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Serum Hormonal, Metabolic and Minerals Profile in Normal Cyclic and Postpartum Anestrus Egyptian Buffaloes

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ABSTRACT

Reproductive performance in buffaloes is greatly affected by postpartum anestrus condition. So, the purpose of the current study was to compare between the normal cyclic and postpartum anestrus Egyptian buffaloes (Bubalus bubalis) in their serum hormonal, metabolic and minerals profile. Blood samples were collected from 20 normal cyclic animals at day 11 of the estrous cycle and other samples were taken from 20 postpartum anestrus animals. Gyneco-clinical and ultrasonographical examinations were performed for confirmation of cyclic and anestrus condition after calving. Anestrus animals had a significant reduction in serum progesterone (P_4), estradiol (E_2), thyroxine (T_4), triiodothyronine (T_3), total cholesterol, phosphorus (P) and zinc (Zn). Nonetheless, blood glucose, protein, triglycerides, HDL - cholesterol, calcium (Ca) and copper (Cu) levels did not vary between regular cyclic and anestrus condition. In conclusion, these findings may improve our understanding of some aspects related to postpartum anestrus buffaloes which may lead to better procedures for reproductive management.

1. INTRODUCTION

Physiological condition as well as health status of animals can be revealed by the changes in blood constituents. There are certain hormonal and metabolic parameters, which influence directly the process of reproduction in animals (Hafez and Hafez, 2000; Ashmawy, 2015). Buffalo bear head significance, contributing to the farming systems, as an origin of milk, meat, draft power, on-farm manure and employment of the agriculturists (Parmar et al., 2015). Anestrus can be defined as the failair or defecincy of the expression of estrus. A period of anestrus after parturition is considered to be a normal physiological event but becomes abnormal condition if its duration exceeds its accepted average (Abraham, 2017). In livestock animals, one of the main factors that have a negative impact on their reproductive performance is the anestrus condition. In Egypt, anestrus remains an important problem for reproduction in buffalo. Its incidence in buffaloes in Egypt and India varies from 31–42% (El-Wishy, 2007; Ashmawy, 2015). Nutritional intake, adverse climatic conditions, energy deficiency, high production, management stress, parasites and diseases can cause the problem of anestrus (Lawania et al., 2017). Also, thyroid gland hormones have been studied as a potential regulators of ovarian activity (Saleh et al., 2011).

Dietary requirements for efficient reproduction are the same as those required for maintenance, growth and lactation, which incorporate protein, energy, mineral and vitamins. Reduction or overabundance of any of these necessities, will influence reproduction and also other physiological processes (Abraham, 2017). Ideal protein level is vital for the improvement of endocrine and sex organs. The diminished protein antagonistic impact on reproduction is through pituitary and sex organs (Patel et al., 2018). Cholesterol is incorporated from acetic acid derivation with a progression of intermediate substances. It is a critical predecessor for the ovary,

testis and adrenal cortex steroid hormones synthesis (Sesh and Meur, 2013).

Certain macro-elements like Ca, P, sodium (Na), potassium (K) and chloride (Cl); and trace elements including Zn, Cu, cobalt (Co), manganese (Mn), selenium (Se), iron (Fe), iodine (I), and molybdenum (Mo) have been found to be crucial for normal animal reproduction (Akhtar et al., 2009). Trace elements, in male and female reproduction, are important for spermatogenesis and folliculogenesis in various animal species (Parmar et al., 2015). Trace elements may act as stimulators of enzymes or as cofactors (Prajapati et al, 2018). Ruminants are as often as possible subjected to serious lacks of trace elements. Concomitant infertility and anestrus condition in ruminant are believed to be associated with enzymatic malfunctions resulting from these deficiencies (Akhtar et al., 2010).

The present study was designed to compare between the serum hormonal, metabolic and minerals parameters in normal cyclic and postpartum true anestrus buffaloes (*Bubalus bubalis*) under the Egyptian farm conditions.

