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Changes in luteal cells distribution, apoptotic rate, lipid peroxidation levels and antioxidant enzyme activities in buffalo (*Bubalus bubalis*) corpus luteum

S. Selvaraju*, B.S. Raghavendra, T. Siva Subramani, R. Priyadharsini, I.J. Reddy, J.P. Ravindra

Reproductive Physiology Laboratory, Animal Physiology Division, National Institute of Animal Nutrition and Physiology, Adugodi, Bangalore 560 030, India

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ABSTRACT

Buffalo (*Bubalus bubalis*) is known for its weak/silent estrous behaviour, lower conception rate and longer inter-calving interval as compared to cattle. Understanding the kinetics and functional properties of luteal cells may be helpful to improve reproductive efficiency in the buffalo. Hence the present study was designed to assess the size and distribution of steroidogenic luteal cells along with biochemical properties during different phases of corpus luteum (CL) in the buffalo. The ovaries collected from the local abattoir were classified into three phases, early, mid and late, based on the morphological appearance of the CL as well as the follicles in the ovary. The proportion (%) of the luteal cells (>10 μm diameter) increased ($P<0.01$) from early (30.7 ± 1.3) to mid (36.30 ± 1.6), and then decreased ($P<0.01$) in late luteal (31.46 ± 1.8) phases. Percentage of small luteal cells (10–20 μm diameter) was higher ($P<0.05$) in early (58.47 ± 0.61) and mid (61.29 ± 0.67) than late luteal (37.18 ± 1.50) phases of CL. However, the percentage of large luteal cells (20–50 μm diameter) was higher ($P<0.05$) only in late (62.82 ± 1.50) than early (41.53 ± 0.61) and mid (38.71 ± 0.67) phases of CL. The average size (μm) of the large luteal cells increased ($P<0.05$) from early (25.46 ± 0.62) to mid (27.15 ± 0.5) and late (28.86 ± 0.47) luteal phases. The percentage of luteal cells expressing *in situ* DNA fragmentation was significantly ($P<0.05$) higher in the late luteal (41.17 ± 5.8) than mid-luteal (21.15 ± 4.9) phase of the CL. In the early stage, half of the steroidogenic luteal cells had significantly ($P<0.05$) less 3β-HSD activity than the other two phases. In the mid stage, the steroidogenic luteal cells had significantly higher ($P<0.05$) intense 3β-HSD activity than the other two phases. Further in the late phase, a significant ($P<0.05$) reduction in intense 3β-HSD activity was observed in the large luteal cells. The lipid peroxidation (μmol/g of CL) levels were significantly ($P<0.05$) higher in late luteal (3.46 ± 0.2) than the mid-luteal (1.43 ± 0.16) phases. The superoxide dismutase and catalase enzyme levels (U/mg of protein) were also significantly ($P<0.05$) higher in late luteal (0.9 ± 0.015 and 3.37 ± 0.45, respectively) than the mid-luteal (0.1 ± 0.01 and 2.34 ± 0.3, respectively) phases. In contrast, the GPx activity (U/mg of protein) decreased significantly ($P<0.05$) from mid-luteal (1.85 ± 0.4) to late luteal (1.22 ± 0.2) phases. The present study suggests that (i) the decrease in progesterone levels in late CL may be associated with loss of 3β-HSD activity in large luteal cells and (ii) demise of the buffalo CL may be mediated by apoptosis despite the high levels of luteal antioxidant enzymes.

* Corresponding author. Tel.: +91 80 25711304; fax: +91 80 25711420.
E-mail address: selvarajuars@rediffmail.com (S. Selvaraju).

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1. Introduction

Mammalian CL contains cells with different anatomical features and biological functions (Fitz et al., 1982; Lei et al., 1991). The mature CL contains both steroidogenic (large and small luteal cells) and nonsteroidogenic (endothelial cells, pericytes, smooth muscle cells, macrophages, leucocytes and occasional plasma cells) cells (Koos and Hansel, 1981). Small and large luteal cells have been reported to originate from theca and granulosa cells, respectively (Alila and Hansel, 1984). During CL development, theca derived small luteal cells also transform into large luteal cells (Alila and Hansel, 1984). During luteolysis, these steroidogenic cells may break up into discrete membrane bound structures containing variable amounts of condensed chromatin or cytoplasm followed by ingestion by the macrophages or neighbouring cells leading to decrease in progesterone concentration (Wyllie et al., 1980).

