

Hemodynamic ultrasound evaluation of the mammary gland of buffaloes at different categories

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[Avaliação hemodinâmica ultrassonográfica da glândula mamária de búfalas em diferentes idades]

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ABSTRACT

The study aimed to evaluate the mammary artery of Murrah crossbred buffaloes at different physiological stages using ultrasonography. Twenty-four pasture-raised animals were divided into four groups (n= 6): heifer calves, yearlings, pregnant animals, and lactating animals. Doppler examinations of the cranial and caudal mammary arteries were performed every 28 days to measure the internal diameter (ID), resistivity index (RI), and pulsatility index (PI). Over one year for heifer calves and yearlings, RI and PI decreased, while ID increased ($P<0.0001$). No significant differences were found between the cranial and caudal arteries. In pregnant animals, the caudal mammary artery showed lower RI and higher ID during the last five and seven months ($P<0.0001$), respectively, while lactating animals had higher ID in the first seven months ($P<0.0001$). These findings suggest the plasticity of the mammary artery during the stages of growth, pre-puberty, pregnancy, and lactation.

Keywords: spectral doppler, mammogenesis, dairy buffalo

RESUMO

O presente estudo teve como objetivo avaliar a artéria mamária de búfalas mestiças Murrah em diferentes estágios fisiológicos, por meio de ultrassonografia. Vinte e quatro animais criados em pasto foram divididos em quatro grupos (n= 6): bezerras, novilhas, animais prenhes e animais lactantes. Exames de Doppler das artérias mamárias cranial e caudal foram realizados a cada 28 dias para medir o diâmetro interno (DI), o índice de resistividade (RI) e o índice de pulsatilidade (PI). Ao longo de um ano, para bezerras e novilhas, o RI e o PI diminuíram, enquanto o DI aumentou ($P<0,0001$). Nenhuma diferença significativa foi encontrada entre as artérias cranial e caudal. Em animais prenhes, a artéria mamária caudal apresentou menor RI e maior DI nos últimos cinco e sete meses ($P<0,0001$), respectivamente, enquanto os animais lactantes tiveram maior DI nos primeiros sete meses ($P<0,0001$). Esses achados sugerem a plasticidade da artéria mamária durante os estágios de crescimento, pré-puberdade, gestação e lactação.

Palavras-chave: Doppler espectral, mamogênese, búfalo leiteiro

INTRODUCTION

The production of buffalo milk has a relevant contribution to the economic and social development of several countries. Currently, 15% of the global production of fresh milk is

from buffalo, with India being the country with the highest production (92 million tons), herd (109.85 million head) and consumption (per capita of 337g/day) (World..., 2021).

Brazil has the largest buffalo herd in the Americas (1,502,482 heads). Animals are

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traditionally bred for meat production, but milk production has been growing and presenting itself as a new alternative to national dairy farming. It is estimated that milk production in the country exceeds 92 million liters per year, especially in the Southeastern region of the country, which has a well-developed production chain for milk and dairy products (Pesquisa..., 2021).

In dairy herds, knowledge of aspects related mainly to the development of the mammary gland is fundamental, since the growth and differentiation of the mammary tissue are determining factors for the productive indices of the herd (Geiger, 2019). The magnitude of mammary gland development in the first years of life will dictate the extent of lobe-alveolar development in the productive life of the animal (Rowson *et al.*, 2012). Therefore, the study of the different stages of development of the mammary gland of Brazilian dairy buffaloes is important for the solidification of the dairy activity in the country, in addition to providing pioneering information to the species.

In this sense, in vivo non-invasive evaluations of animals in real time have been sought in dairy females to estimate cistern size (Caja *et al.*, 2004) and oversee udder health and milk production (Bonelli *et al.*, 2020; Schwarz *et al.*, 2020). Mammary ultrasonography represents a tool of significant importance in animal physiology research and when associated with Doppler provide information about blood flow and make the evaluations more complete (Dantas *et al.*, 2017; Piccione *et al.*, 2018). The mammary artery is the main responsible for the blood supply of the mammary gland in ruminants, so the hemodynamic importance of this vessel for the mammary tissue of dairy cows is due to the knowledge of data that make it possible to describe patterns of blood perfusion and determine the metabolic state of the analyzed organ (Götze *et al.*, 2010). However, reference values for the interpretation of this information in dairy animals are scarce.

