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Quantification of the Ovarian follicles of water Buffaloes (Bubalus bubalis)

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Abstract

Ovarian samples were collected either through ovariectomy or slaughtering of Philippine water buffaloes at the Philippine Carabao Research and Development Centre (now Philippine Carabao Centre), Central Luzon State University, Philippines. From the young buffalo (6 –7 months, 2 pairs of ovaries); pubertal (2 year; n=1 pair); adults (7 –8 year; n = 3 pairs) and old (12 – 14 years; n = 3 pairs) were used for the study. The ovaries were fixed, dehydrated and embedded in paraffin wax and were serially sectioned at 10 microns. The sectioned ovaries were then stained with periodic acid/schiffs-Hematoxylin (PAS-H). Ovarian follices of the following categories (primary, growing, secondary, tertiary and atretic) were quantified in the young, pubertal, adult and old water buffaloes. In the young and pubertal buffaloes, the average follicular population of 75,604 and 85,358 primary follicles were counted, respectively. The numbers of primary follicle decreased as age of the animal increased, meaning the follicles are either ovulated or become atretic during the course of the estrous cycle. The growing follicles were highest in the pubertal animals with an average follicle number of 7,317, which is 8.75 percent of the primordial follicles. The young and adult buffaloes had an average count of 71 and 18 growing follicles respectively. The tremendous increase in the number of growing follicles during the pubertal stage may indicate that follicle are continually being recruited and they grow from stage to stage and end life by becoming atretic or become ovulated. The number of secondary follicles found in each age group decreased with age. The high number of secondary follicles counted in the pubertal revealed that there was a transition of the growing follicles to the secondary stage. Further decline in the number of tertiary follicle occurred, while the average number for tertiary follicles in the young, adult and old were 3,9,7, respectively which is quite low. Data showed that there was a high incidence of atresia occurring in the ovarian follicles of buffaloes ranging from 127 in the young to 142 in the pubertal.

Keywords: Quantification, Ovarian follicle and water buffaloes

Introduction

The water buffalo is a very important source of meat and draft in the Philippine subcontinent. Feeding of the water buffalo is cheap, to a large extent and is based on feeding of roughage with a high proportion of cellulose, which can hardly be digested by other ruminants. This makes the buffalo a very important animal for small-holders and landless labourers and their families. Besides draught power for farming, water buffalo provides milk and meat which when sold yield cash income to the owner (Smith, 1990).

For the owners, it is very important that good fertility be maintained in their buffalo herds as economic production depends on regular calving. However, compared to cattle, especially the exotic breeds, the water buffalo has a number of reproductive problems like late maturity, seasonal variation in fertility, poor heat symptoms and anoestrous conditions, which make it difficult to obtain optimal economy of production (Settergren, 1988). More frustrating is the poor response of buffaloes to superovulatory regimes which have been successfully used in cattle and this can hardly be explained (Cruz, 1988. Personal Communication). It would therefore be of interest to study the reproductive capacity of the water buffalo and one way to do this is to make quantitative investigation of the follicular dynamics of the ovaries.

Materials and Methods

Nine pairs of ovaries were collected either through ovariectomy or slaughter of water buffaloes at the Philipine Carabao Centre (formerly Philippine Carabao Research and Development Centre, PCRDC), Nueva Ecija, Philippines with complete records of births. The animals were classified as young (6 – 7 mo), pubertal (2years), adult (7 –8years) and old (12 – 14 years). From the young, 2 pairs of ovary were obtained, 1 pair from the pubertal, 3 pairs from the adult and 3 pairs from the old. The right and left ovaries were also obtained from each age group of buffalo.

Processing of ovaries for histology

Immediately after collection, the ovaries were trimmed of excess tissues, blotted on a paper towel and were fixed in 10% formol saline. It was then transported to the laboratory for dehydrating, embedding, sectioning and staining according to the procedure outlined by Ruthmann (1970) and Wheater *et al.*, (1985), (although slightly modified depending on available chemicals in laboratory). All ovaries were serially sectioned at 10 microns using a microtome. The tissue sections were placed on a slide and a small amount of Canada balsam was placed on a cover slip and was gently lowered down the slide and pressed to avoid bubbles.

