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Original Research

Transrectal Doppler Ultrasound to Study the Uterine Blood Flow Changes During the Puerperium in the Egyptian Buffaloes

Mohamed Gohar[1,3], Samy Zaabel[1,2], Wael Eldomany[1,2], Asharaf Eldosouky[1,2], Walid Tawfik[3], Heba Sharawy[1,2], Mohammed Elmetwally[1,2*]

[1]Department of Theriogenology, Faculty of Veterinary Medicine; Mansoura University; 35516 Mansoura; Egypt.
[2]Center for Reproductive Biotechnology, Faculty of Veterinary Medicine; Mansoura University; 35516 Mansoura; Egypt.
[3]Veterinary Department, Egyptian Armed Forces, Nasr City, Cairo, Egypt.

***Correspondence**
Mohammed Elmetwally
Department of Theriogenology, Faculty of Veterinary Medicine; Mansoura University; 35516 Mansoura; Egypt.
E-mail address: mmetwally@mans.edu.eg

Abstract

The aim of the current study was to determine the changes in uterine blood flow as well as uterine biometry during the first 5 weeks after parturition in Egyptian buffaloes. The transrectal noninvasive color Doppler ultrasound and uterine biometry were measured at different time points -7, 0 (day of parturition), 7, 14, 21, 28, 35 after the postpartum period. The mean diameter of the uterine horn decreased significantly from day 0 to day 35 after parturition ($P < 0.0001$). The uterine involution was completed on day 28, as demonstrated by transrectal palpation and B-mode sonography. Peak systolic velocity (PSV), timed average maximum velocity (TAMV), blood flow volume (BFV), pulsatility index (PI), resistance index (RI), systolic /diastolic (S/D) as well as the diameter of uterine arteries were the Doppler indices for determination of changes in uterine artery blood flow during different time points after parturition. BFV of uterine arteries decreased linearly during the postpartum period in Egyptian buffaloes. The BFV decreased from 3029.21 ml/min (-7d) to 343.84 ml/min (35 d pp) and moderately ($P < 0.01$) to 731 ml/min on day 28. The TAMV and PSV showed fluctuation in changes during different time points postpartum ($P < 0.05$). But PI, RI, and SD showed significant increases during the different time points postpartum ($P < 0.01$). Altogether, the results show that transrectal color Doppler ultrasound is a successful tool for examining uterine changes during the first 5 weeks after parturition in Egyptian buffaloes. The robust changes in uterine blood flow were demonstrated during the first week of the puerperium, The PI, RI, and S/D were also suitable to investigate alterations in uterine perfusion during the next 8 weeks after parturition.

KEYWORDS
Buffaloes, Puerperium, Transrectal Doppler Ultrasound, Uterine Blood Flow

INTRODUCTION

The domestic buffalo (Bubalus bubalis) plays an important part in agricultural and agricultural economies in many countries (Perera, 2011). It is also used as a source of milk and meat in many nations. The puerperal period in buffaloes is a key stage in the reproductive cycle, during which uterine involution occurs (Gohar *et al*., 2018). Abnormal uterine involution reduces fertility dramatically, so a detailed understanding of the processes involved in uterine involution is critical for successful management of buffaloes and cows during the early postpartum period (Elmetwally, 2018; Lin *et al*., 2020). The reproductive performance of buffaloes influences the success and profitability of the dairy and beef industry, with one calf per cow per year being the optimum goal (Easa *et al*., 2022; Gallab *et al*., 2022). The return of the reproductive cycle and subsequent pregnancy are highly dependent on postpartum uterine involution (Hussien *et al*., 2021).

Previous studies in our lab were published, indicating uterine and vaginal blood flow changes during pregnancy in Egyptian buffaloes (Elmetwally *et al*., 2021a). Moreover, there was another study investigated the ovarian and uterine blood flow changes in pregnant and non-pregnant pluriparous Egyptian buffalos (Abouelela *et al*., 2021).

For many years, Doppler sonographic examination of uterine blood flow has been utilized to detect postpartum uterine involution in cows (Heppelmann *et al*., 2013), small ruminants (Elmetwally and Bollwein, 2017), cats (Blanco *et al*., 2015), dogs (Batista *et al*., 2013), and women (Sohn *et al*., 1988).

