ASSESSING BULL FERTILITY: THE BREEDING SOUNDNESS EVALUATION

Beoordeling van de vruchtbaarheid van de stier: het onderzoek naar de reproductiegeschiktheid

G. Hoflack, G. Opsomer, D. Maes, A. de Kruif, A. Van Soom

Department of Reproduction, Obstetrics and Herd Health,
Faculty of Veterinary Medicine, University of Ghent, Salisburylaan 133, B-9820 Merelbeke, Belgium
Geert.hoflack@UGent.be

ABSTRACT

Fertility is of paramount importance in bovine livestock management. While in Belgium cow fertility is generally monitored quite well, in-depth investigation of bull fertility is only rarely performed, and this sometimes results in poor pregnancy outcomes. Screening bulls for fertility and finally selecting the bulls with the highest fertility potential, however, can easily be done by means of a standardized breeding soundness evaluation in which general health, reproductive health and sperm quality are assessed. Although this screening procedure is not performed in Belgium, there are a number of indications that it might overcome part of the fertility problems encountered in the Belgian Blue breeding herds. This paper describes how to perform a breeding soundness evaluation and how to interpret the results, with some specific considerations concerning Belgian Blue bulls. This procedure makes it possible to exclude bulls with impediments to fertility and to select for bulls with traits favorable for high fertility. However, in spite of recent substantial advances in sperm quality assessment, accurate prediction of the fertility outcome of an ejaculate or of a bull remains an elusive goal.

SAMENVATTING

Een economisch rendabele veehouderij is slechts mogelijk mits de vruchtbaarheid van de koeien en de stier goed is. Daar waar de koeien zeer veel aandacht krijgen, is dit voor de stier, die nochtans voor de helft van de vruchtbaarheid op bedrijfsniveau verantwoordelijk is, in België meestal niet het geval. Dit leidt in een aantal gevallen tot ontgoochelende drachtigheidsresultaten. Vandaar dat het aangewezen is de stieren te onderzoeken alvorens het dekseizoen aanvangt, om zo de stieren die niet voldoen, op voorhand uit de fokkerij te sluiten. Dit kan gebeuren door middel van een onderzoek naar de reproductiegeschiktheid, waarbij de algemene gezondheidstoestand, de gezondheid van het geslachtsapparaat en de spermakwaliteit nauwgezet gecontroleerd worden. Bij Belgisch Witblauwe stieren wordt dit zelden gedaan, al bestaat het vermoeden dat dit meer dan noodzakelijk is. In dit artikel wordt uitgelegd hoe een dergelijk onderzoek dient te gebeuren en hoe de uitkomst geïnterpreteerd moet worden. Daarnaast worden enkele bekende pijnpunten van het Belgisch Witblauwe ras die de vruchtbaarheid van een stier negatief kunnen beïnvloeden, besproken. Met een onderzoek naar de reproductiegeschiktheid kan men stieren met kenmerken die onverzoenbaar zijn met een goede vruchtbaarheid, weren, maar men kan er de vruchtbaarheid van een individuele stier niet mee voorspellen. Het ultieme doel, namelijk het vaststellen van de vruchtbaarheid van een stier of van een ejaculaat, is dus vooralsnog onmogelijk.

INTRODUCTION

Reproduction in cattle livestock is the essential prerequisite for production and thus for potential economic gain. Good fertility both of bulls and of cows is imperative for adequate reproductive performance. Male fertility is a key factor influencing reproductive efficacy since, in cattle, a single bull is generally bred to between 20 and 100 cows (Chenoweth, 1986). Despite the bull's pivotal role in beef reproduction, cows generally receive more interest, both from a scientific and a practical point of view (Chenoweth, 1997a; Parkinson, 2004). However, no individual herd member bears as much responsibility for fertility as the herd sire (Barth, 1997; Hoflack and de Kruif, 2003). Hence, knowledge of a bull's reproductive capacity is of paramount importance to achieve breeding success.

Post-breeding assessments

Several methods can be used to assess bull fertility. The most logical and accurate method is to assess pregnancy rates (Wiltbank and Parish, 1986; Koops *et al.*, 1995). This is more efficacious for estimating male fertility than the assessment of the number of calves produced, since the latter parameter is largely influenced by the cow and her reproductive performance. However, the assessment of pregnancy rates is very labor intensive since it requires a rectal or ultrasonographic examination of a high number of cows sired by a particular bull (Fissore *et al.*, 1986, Phillips *et al.*, 2004). This renders this method rather impractical, particularly in the case of artificial insemination with bulls that sire huge numbers of females (Foote, 2003; Rodriguez-Martinez, 2003).

In order to reduce labor, non-return rates to service (estrus) can be determined instead of pregnancy rates. The non-return rate is the percentage of cows inseminated with semen from a particular bull that do not have to be re-inseminated within a specific time period afterwards; it can be considered a 'preliminary' pregnancy rate (den Daas, 1997). However, this method remains very time consuming since one has to wait until a high number of cows have been inseminated and until the data from subsequent inseminations of these cows become available. Furthermore, this method is imprecise and overestimates the true conception rate, because inseminated non-pregnant cows which are finally culled or naturally served are considered as not returning to estrus and thus pregnant as a result of the insemination (den Daas, 1997). This falsifies the results to some extent. However, to this very day, non-return rates are the golden mean between reliability and practicality, and are therefore used by all artificial insemination centers around the world to quantify bull fertility (CRV Holding, personal communication; Rodriguez-Martinez, 2003; Phillips *et al.*, 2004).

