# Correlation between Uterine Hemodynamics, Sex Steroid Hormone Concentrations, and Enzymatic Antioxidant Levels in Postpartum Buffaloes

Elshymaa A. Abdelnaby[1], Khaled El-Shahat[1,*], Saber Abd-Allah[2] and Hossam R. El-Sherbiny[1]

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

[2]Theriogenology Department, Faculty of Veterinary Medicine, Beni-Suef University, Egypt

**Abstract:** The present investigation aimed to evaluate uterine hemodynamics in six multiparous postpartum buffaloes and their relationship with sex steroid hormone concentrations and enzymatic antioxidant levels. The buffaloes were examined by transrectal Doppler ultrasonography to record the vascular perfusion in uterine arteries of both ipsilateral and contralateral ones. All Doppler indices such as peak (PV) endpoints (EV) of velocity, peak systolic velocity (PSV), blood flow volume (BFV), resistance (RI), and pulsatility index (PI) were recorded from 1$^{st}$ to 6$^{th}$ postpartum. The blood samples were collected starting from 1st-week post-calving and every week thereafter following each ultrasound Doppler examination for assay of steroid hormones (progesterone and estradiol) and antioxidant (superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase, CAT) were measured. PV of the ipsilateral previously gravid arteries showed a linear pattern of significant (P=0.001) decline from the 1$^{st}$ week after parturition till the 6$^{th}$ week. This decline was also associated with a linear decrease in EV from the 1$^{st}$ week till the 6$^{th}$ week after birth. While contralateral PV and EV are not significantly changed throughout weeks after parturition. The levels of SOD and CAT are significantly elevated at 1$^{st}$ week postpartum compared to the 6$^{th}$ week after parturition. In contrast, the GPx levels did not reveal any significant differences during the puerperal period. Estradiol and progesterone declined from 1$^{st}$ to 5$^{th}$ week after parturition. PV of the ipsilateral uterine artery had a significant (P≤0.05) positive correlation with BFV (r=0.49), estradiol 17- α(r=0.98) and progesterone (r=0.85). The same parameter showed a statically (P≤ 0.001) positive correlation with SOD (r=0.87) and CAT (r=0.92). While, Ipsilateral uterine RI showed a significant (P≤0.05) negative correlation with PV(r=-0.85), BFR ((r=-0.62), estradiol 17- α(r=-0.52), and progesterone (r=-0.88), in addition, RI also correlated negatively with both SOD (r=-0.57) and CAT (r=-0.63). Progesterone and estrogen levels are strongly correlated with SOD and CAT. The uterine hemodynamics in buffaloes is affected by the day of the postpartum period. SOD and CAT antioxidants recorded herein, except GPx, increase in the 1$^{st}$ weeks of calving and are affected by the day of the postpartum period.

**Keywords:** Buffaloes, postpartum period, uterine blood flow, SOD, CAT, steroid hormones.

## 1. INTRODUCTION

Buffaloes play an essential role in the animal production of many developing countries and are considered the main source of meat and milk in Egypt. Pregnancy and lactation are major factors in occurring of oxidative stress in water buffaloes [1] and small ruminants [2]. Reactive oxygen and nitrogen species (ROS and RNS) are produced during oxidative stress. They consist of hydroxyl radicals (OH), superoxide ions, hydrogen peroxide ($H_2O$), and nitric oxide radicals (NO), which lead to lipid peroxidation, apoptosis, and infertility [3, 4]. The impact of ROS and RNS on fertility is mediated by influencing folliculogenesis, steroidogenesis, and retained placenta [5, 6]. The antioxidant defense system decreases the damaging effect of ROS by inhibiting it, thus preserving the animal's health [7-9]. This defense system is composed of two segments, enzymatic and non-enzymatic. Catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPX) are enzymatic antioxidants. At the same time, the non-enzymatic segments contain reduced glutathione (R-GSH), vitamin C, vitamin E, β- carotene, and various macro and microelements. In buffaloes, uterine hemodynamics is changed with the estrous cycle [10] and pregnancy [11]. The pro-oxidant and antioxidant balance underline animal reproductive efficiency. Therefore, the current investigation was conducted to investigate the correlation among changes in dynamics of uterine hemodynamics, concentrations of oxidants/ antioxidants, and steroid hormones profile in postpartum buffaloes, as well as to evaluate the influence of stress postpartum on concentrations of blood serum of antioxidants (catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPX).

