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Seasonal changes in some oxidant and antioxidant parameters during folliculogenesis in Egyptian buffalo☆

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ABSTRACT

Knowledge regarding oxidant and antioxidant status in follicular fluid remains limited and its studying *in vivo* should enhance our understanding of the impact of them on fertility and contribute to optimization of in vitro maturation conditions. The present study was conducted on follicular fluid and serum samples obtained from 708 buffaloes. They were examined for Malondialdehyde (MDA) as indicator of lipid peroxidation as well as superoxide dismutase (SOD) and total antioxidant capacity (TAC) as antioxidant markers. The obtained results revealed that MDA levels and SOD activity in follicular fluid decreased significantly as follicle size increased, while TAC increased significantly with the increase in follicular size. Whereas MDA level was significantly higher in summer, the TAC was significantly higher in spring. Moreover, MDA levels and SOD activities were significantly higher in the follicular fluid from different size follicles during the luteal phase than follicular phase. MDA levels in medium follicles in luteal phase and small follicles in follicular and luteal phases were significantly higher in summer than other seasons. Serum MDA levels were significantly increased in summer. In addition, MDA levels, SOD activities and TAC in serum were significantly higher during luteal phase than follicular phase in summer. TAC levels were significantly higher in follicular fluid than serum, while MDA was significantly lower in follicular fluid than serum. In conclusion, the present study revealed that oxidants/antioxidants balance may play a role in normal follicular development and oxidative stress that occur in summer could be related to reproductive seasonality in buffalo.

1. Introduction

Buffalo is an important world-wide species in terms of milk and meat production (Terzano et al., 2012). Within the ovarian follicle, the developing oocyte is surrounded by follicular fluid which is mainly derived from blood besides the locally produced substances (Nandi et al., 2008). Metabolic activity of follicular fluid, together with the 'barrier' properties of the follicular wall, is changing significantly during the growth and expansion of each follicle (Khan et al., 2011) and with the phase of estrous cycle (Kor and Moradi, 2013). Follicular fluid microenvironment can be regarded as a biological window providing a valuable insight into the process of normal follicular development as well as the pathogenesis of some reproductive problems in buffaloes (El-Shahat and Kandil, 2012). Heat stress has been of major concern in reducing buffaloes' productivity

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in tropical and sub-tropical areas (Silanikove et al., 1997).

According to Nour El-Din (2013), summer temperatures in Egypt are extremely high, reaching 38 °C to 43 °C. Based on the historical records over a period of twelve years (1999–2010), the subtropical climate in Cairo is characterized by hot summer season (June–August) with averages 23 °C–35 °C of minimum and maximum temperatures and 74% mean temperature humidity index (THI%). The optimum conditions for buffaloes as suggested by Payne (1990) are 13–18 °C and in terms of the mean temperature–humidity index (THI); values of THI > 72 is considered as stressful and THI > 78 is considered very severe heat stress to this animal.

The ovarian follicles contain their own potential sources of reactive oxygen species (ROS) which are potent stimulators of lipid peroxidation (Filaire and Toumib, 2012). However, ROS must be continuously inactivated by antioxidants to keep the oxidant/antioxidant balance to maintain normal cell function (Basini et al., 2008). Moreover, lipid peroxidation is most often induced by superoxide radical (O^{-}_{2}) and its damage is mainly inhibited by SOD which is an enzyme that contributes to the first line of antioxidant pathway as it removes the O^{-}_{2}, repairs cells and reduces the damage done to them by superoxide, the most common free radical in the body (Ayres et al., 1998). Total antioxidant capacity (TAC) covers both enzymatic and non-enzymatic antioxidants (Gupta et al., 2011) and its measuring considers the cumulative effect of all antioxidants present in plasma and body fluids (Sharma et al., 2013).

El-Sayed et al. (2010) reported that the antioxidant status can be considered as one determinant of reproductive function in bovine. The balance between ROS and antioxidants may have an important role in reproductive processes such as follicular development (Zhang et al., 2006). Megahed et al. (2008) stated that heat stress may affect fertility of animals through increased production of free radicals and according to Lasota et al. (2009), high levels of SOD are needed to neutralize increased lipid peroxidation in follicles that occurs in summer. The current study aimed to investigate the pattern of lipid peroxidation and two relevant antioxidant markers in follicular fluid of different size ovarian follicles and serum of buffalo considering the effect of seasonal changes and phase of estrous cycle.

2. Materials and methods

2.1. Ovaries

Ovaries were collected from 708 non-pregnant female buffaloes (Egyptian breed) in good health and with clinically normal reproductive tracts from local slaughterhouse located near to Cairo (Bahtim abattoir, Al-Qaliobia, Egypt). Immediately after slaughtering, both ovaries from each animal were collected in plastic bags containing 0.9% NaCl and transported in ice tank to be inspected at the laboratory.

2.2. Experimental design

Follicles were collected over one year during different seasons. The averages of minimum and maximum temperatures in summer (June–August) were 22 °C–35 °C with 56.0% mean relative humidity (RH), in autumn (September–November) 18 °C–28 °C with 60.3% RH, in winter (December–February) 10 °C–20 °C, with 58.0% RH, in spring (March–May) 15 °C–28 °C with 48.7% RH. The stage of estrous cycle (follicular or luteal) was identified according to the presence or absence of the corpus luteum on the ovary according to Jaglan et al. (2010) and Mondal et al. (2004). Follicular diameter was measured using a caliper and follicles were divided into three categories: small (≥3 mm), medium (4–9 mm) and large (≥10 mm) according to Dominguez (1995). Ovaries with cystic follicles as well as the morphologically atretic follicles, identified macroscopically by their opaque wall (Ali et al., 2008) were excluded from the study.

2.3. Sampling

2.3.1. Follicular fluid

The contents of the ovarian follicles of different size (small, medium and large) were aspirated using a 10 ml syringe attached to an 18 gauge needle and centrifuged at 3000 rpm for 10 min for separation of the fluid from the cell fraction. Follicular fluids from each group from each pair of the ovaries were pooled in one sample for each individual buffalo. No sample pooling was needed for the large size category. Collected follicular fluid samples were kept at −20 °C until analysis.

2.3.2. Blood

Samples were collected during slaughtering for serum separation after centrifugation at 3000 rpm for 10 min. Collected serum samples were kept at −20 °C until analysis.

2.4. Measured parameters

2.4.1. MDA

MDA level was determined colorimetrically according to the method of Ohkawa et al. (1979) using kits purchased from Biodiagnostic, Egypt.

2.4.2. SOD

SOD activity was estimated kinetically using SOD assay kits (Biodiagnostic, Egypt) according to Nishikimi et al. (1972).

2.4.3. TAC

TAC level was determined colorimetrically according to the method of Koracevic et al. (2001) using kits purchased from Biodiagnostic, Egypt.

2.5. Statistical analysis

The differences among different follicular sizes and seasons of the year were analyzed statistically by one-way ANOVA. The differences between follicular and luteal phases as well as between the average concentrations of