The objective of the present study was to evaluate the hemodynamic parameters of the mammary arteries by Doppler ultrasonography of river buffaloes at different stages of body development.

MATERIAL AND METHODS

All aspects of animal experimentation involved in this study were approved by the Ethics Committee on the Use of Animals (CEUA), São Paulo State University (UNESP), School of Veterinary Medicine and Animal Science, Botucatu, under Protocol No. 79/2013.

The work was carried out with 24 clinically healthy Murrah crossbred buffaloes, belonging to the same herd, divided into 4 groups (n= 6): calves (group 1), heifers (group 2), pregnant primiparous non-lactating (group 3) and multiparous pregnant lactating (group 4). The initial age of the animals in each group was zero, 12, 24 and 48 months. Groups 1 and 2 were evaluated for 12 months and groups 3 and 4 for 10 months.

The inclusion criteria of the animals in the study were: (a) body weight compatible with the physiological state; (b) no history of reproductive or mammary gland diseases; (c) absence of abnormalities in the general physical examination and in the mammary gland (inspection and palpation), as described by Duguma (2016); (d) verification of the gestational primiparity of the females of group 3, through the evaluation of the reproductive history of each animal combined with the chronological age (month), since most of the development of the mammary gland occurs during the first pregnancy; (e) lactation number equal to 3, for females belonging to group 4, since the physiological development of the mammary gland is completed in the third lactation (Akers, 2017).

Throughout the study, the groups were kept in separate batches. Calves (group 1) remained with their mothers (group 4) until seven months of age, when the weaning occurred. All animals were kept under the same conditions as rotational grazing, with water and mineral mixture provided *ad libitum*. Ultrasound examinations were also performed monthly in the buffaloes of groups 3 and 4, confirming the maintenance or absence of pregnancy, respectively (Marques *et al.*, 2020).

Ultrasound examinations of the cranial and caudal mammary arteries were performed at 28-day intervals, at the same time of the day, over a one-year period, by the same operator and

assistant. The examination was performed with an ultrasound Mylab 30 (Esaote® SpA, Firenze, Italy) with a linear transducer (frequency of 13 MHz). The criteria adopted for choosing this time interval between ultrasound evaluations were the availability of infrastructure, financial resources, labor and animal stress, which could negatively influence the results.

It is noteworthy that the females of groups 2, 3 and 4 underwent a period of conditioning to familiarize themselves with the sequence of activities and the team responsible for the study. It started 3 months before the start of the project and consisted of interacting with the animals and

performing weekly ultrasound examinations of the mammary gland.

Each ultrasound examination took up to 15 min, the animals were at a standing position and no anesthetic protocol was applied. Before each examination, udder trichotomy was performed and then gel was applied in the area as contact medium for ultrasonic transmission. The transducer was placed on the cranial surface of the mammary gland, parallel to the teat (transcutaneous ultrasonography), and the images were recorded in sagittal and transverse planes using B-MODE configuration (Figure 1 A, B).

