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Testicular volumetry and prediction of daily sperm output in stallions by orchidometry and two- and three-dimensional sonography

S. Pricking [a, b], H. Bollwein [c], K. Spilker [a, b], G. Martinsson [a, b], A. Schweizer [c], S. Thomas [d], H. Oldenhof [a], H. Sieme [a, *]

[a] Clinic for Horses–Unit for Reproductive Medicine, University of Veterinary Medicine Hannover, Hannover, Germany
[b] National Stud Lower Saxony, Celle, Germany
[c] Clinic of Reproductive Medicine, Vetsuisse Faculty, University of Zuerich, Zuerich, Switzerland
[d] Swiss Institute of Equine Medicine ISME, University of Berne and Agroscope, Avenches, Switzerland

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ABSTRACT

Accurate determination of the testes volume and prediction of the daily sperm output (DSO) is valuable information for reproductive management of a stallion. The aim of this study was to compare different methods for measuring the testes volume, including caliper, 2D and 3D ultrasound. Special emphasis was on feasibility of 3D volume analysis. First, 22 castrated testes were measured and derived volumes were compared with volumes determined via volume displacement in a graded cylinder with saline solution. Then, during the breeding season, testes sizes of 52 stallions were measured *in vivo* and analyzed. With the derived volumes, predicted DSO (pDSO) values were calculated which were compared with actual values (aDSO) determined from semen evaluation. Analyses of castrated testes revealed a discrepancy between volume assessments via the caliper and ultrasound methods and actual volumes as found via volume displacement. The smallest difference was found for 3D volume analysis, followed by caliper and 2D ultrasound. Testicular volumes of breeding stallions were highest if determined via 3D ultrasound, followed by measurements using 2D ultrasound and caliper. Correlation between the total testicular volume (TTV) and aDSO was high with volume assessment via ultrasound (2D: $r = 0.639$, $p < 0.001$, and 3D: $r = 0.604$, $p < 0.001$), and moderate for using caliper ($r = 0.46$, $p < 0.01$). Linear regression analyses of TTV and aDSO values revealed that changes in aDSO in part could be explained by differences in testes volume: 32% and 27% in case of 3D and 2D ultrasound, and 12% with caliper. pDSO values that were predicted from testicular measurements correlated best with aDSO values from semen collection protocols in case of using 3D ultrasound ($r = 0.56$, $p < 0.001$), followed by 2D ultrasound ($r = 0.52$; $p < 0.001$) and caliper ($r = 0.34$, $p = 0.01$). In conclusion, 3D ultrasound can be performed on equine testes for more accurate volume predictions, which in turn may increase precision when determining the breeding potential of a stallion.

1. Introduction

Breeding soundness examination (BSE) is common practice prior to breeding use of a stallion. BSE aims to predict the reproductive capacity and therefore the breeding potential of a stallion, and consists of a physical and reproductive examination, testing of mating ability and biological semen evaluation [1–4]. Reproductive examination includes evaluation of testes normality and size. The size of the equine testes varies with age, season, breed and health status and is correlated with the daily sperm output (DSO). DSO is the number of total sperm that can be collected each day, after extragonadal sperm reserves have stabilized [5–8]. Various authors have described relations between DSO and testicular measurements and values for testicular volumes for different breeds [6,9,10].

Measuring the size of equine testes can be performed by orchidometry using calipers, to measure scrotal dimensions, or by the use of ultrasound imaging [11–13]. The testicular volume (TV) can be calculated by assuming the testes having an ellipsoid shape [5,6]. In 2D ultrasound, testicular dimensions are measured within

* Corresponding author. Clinic for Horses-Unit for Reproductive Medicine, University of Veterinary Medicine Hannover, Bünteweg 15, 30559 Hannover, Germany.
E-mail address: harald.sieme@tiho-hannover.de (H. Sieme).

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the ultrasound image, including only the germinative tissue. Love et al. [6] showed a discrepancy between testicular volumes obtained via volume displacement (in solution in a graded cylinder) compared to volumes derived from 2D ultrasound images. Mean volumes by displacement were higher for the left testis, compared to right testicular volumes. Volumes derived from 2D ultrasound of the same testes (after castration) were always lower as actual volumes determined via volume displacement [6].

3D ultrasound imaging is commonly used in human medicine for different diagnostic clinical applications and its use in veterinary medicine is increasing [14–22]. To the best of our knowledge 3D ultrasound imaging has not yet been used to determine equine testicular volumes. By 3D imaging of the testicular volume and 'Virtual Organ Computer-aided Analysis' (VOCAL); actual volumes can be analyzed. Most of the studies in men compared 2D ultrasound and orchidometer and determined 2D ultrasound to be more accurate than orchidometry [23–25]. However, orchidometry is a rapid and inexpensive alternative for assessment of the testicular volume, revealing volumes that are closely correlated to values found with 2D ultrasound analysis [26]. Studies on animals suggest similar results. For example, in dogs the ultrasound technique was found to be more accurate in predicting the testicular volume [27]. In human medicine, studies on VOCAL analysis of 3D ultrasound images of parathyroid gland and bladder volumes was found to be highly reliable [28–30].

