



OVARIAN AND UTERINE HAEMODYNAMICS AND THEIR RELATION TO STEROID HORMONAL LEVELS IN POSTPARTUM EGYPTIAN BUFFALOES

H. EL-SHERBINY¹, K. H. EL-SHAHAT¹, A. M. ABO EL-MAATY²
& E. A. ABDELNABY¹

¹Theriogenology Department, Faculty of Veterinary Medicine, Cairo
University, Egypt; ²Animal Reproduction and AI Department,
Veterinary Division, National Research Centre, Dokki, Egypt

Summary

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The current study aimed to assess the ovarian and uterine haemodynamics in postpartum (pp) Egyptian buffaloes and their relation to steroid hormonal levels (progesterone and estrogen). Six multiparous buffaloes with normal calving were examined by using trans-rectal Doppler sonography to assess the blood flow in ipsilateral and contralateral ovarian and uterine arteries. Doppler parameters viz. peak systolic velocity (PSV), blood flow volume (BFV), resistance (RI) and pulsatility index (PI) were measured at 1st till 6th postpartum weeks. In addition, diameters (mm) of the ovarian and uterine arteries and the vascularisation area (mm²) of ovary were determined. The blood samples were collected starting from 1st week post calving and every week thereafter following each ultrasound Doppler examination for assay of progesterone and estradiol. Results indicated that PI and RI increased consistently and significantly ($P < 0.05$) till the 6th week post calving as compared to those recorded at 1st week in ipsilateral ovarian and uterine arteries. However, the blood flow volume and peak systolic velocity in both ovarian and uterine arteries decreased gradually from 1st week till the 6th week after calving. There were very strong positive correlations between PSV and BFV, in both ipsilateral and contralateral ovarian and uterine arteries. Blood concentrations of progesterone were lower ($P < 0.05$) during the first four weeks than those recorded at 5th and 6th postpartum weeks. This trend was reversed for blood estradiol 17- β . The results of the present investigation concluded that Doppler ultrasonography is a suitable tool for assessing changes in ovarian and uterine perfusion during the puerperium.

Key words: buffalo, Doppler, ovarian, puerperium, uterine artery

INTRODUCTION

The main constraint of sustainable buffalo production includes long postpartum ano-

estrous intervals and long calving intervals (Singh *et al.*, 2000). So, the solution

for these problems should be directed toward studying the normal uterine involution during postpartum period in buffaloes (Fonseca *et al.*, 1983). Delayed uterine involution will lead to a great decrease in animal fertility (Frazer *et al.*, 2005). The physiological involution of the postpartum uterus includes not only muscle cells and the decidua of the placental site but also the entire branching of the main uterine artery (Andrew *et al.*, 1989; Khong *et al.*, 1993). In previous studies, the assessment of uterine haemodynamics was performed several times in women under different conditions by using Doppler sonography (Jaffa *et al.*, 1996; Nakai *et al.*, 1997). The cyclic uterine hemodynamics and arterial vascular perfusion of estrous cycle was also previously examined in mares (Abdelnaby *et al.*, 2016; Abo El-Maaty & Abdelnaby, 2017), cows (Abdelnaby *et al.*, 2018; 2020a), pregnancy (Bollwein *et al.*, 2002) and during the puerperium (Krueger *et al.*, 2009; Heppelmann *et al.*, 2013; Abdelnaby, 2020a). In buffaloes, a few studies during late gestation were recorded (Hussein, 2013; Varughese *et al.*, 2013). Moreover, a study was conducted on uterine blood supply during the postpartum period in the buffalo (Singh *et al.*, 2016). However, no report was carried on ovarian blood flow correlating with hormonal changes (progesterone and estrogen) Hence, the current investigation was planned to evaluate the ovarian and uterine haemodynamics during the puerperium in normal calving buffaloes in association with hormonal changes (blood progesterone and estrogen).

MATERIALS AND METHODS

Ethical committee approval

The present study was carried in accordance to permission of the ethical commit-

tee of Institutional Animal Care and Use Committee (IACUC) of Faculty of Veterinary Medicine, Cairo University.

Experimental location

The present study was conducted at Faculty of Veterinary Medicine, Cairo University, Egypt (latitude 30°01' N; longitude 31° 21' E) during October – December 2019.

