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

Ahmed Sabry S Abdoon, Mahmoud Z Attia, Nahed E El-Toukhey, Omaima M Kandil, Hussein A Sabra and Seham S Soliman

1Department of Animal Reproduction and Artificial Insemination, Veterinary Research Division, National Research Centre, Dokki 12622, Cairo, Egypt; 2Department of Physiology, Faculty of Veterinary Medicine, Cairo University, Egypt

*Corresponding author: assabdoob@yahoo.com

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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 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 inactive 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.

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 Kandi, 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°C and in cold season was 20°C.

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 anestrus 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
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 sympathetic–adrenal system leading to 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 et al., 2010). 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 Chandershakhar 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 hypothalamic-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, Chandershakhar 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 Chandershakhar 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

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

<table>
<thead>
<tr>
<th>Reproductive status</th>
<th>Cyclic %</th>
<th>Early pregnant%</th>
<th>Inactive ovaries %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot</td>
<td>70 (53.85)</td>
<td>15 (11.54)</td>
<td>45 (34.62)^a</td>
</tr>
<tr>
<td>Cold</td>
<td>137 (72.11)</td>
<td>30 (15.79)</td>
<td>23 (12.11)^b</td>
</tr>
</tbody>
</table>

a, b differs at P<0.05.

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reproductive status</th>
<th>Hot</th>
<th>Cold</th>
</tr>
</thead>
<tbody>
<tr>
<td>P4 (ng/ml)</td>
<td>Cyclic stage</td>
<td>Luteal phase</td>
<td>Smooth inactive ovaries</td>
</tr>
<tr>
<td>Cortisol (n mol/l)</td>
<td>Hot</td>
<td>0.7±0.03</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td></td>
<td>Cold</td>
<td>0.7±0.03</td>
<td>1.4±0.1</td>
</tr>
<tr>
<td></td>
<td>Hot</td>
<td>455.4±1.1.</td>
<td>378.4±4.3</td>
</tr>
<tr>
<td></td>
<td>Cold</td>
<td>425.2±5.4.</td>
<td>377.5±13.2</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>Hot</td>
<td>7.2±0.1</td>
<td>5.6±0.1</td>
</tr>
<tr>
<td></td>
<td>Cold</td>
<td>8.1±0.2</td>
<td>6.1±0.1</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>Hot</td>
<td>4.6±0.2</td>
<td>3.3±0.1</td>
</tr>
<tr>
<td></td>
<td>Cold</td>
<td>4.9±0.1</td>
<td>3.7±0.1</td>
</tr>
<tr>
<td>Globulin (g/dl)</td>
<td>Hot</td>
<td>2.6±0.2</td>
<td>2.3±0.1</td>
</tr>
<tr>
<td></td>
<td>Cold</td>
<td>3.2±0.3</td>
<td>2.4±0.3</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>Hot</td>
<td>106.8±4.1</td>
<td>78.5±2.8</td>
</tr>
<tr>
<td></td>
<td>Cold</td>
<td>87±3.6</td>
<td>43.6±1.9</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>Hot</td>
<td>95.5±4.8</td>
<td>56.2±8</td>
</tr>
<tr>
<td></td>
<td>Cold</td>
<td>76.5±5.2</td>
<td>34±1</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>Hot</td>
<td>84.5±2.7</td>
<td>41.6±2</td>
</tr>
<tr>
<td></td>
<td>Cold</td>
<td>253.5±8.2</td>
<td>320±2.8</td>
</tr>
<tr>
<td>SOD (u/ml)</td>
<td>Hot</td>
<td>290±5.8</td>
<td>347.9±9.3</td>
</tr>
<tr>
<td></td>
<td>Cold</td>
<td>251.5±11.9</td>
<td>429.9±12.7</td>
</tr>
<tr>
<td>Catalase level (U/l)</td>
<td>Hot</td>
<td>364.9±9.8</td>
<td>478±23</td>
</tr>
<tr>
<td></td>
<td>Cold</td>
<td>91±0.1</td>
<td>66.4±0.1</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>Hot</td>
<td>8±0.1</td>
<td>5.6±0.2</td>
</tr>
<tr>
<td></td>
<td>Cold</td>
<td>422±18</td>
<td>530±12</td>
</tr>
<tr>
<td>LDH level (u/l)</td>
<td>Hot</td>
<td>365±12.7</td>
<td>480±22.9</td>
</tr>
</tbody>
</table>

Means with different superscripts a, b, c, d, e, f, g, h are significantly different at P<0.05.
Sinah 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 gonadotrophic and anti-steroidalogenic 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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