

## Biometry of buffalo (*Bubalus bubalis*) ovaries in relation to different stages of the oestrous cycle\*

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Received : August 5, 2003

Accepted : December 6, 2003

### ABSTRACT

Ovaries from non-descript pluriparous buffaloes collected from slaughter house were grouped into four stages based on the characteristics of the corpus luteum and morphometry of the ovary were recorded during different stages of the oestrous cycle. Follicles were grouped according to the diameter as  $\leq 2$ , 3-5, 6-10 and  $>10$  mm. Biometry of ovaries on left and right side was not found to differ significantly and also between stages of the cycle. However, length, diameter and weight of the corpus luteum showed high significant difference during the four stages of the cycle. The mean number of visible follicles between right and left ovaries did not differ significantly. The number of follicles of  $\leq 2$  mm diameter size increased at two times of the cycle indicating two waves of follicular growth in buffalo ovaries.

**Key words :** Biometry, ovary, follicle, corpus luteum, buffalo

Buffalo is a multipurpose animal reared mainly for milk and is also used for meat production and draught purpose. More than half of Indian milk production is obtained from buffalo and hence it plays a pivotal role in the dairy development. Fertility records among buffaloes however, show lower level of reproductive efficiency. Available reports are suggestive of a lower number of follicular population in buffalo ovaries. However, a clear and thorough knowledge of the structure and folliculogenesis to optimize the reproductive efficiency in buffaloes is scanty. Hence, the present investigation was undertaken to study the biometry of the buffalo ovaries at different stages of oestrous cycle.

### MATERIALS AND METHODS

One hundred and eighty one pairs of ovaries of non-descript pluriparous buffaloes at a random stage of reproductive cycle were collected from local slaughter house and were transported to the laboratory in physiological saline within 30 minutes after slaughter at 30-38°C. Based on the characteristics of the corpus luteum

(Zemjanis, 1962), the oestrous cycle was fixed into four stages at stage I (Day 1-4), stage II (Day 5-10), stage III (Day 11-17) and stage IV (Day 18-20). Length, width and height of the ovaries were measured as pole to pole, surface to surface and hilus to free border, respectively with the help of vernier caliper with an accuracy of 0.02 mm. Weight (g) of right and left ovaries were recorded by using a monopan balance after trimming the extraneous tissue of each ovary and the size of the each ovary was calculated using the formula of Singh and Singh (1988).

**Corpus luteum :** After noting the blood supply and colour, the externally visible portion of the corpus luteum was gently squeezed from the ovarian surface and the weight was taken by using monopan balance. After making mid-line section of corpus luteum mass, the colour inside was observed and the final length was measured. The diameter of the corpus luteum at the widest portion was measured. The fluid filled cavities (Lacunae) were recorded, if present, in the cut surface of the corpus luteum.

**Follicle :** The number of visible follicles were identified and counted in each ovary and their diameters were measured, with a dial vernier caliper and the follicles were grouped according to their size as  $< 2$ ; 3-5, 6-10 and 10 mm diameter groups. The data collected were analysed as per the standard procedure described by Snedecor and Cochran (1967).

\*Part of Ph.D. thesis of first author submitted to Tamil Nadu Veterinary & Animal Sciences University, Chennai-600 051

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## RESULTS AND DISCUSSION

The mean length, width and height of right and left ovaries at four stages of oestrous cycle in buffaloes are presented in Table 1. The statistical analysis showed no significant variation in the biometry of ovaries between sides. In both the ovaries, the length was minimum and maximum during the first and second stage of the cycle, respectively. The length increased from first to second stage of the cycle and gradually decreased to the fourth stage. The overall mean length of right and left ovary was  $2.06 \pm 0.03$  and  $2.00 \pm 0.04$  cm, respectively which was found to be shorter when compared to observations of Damodaran (1955),

was  $1.35 \pm 0.03$  and  $1.30 \pm 0.03$  cm, respectively. The width of the ovary of non-descript Indian buffaloes was almost similar to the observations made by Luktuke and Rao (1962). In this study the overall mean weight of the right and left ovaries were  $2.86 \pm 0.06$  and  $2.77 \pm 0.06$  g, respectively with a range of 0.55 to 4.92 g. The mean weight reported by Luktuke and Rao (1962) was more by a gram than the weight observed in this study for both the ovaries, while those reported by Damodaran (1955) was slightly less in non-descript buffaloes. But the results of the present investigations were in agreement with the findings of Napoleon (1989). The ovarian weight was maximum during the second stage of the cycle

