## Title

Uterine, vaginal and placental blood flows increase with dynamic changes in serum metabolic parameters and oxidative stress across gestation in buffaloes



**Journal**

Reproduction in Domestic Animals, 56(1)

**ISSN**

0936-6768

**Authors**

Elmetwally, Mohammed A
Elshopakey, Gehad E
Eldomany, Wael
et al.

**Publication Date**

2021

**DOI**

10.1111/rda.13858

Peer reviewed

Received: 4 October 2020 | Accepted: 6 November 2020
DOI: 10.1111/rda.13858

ORIGINAL ARTICLE

Reproduction in Domestic Animals WILEY

# Uterine, vaginal and placental blood flows increase with dynamic changes in serum metabolic parameters and oxidative stress across gestation in buffaloes

Mohammed A. Elmetwally[1] | Gehad E. Elshopakey[2] | Wael Eldomany[1] | Ashraf Eldesouky[1] | Alaa Samy[3] | Yasser Y. Lenis[4,5] | Dong-bao Chen[6]

[1]Department of Theriogenology, Veterinary Medicine Faculty, Mansoura University, Mansoura, Egypt

[2]Clinical Pathology, Veterinary Medicine Faculty, Mansoura University, Mansoura, Egypt

[3]Surgery, Anesthesiology and Radiology, Veterinary Medicine Faculty, Mansoura University, Mansoura, Egypt

[4]Departamento de Producción Animal, Facultad de Ciencias Agrarias, Universidad Nacional de Colombia, Palmira, Colombia

[5]OHVRI-Group (One Health and Veterinary Innovative Research and Development, School of Veterinary Medicine, Faculty of Agrarian Science, Antioquia University, Medellín, Colombia

[6]Department of Obstetrics and Gynecology, University of California Irvine, Irvine, CA, USA

**Correspondence**
Mohammed A. Elmetwally, Department of Theriogenology, Faculty of Veterinary Medicine, University of Mansoura, Mansoura, Egypt.
Email: mmetwally@mans.edu.eg

## Abstract

The aims of the present study were to determine uterine, vaginal and placental blood flows by Doppler ultrasound cross-buffalo gestation and to evaluate the relationships among reproductive Doppler parameters and serum metabolic parameters as well as oxidative stress. Uterine (UA) and vaginal (VA) arteries were scanned every month, and placentome was scanned from month 4 till 8 in gestation. Time-averaged maximum velocity (TAMV), pulsatility index (PI), resistance index (RI), systolic/diastolic ratio (*SD*) and arterial diameter (AD) were used for accessing UA and VA hemodynamics. Time-averaged maximum velocity positively correlated with and AD, and both negatively correlated with their PI, RI and *SD* in UA and VA. TAMV and AD increased constantly in pregnancy, with maximum increase in months 4 and 9. Pulsatility index, RI and AD of UA decreased between months 4 and 9, while PI, RI and AD of VA decreased between months 5 and 9 and then increased in month 10 in pregnancy. Time-averaged maximum velocity of placentome blood flow increased exponentially from months 4 to 8, but decreased at the last two months in pregnancy. Serum lipids were significantly higher in the first month compared to all other months, while glucose was significantly lower in months 9 and 10. Malondialdehyde increased from month 3 till term, but peaked in month 5 and 10. Glutathione and catalase were highest in the first month and remained after. Time-averaged maximum velocity and AD for both UA and VA negatively correlated with serum lipids, glucose, catalase and glutathione, while positively correlated with malondialdehyde and total protein. Thus, increases in uterine blood flow (UtBF), vaginal blood flow (VaBF) and placental blood flow (PaBF) are associated with increased metabolism and oxidative stress in buffalo pregnancy.

KEYWORDS
buffaloes, colour doppler, metabolism, oxidative stress, pregnancy, uterine artery

# 1 | INTRODUCTION

During normal pregnancy, maternal hemodynamics change dramatically, characterized by mild decreased systemic blood pressure and a larger fall in systemic vascular resistance, associated with increased heart rate, stroke volume, cardiac output and blood volume (Ford et al., 1982; Magness & Rosenfeld, 1989; Rosenfeld et al., 1974). In a non-pregnant ewe, less than 2% total cardiac output is distributed into reproductive organs; when pregnancy proceeds, this number gradually increases up to 15%–20% at the last one third of gestation. The largest portion (~95%) of the increased cardiac output is redistributed to the aggressively developing uteroplacental vascular bed. This is reflected by a substantial increase in uterine blood flow (UtBF) in late pregnancy, which can reach as high as up to 20- to 80-fold of that in non-pregnant ewes (Rosenfeld, 1980; Rosenfeld et al., 1974). Specifically, two peak rises in UtBF that occur during the second and third trimesters, correlating to the completion of placentation and rapid foetal growth, respectively (Rosenfeld et al., 1974; Rosenfeld & Naden, 1989). Aberrant placentation results in reduced UtBF, leading to early pregnancy loss (Cotechini & Graham, 2015; Propst & Hill, 2000).

Previously proposed metabolic biomarkers include serum concentrations of lipids (Abdollahi-Arpanahi et al., 2019; Angeli et al., 2019), total protein, albumin and globulin (Žvorc et al., 2000). In addition, intense mammary gland growth and the onset of copious milk synthesis and secretion demand increase oxygen consumption; this increases the production of reactive oxygen species (ROS), rendering pregnancy as a state of oxidative stress that is often also with decreased production of antioxidants (Trevisan et al., 2001). Indeed, several studies have shown the periparturient period in dairy cows is a state of oxidative stress (Abuelo et al., 2019; Liang et al., 2020; Lykkesfeldt & Svendsen, 2007).

Currently, there are no reports regarding uterine hemodynamic changes in buffalos across gestation. The aims of the study, therefore, were to evaluate gestational age-dependent changes in uterine, vaginal and placental hemodynamics in Egyptian buffaloes and to determine the relationships among reproductive Doppler parameters and serum metabolic parameters as well as oxidative stress. We hypothesized that uterine and virginal artery blood flows alter according to gestational age, in association with changes in serum biochemical biomarkers.

# 2 | MATERIALS AND METHODS

## 2.1 | Animals

This study was conducted at Elmax farm of the veterinary services department of Egyptian Armed Forces, Alexandria Provenance, with an approval 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. A total of thirty-five healthy pregnant buffalo cows, aged 4–9 years old with 2–6 lactation seasons, were used in this study. All buffalo cows used had normal pregnancies without any pathological signs throughout the experiment.

All buffalo cows have inseminated artificially 14 hr after the second GnRH injection of the ovsynch protocol [GnRH (day 0), PGF2α (day 7), GnRH (day 9)] with Italian seminal fluid straws and were milked by machine twice daily for seven months, then dried off milk for the remaining days of gestation.

## 2.2 | Doppler ultrasonography

The uterine arteries in buffaloes were located and examined according to our previous studies (Devender et al. 2018; Varughese et al., 2013). 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 modify the Doppler gate over the uterine artery, adjusted to the diameter of the vessel (Figure 2a,b). The vaginal arteries were located according to (Vittoria, 1997) (Figure 3a,b).

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 4,500 Hz and Doppler angle varying between 0̊ and 40̊. Epidural anaesthesia 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 and vaginal arteries were time-averaged maximum velocity (TAMV), resistance index (RI) and pulsatility index (PI) in addition to the diameters for both arteries (Figures 2 and 3a,b). The diameters of both uterine and vaginal arteries were assessed from a B-mode image, and the average was calculated for each month of pregnancy.

Images for 3–5 placentomes per animal were recorded for each month from months five and ten of pregnancy; the images were used to measure the blood perfusion offline by using NIH Image J. To achieve maximum colour pixels in the placentome, a slight adjustment in the location of the transducer in relation to the placentome was done according to a previous report by Kim-Egloff et al., (2016). The area of the placentome in colour power-mode Doppler, representing the area with measurable blood flow (Figure 4a,b) in the entire area of the placentome, region of interest, was traced in the areas measured (Kim-Egloff et al., 2016).