Increases in pulsatility index (PI) indicate decreases in tissue perfusion and vice versa. As the relation between PI and The resistance index (RI) is positive, one of these indices is assumed to be sufficient. The usefulness of PI and RI as Doppler indices in most color Doppler studies is that they are not affected by both Doppler angle and diameter of the blood vessel, but are affected only by the heart rate (Elmetwally and Meinecke-Tillmann, 2018). As gestation advances, PI and RI decrease progressively, indicating an increase in fetal blood perfusion (Elmetwally *et al*., 2021b). Furthermore, RI and PI are ratios of velocity measurements independent of Doppler angle. The systolic/diastolic ratio (S/D ratio) indicates the impedance of blood flow (S is the maximum Doppler shift frequency and D is the minimum Doppler shift frequency). A decrease in this ratio indicates an increase in blood perfusion of the target tissue (Elmetwally, 2016). The resistance index

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(RI) is one of the most important indices of vascular perfusion of the tissue, and it is usually recommended for low resistance vascular beds with continuous blood flow during diastole. It can be calculated using the equation: RI=PSV-EDV/PSV (Elmetwally, 2016). The importance of the resistance index stems from its negative relationship with vascular perfusion. Namely, decreasing the resistance increases vascular perfusion, and vice versa.

Using this technique, researchers discovered a link between uterine blood flow and delayed uterine involution. Furthermore, there were few studies concerning the evaluation of the uterine blood flow during the early postpartum period in buffaloes. Because clinical studies have shown that uterine and ovarian changes in normal buffaloes occur rapidly during the period of puerperium, this study was conducted to provide normal values for uterine artery blood flow changes at frequent intervals via Doppler sonography in normal parturient buffaloes using noninvasive color Doppler ultrasound. This noninvasive technique may be used as an objective method to assess the uterine involution in Egyptian buffaloes.

MATERIALS AND METHODS

Ethical approval number

This study was conducted at Elmax farm of the veterinary services department of Egyptian Armed Forces, Alexandria Provenance, with an approved animal use protocol (R/35) in accordance with the Guiding Principles for the Care and Use of Research Animals Veterinary Medicine Faculty, Mansoura University, Egypt.

Animal feeding and housing

A total of thirty-five healthy buffalo cows (5 late pregnant buffaloes one week before the expected calving date and 30 post-calving), aged 4-9 years old with 2-6 lactation seasons, were used in this study. All buffalo cows used had normal parturition without any pathological signs throughout the experiment. All buffalo cows have inseminated artificially for 14 h after the second GnRH injection of the ovysnch protocol [GnRH (day 0), PGF2α (day 7), GnRH (day 9)] with Italian seminal fluid straws and were milked twice daily by machine for seven months, then dried off milk for the remaining days of gestation.

These animals were kept in open, hygienic yards that were provided with holding pens for veterinary examination. Animals were offered the available daily ration consisting of concentrates (prepared mainly from ground yellow corn (350kg), wheat bran (235kg), sunflower cake (375kg), and (40kg) food additives with a minerals-vitamins supplement per ton of ration). Analysis of this ration included crude protein (16%), total digestible nutrients (TDN) (76%), calcium (0.1%) and phosphorus (0.5%).

Each one received about (5kg) concentrates per day, in addition to (25kg) Egyptian sweet clover and wheat straw that were also provided as bulk for the diet. Tap water and mineralized salt licks were available ad libitum.

Ultrasound scanning and image analysis

The uterine arteries in buffaloes were located and examined according to previous studies (Elmetwally *et al*., 2021a). The middle uterine artery, a branch originating from the internal iliac artery, was examined near its origin at the rudimentary umbilical artery, which is located cranial to the external iliac artery. The Doppler waveforms were taken at this location by activating the pulsed Doppler function and modifying the Doppler gate over the uterine artery, adjusted to the diameter of the vessel (Fig. 1). The vaginal arteries were located according to Elmetwally *et al*. (2021a) and Sharawy *et al*. (2022).

