Theriogenology Insight: 3(1):37-46. April, 2013

Ovarian follicular dynamics during estrous cycle and its aberrations during certain reproductive disorders in buffalo

G.K. Das[1], B.L. Kumawat[1] and F.A. Khan[2*]

[1]Animal Reproduction Division, Indian Veterinary Research Institute, Izatnagar-243122, India
[2]Department of Animal Sciences and DH Barron Reproductive and Perinatal Biology, Research Program, University of Florida, Gainesville FL 32611, USA

Abstract

Ovarian follicular development in the species follows a wave dynamics involving mostly 2-wave or 3-wave cycles. Although there are still large gaps in our understanding of the etiopathogenic mechanisms underlying reproductive disorders in buffalo, available literature suggests alterations in ovarian dynamics at one or more stages of follicular development. This article gives a brief account of the ovarian follicular dynamics during estrous cycle and aberrations associated with certain reproductive disorders in buffalo.

Keywords: Buffalo, Follicular Dynamics, Ovary, Infertility

Introduction

Buffalo is a multi-purpose species and contributes significantly to rural economy and dairy industry in many developing countries across the world. Due to its notable contribution, buffalo has been rightly designated as the 'Black Gold' of India. Despite its high productive potential, the species has not received considerable attention in the past. Buffalo suffers from several inherent reproductive problems such as seasonality of breeding, delayed puberty and sexual maturity, repeat breeding, prolonged post-partum anestrus, summer anestrus, and silent estrus (Nanda *et al*., 2003; Das and Khan, 2010). Although there has been a surge in the buffalo reproduction research in the recent past, several basic reproductive processes are still far from being elucidated adequately. Anestrus and repeat breeding are two of the most important reproductive problems that need immediate attention. Although the etiopathogenic mechanisms of these disorders are not yet clear, a critical analysis of the available literature indicates their association with derangements in the fundamental process of ovarian follicular

*Corresponding Author: firdousivri@gmail.com
MS Received: 21st April, 2013
MS Accepted: 30th April, 2013

development (Lohan *et al*., 2004; Khan and Das, 2012). Ovarian follicle is central to reproduction and plays an integral part in regulating the estrous cycle. Hence, understanding the mechanism of follicular growth, development and ovulation are essential to improve and control reproductive function in farm and companion animals (Roche, 2004). A good understanding of follicular dynamics can, therefore, help us alleviate various reproductive problems and optimize the efficiency of buffalo reproduction. The present article provides a brief description of the follicular dynamics in buffalo with reference to estrous cycle and some important reproductive disorders.

Antral follicular dynamics during estrous cycle

Historically, studies on ovarian follicular dynamics in large animals were initiated long back by Rajakoski (1960) who for the first time, based on gross and histological observations of ovaries, opined that ovarian follicular growth during estrous cycle occurs in a wave-like pattern. In the normal course of development, the ovarian follicle progresses through growing, static, and regression phases (Ginther *et al*., 1989). This process of continual growth and regression of antral follicles leading to pre-ovulatory size is known as follicular dynamics (Ireland *et al*., 2000).

Study of antral follicular dynamics using ultrasonography in buffalo began in the nineties. However, initial attempts to understand the antral follicular dynamics in buffalo were made during mid to late eighties by histological and clinical examination of buffalo ovaries (Singh *et al*., 1984; Danell, 1987). A preliminary report on antral follicular dynamics in buffalo was documented in early nineties (Manik *et al*., 1994) immediately followed by the first complete description of the process during the estrous cycle (Taneja *et al*., 1996; Baruselli *et al*., 1997). Similar to cattle, the growth of ovarian follicles in buffalo during estrous cycle occurs in a wave-like fashion (Ginther *et al*., 1989). Follicles undergo a dynamic process of development through growth, static, and regression phases. The number of follicular waves varies from 1 to 3 in each estrous cycle (Campanile *et al*., 2010). However, 2-wave follicular dynamics is the most common pattern in buffalo (Taneja *et al*., 1996; Baruselli *et al*., 1997) which constitutes about 63 to 83% cases followed by 25 to 33% cases of 3-waves and 3.3% cases of 1-wave pattern (Manik *et al*., 1994; Baruselli *et al*., 1997; Warriach and Ahmad, 2007). In contrast, a reverse trend was reported in Egyptian buffaloes with a majority (53.6%) showing 3-wave pattern and 46.4% showing 2-wave pattern of follicular development (Barkawi *et al*., 2009). Unlike cattle, there is no report of 4-wave follicular dynamics in buffalo. There are normally one or two non-ovulatory follicular waves followed by an ovulatory wave.

