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Evaluation of changes in blood flow of the uterine artery by Doppler ultrasonography during the estrous cycle in lactating *Bos indicus* cows

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ABSTRACT

The objective of this study was to evaluate the changes in uterine blood flow (UBF) based on the resistance index (RI) and the pulsatility index (PI) by Doppler ultrasonography throughout the estrous cycle in lactating Sahiwal cows (n = 9). Cows were randomly selected during their spontaneous estrus. UBF was examined on alternate days in all cows during the estrous cycle. The results revealed that the mean RI values were higher ($P < 0.05$) on day −1 (estrus) than on day 0 (ovulation). The mean RI values followed a consistent pattern on days 2, 4, 6, and 8 (metestrus to early diestrus) and were lower ($P < 0.05$) than on days 10, 14 16, and 18 (diestrus to early proestrus). The RI and corpus luteum (CL) size negatively correlated during its static phase ($r = -0.99$; $P < 0.05$). The mean RI was greater ($P < 0.05$) in high producers than in low producing cows. The mean PI value was higher ($P < 0.05$) on day 10 than on day −1 and day 0. Similarly, the mean PI values were lower ($P < 0.05$) on days 2 and 4 than on day 10. The PI value remained significantly lower ($P < 0.05$) on day 2 than on day 8 of the estrous cycle. There was a positive correlation between RI and P4 ($r = 0.70$; $P < 0.05$) and PI and P4 ($r = 0.56$; $P < 0.05$) during the estrous cycle. It is concluded that the RI of uterine arteries, as a measure of blood flow, is considerably lower, while the PI is substantially elevated during diestrus compared to estrus and ovulation in Sahiwal cows.

1. Introduction

Bos indicus breeds make a significant contribution to the beef and dairy industries in the tropical and sub-tropical regions (Pegorer et al., 2007). Although, their milk production ability is far less than the *Bos taurus* (Bennett et al., 1985), they have increased resistance to internal and external parasitic infestations (Glass et al., 2005; Khan et al., 2008). Genetic improvement in *Bos taurus* has been primarily based on improved selection methods and the wider use of artificial insemination, although there has been a concomitant decrease in fertility (Lucy, 2001; Pryce et al., 2004). The physiological details of growth and regression of the follicle, corpus luteum (CL), and hormones (Sirois and Fortune, 1988) were essentially required to develop estrus and ovulation synchronization. Moreover, the advent of pre-synchronization and resynchronization protocols resulted in acceptable fertility (Stevenson et al., 1999;

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Wiltbank and Pursley, 2014). These manipulations of *Bos taurus* when attempted in *Bos indicus* do not seem to work well due to physiological differences between these 2 bovine sub-species (Pinheiro et al., 1998; Sartori et al., 2016). Thus, there is a dire need for comprehensive information on physiological events during the estrous cycle of *Bos indicus* cows.

Doppler ultrasonography has extended the scope of imaging from an anatomical to the physiological basis (Herzog and Bollwein, 2007). This new technique was used to measure physiological and pathological changes in uterine blood flow (UBF) (Steer et al., 1990). Early work, using invasive procedures, measured blood flow in the reproductive system of cows which indicated that there are rhythmic changes which were related to concentrations of progesterone (P4) and estrogen (E2) during the estrous cycle (Ford and Christenson, 1979; Ford et al., 1989). Color Doppler ultrasonography, a non-invasive technique developed 2 decades ago, measured changes in blood flow during the estrous cycle of mares (Bollwein et al., 1998), sheep (Roman-Ponce et al., 1983), and Simmental cows (Bollwein et al., 2000). This technique has been used to measure alteration in blood flow during placental separation (Van Camp, 1991), pregnancy (Honnens et al., 2008), uterine torsion (Hussein, 2013), and puerperium (Krueger et al., 2009; Heppelmann et al., 2013). This imaging procedure has provided measurements on the vasculature of the follicle (Acosta et al., 2003; de Tarso et al., 2015), CL (Miyazaki et al., 1998; Acosta et al., 2002), ovulation (Aslan et al., 2011), and uterus (Scully et al., 2015). More recently, it has been used to predict the occurrence of pregnancy on the day of embryo transfer (Kanazawa et al., 2016).