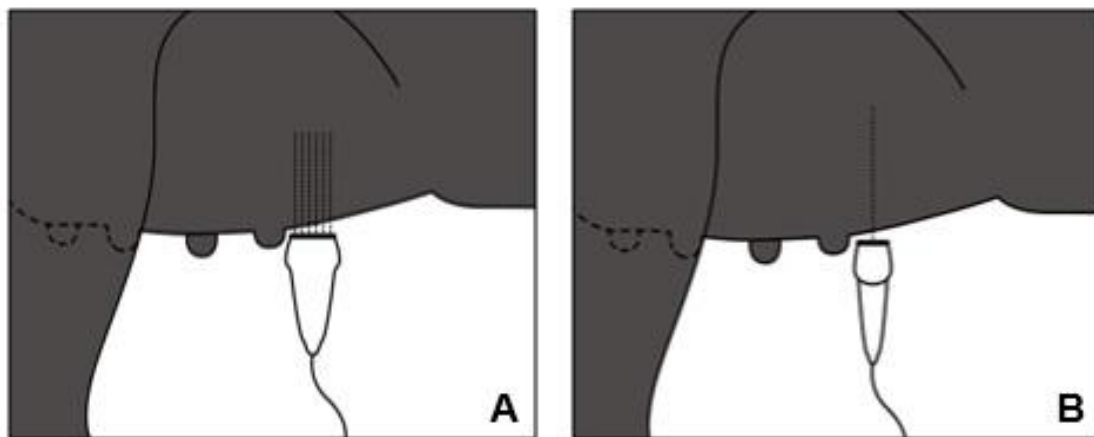


Figure 1. Position of the transducer on the cranial surface of the mammary gland, parallel to the teat (transcutaneous ultrasonography). Images performed in sagittal (A) and transverse planes (B).

Firstly, the cranial mammary artery was located by Color Doppler and then, using spectral Doppler, the cursor was positioned over the target artery. The insonation angle used did not exceed 60°. Cross section images of the artery (initial portion) were obtained and recorded; all evaluations performed for the cranial mammary artery were repeated on the caudal mammary artery. The ultrasound images were independently analyzed by three evaluators with more than 5 years of experience in veterinary ultrasonography, who were blinded to any information of any animal, as well as the review of the other evaluators. All examiners used the same image processing software, and their results were later compared through discussion, with the values with which the three evaluators agreed on being considered the final result. To assess resistivity (RI) and pulsatility (PI) indexes

of each vessel, quantitative evaluations were performed using MyLab software (MyLab®Desk software, Esaote, Genova, Italy), the calculations were based on the spectral waveform morphology of the same systolic and diastolic amplitude, obtained from three consecutive cardiac cycles. The formulas used to calculate RI and PI were respectively: $RI = (PSV - FDV) / PSV$; $PI = (PSV - FDV) / TAMAX$, where PSV is the peak systolic velocity, FDV at final diastolic velocity and TAMAX at time-averaged maximum velocity (Evangelista *et al.*, 2022; Meinecke-Tillmann, 2017). The internal diameter of each vessel (ID) was also measured and expressed in cm based on the processing of the same software.

Analysis of variance (ANOVA) with repeated measures was performed to evaluate the means

of RI, PI and ID (cranial and caudal mammary arteries). Experimental groups (1, 2, 3 and 4) were categorized as covariates and differences were statistically significant when $P < 0.05$. All analysis was performed using SAS® software (Statistical..., 2009). It was recommended only for cranial and caudal mammary arteries, given the absence of statistical difference in the hemodynamic indices of the left mammary arteries in relation to the right.

To study the possible linear relationship between the mean values of RI, PI and ID measured during the experimental period, Spearman's non-

parametric correlations were calculated. The correlation coefficient was considered null ($r = 0$), low ($0 < r \leq 0.30$), moderate ($r = 0.30 < r \leq 0.70$) and strong ($0.70 < r \leq 1$) (Akoglu, 2018).

RESULTS

In calves and heifers, there was a reduction in RI and PI and an increase in ID ($P < 0.0001$) from the first to the last month of evaluation, however, there was no statistical difference for the hemodynamic indices between the cranial and caudal mammary arteries (Figure 2 A, B, C).