Each follicular wave is characterized by emergence, common growth phase, selection or deviation, dominance and atresia or ovulation (Taneja *et al*., 1996; Baruselli *et al*., 1997). However, the physiological basis of these different phases of follicular development in buffalo is not well understood.

Irrespective of the wave patterns (1- or 2- or 3-wave), the number of antral follicles that appear as cohort at the time of each wave emergence (recruitment) is lesser in buffalo than in cattle (Baruselli *et al*., 1997; Gimenes *et al*., 2009). The variation among the number of recruited follicles between the different waves is small in buffaloes. Further, the number of follicles recruited in a follicular wave is relatively constant for individual buffaloes (Baruselli *et al.*, 1997). The maximum diameter attained by the largest follicle in each follicular wave varies from 11.1 to 15.5 mm, depending on the number of follicular waves (Manik *et al*., 1994; Baruselli *et al*., 1997). Buffalo heifers show a higher prevalence of two-wave cycles, whereas buffalo cows tend to have two or three follicular wave cycles. Growth rate of the largest follicle is slower and its size is smaller in heifers than in pluriparous buffaloes for both first and second follicular waves (Baruselli *et al*., 1997; Presicce *et al*., 2004).

Available reports indicate no difference in the growth rate and the maximum size of the dominant non-ovulatory follicle (first wave) and dominant ovulatory follicle (second wave) in cycles with 2- wave pattern of follicular development. However, the growth rate of the dominant ovulatory follicle in the single wave estrous cycle is slower compared to that of a 2-wave estrous cycle. During each wave a single follicle undergoes selection and becomes dominant whereas other follicles in the wave regress. Following dominance phase, this follicle either ovulates (designated as dominant ovulatory follicle) or becomes atretic (dominant non-ovulatory follicle) (Taneja *et al*., 1996).

Follicular dynamics during certain reproductive disorders

Reproductive acyclicity

The real-time follicular dynamics during acyclicity has not been studied in buffalo, however, slaughter-house based investigations on the morphological (Khan and Das, 2012), biochemical (Khan *et al*., 2011) and endocrinological (Khan *et al*., 2012) characteristics of antral follicles have provided leading insights into the derangements in follicular dynamics leading to the condition. Available evidence indicates that follicular dynamics does not cease during acyclicity. Acyclic buffaloes displayed variable degree of ovarian activity, characterized by follicular turnover in one or both ovaries but without expression of estrus signs (Zicarelli, 1997). It seems likely that a normal follicular recruitment is followed by an abnormality during the critical phases of selection and dominance resulting in atresia instead of progression to ovulation (Khan *et al*., 2011; Khan and Das, 2012). Based on the presence of large sized but estrogenically inactive follicles in a small proportion of the acyclic buffaloes, it was concluded that follicles might occasionally attain a size corresponding to morphological dominance but the lack of functional activity deters their progression to ovulation (Khan and Das, 2012). Follicular microenvironment is a reliable indicator of the follicular functional status and an important determinant of selection and dominance (Beg and Ginther, 2006). Alterations in the biochemical and hormonal composition of follicular fluid have been reported during acyclicity in buffalo. The decrease in concentrations of estradiol, insulin, ascorbic acid, glucose and cholesterol, and a concomitant increase in progesterone, nitric oxide and alkaline phosphatase apparently hamper certain key events of follicular dynamics resulting in development of acyclicity (Khan *et al*., 2011; Khan and Das, 2012, Khan *et al*., 2012). However, the temporal and cause-and-effect relationships between the various intra-follicular components during acyclicity need to be studied in future investigations to elucidate the etiopathogenic mechanism of the condition.

Delayed puberty

Delayed puberty has been considered as one of the major inherent reproductive problems in buffalo heifers over many decades. To date, attempts made to combat the problem have not been so successful. One of the reasons could be the inadequate information on the dynamics of follicular growth in delayed pubertal buffaloes in the current literature. A few recent studies suggest that like dairy heifers, the follicle turnover in delayed pubertal buffalo heifers follows a typical wave-like pattern, characterized by variable degree of ovarian activity and follicles >9 mm in diameter on either of the ovaries for about 85% of the daily scanning periods (Ghuman *et al*., 2008). The dominant follicles of delayed pubertal buffaloes may attain preovulatory size (10 to 16 mm) but fail to ovulate and finally undergo atresia (Ghuman *et al*., 2008; 2010). Our personal observations while examining the delayed pubertal buffaloes under field condition indicate that in most of the cases, such buffaloes have small pea-shaped ovaries without any palpable corpus luteum (CL) or follicles. Given the negative impact of delayed puberty and our inadequate knowledge about the underlying mechanisms, studies are warranted to investigate the pattern of follicular dynamics in delayed pubertal buffaloes prior to formulating any strategies to address the problem.