Pre-breeding assessments

The main disadvantage of post-breeding bull fertility evaluations is that they assess the fertility of any given bull after this bull has been bred to his female counterparts. In the case of an infertile bull, damage has been done before fertility results become available. Hence, the prediction of fertility prior to breeding rather than post-breeding could greatly increase the reproductive efficacy (Rodriguez-Martinez, 2003). For this purpose, breeding soundness evaluations of bulls have been used over the past 50 years and are widely accepted (Ott, 1986; Barth, 1997). This manuscript deals with the practical application of breeding soundness evaluations in bulls. Additionally, several laboratory procedures for attempting to predict fertility have also been studied (for a review, see Rodriguez-Martinez, 2003).

THE BREEDING SOUNDNESS EVALUATION

Evaluation of the potential breeding soundness of a bull consists of a general physical examination, a reproductive examination including an examination of the external and internal genitalia (including a scrotal circumference measurement), and a semen quality evaluation (Bruner and Van Camp, 1992; Chenoweth *et al.*, 1994).

General physical examination

The aim of this examination is to ensure that no clinical aberrations which might negatively influence the bull's fertility are present. This includes an examination of the general health, the musculoskeletal system (gait, feet and legs), the eyes and an oral examination (Ott, 1986).

It is obvious that the general health of a breeding bull is important, since sick animals will be less active and fever should be avoided since this will negatively influence semen quality. Moreover, a bull suffering from an infectious disease might infect the female herd, which in turn might harm the reproductive performance of the cows. In addition to the legally obligatory screenings, such as for tuberculosis, brucellosis and leucosis, particular attention should be paid to bovine viral disease virus and infectious bovine rhinotracheitis, since these infections easily spread from bulls to cows with possibly devastating effects on pregnancy results (personal observations).

Besides the general health, a sound conformation of the feet and legs (free of bowleggedness, cow hocks, sickle hocks, and post-leggedness) of a bull is imperative to obtain a good breeding outcome (Larson, 1986). Bulls with rear leg impairment may not move around freely to detect cows in estrus or may be unable to mount succesfully, since during copulation most of the bull's weight is borne on the hind legs and feet (Bruner and Van Camp, 1992; Barth, 1997). Furthermore, it has been demonstrated that even in bulls without clinical symptoms of lameness, joint lesions should be taken into consideration as a contributory cause of reproductive failure (Persson *et al.*, 2004). Subclinical feet and leg problems might result in rear leg discomfort, leading to fewer mounting attempts by these bulls as well as more time spent in recumbency, which may interfere with normal testicular temperature and thus with sperm quality (Ott, 1986; Hopkins and Spitzer, 1997). Even more emphasis should be placed on the fact that quite a number of foot and leg problems (e.g. corkscrew claw defect, interdigital fibromas, weak pasterns, post-leggedness and sickle hocks) have a hereditary basis and will be passed on to the (female) offspring of an affected bull. This effect on the female herd will finally reduce the longevity of the cow herd and increase the labor and veterinary expenses (Ott, 1986, Barth, 1997). Hence, a thorough examination of a bull's hooves and feet prior to the breeding season is an absolute requirement. Furthermore, this examination makes it possible to detect and treat infectious claw disorders, such as digital dermatitis, interdigital dermatitis, and interdigital phlegmon, which could otherwise be passed on to the cow herd (Greenough and Weaver, 1997). A detailed review of the pathological conditions resulting in lameness, which consequently can interfere with fertility, is given in the book *Lameness in Cattle* by Greenough and Weaver (1997).

Since bulls rely mostly on vision to detect cows in heat, it is logical that a thorough examination of the eyes and vision of the bull should be performed. Furthermore, a bull should be free of mouth abnormalities or deformities and have adequate teeth to allow him to sufficiently graze during the breeding season to avoid excessive weight loss. Body condition should therefore be monitored, and both overfed and underfed bulls should be avoided (Ott, 1986, Bruner and Van Camp, 1992; Barth, 1997).

Reproductive examination

The reproductive examination consists of an exam of the external genitalia (i.e. penis, prepuce, scrotum, testes and epididymides), including a scrotal circumference measurement and a rectal palpation to assess the internal genitalia (i.e. prostate, vesicular glands, ampullae ductus deferentes and inguinal rings).

External genitalia

The prepuce

The prepuce should initially be visually inspected for evidence of preputial prolapse. *Bos indicus* bulls and bulls of breeds using Brahman (e.g. Santa Gertrudis, Beefmaster, Brangus) generally have pendulous sheaths, whereas polled breeds are prone to hereditary weakness of the (retractor and protractor) prepuce muscles. All these conditions predispose to preputial eversion, which can result in preputial trauma. This condition may decrease the breeding potential of a bull. When examining the external orifice, careful attention should be paid to precipitated crystals on the hairs, since they suggest the presence of urinary calculi predisposing bulls to urethral obstruction and even rupture, making a bull unsuitable for breeding (Figure 1). Furthermore, the prepuce (and penis) should be examined for balanoposthitis (e.g. caused by the IBR-IPV virus), since this condition finally leads to pain and reluctance to mate (Ott, 1986, Bruner and Van Camp, 1992).