Experimental animals, feeding and design

Investigations were carried out in multiparous buffaloes (n=6), weighing 470–490 kg and 4–7 years of age. The animals received their requirements of a commercially prepared pelleted ration with Egyptian green clover (*Trifolium alexandrinum*) and wheat straw as bulky material. They were subjected to natural daylight and temperature. Colour Doppler sonography studies of the uterine arteries started at 1st week after the parturition and were followed by weekly examinations until 6th week postpartum (pp).

Ultrasonography

A rectal spectral-wave Doppler ultrasound equipped with 7.5 MHz linear-array transrectal transducer (Sonovet R3, Medison, Samsung, South Korea) with the rectal probe was used to examine the ovarian arteries, ovarian vascularisation and the uterine arteries by the aid of colour and spectral modes. The ipsilateral uterine and ovarian artery to the previously gravid horn was considered as the ipsilateral artery. On the other hand, the uterine and ovarian artery contralateral to the above-mentioned gravid horn was the contralateral artery as reported by Abdelnaby & Abo El-Maaty (2017). The cross-sectional diameters of the ovarian and uterine arteries (mm) and the vascularisation area (mm²) of ovary were determined. The

same analyzer traced the Doppler indices pulsatility index (PI) and resistance index (RI). Doppler velocity as peak systolic velocity (PSV), volume of blood flow (BFV, mL/m), ovarian tissue volume (mm³) and ovarian coloured blood flow area (pixels) were measured as previously reported in cows by Bollwein *et al.* (2002) and ewes (Abdelnaby, 2020b).

Image analysis

The stored Doppler images and video clips in the Doppler scanner were exported and analysed at each experiment. The red and blue areas of Doppler images of colour blood flow per pixel were determined by Adobe Photoshop CC (Fouad *et al.*, 2018). The vascularisation areas in the ovary were also counted as previously mentioned in dairy cows (Abdelnaby & Abo El-Maaty, 2017).

Blood sampling and hormone assays

Blood samples were collected via jugular vein punctures in plain vacuum tubes each week following ultrasound Doppler examination. Serum was stored at -20 °C till hormone assay. Progesterone (P4, EIA-1561), and estradiol 17-β (E2 EIA-2693) were analysed using ELISA commercial kits (DRG, Germany). The sensitivity of the assay was 0.045 ng/mL; test intra- and inter-precisions were 5.4% and 9.96% for P4. The sensitivity of the assay was 9.7 pg/mL and test intra- and inter-precisions were 6.81% and 7.25% for E2 as previously measured (Abdelnaby *et al.*, 2016; 2020b).

Statistical analysis

The obtained data were analysed using SPSS® Statistical Software (SPSS® 16.0 for Windows, 2007). There was a normal distribution, so ANOVA was used in evaluation. The mean value of all Doppler

indices (ipsilateral and contralateral ovarian and uterine arteries cross-sectional diameters, Doppler velocities and blood flow volume of ovary) were evaluated in addition to hormonal changes. P values ≤ 0.05 were considered significant.

RESULTS

Doppler indices changes in postpartum buffaloes

Doppler shift spectra were recorded from ipsilateral and contralateral ovarian arteries at different postpartum period starting at 1st till 6th week (Fig. 1). The peak systolic velocity waveform gradually decreased from the first till the 6th week after parturition with continuous flow in diastolic velocity. Moreover, there was an increase in the Doppler indices related to reduction in the systolic velocity. Doppler shift spectra from ipsilateral and contralateral middle uterine arteries at different postpartum periods (Fig. 2) showed the same trend as observed in the ovarian arteries – the peak velocity decreased gradually till the end of puerperium period.