Counting under the microscope

The number of primary, growing, secondary, tertiary and atretic follicles present in the stained ovarian tissues was counted under the microscope at various magnifications (mostly of 20x and 40X) and the values recorded. The number of follicles in every 100th section was counted and the number of follicles per ovary was obtained by multiplication by 100. This procedure was adopted following observations by Zuckerman (1951) that oocytes surrounded by a single layer of follicular epithelium or called primary follicles make up 90% of the follicles in an ovary and can therefore be regarded as a reflection of the total oocyte content of an ovary. To verity the validity of the first count, a second count was done 3 months (later) after the first count. The average number of primary follicles at the second counting was 6% lower than the first count. A minimal difference of 6% between two counting was accepted in the present research.

Results and Discussion

Number of primary follicle. Data on the number of primary follicles in the ovaries of Philippine water buffaloes at different ages are given in Table 1. The data showed that the young and pubertal buffaloes have an average count of 75,604. and 85,385 primary follicles respectively. The adult buffalo had only 5,997 primary follicles which was only 7.93 percent in the young and 7.02 percent of the follicles in the pubertal animal. In the old buffalo, there were only 3,226 primary follicles counted. Theoretically, follicle population decreased as the animal grew older due to many follicles becoming atretic.

Number of growing follicles. Presented in Table 1 are the data on the number of growing follicle for the different age groups of water buffalo. The pubertal animals had the highest number of growing follicles with an average of 7,317, representing 8.57 percent of the number of primary follicles. The young and adult buffaloes had an average of 71 and 18 growing follicles respectively. The tremendous increase in the number of growing follicle during the pubertal stage may indicate that an information or signal was sent to the ovary at this stage of the reproductive life, which elicited such response that culminated in accelerated growth of smaller follicles. Also, in support of this was evident on the number of surface follicles with large amount of follicular fluid. These are ovulatory follicles, which will become atretic because of insufficient gonadotropins. A maximum number 7 – 8 large surface follicles were observed to be bulging on the ovary of the pubertal buffalo.

Table 1 *Average number of primary, growing, secondary, tertiary and artretic follicles in buffalo ovary*

	Age group			
Follicle Class	6 –7 months	2 years	7 –8 years	12-14 years
Primary Follicle	75,604	85,358	5,997	3,226
Growing Follicle	71	7,317	18	17
Secondary Follicle	33	598	14	8
Tertiary Follicle	3	81	9	7
Atretic Follicle	127	142	139	138
Pairs of ovary (N)	2	1	3	3

The advantage of the growing follicle can be efficiently utilized by superovulating the pubertal buffaloes. As was demonstrated in rats (De Reviers and Mauleon, 1979), Sheep (Sonjaya and Driancourt, 1987) and pigs (M.A. Driancourt, unpublished results) massive follicular growth compared to adult numbers occurred in prepubertal and pubertal animals, thus making it possible to superovulate cattle as soon as 1 –2 months of age (Seidel, *et al.*, 1971). A possibility of improving the ovarian response to exogenous gonadotropins in buffaloes would be to superovulate prepubertal and pubertal buffaloes.

Number of secondary follicles. The number of secondary follicles found in each age group decreased with age. The high number of secondary follicles counted in the pubertal buffaloes revealed that there was a transition of the growing follicles to secondary. An average of 598 secondary follicles was counted in the pubertal animals which was only 8.17 percent of the growing follicles. The average number of secondary follicles in the young and adult were 33 and 14 respectively (Table 1) while the old had only 8 follicles. The large number of secondary follicles in the pubertal animal was related to the activity of the hypothalamo-pitutary-ovarian axis previously explained.

Number of tertiary follicle. Further decline in the number of tertiary follicles was observed for respective age group. The number of follicles present in the pubertal buffalo was 81 (13.54%) of the secondary follicles, while the average number of tertiary follicles in the young and old were 3 and 7 respectively (Table 1). This information suggests that pubertal buffalo may be a potential source of greater number of oocytes when treated with superovulatory scheme.