## 2.3 | Biochemical analyses of serum parameters

Serum samples were collected from each animal monthly from month one to ten of pregnancy and stored at −20°C until biochemical analysis could be performed. Total lipids (T. lipid) were measured by using Bio-diagnostic kits (Egypt). Cholesterol, triglycerides, high-density lipoproteins (HDL), low-density lipoproteins (LDL) and glucose were determined using assay kits provided by the Cobas Test Reagent (USA). Total protein and albumin were determined according to the standard protocol of Stanbio Co. (USA). Globulin was calculated by subtracting albumin from total protein. The concentrations of malondialdehyde (MDA), glutathione (GSH) and catalase were measured by using the protocol of Bio-diagnostic Co. (Egypt). All parameters were determined in serum using a spectrophotometer (5,010, photometer, BM Co. Germany).

## 2.4 | Statistical analysis

Data were presented as means ± *SD* for statistical analyses using SAS® (version 9.2, SAS Institute). The Shapiro–Wilk test was used to test for normality of the distribution of all variables. To determine

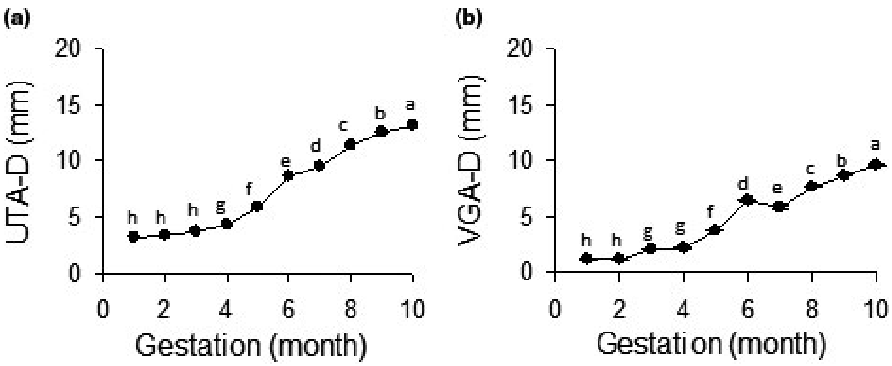


**FIGURE 1** Ipsilateral uterine artery (UA) and vaginal artery (VA) diameter changes in gestation. Values are mean ± standard deviation. Means with different superscripts (a,b,c,d,e,f,g,h) are significantly different ($p < .05$)

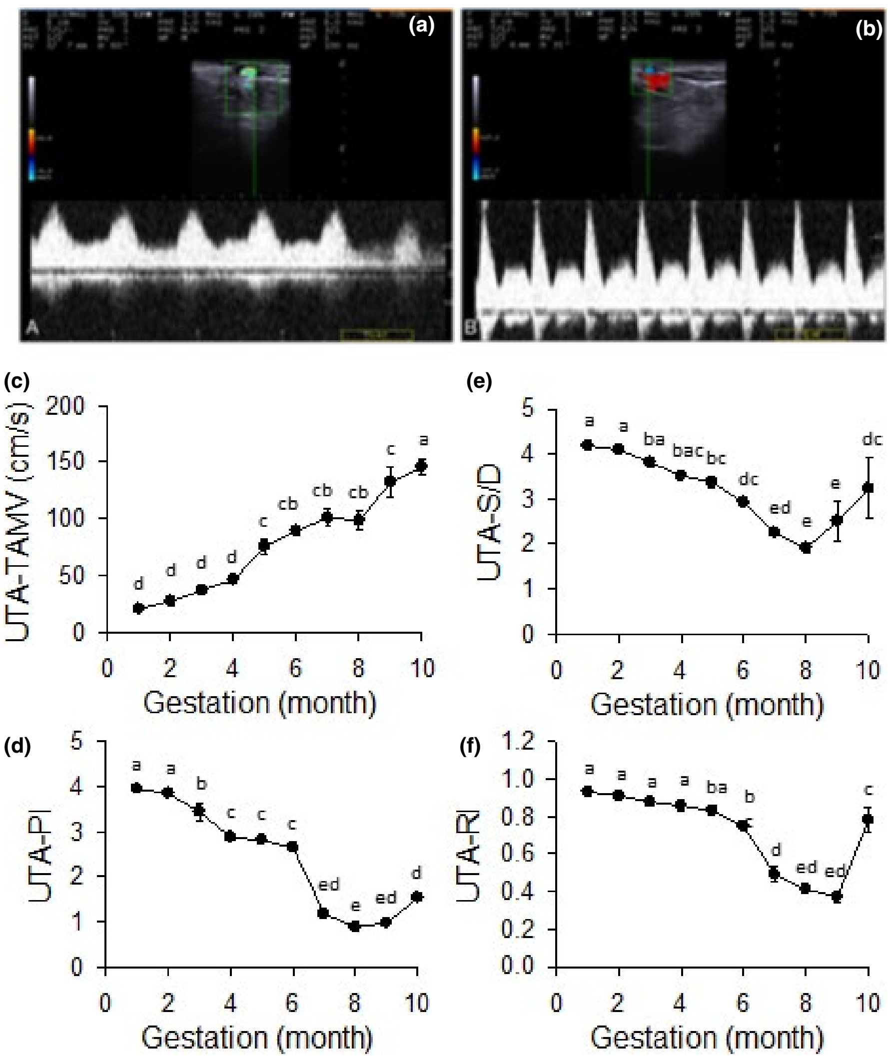


**FIGURE 2** Uterine artery time-averaged maximum velocity, systolic/diastolic velocity, resistance index and pulsatility index (UTA TAMV, S/D, RI, PI) changes during gestation. Values are mean ± standard deviation (*SD*). Means with different superscripts (a,b,c,d,e) are significantly different ($p < .05$)

the effect of time (month) on TAMV, PI, RI and S/D in uterine and vaginal arteries and pixel measurements of placentome, a mixed model one-way analysis of variance, with time points as repeated measurements, was used. Post hoc multiple pairwise comparisons were done according with Duncan's adjustment of error rate.

The relationship between UtBF and VaBF parameters was measured using Pearson's correlation and the significance of correlations was established using Fisher's $r$ to $z$ transformation. Pearson's correlation was used to determine associations between Doppler parameters and pregnancy months as well as changes in the diameter of the arteries. Differences were considered significant at $p \leq .05$.

## 3 | RESULTS

The length of gestation averaged 315.5 ± 0.77 days or 10.52 ± 0.03 weeks in Egyptian buffalo cows. The diameters of UA and VA remained unchanged until months 3 and 2 during early pregnancy, began to significantly enlarge in middle gestation, and continued to increase till month 10 (Figure 1a,b). The diameter of UA was positively correlated ($r = .93$, $p < .001$) with that of VA during gestation.

Changes in UA and VA hemodynamic parameters were dependent on gestational age in Egyptian buffaloes ($p < .001$). The TAMV values for UA and VA blood flow remained unchanged in the first three months, began to significantly ($p < .05$) increase in the 4th month, and continued to increase until reaching its maximum concentration in month 9, and remained highest in month 10 during gestation (Figures 2c and 3c). Meanwhile, S/D, PI and RI of both UA and VA blood flows showed gestation age-dependent changes; they remained constant during early months (one- to five-month) in pregnancy, then significantly ($p < .05$) decreased monthly from month six to reach its nadir in month nine. In the 10th month of gestation, all these values were back up to that of month 7 (Figures 2 and 3d–f).

