Predicting and Promoting Fertility in Beef Bulls

J.P. Kastelic, Professor of Theriogenology and Department Head
J.C. Thundathil, Associate Professor of Theriogenology and Associate Dean, Graduate Education

Department of Production Animal Health, University of Calgary, Faculty of Veterinary Medicine
Calgary, AB, Canada

Introduction

In general, fertility is more important in an individual bull than an individual cow, as one bull may be used to breed up to 40 females (natural service), or potentially hundreds of thousands (artificial insemination). Although 20 to 40% of bulls may have reduced fertility, few are completely sterile (Coulter and Kastelic, 1999). Subfertile bulls delay conception, prolong the calving season, reduce calf weaning weights, and increase numbers of females culled, thereby resulting in economic losses and threatening sustainability of a livestock operation. Furthermore, infertile bulls can have adverse effects of animal welfare, due to repeated breedings and delayed calving (calves are less likely to get close attention). Whereas multiple sire breeding groups, low breeding pressure, and extended (or perpetual) breeding seasons may mask subfertile bulls, in contrast, single-sire mating groups, short breeding seasons and artificial insemination increase the importance of bull fertility.

Evaluation of bull breeding soundness

The two general methods of evaluating the breeding soundness potential of bulls are either breeding a large number of normal, fertile females and determining pregnancy/calving rates, or conducting a bull breeding soundness evaluation (**BBSE**). Although a breeding trial is the ultimate test of fertility, it is expensive, particularly if reproductive performance is poor. Therefore, it is strongly recommended to conduct a standard breeding soundness evaluation before the breeding season. Since bull fertility is influenced by a wide range of factors, no single diagnostic test can accurately predict fertility, although an appropriate combination of tests can be more informative (Kastelic and Thundathil, 2008). A suitable herd sire should be free of obvious genetic defects and infectious diseases, healthy and in good body condition, have sufficient libido and mating ability to identify and mount estrous cows, achieve intromission, and ejaculate large numbers of motile, morphologically normal and fertile sperm. Clearly, a bull lacking any of these characteristics, or one that is marginal in two or more, will have reduced fertility.

A BBSE is not just a semen examination (Kastelic et al., 2012). Although a BBSE is intended to identify bulls expected to have unsatisfactory fertility, it does not guarantee that a bull is highly fertile. Furthermore, it is not a reliable method to predict relative fertility between two or more bulls deemed acceptable. However, a BBSE does identify bulls unlikely to achieve a high pregnancy rate. In that regard, a bull that is ‘breeding sound’ should achieve a pregnancy rate of ~95% in a group of ~25 reproductively normal, cycling females (65 to 70 d breeding season). Although few bulls are completely sterile, in an unselected population, 20 to 40% may have reduced fertility (Kastelic el al, 2012). Subfertile bulls delay conception, prolong the calving season, reduce weaning weights, increase cow culling (delayed or no pregnancy) and increase weight loss and risk of injury in bulls. With multiple sire breeding groups and low bull-to-female

ratios, reproductive performance may be adequate, despite some subfertile bulls. However, single-sire mating groups and high bull-to-female ratios increase the importance of using fertile bulls.

A BBSE begins with assessment of conformation, body condition, and overall physical health. Observe the bull moving (to detect subtle lameness). Feet and legs should be free of defects that limit mobility or mounting. Furthermore, it is essential that a bull have good a normal oral cavity and good eyesight.

Measure scrotal circumference (SC) by forcing the testes downwards, placing a flexible tape around the largest circumference, and creating at least mild compression. Avoid 'inflating' SC by excessive downward pressure or forcing testes apart (for example, with your thumb between the testes). The SC is correlated with paired testis weight, which is correlated with daily sperm production and semen quality traits and puberty. Bulls with a large SC have half-sib heifers and daughters that reach puberty earlier and are more fertile. The heritability of SC in bulls (from 1 to 2 years of age) is ~0.5 and responds quickly to selection.

