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Associations between metabolic profiles, post-partum delayed resumption of ovarian function and reproductive performance in Egyptian buffalo: Roles of IGF-1 and antioxidants

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ABSTRACT

This study was conducted on 47 pluriparous pregnant Egyptian buffalo. Body condition score (BCS) was classified and blood samples were collected pre-partum and post-partum for estimation of IGF-1, hormonal, metabolic and antioxidants values. There was palpation per rectum and ultrasonography in addition to quantitation of progesterone (P_4) and estradiol-17β (E-17β) for monitoring post-partum ovarian resumption. Reproductive indices were calculated 60, 90, 120 and 150 days post-partum. Based on the concentrations of P_4 and E-17β, buffalo were divided into ovulatory and non-ovulatory groups. The P_4 and E-17β were greater ($P < 0.001$) in ovulatory compared to non-ovulatory buffalo. The BCS and IGF-1 post-partum were greater ($P = 0.024$; 0.001, respectively) in ovulatory than non-ovulatory buffalo. Glucose and albumin were greater during pre- ($P < 0.001$; 0.013) and post-partum ($P = 0.005$; 0.003) periods in ovulatory than non-ovulatory buffalo. Post-partum, NEFA and BHBA concentrations were greater ($P < 0.001$) in non-ovulatory than ovulatory buffalo. The BUN concentrations were greater ($P = 0.002$) in non-ovulatory buffalo during pre- and post-partum periods. There were differences in GSH and SOD concentrations between groups ($P < 0.001$; 0.002, respectively). The BCS, albumin, IGF-1, GSH and SOD concentrations post-partum were negatively correlated with the delay of post-partum ovulation. The post-partum NEFA and BHBA concentrations, however, were positively correlated with delayed post-partum ovulation. Ovulatory buffalo had fewer ($P < 0.01$) days non-pregnant and for calving intervals as well as greater pregnancy rates than non-ovulatory buffalo. In conclusion, buffalo with delayed post-partum ovarian resumption were prone to have negative energy balance.

1. Introduction

The bubaline species is prevalent over all continents and is reared mainly in developing countries (Arrighi et al., 2014). Buffalo have unique physiological adaptations that are different than that in cattle, particularly with regard to adaptations in hot

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environmental conditions (Ørskov, 2007). Buffalo, however, have a markedly lesser fertility than cattle (Berdugo-Gutiérrez et al., 2017) due to later ages at puberty, ovulations occurring in the absence of behavioral estrus (Kumar et al., 2013) as well as a greater incidence of anestrum (Kumar et al., 2014). There needs to be more attention, therefore, in developing countries for understanding the physiological adaptations of buffalo (Abd Ellah et al., 2013; Fiore et al., 2017) so as to optimize values for reproductive physiological variables and productivity (Nanda et al., 2003).

After calving, there are great nutritional demands for milk production during a time period when there is less dry matter intake (Drackley, 1999; Sundrum, 2015). During this period, several endocrine, immune and metabolic pathways are functionally altered so that physiological adjustments occur that frequently result in negative energy balance (NEB) (Pande et al., 2016). These alterations in metabolic pathways that occur for adaptation to NEB adversely affect reproductive efficiency and subsequently resulted in economic losses (Panda et al., 2006; Khan et al., 2011; Kalasariya et al., 2017).

The increase in metabolic activities during the peri-partum period is accompanied by modification of animal's energetic status (Fiore et al., 2018) which may lead to generation of reactive oxygen species and minimized antioxidant reserves (Trevisan et al., 2001). Additionally, oxidative stress during the peri-parturient period contributes to some metabolic disorders (Adela et al., 2006) as well as infertility due to impairments in steroidogenesis and folliculogenesis (Ghosh et al., 2015). Furthermore, the oxidative stress has deleterious effects on buffalo gametes (Khalil et al., 2013; Lin et al., 2019) as well as embryo development causing the degeneration and apoptosis of these cells (Sadeesh et al., 2016; Lin et al., 2019). There are several reports in which the usage of antioxidant supplementations is recommended for improvement in gamete quality (Longobardi et al., 2017; Gad et al., 2018) and embryonic development in buffalo (Manjunatha et al., 2009; Zullo et al., 2016; An et al., 2019). The assessment of antioxidant compound concentrations has become a complementary technique for the assessment of metabolic status (Castillo et al., 2005) and consequently reproductive status (Ghosh et al., 2015).