**Table 1. Biometry of ovaries of non-descript she buffaloes**

Ovary	Stage of the cycle	Length (cm)	Width (cm)	Height (cm)	Weight (g)
Right	I (44)	$1.88 \pm 0.05$	$1.32 \pm 0.04$	$1.43 \pm 0.02$	$2.67 \pm 0.09$
	II (46)	$2.27 \pm 0.04$	$1.39 \pm 0.04$	$1.79 \pm 0.04$	$2.97 \pm 0.12$
	III (46)	$2.15 \pm 0.06$	$1.38 \pm 0.04$	$1.64 \pm 0.05$	$3.01 \pm 0.11$
	IV (45)	$1.91 \pm 0.07$	$1.31 \pm 0.05$	$1.52 \pm 0.04$	$2.79 \pm 0.10$
	<b>Overall Mean (181)</b>	<b><math>2.06 \pm 0.03</math></b>	<b><math>1.35 \pm 0.03</math></b>	<b><math>1.60 \pm 0.02</math></b>	<b><math>2.86 \pm 0.06</math></b>
Left	I (46)	$1.89 \pm 0.02$	$1.29 \pm 0.04$	$1.41 \pm 0.06$	$2.66 \pm 0.08$
	II (46)	$2.14 \pm 0.05$	$1.31 \pm 0.04$	$1.51 \pm 0.05$	$2.86 \pm 0.10$
	III (46)	$1.99 \pm 0.07$	$1.35 \pm 0.05$	$1.61 \pm 0.06$	$2.89 \pm 0.13$
	IV (45)	$1.89 \pm 0.09$	$1.25 \pm 0.06$	$1.49 \pm 0.04$	$2.65 \pm 0.13$
	<b>Overall Mean (181)</b>	<b><math>2.00 \pm 0.04</math></b>	<b><math>1.30 \pm 0.03</math></b>	<b><math>1.51 \pm 0.03</math></b>	<b><math>2.77 \pm 0.06</math></b>

Figures in parenthesis are the number of ovaries

Luktuke and Rao (1962) and Napoleon (1989). This might have been due to the variations in the climatic managerial conditions, sample size and sampling variance. Further the changes in four stages of the cycle might be attributed to ovarian cyclical activity. The overall mean height of the right and left ovary was  $1.60 \pm 0.03$  cm, respectively. This was almost similar to the observation made by Luktuke and Rao (1962). Though there was a slight variation in the height of both sides of the ovaries, the statistical analysis revealed no significant difference in height between the stages of the cycle. In both the ovaries height was the least during the I stages of the cycle.

The width was almost equal in all the four stages of the cycle and between the sides. Statistical analysis revealed no significant difference in width between the four stages of the cycle. The overall mean width of the right and left ovaries

and maintained as such till the end of the III stage of the cycle. There was no significant difference in weight of the left and right ovaries in buffaloes in the present study. This was agreement with the findings of Luktuke and Rao (1962) and Khan (1987).

**Corpus luteum** : The colour of the corpus luteum was observed as bright red in the first stage of the cycle, flesh colour with reddish tip in the second stage, tan in the third stage and grey or white in the fourth stage of the cycle. These findings were in concurrence with the findings of Napoleon (1989).

The average length, diameter and weight of the corpus luteum during the four stages of the cycle were shown in Table 2. Analysis of variance of length, diameter and weight of the corpus luteum revealed that differences between stages were highly significant ( $P < 0.01$ ). The overall

**Table 2. Biometry of corpus luteum during four stages of oestrous cycle in she buffaloes**

Stage of the cycle	Length (cm)	Diameter (cm)	Weight (g)
I (44)	0.68±0.02	0.62±0.02	0.75±0.03
II (46)	1.47±0.03	1.01±0.03	1.13±0.01
III (46)	1.027±0.02	1.04±0.02	1.11±0.01
IV (45)	0.94±0.02	0.87±0.02	0.77±0.03
Mean (181)	1.09±0.01	0.89±0.02	0.93±0.01

Figures in parenthesis are the number of pairs of ovaries

mean length, diameter and weight of the corpus luteum was 1.09±0.01, 0.89±0.02 cm and 0.90±0.01 g, respectively. El-wishy *et al.* (1988) found that the mean diameter and weight of the dioestrus corpus luteum were 1.6±4.2 mm and 1.9±0.48 g and were much higher than the values observed in the present study. This indicated that the corpus luteum of Indian buffaloes seemed to be smaller as reported by Luktuke and Rao (1962).

The length, diameter and weights of the corpus luteum were the least in the first stage of the oestrous cycle and it grew significantly in the subsequent stages. The maximum size was reached during the II stage of the cycle and there was no marked reduction in size until the end of the III stage of the cycle. Similar observations were made by Mc-Nutt (1924) and Ireland *et al.* (1979).