## 2. MATERIAL AND METHODS

### 2.1. Ethical Committee Approval

The present study was carried out under the permission of the ethical committee of the Institutional

*Address correspondence to this author at the Theriogenology Department, Faculty of Veterinary Medicine, Cairo University, Giza Square, 12211, Egypt; Mob: 00201064688386; E-mail: attiakh@yahoo.com, attiakh67@cu.edu.eg

Animal Care and Use Committee (IACUC) of the Faculty of Science, Cairo University.

## 2.2. Experimental Animals, Feeding, and Design

### *2.2.1. Animals and Management*

The present investigation was conducted at the Faculty of Veterinary Medicine, Cairo University, Egypt (latitude 30°01″ N; longitude 31° 21′ E) during October –December 2020. Investigations were carried out on six multiparous buffalo cows, aged (4–7 years old) and with a body weight of 470–490 kg. Each animal was fed a commercial concentrate ration (16% crude protein) and *ad libitum* clover (*Trifolium alexandrinum*). Animals underwent a routine physical examination to make a confirmation on the animal health and reproductive status. Rectal and vaginal examinations were performed to exclude any reproductive disorders. Color Doppler sonography studies of the uterine arteries started in 1$^{st}$ week after the parturition and were followed by weekly examinations until the 6$^{th}$ week postpartum.

### 2.2.2. *Ultrasonography*

A trans-rectal pulsed-wave Doppler ultrasound scanner equipped with a 5-7.5 MHz linear-array trans-rectal transducer (Sonovet R3, Medison, Samsung, South Korea) with the linear probe was used to examine the vascularization of the uterine arteries by color and spectral modes. The ipsilateral uterine and ovarian artery to the previously gravid horn is considered the ipsilateral artery. On the other hand, the uterine and ovarian contralateral to the abovementioned gravid horn is the contralateral artery, as reported by [12]. The same analyzer traced The Doppler indices as Pulsatility index (PI) and resistance index (RI), Doppler velocity as peak systolic velocity (PSV), blood volume rate (BFR=blood flow rate, ml/m) were measured as previously reported by [13, 14].

### *2.2.3. Doppler Image Analysis*

The red and blue areas of Doppler images of color blood flow per pixel were determined and analyzed at each examination by using Adobe Photoshop CC software (1990-2013, Adobe Systems) as previously mentioned in dairy cows [15, 16].

### *2.2.4. Blood Sampling and Hormone Assay*

The blood samples were collected following each ultrasound Doppler examination and centrifuged at 3000 rpm for 10 minutes and sera were harvested and stored at -20ºC till further assay. Progesterone ($P_4$, EIA-1561) and estradiol 17-β ($E_2$ EIA-2693) were analyzed using ELISA commercial kits (DRG, Germany). The sensitivity of the assay was 0.045 ng/ml, and the test intra- and inter-precisions were 5.4 and 9.96 for P4. The sensitivity of the assay was 9.7pg/ml, and the test intra- and inter-precisions were 6.81 and 7.25 for E2, as previously measured by [17, 18].

### *2.2.5. Oxidants and Antioxidants Assay*

Superoxide dismutase level was estimated according to [15] using commercial colorimetric kits (Bio Diagnostics). SOD activity was expressed in μ/ml. Glutathione peroxidase was assessed spectrophotometrically according to the method of [19] using commercial colorimetric kits (Bio Diagnostics). Results were expressed as μ/ml. Catalase level was determined using commercial colorimetric kits (Bio Diagnostics, Giza, Egypt) according to the method of [20]. The catalase activity was expressed in μ/ml.

## 2.3. Statistical Analysis

The data were analyzed with SPSS (Version 18.0, SPSS Inc., Chicago, Illinois, 2010). Middle uterine arteries' blood flow pattern and antioxidant levels were measured by one-way ANOVA. Also, the Duncan range test was used. P values ≤ 0.001 were considered highly significant, and P values ≤ 0.05 were considered significant. Pearson correlations between middle uterine arteries' blood flow pattern, enzymatic antioxidant levels, and hormonal measurements were used to determine the positive and negative connection between parameters.

## 3. RESULTS

### 3.1. Vascularity Pattern of Middle Uterine Arteries

As depicted in (Table **1**), ipsilateral uterine arteries PV (cm/sec) showed a linear pattern of significant (P=0.001) decline from the 1$^{st}$ week after parturition (24.32±0.23) till the 6$^{th}$ week (17.34±0.12) and this decline also associated with a linear decrease in EV (cm/sec) from the 1$^{st}$ week till the 6$^{th}$ week after birth (5.96±0.22 to 3.61±0.04) while contralateral PV and EV are not changed throughout weeks after parturition.

