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Effect of breed on testicular blood flow dynamics in bulls

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ABSTRACT

The evaluation of testicular hemodynamics can contribute significantly to the understanding of the thermoregulatory mechanisms and oxygen supply of the testis in domestic animals. The present study aimed to characterize circulatory dynamics using the mean velocity (MV), pulsatility index (PI) and resistive index (RI) of the supratesticular artery in bulls. We evaluated 334 bulls of five different breeds (Nelore, Hereford, Aberdeen Angus, Braford and Brangus) by performing a velocimetry analysis using Doppler ultrasonography. Data were compared by Welch's ANOVA, Games-Howell (post-hoc test) and Spearman correlation with a significance level of 5%. The overall MV of 12.14 ± 0.30 cm/s differed among breeds. In addition, we observed that Brangus bulls showed higher ($P < 0.05$) MV (16.28 ± 1.02 cm/s) compared to Nelore bulls (8.76 ± 0.40 cm/s). The RI had an overall mean of 0.41 ± 0.01 and differed among breeds. We observed higher ($P < 0.05$) RI values in Hereford (0.44 ± 0.01) compared to Brangus (0.36 ± 0.02) animals. Overall, the PI values (0.33 ± 0.01) did not differ ($P > 0.05$) among breeds. The correlation between the PI and RI (0.936; $P < 0.001$) was high and positive; however, the correlations were low and negative between MV and the PI (−0.228; $P < 0.001$) and between MV and the RI (−0.270; $P < 0.001$). We concluded that there are differences in the MV and RI of the bulls' supratesticular arteries among the different evaluated breeds. Moreover, the presented values attributed to blood flow dynamics can serve as parameters in future studies and can be used to identify alternative diagnostic tools for infertility or to understand issues of adaptability in bulls.

1. Introduction

Ultrasonography is a safe, non-invasive technique that provides immediate information on internal structures and allows completely novel imaging for ultrasound-based screening and monitoring. In the field of large animal reproduction, ultrasonography of the reproductive organs can reveal dynamic events and has been used routinely during the past 30 years [1]. In addition, 25% of the ultrasonography used in veterinary medicine has been performed in reproductive tract evaluation and has been used more intensely in females during the breeding season in production animals [2–4]. Recently, color Doppler ultrasonography has provided distinctively different approaches for assessing the vascular system of reproductive organs [1,5,6].

Doppler ultrasonography has commonly been used to evaluate the estrus cycle and changes in reproductive status in cows; however, it has not been used as a routine technique in bull reproductive tract evaluation [4]. The Doppler pulsed-wave system is associated with Doppler color and used for the evaluation of blood supply, the vascular bed and blood flow velocity [7]. In humans, this new technology also allows the evaluation of hemodynamic changes in different organs and tissues, including varicocele and testicular alterations [8,9], testicular torsion [10], azoospermia with normal or altered vascularization [11], and testicular fluid

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alterations and pathologies [12,13]. In addition, this technique is well established and provides new information about physiological changes during reproductive phases in bovine females [14–16].

The production of viable spermatozoa depends on testicular physiological mechanisms, especially thermoregulation, since the temperature of the scrotum should be maintained at 2 to 6 °C below body temperature [17,18]. Among thermoregulatory mechanisms, the contractile action of the cremaster muscle helps to maintain blood flow dynamics [19–22]. The small amount of subcutaneous fat and the high number of sweat glands in the skin covering the testicles, as well as the tunica dartos, are also important contributors to testicular thermoregulation [22,23].

Thus, in addition to the oxygen and nutrient supply functions, blood flow also plays an important role in the maintenance of testicular temperature. However, little is known about the physiological parameters of testicular hemodynamics in bulls. In this study, it was hypothesized that bulls of different genotypes have different supratesticular blood flow dynamics. Therefore, the objective was to determine the hemodynamic parameters, the mean velocity, pulsatility index, and resistive index of the supratesticular artery measured by Doppler ultrasound in Angus, Brangus, Braford, Hereford, and Nelore bulls.

2. Materials and methods

All procedures involving animals in this study were approved by the Animal Ethical Committee from the UEL Institution (CEUA/UEL n. 18656/2014/58).