The correlations among UA and VA blood flow indices were summarized in Table 1. In both UA and VA blood flows, TAMV was negatively correlated with PI (UA: $r = -.49$; VA: $-0.59$, $p < .001$), RI (UA: $r = -.40$; VA: $-0.48$, $p < .001$) and S/D (UA: $r = -.37$, VA: $-0.49$,

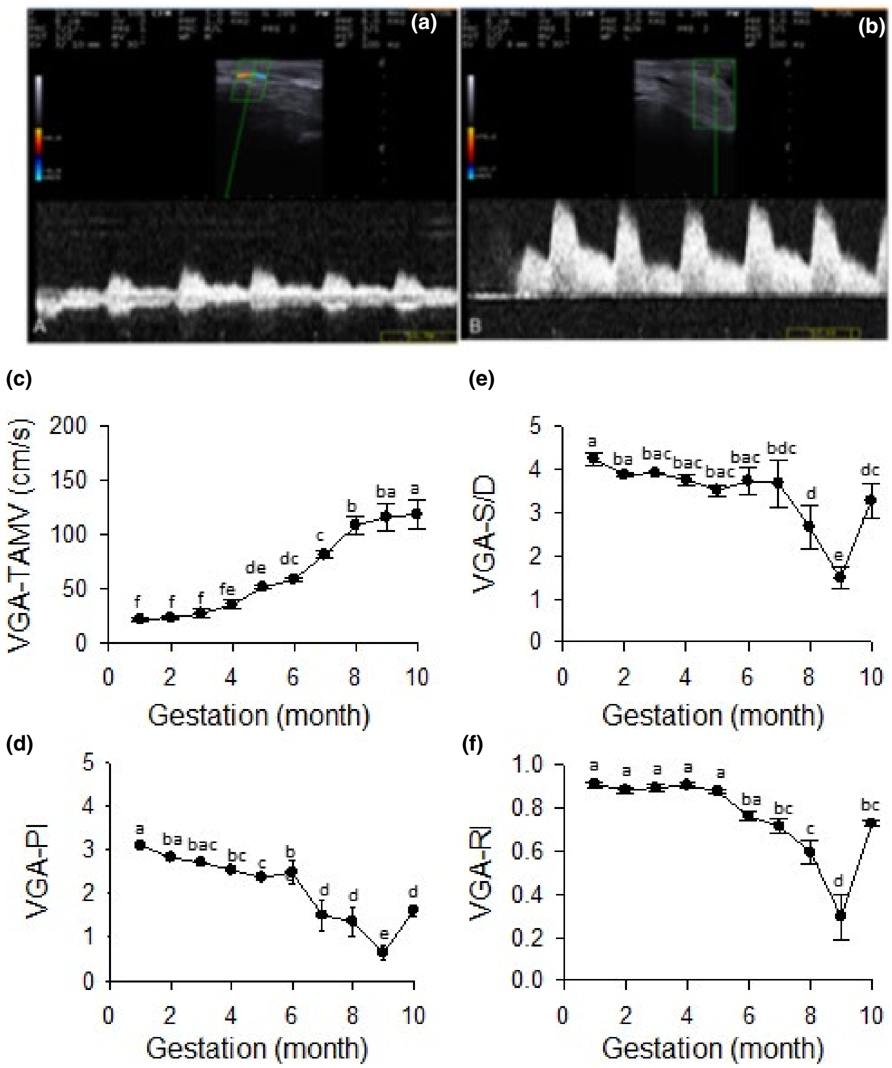


**FIGURE 3** Vaginal artery time-averaged maximum velocity, systolic/diastolic velocity, resistance index and pulsatility index (VGA TAMV, S/D, RI, PI) changes during gestation. Values are mean ± *SD*. Means with different superscripts (a,b,c,d,e) are significantly different ($p < .05$)

**TABLE 1** Pearson's rank correlation coefficients for the relationships between the time-averaged maximum velocity (TAMV), pulsatility index (PI), resistance index (RI), systolic/diastolic velocity (S/D), and diameters (D) of *uterine arteries* and vaginal blood flow Doppler parameters in pregnant Egyptian buffaloes

| Parameters | Doppler parameters | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | UTA_TAMV | UTA_PI | UTA_RI | UTA_SD | UTA_D | VgA_TAMV | VgA_PI | VgA_RI | VgA_SD | VgA_D |
| UTA_TAMV | 1.00000 | −0.49093<br><0.001 | −0.40568<br><0.0004 | 0.83897<br><0.0015 | 0.37077<br><0.001 | 0.78081<br><0.001 | −0.55859<br><0.001 | −0.48027<br><0.001 | −0.48691<br><0.001 | 0.84890<br><0.001 |
| UTA_PI | −0.49093<br><0.001 | 1.00000 | 0.79029<br><0.001 | 0.82977<br><0.001 | −0.52718<br><0.001 | −0.61063<br><0.001 | 0.72146<br><0.001 | 0.70931<br><0.001 | 0.61811<br><0.001 | −0.51236<br><0.001 |
| UTA_RI | −0.40568<br><0.0004 | 0.79029<br><0.001 | 1.00000 | 0.73765<br><0.001 | −0.46837<br><0.001 | −0.57685<br><0.001 | 0.81412<br><0.001 | 0.84231<br><0.001 | 0.69702<br><0.001 | −0.46869<br><0.001 |
| UTA_SD | −0.37077<br><0.0015 | 0.82977<br><0.001 | 0.73765<br><0.001 | 1.00000 | −0.45527<br><0.001 | −0.51634<br><0.001 | 0.67046<br><0.001 | 0.68573<br><0.001 | 0.54955<br><0.001 | −0.42014<br><0.0003 |
| UTA_D | 0.83897<br><0.001 | −0.52718<br><0.001 | −0.46837<br><0.001 | −0.45527<br><0.001 | 1.00000 | 0.88013<br><0.001 | −0.63466<br><0.001 | −0.58652<br><0.001 | −0.61657<br><0.001 | 0.93213<br><0.001 |
| VgA_TAMV | 0.78081<br><0.001 | −0.61063<br><0.001 | −0.57685<br><0.001 | −0.51634<br><0.001 | 0.88013<br><0.001 | 1.00000 | −0.69591<br><0.001 | −0.63297<br><0.001 | −0.62501<br><0.001 | 0.88602<br><0.001 |

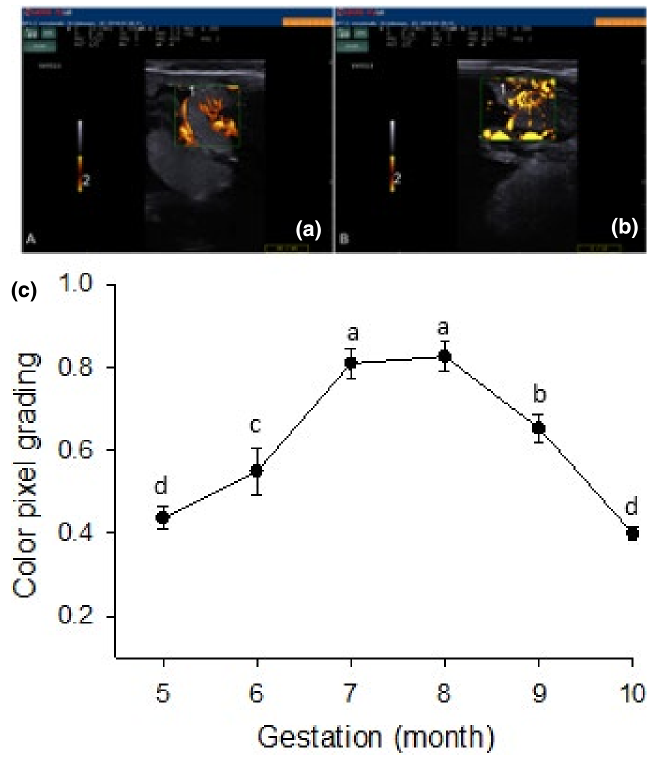


**FIGURE 4** Placentome blood flow changes during gestation. Values are mean ± *SD*. Means with different superscripts (a,b,c,d) are significantly different ($p < .05$). Power Doppler imaging of placentome at month 5 (A) and 7 (B) The perfused portion appears in colour (1) and is demarcated from the rest of the placentome. (2) The scale used to determine the red, green and blue (RGB) colour model values

$p < .001$); however, TAMV was positively correlated with AD (UA: $r = .84$; VA: 0.87, $p < .001$). The resistance Doppler indexes were positively correlated with each other for UA (PI:RI, $r = .79$; PI:*SD*, $r = .79$; RI:*SD*, $r = .73$) and VA (PI:RI, $r = .90$; PI:*SD*, $r = .79$; RI:*SD*, $r = .85$, $p < .001$) blood flows. UA-TAMV positively correlated with VA-TAMV ($r = .78$, $p < .001$) and AD ($r = .84$, $p < .001$), but negatively correlated with PI ($r = -.56$, $p < .001$), RI ($r = -.48$, $p < .001$) and *SD* ($r = -.49$, $p < .001$) in VA blood flow.