Next to the visual inspection, a thorough palpation of the entire external preputial sheath (from the external orifice to the scrotal neck) should be performed to examine whether scars, lacerations, adhesions, stenosis or preputial enlargements are present. Moreover, several penile abnormalities such as fibropapillomas, abscesses and hematomas can often already be detected during this palpation (Bruner and Van Camp, 1992).

The penis

The best way to examine the penis is immediately after natural mating prior to penile withdrawal into the prepuce, or before and after semen collection by means of an artificial vagina (Figure 2), since in the case of electro-ejaculation and/or manual protrusion, artificial deviations may occur. The extended penis should be examined for the presence of fibropapillomas, hair rings, persistent frenula (Figure 3), and penile deviations. Phimosis (inability to extend the penis) and paraphimosis (inability to withdraw the penis), both secondary conditions, are unacceptable for breeding bulls. Urolithiasis with urethral rupture and penile hematoma ('broken' or 'fractured' penis) are both presented as a large subcutaneous swelling cranial to the scrotum and warrant exclusion from breeding (Ott, 1986, Bruner and Van Camp, 1992; Hopkins, 1997).

The scrotum and its contents: the testes and epididymides

Both visual inspection and palpation of the scrotum (and its contents) should be performed. This visual inspection of both scrotal size and shape should be done in a warm environment on a relaxed bull, since under these circumstances the scrotum will be maximally pendulous (Figure 4). In order to provide sufficient thermoregulation, a distinct scrotal neck free of fatty deposits should be present. Straight-sided and wedge-shaped scrotums, as well as normal scrota with fatty deposits in the scrotal neck, are associated with impaired testicular thermoregulation, which can result in abnormal sperm production (Barth, 1997; Johnson, 1997; Van Camp, 1997; Warner, 2004). Furthermore, the external scrotal surface should be free of scabs, lacerations, dermatitis and frostbite since these conditions can alter scrotal temperature and subsequently influence the sperm morphology (Bruner and Van Camp, 1992). Abnormalities such as cryptorchidism, unilateral testicular hypoplasia/atrophy, orchitis, scrotal hernia, and scrotal hematomas and masses will result in abnormal scrotal shape, and such bulls should be classified as unsatisfactory potential breeders (Bruner and Van Camp, 1992).

After the visual inspection, scrotal-, testicular- and epididymal palpation should be performed. The thickness of the scrotal wall and the fat content of the scrotal neck should be assessed, and the testicular cords should be checked for the presence of fat, abscesses, varicoceles or viscera in the case of a scrotal hernia (Barth, 1997). Careful palpation of the testes, examining the size, shape, symmetry and consistency, should be performed to detect possible abscesses, calcification, hematoceles and (rare) neoplasms. During this palpation, both testicular consistency and resilience should be assessed. Healthy breeding bulls should have (very) firm testes, with resilience similar to (soft) rubber. Although testicular palpation remains subjective, tonometers are only rarely used to determine consistency, since tonometer measurements are not strongly correlated to semen quality (Bruner and Van Camp, 1992; Barth, 1997). However, too hard or too soft testes are abnormal and suggest degeneration, which can finally lead to fibrosis. In this case the testes will shrink on subsequent evaluations, and this is in contrast to testicular hypoplasia, in which case the testes are always small (Bruner and Van Camp, 1992). The mean testicular size in mature dairy and beef bulls is approximately 14 – 16 cm length and 7 – 8 cm diameter. Consequently, testicular length is roughly 2 times its diameter (Larson; 1986). Abnormalities, such as cryptorchidism, unilateral testicular hypoplasia/atrophy, orchitis, scrotal hernia, and scrotal hematomas and neoplasms will be accompanied by a loss of testicular symmetry (Bruner and Van Camp, 1992). The testes must move freely within the scrotum and it should be easily possible to slide a testicle upward without invagination of the scrotal tip, which is indicative of testicular adhesions, so that the corpus epididymis of the opposite testicle, which is situated on the medial side of the testes, can be palpated.

Furthermore, the epididymal head (caput epididymidis), a flat and firm structure on the craniodorsal surface of the testicle, and the epididymal tail (cauda epididymidis), ventral on the testicle and generally protruding well beyond the ventral limits of the testicle, should also be carefully palpated for size, shape, symmetry and consistency (Larson, 1986; Ott, 1986, Bruner and Van Camp, 1992; Barth, 1997). The most common abnormalities involve inflammation (epididymitis) or loss of patency. The latter situation can be the result of (inherited) segmental aplasia, tumors, abscesses or spermatoceles, in which case the efferent tubules are defective. The epididymal tail distal to this occlusion will be empty, flaccid and soft. When occlusion is finally accompanied by breakdown of the epididymal lumen, sperm will enter the surrounding tissues and prompt an inflammatory reaction, resulting in the formation of nodular masses or sperm granulomas (Bruner and Van Camp, 1992). Epididymitis is often secondary to orchitis or seminal vesiculitis, and in case of aplasia, the corresponding vesicular gland or ampulla is often also absent (Hopkins, 1997).

