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Research Article

Effect of Reproductive Status and Season on Blood Biochemical, Hormonal and Antioxidant Changes in Egyptian Buffaloes

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ABSTRACT

This work was carried out to study some hormonal, biochemical and antioxidant activities in relation to the reproductive status in buffalo during the hot and cold seasons. Genitalia of mature female buffaloes were collected during hot, (n=130) and cold season (n=190). Genitalia were classified according to their reproductive status into; cyclic, early pregnant and non-cyclic with smooth inactive ovaries. Samples of blood were collected and serum was isolated and stored at −20ºC for hormonal and biochemical analysis. Progesterone and cortisol levels were determined using ELISA. Total protein, albumin, triglyceride, total cholesterol, HDL-cholesterol, malondialdehyde, superoxide dismutase, catalase, and LDH were measured spectrophotometrically. Results indicated that malondialdehyde (MDA), lactate dehydrogenase enzyme (LDH) and cortisol concentrations were significantly (P<0.05) higher in smooth inactive ovaries than in pregnant or cyclic buffalo. Whereas, superoxide dismutase (SOD), catalase, cholesterol, HDL cholesterol, triglyceride, total protein, progesterone (P4) levels, and albumin were significantly (P<0.05) higher in cyclic or pregnant than buffalo with smooth in-active ovaries. During the hot season, MDA, LDH, and cortisol levels were positively correlated (P<0.05) with ovarian inactivity. While, there was a negative correlation (P<0.05) with the concentration of P4 hormones, total protein, albumin, cholesterol, HDL CHO, triglyceride, SOD and catalase and ovarian inactivity in buffalo. Conclusion: reproductive status, hormonal and blood biochemical parameters are affected by seasons in Egyptian buffalo.

Key words: Buffalo, Reproductive status, Season, P4 and cortisol, Blood biochemistry

INTRODUCTION

Buffalo plays an important role in the agricultural economy in numerous developing countries, providing draught power, meat and milk. However, reproduction in buffalo is still low compared with cattle. Reproductive disorders are multifactorial, which include genetics, nutrition, environmental and management conditions (Perera, 2011).

Numerous environmental and physiological stresses encountered by buffaloes and affecting everyday life by disturbing their production and this could be the reason for substantial economic losses. The thermoregulatory capacity of buffaloes is poor compared to cattle. They display distress signs after exposure to high environmental conditions (Bombade *et al*., 2017). High environmental stress contributes to sequences of variation in buffalo's biological functions. This contains food intake depression, oxidative stress and variation in hormonal and blood biochemistry (Bombade *et al*., 2017).

The alteration of hemato-biochemical parameters is major markers for physiological and pathological states of the animal (Hassan *et al*., 2012; Mamun *et al*., 2013). Therefore, standard levels of vital biochemical elements and hormones may affect the reproductive efficiency of buffalo; endocrine imbalances at any point in the sequence may give rise to reproductive failure (Sabasthin *et al*., 2012). Summer and winter stress causes rigorous changes in the blood biochemical and hormonal concentration and thereby reducing the production performance of the animals (Ganaie *et al*., 2013).

The adverse effect of thermal stress and season on reproductive status has been investigated in several recently published reports. However, the mechanism by which the hot season could impair reproductive function in buffalo has not been adequately investigated. Therefore, this study was conducted to compare some of the biochemical, hormonal and antioxidant activity in smooth inactive ovaries, early pregnant and cycling buffaloes under cold and hot environmental conditions.

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MATERIALS AND METHODS

Ethical approval

The work is a part of the 12/1/7 project (NRC) and it follows the guidelines of the Institutional Animal Ethics Committee.

Experimental animals

The display study was conducted on eighty mature and healthy slaughtered buffaloes. Genitalia was collected during the hot season (April–September 2017, n=130) and cold season (October 2017–March 2018, n=190). Genitalia were classified depending on their reproductive status (ovaries macroscopic observation such as presence, size, and shape of CL and follicle, the uterus (color, consistency, size, mucus into cyclic, smooth inactive and early pregnant ovaries according to the methods adopted by (Abdoon and Kandil, 2001) for buffalo. During the study, the temperature and humidity were recorded.

Blood samples

Forty serum samples collected during the hot season and forty serum samples collected cold season were used for hormonal and biochemical analysis.

Measured parameters: (biochemical and hormonal assay)

Progesterone (P4) and cortisol levels were measured using automatic ELISA reader (EZ Read 400, Microplate Reader, biohrome, England). P4 kit **(**Chemux Bioscience, INC), and the assay minimal detection limit was 0.2 ng⁄ml. Cortisol kit was abia Cortisol, AB Diagnostic systems Gmbh, Berlin, Germany), and the assay minimum detection limit was 5 nmol⁄ ml. The variation intraassay coefficients for low and high references were 2.30% and 4.5%.