Weekly scanning of postpartum buffaloes significantly (P≤0.05) affected the diameter of the ovarian, middle uterine arteries and vascularisation changes of ovary (Fig. 3). The highest diameter of the ipsilateral and contralateral sides of ovarian arteries was recorded at 1st week (11.02±0.01 and 11.99±1.86 mm, respectively) and the lowest in 6th week pp (9.85±1.55 and 9.12±0.05 mm, respectively). A similar trend was observed in the diameter of ipsilateral and contralateral uterine arteries. It was significantly higher at 1st week (18.21±2.34 and 17.33±2.65 mm, respectively; P<0.01) vs. 6th week pp (13.25±1.45 and 13.55±1.88 mm, respectively). In addition the ovarian

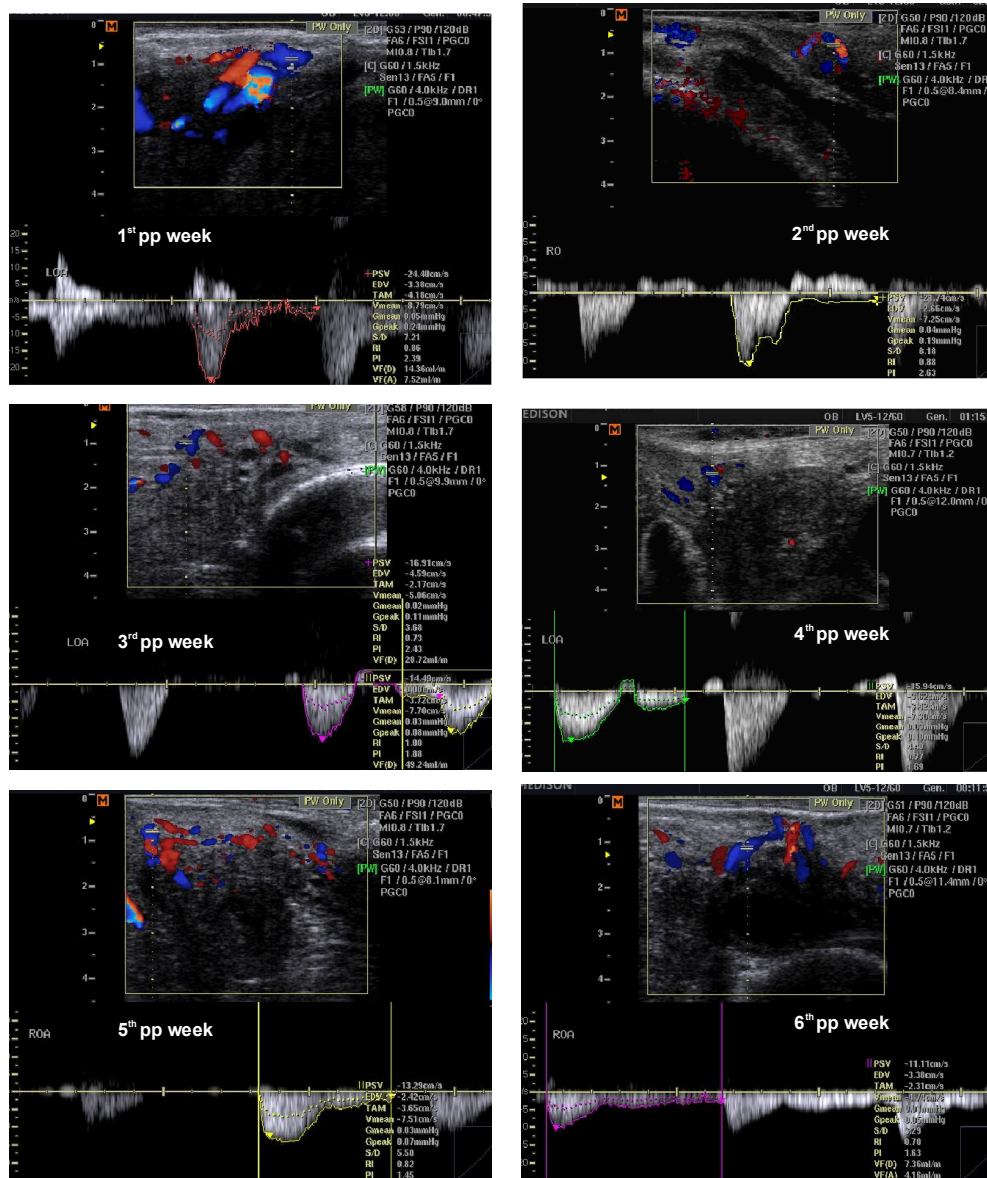


Fig. 1. Doppler sonography showing spectral waveform of ipsilateral and contralateral ovarian arteries in buffaloes at different postpartum (pp) weeks.

area decreased significantly at 1st week reaching the lowest value at 6th week pp.