The exponential relationships of UA and VA-TAMVs with the month of pregnancy (t) was TAMV = 91.50 x $e^{0.209t}$ and TAMV = 92.05 x $e^{0.159t}$, respectively.

Placentome blood flow (PaBF) in the Egyptian buffalo cows can be determined from month 5 till month 10 in gestation by colour Doppler ultrasonography. PaBF increased significantly ($p > .05$) between months five and eight and then decreased until parturition (Figure 4c).

Changes in serum metabolic parameters in Egyptian buffalo cows during pregnancy were summarized in Table 2. Concentrations of serum lipid profiles (mg/dl) (Figure 5) in the first month of gestation were the highest (T. lipid = 668.87 ± 4.09, cholesterol = 204.73 ± 8.97, HDL = 84.33 ± 1.45, and LDL = 85.70 ± 2.07) during pregnancy. With pregnancy proceeded, these lipid profiles tended to decrease continuously, and all reached their nadir

**TABLE 2** Mean values of serum metabolic and antioxidant parameters for each month of pregnancy

| Parameters | Gestation (Month) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| T. Lipid (mg/dl) | 668.87 ± 4.09$^{a}$ | 567.62 ± 8.17$^{cd}$ | 604.79 ± 4.12$^{b}$ | 580.96 ± 7.30$^{c}$ | 684.55 ± 2.18$^{a}$ | 563.05 ± 5.44$^{cd}$ | 524.48 ± 5.62$^{d}$ | 580.14 ± 10.36$^{c}$ | 662.90 ± 7.34$^{a}$ | 542.83 ± 6.71$^{d}$ |
| Cholesterol (mg/dl) | 204.73 ± 8.97$^{a}$ | 116.50 ± 13.24$^{e}$ | 159.64 ± 5.15$^{c}$ | 188.45 ± 5.92$^{b}$ | 151.13 ± 4.85$^{c}$ | 132.78 ± 3.19$^{d}$ | 134.12 ± 5.14$^{d}$ | 113.73 ± 5.95$^{e}$ | 144.63 ± 6.25$^{d}$ | 116.86 ± 4.53$^{e}$ |
| Triglycerides (mg/dl) | 103.52 ± 4.43$^{cd}$ | 122.29 ± 10.98$^{c}$ | 147.05 ± 9.53$^{b}$ | 142.77 ± 4.18$^{b}$ | 140.01 ± 10.09$^{b}$ | 142.76 ± 5.65$^{b}$ | 173.33 ± 13.65$^{a}$ | 200.09 ± 2.96$^{a}$ | 150.19 ± 35.41$^{b}$ | 81.33 ± 6.24$^{d}$ |
| HDL(mg/dl) | 84.33 ± 1.45$^{a}$ | 74.67 ± 1.43$^{ab}$ | 76.00 ± 1.73$^{ab}$ | 66.33 ± 0.88$^{b}$ | 79.33 ± 0.87$^{ab}$ | 62.67 ± 1.76$^{b}$ | 65.67 ± 1.45$^{b}$ | 67.00 ± 1.15$^{b}$ | 69.33 ± 0.85$^{b}$ | 57.33 ± 1.20$^{c}$ |
| LDL (mg/dl) | 85.70 ± 2.07$^{a}$ | 56.60 ± 2.26$^{d}$ | 67.17 ± 1.24$^{c}$ | 66.33 ± 0.96$^{c}$ | 77.33 ± 1.05$^{b}$ | 66.10 ± 1.57$^{c}$ | 67.37 ± 0.66$^{c}$ | 76.43 ± 1.39$^{b}$ | 77.00 ± 1.32$^{b}$ | 55.73 ± 2.46$^{d}$ |
| Glucose(mg/dl) | 100.67 ± 2.19$^{a}$ | 101.33 ± 2.85$^{a}$ | 98.00 ± 5.13$^{a}$ | 98.67 ± 1.45$^{a}$ | 101.67 ± 4.33$^{a}$ | 96.67 ± 2.33$^{ab}$ | 82.33 ± 1.86$^{b}$ | 81.33 ± 3.18$^{b}$ | 73.00 ± 3.79$^{c}$ | 71.67 ± 1.76$^{c}$ |
| T. Protein(g/dl) | 10.76 ± 1.45$^{ab}$ | 12.09 ± 1.04$^{a}$ | 10.15 ± 0.09$^{ab}$ | 10.64 ± 1.20$^{ab}$ | 10.88 ± 1.10$^{ab}$ | 10.23 ± 0.08$^{ab}$ | 9.48 ± 0.28$^{ab}$ | 9.02 ± 0.67$^{b}$ | 10.47 ± 0.37$^{ab}$ | 10.70 ± 0.13$^{ab}$ |
| Albumin (g/dl) | 6.53 ± 0.39$^{ab}$ | 7.89 ± 0.72$^{a}$ | 7.02 ± 0.33$^{a}$ | 6.64 ± 0.31$^{ab}$ | 6.62 ± 0.15$^{ab}$ | 6.45 ± 0.54$^{ab}$ | 5.43 ± 0.30$^{b}$ | 5.37 ± 0.41$^{b}$ | 5.80 ± 0.43$^{b}$ | 7.37 ± 0.23$^{a}$ |
| Globulin(g/dl) | 4.23 ± 1.25 | 4.20 ± 0.70 | 3.13 ± 0.25 | 4.00 ± 1.02 | 4.25 ± 1.10 | 3.77 ± 0.62 | 4.05 ± 0.53 | 3.65 ± 0.38 | 4.67 ± 0.60 | 3.64 ± 0.33 |
| MDA (nmol/ml) | 4.71 ± 0.89$^{d}$ | 4.70 ± 0.33$^{d}$ | 10.32 ± 0.47$^{ab}$ | 11.78 ± 0.87$^{a}$ | 4.05 ± 0.47$^{d}$ | 7.00 ± 1.10$^{c}$ | 7.14 ± 0.27$^{c}$ | 5.93 ± 0.76$^{d}$ | 10.04 ± 0.53$^{ab}$ | 13.32 ± 1.52$^{a}$ |
| GSH(mg/dl) | 2.44 ± 0.35$^{a}$ | 1.38 ± 0.11$^{b}$ | 1.14 ± 0.08$^{b}$ | 1.07 ± 0.02$^{b}$ | 1.73 ± 0.35$^{b}$ | 1.57 ± 0.27$^{b}$ | 1.31 ± 0.14$^{b}$ | 1.24 ± 0.04$^{b}$ | 1.49 ± 0.12$^{b}$ | 1.32 ± 0.11$^{b}$ |
| Catalase (U/l) | 985.23 ± 23.85$^{a}$ | 895.83 ± 56.13$^{ab}$ | 461.09 ± 57.00$^{c}$ | 404.25 ± 38.30$^{c}$ | 786.74 ± 44.58$^{b}$ | 393.84 ± 27.06$^{c}$ | 410.95 ± 41.26$^{c}$ | 434.78 ± 31.51$^{c}$ | 381.17 ± 15.76$^{c}$ | 452.64 ± 25.96$^{c}$ |

*Note:* Within each row, means with varied letters are significantly differ at the 5% level (Tukeys test).
GSH, glutathione; HDL, high-density lipoproteins; LDL, low-density lipoproteins; MDA, malondialdehyde; T. lipid, total lipids.

values (T. lipids = 542.83 ± 6.71, cholesterol = 116.86 ± 4.53, HDL = 57.33 ± 1.20 and LDL = 55.73 ± 2.46) ($p < .05$). However, concentrations of triglycerides (mg/dl) were high in month 7 (173.33 ± 13.65), peaked in month 8 (200.09 ± 2.96) and decreased to the lowest value in month 10 (81.33 ± 6.24) ($p < .05$).