Examine the scrotum for abnormalities, including frostbite, sunburn or irritated skin. The scrotum should have a distinct neck; bulls with a non-existent, short, or extremely long scrotal neck (bottom of scrotum below the hock) are generally not recommended for breeding. Testes should be freely moveable within the scrotum, with ≤10% difference in size. Excessive softness suggests testicular degeneration, whereas excessive firmness is consistent with irreversible testicular damage. A minor rotation of one testis (on its long axis) is generally tolerated.
The sheath should have an appropriate size and configuration. Verify that preputial hairs have no blood or pus and trim them (no shorter than ~ 2 inches, to avoid urine scald). Palpate the entire sheath and penis. During semen collection, the penis should be exteriorized and examined. Empty the rectum and evaluate the internal reproductive organs for size and normality. Gentle longitudinal 'stroking' of the accessory glands should stimulate contractions and improve semen collection. Keep semen (and anything it contacts) warm. Examinations should be done with a good quality microscope, ideally phase contrast. There are drastic differences in semen quality according to age (Table 1). Most producers are unwilling to accept that <50% of yearling bulls will be judged satisfactory, making good communications and education critically important.

Table 1. Percentage of yearling bulls (n=254) of various beef breeds with satisfactory semen quality.

Age (mo ± 15 d)	No. bulls	Mean (range) SC (cm)	Satisfactory semen quality (%)
12	40	33.8 (28.5-39.5)	40
13	100	34.5 (28-41)	55
14	84	34.1 (28-45)	56
15	30	34.9 (27-41)	73

Sperm motility is estimated by examining semen on a clean, warm slide and is reduced by extreme temperatures and environmental contaminants. Mass motility (affected by both individual sperm motility and sperm concentration) is detectable at low power, but progressive motility should be assessed under medium power (~400 x); a cover slip is applied and concentrated samples can be diluted with warm, fresh saline. Sperm with little or no motility are

often due to urine contamination (or much less commonly morphologically normal sperm); poor motility is rarely a reason for a bull to fail a BBSE.

Libido testing and assessing serving capacity have been described, but are rarely done, due to a perceived lack of reliability in virgin bulls, the logistics of conducting these assessments, and negative perceptions regarding animal welfare. Consequently, it is important to remind the end-user of the breeding bull that (s)he should observe the bull early in the breeding season to confirm libido and mating ability.

Bulls with sound confirmation, free of eye and musculoskeletal defects and >70% morphologically sperm, < 20% head defects, and >30% progressively motility are classified as Satisfactory Potential Breeders (Kastelic et al., 2012). Bulls with apparently temporary conditions which are likely to resolve and allow the bull to meet the above thresholds are placed in the category of Classification Deferred. Bulls in this category are usually peripubertal, have an injury or lameness that is likely to resolve or have temporary testicular degeneration (e.g. hot weather, stress, disease). Bulls with undesirable heritable defects, small SC, debilitating injury or disease, or with permanent testicular degeneration, should be classified Unsatisfactory Potential Breeder.

Infrared thermography and ultrasonography

Bull testes must be a few degrees cooler than body temperature to produce morphologically normal, fertile sperm (Kastelic 2013). Infrared thermograms of the scrotum of bulls with apparently normal scrotal thermoregulation were symmetrical left-to-right, with the temperature at the top 4 to 6 degrees Celsius warmer than at the bottom (Kastelic et al., 2012). More random temperature patterns, including a lack of horizontal symmetry and areas of increased scrotal surface temperature, were interpreted as abnormal thermoregulation of the testes or epididymides. Nearly every bull with an abnormal thermogram has reduced semen quality (Kastelic 2013); however, it is noteworthy that not every bull with poor quality semen has an abnormal thermogram. As a consequence, although infrared thermography is a useful tool for breeding soundness evaluation of bulls, it does not replace collection and evaluation of semen. In one study, 30 yearling beef bulls, all deemed breeding sound on a standard breeding soundness examination, were individually exposed to ~18 heifers for 45 days (Lunstra and Coulter, 1997). Pregnancy rates 80 days after the end of the breeding season were similar (83 versus 85%) for bulls with a normal or questionable, scrotal surface temperature pattern, respectively, but were higher ($P<.01$) than pregnancy rates for bulls with an abnormal scrotal surface temperature pattern (68%).

Diagnostic ultrasonography can be used to assess the reproductive tract of bulls, particularly to provide further insights into tissues or structures that are grossly abnormal (Kastelic and Brito, 2012). Testicular echogenicity increased (parenchyma was more white) as a bull approached puberty, but echogenicity was not superior to scrotal circumference as a predictor of puberty (Brito et al., 2012a). Areas of increased echogenicity in testes (due to fibrosis) are common, especially in young bulls, but are not associated with decreased semen quality (Kastelic and Brito, 2012). Neither visual evaluation nor computerized pixel analysis of testicular ultrasonic echotexture were consistently predictive of semen quality in bulls (Brito et al., 2012a). Ultrasonography can also be used to assess the penis, prepuce and accessory sex glands. The

most common use of ultrasonography in a BBSE is to investigate tissues that are known or suspected to be abnormal.