The results from the uterine and ovarian haemodynamics of postpartum buffa-

loes are illustrated on Fig. 4. There was an increase ($P \leq 0.05$) in blood flow volume (BFV) of ipsilateral and contralateral ovarian arteries at 1st week (12.32 ± 0.16

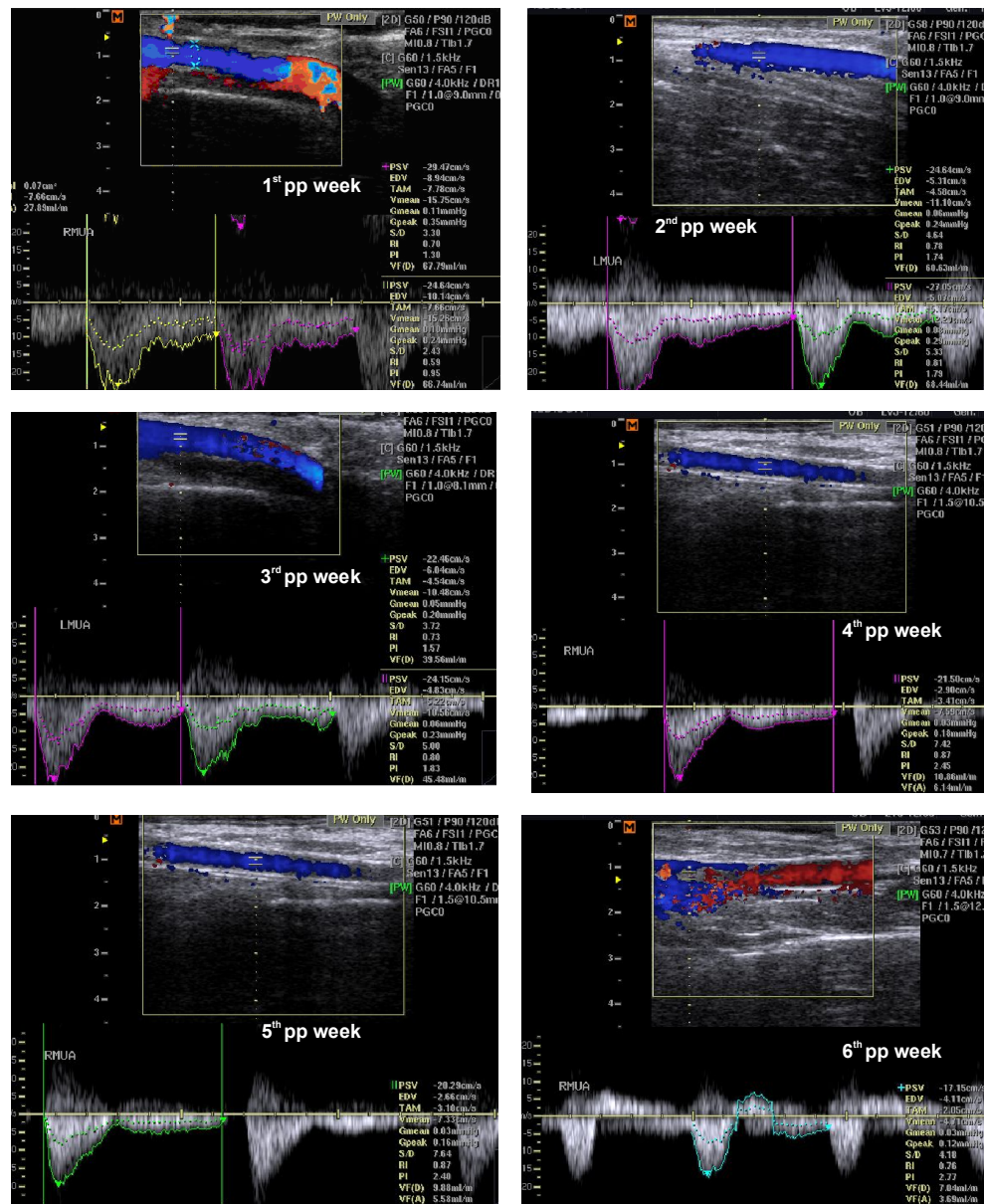


Fig. 2. Doppler sonography showing the spectral waveform graph of ipsilateral and contralateral uterine artery in buffaloes at different postpartum (pp) weeks.