The aim of this study was to evaluate the use of different techniques for determining the equine testicular volume. In a first experiment, this was done using castrated testes to identify the method closest to actual volumes. Testicles of 11 stallions were measured after castration with caliper, 2D ultrasound and 3D VOCAL analysis. In addition, actual volumes were determined as saline displacement in a graded cylinder. In a second experiment, testicular dimensions of 52 breeding stallions were measured using caliper as well as 2D and 3D ultrasound scanning, and testicular volumes were calculated via VOCAL analysis. The derived testicular volumes were used to calculate DSO values, which were compared with values listed in semen protocols via regression analysis (coefficient of variation, intra class correlation, Bland-Altmann plots).

2. Materials and methods

2.1. Determination of equine testicular volumes using caliper, 2D and 3D sonography/ultrasound on castrated testes

Testes of 11 stallions were used, directly after castration. First, volume displacement of the testes was measured using a graded cylinder containing saline (0.9 w-% NaCl). Then, for testicles with d > 5 cm, caliper measurements were performed with a commercially available 'Stallion Scrotal Caliper' (Animal Reproduction Systems, Chino, CA) whereas a self-made caliper was used for smaller testicles. Ultrasound imaging was performed in a waterbath filled with saline. 2D ultrasound was carried out using a LOGIQ e (GE-Healthcare, Wauwatosa, WI) device equipped with an 8C micro convex probe and 4–10 MHz frequency range. 3D ultrasound was performed using a Voluson I device with a RealTime-4D-convex-transducer RAB4-8-RS (GE-Healthcare, Wauwatosa, WI) at 2–8 MHz Testicular dimensions obtained by caliper and 2D ultrasound were used to calculate the volume via assuming an ellipsoid shape as previously described [13]. Testicular volumes (TV) were calculated for each testis, the total testicular volume (TTV) was determined as the sum of the left and right testicular volume.

$$TV = 4/3(L/2)(W/2)(H/2) = volume\ in\ cm^3$$

$$= 0.5233(L)(W)(H) = volume\ in\ cm^3$$

$$TTV = TV(left\ testis) + TV(right\ testis)$$

(L = length of the testis in cm, W = width of the testis in cm, H = height of the testis in cm).

In vivo measurements of testicular volumes and comparisons with the daily sperm output.

2.1.1. Animals and semen collection

Fifty-two stallions of different breeds (Hanoverian, Westfalian, Oldenburg, Thoroughbred) ages ranging from 3 to 24 years were examined at the State Stud of Lower Saxony (Celle, Germany) in 2016. All 52 stallions participated in an artificial insemination program. Stallions were housed in single boxes bedded with straw and were fed hay, oat and pellets three times a day while having free access to water. Stallions were held according to national regulations and institutional animal care and use protocols. Semen collections took place once every day from Monday to Saturday, from February through July. A 'Hannover' model artificial vagina was used, with a disposable inner liner and a non-woven semen filter, while stallions mounted a dummy (Minitüb, Tiefenbach, Germany). The volume of the gel-free semen fraction was measured in a graded sterilized cylinder. The sperm concentration was measured photometrically (SpermaCue, Minitüb, Tiefenbach, Germany). It should be noted that with photometric assessments the sperm concentration is not determined directly and can be complicated with increased numbers of non-sperm particles present in an ejaculate and in case of low or high sperm concentrations. All ejaculates were processed by diluting with an equal volume of skimmed milk extender (INRA82 [31]), followed by centrifugation (10 min, 400 × g), removal of the supernatant and resuspension of the pellet to 30×10^6 progressive motile sperm/mL and cooling to +5 °C. Data from each ejaculate that was processed in the breeding season were documented using specific studsoftware (easyhorse, Ostendorf Büroorganisation GmbH, Cloppenburg, Germany); and included the ejaculate volume, sperm concentration and percentage of progressively motile sperm. Staff for semen collection and processing was trained and did not change throughout the trials, nevertheless different staff members performed semen collections in different occasions.

2.1.2. Testicular volumetry

Each stallion was examined for its general health, followed by an investigation of the genital tract. For this, stallions were placed in stands with one body side facing the examiner, an assisting person holding the stallion using a halter. The examiner (always the same person) positioned himself directly next to the stallion, allowing him to be in a comfortable position for the examination. The ultrasound was positioned close to the examiner, while being easily accessible to operate yet far enough to be out of the stallion's range. Examination was carried out in dimmed light conditions. Both testes were examined from the left body side of the stallion. The scrotum and testes were palpated before examination to identify physiological characteristics and to determine the temperament and resistance each stallion might show. If stallions were not cooperative and did not tolerate examination, a nose-twitch was used. While palpating the testes, the other hand was placed on the withers of the stallion.

2.1.2.1. Caliper. The size of each testis was measured using a 'Stallion Scrotal Caliper' (Animal Reproduction Systems, Chino, CA, USA)