Nutrition and reproductive development of bulls

A series of four experiments were conducted to evaluate the effects of nutrition during calfhood (10 to 26-30 weeks of age) and peripubertal period (approximately 30 to 70 weeks) on sexual development and reproductive function in beef bulls (reviewed by Barth et al., 2008). In these studies, increased nutrition during calfhood resulted in a more sustained increase in luteinizing hormone (LH) pulse frequency early in life (prior to 25 weeks) and greater testicular development at maturity. Conversely, low nutrition during calfhood suppressed LH secretion before 25 weeks, delayed puberty and reduced testicular development at maturity. For example, for bulls fed low, medium or high nutrition from 10 to 70 weeks of age, age at puberty was (mean ± SEM) 326.9 ± 5.5, 304.7 ± 7.4, and 292.3 ± 4.6 days respectively, and paired testis weights were 523.9 ± 25.8, 552.4 ± 21.1 and 655.2 ± 21.2 grams. Furthermore, in bulls fed reduced nutrition prior to approximately 26 weeks of age, and subsequently fed high nutrition, suppression of testicular development and delayed puberty are not overcome. Therefore, it is not possible to compensate for the effects of early-life low nutrition by subsequently giving supplemental feed.

Sexual development and reproductive function were studied from 6 to 16 months of age in 22 Angus Charolais and 17 Angus bulls (Brito et al., 2012b). Associations of average daily gain (ADG) and body weight with ages at puberty and at maturity (satisfactory semen quality), scrotal circumference, paired-testes volume and weight, testicular vascular cone diameter and fat thickness, scrotal temperature, sperm production and morphology, and testicular histology, were determined. There were no significant correlations between cumulative average daily gain and any of the end points investigated. Body weight at various ages was negatively correlated with ages at puberty and maturity in Angus Charolais bulls, positively correlated with paired-testes weight in Angus Charolais and Angus bulls, and positively correlated with seminiferous tubule volume in Angus bulls ($P < 0.05$). Semen quality improved gradually with age and the interval between puberty and maturity (mean + SD; 309.4 + 29.7 and 357 + 42 days of age) was approximately 50 days. Age, weight, scrotal circumference, and paired-testes volume were all good predictors of pubertal and mature status, with moderate to high sensitivity and specificity (71.6 to 92.4%). In summary, growth rate between 6 and 16 months of age did not affect sexual development and reproductive function in beef bulls. However, greater body weight at various ages was associated with reduced age at puberty and maturity, and with larger testes at 16 months of age. Therefore, improved nutrition might be beneficial, but only when offered before 6 months of age. Average daily gains of approximately 1.0 to 1.6 kg/day did not result in excessive fat accumulation in the scrotum, increased scrotal temperature, or reduction in sperm production and semen quality, and could be considered “safe” targets for growing beef bulls.
In a series of experiments, Angus, Hereford, and Simmental bulls were fed high (80% grain and 20% forage) or medium nutrition (primarily forage) from weaning (6-7 months) to 12-24 months of age (reviewed in Coulter and Kastelic, 1999). In general, bulls receiving high nutrition had greater body weight and backfat, but paired testes weight was not affected by diet. Moreover, bulls receiving high nutrition had lower daily sperm production and epididymal sperm reserves, and greater proportion of sperm abnormalities. It was speculated that increased dietary energy may adversely affect sperm production and semen quality due to fat deposition in the scrotum,

thereby reducing heat radiated from the scrotal skin, and increasing scrotal and testicular temperatures (reviewed in Coulter and Kastelic, 1999). In another study, bulls fed high-nutrition diets had greater scrotal circumference than those fed medium-nutrition diets, but paired testes weight was the same (Coulter and Kastelic, 1999). Since scrotal weight was greater in bulls fed high nutrition, perhaps fat deposition in the scrotum increased scrotal circumference in these bulls. In addition to the deleterious effects of high-energy diets on reproductive function, these diets may also result in abnormal foot growth due to laminitis, as well as adverse effects on bone and cartilage growth, and may increase the risk of rumen inflammation, liver abscess, and vesicular adenitis (Coulter and Kastelic, 1999).