Summer anestrus

Studies on summer anestrus buffaloes particularly in relation to the ovarian functional structures are inconsistent in literature (Das and Khan, 2010). Some early studies indicated that buffalo ovaries remain active during summer months (Roy *et al*., 1968; Pandey and Raizada, 1979), characterized by the presence of both follicular growth and atresia (Takkar *et al*., 1983). On the contrary, some early and recent reports indicate that most of the buffalo ovaries remain smooth and inactive without any maturing Graafian follicle during summer (Das and Khan, 2010). Our preliminary observations made from slaughter house ovaries revealed that the buffalo ovaries have lower number of visible surface follicles during summer (2.4 per ovary; Das, unpublished data) than winter (6.1-12.6 per ovary, Jaglan, unpublished data). Recently, we have examined the follicular structures in buffalo ovaries obtained from slaughter house during summer. Acyclic buffaloes during summer show suppression of follicular growth resulting in a reduction in the largest follicle size (Khan *et al*., 2009; Jan *et al*., 2011). Moreover, two recent ultrasonographic studies on follicular dynamics in summer anestrus buffaloes (Rohilla *et al*., 2005; Ghuman *et al*., 2010) have demonstrated that follicular growth and atresia continues in such animals. Two types of ovarian follicular dynamics were observed in postpartum anestrus buffaloes during summer; one with the presence of CL indicating silent or unobserved estrus and the second type, a true anestrus characterized by a smaller population of follicles, failure of the largest follicle to reach the pre-ovulatory size, and anovulation in majority of the animals (Rohilla *et al*., 2005). However, anovulatory follicles that attained a diameter approaching the preovulatory size in cyclic buffaloes were reported in another study (Ghuman *et al*., 2010).

Silent estrus

Silent estrus constitutes the single largest problem affecting reproductive efficiency in water buffaloes (Awasthi *et al*., 1998). The incidence of this problem is greater in postpartum buffaloes (Lohan *et al*., 2004) and during summer months (Rohilla *et al*., 2005). About 63 per cent of the buffalo population showed silent ovulation without estrus behavior during the postpartum period (Lohan *et al*., 2004). A recent study on the follicular dynamics of silent estrus buffaloes demonstrated smaller size and slower growth rate of the dominant ovulatory follicle associated with lesser concentrations of estradiol (Awasthi *et al*., 2007). Although the real-time hormonal dynamics during silent estrus in buffalo is not known, based on the observation that ovulation occurs without manifestation of estrus it has been hypothesized that the condition most likely results from an increased threshold of estradiol for the neural mechanism controlling behavioral exhibition of estrus signs than for stimulation of luteinizing hormone (LH) surge (Das *et al*., 2011).

Postpartum anestrus

Information on the wave pattern of ovarian follicle development in postpartum buffaloes could be helpful for improving the treatment protocols used to induce estrus and ovulation in anestrus condition. Available reports suggest that buffaloes show dynamic follicular activity characterized by growth, static and regression phases during postpartum period (Presicce *et al.*, 2005; Malik *et al.*, 2010). Following calving, both the ovaries show a high number of antral follicles greater than or equal to 2.0 mm but the number reduces with advancement of time till 60 days postpartum while follicles greater than or equal to 3.0 mm in diameter gradually increase in number until stabilizing at around 15-20 days from calving (Presicce *et al.*, 2005). During early postpartum period between days 10-23, follicles grow but do not reach >8.0 mm size (Lohan *et al.*, 2000; Lohan *et al.*, 2004; Malik *et al.*, 2010). Follicles may attain a size over 10 mm as early as 26 days postpartum but still remain sub-optimal for initiation of cyclicity and ovulation that occurs on an average 52 days postpartum (Malik *et al.*, 2010).

