Nig. J. Anim. Prod. 2005, 32(2): 308 - 314

Growth and replacement of the ovarian follicles during the estrous cycle in philippine water buffaloes

O.F. Smith

Dept. Forestry and Wildlife Management University of Agriculture, Abeokuta, Nigeria.

Abstract

Six normally cycling Philippine buffaloes were selected from a herd after showing normal estrous cycle length. Follicular development was monitored using a simple fiberscope. This procedure allowed the chance to follow the pattern of growth and the population of the surface follicles at specified days (3,6,9,12,15,18 and 21) of the estrous cycle. The following endpoints were used for each ovary: diameter of surface follicles and population of the various sized follicles. The follicles were classified into small-sized follicles (1-3mm), medium-sized follicles (4-7mm) and large sized follicles (>8mm). The results of the growth and replacement of follicles showed that the mean number of small – sized follicles (1-3mm) was greater (P<.01) on days 3,9,12,15,18 and 21. The medium-sized follicles (4-7mm) were greater in number on days 12,15,18 and 21 while the large-sized follicles (>8mm) were observed to be different (P<.01) on days 15,18 and 21.

Keywords: Simple fiberscope, growth, ovarian follicles, buffaloes.

Introduction

Considering the growing population of Asia and the world, an exiguous technology that could multiply the buffaloes within a shorter period is importunately needed. The focus on ovary is necessary because the total ovarian follicular populations also relates with ovulation rate in the adult buffalo. Understanding the mechanisms that regulate selection of species specific number of follicles to ovulate may require a thorough knowledge in changes that occur in populations of follicles during an estrous cycle.

In follicular growth, signals are sent to the ovary to stimulate the growth of the follicles from the total number of follicular population that is developed during fetal life or soon after birth. This means that the follicles depend on hormones (gonadotropins) to reach ovulatory size. However, not all follicles presented (whether the follicles destined to ovulate were already enlarged at the beginning of the cycle and that they only underwent little increase in size until preovulatory enlargement or the follicles went through waves of follicular growth during the estrous cycle) are destined to ovulate at the day of estrus. Only one of the many follicles ovulate while the rest become atretic during the course of growth.

Previous studies have generated hypotheses about the pattern of growth and regression of ovarion follicles during the estrous cycle in cattle (Spicer and Echternkamp, 1986). Follicular development during the estrous cycle in cattle occurs in a pattern of two or three waves (Savio *et al.*, 1988; Knopf *et al.*, 1989 and Townson *et al.*, 2002).

The focus on ovary is necessary because the total ovarian follicular population also relates with ovulation rate in the adult buffalo. To understand the mechanisms that regulate selection of specific number of follicles for ovulation, each species may require a thorough knowledge of the relationship between morphometric and endocrine changes that occur in populations of follicles during an estrous cycle. In cattle, a single follicle is ovulated which can be identified by its size about 3 days before the onset of estrus when there are one or two large follicles in the ovaries. In sheep, one or two large follicles secrete more estrogens and bind more gonadotropins or granulose cells than smaller follicles. In sows, recruitment of follicles into the ovulatory population continues during the follicular phase. Thus, the development of smaller follicles may be promoted rather than inhibited by larger "dominant" follicles. The objective of the study was to determine the pattern of growth and regression of ovarian follicles during the estrous cycle in Philippine water buffaloes using a simple fiberscope.

Materials and Methods

Location of study.

The experiment took place at the experimental station of the Philippine Carabao Centre (PCC) Carranglan, Digdig, Philippines (PCC was formerly PCRDC, Philippine Carabao Research and Development Centre). Carranglan town is in the Northern part of the Philippine Islands, South-Asia.

Experimental animals.

Six normally cycling Philippine buffaloes with an average age of 8.58$\pm$1.17 years old and average live weight of 365.67$\pm$ 62.51 kg were selected from a herd. The animals were selected after showing normal estrous cycle length for two consecutive cycles having a mean of 21.75$\pm$0.75 days. The animals were allowed to graze freely during daytime and were confirned at night and were also given concentrate-mineral supplement and molasses at 250g/kg of total dry matter intake. The pasture grazed in this experiment was dominated by perennial grasses, *mainly Andropogon species, Bracharia* and *Sporobolus species, Cenchrus ciliaris, Panicum phrimitoides and Elionurus pubigunii.* The native legumes occurring in the area were *Tephrosia practeolata, Cassia rotindofolia, Desmodium velitinum and Alyscarpus species.* It was during the dry season that the animals were grazed from 6.00a.m to 6.00p.m each day

Observation of ovarian structures during the estrous cycle.