Doppler ultrasound examinations were performed by using Esaote MyLab 30 (Esaote MyLab 30X Vision, Esaote, Genova, Italy) with high-frequency linear transducers: 6–12 MHz with a filter of 100 Hz, power of 50%, pulse repetition frequency (PRF) of 4500 Hz and Doppler angle varying between 0̊ and 40̊. Epidural anesthesia using 4 mL procaine hydrochloride (Procasel 2%, Selectavet, Weyarn-Holzolling, Germany) was administered immediately before measuring blood flow in order to avoid continuous straining by the buffalo cows. The Doppler indices that the device displayed for each waveform by applying the automatic mode for uterine arteries were time-averaged maximum velocity (TAMV), resistance index (RI), and pulsatility index (PI) in addition to the diameters for arteries. The diameters of both uterine arteries were assessed from a B-mode image, and the average was calculated for each animal group. The most regularly used color Doppler indices are described by Elmetwally (2012); Elmetwally (2016) and Elmetwally *et al*. (2016). The setting for the range of flow velocity was adjusted to obtain the spectral graph. A Doppler spectrum is generated across at least 3 to 5 uniform cardiac cycles, and one of the waves is used for spectral measurements.

The means of two to three measurements are used for statistical analysis (Fig. 1).

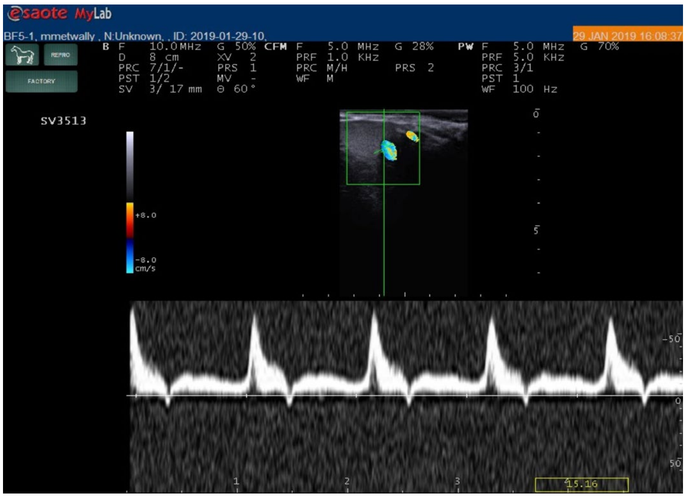

Figure 1. Location of uterine artery in buffaloes: Color Doppler waves of postpartum uterine artery in buffalo cows (Day 21).

Statistical Analysis

The data were presented as means±SD for statistical analysis using SAS® (version 9.2, SAS Institute). The Shapiro–Wilk test was used to determine the normality of all variables' distributions. To determine the effect of time after parturition on Doppler indices in uterine and vaginal arteries, as well as steroid hormone concentrations, a mixed model one-way analysis of variance was used, with time points as repeated measurements. Multiple pairwise comparisons were performed post hoc using Duncan's error rate adjustment.

RESULTS

All Buffalo cows used in the present experiment were normal and did not show any pathological abnormalities at the time of parturition. The range of the gestational period in pregnant buffaloes was 314.4±5.06. The results of the mean values of the uterine horn diameter in the studied buffaloes during the different time points after parturition indicated a significant decrease (P<0.001) in the uterine horn diameter. As shown in Table 1, the gravid uterine horn decreased by about 30 % from days 7 to 14 postpartum (D 7: 8.6±0.36 vs. D14: 6.25±0.28). The maximum decrease of the gravid uterine horn diameter was investigated by day 35 (61%: D 7: 8.6±0.36 vs. D28: 3.42±0.13, P < 0.001) and 35 (64%: D 7: 8.6±0.36 vs. D28: 3.18±0.17, P < 0.001) postpartum. The mean diameter of the dominant follicle at day 7 postpartum in was 5.4±0.4 mm and by days 21 and 38 we investigated a significant change in the dominant follicle diameter, which was 12.9±0.6 and 14.06±0.56 mm respectively. Up to day 21 postpartum, the size of the dominant follicle did not exceed about 9 mm.