A healthy post-partum buffalo undergo complete uterine involution, have ovulations from a dominant follicle that develop early in the post-partum interval and have estrous cycles of typical length for the species, accompanied with homeostatic concentrations of insulin and insulin-like growth factor-1 (IGF-1) (Roche, 2006; Bakr et al., 2015). Insulin-like growth factor-1 is a primary endocrine factor in regulation of NEB as well as being an integral factor associated with reproductive disorders (Rhoads et al., 2008). An integral factor related to reproductive performance in the post-partum period is early resumption of ovarian functions (i.e., follicular development to the dominant state and ovulation) (Jainudeen et al., 1983). Usually NEB is the causal factor associated with longer post-partum periods of anestrus. Furthermore, relatively greater serum concentrations of non-esterified fatty acids (NEFA) that result from NEB have a negative effect on uterine immunity and ovarian functions; either steroidogenesis (Roche et al., 2000) and/ or folliculogenesis (Vanholder et al., 2006). Also relatively greater concentrations of NEFA are associated with lipolysis when there is a NEB, severely disrupting the IGF-1 hepatic synthesis that leads to decreased ovarian follicular development and ultimately ovulations (Hussein et al., 2013; Dupont et al., 2014; Ramoun, 2016).

The aim of the current study is to investigate the concentrations of IGF-1, metabolic profiles and antioxidant markers before and after calving and the associations with the incidence of delayed post-partum ovarian resumption and reproductive performance in pluriparous Egyptian buffalo.

2. Material and methods

2.1. Animals

A total of 47 pluriparous pregnant Egyptian buffalo were used in the study. Buffalo were examined regularly by veterinarians. All the experimental buffalo were apparently clinically healthy. Additionally, fecal, blood samples as well as skin scrapes were collected to ensure that the experimental buffalo did not have external and internal parasite infestations. The animals were maintained in an open paddock and were managed similarly. All animals were fed a diet that fullfilled their nutritional recommendations of the NRC (1978). Buffalo were manually-milked twice daily. The mean 305 day milk yield of buffalo in the previous lactation was 1540.9 ± 43.6 kg. They were calved in the autumn season. The animals were owned by the Veterinary Educational Farm, Suez Canal University, Egypt. Ethics and Animal Experimentation Committee of Suez Canal University approved for all procedures of the study with regestration number (2019005).

2.2. Blood sampling

Duplicate jugular vein blood samples were collected at 4 (W-4), 3 (W-3), 2 (W-2) and 1 (W-1) weeks pre-partum and also at 1 (W1), 2 (W2), 3 (W3), 4 (W4), 5 (W5), 6 (W6) and 7 (W7) weeks post-partum. One blood sample was collected in a tube containing Na flouride for glucose quantification and the other in a tube that did not contain reagents. Blood collected in these tubes were left to clot then centrofugation occured at 3000 rpm for 15 min for sera separation. The harvested sera were stored at -80 ˚C until there was analysis for concentrations of hormones, as well as metabolic and antioxidant compounds.

2.3. Reproductive hormones and resumption of ovarian functions

Palpation per rectum and ultrasonography examination (Honda Electronics Co., Japan) in addition to estimation of serum progesterone (P_4) and estradiol 17-β (E-17β) were performed for monitoring of post-partum resumption of ovarian functions such as follicular development and ovulations. Serum P_4 and E-17β were measured at W2 (12.74 ± 0.39 days), W3 (19.33 ± 0.28 days),

W4 (26.81 ± 0.24 days), W5 (33.37 ± 0.32 days), W6 (38.41 ± 0.39 days) and W7 (45.85 ± 0.41 days) post-partum using bovine enzyme linked immunosorbent assay (ELISA) kits (MyBiosource, USA).

Luteal function was considered to have been initiated when there was detection of serum P_4 concentrations of ≥1.0 ng/ml (Palta and Madan, 1995). Buffalo with serum P_4 concentrations of < 1.0 ng/ml were considered to be either in the follicular phase of the estrous cycle or there had not been resumped estrous cycles during the post-partum period. Ovulation was considered to have occured 5 days before the day of detection first outset of circulating P_4 ≥1.0 ng/ml (Usmani et al., 2001). Normal resumption of post-partum ovarian functions occurred when buffalo had ovulations during the period of ≤45 days post-partum while there was considered to be a delay in resumption of ovarian function when there was not an ovulation until > 45 days after calving (Shrestha et al., 2004).

2.4. Body condition score (BCS)

Records of BCS were assessed at W-4, W-3, W-2, W-1, W1, W2, W3, W4, W5, W6 and W7 using the methods previously described by Ezenwa et al. (2009). The scale of BCS classifications ranged from 1 to 5 with 0.25 increments.

2.5. Insulin-like growth factor-1

Bovine ELISA kit was used for assessement of circulating IGF-1 (Chongqing Biospes Co., China). Serum concentrations of IGF-1 were estimated at W-4, W-3, W-2, W-1, W1, W2, W3, W4, W5, W6 and W7 post-partum according to instructions of the manufacturer.