The current information on follicular dynamics in postpartum anestrus buffaloes is scarce. A recent study indicated that a delayed development of dominant follicle (>10 mm) and subsequent ovulation might be one of the reasons for postpartum anestrus in buffaloes (Malik *et al.*, 2010). In a different study, the presence of anovulatory follicles in the ovaries was reported during 60-240 days after calving in anestrus buffaloes (Dahiya *et al.*, 2003). However, in postpartum anestrus buffaloes during summer season, two types of dynamics were found: one in which majority of the buffaloes showed dynamic follicular activity with a smaller population of follicles and failure of the largest follicle to reach preovulatory size without ovulation; second type showed normal follicular development and presence of corpus luteum indicating silent estrus condition (Rohilla *et al.*, 2005). A higher rate of atresia of preantral and/or antral follicles is associated with anestrus condition in the species following calving. Other observations were development of dominant follicle associated with longer time span of growth, static and regression phase in some buffaloes. The largest follicle in such cases attained the size similar to the limits of a typical dominant (12-14 mm) follicle (Presicce *et al.*, 2005).

Uterine infection

Information pertaining to follicular dynamics in relation to uterine infection is relatively scanty in buffalo than in cattle. It is speculated that sub-clinical uterine infections may be part of the cause of reduced ovarian activity during postpartum period in buffaloes (Presicce *et al.*, 2005). A high incidence (71.9%) of ovarian inactivity with an apparent absence of large follicles was reported in the recent past (Hanafi *et al.*, 2008). Very recently, using buffalo ovaries of slaughter house origin, we have shown that growth of the largest follicle growth is suppressed without any alteration in the total number of follicles during uterine infection (Pande *et al.*, 2012).

Cystic ovarian disease

Buffaloes suffering from cystic ovarian disease exhibit wave pattern of follicular growth. However, the number of follicular waves in each cycle is highly variable and ranges from 1 to 6 (5.2±0.5, mean) waves. Estrous cycle length is considerably extended (55.3 ± 4.3 days, Mean) in buffaloes with cystic ovarian disease. Cystic follicles have an extended life-span and grow either alone or may be accompanied by low number of subordinate follicles (Barkawi *et al.*, 2009). Recent studies on follicular fluid from cystic follicles have revealed altered biochemical and hormonal intra-follicular compositions compared to those of normal follicles. Notable changes included increase in nitric oxide, progesterone, cortisol, and tri-iodothyronine concentrations concurrent with decrease in ascorbic acid, insulin and glucose concentrations (Khan *et al.*, 2011).

Conclusion

Buffalo follicular dynamics has received a considerable attention since last two decades considering the importance of the animal in food production. Although there have been advancements in certain research areas, many still remained to be addressed. During each estrous cycle, antral follicles develop in wave-like pattern. This wave pattern of follicular development occurs during various reproductive disorders as well. However, there are indications about aberration in various events of the wave development, which can be hypothesized to be responsible for the development of such reproductive ailments. Only a limited number of studies are available on the follicular wave derangements during the reproductive problems. Future investigations are, therefore, warranted in order to elucidate the etiopathogenic basis of these problems and to formulate effective strategies for their management.

References

Awasthi, M.K., Kavani, F.S., Dhami, A.J., and Panchal, M.T., 1998. Induction of estrus and fertility with low dose of prostaglandin F2alpha in subestrus buffaloes. *Indian J. Anim. Reprod.*, **30:** 71-74.

Awasthi, M.K., Kavani, F.S., Siddiquee, G.M., Sarvaiya, N.P. and Derashri, H.J., 2007. Is slow follicular growth the cause of silent estrus in water buffaloes? *Anim. Reprod. Sci.*, **99:** 258-268.

Barkawi, A.H., Hafez, Y.M., Ibrahim, S.A., Ashour, G., El-Asheeri, A.K. and Ghannem, N., 2009. Characteristics of ovarian follicular dynamics throughout the estrous cycle of Egyptian buffaloes. *Anim. Reprod. Sci.*, **110:** 326-334.

Baruselli, P.S., Mucciolo, R.G., Visintin, J.A., Viana, W.G., Arruda, R.P., Madureira, E.H., Oliveira, C.A. and Molero-Filho, J.R., 1997. Ovarian follicular dynamics during the estrous cycle in buffalo (Bubalus bubalis). *Theriogenology*, **47:** 1531-1547.

Beg, M.A. and Ginther, O.J., 2006. Follicle selection in cattle and horses: role of intrafollicular factors. *Reproduction*, **132:** 365-377.

Campanile, G., Baruselli, P. S., Neglia, G., Vecchio, D., Gasparrini, B., Gimenes, L. U., Zicarelli, L. and D'Occhio, M. J., 2010. Ovarian function in the buffalo and implications for embryo development and assisted reproduction. *Anim. Reprod. Sci*., **121:** 1-11.