and 12.38 ± 0.99 mL/min, respectively) than those recorded at 6th week (4.36 ± 1.01 and 5.55 ± 0.87 mL/min respectively). The BFV in the ipsilateral and contralateral middle uterine arteries declined to a minimum value on 6th week pp (9.01 ± 0.11 , 9.36 ± 0.54 mL/min). In the ipsilateral and contralateral of ovarian arteries, the PSV was higher ($P \leq 0.05$) at 1st week (24.33 ± 0.36 , 22.01 ± 1.33 cm/sec, respectively) compared to their counterparts at 6th week pp (14.25 ± 1.22 and 15.01 ± 0.14 cm/sec, respectively). The same trend was observed in ipsilateral and contralateral uterine arteries. Peak systolic velocity (PSV) value in the ipsilateral and contra-

lateral uterine arteries declined gradually ($P \leq 0.05$) to reach a minimum value at 6th week postpartum (20.33 ± 0.45 and 18.65 ± 1.99 cm/sec, respectively). There were very strong positive correlations between PSV and BFV in both ipsilateral and contralateral ovarian and uterine arteries. The ipsilateral and contralateral ovarian volume decreased linearly reaching lowest values at 6th week of puerperium (15.44 ± 0.01 , 13.59 ± 0.02 , respectively). In addition the blood flow area of both ovarian arteries decreased gradually to a minimum value at 6th week pp (1987 ± 46.78 and 1600 ± 21.52 , respectively) (Table 1).

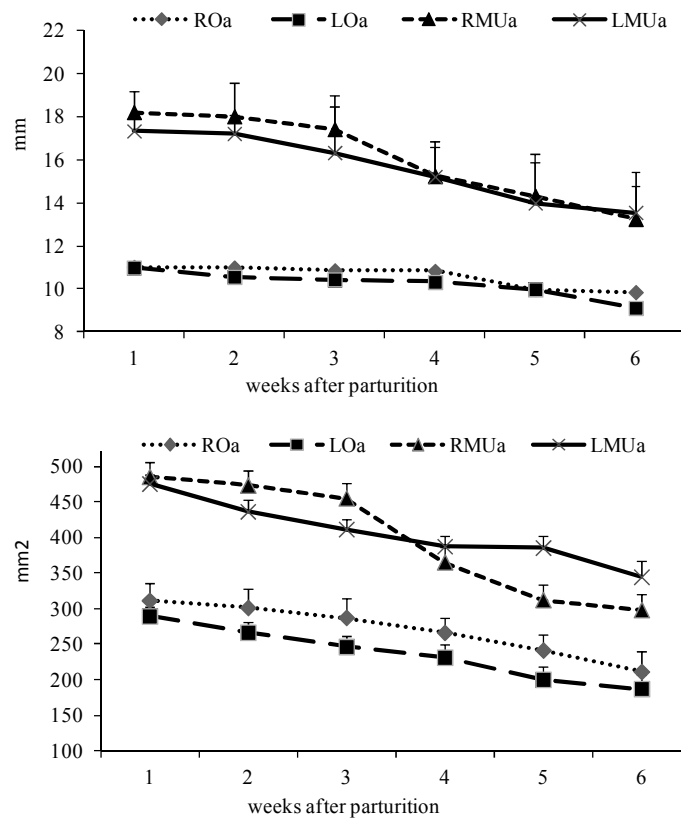


Fig. 3. Cross-sectional diameter (mm) (top) and area (mm²) (bottom) of right ipsilateral and left contralateral (ROa; LOa) ovarian arteries and right ipsilateral and left contralateral middle uterine arteries (RMUa; LMUa) in postpartum buffaloes. Data are presented as mean \pm SD (n=6).