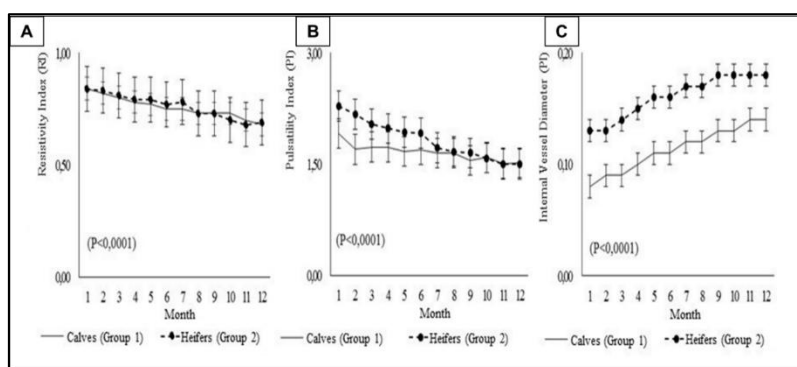


Figure 2. Mean of the resistivity index (A), pulsatility index (B) and internal diameter of the vessel (C) of the mammary arteries of crossbred Murrah calves (group 1) and heifers (group 2), evaluated for 12 months.

In groups 1 and 2, vascular indices were significantly correlated with each other. In calves, RI was strongly and positively correlated with PI ($r = 0.94$; $P < 0.0001$) and negatively with ID ($r = -0.98$; $P < 0.0001$), in addition to PI that also had a strong and negative correlation with ID ($r = -0.98$; $P < 0.0001$). Likewise, in the females of group 2 there was a significant and strong correlation, being positive between RI and PI ($r = 0.99$; $P < 0.0001$) and negative, between RI and ID ($r = -0.95$; $P < 0.0001$), as well as between PI and ID ($r = -0.98$; $P < 0.0001$) (Table 1).

Table 1. Spearman's correlation coefficients and the significance levels obtained between the hemodynamic parameters of calves, heifers, pregnant and lactating of crossbred Murrah

	Calves			Heifers			Pregnant			Lactating		
	RI	PI	ID	RI	PI	ID	RI	PI	ID	RI	PI	ID
RI	1.000	0.94* <0.0001	-0.98* <0.0001	1.000	0.99* <0.0001	-0.95* <0.0001	1.000	0.98* <0.0001	-0.98* <0.0001	1.000	0.91* 0.0002	-0.95* <0.0001
PI	0.94* <0.0001	1.000	-0.98* <0.0001	0.99* <0.0001	1.000	-0.98* <0.0001	0.98* <0.0001	1.000	-0.98* <0.0001	0.91* 0.0002	1.000	-0.82* 0.0034
ID	-0.98* <0.0001	-0.98* <0.0001	1.000	-0.95* <0.0001	-0.98* <0.0001	1.000	-0.98* <0.0001	-0.98* <0.0001	1.000	-0.95* <0.0001	-0.82* 0.0034	1.000

*Statistically significant when $P < 0.05$.

In group 3, there was a progressive decrease in RI and PI values and an increase in ID in both mammary arteries, from the first to the last month of the study. However, in the caudal mammary artery, lower RI ($P < 0.0001$) and

higher ID ($P < 0.0001$) were observed in the last five and three months of the experimental period, respectively (Figure 3 A-F). Correlation was also found between all hemodynamic parameters, being positive and high between RI and PI ($r =$

0.98; $P < 0.0001$) and negative and high between RI and ID ($r = -0.98$; $P < 0.0001$), as well as

negative and high PI with ID ($r = -0.98$; $P < 0.0001$) (Table 1).

