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QUANTITY AND QUALITY OF HARVESTED BUFFALO OOCYTES IN RELATION TO OVARIAN STRUCTURES AND METHODS OF COLLECTION

(With 4 Tables)

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كمية ونوعية البويضات المجموعة من مبايض الجاموس وعلاقتها بتركيبات المبيض وطريقة الجمع

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لدراسة تأثير تركيبات المبيض على نوعية وكمية البويضات المتحصل عليها من مبايض الجاموس المذبوح لاستعمالها فى التلقيح خارج الرحم تم فى الجزء الأول من الدراسة جمع ٤٠ مبيض وتقسيمهم الى أربعة مجموعات : تلك التى تحوى جريبة سائدة (١٤ مبيض)، والتى تحوى جسم أصفر(٩ مبايض) ، والتى تحوى كلتا التركيبتين (٥ مبيض)، والأخيرة كانت لا تحوى أى من التركيبتين (١٢ مبيض). تم عد الجريبات السطحية والتى يتراوح قطرها بين ٣-٨ مم ثم تم سحب السائل الجريبى منها بواسطة محقن وفحصه مجهريا لجمع البويضات. تم عد البويضات المتحصل عليها وتقييمها طبقا لعدد وتماسك خلايا طبقات الركام المحيطة بها وكذلك طبيعة السيتوبلازم. فى الجزء الثاني من الدراسة تم مقارنة عدد ونوعية البويضات التى تم الحصول عليها فى التجربة السابقة (طريقة السحب بالمحقن) بتلك التى تم الحصول عليها بتقطيع المبيض الى اجزاء صغيرة بواسطة سلاح مشرط ثم غسل تلك الأجزاء فى محلول فسيولوجى (PBS). تم فحص ذلك المحلول لجمع البويضات وتقييمها. وقد وجد من النتائج ان عدد البويضات التى تم الحصول عليها من المبايض التى تحمل جريبة سائدة كان اقل فى العدد وكذلك كانت مواصفات البويضات اقل فى جودتها من تلك التى جمعت من المجموعات الأخرى. كما وجد أيضا ان عدد البويضات التى تم الحصول عليها بتقطيع المبيض كانت اكثر فى العدد وذات جودة عالية عن تلك التى تم الحصول عليها بواسطة سحب السائل الجريبى.

SUMMARY

A total number of 68 buffalo-cows ovaries were collected from slaughterhouse. In the first part of this study, 40 ovaries were classified according to the ovarian structures into: those with dominant follicle (DF, n = 14) , with corpus luteum (CL, n=9), with DF and CL (n=5)

and those without DF or CL (no dominant structure: nDS, n = 12). Surface follicles between 3 and 8 mm were enumerated and aspirated. The aspirated follicular fluids were examined microscopically for the oocytes. The recovered oocytes were counted and evaluated according to number and quality of the cumulus layers and character of the ooplasm. In the second part of the study, the obtained oocytes were compared with those obtained by slicing technique applied on 28 ovaries. The results revealed that, the average number of aspirated follicles/ovary was higher from ovaries with nDS (7.33 ± 3.8), than from ovaries with DF (3.67±2.2), $p<0.5$. Also, the average number of recovered oocytes/ovary was higher from ovaries with nDS (5.75 ± 2.4), than from those with DF (2.79 ± 2), $p<0.05$. Ovaries which carried CL showed in-between results (4.0±3.7 aspirated follicles and 3.22±3.1 recovered oocytes). Higher number of oocytes with more than 5 cumulus layers was collected from ovaries with nDS (28.9%) than from ovaries with DF (5.1%), $p<0.05$. Slicing technique increased significantly the average number of recovered oocytes/ovary than the aspiration technique, (5.00 ± 2.3 vs 3.90±2.4) and the number of oocytes with more than 5 cumulus layers (62.1% vs 15.3%) $p<0.05$. It is concluded that 1) DF adversely affected the number and quality of recovered oocytes in buffalo, 2) ovarian slicing technique increased significantly the quantity and quality of the harvested oocytes.

Key words: *Buffalo, oocyte, dominant follicle, cumulus.*

INTRODUCTION

Nowadays, there is a considerable interest in developing the technology of in-vitro-fertilization (IVF) for embryo production in buffaloes (Kruel, 1991 and Gordon, 1997). However, in comparison to cattle and camels, buffaloes produced lower number and less culturable oocytes (Ali and Abdel-Razek, 2001). The number and quality of the oocytes may be affected by the method of oocyte collection as well as by the structures present on the ovary (Kumar *et al.,* 1997 and Ali *et al.,* 1999). As the oocyte grows within the follicles, a number of factors may influence its health and developmental competence (Abdoon and Kandil, 2001). These factors include stage of estrous cycle, follicle size and the predominant ovarian structure (Hagemann *et al.,* 1999).