2.6. Biochemical analysis

Plasma glucose concentrations were quantified using a caloremetric estimation kit (Diamond Diagnostic Co., Egypt). Serum concentrations of NEFA (Wako Co., Japan), beta-hydroxybutyric acid (BHBA) (Biospes Co., China), albumin (Spectrum Co., Germany) and blood urea nitrogen (BUN) (Diamond Co., Egypt) were quantified using spectrophotometeric procedures (LT-291 LABTRONICS, India) according to manufacturers' protocols.

Serum concentrations of antioxidant markers, reduced glutathione (GSH) and superoxide dismutase (SOD), were calorimetrically assayed (BioAssay Systems Co., USA). The procedures were performed as described in the pamphlet provided by the manufacturer of the assay materials.

2.7. Assessement of reproductive performance

Buffalo that expressed symptoms of behavioral estrus were naturally mated by superior proven sire. Days non-pregenant (the interval from calving to pregnancy) and pregnancy rate were recorded. Pregnancy was diagnosed by rectal palpation and ultrasonography (Honda Electronics Co., Japan) at 60, 90, 120 and 150 days post-partum. The interval between two successive calvings was recorded to calculate the calving interval for each buffalo.

2.8. Statistical analysis

Repeated measures of variance (IBM SPSS software computer program version 20, USA) was used for analysis of BCS and concentrations of IGF-1, glucose, NEFA, BHBA, albumin, BUN, GSH and SOD in ovulatory and non-ovulatory buffalo during the different weeks of the peri-parturient period. Comparision between groups for BCS, IGF-1 concentrations, blood metabolic profiles and concentrations of antioxidant biomarkers during the pre- and post-partum periods were conducted using the *t*-test as well as the differences between buffalo in days non-pregnant were analyzed using Graphpad prism software (version 7, San Diego, USA). Additionally, correlations between values for BCS, IGF-1 concentrations, metabolic biomarker concentrations and antioxidant biomarker concentrations with delayed post-partum ovarian resumption were assessed using Pearson correlation coefficients. The obtained data are presented as means ± SEMs. Significance was designated at a probability value $P < 0.05$.

3. Results

3.1. Classification of buffalo based on P4 and E-17β concentrations

Based on the circulating concentrations of P_4 and E-17β post-partum, buffalo were divided into two groups: an ovulatory group (63.83%, $n = 30$) that had normal resumption of ovarian functions (i.e., dominant follicle development and ovulation) and a non-ovulatory group (36.17%, $n = 17$) that had delayed resumption of ovarian functions. Serum concentrations of P_4 and E-17β were greater ($P = 0.001$ and $P < 0.001$) in ovulatory as compared to non-ovulatory buffalo. Furthermore, there was an interaction between group and week ($P < 0.001$ and $P < 0.001$, respectively; Fig. 1A, B).

3.2. BCS, IGF-1, metabolites and antioxidants

The overall mean of values are reported in Table 1 for BCS, IGF-1, metabolic biomarkers as well as antioxidant markers concentrations in ovulatory and non-ovulatory buffalo at the pre- and post-partum weeks. Post-partum BCS was greater ($P = 0.024$;

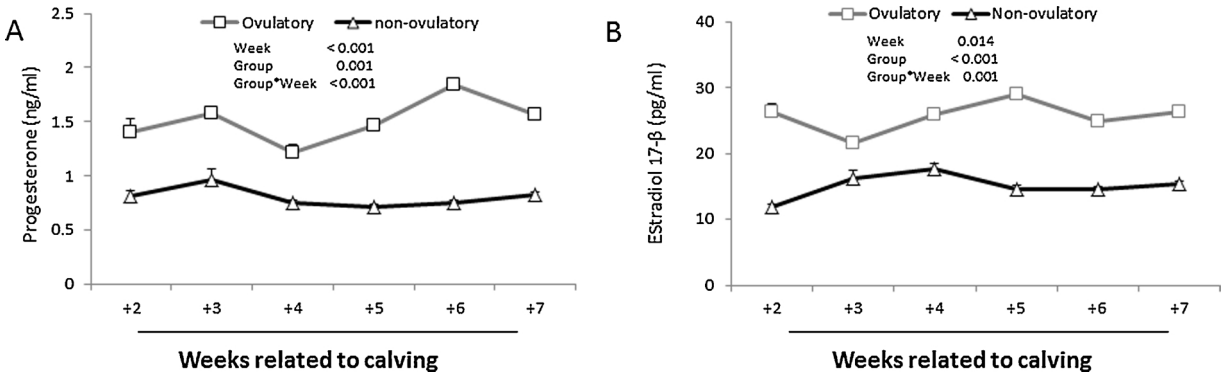

Fig. 1. Serum concentrations of A) progesterone (ng/m) and B) estradiol-17β (pg/ml) in ovulatory (n = 30) and non-ovulatory buffalo (n = 17) from W2 until W7 post-partum; Statistical analyses were conducted separately for pre- and post-partum weeks in both groups, weeks around calving and the interaction between groups and weeks relative to calving.