Sahiwal is an established milch breed of zebu cattle, representing *Bos indicus* (Shah, 1994). Information on this breed's reproductive aspects is lacking (Layek et al., 2011; Hassan et al., 2016). Therefore, the objective of this study was to evaluate the changes in UBF based on the resistance index (RI) and the pulsatility index (PI) by Doppler ultrasonography during the estrous cycle in Sahiwal cows. Additionally, the correlation of UBF with blood P4 concentrations was investigated.

2. Materials and methods

2.1. Animal management and study design

This study was carried out during breeding season (March–June 2015) at Livestock Experiment Station Jahangirabad in the Khanewal district of Punjab, Pakistan. This study was conducted on eleven (n = 11), adult, healthy, cycling, and lactating Sahiwal cows with body condition score (BCS, scale 1 = poor and 5 = excellent) of 3.38 ± 0.33, and body weights of 430 ± 18 kg maintained under uniform conditions of feeding and management. The cows were enrolled at their spontaneous estrus and ultrasonography was performed on alternate days on each cow during the estrous cycle. Estrus detection was carried out twice daily (6:00 a.m. and 6:00 p.m.) using penile-deviated teaser bulls to determine the onset of standing heat.

2.2. Observation of follicular and luteal dynamics

Each cow underwent transrectal gray scale ultrasonography (My Lab30 Gold VET, Esaote, Genoa, Italy) attached to a linear array probe of 7.5 MHz frequency from 1 estrus (day − 1) to subsequent estrus, on alternate days by the same operator under optimized conditions as described by (Pierson and Ginther, 1984). Detailed drawings of the ovaries were made to record the number, diameter and relative position of the follicle and CL. Follicular diameters and the sequential identification of individual follicles ≥4 mm were recorded as described by (Knopf et al., 1989). The day on which signs of estrus and dominant ovulatory follicle observed was designated as day −1 (estrus), while the day on which the dominant follicle disappeared was designated as day 0 (ovulation). The ovulation time and size of the follicle just before ovulation were determined. The diameter of the dominant follicle was measured each time. Ovulation was based on when a previously observed large follicle was no longer present at the subsequent ultrasonographic examination. This was up until 12 h after the onset of standing estrus. Mid-point from the first finding of the ruptured follicle and the previous check of the intact follicle was considered ovulation time (Sá Filho et al., 2010). The onset of luteal regression was defined as the first day; the CL diameter began to decline and continued to decrease thereafter. The CL diameter was calculated as described by (Kastelic et al., 1990). The diameter of the CL was measured throughout the estrous cycle and was divided into 3 phases (Tom et al., 1998): growth, static, and regression between days 4–9, 10–14, and 15–18 of the cycle, respectively.

2.3. Measurement of UBF using color doppler ultrasound

Doppler measurements were determined using color Doppler ultrasound (My Lab 30 Gold Vet, Esaote, Genoa, Italy) attached to a linear array probe of 7.5 MHz frequency by the same operator for at least 40 min per cow on alternate days. All observations were obtained in the morning and the cows were restrained properly. Pulse wave Doppler function was used to measure the velocity of waveform. To minimize variations, a standardized preset was used throughout the examination of both the color and pulse wave Doppler; an integration angle between the Doppler ultrasound beam and the flow direction was set at 45 ° and a color gain of approximately 70 was used. To eliminate the signal from moving tissue and vessel wall movements in the path of the Doppler ultrasound pulse, the high pass filter was set at 100 Hz. For flow evaluation a small color box with a depth adequate for maximizing the number of frame per minutes (frame rate), a pulse repetition frequency (PRF) of 1.3 KHz and a sample size of 1 mm were maintained. Each observation was recorded on the hard drive of the Doppler unit. The analysis was based on the Doppler spectrum. UBF was examined in the left and right uterine arteries using the method published previously (Bollwein et al., 2000). The uterine artery is a moveable vessel found within the mesometrium. Once the uterine artery was identified through color mapping the electronic marker (gate) was placed on the central region of the vessel and the pulsed Doppler tool was started. The RI and PI were measured to reflect changes in blood flow. The RI and PI were calculated from the built in caliper for the values of the UBF