**Follicle:** Mean number of follicles of ≤ 2, 3-5, 6-10 and >10 mm diameter in four stages of the cycle are presented in Table 3. The distribution of total number of follicles during the different stages of the cycle was comparable to the

observations made by Skyer *et al.* (1987) in cows. The mean number of follicles on the ovarian surface was 3.83 and 3.45 in the right and left ovaries, respectively which was not significantly different but less than those reported by Kruij (1982) and Moor *et al.* (1984) in cows. This less number of follicles per ovary in buffalo might be due to the presence of low number primordial and Graafian follicular population in buffalo ovaries (Madan, 1990 and Totey *et al.*, 1991). The mean number of follicles was more (4.18±0.11) during the IV stage than in other stages of the oestrous cycle which was comparable to the observations made by Skyer *et al.* (1987) in cow. Total number of follicles were less during the second and third stage of the cycle as compared with the IV stage. In I stage, the mean number of follicles per ovary was slightly lower when compared with the IV stage of the cycle.

The mean number of < 2 mm follicles was more during the IV stage of the cycle which gradually decreased to I and II stage and there was an increase in the III stage of the cycle indicating two waves of follicular activity in buffalo

**Table 3. Mean number of different stages of follicles in four stages of the estrous cycle**

Signature of the cycle	Ovary	Follicular diameter ranges (mm)				No. of follicles/ovary
		≤ 2	3 - 5	6 - 10	> 10	
I (21)	Right	0.91±0.18	1.86±0.19	0.91±0.18	0.13±0.01	3.78±0.17
	Left	1.00±0.12	1.62±0.14	0.67±0.13	0.14±0.01	3.39±0.12
	Mean	0.96±0.12	1.74±0.13	0.79±0.13	0.14±0.08	3.59±0.13
II (26)	Right	0.42±0.14	1.96±0.16	1.00±0.14	0.16±0.02	3.54±0.12
	Left	0.35±0.12	1.85±0.15	0.89±0.13	0.19±0.02	3.28±0.14
	Mean	0.39±0.11	1.91±0.11	0.95±0.12	0.18±0.07	3.41±0.11
III (42)	Right	0.72±0.11	1.62±0.13	0.95±0.10	0.33±0.01	3.62±0.11
	Left	0.62±0.12	1.48±0.11	0.81±0.06	0.24±0.10	3.31±0.09
	Mean	0.67±0.08	1.55±0.09	0.88±0.09	0.29±0.05	3.39±0.12
IV (32)	Right	1.37±0.16	1.67±0.13	1.00±0.19	0.40±0.11	4.38±0.14
	Left	1.22±0.12	1.40±0.09	1.13±0.19	0.23±0.11	3.98±0.12
	Mean	1.27±0.09	1.54±0.10	1.07±0.11	0.31±0.06	4.18±0.11

Figures in parentheses are the number of ovaries

ovary. The present investigation confirmed the findings of Matton *et al.* (1981) and Skyer *et al.* (1987) who reported that a large pool of small antral follicles developed during the preovulatory and early luteal periods, and might be associated with the rise in both FSH and LH prior to ovulation. The present investigation showed that the mid luteal phase was a period of diminished ovarian activity and there was significantly fewer follicles of smaller sizes compared with follicular phase. Similar observations were made by Matton *et al.* (1981) and Ireland and Roche (1988) in cows. The ovary bearing corpus luteum had more larger follicles than the ovary bearing the corpus albicans. Several authors have reported similar findings in cows (Dufour *et al.*, 1971; Fogwell *et al.*, 1977; Dailey *et al.*, 1982) and in buffaloes (Hafez, 1955).

In this present investigation, there was no significant difference in the distribution of follicles between the sides of the ovaries. This clearly indicated that both the ovaries were active which was in agreement with the findings of Elsawaf and Schmidt (1963). The largest diameter of the Graafian follicle of 2.1 cm recorded in the study was supported by the findings of El-Sheikh and Abdel-Hadi (1970) and El-wishy *et al.* (1988), where as Hafez (1955) recorded the diameter of the largest follicle as 0.5 to 0.7 cm and 0.8 to 1.3 cm in ovary with corpus luteum and without corpus luteum, respectively in Egyptian buffaloes. The difference might have been due to the breed variation and environmental differences between the places of study.