Table 1
Total overall means ± SE of BCS, metabolic profiles, IGF-1 and antioxidants in ovulatory and non-ovulatory buffalo in pre- and post-partum periods.

Variables	Pre-partum		Post-partum	
	Ovulatory	Non-ovulatory	Ovulatory	Non-ovulatory
BCS	3.19 ± 0.03	3.08 ± 0.02	3.15 ± 0.04*	3.03 ± 0.03
IGF-1 (μg/l)	80.98 ± 7.25	96.32 ± 8.35	132.20 ± 4.89***	69.18 ± 4.88
Glucose	61.21 ± 0.96***	54.36 ± 0.83	65.76 ± 0.64**	61.30 ± 0.73
NEFA (mlEq/l)	0.48 ± 0.02	0.47 ± 0.03	0.40 ± 0.01	0.64 ± 0.03***
BHBA (mg/ dl)	55.64 ± 3.56	60.20 ± 3.83	46.18 ± 2.73	90.69 ± 8.21***
Albumin (g/dl)	3.59 ± 0.06**	3.39 ± 0.03	3.68 ± 0.02**	3.58 ± 0.03
BUN (mg/dl)	49.40 ± 0.97	51.33 ± 1.18**	54.19 ± 0.90	59.60 ± 0.60**
GSH (μMol/l)	17.07 ± 0.84	18.46 ± 0.97	20.30 ± 0.13***	17.05 ± 0.55
SOD (IU/ml)	7.10 ± 0.03	7.12 ± 0.03	6.54 ± 0.03**	6.27 ± 0.01

Statistical analyses were performed separately between ovulatory and non-ovulatory buffalo during pre- and post-partum periods using a $t-$test; Different superscripts between rows indicate differences $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Fig. 2A) in ovulatory than non-ovulatory buffalo, while there tended to be an increase in BCS during the pre-partum period in the ovulatory group ($P = 0.060$). There was an interaction for BCS between group and week ($P = 0.011$; Fig. 2A) during the post-partum period only.

Serum IGF-1 concentrations of ovulatory buffalo were different ($P < 0.001$; Fig. 2B) compared with those of non-ovulatory buffalo during post-partum period and there was a group x week interaction ($P = 0.021$). During the pre-partum period there were no differences in IGF-1 concentrations between or an interaction among values between groups.

There were great concentrations of glucose in the ovulatory group (Fig. 2C) during the pre- and post-partum periods ($P < 0.001$ and $P < 0.01$, respectively). There was no group x week interaction during pre- nor post-partum periods.

Serum NEFA concentrations were different in non-ovulatory than ovulatory buffalo during post-partum period ($P < 0.001$; Fig. 2D). There, however, were no differences during the pre-partum period in NEFA concentrations between groups. For NEFA concentrations, during the pre- and post-partum periods there was a group x week interaction ($P = 0.027$ and $P < 0.001$, respectively; Fig. 2D).

The BHBA concentrations were greater ($P < 0.001$; Fig. 2E) post-partum in non-ovulatory than ovulatory buffalo with there being no differences between groups during the pre-partum period ($P = 0.660$). There, however, was a group x week interaction for BHBA concentrations during pre- and post-partum periods ($P = 0.039$ and $P < 0.05$, respectively; Fig. 2E).

Albumin concentrations during the pre- and post-partum periods in ovulatory buffalo were different ($P = 0.013$ and $P = 0.003$, respectively; Fig. 3A) compared with the non-ovulatory group. Furthermore, there was an interaction for albumin concentrations between group and week during pre- and post-partum periods ($P = 0.001$ and $P = 0.041$, respectively; Fig. 3A).

There were greater ($P = 0.002$; Fig. 3B) serum concentrations of BUN in non-ovulatory than ovulatory buffalo during the pre- and post-partum periods. There was a similar trend for BUN concentrations with there being a group x week interaction during the pre- and post-partum periods ($P = 0.013$ and $P = 0.025$, respectively; Fig. 3B).

Concentrations of post-partum antioxidants markers, GSH and SOD, were different between groups ($P < 0.001$ and $P = 0.002$, respectively; Fig. 3C, D). There were no differences in concentrations of these antioxidants biomarkers between groups in the pre-partum period. Furthermore, there was no group x week interaction in concentrations of these biomarkers during the pre- and post-partum periods.