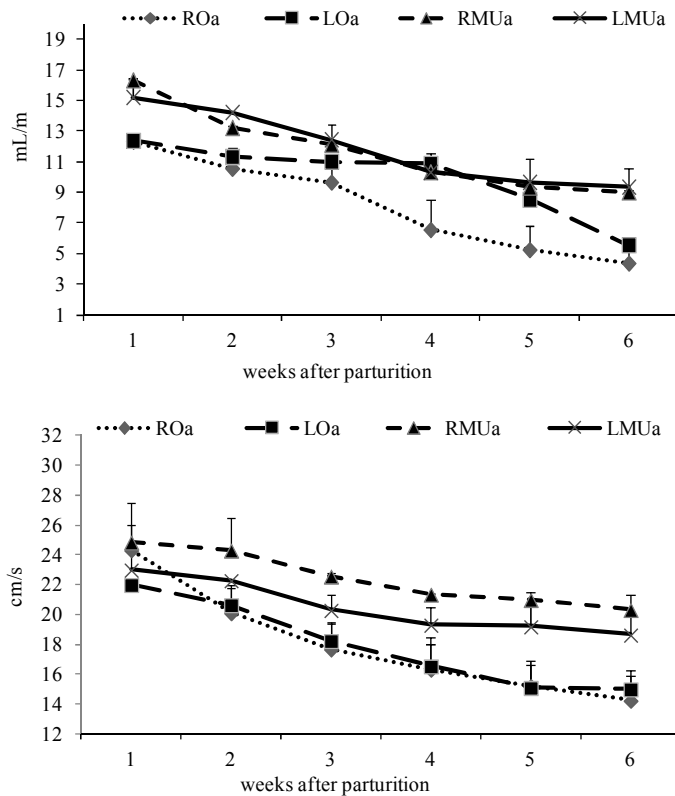


Fig. 4. Volume of blood flow (ml/m) (top) and peak systolic velocity (PSV, cm/s) (bottom) of right ipsilateral and left contralateral (ROa; LOa) ovarian arteries and right ipsilateral and left contralateral middle uterine arteries (RMUa; LMUa) in postpartum buffaloes. Data are presented as mean±SD (n=6).

Table 1. Ipsilateral ovarian volume (mm³), contralateral ovarian volume (mm³), ipsilateral ovarian blood flow area (pixels), contralateral ovarian blood flow area (pixels) in postpartum buffaloes. Data are presented as mean ± standard deviation (SD) (n=6).

Post-partum weeks	Ipsilateral ovarian volume	Contralateral ovarian volume	Ipsilateral ovarian blood flow area	Contralateral ovarian blood flow area
1	17.01±1.02 ^c	15.66±0.02	2763±17.62 ^c	2113±24.52 ^c
2	16.87±0.02 ^{bc}	15.32±0.33	2415±15.62 ^{bc}	2101±33.52 ^c
3	16.54±0.55 ^{ab}	15.01±0.01	2235±25.66 ^b	2014±40.31 ^{bc}
4	16.22±1.33 ^{ab}	14.33±0.11	2166±22.31 ^{ab}	1954±18.22 ^b
5	16.19±0.03 ^{ab}	13.99±0.01	2133±36.25 ^{ab}	1625±44.82 ^{ab}
6	15.44±0.01 ^a	13.59±0.02	1987±46.78 ^a	1600±21.52 ^a

Means with different superscripts within column are significantly different at P≤0.05.

Table 2. Pulsatility index (PI) and resistance index (RI) values of the ovarian arteries in postpartum buffaloes. Data are presented as mean±standard deviation (n=6).

Post-partum weeks	Ovarian arteries			
	Ipsilateral		Contralateral	
	PI	RI	PI	RI
1	1.44±0.16 ^a	0.71±0.04 ^a	1.62±0.23 ^a	0.75±0.05 ^a
2	1.49±0.18 ^a	0.75±0.04 ^a	1.65±0.17 ^{ab}	0.79±0.04 ^{ab}
3	1.53±0.33 ^b	0.79±0.07 ^{ab}	1.87±0.33 ^b	0.81±0.11 ^{ab}
4	1.58±0.17 ^b	0.8±0.05 ^{ab}	2.31±0.21 ^{bc}	0.86±0.07 ^{ab}
5	2.04±0.28 ^c	0.86±0.08 ^b	2.43±0.26 ^{bc}	0.89±0.11 ^b
6	2.22±0.31 ^c	0.89±0.06 ^b	2.61±0.66 ^c	0.89±0.07 ^b

Means with different superscripts within column are significantly different at P≤0.05.