Scrotal circumference measurement

The most important part of the scrotal examination is the scrotal circumference measurement (Hopkins and Spitzer, 1997), as scrotal circumference, a highly heritable trait, is positively correlated to daily sperm output, normal sperm morphology and sperm motility, and consequently to pregnancy rates (Makarechian and Farid, 1985; Ott, 1986; Bruner and Van Camp, 1992; Barth, 1997). Each gram of functional testicular tissue has the same amount of tubular epithelium resulting in a constant sperm production per gram of testis weight without breed differences. Hence, in order to predict the potential sperm production of a bull, it suffices to weigh his testicles. However, high correlations between paired testis weight and scrotal circumference have been demonstrated. Consequently and for practical purposes, measuring the scrotal circumference of a bull is, in essence, equivalent to weighing his testicles (Spitzer and Hopkins, 1997). This can be done by pulling a scrotal tape around the testicles until snug at the site of maximal circumference, after both testicles are carefully forced ventrally into the bottom of the scrotum until no scrotal wrinkles are any longer evident (Figure 5). During this measurement, the testicles should be immobilized by placing the thumb and fingers on opposite sides of the scrotal neck, avoiding putting thumbs or fingers between the testes, which results in separation of the testes, thus falsifying the measurement (Bruner and Van Camp, 1992). It is recommended to repeat this measurement to check for accuracy. The scrotal circumference thresholds for all breeds, regardless of genotype or environment, are listed in Table 1 (Chenoweth *et al*., 1992; 1994; Hopkins and Spitzer, 1997). These are the minimal acceptable measurements suitable for all breeds, notwithstanding the fact that breed differences in scrotal circumference at a given age have been demonstrated (Michaux and Hanset, 1981; Chenoweth *et al*., 1984; 1996; Coulter *et al*., 1987; Bruner *et al*., 1995).

Internal genitalia

The last step in the reproductive examination of a bull is a rectal palpation of the internal genital tract, during which the dorsal transverse ridge of the prostate gland, the ampullae of the ductus deferens, the vesicular glands, and the internal inguinal rings are examined. Immediately

Figure 1. Urinary calculi, which can finally lead to urethral rupture, are a common finding in young Belgian Blue bulls. Specific management practices, such as access to free water, NaCl supplementation, water quality assessment, and higher roughage diets can help to prevent this problem.

Figure 2. The bull's penis can be thoroughly examined for abnormalities, such as fibropapillomas, hair rings, persistent frenula, and penile deviations, during false mounts or immediately prior to (or after) semen collection by means of an artificial vagina.

Figure 3. Persistent frenulum of the penis of a Belgian Blue breeding bull. This condition is encountered in approximately 4 – 7% of young Belgian Blue bulls.

Figure 4. Both a visual inspection of scrotal size and shape, and a palpation of the scrotum and its contents should be performed. A distinct scrotal neck free of fatty deposits should be noticeable.

Figure 5. Scrotal circumference measurement of a Belgian Blue breeding bull.

Figure 6. Apart from penile abnormalities, both libido and mating ability can be assessed during semen collection by means of an artificial vagina, which results in the most reliable semen quality compared to other collection methods.

Table 1. Minimum scrotal circumference (in cm) in bulls relative to the bull's age, as advised by the 1993 society for Theriogenology guidelines, to be classified as a satisfactory potential breeder.

Age (months)	Minimum scrotal circumference (cm)
> 12 - 15	30
> 15 - 18	31
> 18 - 21	32
> 21 - 24	33
> 24	34

after entering the rectum, the examiner can identify the pelvic urethra as a firm cylindrical structure on the pelvic floor.

The prostate

Approximately 7 cm cranial to the anus, the corpus of the prostate can be identified as a transverse ridge crossing the pelvic urethra. It is very rare to detect prostatic abnormalities (Larson, 1986; Ott, 1986; Bruner and van Camp 1992).

The seminal vesicles

Craniolateral to the prostate, on both sides, the seminal vesicles can be palpated as grape-like turgid, easily mobile clusters, approximately 2 to 6 cm in width and 6 to 15 cm in length. A common finding here, which can result in high numbers of white blood cells and even in pus in the semen, is seminal vesiculitis, which is generally unilateral but can be bilateral. This generally produces no external signs of illness, but results in increased size and firmness of the glands (finally leading to fibrosis), loss of lobulation and pain on palpation. This condition is very difficult to treat and can evolve to abscessation, intrapelvic adhesions and even, although extremely rarely, peritonitis. Secondary infections of the ampullae, epididymides and testes can result from vesiculitis.

Exceptionally, congenital defects such as aplasia or hypoplasia can occur, but this is often accompanied by aplasia of other segments of the reproductive tract (e.g. the epididymis) (Larson, 1986; Ott, 1986; Bruner and van Camp 1992; Barth, 1997; Cavalieri and Van Camp, 1997; Hopkins, 1997).

The ampullae of the ductus deferens

These smooth tubular structures of approximately 0.5 – 0.8 cm in diameter and 6 – 15 cm in length can be found directly cranial to the prostate and in between the vesicular glands by rubbing the fingers over the pelvic floor. This procedure should be painless, but in the case of a rare ampullitis, generally secondary to seminal vesiculitis, this will no longer be true. Hypoplasia or aplasia of the ampullae very seldomly occur (Larson, 1986; Bruner and van Camp 1992; Barth, 1997).