Biochemical analysis

Biochemical values in serum were measured using UV spectrophotometer (Jasco, V-730, Japan), Biochemical study included measurements of serum total proteins, albumin, triglycerides (TG, mg/dl), total cholesterol (CHO, mg/dl), high density lipoprotein (HDL-C) (MG, Salus a, Holland kit), calculated globulin, SOD activity (using SOD assay kits (Bio diagnostic, Egypt)) according to (Nishikimi *et al*., 1972), catalase activity was measured using a Catalase Assay Kit (Bio diagnostic, Egypt) in line with (Aebi, 1984). Lactate dehydrogenase enzyme activity (by a Lactate dehydrogenase Assay Kit (Spectrum, Egypt)) according to (Young DS, 1990). Malondialdehyde value was measured calorimetrically along with the method of (Ohkawa *et al*., 1979) using Bio diagnostic kits.

The temperature was recorded during the whole year. The mean of maximum temperatures in the hot season was 35℃ and in cold season was 20℃.

Statistical analysis

Results were offered as Mean±SEM and statistically computed by one-way analysis of variance (ANOVA) using SPSS (Statistical Package for the Social Sciences) Program Ver. 20.

RESULTS

The effect of season on reproductive status in buffalo is presented in Table 1. Morphological examination of buffalo genitalia revealed that 34.625% of buffalo showed smooth inactive ovaries during hot compared to cold season ($P<0.05$) and incidence of pregnant and cyclic buffaloes were significantly ($P<0.05$) higher in cold than a hot season (Table 1).

Data in Table 2 represent the effect of season and reproductive status on P4, cortisol, total protein, albumin, globulin, triglycerides, cholesterol, HDL-cholesterol, SOD, Catalase, MDA, LDL concentrations in buffalo. The obtained results indicated that P4 level, total protein, triglycerides, albumin, total cholesterol, HDL-cholesterol, SOD and catalase were significantly ($P<0.05$) higher in cold than in hot season, whereas, cortisol, MDA and LDH values were significantly ($P< 0.05$) higher in the hot season than cold one.

Also, results showed that mean serum levels of progesterone, total protein, albumin, triglycerides, total cholesterol, HDL-cholesterol, SOD and catalase were significantly ($P<0.05$) higher in cyclic and early pregnant buffalo than in buffalo with smooth inactive ovaries. On the other hand, cortisol, MDA and LDH values were higher ($P<0.05$) in buffalo with smooth inactive ovaries than pregnant and cyclic animals.

DISCUSSION

Seasonality in buffalo reproduction has been attributed to environmental factors more directly than the genetic factors (Zicarelli 1994). A blood profile is an important indicator of the diagnosis, treatment, and prognosis of reproductive disorders. The present work revealed that 34.625% of buffalo showed smooth inactive ovaries through hot than in the cold months. These results concur with other reports in which the incidence of anestrus was higher in buffalo in summer than in winter (Ali *et al.*, 2009; Soliman *et al.,* 2016)**.** The summer stress results in a reduction of feed intake which in turn leads to higher ovarian inactivity and poor estrus expression.

In addition, numerous studies have designated that the P4 level reflects the corpus luteum functions and even any alteration in the P4 level might have a fundamental physiological implications on reproduction. The current study showed that in buffalo, serum progesterone levels were significantly ($P<0.05$) higher in early pregnant animals than cyclic and the lowest concentration was recorded in smooth inactive ovaries. These results are parallel to those reported in buffalo (Khan *et al*., 2011; Hussein *et al*., 2013) and cattle (Gebhardt *et al*., 2012). Also, P4 values were significantly ($P<0.05$) higher in cold than in the hot season. Similarly, Soliman *et al.* (2016) recorded that the hot season harms progesterone levels in buffalo and cattle. It was reported that high environmental condition joined with under-nutrition might be the reason for buffalo's long anestrous phases besides delayed ovulation causes changes in the preovulatory follicle microenvironment (Bage *et al*., 2002).

In this work, hot season and animals with smooth inactive ovaries showed significant ($P<0.05$) increase in cortisol compared with the cold season or for cyclic or early

Table 1: Morphological assessment of buffalo genital tract during hot and cold seasons.

Season	No. genitalia	Reproductive status		
		Cyclic %	Early pregnant%	Inactive ovaries %
Hot	130	70 (53.85)	15 (11.54)	45 (34.62) [a]
Cold	190	137 (72.11)	30 (15.79)	23 (12.11) [b]

a, b differs at P<0.05.