### 3.2. Enzymatic Antioxidants and Steroids Hormonal Levels in Postpartum Buffaloes

The levels of SOD and CAT are significantly elevated at 1$^{st}$ week postpartum than those estimated at the 6$^{th}$ week after parturition (47.21±2.25 vs.

**Table 1: Middle Uterine Arteries (Ipsilateral and Contralateral) Blood Flow Pattern during the Postpartum Period in Buffaloes**

| Weeks after parturition | Ipsi PV (cm/sec) | Ipsi EV (cm/sec) | Contra PV (cm/sec) | Contra EV (cm/sec) |
|---|---|---|---|---|
| $1^{st}$ week | $24.32 \pm 0.23^{d}$ | $5.96 \pm 0.22^{d}$ | 22.42±0.04 | 4.22±0.05 |
| $2^{nd}$ week | $22.44 \pm 0.42^{c}$ | $5.31 \pm 0.24^{c}$ | 22.44±0.47 | 4.69±0.47 |
| $3^{rd}$ week | $19.90 \pm 0.52^{b}$ | $4.34 \pm 0.07^{b}$ | 21.90±0.55 | 4.14±0.18 |
| $4^{th}$ week | $17.94 \pm 0.54^{a}$ | $3.83 \pm 0.16^{ab}$ | 22.54±0.47 | 4.18±0.41 |
| $5^{th}$ week | $17.32 \pm 0.33^{a}$ | $3.65 \pm 0.21^{a}$ | 22.14±0.47 | 4.68±0.05 |
| $6^{th}$ week | $17.34 \pm 0.12^{a}$ | $3.61 \pm 0.04^{a}$ | 21.82±0.11 | 4.99±0.11 |
| P-value | 0.001 | 0.001 | 0.587 | 0.235 |

Data are expressed as mean ±standard error of the mean (SEM). Means with (a, b, and c) superscripts within a column are significantly different at $P<0.05$.

35.26±2.81 U/ml, respectively for SOD) and (511.21±8.27 vs. 454.17±19.91 U/ml, respectively for CAT). In contrast, the GPx levels s did not reveal any significant differences during the puerperal period (Table **2**). Estradiol and progesterone declined from $1^{st}$ week to $5^{th}$ week after partition, as shown in (Figure **1**).

### 3.3. Pearson Correlations between Middle Uterine Arteries Blood Flow Pattern, Enzymatic Antioxidants Levels, and Hormonal Measurements

The ipsilateral uterine artery PI showed statistically significant (P≤ 0.001) strong negative correlation with PV(r=-0.64), BFR ((r=-0.58), estradiol 17- α(r=-0.69),

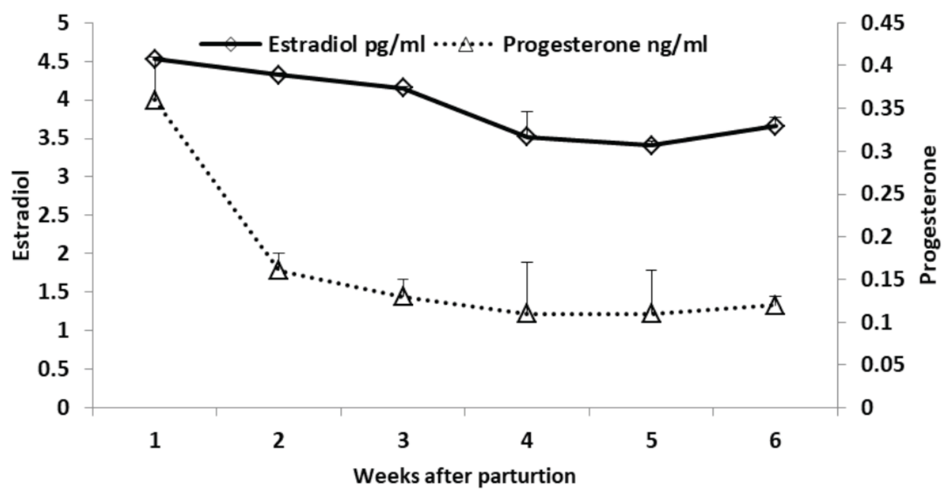


**Figure 1:** Mean ± SE (n=6) estradiol and progesterone levels in postpartum buffaloes.

**Table 2: Enzymatic Antioxidants Levels Expressed by Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx) during the Postpartum Period in Buffaloes**