Dahiya, V., Lohan, I.S., Saini, M.S., Kaker, M.L. and Malik, R.K., 2003. Ultrasonographic assessment of ovarian changes in anoestrus buffaloes treated with norgestomet. *Indian J. Anim. Sci*., **73:** 1033-1036.

Danell, B., 1987. Oestrus behavior, ovarian morphology and cyclical variation in the follicular system and endocrine pattern in Water Buffalo heifers. PhD Thesis. Uppsala, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, pp 124.

Das, G.K. and Khan, F.A., 2010. Summer Anoestrus in Buffalo – A Review. *Reprod. Dom. Anim*., **45:** e-483-e494.

Das, G.K., Kumar, Brijesh, Jan, Mustafa Hasan, Pande, Megha and Khan, F.A., 2011. In: Advances in Reproductive Technologies to Augment Fertility in Farm Animals. S. K. Singh, S. K. Ghosh, H. Kumar and S. K. Agarwal (eds.), I. V. R. I., Izatnagar, pp.45-49.

Ghuman, S.P.S., Singh, J. and Dhaliwal, G.S., 2008. Recent concepts in ovarian activity of buffaloes (Bubalus bubalis) with prepubertal anestrus and its alleviation through endocrine interventions. In: Proceedings of XXIV annual convention and national symposium of ISSAR, Bangalore. December 11-13, 2008. pp 75-84.

Ghuman, S.P.S., Singh, J., Honparke, M., Dadarwal, D., Dhaliwal, G.S. and Singh, S.T., 2010. Fate of Dominant follicle in Summer anestrus buffaloes. *Indian J. Anim. Reprod*., **31:** 7-10.

Gimenes, L.U., Fantinato, N.P., Arango, J.S.P., Ayres, H. and Baruselli, P. S., 2009. Follicular dynamics of Bos indicus, Bos taurus and Bubalus bubalis heifers treated with norgestomet ear implant associated or not to injectable progesterone. *Anim. Reprod*., **6:** 256.

Ginther, O. J., Knopf, L., and Kastelic, J. P., 1989. Temporal association among events in cattle during estrous cycles with two and three follicular waves. *J. Reprod. Fert*., **87:** 223-230.

Hanafi, E.M., Ahmed, W.M., Abd El Moez, S.I., El Khadrawy, H.H., Abd EL Hameed, A.R., 2008. Effect of Clinical Endometritis on Ovarian Activity and Oxidative Stress Status in Egyptian Buffalo-Cows. *Am. Eurasian J. Agric. Environ. Sci*., **4:** 530-536.

Ireland, J.J., Mihm, M., Austin, E., Diskin, M.G. and Roche, J.F., 2000. Historical Perspective of Turnover of Dominant Follicles during the Bovine Estrous Cycle: Key Concepts, Studies, Advancements, and Terms. *J. Dairy Sci*., **83:** 1648-1658.

Jan, M.H., Khan, F.A., Pande, Megha, Kumar, Brijesh, Das, G.K. and Sarkar, M., 2011. Follicular attributes and intra-follicular nitric oxide and ascorbic acid concentrations in cyclic and acyclic buffaloes during summer season. *Theriogenology Insight*, **1:** 83-88.

Khan, F.A. and Das, G.K., 2012. Follicular characteristics and intrafollicular concentrations of nitric oxide and ascorbic acid during ovarian acyclicity in water buffalo (Bubalus bubalis). *Trop. Anim. Health Prod*., **44:**125-131.

Khan, F.A., Das, G.K. and Gogoi, D., 2009. Ovarian attributes of cyclic and non-cyclic buffaloes during summer. In: Proc. XXVth Annual Convention of ISSAR, Namakkal, India, Dec. 10 to 12, pp. 115.

Khan, F.A., Das, G.K., Pande, M., Mir, R.A., Shankar, U., 2011. Changes in biochemical composition of follicular fluid during reproductive acyclicity in water buffalo (Bubalus bubalis). *Anim. Reprod. Sci*., **127:** 38-42.

Khan, F.A., Das, G.K., Pande, M., Sarkar M., Mahapatra R.K., Shankar, U., 2012. Alterations in follicular fluid estradiol, progesterone and insulin concentrations during ovarian acyclicity in water buffalo (Bubalus bubalis). *Anim. Reprod. Sci*., **130:** 27-32.

Khan, F.A.., Das, G.K., Pande, M., Pathak, M.K., Sarkar, M., 2011. Biochemical and hormonal composition of follicular cysts in water buffalo (*Bubalus bubalis*). *Anim. Reprod. Sci*., **124:** 61-64.