Blood flow volume (P < 0.001, Fig. 2); UtA-TAMV (P < 0.01, Fig. 3) and UtA-PSV(P < 0.05, Fig. 4) increased at the time of parturition when compared to the other time points after parturition. A significant, robust decrease was investigated at day 7 postpartum (P < 0.05). Furthermore, a significant increase in BFV was recognized at days 21 and 28 after parturition (P < 0.05). The PSV of uterine blood flow showed an increase between days 21 to 35 postpartum (Fig. 4: P < 0.01). However, UtA-PI increased steadily after parturition and decreased at days 21 and 28 postpartum (P < 0.01) as shown in Fig. 5. The uterine artery resistance index and impedance showed increased values except at days 21 and 28 postpartum, respectively (Fig. 6 and 7; P < 0.05). The uterine artery diameter decreased continuously during all postpartum time points (P < 0.0001).

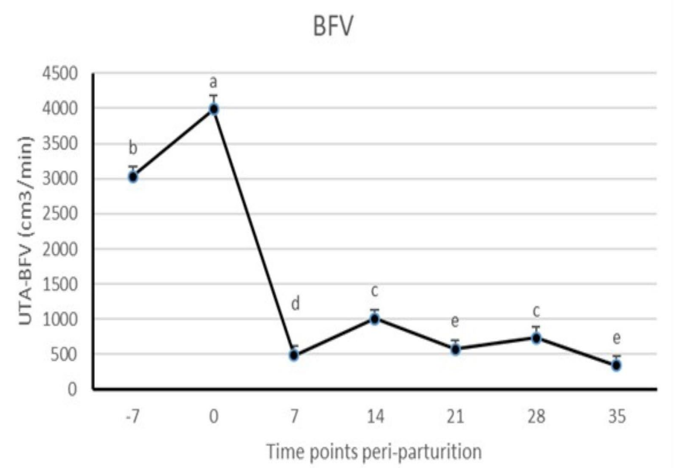

Figure 2: Mean ± SD of Blood flow volume (UtA_BFV) of uterine arteries ipsilateral to gravid uterine horn between one week before parturition (D -7), immediately after parturition (D 1) until day 35 (D 35) postpartum in Egyptian buffaloes. Different letters denote a difference compared to the preceding time point, *denotes P < 0.05.

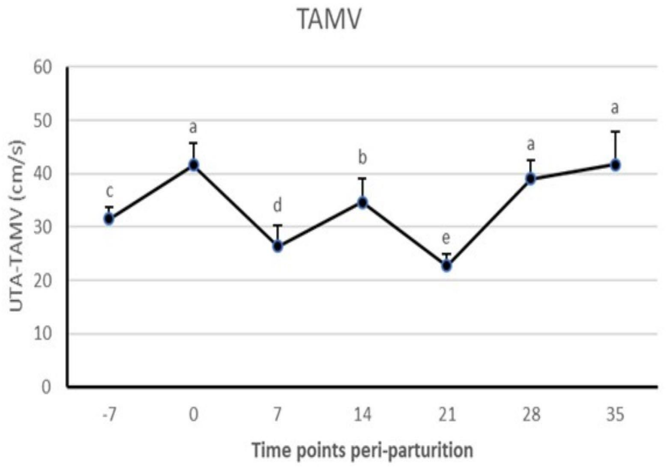

Figure 3: Mean ± SD of the timed average maximum velocity (UtA_TAMV) of uterine arteries ipsilateral to gravid uterine horn between one week before parturition (D -7), immediately after parturition (D 1) until day 35 (D 35) postpartum in Egyptian buffaloes. Different letters denote a difference compared to the preceding time point, *denotes P < 0.05.

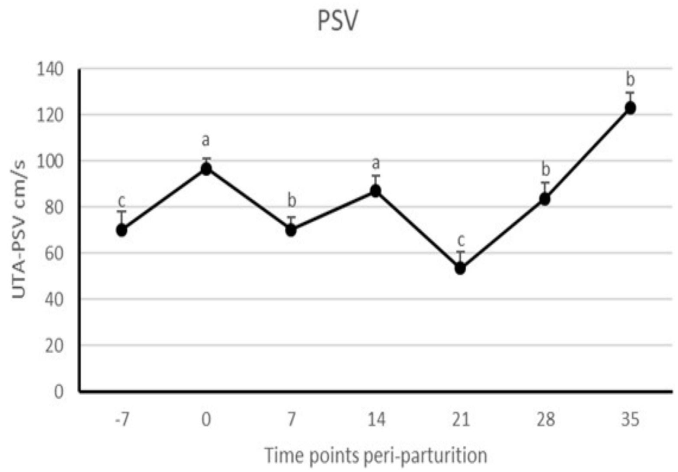

Figure 4: Mean ± SD of the peak systolic velocity (UtA_PSV) of uterine arteries ipsilateral to gravid uterine horn between one week before parturition (D -7), immediately after parturition (D 1) until day 35 (D 35) postpartum in Egyptian buffaloes. Different letters denote a difference compared to the preceding time point, *denotes P < 0.05.