Serum glucose and protein concentrations (mg/dl) also decreased with advanced gestational age (Figure 6). Serum glucose concentrations were high and unchanged ($p > .05$) in the first five months of gestation and were significantly lower ($p < .05$) in the last two months of gestation (73.00 ± 3.79 and 71.67 ± 1.76, months 9 and 10, respectively) compared to early gestation months. Albumin concentrations (g/dl) were significantly lower ($p < .05$) in months 7, 8 and 9 (5.43 ± 0.30, 5.37 ± 0.41 and 5.80 ± 0.43, respectively) compared to month 2 (7.89 ± 0.72) in gestation and was back up to the high concentration (g/dl) at month 10 (7.07 ± 0.23). Globulin concentration was unchanged during pregnancy. Total protein concentration remained steady, only with a decrease ($p < .01$) in month 8 (9.02 ± 0.67) compared to month 2 (12.086 ± 1.04) in gestation.

Serum concentrations of the oxidative stress marker MDA and antioxidants in Egyptian buffalo cows during pregnancy were summarized in Figure 7. Serum MDA concentrations peaked in month 5 (11.78 ± 0.87 nmol/ml) and month 10 (13.32 ± 1.52 nmol/ml), and also, concentrations in months 4 and 9 were also greater compared to other months in gestation. On the contrary, serum concentrations of the antioxidants, that is GSH (2.44 ± 0.35 mg/dl) and catalase (985.23 ± 23.85 U/l), in the 1st month of gestation were the highest among all months in gestation. GSH and catalase both sharply reduced to lowest concentrations ($p < .05$) in months 3–4, increased in month 5 and then maintained at lowest concentrations the rest of gestation.

As summarized in Table 3, TAMV and AD for both UA and VA (UA-TAMV, UA-D, VA-TAMV and VA-D) were negatively correlated with cholesterol ($r$ = −.417, −.410, −.405, and -.400, $p < .01$), HDL ($r$ = −.642, −.579, −.469, and −.569, $p < .01$), glucose ($r$ = −.762, −.890, −.781 and −.858, $p < .01$), GSH ($r$ = −.269, −.269, −0.240 and −.272, $p < .05$) and catalase ($r$ = −.474, −.607, −.563 and −.613, $p < .01$); all were positively correlated with MDA ($r$ = .572, .632, .537 and .614, $p < .01$). In addition, UA-TAMV, UA-D and VA-D were positively correlated with total protein [$r$ = .254 ($p < .05$), 0.323 ($p < .01$) and 0.309 ($p < .01$)]. Moreover, UA-TAMV was negatively correlated with T. Lipid ($r$ = −.334, $p < .01$), triglycerides ($r$ = −.264, $p < .05$) and LDL ($r$ = −.295, $p < .05$).

## 4 | DISCUSSION

The current study is the first to use colour Doppler ultrasonography to determine gestational age-dependent changes in hemodynamics of the lower female reproductive tract, including UtBF, VaBF and PaBF, in Buffalo cows. We found that compared to the 1st month in pregnancy, TAMV of UtBF in Egyptian Buffalo cows increases exponentially along with decreased PI and PS, resulting in an approximate

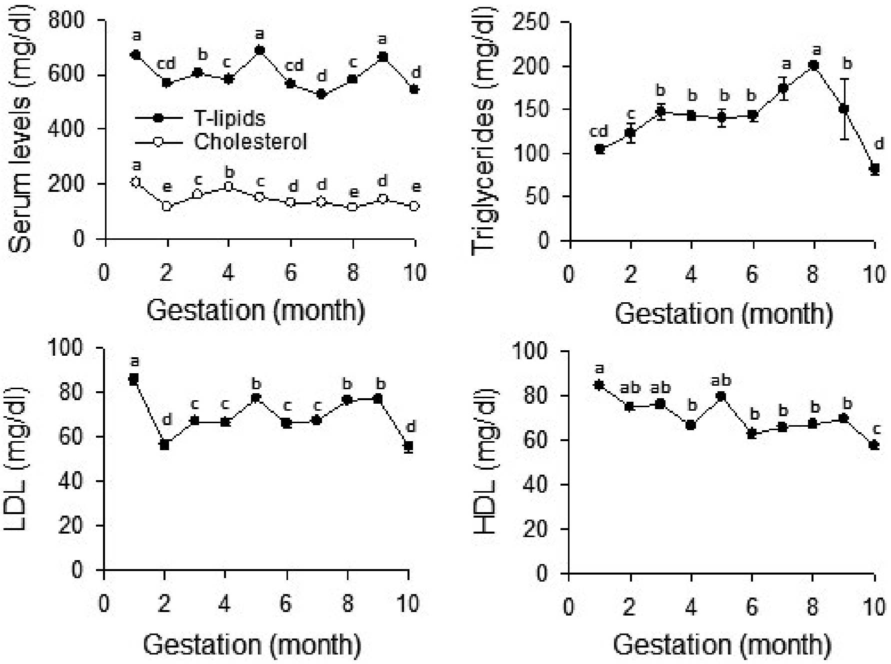


**FIGURE 5** Lipids profiles in pregnancy (total lipids, cholesterol, triglycerides, low-density (LDL) and high-density (HDL) lipoproteins). Values are mean ± *SD*. Means with different superscripts (a,b,c,d,e) are significantly different ($p < .05$)

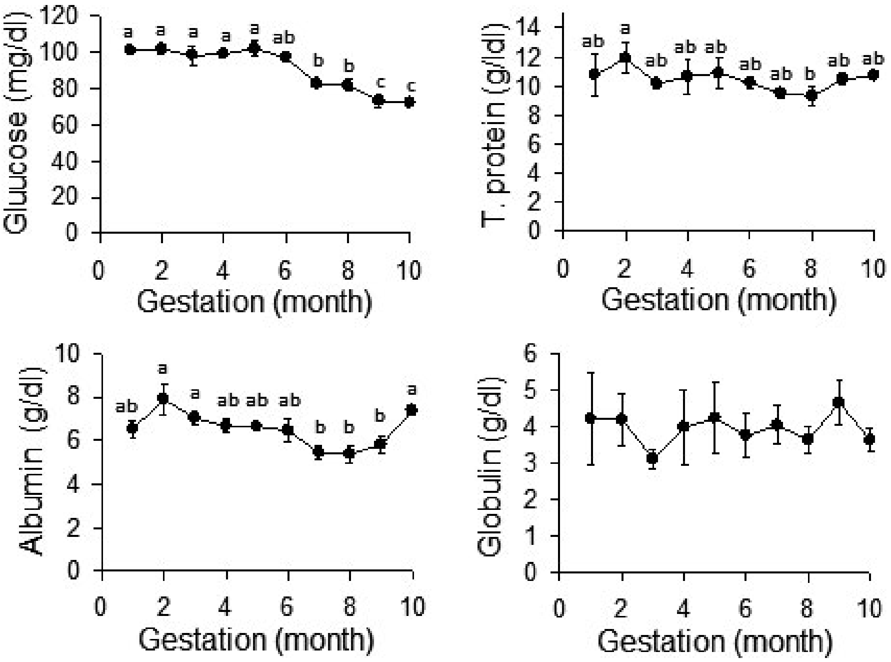


**FIGURE 6** Glucose/protein levels (total protein, albumin and globulin) in pregnancy. Values are mean ± *SD*. Means with different superscripts (a,b,c) are significantly different ($p < .05$)