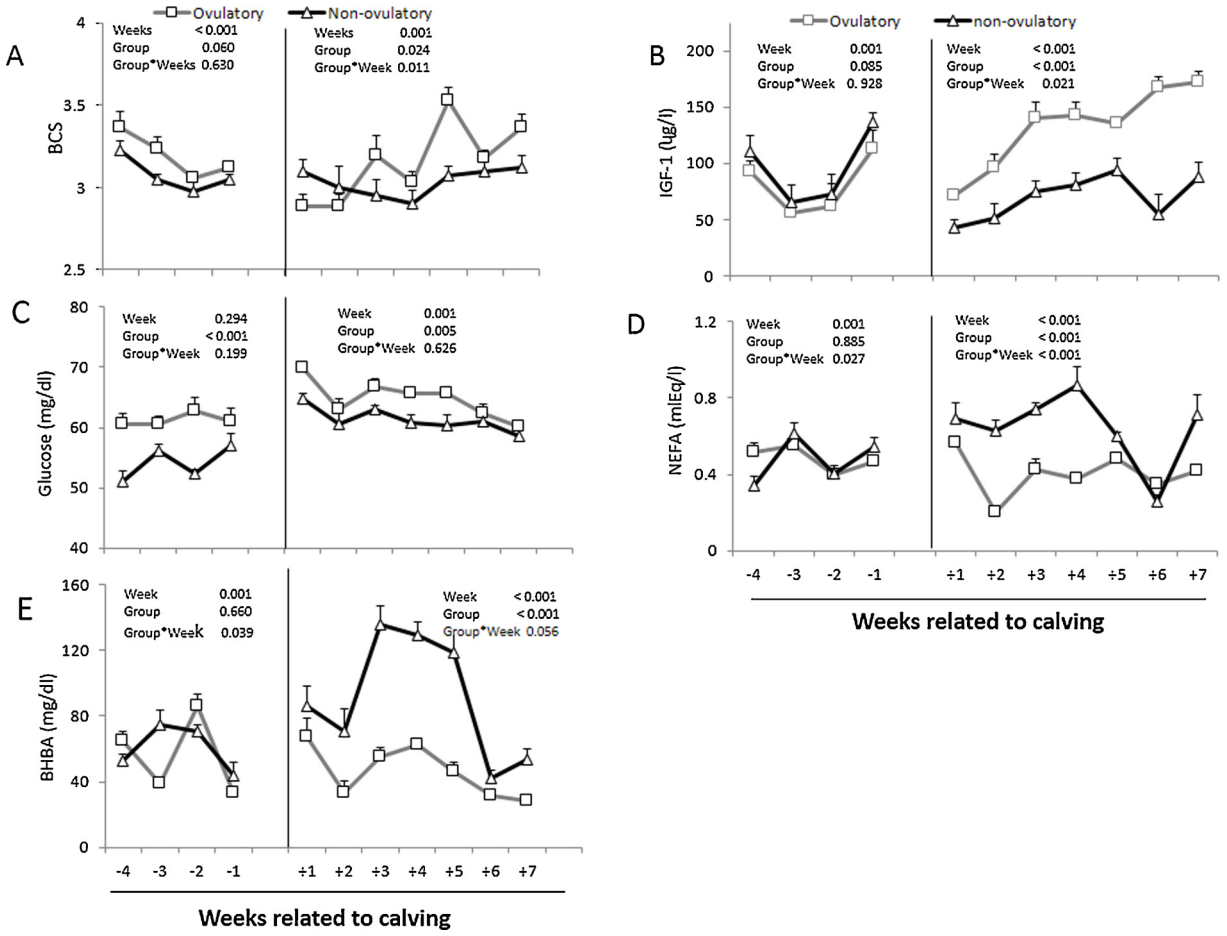

Fig. 2. A) Body condition score, B) serum IGF-1 (µg/l) and blood metabolites concentrations; C) glucose (mg/d), D) NEFA (mlEq/l) and E) BHBA (mg/dl) in ovulatory ($n = 30$) and non-ovulatory buffalo ($n = 17$) during the peri-parturient period from W-4 pre-partum until W7 post-partum; Statistical analyses were conducted separately for pre- and post-partum weeks in both groups, weeks around calving and the interaction between groups and weeks relative to calving.

3.3. Correlations of BCS, metabolites, IGF-1 and antioxidants with delayed post-partum ovulation

Pre-partum BHBA was positively correlated ($P < 0.001$) while, albumin was negatively correlated ($P < 0.01$) with delayed post-partum ovulation of buffalo. Post-partum BCS, as well as IGF-1, albumin, GSH and SOD concentrations, however, were negatively correlated with delayed post-partum ovulation. Post-partum NEFA, BHBA and BUN concentrations, however, were positively associated with the non-ovulatory status (Table 2).

3.4. Reproductive indices in ovulatory and non-ovulatory buffalo

Ovulatory buffalo had fewer ($P < 0.01$ and $P < 0.05$) days non-pregnant and lesser calving intervals than non-ovulatory animals. In addition, pregnancy rates were greater in ovulatory than non-ovulatory buffalo (Table 3).