2.1. Animals, location and climate

This experiment was performed in farms located in the states of Tocantins (TO), Mato Grosso (MT), Mato Grosso do Sul (MS), Paraná (PR), Santa Catarina (SC), and Rio Grande do Sul (RS). The climatic conditions in PR and RS, according to the classification of Köppen, are the Cfa type (humid subtropical climate), which is characterized by four seasons well defined, being very hot during summer and potentially dry during winter. The climate in the states of MT, TO and MS is classified as Aw (tropical hot and humid climate), which is characterized by intense annual precipitation, especially during summer, and absence of winter. In the state of SC, the climate was considered Cfb (subtropical humid climate with mild summers) which is characterized by four seasons well defined, abundant precipitation throughout the year and no dry season defined [24].

The ambient temperature and relative humidity were obtained from INMET [25] for each farm location throughout the experimental period and were used to calculate daily values of the Temperature Humidity Index (THI). The THI data were estimated using the following equation described by Buffington et al. [26]:

$$THI = Tdb + 0.36 \times Tdp + 41.5$$

in which Tdb = dry-bulb air temperature (°C) and Tdp = dew-point temperature.

A total of 334 bulls aged from 18 to 36 mo were kept under extended conditions, all animals were kept in the field and fed in a diet system based on natural pasture (1.5AU/ha) and free access to shadow. Mineral supplementation and water were provided *ad libitum*. During the winter, the animals were submitted to a supplementary feeding program established at the farm. The breeds represented were Aberdeen Angus (AA; n = 57), Braford (BF; n = 94), Brangus (BR; n = 29), Hereford (HF; n = 109), and Nelore (NL; n = 45; Table 1). All animals were clinically healthy and had no pathologies in the reproductive tract at the time of evaluation. Prior to examination and reproductive evaluation, all bulls were held for a minimum period of two hours at the paddock. The animals were then restrained in the standing position in a chute suitable for bulls without sedation or tranquilization. To measure the scrotal circumference, the testes were pulled down to the bottom of the scrotum, and a millimeter tape was positioned around the largest circumference. An immediate repetition of this operation was performed to confirm the results.

2.2. Doppler ultrasonography evaluation

A real-time, portable ultrasound scanner with a 7.5-MHz linear array transducer (L741V) was used for data collection (SonoScape model A6V, DOMED, Valinhos, Brazil). The probe was positioned distally to the spermatic cord and together with the testicular parenchyma. This position was used as a standard anatomical position for all animals, allowing a perpendicular position within the supratesticular artery (Fig. 1A). A small amount of ultrasound gel was used to allow probe-skin contact, and measurements were then performed using a B-mode scan at the established site with minimum operator pressure (Fig. 1B). The primary structure visualized was the tortuous spermatic artery as it branches towards the testis. Once the supratesticular artery was identified, the Doppler color system was initiated, and the PW (Pulsed-Wave) mode was activated to capture at least four cardiac cycles. The image was then frozen, and one of the cycles was manually measured to obtain the chosen parameters.

Table 1
Data collected and parameters evaluated from bulls in Brazil.

Location	Animals (n)	Breeds[a]	Age (mo)	SC (cm)[b,*]	Climate[c]	Air temperature (^{0}C)*	Humidity (%)*	THI[d]
Mato Grosso	17	BF	30	36.3 ± 0.69	Aw	32.6	63	82.99
Mato Grosso do Sul	29	BR	24	39.1 ± 0.71	Aw	28.4	78	73.73
Paraná[e]	33	AA	24	39.3 ± 0.51	Cfa	25.6	70	74.15
	59	BF	36	35.5 ± 0.30				
Rio Grande do Sul[f]	94	HF	18	35.7 ± 0.21	Cfa	22.9	83	70.09
Santa Catarina	24	AA	28	40.7 ± 0.52	Cfb	23.6	77	75.33
	15	HF	24	38.3 ± 0.57				
Tocantins	18	BF	36	35.2 ± 0.82	Aw	28.2	75	78.04
	45	NL	30	32.6 ± 0.35				
Total number of bulls	334							

[a] AA: Aberdeen Angus, BF: Braford, HF: Hereford, BR: Brangus, NL: Nelore.
[b] Scrotal circumference.
[c] According to IBGE (Aw - tropical hot and humid climate, Cfa - subtropical humid climate and Cfb - subtropical humid climate with mild summers).
[d] Temperature humidity index.
[e] Data were collected at different periods.
[f] Data were collect from two farms at the same municipality at the same day;* Data showed as mean ± SEM.