The internal inguinal rings

These slit-like openings can be palpated by examining both sides of the abdominal wall approximately 15 cm downwards, after passing the hand over the pelvic brim. No structures other than the spermatic cord, which leaves the abdomen through these openings, should be palpable in these rings. These rings should admit no more than 2 (4 cm) to 3 (6 cm) fingers in yearling and adult bulls, respectively. Large rings predispose the bulls to inguinal hernias, a condition that can be diagnosed through rectal examination. Due to the risk for genetic transfer of this condition, such bulls should be excluded from breeding (Larson, 1986; Bruner and van Camp 1992).

Semen collection and evaluation

Semen collection

The final part of the breeding soundness evaluation is the collection and analysis of a semen sample. Several methods for the collection of semen exist, such as transrectal massage, electro-ejaculation, and the use of external and internal artificial vaginas (Barth, 1997; Barth *et al*., 2004). The method most commonly used for bull semen collection during breeding soundness evaluations throughout the world is electro-ejaculation (Elmore, 1994). However, in Belgium there is little expertise with this technique. Moreover, this practice has been banned in several countries due to animal welfare concerns (Barth *et al*., 2004). Collection by means of an artificial vagina yields the best sperm quality (Spitzer and Hopkins, 1997). In addition, this method allows for an evaluation of libido and mating ability, which are very important attributes necessary for adequate breeding efficiency that are not routinely tested during the breeding soundness evaluation (Ott, 1986; Barth, 1997). Hence, collection of semen by means of an artificial vagina is preferable to all other collection techniques (Larson, 1986). However, in some cases of injured bulls, the only humane sperm collection technique is transrectal massage (Barth, 1997). This can easily be accomplished by massaging the ampullae, prostate and urethra until urethral contractions begin, after which one tries to massage in synchrony with these contractions. In general, semen can be collected in a 37°C sperm collection vial by a second person within a few minutes after the start of the massage procedure. However, lack of penile protrusion frequently occurs, resulting in contaminated samples. Furthermore, not all bulls can be collected with this technique, and semen quality is generally poorer compared to other collection techniques (Barth, 1997; Palmer *et al*., 2005).

Semen evaluation

Several methods can be used to evaluate the quality of a semen sample, but subjective evaluation using standard optical microscopy is by far most commonly used. The semen parameters that are routinely examined using standard optical microscopy are the volume, the concentration, the percentage of motile spermatozoa and the morphological grading of the sperm cells (Neuwinger *et al*., 1990; Rodriguez-Martinez, 2003; Phillips *et al*., 2004). However, volume and density of the sperm are unreliable characteristics when semen is collected by electroejaculation, since they are largely influenced by many factors other than the bull. Nevertheless, when bulls produce several ml of highly concentrated semen, it assures the fact that the bull is capable of producing good ejaculates (Barth, 1997).

The volume of each ejaculate can be read from the graded collection tube immediately after collection.

Concentration is generally estimated by evaluating the color, opacity and viscosity of the sample. A creamy, thick and viscid ejaculate is considered very good and corresponds to a concentration of = 750 million sperm per ml. Good ejaculates, containing 400 – 750 million spermatozoa per ml, look like slightly viscid milk. Skim-milk like, non-viscid samples contain 250 – 400 million sperm per ml and are considered fair, whereas poor ejaculates (< 250 million sperm per ml) have a watery translucent appearance (Elmore, 1994; Barth, 1997). However, concentration can also be more accurately determined by means of a counting chamber, for example a Bürker counting chamber (Merck, Leuven, Belgium). In this case, the concentration of the ejaculates is determined by diluting 10 µl of semen in 990 µl 1M HCl and by counting the number of sperm cells in 1/100 mm^3, which corresponds to 40 small squares.

Semen motility is best evaluated immediately after collection. Gross motility can be determined on a wet mount of neat semen at 100 x magnification. Generally, the following scoring system is used: 1 = cells present without of with very little motion; 2 = prominent individual cell motion without swirls; 3 = slow swirls; 4 = rapid dark swirls (Barth, 1997). Individual cell motion should be discernable when assessing gross motility (≥2). Total and progressive individual motility are subjectively assessed to the nearest 5% by placing 10 µl of diluted semen (10 µl aliquot of pure semen in 790µl physiological saline solution) on a pre-warmed glass slide at 37°C under a coverslip, and by examining 5 different microscopic fields all in the centre of the coverslip, under a 200 x phase-contrast microscope (Hopkins and Spitzer, 1997). This procedure is best repeated twice to ensure the correctness of the estimate. Progressive motility should be at least 30% (= fair), ≥50% to be good and ≥70% to be very good (Chenoweth *et al*., 1994). In addition to these percentages, a velocity score (1 – 4) can also be attributed to the sample: 1 = very slow semen, 2 = slow semen, 3 = rapid semen, 4 = extremely rapid semen.

