EVALUATION OF FOLLICULAR ATRESIA AND ELECTROPHORETIC PATTERN OF FOLLICULAR FLUID PROTEINS IN ACYCLIC BUFFALO (*Bubalus bubalis*)

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ABSTRACT

Histological examination of H&E stained sections of ovaries collected from cyclic and acyclicbuffaloes (n=6/group) was done in order to evaluate the degree of follicular atresia. The percentages of healthy and atretic follicles were different (P<0.05) between cyclic (53.1% and 46.9%, respectively) and acyclic (10.7% and 89.3%, respectively) buffaloes. Electrophoretic patterns of follicular fluid proteins, studied by SDS-PAGE of follicular fluid samples aspirated from small (5.0-6.9 mm), medium (7.0-9.9 mm) and large (≥ 10 mm) follicles of cyclic and acyclic buffaloes (n= 6/group), did not reveal any apparent differences between the groups. DNA fragmentation patterns evaluated by using DNA isolated from the cell pellets obtained after centrifugation of the follicular fluid samples from small-, medium- and large-sized follicles of cyclic and acyclic buffaloes (n=5/ group) showed a typical apoptotic oligonucleosome ladder pattern in the acyclic group. In conclusion, these results indicate an increased rate of follicular atresia without any qualitative changes in the follicular fluid proteins during ovarian acyclicity in buffalo.

Keywords: buffaloes, *Bubalus bubalis*, ovary, follicle, atresia, follicular fluid

INTRODUCTION

Follicular development in buffalo is a continuous process with concurrent growth and regression of ovarian follicles occurring during the reproductive cycle. Similar to those in cattle, follicular dynamics in buffalo follows a wave pattern with predominance of two-wave cycles (Baruselli *et al.,* 1997). During each follicular wave, there is recruitment of a group of follicles (cohort) out of which usually one is selected for progressive growth to become the dominant follicle while the others undergo regression. Dominant follicles can have variable fates-ovulation, persistence or atresia, depending upon several factors like physiological state, stage of the cycle, pathological conditions or hormonal treatments etc. An increased rate of follicular atresia has been implicated as one of the major factors for reproductive failure in buffalo (Rajesha *et al.,* 2001). Atresia of ovarian follicles is associated with various morphological, biochemical and histological changes (Kaipia and Hsueh, 1997). A recent study on the follicular characteristics during ovarian acyclicity in buffalo showed that all the large-sized (≥ 10 mm) follicles

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are estrogenically inactive, which indicates a derangement in the normal follicular development culminating in follicular atresia rather than ovulation (Khan and Das, in press). Studies on biochemical composition of the follicular fluid demonstrated alterations in certain components like nitric oxide, ascorbic acid, glucose, cholesterol and alkaline phosphatase during acyclicity in buffalo leading to the conclusion that oxidative stress caused by the imbalance of nitric oxide and ascorbic acid possibly results in follicular atresia manifested as inactive estrogen status and increased alkaline phosphatase levels in the follicular fluid (Khan and Das, in press; Khan *et al.,* 2011). Available evidence suggests that follicular fluid protein concentrations are not affected by the reproductive state of the animal (Khan *et al.,* 2011). However, the effect of acyclicity on the electrophoretic pattern of follicular fluid proteins remains unknown. The objectives of the present study were to evaluate follicular atresia in ovarian follicles of acyclic buffaloes using ovarian histology and DNA fragmentation patterns (oligonucleosome ladder pattern) characteristic of apoptosis, and to examine the changes in the electrophoretic pattern of follicular fluid proteins during acyclicity in buffalo.

MATERIALS AND METHODS

Ovaries were collected from cyclic and acyclic buffaloes (n=6/group) at a local abattoir and transported to the laboratory on ice within 30 minutes. The ovaries were fixed in 10 % formalin, embedded in paraffin wax, cut into thin sections of 5-7 µm thickness and stained with haematoxylin and eosin (H&E) using routine histological staining procedure (Luna, 1968). The stained slides were examined by light microscopy for histological characteristics of the ovarian follicles, especially the granulosa and theca cell layers. Classification of follicles into healthy and atretic was done as described previously (Guraya, 1979).

Follicular fluid was aspirated from small, medium-sized and large follicles of cyclic and acyclic buffaloes (n=6/group), centrifuged at 1000 g and 4°C for 10 minutes and the supernatant was collected for analysis. The electrophoretic pattern of follicular fluid proteins was studied by a standard protocol (Laemmli, 1970) using 10% polyacrylamide gel in a vertical slab gel apparatus (Bangalore GENEI, India).

For studying the apoptotic oligonucleosome pattern, the cell pellets obtained after centrifugation of the follicular fluid samples from small-, medium- and large-sized follicles of cyclic and acyclic buffaloes (n=5/group) were used. DNA was isolated using a DNeasy Kit as per the protocol recommended by the manufacturer (Quiagens Ltd.). For visualizing the probable presence of the oligonucleosome ladder pattern characteristically shown by apoptotic DNA fragments (Hermmann *et al.,* 1994), 10 µl of DNA sample obtained was electrophoresed in 2% agarose gel containing 1 µg/ml of ethidium bromide and visualized in a UV-Gel Documentation system (Syngene, UK).

Comparison of percentage normal and atretic follicles between cyclic and acyclic groups was done by Chi-square test using STATS™ Version 1.1, Decision Analyst Inc., USA.