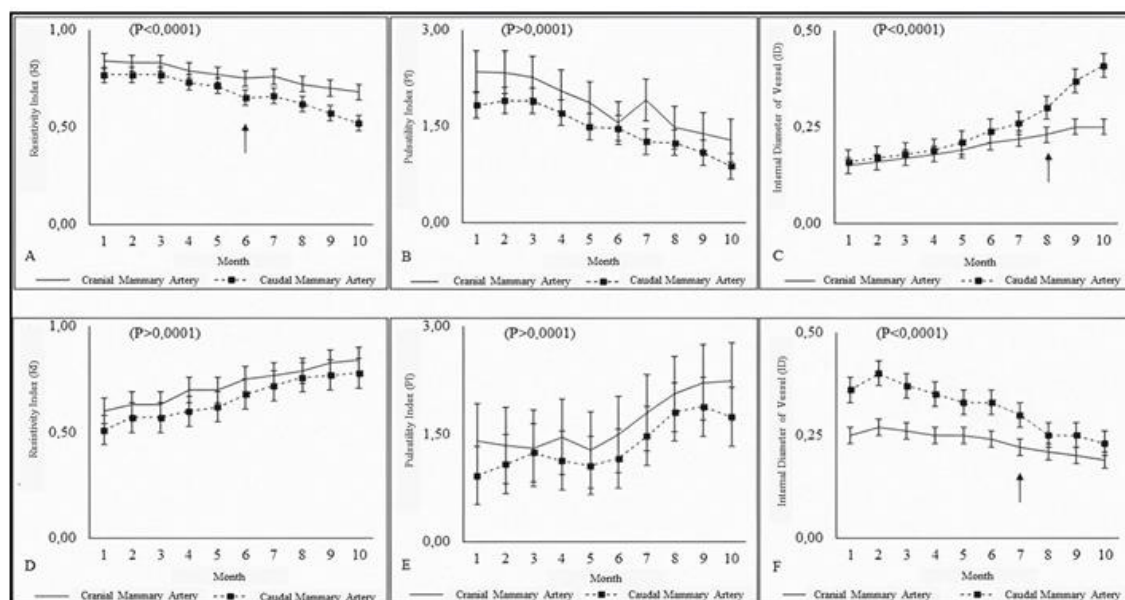


Figure 3. Mean resistivity index (A), pulsatility index (B), internal diameter (C) of the left cranial and caudal mammary arteries of pregnant Murrah crossbred buffaloes (Group 3). Resistivity index (D), pulsatility index (E), internal diameter (F) of the left cranial and caudal mammary arteries of lactating Murrah crossbred buffaloes (Group 4), evaluated monthly throughout the study. (↑) They indicate lower RI and higher ID in the final five and three months of pregnancy and higher ID in the first seven months of lactation, shown in tables (A), (C) and (F), respectively.

In group 4, there was a gradual increase in RI and PI values and a decrease in ID in both mammary arteries, from the first to the last month of evaluation. However, there was a significant difference for the Doppler indices of the mammary arteries, being observed during the first seven months of the experiment, greater ID for the caudal mammary artery ($P < 0.0001$) (Figure 3 A-F). The correlation between hemodynamic indices was strong, being positive between RI and PI ($r = 0.91$; $P = 0.0002$) and negative between RI and ID ($r = -0.95$; $P < 0.0001$) and between PI and ID ($r = -0.82$; $P = 0.0034$) (Table 1).

DISCUSSION

In calves and heifers, the gradual decrease in RI and PI and the increase in ID throughout the study, imply a reduction in vascular resistance and a continuous increase in blood perfusion. According to Wood *et al.* (2010), the lower the RI value, the greater the perfusion required by the tissue and second (Castelló *et al.*, 2015) the

reduction in PI infers, in addition to increased tissue perfusion, vasodilation and increased cardiac output. Thus, vessels that supply organs that need constant perfusion generally have low RI and PI. Likewise, the increase in ID observed in both groups throughout the study contributed to the increase in arterial lumen and mammary blood supply.

The results obtained in the present work indicate that groups 1 and 2 showed an increase in the amount of blood transferred to the mammary gland during the experimental period. This was probably due to the increase in nutrient demand in response to the expected development of mammary tissue for both groups, which corroborates with Hardy *et al.* (2021), who observed that in prepubertal calf, the importance of a non-limiting blood supply is emphasized, as the supply of mammary blood flow establishes a direct relationship with the intensity of gland development, being in tune with changes in this organ.

Also, in group 3, the decline in RI and PI and increase in ID during pregnancy, infers an increase in the vascular system's ability to adapt to the gland's energy requirement due to its preparation for subsequent lactation. Most of the mammary incorporation occurs in the last months of pregnancy (~80%) and during this period, the mammary gland undergoes significant physical (size and shape) and functional transformations (Davis, 2017), requiring an increase in arterial caliber and blood perfusion. In this same group, the caudal mammary artery presented higher hemodynamic values (RI and ID) at the end of pregnancy than the cranial artery, reinforcing the hyperdynamic state of blood flow with the advancement of lactogenesis, probably due to the importance that this vessel represents for the gland, as milk production is higher in the hind quarters (Pandey *et al.*, 2018).