| Weeks after parturition | SOD U/mL | CAT U/mL | GPx U/L |
|---|---|---|---|
| 1st week | $48.56 \pm 1.25^{c}$ | $511.21 \pm 8.27^{b}$ | 0.52±0.04 |
| 2nd week | $48.78 \pm 1.46^{c}$ | $508.25 \pm 3.25^{b}$ | 0.56±0.03 |
| 3rd week | $47.21 \pm 2.25^{c}$ | $506.69 \pm 9.05^{b}$ | 0.48±0.01 |
| 4th week | $43.07 \pm 2.01^{bc}$ | $501.5 \pm 3.44^{b}$ | 0.46±0.02 |
| 5th week | $38.19 \pm 2.39^{ab}$ | $458.21 \pm 16.68^{a}$ | 0.45±0.01 |
| 6th week | $35.26 \pm 2.81^{a}$ | $454.17 \pm 19.91^{a}$ | 0.54±0.03 |
| P-value | 0.002 | 0.008 | 0.372 |

Data are expressed as mean ±standard error of the mean (SEM). Means with (a, b, and c) superscripts within a column are significantly different at $P<0.05$.

**Table 3: Pearson Correlations between Middle Uterine Arteries Blood Flow Pattern, Enzymatic Antioxidants Levels, and Hormonal Measurements during the Postpartum Period in Buffaloes**

| Parameter | Ipsi U. RI | Ipsi PV | Ipsi BFR | E2 | P4 | SOD | CAT | GPx |
|---|---|---|---|---|---|---|---|---|
| Ipsi U. PI | 0.652* | -0.64** | -0.58** | -0.69** | -0.94** | -0.48* | -0.85* | 0.49 |
| Ipsi U. RI | 1 | -0.85* | -0.62* | -0.52* | -0.88* | -0.57* | -0.63* | 0.32 |
| Ipsi PV | | 1 | 0.49* | 0.98* | 0.85* | 0.87** | 0.92** | 0.25 |
| Ipsi BFR | | | 1 | 0.86* | 0.55* | 0.76 | 0.62 | 0.44 |
| E2 | | | | 1 | 0.66* | 0.63** | 0.48** | 0.68 |
| P4 | | | | | 1 | 0.88** | 0.96** | 0.82 |
| SOD | | | | | | 1 | 0.85* | 0.69 |
| CAT | | | | | | | 1 | 0.22 |
| GPx | | | | | | | | 1 |

** Means correlation is significant at $P<0.001$, * means the correlation is significant at $P<0.05$.
Ipsi =ipsilateral, U=uterine, PI=pulsatility index, RI=resistive index, BFR=blood flow rate (bpm), PV= peak velocity of contraction, E2=estradiol 17α, P4=progesterone, SOD=superoxide- dismutase, CAT=catalase, and GPx=Glutathione peroxidase.

progesterone (r=-0.94), SOD (r=-0.48), and CAT (r=-0.85), while there was a positive correlation between PI and between PI and RI (r=0.652). RI of Ipsilateral uterine had a significant (P≤0.05) negative correlation with PV(r=-0.85), BFR ((r=-0.62), estradiol 17- α(r=-0.52), and progesterone (r=-0.88), in addition, RI also correlated negatively with both SOD (r=-0.57) and CAT (r=-0.63) as illustrated in (Table **3**). PV of the ipsilateral uterine artery had a significant (P≤0.05) positive correlation with BFV (r=0.49), estradiol 17- α(r=0.98) and progesterone (r=0.85), but the same parameter showed a statically (P≤ 0.001) positive correlation with SOD (r=0.87), and CAT (r=0.92). In the case of ipsilateral uterine BFR, a positive significant (P≤0.05) correlation existed between BFR and estradiol 17-α(r=0.86) and between BFR and progesterone (r=0.55). No correlation has existed between BFR and antioxidant levels. Estradiol17α levels showed a pattern of statistically significant (P≤ 0.001) positive correlation with SOD (r=0.63), and CAT (r=0.48), as well as E2 correlates positively with progesterone levels (r=0.66; P<0.05). In addition to estradiol alterations during this stage, progesterone levels had a positive correlation with SOD (r=0.88; P<0.001) and CAT (r=0.96; P<0.001), while on the contralateral side, neither positive nor negative correlation was detected.

