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Effect of the stage of estrous cycle on follicular population, oocyte yield and quality, and biochemical composition of serum and follicular fluid in Anatolian water buffalo

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ABSTRACT

This study was designed to investigate the effects that the different stages of the estrous cycle had on the number of surface ovarian follicles and oocyte yield and quality of Anatolian water buffalo during peak breeding season. Assessments were made on the basis of ovarian morphology, serum and follicular fluid concentrations of variety of biochemical parameters. Following slaughter, blood samples were collected from each animal. The stage of estrous cycle was classified as either the luteal or follicular phase, and surface ovarian follicles were classified as small, medium, or large. The follicular fluid was aspirated, and oocytes were evaluated microscopically for classification into four categories. No statistical differences ($p > 0.05$) were observed regarding the total number of follicles or quality of oocytes relative to the stage of the estrous cycle. Serum high-density lipoprotein cholesterol (HDL), alkaline phosphatase (ALP) and progesterone (P4) concentrations were significantly higher in the luteal phase than in the follicular phase ($P < 0.05$). Significant correlations were observed in the luteal phase between the total number of oocytes and cholesterol (Cho), HDL, sodium (Na), chloride (Cl); A-quality oocytes and Na, Cl, Mg; C-quality oocytes and Cho, HDL, and Mg in follicular fluid. These results offer new information concerning Anatolian water buffalo reproductive physiology, which may be useful for improving oocyte quality in buffalo. This is the first study to describe the number of ovarian follicles, oocyte yield and quality, and a variety of biochemical parameters in the serum and follicular fluid of Anatolian water buffalo during peak breeding season in Turkey.

1. Introduction

Buffaloes are an economically important livestock species in many Asian, Mediterranean, Latin American and European countries (Azawi et al., 2009; Perera, 2011). In sub-tropical environments and at higher latitudes the reproductive function of buffalo is similar to that of small ruminants (Zicarelli, 1994) in that it is positively influenced by decreases in daylight hours, thereby classifying these animals as polyestrous short-day breeders (Zicarelli, 1990; Baruselli, 1994; Boni et al., 1994). Anatolian water buffalo are classified as a river-type buffalo belonging to a Mediterranean group (Cocrill, 1974), and are raised primarily for milk and meat production by subsistence farmers. The milk is typically used for making cream (kaymak) and certain kinds of cheese, and the meat is preferred for making a Turkish style of sausage because of its color (GDAR, 2011).

High environmental temperatures are known to have a detrimental effect on the overall oocyte yield, quality, and developmental competence of buffalo oocytes recovered

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from slaughterhouse ovaries (Nandi et al., 2001). In Italian Mediterranean buffalo, it has been demonstrated that in the spring-summer season (between March and July) there is a decrease in both the number of follicles and cumulus oocyte complexes (COC's) compared with the winter–spring season (between November and April) (Di Francesco et al., 2011). Interestingly, ovarian morphology, presence of the corpus luteum (Boediono et al., 1995) and reproductive status (Leroy et al., 2004a) have also been shown to affect the oocyte yield and quality (Gandolfi et al., 1997).

Oocytes are located in the follicular fluid, where oocyte growth and maturation takes place (Iwata et al., 2004). The follicular fluid provides a very important microenvironment for the development of oocytes, because it is the product of both the transfer of blood plasma constituents that cross the blood follicular barrier and of the secretory activity of granulosa and theca cells (Fortune, 1994). Therefore, metabolic changes in blood serum affect in the biochemical composition of the follicular fluid and indirectly influence oocyte quality (Ali et al., 2008) and because, reproductive status and season is known to affect the content of follicular fluid (Eissa, 1996) with certain metabolites changing significantly during estrous cycle (Abd Ellah et al., 2010), this may also affect the overall quality of buffalo oocytes (Kruip and Dieleman, 1985).

To the authors' knowledge, this is the first study on ovarian morphology, oocyte yield, oocyte quality, and follicular fluid contents in Anatolian water buffalo. Therefore, the aim of this study was to evaluate Anatolian water buffalo during peak breeding season for data regarding (1) the effects of the stage of estrous cycle on the number of surface ovarian follicles, oocyte yield, oocyte quality, and the oocyte quality index (OQI) in the ovaries; (2) blood serum and follicular fluid concentrations of variety of biochemical parameters; and (3) a correlation between the biochemical composition of the follicular fluid and oocyte quality.