4. Discussion

Metabolic status and BCS during the peri-partum period are important indicators of several physiological adaptations that affect animal reproduction. Studying these metabolic effects on buffalo reproduction is important for prediction of fertility and productivity of buffalo. Data from the present study indicate ovulatory buffalo had a markedly greater BCS than the non-ovulatory group. Body condition scoring provides information about the interaction between body fat reserves and animal appearance thus it is related to energy balance (Garnsworthy, 1988). The greater BCS of ovulatory buffalo provided insights about the energetic status that was positively reflected in the post-partum reproductive performance of these buffalo. This information was augmented by there being positive correlation between post-partum resumption of ovarian functions and BCS at W5 and W7. Results of the present study are consistent with those of Raj et al. (2016) and Patel et al. (2018).

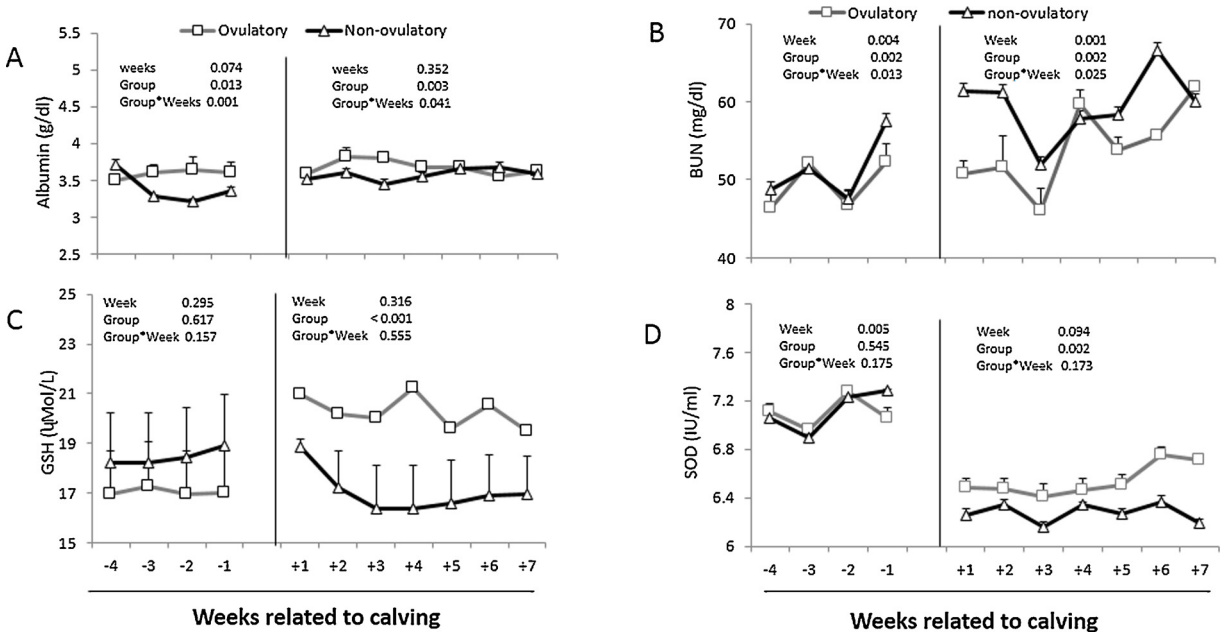

Fig. 3. Serum concentrations (mean ± SEM) of A) albumin (g/d), B) BUN (mg/dl), C) GSH (µMol/L) and D) SOD (IU/ml) in ovulatory (n = 30) and non-ovulatory (n = 17) buffalo during the peri-parturient period from W-4 pre-partum until W7 post-partum; Statistical analyses were conducted separately for pre- and post-partum weeks in both groups, weeks around calving and the interaction between groups and weeks relative to calving.

Table 2
Pearson correlation coefficients between values for BCS, metabolic variables, IGF-1 and antioxidants in buffalo that were classified as ovulatory and non-ovulatory during the pre- and post-partum periods.

Variables	Pre-partum weeks				Post-partum weeks					
	W-3		W-1		W3		W5		W7	
	r	*P*	r	*P*	r	*P*	r	*P*	r	*P*
BCS	−0.3	NS	−0.3	NS	−0.26	NS	−0.6	0.001	−0.4	0.05
IGF-1	0.1	NS	0.2	NS	−0.5	0.01	−0.7	0.001	−0.7	0.001
Glucose	−0.4	0.05	−0.2	NS	−0.4	NS	−0.4	0.05	−0.1	NS
NEFA	0.2	NS	0.2	NS	0.6	0.001	0.6	0.01	0.5	0.05
BHBA	0.7	0.001	0.3	NS	0.5	0.01	0.6	0.001	0.6	0.001
Albumin	−0.6	0.01	−0.4	NS	−0.7	0.001	−0.07	NS	−0.06	NS
BUN	−0.2	NS	0.3	NS	0.3	NS	0.4	0.05	−0.3	NS
GSH	0.1	NS	0.1	NS	−0.5	0.05	−0.4	0.05	−0.4	NS
SOD	−0.1	NS	0.4	NS	−0.3	NS	−0.4	0.05	−0.7	0.001

NS = non-significant; Values of $P < 0.05$ indicate differences; W-3 and W-1 were chosen as representative pre-partum weeks; W3, W5 and W7 were chosen as representative post-partum weeks.