Sperm morphology is the most reliable criterion for qualifying an ejaculate, since it is least influenced by the collection process (Garner, 1997), and since no other sperm criterion is more closely related to fertility than morphology (Elmore, 1994). Morphology can be assessed using different techniques, but supravital staining procedures such as eosin-nigrosin staining are commonly used and allow both a morphology differentiation and a live-dead assessment (Barth and Oko, 1989; Elmore, 1994; Hopkins and Spitzer, 1997). This live-dead assessment is based on the physical intactness (i.e. structural integrity) of the membranes: the fact that the stain is able to penetrate only the damaged sperm cells results in a clear distinction between eosin penetrated (dead) and unstained (live) spermatozoa. For this purpose, a drop of semen on a glass slide is mixed with a few drops of stain, after which a smear of this mixture is prepared. Then, the eosin-nigrosin stained smears are air-dried and assessed under a 1000 x light microscope, using immersion oil. At least 100 spermatozoa should be evaluated for the live-dead assessment and the morphology evaluation (Barth, 1997; Kuster *et al*., 2004). Individual spermatozoa should be classified on the basis of the 1993 Society for Theriogenology guidelines (Chenoweth *et al*., 1992; 1994; Hopkins and Spitzer, 1997). This classification systems logs abnormalities and classifies sperm cells as either normal or abnormal. Abnormal sperm cells are further classified in terms of primary abnormalities (i.e. underdeveloped forms, double forms, acrosome defects, narrow heads, nuclear pouches or vacuoles or diadem defects, pear-shaped heads, abnormal contour, small and free abnormal heads, abnormal midpieces [pseudodroplets, rough midpieces and segmental aplasia], proximal droplets, folded or coiled tails and accessory tails), which are of testicular origin, and secondary abnormalities (i.e. small normal heads, giant or short broad heads, free normal heads, detached or folded or loose acrosomal membranes, simple bent tails, abaxial tails, terminally coiled tails and distal cytoplasmic droplets), which are considered to originate after the sperm cells have left the testis (Barth and Oko, 1989; Hopkins and Spitzer, 1997). When multiple abnormalities are observed in the same sperm cell, only one abnormality is logged. Primary abnormalities are given first priority in classification. At least 70% of the spermatozoa should have a normal morphology (Chenoweth *et al*., 1994; Elmore, 1994). Other cells should also be determined, but are generally not discernable on eosin–nigrosin stains. When neutrophils are present in the ejaculate, white irregular bodies three times the size of a sperm head are noticeable, and their presence can easily be confirmed using a white blood cell stain, such as Diff Quick (Barth, 1997; Hopkins, 1997).

When bulls pass the general physical and reproductive examination, and when they also equal or exceed the minimum thresholds for scrotal circumference, sperm motility (gross motility ≥ 2, progressive motility ≥ 30%) and sperm morphology (≥ 70% normal spermatozoa), they are classified as satisfactory potential breeders. When they fail for one or more reasons, the bulls are classified either as deferred or unsatisfactory potential breeders. Bulls are only attributed to the latter category when genetic faults or one or more other severe problems occur, or when a problem is irreversible. Deferred bulls are likely to improve with time or therapy and should be scheduled for a retest (Chenoweth *et al*., 1994; Hopkins and Spitzer, 1997).

And what about libido?

Although the breeding soundness evaluation assesses several important characteristics necessary for good fertility, it does not at all deal with the willingness and eagerness of a bull to mount and attempt service (= libido) or with the ability to complete service (= mating ability), since semen is generally collected by means of electro-ejaculation. Notwithstanding the fact that bulls are classified as satisfactory potential breeders, it is very well possible that such bulls are incapable of impregnating cows when the will and ability to service cows is absent (Chenoweth, 1986, Barth *et al*., 2004). Furthermore, it has been demonstrated that bulls with higher sex drive obtain better pregnancy rates compared to bulls with lower sex drive (Blockey, 1978; Makarechian and Farid, 1985; Blockey, 1989, Farin *et al*., 1989), although this effect is most evident over short mating periods and is partially nullified in the case of a long breeding season (Silva-Mena *et al*., 2002; Parkinson, 2004). Hence, bull libido and mating ability can be considered important contributing factors to good male fertility. Consequently, in addition to the breeding soundness evaluation, and to avoid the selection of bulls unwilling or unable to serve cows, tests for bull libido and/or serving capacity should also be included (Chenoweth, 1986; Chenoweth *et al*., 1994; Barth, 1997; Garner, 1997; Parkinson, 2004).

Observing natural mating is the simplest and least expensive method to assess willingness and ability to service cows, but these traits can also be evaluated to some extent when semen is collected by means of an artificial vagina (Ott, 1986; Bruner and Van Camp, 1992; Figure 6). In the case of semen collection by electro-ejaculation or massage, neither libido nor mating ability can be evaluated and other methods to test these characteristics should be implemented in order to fully evaluate reproductive potential. For this purpose, various testing methods have been investigated, such as pasture and corral trials with restrained or unrestrained, estral and nonestral females, where the bulls were tested either individually or as a group for different periods of time (Barth, 1997). In general, several bulls (e.g. 5) of comparable age, to avoid invalid results due to social dominance, are tested simultaneously in a small corral with several (e.g. 2 or 3) sedated and restrained, nonestral cows of which the vagina was lubricated with a sterile lubricating jelly to reduce vaginal trauma as a result of repeated breedings. The expression of sex drive, the ability to serve, the reaction time (the elapsed time between exposure of the bull to suitable stimuli and first service), the number of mounts (without ejaculation) and the number of services (= serving capacity) completed within a stipulated time period can then be observed (Chenoweth, 1986; Barth, 1997). It is obvious that a serving capacity test, counting the number of services within a short period of time, yields sufficient information, since a successful test requires both good libido and mating ability (Chenoweth, 1997b). The simultaneous testing of several bulls is done to assess social interactions between dominant and more timid bulls, which is important in multi-sire breeding programs as dominant bulls can exert an inhibitory effect on submissive bulls from a distance, which results in more services being performed by the dominant bulls (Chenoweth, 1997b, Fordyce *et al*., 2002). However, this can negatively influence pregnancy outcome when the dominant bull is subfertile or becomes subfertile through over-use (Parkinson, 2004). Since dominance is related to bull age and weight, the use of mixed age bulls should be avoided (Makarechian and Farid, 1985; Barling *et al*., 1997). Contrastingly, beneficial social effects on bull sex drive expression can also occur, but primarily in young bulls, in which dominance is of little or no significance (Chenoweth, 1997b; Blockey, 1979; Barling *et al*., 1997). However, in Belgium single-sire mating systems are the general rule, so the individual testing of bulls is just as appropriate. However, since corral trials with restrained females are impossible in the Benelux countries for practical, sanitary and ethical reasons, other methods to estimate libido and mating ability have to be used. The measurement of the reaction time, defined as the elapsed time between exposure to stimuli and first service, can be used to test libido (Chenoweth, 1986), while mating ability can be assessed when semen is collected with an artificial vagina (Ott, 1986, Barth *et al*., 2004). Older bulls tend to mount less, although no difference in the number of services can be demonstrated. This probably is a result of their greater mating experience (Chenoweth *et al*., 1984).