The structure, histochemistry and regulatory mechanisms of the CL have been well documented in bovine (Lei et al., 1991; Zheng et al., 1994), but information as such on the above aspects is meagre in buffaloes, a species of great economic importance in Southeast Asia and the Middle East. In buffaloes, there is a high incidence of embryonic losses resulted in lower conception rate and longer inter-calving interval as compared to cattle (Agarwal and Tomer, 2003), understanding the kinetics and functional properties of luteal cells may provide information on how to improve reproductive efficiency in this species. Buffalo CL is smaller in size, does not protrude well over the surface of ovary and secretes less progesterone as compared to cattle (Agarwal and Tomer, 2003). Distribution of steroidogenic luteal cells has been characterized in human (Lei et al., 1991), bovine (Lei et al., 1991; Zheng et al., 1994), horse (Al-Zi'abi et al., 2002), sheep (Fitz et al., 1982) and goat (Kalender and Arikan, 2007), but not in buffalo.

The corpus luteum contains high levels of antioxidant enzymes including superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione-*S*-transferase and glutathione reductase (GPx). The antioxidants protect luteal cells against oxygen radicals produced during steroidogenesis (Chew et al., 1984). These oxygen radicals may also be functional in leading to luteolysis and apoptosis in corpus luteum during each reproductive cycle (Riley and Behrman, 1991a). The reactive oxygen species does not always cause luteal cell apoptosis and reactive oxygen species-induced apoptosis may be dependent on animal species (Sugino, 2006). It has been reported that the steroidogenic capacity of the bovine corpus luteum shows major changes during the estrous cycle (Rapoport et al., 1998), but whether the same protective mechanisms against oxidative damage prevail at different phases of corpus luteum development in the buffalo has not been determined. Buffalo is known for its weak/silent estrous behaviour, longer inter-calving interval and low conception rate as compared to cattle (Agarwal and Tomer, 2003). Studies, therefore, on the corpus luteum may provide for better understanding of kinetics of luteal cells to enhance reproductive efficiency in buffaloes.

Hence, the present investigation was designed to study the distribution of small and large luteal cells, histomorphological characteristics and biochemical properties of the CL in buffalo.

2. Materials and methods

2.1. Collection of samples

Ovaries from non-pregnant buffaloes bearing CL were collected from the local abattoir and classified into three phases i.e. early (days 3–5, $n = 6$) mid (days 6–15, $n = 11$) and late luteal phase (days 16–20, $n = 9$) as described earlier in cattle (Ireland et al., 1980) with a modification. The corpus luteum with numerous blood vessels and ovulatory depression was classified into early stage. The CL having a clear neck with ovarian stroma was classified into mid stage. Since in buffalo, embedded CL is characteristic feature, the brownish appearance of the surface of the CL after decapsulation was also considered as a mid stage. The corpus luteum with invariably less blood vessels associated with loss of brownish pigmentation and appearance of the whitish colour was considered as late phase CL. The CL was dissected, decapsulated and its fresh weight was recorded.

2.2. Distribution of luteal cells

2.2.1. Dissociation of luteal cells

The CL was digested with 0.2% collagenase in TCM-199 containing BSA (0.5%), HEPES (25 mM), DNase (0.02%), gentamicin (30 μg/ml) and streptomycin–penicillin (100 units/ml) for 3 h in a orbital shaker incubator at 37 °C. The dispersed luteal cells were filtered through a 70 μm pore size cell strainer (BD Falcon, Bedford, MA, USA) to remove tissue debris. The filtrate was centrifuged ($900 \times g$ for 10 min) to pellet the luteal cells. The cells were washed with fresh medium to remove the collagenase by centrifugation ($900 \times g$ for 10 min). The supernatant was discarded and the cells were resuspended in fresh medium.

2.2.2. Identification of luteal cell types

Initial identification of steroidogenic luteal cells was based upon the characteristic granular appearance of the cytoplasm (Fraser et al., 1999) under the phase contrast microscope (Nikon, Eclipse E200, Tokyo, Japan). Morphological identification was also confirmed by 3β-HSD activity and Oil red O staining.

2.2.3. Cell counting

The luteal cells fraction was transferred to a hemocytometer and the total number of cells was counted. Nonluteal cells were eliminated based on their features of not being round, smaller in size and highly condensed nuclei compared to luteal cells. Cells having >10 μm size were considered as luteal and <10 μm as nonluteal (Lei et al., 1991). The diameters of randomly selected 500 steroidogenic luteal cells were measured for each corpus luteum. The exact size of the small and large luteal cells was calculated with Image J version 1.3 analysis software distributed in the public domain by the NIH (NIH, Bethesda, MD, USA, http://rsb.info.nih.gov/ij/).