Table 2: Effect of season and different reproductive status on hormonal, biochemical and antioxidant activity.

Parameter	Season	Reproductive status			
		Follicular stage	Luteal phase	Smooth inactive ovaries	Early pregnant
P4 (ng/ml)	Hot	0.5±0.02 [f]	1.2±0.1 [d]	0.1±0.01 [h]	1.7±0. 1 [b]
	Cold	0.7±0.03 [e]	1.4±0.1 [c]	0.3±0.01 [g]	2±0.1 [a]
Cortisol (n mol/l)	Hot	455.5±11.1 [c]	378.4±4.3 [e]	527.5±8.4 [a]	333.6±3.9 [f]
	Cold	425.2±5.4 [d]	377.5±13.2 [e]	481.5±6.1 [b]	292.4±11.7 [g]
Total protein (g\dl)	Hot	7.2±0.1 [b]	5.6±0.1 [f]	4.7±0.1 [h]	6.5±0.1 [d]
	Cold	8.1±0.2 [a]	6 .1±0.1 [e]	5.2±0.1 [g]	6.9±0.1 [c]
Albumin (g\dl)	Hot	4.6±0.2 [b]	3.3±0.1 [f]	2.4±0.1 [h]	4±0.1 [d]
	Cold	4.9±0.1 [a]	3.7±0.1 [e]	2.8±0.1 [g]	4.3±0.1 [c]
Globulin (g\dl)	Hot	2.6±0.2 [b]	2.3±0.1 [b]	2.2±0.1 [b]	2.5±0.1 [b]
	Cold	3.2±0.3 [a]	2.4±0.3 [b]	2.4±0.1 [b]	2.5±0.1 [b]
Triglycerides (mg/dl)	Hot	92.2±2.8 [d]	65.4±2.8 [f]	39±2.2 [h]	119.2±4.6 [b]
	Cold	106.8±4.1 [c]	78.5±2.8 [e]	52.2±2.3 [g]	129.4±4.6 [a]
Total cholesterol (mg/dl)	Hot	87±3.6 [b]	43.6±1.9 [f]	16±1.5 [h]	67.3±1.8 [d]
	Cold	95.5±4.8 [a]	56±2.8 [e]	29.3±2.2 [g]	77.3±2 [c]
HDL cholesterol (mg/dl)	Hot	76.5±5.2 [b]	34±1 [f]	18.2±1.5 [h]	50±2.3 [d]
	Cold	84.5±2.7 [a]	41.6±2 [e]	26.2±0.7 [g]	58±2 [c]
SOD (u/ml)	Hot	253.5±8.2 [f]	320±2.8 [d]	147.4±12.5 [h]	375.8±3.8 [b]
	Cold	290.5±4.8 [e]	347.7±9.3 [c]	212.5±0.01 [g]	402.1±16.9 [a]
Catalase level (Ul)	Hot	251.5±11.9 [f]	429.9±12.7 [d]	104.8±3.1 [g]	628.5±15.4 [b]
	Cold	364.9±9 [e]	478±23 [c]	138.9±3 [g]	742.2±13 [a]
MDA (nmol/ml)	Hot	9.1±0.1 [c]	6.6±0.1 [e]	10.8±0.3 [a]	4.8±0.1 [g]
	Cold	8±0.1 [d]	5.6±0.2 [f]	9.9±0.3 [b]	4.3±0.1 [h]
LDH level (ul)	Hot	422±18 [e]	530±11.2 [c]	627.4±18.5 [a]	257.2±7.9 [g]
	Cold	365.4±12.7 [f]	480.6±28.9 [d]	578.6±15.4 [b]	195.2±12.5 [h]

Means with different superscripts a, b, c, d, e, f, g, h are significantly different at P<0.05.

pregnant buffaloes. These results are concurrent with other reports in which cortisol levels were higher in high environmental temperatures (Lakhania *et al*., 2018). The high cortisol levels in hot seasons maybe stimulate the hypothalamic–pituitary–adrenal axis plus the sympatho-adrenal system leading to increase the circulatory concentration of cortisol and allowing the animal to regulate its physiology and keep homeostasis but this limit secretion of the gonadotrophins and infertility (Alhussien and Dang, 2017). The higher level of serum corticoids leads to an altered gonadotropin secretion, which ultimately triggers the state of anoestrus (Singhal *et al.*, 1984).

