ANTIOXIDANT/OXIDANT STATUS, METABOLIC PROFILE STATUS AND CONCENTRATION OF SOME INFLAMMATORY CYTOKINES IN EGYPTIAN BUFFALO-COWS SUFFERED FROM OVARIAN INACTIVITY

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ABSTRACT

Anestrous condition due to inactive ovaries is a major problem in buffalo-cows especially in summer season, which may decrease the profitability due to prolonged calving interval. The aim of the study was to evaluate the serum concentration of some oxidative biomarkers, metabolic and inflammatory indicators in Egyptian buffalo-cows suffered from inactive ovaries during summer season. Thirty pluriparous Egyptian buffalo-cows were included in the current study, fifteen cows were cyclic and the remaining 15 were, suffering from inactive ovaries. Blood samples were collected from both of the experimental groups for measurement of serum total anti-oxidant capacity (TAC), advanced oxidized protein products (AOPP), non-esterified fatty acid (NEFA), leptin hormone, inflammatory cytokines; tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and interleukin-1 beta (IL-1β) and inflammatory proteins (C-reactive protein and ceruplasmin). In comparison to normal cyclic buffalo-cows, buffalo cows suffered from ovarian inactivity showed higher concentration of AOOP, TNF-α, IL-1ß, IL-6, C- reactive protein, ceruplasmin and NEFA, but it showed lower concentration of TAC and leptin. The antioxidant/oxidant status and informatory cytokines may be implicated fundamentally in the development of ovarian inactivity.

Keywords: *Bubalus bubalis*, buffaloes, inactive ovaries, heat stress, oxidative stress, inflammatory indicators

INTRODUCTION

In buffaloes, anestrous is refereed to cessation of estrous cycle or poor expression of estrus signs. Summer anestrous is a major problem which may impair efficient reproduction and production in buffalo (Das and Khan, 2010), because it results in high economic loss as result of prolonged calving interval (Borghese *et al*., 1993). Ovarian inactivity is one of the most important causes of anestrous condition in buffalo-cows during the postpartum period (Singh *et al*., 2000; Gad El-Karim and Ghallab, 2020). It mostly occurs due to insufficient synthesis and/or secretion of the gonadotropins or failure of the ovarian stroma to respond to the gonadotropins. (Abraham, 2017). It is plausible that

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the imbalance between reactive oxygen species (ROS) production and antioxidants state inside the animal body may have a deleterious effect on different reproductive events including follicular development and steroidogenesis (Zhang *et al*., 2006; Al-Gubory *et al*., 2010). Moreover, excessive production of ROS may lead to lipid peroxidation, cell membrane alterations and disturbances, and DNA damage (Sugino, 2006). The previous scenario may result in activation of an immune reaction and inflammation (Chou *et al*., 2008). High concentration of inflammatory cytokines as TNF-α may impair many reproductive functions (Spicer and Alpizar 1994; Sheldon *et al*., 2009). Exposure of the animals to a high temperature as in the case of summer season in tropical and sub-tropical areas constitute an important form of stressful conditions that may result in oxidative stress in buffaloes (Kumar *et al*., 2014; Singh *et al*., 2016). Several studies have discussed the occurrence of summer infertility in buffalo-cows at its relation to the hormonal disturbance (Wakayo *et al*., 2015; Gad El-Karim and Ghallab, 2020), but, few of them have compared the concentration of the oxidative stress biomarkers and inflammatory cytokines between cyclic and cows with ovarian in activity (insert a reference). Therefore, the current study was designed to compare the serum concentration of some oxidative stress biomarkers, metabolic indicators, some inflammatory cytokines between cyclic buffalo-cows and that suffered from ovarian activity reared under heat stress condition.

MATERIAL AND METHODS:

Animals

Thirty pluriparous buffalo-cows weighting about 500 to 700 kg, at the age of 5 to 7 years, belonging to several small holders in Abis district, Alexandria province, Egypt were used in the current study during a period extend from June 2020 to August 2020. All cows were examined physically, trans-rectally and ultrasonographically to prove their freedom from any reproductive-related pathological condition. All cows were examined trans-rectally and ultrasonographically once weekly for three successive weeks to evaluate the ovarian activity The cows were assigned according to their ovarian activity into two groups (15 animals/each) as follow: Cyclic cows: This group include cows that showed a luteal tissue at least in one examination. Cows with Inactive ovaries: this group includes the cows that never showed any luteal tissue at any of the examinations. The ovaries of these cows were found small, spindle-shaped without any palpable structure. All of the animals were reared in small corrals made from bricks with a concrete ceiling and floor and have one door and window for ventilation. They were fed on wheat or rice straws basically, in addition to wheat or corn bran, corn silage was introduced sometimes when available. The water was provided *ad-libitum*. They were milked by hands two times/day once at the morning and the other at the afternoon with an average production of 5 to 7 kg of milk/day.

Blood sampling

Blood samples were collected from left jugular vein in plain vacutainers. After complete clotting, samples were centrifuged at 3000 rpm for 10 minutes for serum separation. Serum aliquots were transferred immediately and stored at -20°C till analysis time.