During lactation, the variation of hemodynamic parameters presented a behavior contrary to that observed in the other groups (increase in RI and PI and decrease in ID) throughout the study, characterizing a reduction in blood perfusion and arterial caliber, as tissue involution occurs mammary. Differently from what was observed by Piccione *et al.* (2018) and Götze *et al.* (2010), who despite having used Doppler ultrasonography to assess the velocity of blood flow in the mammary vein of cows at various stages of lactation, did not obtain significant changes. According to Zhao *et al.* (2019), the decrease in mammary blood flow occurs due to the decrease in the demand for metabolites, due to the decrease in the activity of milk synthesis, and this decrease is associated with the decrease in mammary volume.

In addition, the caudal mammary artery differed from the cranial one until the seventh month of lactation, presenting higher ID. Changes in hemodynamic indices are directly influenced by the importance of the blood contribution that the vessel exhibits in relation to a given organ (Meinecke-Tillmann, 2017), possibly the volume of blood flow in the caudal mammary artery was greater, corresponding to the physiological merit that it represents for the gland, since the hind quarters are more developed and produce more milk than the fore quarters (60 and 40%, respectively), requiring more intense blood perfusion (Akers, 2017). It is believed that this

difference between the cranial and caudal mammary arteries was not maintained until the end of lactation, due to the weaning of the calves that took place in the seventh month of lactation. The cessation of suckling stimulation intensifies the involution of mammary tissue, in agreement with Davis and Collier (1985) and Hurley (1989), who state that the blood supply of the mammary gland and the regulation of milk production are closely related.

The RI assessment also provides information linked to the metabolic state of the organs, where decreasing RI values suggest increased metabolism (Jenderka and Delorme, 2015; Revzin *et al.*, 2019). Thus, the continuous decrease in RI observed in calves, heifers and pregnant is related to the increase in metabolic activity in the tissue, showing consistency between the different phases of mammary development. According to Cai *et al.* (2018), the increased blood supply to the mammary gland of dairy animals favors mammary structural development and occurs according to the dynamics of the magnitude of their physiology. And the opposite is also true, the progressive increase in RI indicates a decrease in the metabolic activity of the gland because of the decline in milk production. According to Gross and Bruckmaier (2019), during the normal course of lactation, blood flow volume and mammary metabolic activity are closely associated, so the decrease of one trait leads to the reduction of the other.

In groups 1, 2 and 3, RI and PI were significantly correlated with each other, since the decrease in one variable was closely associated with the decrease in the other. Furthermore, the decrease in RI and PI was related to the corresponding increase in ID. Several Doppler indices allow semi-quantitative assessments. The two highly correlated indices that are most used clinically for the description of arterial flow velocity waveforms are the RI and the (PI), since neither the vessel size nor the angular correction need to be known for their calculation (Nelson and Pretorius, 1988).

According to Ochi *et al.* (1995), vascular resistance presents a linear correlation with PI and, often, quadratic with RI. Thus, the correspondence between the hemodynamic indices evaluated reflected the increase in

intramammary blood perfusion that occurred due to its morphological development, being, therefore, good parameters to analyze in real time the blood regulation of the mammary gland of growing and pregnant buffaloes. What contrasts with Dantas *et al.* (2017), who observed that the mammary hemodynamic values remained constant, regardless of the body development presented by the growing females.

In the group of lactating, RI and PI were also highly and positively correlated, however, the increase of the two and the decrease in ID reproduced the scenario of the physiological decrease in blood flow volume with the advancement of lactation with a consequent decrease in vessel amplitude, characteristics associated with regression of secretory tissue and decline in milk production during lactation (Capuco and Akers, 1999).

CONCLUSION

In conclusion, the hemodynamic indexes of the mammary arteries reflect the ages studied and can be considered predictors of the progression of the basal mammary development of healthy crossbred Murrah buffaloes during the first two years of life.

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