The breeding soundness evaluation is generally used for naturally serving beef bulls, although it also applies to dairy and beef bulls used for artificial insemination purposes with cryopreserved semen (Ott, 1986; Barth, 1997). It provides a reliable, quick and cost-effective method for screening and classifying bulls in terms of fertility in order to minimize the use of subfertile bulls and bulls of questionable fertility (Chenoweth *et al*., 1994). These evaluations are reliable for detecting bulls that have the potential for high fertility and those that are clearly unsatisfactory (Barth, 1997). However, it does not predict the fertility of bulls classified as satisfactory potential breeders; it merely identifies bulls with a high or low probability of having reduced fertility, thus classifying bulls as having a high or low risk of developing fertility problems. Hence, other more specialized semen evaluation tests have been developed in an attempt to predict the fertility outcome on the basis of qualitative aspects of the ejaculate (Rodriguez-Martinez, 2003). The goal of these tests is to evaluate one or more sperm attributes that are necessary to reach, bind, penetrate and fertilize an oocyte, such as motility, normal morphology, capacitation, hyperactivation, zona pellucida binding, acrosome reaction, zona pellucida penetration, fusion with the oocyte, and DNA decondensation. Although significant correlations between the respective tests and fertility outcome can be demonstrated in many cases, accurate prediction of the fertility of a bull is still not possible on the basis of these tests, which can provide no more than an estimate (Zhang *et al*., 1999). Male fertility by itself is already a very complex matter as it depends on a heterogeneous population of spermatozoa (Rodriguez-Martinez, 2003). These spermatozoa are furthermore greatly influenced by the female animals, since they interact with a variety of environmental conditions at different levels of the female genital tract. A recent comprehensive study revealed that differences between bulls and between the ejaculates of any given bull accounted for only 0.38 % of the total variation in non-return rates (Christensen *et al*., 2005). The combining of several laboratory semen assessments, and thus the testing of as many different sperm attributes as possible that are relevant for fertilization and embryo development, results in better correlations and hence more accurate predictability of in vivo fertility compared to the testing of a single attribute. Nevertheless, the prediction of bull fertility on the basis of the laboratory assessment of semen still remains a utopian goal (Rodriguez-Martinez, 2003; Parkinson, 2004).

BREEDING SOUNDNESS EVALUATIONS OF BELGIAN BLUE BULLS

Since breeding soundness examinations and libido evaluations are rarely performed in Belgium, there is a lack of data concerning the fertility of naturally serving bulls. The two predominant breeds in the country are the Belgian Blue (BB) beef breed and the Holstein Friesian (HF) dairy breed. The BB breed stems from the Durham Shorthorn, which was introduced in Belgium in 1841 and crossed with local dairy breeds, resulting in a breed called the "Blue of Limon", which was further mixed with local breeds. In 1938, selection for a white color was started, resulting in the "White breed of Middle and High Belgium". Almost simultaneously, and from a limited number of ancestors, selection for a better muscularity was begun, which eventually led to the present, hyper muscled BB breed, which is famous for its low feed conversion ratio, its high percentage of lean meat and its advantageous carcass classification (Coopman *et al*., 2001). Natural service and artificial insemination are both in use for the BB, while for the HF it is mainly artificial insemination that is used for reproduction. Because BB bulls are used for natural service without a preceding breeding soundness evaluation, herd fertility problems frequently occur (Bombeek, 2004). Bull turnover at the farm level is consequentially high, which is due in part to the susceptibility of the bulls to injuries, as well as to the fact that quite a number of bulls are finally culled due to poor pregnancy rates. Usually, when natural service BB bulls are used, specific breeding management practices based on empirical farming experience with BB livestock rather than on scientific research are applied to avoid the frequently noted disappointing pregnancy results. This includes practices such as lowering the breeding pressure by limiting the number of females to 15 – 25 per bull (depending on the bull's age), applying a prolonged breeding season from