There is a growing tendency to select beef cattle for improved nutritional efficiency, based on residual feed intake (RFI), the difference between actual and expected feed consumption (based on body weight and rate of gain (Bezerra et al., 2013). That reproduction is a low priority, it is very likely that bulls with a genetic background for negative RFI (improved feed efficiency) may have compromised reproductive development.

Improved methods of assessing semen

Visual assessment of sperm motility is quick and inexpensive. However, it is highly subjective and often has limited repeatability (DeJarnette, 2005). There are numerous computer-assisted sperm analyzer (CASA) machines that are commercially available. These provide an objective and much more repeatable method of assessing sperm motility, yielding numerous end points characterizing sperm motion (Kathiravan et al., 2011).

Acrosomal integrity and sperm viability are commonly measured to predict fertility. The acrosome can be stained, or assessed without staining with appropriate optical systems (e.g. Differential Interference Contrast microscopy). There are numerous live/dead stains (including eosin/nigrosin). Furthermore, there are several stains that enable assessments to be made with flow cytometry, thereby allowing rapid and objective evaluation of large numbers of sperm. Frozen-thawed sperm are often maintained at 37 ^{0}C for 2 to 4 hours after thawing, and then evaluated (stress test); this mimics exposure to the female reproductive tract and facilitates detection of latent sperm abnormalities that may not be apparent immediately post-thaw (DeJarnette, 2005). Furthermore, the hypo-osmotic swelling test (HOST) can be used to determine membrane viability.

Use of genetics and genomics for bull selection

Genetic selection reduces generation interval, and increases prediction accuracy and selection intensity (Schaeffer, 2006; Neves et al., 2014). Genome-based selection requires quantification of effects of genome-wide Single Nucleotide Polymorphism (SNP) markers on phenotype (estimated breeding value, EBV) from a reference population large enough to make accurate measurements (Calus et al., 2013; Berry et al., 2014). These data can be used to estimate direct genomic breeding values (DGV), enhance selection of specific genotypes (Meuwissen et al., 2001; Hayes et al., 2009) and hasten genetic progress (Schaeffer, 2006). Genome-based selection is much more advanced in dairy than beef; challenges include development of genome-based strategies useful across breeds, lack of data and quantitative trait loci (QTL) validation (Fortes et al., 2013). For example, Nelore cattle have a critical role in beef production in tropical countries. Therefore, genetic improvement of production and fertility of this breed will substantially

improve tropical beef cattle production. Accuracies of genomic predictions in Nelore cattle were influenced by genetic relatedness between reference and tested populations (Fortes et al., 2013) and the EBV of bulls were determined with a high-density SNP panel to identify loci affecting SC (Utsunomiya et al., 2014).

Early-life predictors of bull fertility and use of bull fertility traits to improve herd fertility

Various reproductive traits of bulls and its relationship to other measures were reviewed (Burns et al., 2011); these could be used as early-life predictors of bull fertility and some traits have implications for enhancing herd fertility by improving female reproductive performance. It is well known that SC is associated with sperm characteristics (Brinks et al., 1978) and fertility (Mackinnon et al., 1990), as sire SC has strong negative (age at puberty, age at first estrus and age at first calf) and positive (yearling pregnancy rate, life time pregnancy rate, ovulation rate) associations with several reproductive traits of female progeny (Burns et al., 2011). Furthermore, Nelore bulls selected for larger SC at 12 mo of age achieved higher rates of heifer pregnancy at 16 mo of age and younger age at first calving (Terakado et al., 2015).

Prepubertal serum FSH concentrations are associated with testicular function and Sertoli cell numbers (Moura and Erickson, 1997). Prepubertal serum LH concentrations are associated with age at puberty (Aravindakshan et al., 2000) and GnRH-induced LH release is related to testosterone concentrations and fertility (Post et al., 1987) In addition to predicting testicular development and function, GnRH-induced LH is predictive of reproductive function in female progeny of various species (Burns et al., 2011). Serum IGF-1 concentrations in pre-pubertal bulls are positively correlated with adult SC and sperm motility, and this trait is genetically related to age at first calving of female progeny and calving rate (Burns et al., 2011). Therefore, these reproductive traits may serve as markers for fertility and reproductive potential of bulls and their female progeny. Identification and use of genetic markers associated with these reproductive traits for selection have implications for improving herd fertility (Burns et al., 2011).