## 4. DISCUSSION

The present investigation aimed to determine, for the first time, the correlation between antioxidant levels and uterine Doppler parameters during the postpartum period in buffaloes. During the postpartum period, many changes occur in the blood flow pattern as now the blood supply requests of pregnancy are no longer needed. This process involves many changes in the middle uterine artery due to wall changes and thrombosis, which results in a decrease in the diameter of the main artery [21] that is associated with a marked decline in the peak velocity point due to the maximum contraction of the artery. Moreover, this process leads to a linear decrease in the end-diastolic notch that is associated with the cardiac cycle in the spectral graph by Doppler analysis [22]. All those changes were accompanied by an elevation of the vascular resistive index that was inversely correlated with all blood flow velocities parameters [23, 24]. However, those found in this current study may be reflected in the general circulatory alterations [25], with the presence of mechanical pressure forced by the tense postpartum myometrium [26, 27]. Similarly, it is noticed that in the first two months postpartum at the stage of uterine involution, there was a significant decline in both Doppler velocities that were associated with an elevation in both UtA-PI and RI [28]. Like present research findings, in anestrus cows, both uterine arteries RI and PI increased when compared to normal cyclic cows [22], and the increase in both indices correlates negatively with the amount of blood supply to the genital tract [15, 17, 29]. The same finding was recorded during the first month of postpartum cows; there was the same increase in Doppler indices which are negatively correlated with estradiol and progesterone levels [30]. As recognized, estrogen hormones have a vasodilator action on all genital vessels leading to an increase the uterine circulation [31], and the levels of estradiol are decreased gradually after the end of parturition [32]. Also, there was a decline in progesterone levels [33] in the postpartum buffalos. The postpartum period in the current study is characterized by a marked increase in the enzymatic antioxidant levels of SOD and CAT at 1st week postpartum than those recorded at the 6th-week pp. This agrees with [34], who reported that the early puerperium is characterized by an imbalance between pro and antioxidants (oxidative stress) that lead production of reactive oxygen species (ROS). This could be one of the main reasons for the incidence of many diseases after parturition that could adversely affect milk production [35, 36]. The higher serum SOD activity in the first weeks of postpartum buffaloes herein indicates higher oxidative stress, which was probably caused by a response of the organism to higher superoxide generation, especially after calving. A similar finding was observed in dairy cows, where the SOD activity increases after calving in dairy cows [37]. In our study, there is a reduction in GSH-Px activity at 1st-week post-calving. A similar finding was recorded by [38], who reported that the decline of GSH-Px levels in dairy cows in 1st week after parturition might attribute to a loss of homeostatic control in the postpartum period. At calving, SOD and GSH-Px enzymes represent the major antioxidant defense components in protecting the cells against increased ROS. Superoxide dismutase converts the superoxide radicals to hydrogen peroxide, which is further metabolized to water by the GSH-Px enzyme. The SOD activity is higher on the day of parturition; this is maybe indicating higher oxidative stress and lower antioxidant status [37]. In postpartum buffaloes, there is a marked elevation in lipid peroxidation with a marked linear decline in the enzymatic antioxidants [39]. This could be since, in the postpartum period with early lactation, the increasing demand for some micronutrients doesn't fill the deficiency due to the presence of reactive metabolites (ROMs) that adversely lead to a decline in the levels of antioxidants as SOD and CAT [40].This decline in the levels of antioxidants correlates positively with ipsilateral uterine peak velocity, which may be due to the increase in stress during this period .another study following our result [41] evaluated the levels of enzymatic antioxidants, especially SOD and CAT, and they observed a marked decline in both antioxidants which associated with a marked elevation in oxidative stress. In cows, [42]concluded that the levels of antioxidants are significantly increased at estrus rather than in the postpartum period. The present findings confirm that the negative correlation between Doppler indices and peak velocity at the first six weeks of puerperium is like those results in cows suffering from anestrum related to decreasing the uterus circulation [22]. The negative correlation between Doppler indices and BFV was also determined in women's uterine vascularization [43]. In contrast to our findings regarding no correlation between blood flow and Doppler indices on the contralateral side, a study reported a weak correlation between both parameters [44]. In conclusion, the vasculature of the uterus recorded in buffaloes is significantly affected by the day of the postpartum period. SOD and CAT antioxidants were increased in the present study, except GPx, at the 1st week of calving and are affected by the day of the postpartum period. This indicates buffaloes are suffering from stress which may be because of production or reproduction.

## DATA AVAILABILITY

Data sharing is not applicable.

## DECLARATION OF INTEREST

All authors declare any conflict of interest.

## AUTHORSHIP CONTRIBUTION STATEMENT

Elshymaa A. Abdelnaby: Data curation, Formal analysis, Investigation, Methodology, Writing-review & editing. Khaled. H. EL-Shahat, methodology, writing the draft and supervising, Hossam R. El-Sherbiny: Methodology, analyzing hormones and enzymatic antioxidants, and involved in manuscript writing.

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Received on 07-07-2022 Accepted on 22-09-2022 Published on 01-11-2022

https://doi.org/10.6000/1927-520X.2022.11.07