The experimental animals were monitored for the onset of natural estrus with the aid of a vasectomized bull. Onset of estrus was marked as day O. The observation of the structures on the ovary were performed on every 3 days apart initiated on day 3 of the estrous cycle until the end of subsequent estrus. At every observation period, the animals were confined in the pen and were taken off feed for at least 6 hours prior to actual observation. After completing the serial observations on the first estrous cycle in 6 animals, 3 buffaloes were randomly identified and were again subjected to the same serial observations until the onset of another subsequent estrus.

Observations of the ovarian structures were performed with the use of a simple fiberscope (Model FS-100, Scott Fiber Optics Inc., 122

Charlton St., Southbridge, MA 01550). The device had tow fiber bundles, the inner bundle serves as the image bundle while the illuminating bundle surrounds it and carries the light from the bulb located in the bundle to the viewing area (Smith and Cruiz, 2002). The fiberscope was guided into the pelvic cavity close to the position of the ovary by inserting it through the cannula puncturing the floor of the vagina, right at the base of the cervix.

Puncture at the vaginal floor was made with the trocar and cannula after treating the animas with local anesthesia. The trocar was withdrawn leaving the cannula with its tip touching the ovary. To facilitate the observation of ovarian structures, another individual palpated the ovary gently through the rectum and brought it close to the fiberscope tip. The use of the fiberscope in observing ovarian structures has been previously validated in the laboratory (Smith and Cruz, 2002). This was done by measuring different objects and also measuring follicles from ovaries collected from the slaughterhouse. *In vitro*, efforts were made to view both sides of the ovaries. At any given observation period, the size and relative positions of the structures were recorded. In this manner, any particular follicle in the ovary could be monitored with near accuracy. This procedure allowed the chance to follow the pattern of growth and the population of the surface follicles at specified day (3,6,9,12,15,18 and 21) of the estrous cycle. The following end points were used for each ovary; diameters of surface follicles and population of the various sized follicles. The follicles were classified into small-sized follicles (1-3mm), medium-sized follicles (4-7mm) and large-sized follicles (>8mm).

Statistical analysis

Data collected were analysed using the standard analysis of variance (SAS, 1988). Differences between treatment means were separated by Duncan Multiple range test (Duncan, 1955).

Results

Based on the analysis of follicular growth pattern on the various days of observation, there were distinct peaks within the cycle particularly with reference to the number of large follicles. Since the peaks may be considered the point of maximal growth, which are followed by the decline in follicular number, each rise and decline of follicular population indicates a continuous growth and replenishment of follicles. The mean number of the small follicles (1-3mm) was greater ($P<0.01$) on days, 3,12,15,18 and 21 than on days 6 and 9 of the estrous cycle (Table 1) with the largest number on day 3.

The mean number of medium follicles (4-7mm) increased numerically on the same days as that of small follicles, however, without significant differences between days. The mean number of large follicles was more pronounced ($P< 0.01$) on days 3,15,18 and 21. These were results obtained for the first 6 animals observed.

The second set of observations involving three animals showed that the follicular growth pattern in the small follicles (1-3mm) increased on days 3,9,12,15, 18 and 21 while in the medium-sized follicles (4-7mm), the increase in number was observed on days 12, 15, 18 and 21. These were significant at $P<0.01$. For the large-sized follicles, although no significant differences were obtained in the result, follicles size differed slightly on days 3,15 and 21.

Discussion

In the first 6 animals observed, the pool of small-sized follicles on days 3,12,15,18 and 21 of the cycle apparently indicated a continuous replenishment of the follicular sizes. Since all other follicle sizes depended on how many small-sized follicles could be recruited from the pool of preantral follicle in the ovary, it was possible

Table 1: The growth pattern and mean number of follicles throughout the estrous cycle in water buffaloes.

SIZE OF FOLLICLES	**DAYS OF CYCLE** 3	6	9	12	15	18	21
				N=6 animals			
Small							
(1 to 3mm)	12.00 ± 1.63^{a}	6.67 ± 1.79^{a}	8.67 ± 2.13^{da}	9.67 ± 1.75^{abcd}	10.83 ± 0.90^{abcd}	11.83 ± 0.90^{abcd}	11.83 ± 1.07^{ab}
Medium							
(4 to 7mm)	3.67 ± 1.25 3	$.67\pm2.43$	4.5 ± 1.5	5.5 ± 1.38	6.17 ± 1.95	6.5 ± 2.69	6.67 ± 1.79
Large							
(>8mm)	1.5 ± 0.5^{abcd}	0.83 ± 0.69^{d}	0.33 ± 0.47^{d}	0.8 ± 0.69^{d}	2.17 ± 0.37^{abc}	2.5 ± 0.5^{ab}	2.5 ± 0.9^{a}
				N=3 animals			
Small							
(1-3mm)	12.00 ± 0.82^{a}	8.67 ± 0.47^{a}	11.33 ± 0.94^{ab}	11.33 ± 0.94^{abc}	10.00 ± 0.82^{abcde}	11.33 ± 0.94^{abc}	11.33 ± 0.47^{ab}
Medium							
(4-7mm)	5.00 ± 1.41^{d}	6.00 ± 0.00^{bcd}	6.00 ± 0.82^{bcd}	7.67 ± 0.47^{abcd}	8.33 ± 0.94^{abc}	9.33+1.25a	9.00 ± 0.82^{ab}
Large							
(<8mm)	1.67 ± 0.47	0.67 ± 0.47	1.00 ± 0.82	0.67 ± 0.94	2.33 ± 1.25	1.67 ± 0.94	2.33 ± 0.47