eightfold increase in flow rate near term. In parallel, TAMV for vaginal artery blood flow also increases fivefold in flow rate, with significantly decreased PI and PS, compared with that in the 1st month in gestation. PaBF only can be measured from the 4th month till term in gestation, which reaches the maximum in the 7-8th months in gestation, then gradually decreased and returned to the 4th month level in the 10th month in gestation. Maternal circulating metabolic parameters tend to increase and antioxidants tend to decrease with advanced gestational age. Correlation analyses demonstrate that the inverse relationship between metabolic parameters and antioxidants correlates with gestation age-dependent increases in UtBF, VaBF and PaBF during pregnancy.

Both uterine and vaginal arteries constantly enlarge with advanced gestational age; UA and VA were approximately fivefold and eightfold greater in AD, respectively, at the end of pregnancy when compared with month one of pregnancy. The increase in UA diameters with advanced gestation age show UA remodelling, along with increased uterine TAMV, to facilitate the delivery of increased blood supply to meet the needs of a higher amount of nutrients and oxygen by the growing foetus during different stages of pregnancy. Similar changes in compliance were detected in cows and buffaloes during pregnancy (Bollwein et al., 2002; Panarace et al., 2006; Varughese et al., 2013).

Vaginal arteries blood flow changes revealed in this study are perhaps the first record of such kind in large animals. In humans, a high

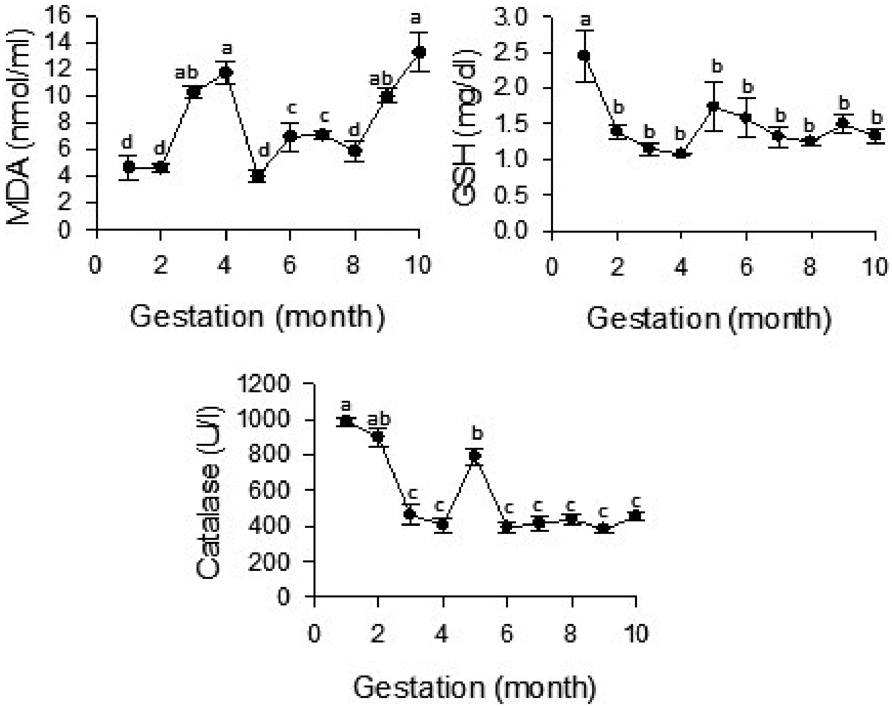


**FIGURE 7** Antioxidants and oxidant stress markers (Malondialdehyde (MDA), Glutathione (GSH) and catalase) in pregnancy. Values are mean ± *SD*. Means with different superscripts (a,b,c) are significantly different ($p < .05$)

**TABLE 3** Correlations among metabolic and antioxidant parameters and uterine (UTA) and vaginal (VgA) hemodynamic changes in gestation in Egyptian buffalos

| | Doppler parameters | | | |
|---|---|---|---|---|
| | UTA | | VGA | |
| Biochemical parameters | TAMV | D | TAMV | D |
| T. Lipid | −0.334 | −0.161 | −0.099 | −0.136 |
| Cholesterol | −0.417 | −0.410 | −0.405 | −0.400 |
| Triglycerides | −0.264 | −0.213 | −0.067 | −0.211 |
| HDL | −0.642 | −0.579 | −0.469 | −0.569 |
| LDL | −0.295* | −0.210 | −0.090 | −0.211 |
| Glucose | −0.762 | −0.890 | −0.781 | −0.858 |
| T. Protein | 0.254 | 0.323 | 0.206 | 0.309 |
| Albumin | 0.016 | 0.048 | −0.147 | 0.033 |
| Globulin | 0.075 | 0.073 | 0.114 | 0.044 |
| MDA | 0.572 | 0.632 | 0.537 | 0.614 |
| GSH | −0.269 | −0.269 | −0.240 | −0.272 |
| Catalase | −0.474 | −0.607 | −0.563 | −0.613 |

D, artery diameter; TAMV, time-averaged maximum velocity.
*$p < .05$;
**$p < .01$.

correlation was found between UtBF and VaBF throughout gestation (Craig & Billow, 2020; Wright & Burns, 2020). The increase of VaBF after month four of pregnancy indicates the contribution of VaBF in the nourishment of gravid uterus in the second half of pregnancy that indicates the descending of the gravid uterus to the caudal abdominal cavity. UtBF also increases monthly in parallel with VaBF during pregnancy. The further rises in VaBF and UtBF at the end of pregnancy are not surprising, as this is expected to meet the demand of the exponential growth rate of the foetus at the last one third of gestation, consistent with previous studies in cows and buffaloes (Bollwein et al., 2002; Varughese et al., 2013).

The PI, RI and S/D values of the uterine arteries significantly decrease between months four and eight of pregnancy, while RI and S/D increase at month 10 of pregnancy. The values of vaginal arteries' resistance impedance were similar to that of uterine arteries. The decrease of resistance impedance for either uterine and vaginal arteries means an increase in UtBF (Elmetwally et al., 2016; Panarace et al., 2006; Varughese et al., 2013).

In the current study, the power Doppler ultrasonography was used for the first time to investigate the placentomes blood perfusion in buffalo cows between months five and ten of pregnancy. The blood flow to the placentomes increase between months five and eight and then decreased during the months nine and ten of pregnancy. The increased placental perfusion is essential for meeting the high nutrient and oxygen demands of the foetus. In dairy cows, a previous study reported placentome blood flow does not change significantly in pregnancy; however, this study only measured PaBF in the last month of pregnancy (Kim-Egloff et al., 2016). Compared to earlier months, the decrease in PaBF during the last two months of gestation in buffalo cows revealed in our study may be due to an increase of placental oedema and implicates that PaBF may decrease prior to the process of parturition (Botta et al., 2019).