Genetics of semen quality

Based on a recent genome-wide association study on Holstein bulls with various motility classes [i.e. poor (26%) vs normal (73%)], several SNPs close to a cohort of genes involved in regulation of sperm motility were reported (Hering et al, 2014a) This study highlighted the complex genetic regulation of sperm motility and existence of genetic markers useful in marker-assisted selection. These authors also identified markers and candidate genes associated with semen volume and total number of sperm (Hering et al., 2014b). Similarly, estimated effects of SNPs on semen production traits were reported, including candidate genes affecting sperm concentration, semen volume, number of sperm, and motility score (Suchocki and Szyda, 2015). Therefore, semen quality is apparently genetically controlled and associated genetic markers could be used for genomic selection. Availability of such genomic approaches for early detection of bull calves unsuitable for semen production will substantially reduce production costs. However, high accuracy of these genomic approaches must be ensured before culling calves based exclusively on genomic approaches. In addition to establishing markers and associations with candidate genes and production traits, accurate genetic correlations between reproductive and performance traits in beef cattle are needed. Understanding these correlations are important to predict changes in production due to selection for reproductive traits, or expected changes in reproductive performance due to selection for other traits.

References

Aravindakshan, J. P., Honaramooz, A., Bartlewski, P.M., Beard, A. P., Pierson R. A., and N. C. Rawlings. 2000. Pattern of gonadotropin secretion and ultrasonographic evaluation of developmental changes in the testis of early and late maturing bull calves. Theriogenology 54:339-354.

Barth, A. D., Brito, L. F .C., and J. P. Kastelic. 2008. The effect of nutrition on sexual development of bulls. Theriogenology 70:485-495. 10.1016/j.theriogenology.2008.05.031

Berry, D. P., Wall, E., and J. E. Price. 2014. Genetics and genomics of reproductive performance in dairy and beef cattle. Animal 8, Suppl. 1:105-121. doi: 10.1017/S1751731114000743

Bezerra, L. R. , Sarmento, J. L., Neto, S. G., de Paula, N. R., Oliveira, R. L., and W. M. do Rego. 2013. Residual feed intake: A nutritional tool for genetic improvement. Trop. Anim. Health. Prod. 45:1649-1661. 10.1007/s11250-013-0435-y.

Brinks, J., McInerney, M. J., and P. J. Chenoweth. 1978. Relationship of age at puberty in heifers to reproductive traits in young bulls. Proc. West. Sect. Amer. Soc. Anim. Sci. 29:28-30.

Brito, L. F., Barth, A. D., Wilde, R.E., and J. P. Kastelic. 2012a. Testicular ultrasonogram pixel intensity during sexual development and its relationship with semen quality, sperm production, and quantitative testicular histology in beef bulls. Theriogenology 78:69-76. doi: 10.1016/j.theriogenology.2012.01.022.

Brito, L. F., Barth, A. D., Wilde, R.E., and J. P. Kastelic. 2012b. Effect of growth rate from 6 to 16 months of age on sexual development and reproductive function in beef bulls. Theriogenology. 77:1398-1405. doi: 10.1016/j.theriogenology.2011.11.003.

Burns, B. M., Gazzola, C., Holroyd, R. G., Crisp, J., and M. R. McGowan. 2011. Male reproductive traits and their relationship to reproductive traits in their female progeny: A systematic review. Reprod. Dom. Anim. 46:534-553. 10.1111/j.1439-0531.2011.01748.x.

Calus, M. P., de Haas, Y., and R. F. Veerkamp. 2013. Combining cow and bull reference populations to increase accuracy of genomic prediction and genome-wide association studies. J. Dairy Sci. 96:6703-6715. doi: 10.3168/jds.2012-6013.

Coulter, G.H., and J.P. Kastelic. 1999. Management programs for developing bulls. In: Current Veterinary Therapy-Food Animal Practice 4. W.B. Saunders Co., Edited by J.L. Howard and R. Smith, p. 127-136.

DeJarnette, J.M. 2005. The effect of semen quality on reproductive efficiency. Vet. Clinics. North. Am. Food. Anim. Practice. 21:409-418.