Table 3
Post-partum reproductive indices in ovulatory and non-ovulatory buffalo.

Variables	Ovulatory	Non-ovulatory
Number (n = 47)	30 (63.83%)	17 (36.17%)
Days not pregnant	121.60 ± 15.00	170.50 ± 16.20**
Pregnancy rate at 60 days	30 % (n = 9)	0
Pregnancy rate at 90 days	36.66% (n = 11)	11.76% (n = 2)
Pregnancy rate at 120 days	63.33% (n = 19)	17.64% (n = 3)
Pregnancy rate at 150 days	83.33% (n = 25)	29.41% (n = 5)
Calving interval (days)	436.20 ± 14.82	489.10 ± 15.73*

Values with different superscripts differ *$P < 0.05$, **$P < 0.01$.

The results of the present study indicate there was a positive correlation of glucose concentrations on whether buffalo were classified as ovulatory or non-ovulatory with those animals having greater glucose being ovulatory. Glucose is an important signal molecule for release of hypothalamic GnRH (Foster and Nagatani, 1999) that affects the resumption of post-partum ovarian functions (Doijad et al., 2018) and is associated with greater concentrations of estrogen as a result of enhanced folliculogenesis. These findings are consistent with those of Hussein et al. (2013) where there was a positive correlation between post-partum glucose concentrations and resumption of ovarian functions after calving in buffalo.

The concentrations of NEFA and BHBA were considered to be reliable markers for NEB during the post-partum period. Concentrations of both NEFA and BHBA were greater in non-ovulatory buffalo during the post-partum period. These results may be attributed to the fat mobilization to meet the greater reliance on metabolic energy for milk production thus resulting in greater serum NEFA and BHBA concentrations. Additionally, the lack of ovarian functions (i.e., development of dominant follicules and ovulation) is closely associated with there being a NEB status (Llewellyn et al., 2007; Bicalho et al., 2017) that is evident from the greater concentrations of NEFA and BHBA. The greater concentrations of NEFA and BHBA may directly impair the development of ovarian follicles and the capacity for ovulation (Beam and Butler, 1999) and ultimately the future reproductive performance of buffalo (Ranjan et al., 2012) as ascertained in the present study. Furthermore, a NEB status could disrupt the post-partum pulsatile LH secretion and reduce ovarian sensitivity to this gonadotropin which is necessary for steroidogenesis and pre-ovulatory follicle development (Beam and Butler, 1999) with ultimately there being a lesser P_4 and E-17β production in non-ovulatory buffalo. Furthermore, the relatively greater NEFA and BHBA concentrations directly inhibit granulosa (Jorritsma et al., 2003; Vanholder et al., 2005) and theca (Vanholder et al., 2006) cell functions that results in a suppressed steroidogenesis (P_4 and E-17β) in non-ovulatory buffalo as indicated by results in the current study. These results are consistent with the lesser BCS status as well as lesser glucose concentrations in non-ovulatory buffalo which could result from lipid catabolism and a NEB. The ovulatory buffalo had enhanced physiological and metabolic adaptations to normalize metabolic status during the post-partum period.

Albumin concentrations were markedly greater in ovulatory than non-ovulatory buffalo in the present study. The NEB manifested by oxidizing of NEFA (Drackley, 2000) and production of BHBA (Drackley and Andersen, 2006) could impair the hepatic synthetic function of albumin (Youssef et al., 2010). Furthermore, the lesser serum albumin concentrations and increased albumin catabolism due to the energetic deficient status (Bell et al., 2000) is associated with a delay in resumption of ovarian functions (i.e., dominant follicule development and ovulation) in non-ovulatory buffalo.

Pre- and post-partum BUN concentrations were greater in non-ovulatory buffalo and there was a significant group x week interaction during both periods. These results are consistent with those of Campanile et al. (2006). The greater BUN concentrations in non-ovulatory buffalo is consistent with the NEB status of these animals where as a result of this NEB status there was excessive deamination of protein (Oliva et al., 1991). The greater BUN concentrations in the present study in non-ovulatory buffalo are consistent with findings where there was a lesser development of ovarian follicles in animals with relatively greater BUN concentrations (Leroy et al., 2008; Khan et al., 2011). Furthermore, there was an association of greater BUN concentrations and disrupted oviduct and uterine environments that interfere the development of ova and embryo (Qureshi et al., 2002). The greater concentrations of BUN are negatively correlated with post-partum resumption of ovarian functions and reproductive indices.