abcde Means with the same letter within the row are not different (P>.01).

that these follicles might have encountered gonadotropin in the course of their development, especially follicle stimulating hormone (FSH). The gonadotropin follicle stimulating hormone responsible for recruitment of follicle from the pool of preanral follicles and FSH which was observed to increase and decrease abruptly immediately after luteinizing hormone (LH) peak in cows might have been the reason why a greater number of small-sized follicles were observed on day 3. In cows (Matton *et al.*, 1981) showed that small-sized follicles were more numerous at the beginning of the estrous cycle (day 3) than at other stages. The increase in numbers of small-sized follicles seen after estrus in buffaloes in the present study might be because of follicle stimulating hormone (FSH), which have been observed in the cow. Hirsclifield and Midgley (1978a) have shown in rats that FSH peak promotes development of antral follicles from preantral stages. An alternative working hypothesis was that growth of preantral follicles into the pool of follicles observable by fiberscope might have occurred at a relatively constant rate and that the increased number of 1-3mm follicles on day 12 and at the time of ovulation may have been due to atresia of follicle in the larger follicular categories.

This was supported by the observation of large-sized follicles presented between days 9 to 12 which later regressed and were sometimes accompanied by observable mucus discharge. This may be understood from two stand points namely: the estrogen peak on days 8 to 11 of the estrons cycle in cows (Shemesh *et al.*, 1972 and Glencross *et al.*, 1973) and secondly, Sirois and Fortune (1988) which showed that ovarian follicle >5mm in heifers occurred in waves meaning that follicular turnover was characterized by the presence of waves of follicular growth beginning at different times during the estrous cycle. The typical symptoms of heat in the middle of an estrous cycle in-spite of a functioning corpus luteum are a well-known problem in buffaloes. This was described in the recent literatures (Kavani and Kodagali, 1984; Danell et al., 1984; Danell, 1987). In these studies, the buffalo heifers had plasma progesterone levels, which were within normal limits for this stage of the cycle. The estrogen levels were not measured but the heifers had at least one large follicle each, which could have produced an increased amount of estrogen. The progesterone levels proved that the corpus luteum was functioning. On the other hand, buffaloes are known to be sensitive to estrogens and therefore the midcycle estrus may be caused by the estrogens produced by the large follicles at midcycle. For buffalo owners as well as for the artificial inseminators (AI) this might be very confusing. If AI is carried out at this stage of the cycle it might lead to endometritis and missed opportunities of fertilization.

In the second set of observations (n=3 animals), the medium-sized follicles showed an increase on days 12,15,18 and 21. This might have resulted from the growth of the large pool of small-sized follicles presented earlier in the cycle. The growth stimulus of small follicles might have been the result of a systemic or local (intraovarian) action of estrogens. Several authors have shown that estrogen has a positive effect on follicular growth (Harman *et al.*, 1975; Batta, 1976) and that in cows, there is an estrogen peak between days 8 and 11 (Shemesh *et al.*, 1972; Glencross *et al.*, 1973). Another thesis to support this observations is that the period from days 0 to 6 is associated with growth of the corpus luteum and increasing plasma progesterone concentrations, while the period from days 16 to 20 is associated with luteal regression and decreasing progesterone concentrations (Henricks *et al.*, 1970). That is, the growth in terms of the increase in mean diameter of the antral follicle and preovulatory follicle occurred immediately before and after ovulation, respectively, when progesterone concentrations were low.

The process by which the follicle destined to ovulate is selected is not properly understood. However, under this present study, the process of selection of the follicle destined to ovulate appeared to become manifested as selective growth of the medium-sized follicles increase in mean number of large-sized follicle. A similar process apparently occurred in the luteal phase, days 9 to 12. There was apparent selective growth to preovulatory size, coincident with little increase in the mean number of medium and large-sized follicles. The only apparent difference was that the follicle which attained preovulatory diameter early in the interovulatory interval or luteal phase remained in the ovary for sometime, maximum of two days, then regressed. Whereas, the follicle which attained preovulatory diameter at approximately day 18 ovulated a few days later. Pierson and Ginther (1988) similarly concluded that selection of the follicle destined to ovulate occurred between days 16 and 17 (equivalent to day -4 to day –3, preovulation).