Lohan, I.S., Malik, R.K. and Kaker, M.L., 2004. Asian-Aust. Uterine involution and ovarian follicular growth during early postpartum period of Murrah buffaloes (Bubalus bubalis). *J. Anim. Sci*., **17:** 313-316.

Lohan, I.S., Malik, R.K., Saini, M.S., Dhanda, O.P. and Singh, B., 2000. Uterine and ovarian changes during the early postpartum period in Murrah buffaloes. *Buffalo Bull*., **19:** 20-23.

Malik, R.K., Singh, P., Tuli, R.K., Chandolia, R.K., Malik, V.S., Malik, D.S. and Lathwal, S.S., 2010. Ovarian changes and follicular dynamics during postpartum period in Murrah buffaloes. *Indian J. Vet. Res*., **19:**43-50.

Manik, R.S., Madan, M.L., and Singla, S.K., 1994. Ovarian follicular dynamics in water buffaloes (Bubalus bubalis): ultrasonically monitoring individual follicles for wave hypothesis. *Theriogenology*, **41:** 247 (abstr.).

Nanda, A.S., Brar, P.S. and Prabhakar, S., 2003. Enhancing reproductive performance in dairy buffalo: major constraints and achievements. *Reproduction* (Suppl.) **61:** 27-36.

Pande, M., Das, G.K., Khan, F.A., Sarkar, M., Prasad, J.K., Pathak, M.C. and Kumar, H., 2013. Uterine infection influences size and follicular fluid composition of the largest follicle in buffalo (Bubalus bubalis). *Reprod. Dom. Anim*., **48:** 79-84.

Pandey, M.D. and Raizada, B.C., 1979. Overcoming summer sterility in buffalo bulls and cows. In: Buffalo Reproduction and Artificial Insemination. FAO, Rome, FAO Animal Production and Health Paper No. 13, pp 235–246.

Presicce, G.A., Bella, A., Terzano, G.M., De Santis, G., and Senatore, E.M., 2005. Postpartum ovarian follicular dynamics in primiparous and pluriparous Mediterranean Italian buffaloes (Bubalus bubalis). *Theriogenology*, **63:** 1430-1439.

Presicce, G.A., Senatore, E.M., Bella, A., De Santis, G., Barile, V. L., De Mauro, G. J., Terzano, G.M., Stecco, R. and Parmeggiani, A., 2004. Ovarian follicular dynamics and hormonal profiles in heifer and mixed-parity Mediterranean Italian buffaloes (Bubalus bubalis) following an estrus synchronization protocol. *Theriogenology*, **61:** 1343-1355.

Rajakoski, E., 1960. The ovarian follicular system in sexually mature heifers with special reference to seasonal, cyclical, end left-right variations. *Acta Endocrinol*., **52 (suppl.):**1- 68.

Roche, J.F., 2004. Follicular Waves in Cattle. Follicular Waves in Cattle. *Vet. Res. Commun*., **28:**107-110.

Rohilla, N., Singh, U., Sharma, R.K. and Singh, I., 2005. Ultrasonic ovarian status in summer anestrus postpartum Murrah buffaloes. *Indian J. Anim. Reprod*., **26:** 95–98.

Roy, A., Raizada, B.C., Tiwari, R.B., Pandey, M.D., Yadav, P.C. and Sengupta, B.P., 1968. Effect of management on fertility of buffalo cows bred during summer. *Ind J. Vet. Sci*., **38:** 554-560.

Singh, G., Singh. G.B. and Sharma, S.S., 1984. Studies on follicular patterns in buffalo heifers. *Theriogenology*, **22:** 453-462.

Takkar, O.P., Singh, M. and Varman, P.N., 1983. Progesterone levels vis a vis anoestrum in buffaloes concurrent with profiles during stages of estrous cycle. *Indian J. Dairy Sci*., **36:** 125–128.

Taneja, M., Ali, A. and Singh, G., 1996. Ovarian follicular dynamics in water buffalo. *Theriogenology*, **46:** 121-130.

Warriach, H.M. and Ahmad, N., 2007. Follicular waves during the oestrous cycle in Nili Ravi buffaloes undergoing spontaneous and PGF2á-induced luteolysis. *Anim. Reprod. Sci.,* **101:** 332-337.

Zicarelli, L., 1997. Reproductive seasonality in buffalo. In: Proc. 3rd Course of Biotechnology of Reproduction in Buffaloes (Issue II), October 6-10, 1997, Caserta, Italy, 29-52.