DISCUSSION

In the current study, provided values for the normal changes in blood flow dynamics of the uterine arteries during the first five weeks after parturition. Also, it showed significant changes in the uterine biometry throughout the postpartum period in Egyptian buffaloes. It was investigated that the changes that occur during the postpartum period of the uterus and uterine blood flow indices play an important role in the improvement of the reproductive performance of cows and small ruminants (Elmetwally and Bollwein, 2017; Elmetwally, 2018).

In the current study, the uterus completely involuted by day 28 postpartum. In the same line, other studies reported time intervals for complete involution of the uterus as 25.6±1.0 days (Usmani *et al.*, 2001), 28.37±1.36 days (Atanasov *et al.*, 2012) in the Nili-Ravi breed, 40.3±1.2 days (Ilieva *et al.*, 2021), 36.20±1.65 days (Ramoun *et al.*, 2006) in the Egyptian buffalo, in mediterranean Italian Buffalo (Presicce *et al.*, 2005), was 31.12±0.9 days, and 31.1±1.0 and 33.1±1.3 days in primiparous or multiparous. In contrast, other studies reported a longer time for the uterine involution, which was completed by day 45 postpartum (El-Wishy, 2007). A robust uterine involution, evident estrous expression, and fertilization between 80 and 115 days following the last parturition are required for the optimum intercalating interval of 13 to 14 months to be reached (Atanasov *et al.*, 2012; Japheth *et al.*, 2021). The fact that each of the aforementioned studies was carried out on various buffalo breeds without the use of oxytocin

Table 1. The dominant follicle (DF) diameter (mean ± SD) in mm2 as measured by ultrasound imaging in Days 7, 14, 21, 28 and 35 (D) postpartum in Egyptian buffaloes.

Days pp	7	14	21	28	35
Dominant Follicle	5.40±0.40	6.63±0.08*	8.20±0.20**	12.90±0.60**	14.06±0.56***
Uterine Horn diameter	8.60±0.36^{a}	6.25±0.28^{b}	4.33±0.35^{c}	3.42±0.13^{d}	3. 18±0.17^{d}
	-100	-72.63	-47.48	-39.73	-36.98
Milk eject reflex	-	+	++	+++	-

therapy and in various seasons may help to explain the variations in the time frame for uterine involution.

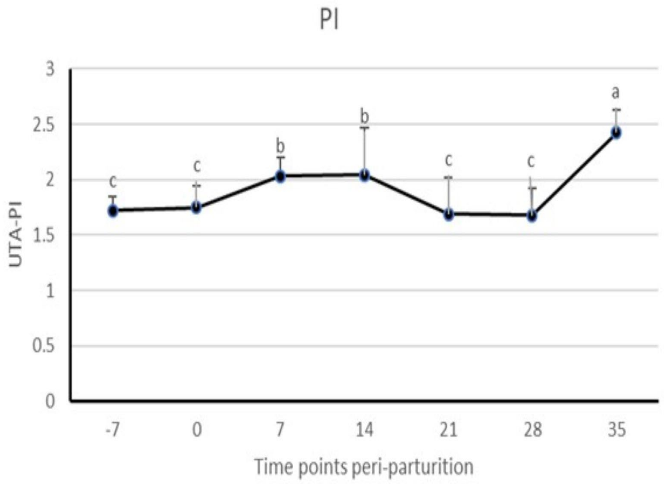

Figure 5: Mean ± SD and ranges of the pulsatility index (UtA_PI) of uterine arteries ipsilateral to gravid uterine horn between one week before parturition (D -7), immediately after parturition (D 1) until day 35 (D 35) postpartum in Egyptian buffaloes. Different letters denote a difference compared to the preceding time point, *denotes $P < 0.05$.