Schaeffer, L. R. 2006. Strategy for applying genome-wide selection in dairy cattle. J. Anim. Breed. Genet. 123:218-223.

Fortes, M. R., Deatley, K. L., Lehnert, S. A., Burns, B. M., et al. 2013. Genomic regions associated with fertility traits in male and female cattle: advances from microsatellites to high-density chips and beyond. Anim. Reprod. Sci.;141:1-19. 10.1016/j.anireprosci.2013.07.002.

Hayes, B. J., Bowman, P. J., Chamberlain, A. J., and M. E. Goddard. 2009. Invited review: genomic selection in dairy cattle: progress and challenges. J. Dairy Sci. 92:433-443. 10.3168/jds.2008-1646.

Hering, D. M., Olenski, K., and S. Kaminski. 2014a. Genome-wide association study for poor sperm motility in Holstein-Friesian bulls. Anim. Reprod. Sci.146:89-97. 10.1016/j.anireprosci.2014.01.012.

Hering, D. M. , Olenski, K., Ruść, A., and S. Kaminski. 2014b. Genome-wide association study for semen volume and total number of sperm in Holstein-Friesian bulls. Anim. Reprod. Sci. 151:126-130.

Kastelic J. P., and L. F. Brito. 2012. Ultrasonography for monitoring reproductive function in the bull. Reprod. Domest. Anim. 47 Suppl 3:45-51. doi: 10.1111/j.1439-0531.2012.02042.x.

Kastelic, J. P. 2013. Thermoregulation of the testes. In: Hopper, R.M., editor. Bovine Reproduction. Hoboken: Wiley-Blackwell.

Kastelic, J. P., and J. Thundathil. 2008. Breeding soundness evaluation and semen analysis for predicting bull fertility. Reprod. Dom. Anim. 43(Supplement 2):368-373. 10.1111/j.1439-0531.2008.01186.x.

Kastelic, J.P., Thundathil, J.C., and L. F. C. Brito. 2012. Bull BSE and semen analysis for predicting bull fertility. Clinical Theriogenology. 4:277-287.

Kathiravan, P., Kalatharan, J., Karthikeya, G., Rengarajan, K., and G. Kadirvel. 2011. Objective sperm motion analysis to assess dairy bull fertility using computer-aided system--a review. Reprod. Domest. Anim. 461:165-172. doi: 10.1111/j.1439-0531.2010.01603.x.

Lunstra, D. D., and G.H. Coulter. 1997. Relationship between scrotal infrared temperature patterns and natural-mating fertility in beef bulls. J. Anim. Sci. 75:767-774.

Mackinnon, M. J., Hetzel, D. J. S., Corbet, N.J., Bryan, R. P., and R. Dixon. 1990. Correlated responses to selection for cow fertility in a tropical beef herd. Anim. Prod. 50:417-424.

Meuwissen, T. H., Hayes, B. J., and M. E. Goddard. 2001. Prediction of total genetic value using genome-wide dense marker maps. Genetics 157:1819-1829.

Moura, A. A., and B. H. Erickson. 1997. Age-related changes in peripheral hormone concentrations and their relationships with testes size and number of Sertoli and germ cells in yearling beef bulls. J. Reprod. Fertil. 111:183-190.

Neves, H. H., Carvalheiro, R., O'Brien, A. M., Utsunomiya, Y. T., et al. 2014. Genet. Sel Evol. 46:17. doi: 10.1186/1297-9686-46-17.

Post, T. B., Christensen, H. R., and G. W. Seifert. Reproductive performance and productive traits of beef bulls selected for different levels of testosterone response to GnRH. Theriogenology 27:317-28.

Suchocki, T., and J. Szyda. 2015. Genome-wide association study for semen production traits in Holstein-Friesian bulls. J Dairy Sci 98:5774-80. doi: 10.3168/jds.2014-8951.

Terakado, A. P., Boligon, A. A., Baldi, F., Silva, J. A., and L. G. Albuquerque. 2015.Genetic associations between scrotal circumference and female reproductive traits in Nelore cattle. J. Anim. Sci. 93:2706-2713. 10.2527/jas.2014-8817

Utsunomiya, Y. T., Carmo, A. S., Neves, H. H., and R. Carvalheiro. 2014. Genome-wide mapping of loci explaining variance in scrotal circumference in Nellore cattle. PLoS One. 18;9:e88561. doi: 10.1371/journal.pone.0088561