of the testes and ultrasonography. However, this bull had the
lowest scrotal circumference (35 cm, age 3½ yrs) compared with the rest of the bulls. This value is slightly higher than the minimum standard set by SFT for fertility soundness (minimum of 34 cm for age ≥ 2 yrs). It has shown a 1 cm increment only in its additional 1½ yr life time. Being the youngest of all the bulls examined, the age may have played a role for the high percentage of abnormal sperm morphology obtained.

The use of trans-scrotal ultrasonography in the assessment of bull’s soundness for fertility was evaluated in this study. Out of the total eight bulls examined, the ultrasonography of the testes revealed the presence of lesions in three bulls. Unilateral testicular degeneration in bull 3770 was one of the lesions found. Meanwhile, the ultrasonogram of this testicle showed the presence of a hyperechogenic fibrotic tissue with diminished size and loss of homogeneity in the parenchyma, compared with the normal contralateral testicle. This finding of ultrasonography enhanced the fertility assessment of bull 3770 which was found to be unfit for breeding, based on the routine BSE procedure. Ultrasonography has given a more detailed explanation for the poor quality of semen obtained from bull 3770 which was likely due to the increased fibrotic tissue in the parenchyma of the affected testis. This is in accordance with Chapwanya et al. (2008) who have reported that there is an agreement between trans-scrotal ultrasonography and BSE in classifying a bull’s fertility. It has been described that a decrease in the sperm concentration and motility, in combination with an increase in morphological abnormalities demonstrated in several Belgian Blue bulls, is associated with testicular degeneration (Hoflack et al., 2008). Moreover, Hahn et al. (1999), who compared ultrasonogram of Holstein bulls testes with high semen quality and bulls with low semen quality using low frequency transducers (1.6 and 2.25 MHz), discovered the presence of substantial fibrotic tissue (more parenchyma tissue replaced with connective tissues) in the testes of bulls with poor quality semen.

The other two testicular lesions revealed in the current study were idiopathic scrotal hydrocele (seen as unilateral fluid filled anechoic space in the vaginal cavity on ultrasound) and bilateral scattered fibrotic foci found in a Brahman-KK cross (3568) and Friesian-Sahiwal (1545) bulls, respectively. Despite the lesions found in the two bulls, BSE showed a sound fertility status with satisfactory semen quality and scrotal circumference above the minimum standard. The larger the testicular size in both bulls (3568 & 1545) and the unilateral nature of the hydrocele found in bull 1545, thought to have played a role for the quality of semen not to be compromised. A hydrocele is an abnormal accumulation of serous fluid in the vaginal cavity (the space between the parietal and visceral layer of tunica vaginalis layer found immediately covering the testes) (Bartholomew et al., 2003). In normal bulls, the parietal and visceral layers of the vaginal cavity were not observed separately as examined by ultrasonography. However, a space that does not exceed 2 mm width has also been reported as normal (Pechman & Eilts, 1987). Temperature induced dysfunction of spermatogenesis is generally thought to occur because of the insulating effects of the fluid in a hydrocele. Consequently, fertility can be guarded especially in cases where bilateral hydroceles persist (Schumacher & Varner, 2007). Brito et al. (2003) reported whole scrotal insulation resulted in decreased sperm production and semen quality in B. indicus and B. indicus x B. taurus bulls without changes in testicular echotexture. Similarly in this study, there was no change in the echo texture in the testicle of the bull with hydrocele. Shore et al. (1995), who did a follow up study on the scrotal sonogram of hydrocele and semen quality, reported spontaneous resolution of hydrocele within 120 days in 85% of bulls (22/26). The percentage of bulls with unilateral hydrocele resolved was higher than those with bilateral hydrocele (94 vs. 62.5). The researchers also reported that at 90 days, semen quality was satisfactory in most bulls with unilateral hydrocele which was higher than those with bilateral problem. The hydrocele observed in this study was reduced significantly after 2 months’ period.
Meanwhile, the lack of correlation between the findings of multifocal echodensities in the testicular parenchyma and poor breeding soundness score or with a high number of spermatozoa abnormalities have been reported by Shore et al. (1995). Similarly in this study, BSE showed an acceptable fertility status with no adverse effect of the foci lesion on semen quality despite the scattered foci lesion in one of the bulls examined (1545). Nevertheless, racket-shaped sperm head abnormal morphology, which was frequently examined in bull-1545 (data not shown), might be associated with the scattered hyperechoic foci lesion found in the testes. In general, the lesions revealed by ultrasonography in this study were not detected through palpation in a routine BSE. This shows the significance of trans-scrotal ultrasonography in the assessment of bulls for the soundness of fertility although semen quality is dependent on the severity of the lesions and the overall scrotal/testicular size of the bull.

CONCLUSION

The current study clearly showed the consequence of underestimating BSE for breeding bulls of unknown fertility status which is demonstrated by the occurrence of 3/8 bulls with unsound fertility that lead to the drop in the overall reproductive efficiency of the herd. Moreover, the study enabled to evaluate the use of TSU in the assessment of bull’s soundness for fertility. TSU using a 5MHz transducer was found to be a useful non invasive and easily applicable technique in assessing the health status of the testicle in detail, revealing lesions which were difficult to be detected by palpation, consequently enhancing the routine BSE technique. Despite the presence of the lesions in the testes of the two bulls (bull 1545 and 3568), their semen quality was not affected and BSE result showed a sound fertility status. The authors are of the opinion that the larger size of scrotal circumference (43 and 46 cm) or testicular size, as a compensatory factor in both bulls and the unilateral existence of the hydrocele in bull 3568, have played a role for the semen quality (normal morphology and motility) not to be affected compared to the standards. However, there is a need of further research to estimate the extent of lesion obtained by US that can produce substantial effect on the quality of semen below the standard set by SFT. In this study, it was generally noted that ultrasonographic findings of a unilateral hydrocele (width, 1.6 cm and scrotal circumference, 43 cm) in a Brahman-KK cross bull and bilateral presence of scattered fibrotic foci with acoustic shadow (SC, 46 cm) in a Friesian Sahiwal bull have no significant effect on the quality of semen and soundness for fertility.

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