May to October, and supplementing the bull's diet with a daily portion of approximately 700 g concentrates / 100 kg body weight (Hoflack and de Kruif, 2003). These recommendations, which are implemented to avoid poor pregnancy rates, suggest that subfertility is a problem in the BB bulls, although this issue has never been studied in detail. However, Hanset (2000) already demonstrated that the average scrotal circumference of 13-month-old BB bulls was rather low, namely 31.3 cm, with a median scrotal circumference of 32.0 cm, both of which are low compared to other beef breeds (Coulter *et al*., 1987; Hanset, 2000). Breed differences in scrotal circumference at a given age are not uncommon however (Coulter *et al*., 1987; Chenoweth *et al*., 1996; Michaux and Hanset, 1981; Chenoweth *et al*., 1984; Bruner *et al*., 1995). The BB breed is indeed a specific breed, with extreme muscularity but reduced organ size. This has been demonstrated for several organ systems (Ansay and Hanset, 1979). Hence, it might be possible that this is also the case for the reproductive organs, which would result in smaller testicles compared to other breeds and, as a consequence, a lower scrotal circumference relative to the bull's age (Michaux and Hanset, 1981). However, the scrotal circumference thresholds set by the Society for Theriogenology are minimum values suitable for all bulls, regardless of genotype or environment, and consequently they also apply to the BB breed (Chenoweth *et al*., 1992; 1994; Hopkins and Spitzer, 1997). Moreover, high circumference bulls produce more highly fertile offspring, both male and female, that attain puberty at an earlier age, which results in economic profit (Coulter and Foote, 1979; Bruner and Van Camp, 1992; Barth, 1997; Spitzer and Hopkins, 1997). Selection for higher scrotal circumference in the BB breed is therefore advisable. Since scrotal circumference is positively correlated to daily sperm output, normal sperm morphology and motility, and consequently to pregnancy rates, Hanset's data (2000) are somewhat worrying (Makarechian and Farid, 1985; Ott, 1986; Bruner and Van Camp, 1992; Barth, 1997). In view of these findings, it might very well be possible that a high proportion of the BB bulls have substandard semen quality and are consequently subfertile. As scrotal circumference is correlated to the fertility of the female offspring, a negative effect of the use of these subfertile bulls on the reproductive performance of female offspring in the BB breed seems inevitable (Barth, 1997). Although no reliable data on this subject are yet available, based on limited data, a general tendency toward a higher age at first calving is noticeable in BB heifers (AWE, personal communication).

Moreover, several other abnormalities which have been noted to cause problems for breeding soundness in other beef breeds are also encountered in the BB breed. It is, for example, common knowledge that the inbred BB breed is susceptible to several heritable feet and leg abnormalities, such as sickle hocks and post-leggedness, two problems which can interfere with the bull's ability to mate (Larson, 1986; Hanset *et al*., 2003). Furthermore, embryo transplantation was extensively used in this breed to disseminate the best genetics throughout the entire Belgian beef population. This not only led to even higher inbreeding coefficients and subsequent feet and leg problems in BB animals, but indirectly also is the reason that infectious claw disorders, which were otherwise rare in this breed, entered the BB population (personal observations). The predominantly used Holstein Friesian embryo receptors are partially responsible for the introduction of these infectious problems in the BB breed. Additionally, mouth abnormalities, such as brachygnatia inferior and superior, hyper muscled and long tongue, and crooked jaw are frequently encountered heritable abnormalities that can interfere with the ability to sufficiently graze, and intensive selection procedures against these defects have been undertaken (Hanset and de Tillesse, 2000).

In view of these findings, fertility in the BB breed deserves special attention and should therefore be monitored with the utmost care. Breeding soundness examinations of BB bulls seems essential to minimize herd fertility problems, since no individual herd member bears as much responsibility for fertility as the herd sire (Barth, 1997; Hoflack and de Kruif, 2003). Nevertheless, the accurate prediction of bull fertility remains utopian (Rodriguez-Martinez, 2003). Hence, bovine fertility management to date consists essentially of minimizing the risk for infertility (Parkinson, 2004).

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Uit het verleden

EEN PRIMITIEVE VORM VAN "LEVENDE" KEURING OP KALVER- EN RUNDERMARKTEN IN 1803

Uit het *"Besluyt rakende de policie der Kalver- en Beeste-merkten",* Gent, 11 maart 1803

Er zullen geoeffende persoonen zyn, benoemd door den meyer (Fr. maire) der stad, om zig te verzekenen of de te koop gestelde beesten gezond en bekwaem zyn om ter sleet (Fr. concomation) geleverd te worden.

Deeze persoonen zullen moeten volkomen aen het gebod 'tgeen hun van officie-wege (van hogerhand) zoude gedaen worden; de kosten van warrandatie (Fr. expertise) zullen zyn tot last der koopers.

Wie die *geoeffende persoonen* wel waren, werd niet gespecificeerd. Er waren in die tijd nog maar enkele in Frankrijk opgeleide dierenartsen en ook de taken van hun voorlopers, de 'paardenmeesters', waren nog niet vastgelegd. Het is overigens weinig waarschijnlijk dat dergelijke figuren daarbij betrokken waren. Wellicht waren het beenhouwers. De gebruikte terminologie lijkt nog op deze gebruikt door de enkele jaren eerder afgeschafte ambachtsbesturen. Verschillende ambachten stelden al sinds de middeleeuwen warrandeerders, waardeerders of waarders (E. to warrant) aan, om de op de markt gebrachte goederen te keuren.

Luc Devriese