Our results demonstrated significantly higher values of T. lipid, cholesterol, HDL and LDL in the first month compared to the other months of pregnancy. The triglycerides are the highest in months 7, 8 and 10 of pregnancy, when the significantly lowest values of all other lipids are recorded. Effectively, the alterations in serum cholesterol and LDL levels in the peripartum period indicate their utilization during pregnancy by steroidogenic endocrine organs, particularly the ovaries and placenta for synthesizing steroid hormones, and mammary glands for milk production (Arfuso et al., 2016). The disturbances in triglycerides probably reflect their consumption by the mammary gland in the synthesis and secretion of milk fat (Bernard et al., 2008; Mantovani et al., 2010).

Glucose is considered the primary metabolic fuel for maintaining foetal growth and milk production in dairy cows (Aschenbach et al., 2010; Wankhade et al., 2017). The significantly lower serum glucose level at the last two months of gestation (9 and 10) compared to the first five months in buffalo cows may be related to insulin uptake by the pregnant uterus, mammary gland and liver changes, which have been observed in lactation and pregnancy dairy cows previously (Chalmeh et al., 2015).

Serum proteins may serve as potential diagnostic markers for many pathological conditions and be used as indicatives of many health problems (Ghaffari et al., 2020; Pinedo et al., 2020). Mobilization of body protein, fat and mineral stores has been recorded in periparturient and postparturient periods, providing materials for milk production (van Dorland et al., 2009). We show that albumin level is significantly lower in months 7, 8 and 9 of pregnancy, compared to month 2 and is back to the high level at month 10, while globulin level remains constant during pregnancy, and total protein only decrease in month 8 compared to that in all months of pregnant in buffalo cows. Serum albumin level reduces and reaches its lowest point at the middle of gestation and then gradually increases to a point within the normal range until foaling (Kaneko et al., 1997). However, in dairy cows, Piccione et. al. (2011) reported a similar albumin concentration at 60, 30 and 7 days before calving, with a small increase at calving.

Our results show an elevation in the MDA level, starting from month 3 until the end of pregnancy in a fluctuated manner; the highest MDA value is recorded at months 5 and 10. Higher GSH and catalase concentrations are only observed in 1st month of pregnancy in buffalo cows, while their values constantly are at significantly lower concentrations in all the rest months of pregnancy. In dairy cows, the MDA level tends to transiently increase at 6 weeks before calving with a reduction in GSH-Px activity one week after calving (Konvičná et al., 2015). Furthermore, total antioxidant capacity significantly increases in early lactating compared with late pregnant dairy cows (Gong & Xiao, 2018). Thus, the results support the idea that pregnancy is a physiological condition of oxidative stress. In this study, some of the metabolic parameters and oxidative stressors are also correlated with both uterine and vaginal TAMV and AD in buffalo cows. The significantly increased UtBF and PaBF may be attributed to the significantly increased steroid hormones, that is, oestrogen, and progesterone, throughout pregnancy, which increases cardiac output and UtBF to subsequently affect biochemical and oxidative markers (Ford, 1982; Kim-Egloff et al., 2016).

Altogether, our current study demonstrates that UtBF, VaBF and PaBF increase with advanced gestational age in buffalo cows to facilitate the delivery of blood supplies of nutrient and oxygen supply to meet foetal demands. Changes in blood flows are also associated with dynamic changes in serum metabolic parameters and increased oxidant and decreased antioxidant production, showing that pregnancy is a physiological state of increased metabolism and oxidative stress.

## CONFLICT OF INTEREST

There is no conflict of interest to report.

## AUTHOR CONTRIBUTIONS

Mohammed A Elmetwally: conceptualization, study design, Doppler imaging, statistical analysis, manuscript preparation, editing and finalizing. Gehad Elshopaky: conceptualization, analyses for blood, data analyses, writing, review and editing. Wael Eldomany, Ashraf El-Desouky, Alaa Samy and Yasser Lenis: conceptualization, review and editing. Dong-bao Chen: data interpretation, manuscript preparation, editing and finalizing.

## DATA AVAILABILITY

The authors state that there are no data available to be shared.

## ORCID

*Mohammed A. Elmetwally* https://orcid.org/0000-0001-7486-7756

## REFERENCES

Abdollahi-Arpanahi, R., Carvalho, M. R., Ribeiro, E. S., & Peñagaricano, F. (2019). Association of lipid-related genes implicated in conceptus elongation with female fertility traits in dairy cattle. *Journal of Dairy Science*, *102*(11), 10020–10029. https://doi.org/10.3168/jds.2019-17068

Abuelo, A., Hernández, J., Benedito, J. L., & Castillo, C. (2019). Redox biology in transition periods of dairy cattle: Role in the health of periparturient and neonatal animals. *Antioxidants (Basel, Switzerland*, *8*(1), 20. https://doi.org/10.3390/antiox8010020

Angeli, E., Rodríguez, F. M., Rey, F., Santiago, G., Matiller, V., Ortega, H. H., & Hein, G. J. (2019). Liver fatty acid metabolism associations with reproductive performance of dairy cattle. *Animal Reproduction Science*, *208*, 106104. https://doi.org/10.1016/j.anireprosci.2019.06.016

Arfuso, F., Fazio, F., Levanti, M., Rizzo, M., Di Pietro, S., Giudice, E., & Piccione, G. (2016). Lipid and lipoprotein profile changes in dairy cows in response to late pregnancy and the early postpartum period. *Archives Animal Breeding*, *59*(4), 429–434. https://doi.org/10.5194/aab-59-429-2016

Aschenbach, J. R., Kristensen, N. B., Donkin, S. S., Hammon, H. M., & Penner, G. B. (2010). Gluconeogenesis in dairy cows: The secret of making sweet milk from sour dough. *IUBMB Life*, *62*(12), 869–877. https://doi.org/10.1002/iub.400

Bernard, L., Leroux, C., & Chilliard, Y. (2008). Expression and nutritional regulation of lipogenic genes in the ruminant lactating mammary gland. *Advances in Experimental Medicine and Biology*, *606*, 67–108. https://doi.org/10.1007/978-0-387-74087-4_2

Bollwein, H., Baumgartner, U., & Stolla, R. (2002). Transrectal Doppler sonography of uterine blood flow in cows during pregnancy. *Theriogenology*, *57*(8), 2053–2061. https://doi.org/10.1016/s0093-691x(02)00706-9

Botta, C., Pellegrini, G., Hässig, M., Pesch, T., Prähauser, B., Wunderlin, S., Guscetti, F., Schneeberger, M., Schmitt, S., Basso, W., Hilbe, M., Schuler, G., & Borel, N. (2019). Bovine Fetal Placenta During Pregnancy and the Postpartum Period. *Veterinary Pathology*, *56*(2), 248–258. https://doi.org/10.1177/0300985818806453

Chalmeh, A., Pourjafar, M., Nazifi, S., Momenifar, F., & Mohamadi, M. (2015). Study on serum glucose, insulin, NEFA, BHBA and lipid profile in different productive status of high producing Holstein dairy cows. *Iranian Journal of Veterinary Medicine*, *9*(3), 171–178. https://doi.org/10.22059/IJVM.2015.55285

Cotechini, T., & Graham, C. H. (2015). Aberrant maternal inflammation as a cause of pregnancy complications: A potential therapeutic target? *Placenta*, *36*(8), 960–966. https://doi.org/10.1016/j.placenta.2015.05.016

Craig, M. E., & Billow, M. (2020). Anatomy, abdomen and pelvis, broad ligaments. In *StatPearls*. Treasure Island (FL): StatPearls Publishing.

Devender, X., Chandolia, R. K., Singh, G., Pandey, A. K., &Kumari, S. (2018). Evaluation of blood flow in middle uterine artery by color doppler ultrasonography in uterine torsion buffaloes before and after detorsion. *Buffalo Bulletin*, *37*, 495–502.