Table 3. Pulsatility index (PI) and resistance index (RI) of the uterine arteries in postpartum buffaloes. Data are presented as mean±standard deviation (n=6).

Post-partum weeks	Middle uterine arteries			
	Ipsilateral		Contralateral	
	PI	RI	PI	RI
1	1.33±0.15 ^a	0.71±0.01 ^a	1.36±0.11 ^a	0.76±0.05 ^a
2	1.78±0.21 ^b	0.82±0.02 ^{ab}	1.44±0.09 ^a	0.79±0.05 ^a
3	1.83±0.15 ^{bc}	0.89±0.01 ^{ab}	1.63±0.25 ^{ab}	0.83±0.05 ^{ab}
4	2.31±0.25 ^c	0.91±0.11 ^{ab}	1.89±0.18 ^{bc}	0.87±0.05 ^{ab}
5	2.48±0.14 ^c	0.93±0.07 ^b	2.33±0.26 ^c	0.88±0.05 ^{ab}
6	2.77±0.31 ^d	0.95±0.02 ^b	2.45±0.11 ^c	0.96±0.05 ^b

Means with different superscripts within column are significantly different at P≤0.05.

Pulsatility index (PI) and resistance index (RI) during normal puerperium in buffaloes are presented in Table 2. PI showed lowest values at 1st week postpartum (P≤0.05) – 1.44±0.16 in ipsilateral and 1.62±0.23 in contralateral ovarian arteries. The highest values were recorded at 6th week postpartum (2.22±0.31, 2.61±0.66, for ipsilateral and contralateral respectively). The RI of ipsilateral and contralateral ovarian arteries ranged from 0.71±0.04 to 0.79±0.04 during the first two weeks post calving but was signifi-

cantly higher (P≤0.05) for 5th and 6th weeks postpartum (from 0.86±0.06 to 0.89±0.11).

The PI and RI were not dependent on the location of middle uterine arteries (ipsilateral and contralateral). The PI and RI showed a significant (P<0.05) linear marked elevation from first week till the end of puerperium period as shown in Table 3. In addition, there was a positive correlation between blood flow variables (r=0.75, P≤0.05).

Circulating steroid hormonal levels in postpartum buffaloes

During the first four post-partum weeks, there were no significant differences in the concentration of serum progesterone. The circulating serum progesterone ranged from 0.11±0.05 to 0.13±0.01 ng/mL. Two weeks later, e.g. 5th and 6th week postpartum; serum progesterone concentrations were significantly elevated (P≤0.05) to average 0.16 ng/mL (Table 4). The levels of estradiol 17-β showed a pattern of decrease from 1st week (4.32±0.01 pg/mL) till 6th week postpartum (3.88±0.08 pg/mL). The levels of progesterone were positively correlated with PI and RI of both ipsilateral ovarian and uterine arteries (r=0.65, all P≤0.05) and negatively correlated with the other blood flow variables including D, BFV, and PSV.

DISCUSSION

The current study reported for the first time that transrectal Doppler sonography could be performed on buffaloes to study the small ovarian branch vascularisation associated with uterine blood supply and hormonal changes (progesterone and estrogen) during first 6 weeks post-partum. In the current study, Doppler ultrasonography indicated that the recorded blood flow and peak systolic volumes of the

ovarian and uterine arteries gradually declined from the 1st week till the end of study (6th week) after calving. In this respect, previous studies have shown that the values of time average peak velocity (TAMV) and blood flow volume (BFV) in the uterine artery recorded at 30 min post-calving showed a trend towards significant subsequent decrease till the 6th h in postpartum buffaloes (Singh *et al.*, 2016). A similar trend was observed in dairy cows where the uterine perfusion was significantly decreased at the 1st week postpartum (Krueger *et al.*, 2009; Heppelmann *et al.*, 2013). The decrease in PSV and BFV recorded herein could be attributed to an abrupt decrease in blood flow in the placenta with subsequent shrinkage of chorionic villi after expulsion of the foetus. Another explanation for the most pronounced decrease in BFV and PSV is the rapid uterine involution during the first 6th weeks postpartum (Laven & Peters, 1996). In buffaloes, a rapid involution of the uterus including retraction and regression of diameter of ventral uterine curvature, uterine lumen, and total uterine horn of postpartum buffaloes were completed by first 6 weeks (Gad *et al.*, 2017). Beside, strong, rhythmic, peristaltic waves of myometrium contractions during the first three days postpartum lead to a great reduction of muscle fibre length from about 750 µm to 200 µm (Van Camp, 1991).