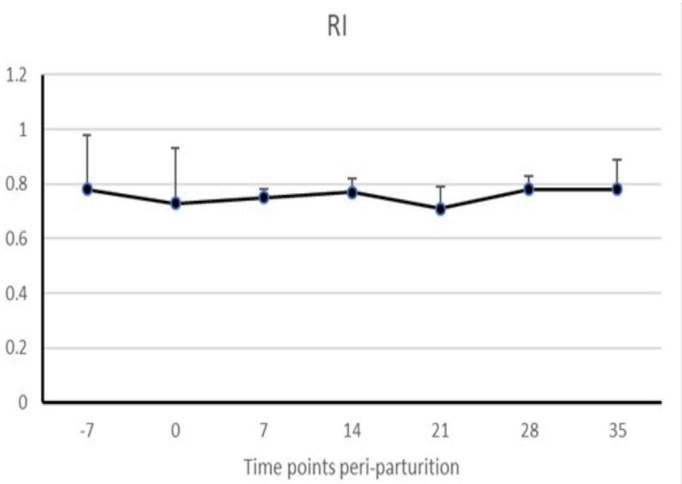

Figure 6: Mean ± SD and ranges of the resistance index (UtA_RI) of uterine arteries ipsilateral to gravid uterine horn between one week before parturition (D -7), immediately after parturition (D 1) until day 35 (D 35) postpartum in Egyptian buffaloes. Different letters denote a difference compared to the preceding time point, *denotes $P < 0.05$.

In the current study, the dominant follicles at day 35 postpartum were larger than the other postpartum time points. In buffaloes, the estrous cycle is characterized by a wave-like follicular pattern, just like in other species. The two follicular wave patterns were documented in previous descriptions of the follicular dynamics in pure riverine and swamp-type buffaloes (Gaur and Purohit, 2019; Peralta-Torres *et al*., 2020; Murtaza *et al*., 2021). The results of the present study were similar to other studies (Presicce *et al*., 2005; Yindee *et al*., 2011). Within 7–10 days of calving, 6–8 mm follicles start to form in the postpartum buffalo. From this group of follicles, one dominant follicle arises, and between days 10 and 30 following calving, 75–80% of cows ovulate. Buffalo that ovulate after day 30, but typically not before, are more likely to have a brief cycle (8–13 days in length) where the first dominant follicle ovulates (Presicce *et al*., 2005; Yindee *et al*., 2011). The increased dominant follicle diameter in the present study was associated with the milk ejection reflex, which indicates the increased level of serum estrogen. We did not find enough literature that described the milk ejection reflex, but it depends mainly on the owner's history. These changes were associated with robust changes in blood flow indices of the uterine arteries in the current study.

Regarding the uterine blood flow changes during the first 5 weeks after parturition, we investigated characteristic changes during the different time points of the present study. The blood flow volume and peak systolic velocity of uterine arteries showed a great change during the first week postpartum in Egyptian buffaloes. The significant reduction in uterine mass and size during this time was most likely responsible for the noticeable changes. Myometrial contractions, which manifest as potent, regular, peristaltic waves during the first three days following calving, may help to explain this also. These contractions reduce muscle fiber length from roughly 750 to 200 by day 3 postpartum (Sd, 1991). Directly after parturition, expulsion of the lochia in the first few days postpartum is another important factor that helps reduce the size and weight of the uterus (Gier and Marion, 1968). The present results are similar to those reported in Simmental heifers (Heppelmann *et al*., 2013). The blood flow volume was decreased by about 84 % during the first week of parturition. A linear decrease in uterine blood flow was reported in previous studies (Magata *et al*., 2013; Abdelnaby, 2020).