#### REFERENCES

- Dailey, R.A., Fogwell, R.L. and Thyne, W.V. (1982). Distribution of visible follicles on the ovarian surface in ewes. *J. Anim. Sci.*, **54**: 1196-1204.
- Damodaran, S. (1955). Some observation on ovarian activity, cornual pregnancy and sex ratio in the Indian buffalo (*Bubalus bubalis*). *Indian Vet. J.*, **32**: 227-231.
- Dufour, J., Ginter, O.J. and Casida, L.E. (1971). Corpus luteum action on ovarian follicular development after destruction of macroscopically visible follicles in ewes. *Exp. Biol. Med.*, **138**: 475.
- Elsawaf, S. and Schmidt, K. (1963). Morphological changes in normal and abnormal ovaries of buffaloes with special reference to their function. *Vet. med. J. (Cairo)*, **8**: 249-273 (c.f. *Anim. Bred. Abstr.*, 1965; **33**: 227).
- El-Sheikh, A.S. and Abdel-Hadi, H.A. (1970). Anatomy and histology of the ovary in Egyptian buffalo. *Indian J. Anim. Sci.*, **40**: 9-14.
- El-Wishy, A.B., El-Sayed, M.A.I., Seida, A.A. and Ghallab, A.M. (1988). Observation of pregnancy ovarian activity and genital abnormalities of slaughtered buffaloes. *Buffalo J.*, **1**: 39-49.
- Fogwell, R.L., Lewis, G.S., Butcher, R.L. and Inskeep, E.K. (1977). Effects of ovarian bisection on response to intrafollicular injection of PGF<sub>2a</sub> and on follicular development in ewes. *J. Anim. Sci.*, **45**: 328.
- Hafez, E.S.E. (1955). Puberty in the buffalo-cow. *J. Agric. Sci.*, pp 137-142.
- Ireland, J.J., Murphee, R.L. and Corvicon, P.B. (1979). Accuracy of predicting stages of bovine estrous cycle by gross appearance of the corpus luteum. *J. Dairy Sci.*, **63**: 155-160.
- Ireland, J. and Roche, J.F. (1988). Development of non-ovulatory antral follicles in Heifers : changes in steroids in follicular fluid and receptors for gonadotropins. *Endocrinology*, **29**: 21-37.
- Khan, M.Z. (1987). Biometrical studies on sex organs in Nili-Ravi buffalo. *Buffalo J.*, pp 161-167.
- Kruip, Th. A.M. (1982). Macroscopic identification of tertiary follicles > 2 mm in the ovaries of cycling cows. In: *Factors Influencing Fertility in the Post Partum Cows*. (Ed.) H. Karg and E. Schallenbera, Martinus Nighotfts, Boston, pp 95-101.
- Luktuke, S.N. and Rao, A.S. (1962). Studies on the biometry of the reproductive tract of the buffalo cow. *Indian J. Vet. Sci.*, **32**: 106-111.
- Madan, M.L. (1990). Factors limiting super-ovulation response in embryo transfer programme among buffaloes. *Theriogenology*, **33**: 280.
- Matton, P., Adelakan, V., Couture, Y. and Dufour, A. (1981). Growth and replacement of the bovine ovarian follicles during the oestrous cycle. *J. Anim. Sci.*, **52**: 813-820.
- McNutt, G.W. (1924). The corpus luteum of the ox ovary in relation to the estrous cycle. *J. Am. Vet. Med.*, **53**: 556-597.
- Moor, R.M., Kruip, A.M. and Green, D. (1984). Intraovarian control of folliculogenesis : Limits to superovulation. *Theriogenology*, **21**: 103-115.
- Napolean, R.E. (1989). Morphometrical and Histometrical changes of the ovary and uterus in relation to the gross appearance of the corpus luteum of buffalo (*Bubalus bubalis*). Thesis submitted for the award of M.V.Sc. to the Tamil Nadu Agricultural University.
- Singh, G. and Singh, G. (1988). Studies on development of ovaries by prepubertal buffalo heifers. *Indian J. Anim. Reprod.*, **9**: 32-35.
- Skyer, D.M., Garverick, H.A., Youngquis, R.S. and Krause, G.F. (1987). Ovarian follicular populations and *in vitro* steroidogenesis on three different days of the bovine estrous cycle. *J. Anim. Sci.*, **64**: 1710-1716.
- Snedecor, G.W. and Cochran, W.G. (1967). *Statistical Methods*. 6th edn., Oxford & IBH Publishing Co., Calcutta.
- Totey, S.M., Singh, G., Taneja, M. and Talwar, G.P. (1991). *In vitro* maturation and fertilization of follicular oocytes from buffalo. *Theriogenology*, **35**: 284.
- Zemjanis, R. (1962). *Diagnostic and Therapeutic techniques in Animal Reproduction*. The Williams and Wilkins Company, Baltimore, 55 p.