Table 4. Hormonal concentrations of estradiol (pg/mL), progesterone (ng/mL) in Egyptian buffaloes during the puerperium. Data are presented as mean ± standard deviation (n=6).

Post-partum weeks	Estradiol	Progesterone
1	4.32±0.01 ^c	0.11±0.05 ^a
2	4.12±0.02 ^b	0.12±0.03 ^a
3	4.11±0.01 ^b	0.12±0.02 ^a
4	4.01±0.01 ^{ab}	0.13±0.01 ^{ab}
5	3.55±0.06 ^a	0.16±0.02 ^b
6	3.88±0.08 ^{ab}	0.16±0.01 ^b

Means with different letters within a row are significantly different at P≤0.05.

The decrease in uterine size was also attributed to expulsion of the lochia after birth (Gier & Marion, 1968).

The present finding revealed that PI and RI increased gradually and significantly thereafter till 6th week postpartum as compared to those obtained at 1st weeks in the ipsilateral and contralateral ovarian and uterine arteries. A similar finding was obtained by Singh *et al.* (2016) in buffaloes. In contrast, the PI and RI were significantly elevated at first weeks till the end of two week postpartum in cattle (Krueger *et al.*, 2009). This may be due to species differences. The present study demonstrated that there were positively significant correlations between PI and RI and negative relationships with the other two blood flow variables (BFV and PSV) in ipsilateral ovarian and uterine arteries throughout the study period. Moreover, PI and RI were positively significantly correlated between ipsilateral and contralateral ovarian and uterine arteries. The recorded PI and RI herein significantly ($P < 0.05$) increased continuously until 6th week postpartum in ovarian and uterine arteries. This may be attributed to the rapid decrease in uterine size and thereafter, vasoconstriction of caruncular blood vessels during 1st week postpartum. These alterations in the caruncular vascular bed are finished by day 30 pp with subsequent ischemic necrosis of the caruncles (Gier & Marion, 1968; Van Camp, 1991). Results obtained in current investigation indicate that the puerperium of buffaloes had a significant effect on the circulating steroid hormonal levels (progesterone and estrogen). The concentrations of progesterone were lower ($P < 0.05$) during the first four weeks than those recorded at 5th and 6th weeks pp. The reverse was true for estradiol 17- β . The levels of estradiol 17- β were higher ($P < 0.05$)

in the first weeks. Stimulation of the ovarian activity, early during the post-calving period, essentially improved uterine clearance and involution in buffaloes (Kandiele *et al.*, 2013). This could be due to the effect of estradiol on the uterus (endometrium and/or myometrium) (Sheldon *et al.*, 2003). The release of gonadotropic and gonadal hormones under the effect of hypothalamic-pituitary-ovarian axis will lead to regular estrous cycle and ovulation and hasten the uterine involution (Peter *et al.*, 2009). The uterine involution and ovarian rebound were greatly influenced by the high level of prostaglandin F2 α secreted by the endometrium (Kindahl *et al.*, 1992). Strong correlations between PI and RI of both ipsilateral ovarian and uterine arteries and progesterone were recorded herein. This phenomenon was the opposite of estradiol 17- β patterns and could be explained by the hormonal changes (Tekay *et al.*, 1993).

In conclusion, the present study provided reference values of Doppler indices for ovarian and uterine arteries during the first six weeks in postpartum Egyptian buffaloes. The results of the present investigation suggested that Doppler ultrasonography is a suitable tool for assessing changes in ovarian and uterine perfusion during puerperium.

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Correspondence:

K. H. El-Shahat
Theriogenology Department,
Faculty of Veterinary Medicine,
Cairo University, Giza square, 12211, Egypt
mobile: 00201064688386,
e-mail: attiakh@yahoo.com