2. MATERIALS AND METHODS

2.1. Animals:

The present study was conducted on healthy buffaloes-cows belonging to small farmers at El-Behera Province in Egypt. They were between the second and fourth calving, aged from 5-8 years and their body weight ranged from 500-600 Kg. This study was carried out from Februry to April 2018. Animals were maintained on green fodder and supplemented with concentrate ration and drinking water was provided ad libitum. All animals were examined gyneco-clinically twice at ten days intervals. Twenty regular cycling buffalo-cows coming in estrous before 65 days of postpartum for more than three consecutive lactations including present lactation were selected as the control group (Group A). Twenty buffalo-cows, which did not express estrus signs for more than 120 days postpartum, having smooth ovaries with no palpable structures, having no clinically detectable abnormalities in their genital tract and no treatments with hormones were administered during the last three months; were categorized as true anestrus animals (Group B). Ultrasonography was used to confirm the diagnosis, which was done by linear array scanner that produces a real time B-mode image (scanner 480-Vet-scan, Pie medical Co.). Scanner was equilibrated a five MHz transducer designed for intra-rectal insertion in buffalo-cow.

2.2. Blood samples:

Blood samples were collected from the regular cycling buffaloes (Group A) at day 11 of the estrous cycle (during diestrus phase) and from the true anestrus buffaloes (Group B) at any time from jugular vein using a 16 G needle in sterile centrifuge tube (Akhtar et al., 2010). The samples were left on oblique position for two hours at room temperature to allow proper clotting. Serum was separated out by centrifugation at 3000 rpm for 15 minutes and stored at −20 ºC in a deep freezer until analysis of P_4, E_2, T_4, T_3, glucose, total protein, total cholesterol, triglyceride, high-density lipoproteins (HDL-cholesterol), Ca, P, Zn and Cu.

2.3. Hormonal, metabolic and macro-minerals assay:

Serum P_4 and E_2 levels were determined by Enzyme-linked Immunosorbent assay (ELISA) micro-well technique according to the manufacturer's instructions using kits supplied by Hellabio biokits company (USA). Radioimmunoassay (RIA) using commercial kits was used for the measurement of serum concentrations of T_4 and T_3 supplied by Synbiotics Corporation, 11011 via Frontera, San Diego. Estimation of serum glucose, total protein, total cholesterol, triglyceride, HDL-cholesterol, Ca and P levels were determined spectrophotometrically (Perkin Elmer Model No. 186, Germany) using standard diagnostic kits (Biodiagnostics Company, Egypt).

2.4. Micro-minerals estimation:

Serum samples were digested by concentrated acids (3 ml perchloric + 2 ml nitric acid per ml serum) for 24 hours (Simsek et al., 2016). Then all samples were diluted, filtered and analyzed for micro-minerals (Zn and Cu) using flame emission atomic absorption spectrophotometer (Model 210 VGP Buck Scientific, USA). The flame conditions were these as recommended by the instrument manufacturer. All micro-minerals concentrations were calculated as µg/dL.

2.5. Statistical analysis

Data were analyzed using the SPSS package (SPSS Inc., Chicago, IL). Results are expressed as mean ± SE with the experiment repeated at least three times. Statistical evaluations were done using paired Student's t-test to compare between the two groups **(Snedecor and Cochran, 1967).**

3. RESULTS

3.1. Serum hormonal and metabolic levels in normal cyclic and postpartum anestrus buffaloes:

Serum P_4, E_2, T_4, T_3 and total cholesterol were significantly reduced ($p < 0.05$) in postpartum anestrus buffaloes relative to the normal cyclic buffaloes. Non-significant decreases were recorded in serum glucose, total protein, triglyceride and HDL-cholesterol between the two groups (as shown in Table 1).

1.1. Serum macro- and micro-minerals levels in normal cyclic and postpartum anestrus buffaloes:

Serum P, Zn and Zn/Cu ratio were significantly lower ($p < 0.05$) in postpartum anestrus buffaloes relative to normal cyclic buffaloes. While the Ca/P ratio was significantly higher ($p < 0.05$) in postpartum anestrus buffaloes compared to normal cyclic buffaloes. There were no significant decrease in serum Ca, and Cu between the two groups (as shown in Table 2).

Table 1: Serum hormonal and metabolic levels in normal cyclic and postpartum anestrus buffaloes.