2. Materials and methods

2.1. Collection of ovaries and blood

Ovaries from non-pregnant Anatolian water buffalo over 4 years of age, in good health, and having clinically normal reproductive tracts upon macroscopic examination post-slaughter were used for this study. Ovaries completely absent of any stage of corpus luteum (including corpus albicans) or having cystic follicles (greater than 20 mm in diameter) were excluded from the investigation. Collection of all samples was performed between November and March of the peak breeding season of buffaloes in abattoirs located in the cities of Afyonkarahisar (latitude, 38°45′2″N; longitude, 30°32′3″E; altitude, 1015 m; mean temperature, 11.2 °C) and Samsun (latitude 41°34′16″N; longitude, 35°51′56″E; altitude, 20 m; mean temperature, 16 °C). Approximately 10 mL of jugular blood was collected from each animal after slaughter, and the serum was separated by centrifugation and stored at −20 °C until used for biochemical analysis.

2.2. Morphological study

This experiment examined the effect that the stage of estrous cycle had on the number of surface ovarian follicles and also oocyte yield, oocyte quality, and oocyte quality index (OQI) in the ovaries of Anatolian water buffalo ($n = 31$ pairs). Ovaries were transported to the laboratory in pairs in numbered plastic bags stored in an ice box. In the laboratory, the stage of the estrous cycle was classified according to Nandi et al. (2008) as luteal phase (new, active CL, red to pink in color, with dominant and subordinate follicles present) or follicular phase (regressing CL, orange, yellow or white, with a dominant follicle up to preovulatory size). Follicular diameter was measured on the ovarian surface using calipers, and the follicles were classified into 3 categories as small (<3 mm), medium (3–8 mm) or large (>8 mm) in accordance with to Nandi et al. (2008). For each buffalo, all follicles of each size in both ovaries were counted.

Follicular fluid was aspirated from the surface ovarian follicles using a 20 G needle connected to a 10 mL sterile syringe. The follicular contents were then transferred into 60 mm glass petri dishes. Oocytes were collected and evaluated under a stereomicroscope by the same technician each time. The oocytes were classified on the basis of their morphological appearance (Brackett and Zuelke, 1993) as follows: Grade A, oocytes with more than 4 layers of cumulus cells and homogenous cytoplasm; Grade B, oocytes with complete or partial (2–4) layers of cumulus cells and homogenous cytoplasm; Grade C, denuded oocytes with non-homogenous cytoplasm. Non-spherical, granulated, vacuolated, or fragmented oocytes were classified as degenerated. Total oocyte recovery and quality were recorded. An index was calculated to reflect overall oocyte quality ([Grade A*1 + Grade B*2 + Grade C*3 + Degenerated*4]/Total oocytes recovered) (Sales et al., 2008). For example: 8*1 + 4*2 + 3*3 + 0*4/15 = 1,66. Values around 1 reflect good overall oocyte quality.

2.3. Biochemical study

This investigation was designed for 2 purposes: (1) to evaluate serum and follicular fluid concentrations of various biochemical parameters including glucose, cholesterol (Cho), total proteins (TP), triglyceride (TG), blood urea nitrogen (BUN), creatinine (Cre), high-density lipoprotein cholesterol (HDL), alkaline phosphatase (ALP), calcium (Ca), magnesium (Mg), phosphorus (P), sodium (Na), potassium (K), chloride (Cl), and progesterone (P4) during the peak breeding season in 22 healthy Anatolian water buffalo; (2) to identify any correlation between biochemical composition of follicular fluid and overall oocyte quality. Follicular fluid was collected from follicles measuring 3–8 mm in diameter by aspiration with a 20 G needle connected to a 10 mL sterile syringe. A different needle and syringe were used for each individual buffalo. The oocytes were collected and evaluated under stereomicroscope. The aliquots of follicular fluid were kept in a test tube and were stored at −20 °C for subsequent biochemical analysis. The