Biochemical analysis

The serum concentration of total antioxidant capacity (TAC) was measured calorimetrically using commercially kits (Biodiagnostic, Egypt)

according to the method of Koracevic *et al.* (2001). The serum concentration of tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β) and interleukin-6 (IL-6) were measured using highly specific enzyme-linked immune-sorbent assay based kits (Abcam, USA).The serum concentration of advanced oxidized protein product (AOPP) was assessed according to the method described by Witko-Sarsat *et al.* (1998). The serum concentration of C-Reactive protein (CRP) was evaluated using rapid latex slide test commercial kit (Spectrum, Egypt). The serum ceruplasmin concentration was measured by immuno-turbidimetry method using a commercial kit (Spectrum, Egypt).The serum concentration of non-stratified fatty acid (NEFA) was measured using commercially kits (Zen-bio, Inc., USA).The serum concentration of leptin hormone in serum was assessed using species specific double-antibody sandwich ELISA kits(Cloud-Clone Corp, USA).

Statistical analysis

The statistical differences in the serum concentration of the evaluated parameters between both of the experimental groups were measured using independent samples *t*-test by the aid of SPSS 16.0 for windows.

RESULTS AND DISCUSSIONS

The concentration of TAC indicator in serum was significantly lower and the concentration of the AOPP was significantly higher($P<0.001$) in buffalos suffering from ovarian inactivity condition when compared to regularly cycling buffalos (Table 1). The serum concentration of the TAC gives a cumulative view about both of enzymatic and non-enzymatic antioxidants state in the body (Gupta *et al*., 2011), which would deactivate reactive oxygen species and prevent its deleterious effects (Cheeseman and Slater, 1993). The result of the current study indicate that cows with ovarian inactivity was under a condition that may result in over-production of free radicals or reactive oxygen species and exhaustion of anti-oxidant defense systems that ended with a status of antioxidant/ oxidant imbalance (oxidative stress) (Trevisan *et al*., 2001; Williams *et al*., 2002). The status of the antioxidant/oxidant imbalance may disturb the physiological events responsible for controlling the estrous cycle and ended with ovarian inactivity as previously reported in the case of ovarian follicular cyst (Talukder *et al*., 2014). The serum concentration of pro-inflammatory cytokines TNF-α, IL-1β and IL-6 recorded were significantly higher ($P<0.001$) in the buffalo cows affected with ovarian inactivity compared to cyclic buffalo cows. This higher concentration of pro-inflammatory cytokines in serum of buffalo cows with ovarian inactivity indicate that theses cows are suffered from inflammatory or even stress condition. Higher concentration of inflammatory cytokines in buffalo-cows with ovarian inactivity is supported with higher concentration of acute phase proteins (ceruplasmin and C-reactive protein) in comparison to cyclic buffalo cows ($P<0.001$, Table 1). High concentration of TNF-α and IL-1ß has been found to suppress the release of gonadotropin-releasing hormone and the luteinizing hormone (Rivest and Rivier 1993; Yoo *et al*., 1997; Kalra *et al*., 1998; Watanobe and Hayakawa, 2003). In contrast, IL-6 had no inhibitory effect on the release of the GnRH (Watanobe and Hayakawa, 2003), however; it is a strong inflammatory inducer which can exaggerate the inflammatory state and the release of other inflammatory cytokines (Cronstein, 2007). The result of the current study showed that the

Table 1. Serum concentration of TAC, Aopp, TNF, IL-1ß, IL-6, CRP, Ceruplasmin, NSFA and leptin normal cyclic and cows with ovarian in activity:

Parameter	Cyclic cows (mean ± SE)	Cows with ovarian inactivity (mean ± SE)	P-value
Total antioxidant capacity (mM/L)	0.72±0.03***	0.50±0.02	0.001
Advanced oxidized protein product (µM/L)	61.70±2.94	91.16±2.26***	0.001
Tumor necrosis factor alpha (pg/ml)	165.76±6.90	245.34±12.00***	0.001
Interleukin-1 beta (pg/ml)	5.18±0.25	8.27±0.25***	0.001
Interleukin-6 (pg/ml)	159.23±9.06	239.10±10.65***	0.001
C-Reactive protein (mg/L)	2.16±0.27	5.37±0.47***	0.001
Ceruplasmin (mg/dl)	32.80±1.86	67.47±2.76***	0.001
Non-stratified fatty acid (µM/L)	288.46±9.94	415±17.88***	0.001
Leptin (ng/ml)	5.74±0.34***	3.86±0.15	0.001

serum concentration of NEFA was significantly higher, and the serum concentration of leptin was significantly lower in cows with ovarian inactivity compared with cyclic cows (Table 1). This indicates that the state of energy balance in cows with ovarian in activity was worse than that in normal cyclic cows. The state of negative energy balance mostly occurs during the early postpartum period as a result of imbalance between the food intake and the requirement. This condition results in rapid turn-over and mobilization of body fat and production of NEFA (Herdt, 2000). Negative energy balance is associated with delayed ovarian re-bound and ovarian dysfunction after calving (Beam and Butler, 1999; Vanholder *et al*., 2005). Lower concentration of leptin hormone in cows with ovarian inactivity indicates that these animals were under a state of food restriction or negative energy balance (Maffei *et al*., 1995). Leptin deficiency may inhibit the release of GnRH from the hypothalamus which ended with ovarian inactivity (Caprio *et al*., 2001). In conclusion, the result of the current study showed that in comparison to cyclic buffalo-cow, buffalo-cows with ovarian inactivity showed worse antioxidant/oxidant status, worse metabolic profile and higher concentration of inflammatory cytokines. All the previous factors may play a role in induction of the ovarian inactivity in buffalo-cows.

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