	Group A (Normal cyclic)	Group B (Anestrus)
P_4 (ng/mL)	2.83 ± 0.31	0.32 ± 0.02*
E_2 (pg/mL)	11.48 ± 0.47	6.11 ± 0.27*
T_4 (µg/dL)	5.92 ± 0.96	3.41 ± 0.72*
T_3 (ng/dL)	208.17 ± 8.57	171.67 ± 6.19 *
Glucose (mg/dL)	56.90 ± 3.15	52.24 ± 2.87
Total protein (g/dL)	7.23 ± 0.48	6.76 ± 0.36
Total cholesterol (mg/dL)	138.78 ± 9.73	76.47 ± 4.82*
Triglycerides (mg/dL)	26.71 ± 1.18	25.45 ± 0.89
HDL-cholesterol (mg/dL)	58.33 ± 4.54	55.14 ± 3.07

Values are expressed as mean ± S.E. of 20 animals per group. Significant differences in the values between the two groups were indicated by *P < 0.05.

Table 2: Serum macro- and micro-minerals levels in normal cyclic and postpartum anestrus buffaloes.

	Group A (Normal cyclic)	Group B (Anestrus)
Calcium (mg/dL)	8.12 ± 0.25	7.87 ± 0.32
Phosphorus (mg/dL)	5.29 ± 0.28	3.24 ± 0.03*
Ca/P ratio	4.55 ± 0.09	6.35 ± 0.31*
Zinc (µg/dL)	91.21 ± 4.23	57.94 ± 2.42*
Copper (µg/dL)	68.87 ± 3.51	64.14 ± 3.87
Zn/Cu ratio	1.32 ± 0.13	0.90 ± 0.02*

Values are expressed as mean ± S.E. of 20 animals per group. Significant differences in the values between the two groups were indicated by *P < 0.05.

4. DISCUSSION

The low reproductive efficiency in buffalo is a major restriction in obtaining maximum production potential which sets a perfect platform for the present research input. Ovarian inactivity is still one of the most common reproductive disorders in this species (El-Wishy, 2007). The reason for the ovarian inactivity might be insufficient synthesis or secretion of gonadotropins for follicular development or it may indicate the failure of ovaries to respond to gonadotropins (Abraham, 2017). The present study aims to compare between the serum hormonal, metabolic and minerals profile in normal cyclic and postpartum anestrus Egyptian buffaloes.

The significantly low serum P_4 and E_2 levels in postpartum anestrus animals in the present study agree with the findings of Ahmed et al. (2010), Akhtar et al. (2010) and Kalasariya et al. (2017), in buffaloes and Saleh et al. (2011) in cattle. It was reported that the recent diagnostic tools of ovarian inactivity not depend only on rectal palpation but also can use blood P_4 and E_2 profile, as serum P_4 and E_2 level reduce in anestrus animals due to the absence of LH surge and impaired follicular growth accompanied by ovarian inactivity (Terzano et al., 2012). In anestrus animals, a broad variety has been recorded in serum P_4 and E_2 concentration demonstrating continual follicular growth and atresia (Ahmed et al., 2010). It is an established fact that the chief function of estrogen is the manifestation of estrus by regulating the functionality of the tubular genital tract; it also coordinates sexual behavior and receptivity to the male animals by sensitizing the central nervous system (Hafez and Hafez, 2000).

In regard to the thyroid hormones, the present results revealed that significantly lower values of T_4 and T_3 were observed in anestrus buffalo-cows which agree with the findings of Kumar et al. (2010b) in buffaloes and Saleh et al. (2011) in cattle. An association between thyroid hormones and reproductive efficiency was plausible. Normal levels of T_3 are required for expression of estrus. So, low levels of T_3 can depress the reproductive performance in the buffaloes (Hafez and Hafez, 2000). In buffalo cows, hypothyroidism was associated with the absence of behavioral signs of estrus as well as low blood progesterone levels (Kumar et al., 2010b). It was supported that thyroid hormones may activate the ovarian function through the synergistic action with the follicular stimulating hormone to stimulate the differentiation of granulosa cells (Saleh et al., 2011). It is still not well known the exact mechanism by which the thyroid hormones regulate steroidogenesis. Although T_3 and T_4 had little or no effect on aromatase activity, they could provide important estrogen precursors to granulosa cells and thus indirectly increase E_2 (Akhtar et al., 2010). While others suggested that T_3 and T_4 hormones may interfere directly with the reproductive functions only if there is a systemic influence (Jena et al., 2016).