Elmetwally, M., Rohn, K., & Meinecke-Tillmann, S. (2016). Noninvasive color Doppler sonography of uterine blood flow throughout pregnancy in sheep and goats. *Theriogenology*, *85*(6), 1070–1079. https://doi.org/10.1016/j.theriogenology.2015.11.018

Ford, S. P. (1982). Control of uterine and ovarian blood flow throughout the estrous cycle and pregnancy of ewes, sows and cows. *Journal of Animal Science*, *55*(Suppl 2), 32–42.

Ford, S. P., Reynolds, L. P., & Magness, R. R. (1982). Blood flow to the uterine and ovarian vascular beds of gilts during the estrous cycle or early pregnancy. *Biology of Reproduction*, *27*(4), 878–885. https://doi.org/10.1095/biolreprod27.4.878

Ghaffari, M. H., Schuh, K., Kuleš, J., Guillemin, N., Horvatić, A., Mrljak, V., Eckersall, P. D., Dusel, G., Koch, C., Sadri, H., & Sauerwein, H. (2020). Plasma proteomic profiling and pathway analysis of normal and over-conditioned dairy cows during the transition from late pregnancy to early lactation. *Journal of Dairy Science*, *103*(5), 4806–4821. https://doi.org/10.3168/jds.2019-17897

Gong, J., & Xiao, M. (2018). Effect of Organic Selenium Supplementation on Selenium Status, Oxidative Stress, and Antioxidant Status in Selenium-Adequate Dairy Cows During the Periparturient Period. *Biological Trace Element Research*, *186*(2), 430–440. https://doi.org/10.1007/s12011-018-1323-0

Kaneko et al.,Kaneko Kaneko, J., Harvey, J., & Bruss, M. (1997). *Clinical Biochemistry of Domestic Animals* (5th Edition.)

Kim-Egloff, C., Hässig, M., Bruckmaier, R., & Bleul, U. (2016). Doppler sonographic examination of uterine and placental perfusion in cows in the last month of gestation and effects of epidural anesthesia and isoxsuprine. *Theriogenology*, *85*(5), 986–998. https://doi.org/10.1016/j.theriogenology.2015.11.010

Konvičná, J., Vargová, M., Paulíková, I., Kováč, G., & Kostecká, Z. (2015). Oxidative stress and antioxidant status in dairy cows during prepartal and postpartal periods. *Acta Veterinaria Brno*, *84*(2), 133–140. https://doi.org/10.2754/avb201584020133

Liang, Y., Alharthi, A. S., Bucktrout, R., Elolimy, A. A., Lopreiato, V., Martinez-Cortés, I., Xu, C., Fernandez, C., Trevisi, E., & Loor, J. J. (2020). Body condition alters glutathione and nuclear factor erythroid 2-like 2 (NFE2L2)-related antioxidant network abundance in subcutaneous adipose tissue of periparturient Holstein cows. *Journal of Dairy Science*, *103*(7), 6439–6453. https://doi.org/10.3168/jds.2019-17813

Lykkesfeldt, J., & Svendsen, O. (2007). Oxidants and antioxidants in disease: Oxidative stress in farm animals. *Veterinary Journal*, *173*(3), 502–511. https://doi.org/10.1016/j.tvjl.2006.06.005

Magness, R. R., & Rosenfeld, C. R. (1989). The role of steroid hormones in the control of uterine blood flow. In C. R. Rosenfeld (Ed.), *Reproductive and perinatal medicine* (pp. 239–271). :X. Perinatology Press.

Mantovani, R., Sgorlon, S., Marinelli, L., Bailoni, L., Bittante, G., & Gabai, G. (2010). Oxidative stress indicators and metabolic adaptations in response to the omission of the dry period in dairy cows. *The Journal of Dairy Research*, *77*(3), 273–279. https://doi.org/10.1017/S0022029910000117

Panarace, M., Garnil, C., Marfil, M., Jauregui, G., Lagioia, J., Luther, E., & Medina, M. (2006). Transrectal Doppler sonography for evaluation of uterine blood flow throughout pregnancy in 13 cows. *Theriogenology*, *66*(9), 2113–2119. https://doi.org/10.1016/j.theriogenology.2006.03.040

Pinedo, P., Santos, J., Chebel, R. C., Galvão, K. N., Schuenemann, G. M., Bicalho, R. C., Gilbert, R. O., Rodriguez-Zas, S. L., Seabury, C. M., Rosa, G., & Thatcher, W. (2020). Associations of reproductive indices with fertility outcomes, milk yield, and survival in Holstein cows. *Journal of Dairy Science*, *103*(7), 6647–6660. https://doi.org/10.3168/jds.2019-17867

Propst, A. M., & Hill, J. A. (2000). Anatomic factors associated with recurrent pregnancy loss. *Seminars in Reproductive Medicine*, *18*(4), 341–350. https://doi.org/10.1055/s-2000-13723

Rosenfeld, C. R. (1980). Responses of reproductive and nonreproductive tissues to 17 beta-estradiol during ovine puerperium. *The American Journal of Physiology*, *239*(5), E333–E339. https://doi.org/10.1152/ajpendo.1980.239.5.E333

Rosenfeld, C. R., Morriss, F. H., Makowski, E. L., Meschia, G., & Battaglia, F. C. (1974). Circulatory changes in the reproductive tissues of ewes during pregnancy. *Gynecologic Investigation*, *5*(5–6), 252–268. https://doi.org/10.1159/000301658

Rosenfeld, C. R., & Naden, R. P. (1989). Uterine and nonuterine vascular responses to angiotensin II in ovine pregnancy. *The American Journal of Physiology*, *257*(1 Pt 2), H17–24. https://doi.org/10.1152/ajpheart.1989.257.1.H17

Trevisan, M., Browne, R., Ram, M., Muti, P., Freudenheim, J., Carosella, A. M., & Armstrong, D. (2001). Correlates of markers of oxidative status in the general population. *American Journal of Epidemiology*, *154*(4), 348–356. https://doi.org/10.1093/aje/154.4.348

van Dorland, H. A., Richter, S., Morel, I., Doherr, M. G., Castro, N., & Bruckmaier, R. M. (2009). Variation in hepatic regulation of metabolism during the dry period and in early lactation in dairy cows. *Journal of Dairy Science*, *92*(5), 1924–1940. https://doi.org/10.3168/jds.2008-1454

Varughese, E. E., Brar, P. S., & Dhindsa, S. S. (2013). Uterine blood flow during various stages of pregnancy in dairy buffaloes using transrectal Doppler ultrasonography. *Animal Reproduction Science*, *140*(1–2), 34–39. https://doi.org/10.1016/j.anireprosci.2013.05.011

Vittoria, A. (1997). Anatomy of the female genital tract in the buffalo. Bubalis bubalis, 4 (supp 1) . In *Third Course on Biotechnology of Reproduction in buffaloes* (Vol. 4 (1), pp. 15–20). Caserta, Italy.

Wankhade, P. R., Manimaran, A., Kumaresan, A., Jeyakumar, S., Ramesha, K. P., Sejian, V., Rajendran, D., & Varghese, M. R. (2017). Metabolic and immunological changes in transition dairy cows: A review. *Veterinary World*, *10*(11), 1367–1377. https://doi.org/10.14202/vetworld.2017.1367-1377

Wright, N., & Burns, B. (2020). Anatomy, abdomen and pelvis, posterior abdominal wall arteries. In *StatPearls*. Treasure Island (FL): StatPearls Publishing.

Žvorc, Z., Matijatko, V., Beer, B., Foršek, J., Bedrica, L., & Kučer, N. (2000). Blood serum proteinograms in pregnant and non-pregnant cows. *Veterinarski Arhiv*, *70*, 21–30.

**How to cite this article:** Elmetwally MA, Elshopakey GE, Eldomany W, et al. Uterine, vaginal and placental blood flows increase with dynamic changes in serum metabolic parameters and oxidative stress across gestation in buffaloes. *Reprod Dom Anim*. 2021;56:142–152. https://doi.org/10.1111/rda.13858