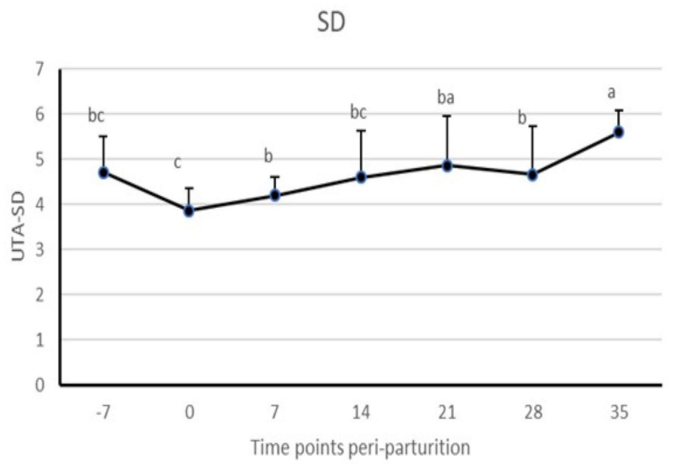

Figure 7: Mean ± SD and ranges of the systolic/diastolic (UtA_S/D) of uterine arteries ipsilateral to gravid uterine horn between one week before parturition (D -7), immediately after parturition (D 1) until day 35 (D 35) postpartum in Egyptian buffaloes. Different letters denote a difference compared to the preceding time point, *denotes $P < 0.05$.

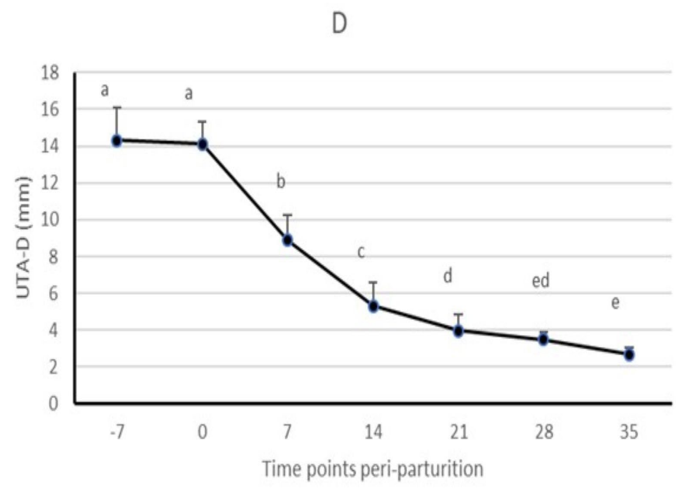

Figure 8: Mean ± SD and ranges of the diameter (UtA_BFV) of uterine arteries ipsilateral to gravid uterine horn between one week before parturition (D -7), immediately after parturition (D 1) until day 35 (D 35) postpartum in Egyptian buffaloes. Different letters denote a difference compared to the preceding time point, *denotes $P < 0.05$.

The decrease of the uterine blood flow in the present study is associated with an increase in PI, RI, and S/D in a linear manner during the first 5 weeks after parturition in Egyptian buffaloes. These discrepancies could be attributed to the various processes involved in uterine involution. As previously stated, the most noticeable decrease in uterine size happens in the first week following parturition, followed by vasoconstriction of the uterine veins and ischemic necrosis of the caruncles (Krueger *et al*., 2009). The

PI, RI, and S/D show the resistance in the vascular bed distal to the measuring point. As a result, the increase in PI in the uterine arteries could be due to vasoconstriction of the caruncular blood vessels. These changes in the caruncular vascular bed are complete by day 30 postpartum (Gier and Marion, 1968). Similar circumstances can be seen in women, where the period of time needed to change from a pregnant to a non-pregnant condition in peripheral resistance of uterine arteries takes longer than originally thought (Mulic-Lutvica *et al*., 2007) : The maximum PI in the uterine arteries was detected much later, at day 101 after parturition, at the end of the examination period, even though the uterine involution in women is expected to be complete 6-8 weeks after parturition (Salamonsen, 2003). Additionally, these were still much below the PI of non-pregnant women's uterine arteries (Trongpisutsak and Phupong, 2021; Besimoglu *et al*., 2022; Cahuana-Bartra *et al*., 2022;)

CONCLUSION

The findings of this study demonstrate that transrectal color Doppler sonography is an effective method for determining changes in uterine perfusion in cows during the first 6 weeks following parturition. This method will be used in future research as a method to obtain oblique but accurate data for the description of uterine involution. The amount of uterine blood flow declines in the first four weeks following delivery, but the PI, RI, S/D which measures uterine blood flow resistance, exhibits distinct changes over the course of the entire study period, increasing in the first four weeks. Although the changes in blood flow volume stopped concurrently with uterine involution, there were still additional alterations in the uterine vascular bed even after the completion of the assumed uterine involution.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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