Serum glucose level did not significantly decrease between cyclic and anestrus buffalo-cows. This concurs with the observations of Kumar et al. (2010a), Jayachandran et al. (2013) and Kumar et al. (2015). Singh et al., (2010) observed that blood glucose was not a metabolic regulator responsible for the beginning of the ovarian cycle. Others reported that low blood glucose in buffaloes decreased the hypothalamic-hypophyseal-ovarian axis signal communication leading to the condition of anestrus (Kumar et al., 2015). Fontana and Torre (2016) suggested that the reduced concentration of glucose in blood was associated with nutritional anestrus. On the contrary, Ahmed et al. (2010) reported that unless severe energy restriction happened, the energy deficiency can delay puberty but could not affect estrus activity after puberty.

In the current study the serum total protein level, of anestrus buffaloes did not significantly decrease from the normal cyclic animals, which was in agreement with the results of Parmar et al. (2015) and Kalasariya et al. (2017) in buffaloes and Saleh et al. (2011) in cattle, but disagreed with the results of Khasatiya et al. (2005). Depending upon the feed intake of the animal the serum protein levels change with different phases of reproduction. Protein lack retarded the reproductive organs improvement and was regarded as a factor responsible of the postponement in beginning or failure of postpartum estrus (Patel et al., 2018). As there was no difference in glucose and protein level between cyclic and anestrus animals, glucose and protein level may not be an essential aspect for the anestrus condition in buffalo-cows of this study area.

Reduced total cholesterol levels in the present study concur with the observations of Ali and Shukla (2012), Jayachandran et al. (2013) and Parmar et al. (2015). On the contrary, Kumar et al. (2015) did not report any variation in total cholesterol levels between normal cyclic and anestrus buffaloes. High cholesterol level in normal cyclic animals compared to anestrus ones might be a sign of improved steroid secretion due to increased ovarian activity (Sesh and Meur, 2013). Cholesterol acts as a precursor for the formation of steroid hormones in thica and luteal cells in the ovarian (Hafez and Hafez, 2000). The mechanism by which estrogens affect the inter-relationships of pituitary-thyroid-adrenal functions is by affecting the carbohydrate metabolism that in turn increase the production of cholesterol in endocrine gland tissue from acetate, and that explains the increase in serum cholesterol during estrus (Yadav et al., 2006). During postpartum anestrus in the current work, the low concentration of total cholesterol suggests that it might have led to the insufficient synthesis of steroid sex hormones prompting the anestrus state.

Serum triglyceride concentration had no significant variation between cyclic and anestrus buffaloes. This was in agreement with the report of Jayachandran et al. (2013) and Kumar et al. (2015) in buffaloes and Saleh et al. (2011) in cattle. The serum triglyceride level was not associated with the resumption of the ovarian cycle after parturition in cattle (Nogalski et al., 2012). It was reported that triglycerides act as a metabolic regulator for bovine oocyte maturation but they do not have a direct role in ovarian steroidogenesis (Fontana and Torre, 2016). In the current study HDL-cholesterol levels of the normal cyclic and anestrus animals did not vary significantly. HDL-cholesterol is the principle class of cholesterol in follicular fluid of bovines and it is essential for steroidogenesis in luteal cells in vitro and in vivo (Terzano et al., 2012). As there was no significant difference in triglycerides and HDL-cholesterol values in animals of both groups in the present work suggests

that the energy level may not be a reason for the ovarian inactivity in these animals.