Number of atretic follicle. Atretic follicles could be identified by the presence of pyknotic granulosa cell nuclei and the pulling away of the follicular epithelium from the theca interna. Both of these histological features were symptoms of granulosa cell death. Data presented in Table 1 show that there was a high incidence of atresia occurring in buffaloes ranging from 127 in the young to 142 in the pubertal. Data have shown that under normal physiological conditions, once a follicle began to degenerate in vivo, it would probably not return to the ovulatory pathway (Hirschfield, 1989). It is surprising to note that the numbers of growing, secondary and tertiary follicles in the young, adult and old were quite low while the incidence of atresia was high. Danell (1987) reported that in buffalo heifers of Surti breed the atresia frequency was much higher than in cattle.

About two-thirds (66.67%) of all follicles >1mm were atretic. There was relatively more atresia in the smallest follicles than in the larger ones. The combined effect of a low number of follicles and higher frequency of follicular atresia in buffaloes means that the number of available normal follicles >1mm is only about 30% of what it is in cattle. Also, the high incidence can be supported by the observation of Sirois and Fortune (1988) that ovarian follicles > 5mm in heifers occurred in waves. If ten, follicles that were still growing in size were commited to atresia due to may be insufficient support from gonadotropins, then the large numbers of atretic follicles observed in the different ages of buffaloes might have been contributed by such follicles.

The observation that ovarian follicle > 5mm occurred in waves could also be an effect of inhibition from other large-sized follicles. In the wave hypothesis results of daily ultrasonic monitoring of individually identified follicles in heifers Knopf *et al.*,(1980) shows that the first wave was identified retrospectively on the average, day = 0 day of ovulation and the second wave on day 10 when the follicles in the waves were 4 to 6mm in diameter. A follicle in the first wave became dominant, grew to a mean diameter of 16mm on average on day 7, and began to regress on day 11. The dominant follicle of the second wave was the ovulatory follicle. The surbodinate follicles in each wave increased in diameter for a few days after first detection of the wave and then ceased growing or regressed. It thus seemed that the largest dominant follicles present in the ovary would inhibit the growth of the subordinate follicle and of smaller follicles, and would promote atresia without allowing any of these to become the largest follicle.

In general, the number of primary follicles tended to decrease at a very fast rate from puberty onwards and the decrease rate appeared to decline with age. Such pattern of change could be explained by the fact that significantly more primary follicles tended to grow from the pool. This was clearly indicated in Table 1 in which the number of growing follicles in pubertal animal was about 100 fold more than that noted in young buffaloes. Since the follicles recruited to grow underwent the development sequence, the significantly more number of secondary and tertiary follicles in the pubertal animals only reflected the transformation of the growing follicles into the subsequent stages. However, what was apparent in the process seems to be very low efficiency of transformation indicating that tremendous losses occurred from one stage to another. This high rate of recruitment from the pool and the high rate of loss from one stage to the other may accounts for the fast decline rate of follicle noted to start at puberty age.

Recruitment of follicles from the pool appeared to be highly active at puberty than any of the other age groups covered in the study. In fact, at this stage, the number of growing follicles represented about 8.5 percent of the follicular pool and was significantly greater than that noted in the young animal (0.09%) or older groups representing only about 0.3 percent to 0.52 percent. The shifts in the transformation rate of the follicles as the animal reached puberty was readily understood. What is interesting to note was the implied high degree of responsiveness of the primordial follicles to the recruitment signal at puberty than at any other age thereafter. A review of the hormonal yielded no clear explanation as the levels of gonadotropins tended to become even a little higher with age. The implied high degree of responsiveness to the recruitment signal in younger animal was also seen in the transformation of follicles to growing stage despite the relatively weak signal reaching the ovary. This observation may also imply that the transformation of follicles may constitute some inherent signal as well as the signal requiring maturity of the hypothalamic-pitutary-gonadal axis.

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