REFERENCES AND DISCUSSION

The percentages of healthy and atretic follicles were different ($P<0.05$) between cyclic (53.1% and 46.9%, respectively) and acyclic (10.7% and 89.3%, respectively) buffaloes. The concomitant

presence of both healthy and atretic follicles in cyclic buffalo ovaries indicates that growth and regression of follicles occur concurrently further confirming the previous observations that follicular development is a continuous process in buffalo (Baruselli *et al.,* 1997). Based on the presence of an almost equal proportion of healthy and atretic follicles in cyclic buffaloes, it seems apparent that growth and regression of follicles occur at a similar rate during the estrous cycle. The presence of both healthy and atretic follicles in acyclic buffaloes provides support to our earlier notion that follicular development does continue during acyclicity as well (Khan and Das, in press). The predominance of atresia in acyclic buffalo ovaries is in line with observations during acyclicity based on estrogenic status (Khan and Das, in press) and the findings of Guraya (1979) who reported an increased incidence of atresia in buffalo ovaries during anestrus. A prominent apoptotic DNA fragmentation (oligonucleosome ladder) pattern was observed in DNA isolated from the granulosa cells and oocytes of all the three follicle size categories (Figure 1; lanes 1, 2 and 3) in acyclic buffaloes. In contrast, no such pattern was evident in the cyclic group (Figure 1; lanes 4, 5 and 6). The higher percentage of atresia indicated by the above results compared to the observations on histology can be inferred to be a reflection of changes occurring in the DNA earlier than the manifestation of the morphological alterations during follicular atresia.

SDS-PAGE analysis of follicular fluid proteins did not reveal any apparent difference in the overall electrophoretic pattern between acyclic and cyclic groups (Figure 2). Pertinently, in an earlier study on the biochemical composition of follicular fluid (Khan *et al.,* 2011), no difference in the total protein concentration was recorded between cyclic and acyclic buffaloes. Based on these observations, it can be concluded that there are no qualitative or quantitative alterations in the protein component of the follicular fluid during acyclicity in buffalo.

In summary, results of the present study indicate an increased rate of follicular atresia characterized by histological alterations in the follicle and fragmentation of granulosa cell DNA without any characteristic change in the electrophoretic pattern of follicular fluid proteins during ovarian acyclicity in buffalo.

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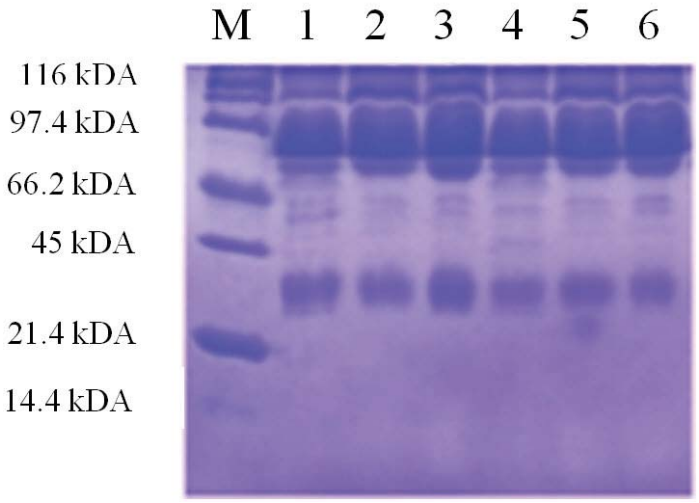

Figure 1. Electrophoretic pattern of follicular fluid proteins (Lane M-Marker; Lanes 1-3: small, medium-sized and large follicles of the acyclic group, respectively; Lanes 4-6: small, medium-sized and large follicles of the cyclic group, respectively).

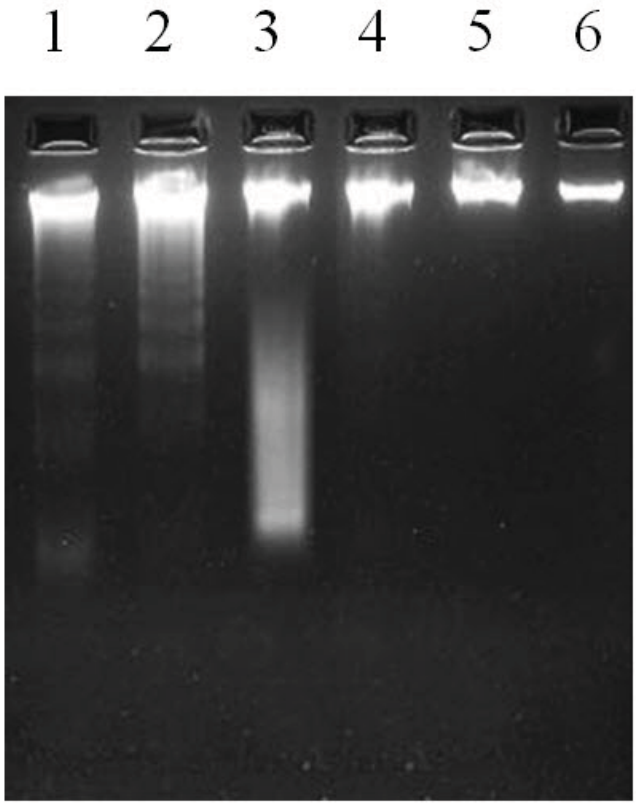

Figure 2. Granulosa cell DNA fragmentation pattern (Lanes 1-3: small, medium-sized and large follicles of the acyclic group, respectively; Lanes 4-6: small, medium-sized and large follicles of the cyclic group, respectively).

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