In the current work, the non-significant decrease in serum Ca agrees with the observations of Yadav et al. (2006), Kumar et al. (2010a) and Kumar et al. (2015) who reported a non-significant difference in Ca level between cyclic and anestrus buffaloes, whereas Chaurasia et al. (2010) observed relative to the cyclic buffaloes, significant lower Ca level in anestrus buffaloes. Ca is essential in the field of reproduction for contraction of the uterine muscle, LH secretion from the pituitary gland and fertilization (Abraham, 2017). Ca is also known to affect the animal's ability to utilize other trace elements; it may disrupt reproductive functions through its influence on certain enzyme system (Hafez and Hafez, 2000).

Serum phosphorus (P) level of buffaloes in anestrus state showed significantly lower level compared to regular cyclic buffaloes, this concur with Chaurasia et al. (2010), Kumar et al. (2016) and Mourad (2017). Whereas, Khasatiya et al. (2005) between cyclic and anestrus buffaloes did not record any variation in P level. The participation of P in phospholipids and cAMP formation may be an essential factor for its effect on the reproductive performance. Marginal P insufficiency may prompt anestrus condition because of the close connection amongst P and reproductive hormones (Chaurasia et al., 2010). A phosphorus deficiency causes impaired ovarian activity, irregular estrus, anestrus, reduced conception rate, high incidence of cystic follicles, and generally depressed fertility (El-Wishy, 2007; Abraham, 2017). Administration of P has been found to alleviate the anestrus conditions in buffalo heifers (Khan et al., 2015). Significantly enhanced Ca/P ratio was revealed in the postpartum anestrus buffaloes compared to the normal cyclic buffaloes. This is due to the decreased P level in the serum of postpartum anestrus buffaloes. Literatures also support the disturbed Ca/P ratio in anestrus animals (Chaurasia et al., 2010; Kumar et al., 2010a). Predominantly, the changes of Ca/P ratio can affect the ovarian activity by blocking the pituitary gland and this lead to prolongation of ovulation and subsequently the estrus cycle but serum Ca concentration alone won't affect the reproductive physiology of animals directly (Kumar et al., 2015).

The results of this study for a significant decrease in Zn agrees with the findings of Yadav et al. (2006), Akhtar et al. (2009) and Mourad (2017), whereas Jayachandran et al. (2013) did not find any variation in Zn level between cyclic and anestrus buffaloes. It was reported that Zn level is related with the steroid reproductive hormone concentrations which indicated that there was a close relationship between blood Zn level and progesterone-estrogen levels for optimum reproductive performance (El-Wishy, 2007). This element plays an important role in the repair of the endometrium following parturition, speeding the return to normal reproductive function and ovarian activity (Abraham, 2017). Zn deficiency has been shown to reduce the synthesis and secretion of FSH and LH, disruption of the estrus cycle in buffalos and impair the ovarian development (Ahmed et al., 2010). Supplementation with Zn and other elements has been found to improve anestrus condition in buffalo heifers (Khan et al., 2015).

The non-significant decrease in serum Cu levels of the present study, which did not show a significant difference between normal control and anestrus buffaloes, are in agreement with Jayachandran et al. (2013) and Kumar et al. (2016). On the other hand, low Cu level in anestrus buffaloes compared with normal cyclic buffaloes was reported by Yadav et al. (2006) and Akhtar et al. (2009). The importance of Cu for growth, production and reproductive functions has been well established; ceruloplasmin and superoxide dismutase are a number of Cu-containing proteins that are essential for the physiological functions of Cu (El-Wishy, 2007). It was reported that Cu deficiency may have adverse effects on female reproduction via the hypothalamus-pituitary-gonadal axis on LH secretion leading to a decrease in the ovarian oestradiol secretion and absence of the expression of estrus in animals (Prajapati et al, 2018). The Zn/Cu ratio was significantly reduced in postpartum anestrus animals compared to normal cyclic ones; this might be due to the decreased Zn level in the serum of postpartum anestrus buffaloes.

4. CONCLUSION

It was concluded from the present study that the reduction of serum P_4, E_2, T_4, T_3, total cholesterol, P and Zn could be responsible for the anestrus condition in buffalo-cows under Egyptian farm conditions which could be reversed by improving the nutritional status to enhance fertility in this species.

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Conflict of Interest: The author proclaims that they have no contending interests.

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