Furthermore, the present investigation demonstrated that serum protein and albumin concentrations were lower (P<0.05) in buffaloes with smooth inactive ovaries than cyclic or early pregnant ones. The present findings corroborate with the findings (Al-Saeed *et al*., 2009; Kumar *et al*., 2010) in buffalo and Muna *et al*. (2009) and Chandershekhar *et al*. (2017) who also reported significantly higher total serum proteins during the winter season in cattle. However other studies reported higher serum total protein during summer as compared to winter season (Shrikhande *et al*., 2008; Cozzi *et al*., 2011; Das *et al*., 2014). This discrepancy could be related to the severity of high temperature or due to species differences.

Blood proteins optimum level encourages estrus cyclicity via hypothalamo-hypophyseal system (Tandle *et al*., 1998**).** Meanwhile, protein and albumin concentration was significantly (P<0.05) higher in cold than in the hot months. High environmental temperature has a negative impact on serum total protein and albumin values (Marai *et al*., 2007). The high THI in summer leads to a reduction in feed intake which leads to lower serum protein concentration during summer (Dar *et al*., 2019).

Although, in this work serum triglycerides were significantly (P<0.05) higher in cold than a hot season and in early pregnant or cyclic buffaloes than smooth inactive ovaries and. This is concomitant with Hussein *et al*. (2013) who found that the level of triglyceride was lower in non-pregnant buffalo than the pregnant one. Similar to the present findings, Chandershekhar *et al*. (2017) and Ahmed and Abdalla (2012) also reported higher triglyceride levels during the winter season. Contrarily, Giuseppe *et al*. (2014) reported higher concentration of serum triglycerides in dairy cows during summer season.

In the current investigation, serum cholesterol and HDL-cholesterol levels were significantly (P<0.05) lower in hot than in the cold season and in buffaloes with smooth inactive ovaries than cyclic or early pregnant animals. These findings are in complete with Sandhya *et al*. (2015). Also, the present findings were in agreement with that of Ahmed and Abdalla (2012) and Chandershekhar *et al*. (2017) who also reported significantly higher cholesterol levels during the winter season. The lower cholesterol values during summer may be attributed to lower liver activity during this period (Rasooli *et al*., 2004). The E2 stimulates lipid metabolism through lipogenesis, which in turn reasons for increasing production of cholesterol. This may be directly relational to the production of cholesterol plus variations in the physiological status of buffalo (Hafez *et al*., 2000). Also, decrease cholesterol may be due to lessening in feed intake (Scharf *et al*., 2010). Conversely

Sinha *et al*. (1981) reported that the serum cholesterol levels were high in cattle during summer season. This difference could be due to feeding system or the severity of environmental temperature.

The mean of SOD activity in buffalo serum decrease when the temperature increases. SOD values were significantly ($P<0.05$) higher in cold than compared hot season representing. Also, there were significant differences among mean serum activity of SOD with different reproductive status. It shows high level in early pregnant and normal cycling than buffalo with smooth inactive ovaries. This indicates that hot season is extra stressful to buffalo besides leads to amplify of free radicals' production (Sandhya *et al*., 2015) leading to extreme oxidative stress (Arjun *et al*., 2016).

There were significant differences among mean serum activity of catalase during different seasons. Its activity was high in cold than in hot season. Also, higher catalase levels in early pregnant and normal cycling than in buffaloes with smooth inactive ovaries may indicate the link between oxidative stress and ovarian function. There was a connection among ovarian function (such as estradiol-17b concentration), oxidative stress levels, pregnancy rates and oocyte quality. Catalase keep the genome from oxidative damage also, catalase has role in follicular development regulation and differentiation (Park *et al*., 2016).

There were significant differences among mean serum activity of MDA during different seasons and reproductive status. MDA activity was higher ($P<0.05$) in hot period than cold periods and in buffalo with smooth inactive ovaries than cyclic or pregnant one. This result agrees with Ahmed *et al*. (2010) who stated that MDA values high in buffalo-cows that displayed impaired fertility because of inactive ovaries. Lakhania, *et al*. (2017) reported that the high MDA levels occur due to high thermal temperature. The increased lipid peroxidation detected in in hot summer season may be one of the foremost reasons for oxidative stress coming from decrease in antioxidant defense and amplified production of free radicals and that has anti gonadotropic and anti-steroidogenic actions (Williams *et al.*, 2002).

Conclusions

Reproductive potentials in buffalo are severely affected by thermal stress via antioxidant (SOD, catalase) and increase MDA and (LDH) levels, conversely, they were still showing stress signs which may be reflected by higher cortisol levels. Protein, albumin, triglyceride, and CHO play an important role in regulating buffalo reproduction.

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