Conclusion

In conclusion, it would be mentioned that a number of follicle observed in water buffaloes in the small-sized follicle category (1-3mm) were quiet low than those obtained in cows. In cows, average of 20 small-sized follicles were counted compared with 12.00$\pm$1.5 on the average in this study with buffaloes. This means there is a low exit of preantral follicles to the small-sized follicles. The number of large-sized follicles are highly dependent on the number of small-sized follicles recruited from the preantral stage.

References

Batta, K.S. 1976. A study of follicular atresia in organ culture. *Soc. Study Reprod. 9th Annu. Meet. Abstr. 87.*

Danell, B. 1987. Estrous behaviour, ovarian morphology and cyclical variation in follicular system and endocrine pattern in water buffalo heifers. Thesis, Department of Obstetrics and Gynecology. Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala, Sweden. Pp 124.

Danell, B., N. Gopakumar, M.C.S. Naiv and K. Rajagopalan, 1984. Heat symptoms and detection in Surti buffalo heifers. *Indian J. Anim. Sci. Reprod* 5:1-7

Duncan, D. B. 1955. Multiple range and Multiple F. Test. *Biometrics* 11:1-42

Glencross, R.G., I.B. Munro, G.E. Senior and G.S. Pope, 1973. Concentrations of estradiol -17, estrone and progesterone in jugular venous plasma of cows during estrous cycle and in early pregnancy. *Acta Endocrinology.* 73:374

Harman, S.M., J.P. Louvet and G.T. Ross. 1975. Interaction of estrogen and gonadotropins on follicular atresia. *Endocr.* 96:1145.

Hanricks, D.M., J.F. Dickey and G.D. Niswender. 1970. Serum luteinizing hormone and plasma progesterone levels during the estrous cycle and early pregnancy in cows. *Biol. Reprod.* 2:346.

Hirschfield, A.N. and A.R. Midgley. 1978a. Morphometric analysis of follicular development in the rat, *Biol. Reprod.* 19:597-605

Kavani, F.S. and S.B. Kodagali. 1984. clinical investigation of repeat breeder buffaloes for ovulatory disturbances. *Indian J. Anim. Reprod.* 5:38-42

Knopfi, L., J. P. Kastelic, E. Schallenberger and O. J. Ginther. 1989. Ovarian follicular dynamics in heifers: Test of two-wave hypothesis by ultrasonically monitoring individual follicles. *Domest. Anim Endocrinol.* 8:209-221.

Matton, P., V. Adelakoun, Y. Couture and J.J. Dufour 1981. Growth and replacement of the bovine ovarian follicles during the estrous cycle. *J. Anim. Sci.* 52 (4): 813-820.

Pierson, R.A. and O.J. Ginther. 1988. Follicular population during the estrous cycle in heifers. III. Time of selection of the ovulatory follicle. *Anim. Reprod. Sci.* 16:81-95.

SAS, 1988. SAS Users guide, Release 603 Edition SAS Institute Inc. Gary NC. U.S.A.

Savio, J. D., L. Keenan, M.P. Boland and J. F. Roche. 1988. Pattern of growth of the dominant follicles during the estrous cycle of heifers. *J. Reprod. Fertil.* 83:663-671

Shemesh, M., N. Ayalon, and H.R. Lindner. 1972. Estradiol levels in the peripheral blood of cows during the estrous cycle. *Endocr.* 55:73

Sirois, J. and J.E. Fortune, 1988. Ovarian follicular dynamics during the estrous cycle in heifers monitored by real-tie ultrasonography. *Biol. Reprod.* 39:308-317.

Smith, O.F. and L.C. Cruz. 2002. Development of per vaginal route of serial observations of ovarian activities in buffalo using a simple fiberscope. *Nig. J. Anim. Prod.* 29 (1): 132-137

Spicer L. J. and S. E. Echternkamp. 1986. Ovarian follicular growth, function and turnover in cattle: A review. *J. Anim Sci.* 62:428.

Townson, D. H., P. C.W. Tsang, W. R. Butter, M. Frajblat, L. C. Griel, Jr., C. J. Johnson, R. A. Milvae, G. M. Niksic and J. L. Pate 2002. Relationship of fertility to ovarian follicular waves before breeding in dairy cows. *J. Anim. Sci. 80:1053-1058.*

(Received 15 January 2003 ; Accepted 15 October 2004).