The serum concentrations of IGF-1 were markedly less in non-ovulatory than ovulatory buffalo with there being a significant group x week interaction during the post-partum period. This result was a consequence of the NEB status of the animals in the non-ovulatory group which led to greater concentrations of growth hormone (GH). The production of GH disrupts lipolysis and NEFA liberation that suppresses hepatic metabolism and oxidation resulting in a suppressed IGF-1 synthesis followed by disruption of the hepatic somatotropic axis coupling (Lucy et al., 2001; Lucy, 2003). These metabolic changes will lead to a suppression of synthesis of albumin by hepatocytes (Ballmer et al., 1995) and lead to a state of insulin resistance (Morigny et al., 2016) in non-ovulatory buffalo. With ovulatory buffalo, however, there were metabolic alterations during the peri-parturient period especially during the post-partum period that led to the positive correlation between IGF-1 concentrations and resumption of post-partum ovarian functions. In ovulatory buffalo, receptors for GH are functional and hence the IGF-1 secretions from hepatocytes increases which lead to a further decrease in pituitary GH secretion leading to greater insulin responsiveness (Lucy, 2008). Both IGF-1 and insulin function to improve ovarian sensitivity to gonadotropins (Butler et al., 2003; Lucy, 2008) that results in an enhanced follicular development with subsequent estrogen production. Furthermore, IGF-1 promotes luteal cell functions as well as angiogenesis (Chouhan et al., 2015), therefore, there are greater P_4 concentrations in ovulatory buffalo.

Similarly, post-partum serum GSH and SOD are in greater concentrations in ovulatory than non-ovulatory buffalo with there being a significant positive correlation with time of resumption of ovarian functions. The non-ovulatory buffalo had a greater tissue catabolism with this metabolic status occurring as a result of adaptions to the NEB and greater energetic requirements during post-partum period. The fatty acid oxidation resulted from lipolysis in the non-ovulatory buffalo in the present study and was a source of free radicals that exceed the normal antioxidant scavenging capacity of the body leading to a depletion in GSH and SOD (Li et al., 2016). The consequence of this metabolic state is a negative correlation between antioxidant markers and longer post-partum period before resumption of ovarian functions. The antioxidants biomarkers have been used as indicators for oxidative stress and actions of these compounds can lead to metabolic disorders and pathological conditions. Free radicals are implicated in these alterations which could damage the individual cells (Castillo et al., 2005) especially those of the ovary thus impairing steroidogenic capacity. Furthermore, the greater oxidative load in non-ovulatory buffalo during the post-partum period in the present study was consistent with the lesser concentrations of serum albumin than the ovulatory buffalo; where albumin is considered to possess antioxidant properties (Quinlan et al., 1998; Rostoker et al., 2011; Taverna et al., 2013). The consequences of NEB, therefore, were an increase in the oxidative load in non-ovulatory buffalo.

The NEB resulted in impairments in ovarian steroidogenesis that was manifested by lesser P_4 and E-17β concentrations in non-

ovulatory buffalo. Ovulatory buffalo could physiologically compensate for the NEB and this was reflected by the greater glucose concentrations which ultimately led to resumption in ovarian functions and steroidogenesis earlier in the post-partum period. The concentrations of P_4 and E-17β have been considered to be predictors for efficient reproductive performance in buffalo (Hussein et al., 2013). The results of the current study indicate that there are fewer days in the non-pregnant status following calving and greater pregnancy rates in ovulatory than non-ovulatory buffalo. These results were mainly attributed to the greater serum P_4 and E-17β concentrations resulting as a consequence of the physiologically hemostatic condition. The interval of days in which animals were not pregnant in the current study is consistent with results from previous studies in buffalo (Takkar et al., 1999; Hussein et al., 2013).

5. Conclusion

Buffalo that had longer periods before resuming post-partum ovarian functions (i.e., development of dominant follicules and ovulation) in the present study had a lesser BCS, as well as glucose, albumin, IGF-1, GSH and SOD concentartions and greater NEFA, BHBA and BUN concentartions than those with earlier resumption of ovarian functions during the post-partum period. The results from the present study provide evidence that there was a NEB in non-ovulatory buffalo that led to lesser production of P_4 and E-17β, delayed ovarian function resumption, more days when animals were not pregnant and lesser pregnancy rates. Animals in the ovulatory group were able to compensate for the NEB and their reproductive performance was greater as a consequence.

Declaration of Competing Interest

All the authors confirm that they did not receive any fund for the current study.

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