Although, dominant follicle (DF) has been proved to adversely affect the developmental competence of the oocytes in cattle (Smith *et*

al., 1996), little is known about its effect in buffaloes. Like cattle, follicles in buffalo develop in a wave like pattern, with two to three waves per a cycle (Ali *et al.*, 2003). Each wave is characterized by recruitment of many small follicles, followed by selection of one of them (DF) and regression of the others (subordinate follicles) (Taneja *et al.*, 1996). In cattle, there is evidence that the DF suppress the development of the subordinate follicles.

The aim of the current study was to: 1) Clarify the effect of the presence of DF on the number and quality of the oocyte obtained from subordinate follicles in buffalo-cows. 2) Compare between the aspiration and slicing techniques on the quantity and quality of harvested oocytes.

MATERIALS and METHODS

Female reproductive tracts of native buffalo-cows were collected from local slaughterhouse shortly after evisceration. The ovaries (n = 68) were dissected from the tracts and transferred in a previously warmed thermos (35 °C) within about one hour to the laboratory.

Part 1

In the laboratory, a number of 40 ovaries were classified according to the predominant ovarian structure into those with DF (defined as that which reach the diameter of 8 mm or more and exceed the diameter of other follicles on the ovary) (DF, n = 14) , those with corpus luteum (CL, n=9), those with DF and CL (DF &CL, n=5) and those without DF or CL (no dominant structures: nDS, n = 12). Subordinate follicles between 3 and 8 mm in diameter were enumerated and aspirated from groups. Aspiration of follicles was performed using 19 G needle attached to 5 ml syringe. The aspirated follicular fluids were collected in conical glass centrifuge tubes. After about 15 minutes, the sediment were aspirated with pipette and poured in small Petri dishes, diluted with dulbeccos phosphate buffer saline (PBS) supplemented with 3mg/ml bovine serum albumin (BSA) and examined under stereomicroscope. The recovered oocytes were counted and evaluated according to the number of cumulus cell layers (< 3, 3-5 and > 5 layers), compactness of cumulus cells (compact or expanded) and homogeneity of the ooplasm (homogenous or heterogeneous) according to criteria used by Ali and Abdel-Razek (2001).

Part II

A second group (n = 28) of ovaries were sliced in a Petri dish (7.5 cm in diameter) containing buffer solution (PBS and BSA). The

slicing process was performed with sterile, clean and sharp scalpel. The sliced ovarian tissues were thoroughly washed in the buffer solution. The buffer solution then examined under stereomicroscope for the presence of oocytes (Sharma and Taneja, 2000). Oocytes were evaluated as in experiment I. The number and quality of the obtained oocytes were compared with those obtained in the first part.

Data are expressed in Mean ± SD. The Data were statistically analyzed using ANOVA-test to compare between means of the recovered oocytes during the different estrous cycle stages. CHI-test was used to compare between the percentages of the oocyte quality, while t-test was used to compare between the two recovery methods (SAS, 1992).

RESULTS

The number of aspirated follicles and oocyte recovered from ovaries with DF, CL, DF&CL and with nDS are illustrated in table 1. More follicles were aspirated from ovaries with nDS (mean of 7.33±3.8 follicles/ovary) than from those with DF, CL and CL&DF (mean of 3.67±2.2, 4±3.7 and 4.8±2.3 follicles/ovary, respectively), $p< 0.05$.

Also, a higher number of oocytes were obtained from ovaries with nDS (average of 5.75±24 oocyte/ovary) than from those with DF, CL and CL&DF (average of 2.79±2.0, 3.22±3.1 and 3.8±1.9 oocytes/ovary), $p< 0.05$.

Effect of the predominant ovarian structures on the quality of the recovered oocytes is showed in Table 2. Higher number of oocytes with more than 5 cumulus layers was collected from ovaries with nDS (28.9%) than from ovaries with DF (5.1%), $p< 0.05$. However, there was no difference between the oocytes from ovaries without or with DF and/or CL with respect to the compactness of the cumulus or the homogeneity of the ooplasm.

Effect of the recovery method (aspiration vs. slicing) on the number of the harvested oocytes is shown in Table 3. The slicing technique increased significantly ($p< 0.05$) the recovered oocytes (mean of 5.00±2.3 oocyte/ovary) than the aspiration method (mean of 3.90±2.4 oocytes/ovary).

Moreover, slicing technique increased significantly ($p< 0.05$) the number of oocytes with more than 5 cumulus layers (62.1%) than the aspiration technique (15.3%) (Table 4). The incidences of harvesting denuded oocytes or those with less than 3 cumulus layers as well as

aspiration method than in the slicing technique (Table 4). However, there was no difference between the two recovery methods in concerning to the compactness of the cumulus layer or the homogeneity of the ooplasm.

Table 1: Effect of ovarian structures on the number of aspirated follicles and the recovered oocytes

Different criteria	Ovarian Structures			
	DF	CL	CL + DF	NDS
Number of ovaries	14	9	5	12
Aspirated follicles/ovary	$3.67^{a}\pm2.2$	$4.0^{ab}\pm 3.7$	$4.8^{ab}\pm 2.3$	$7.33^{b}\pm 3.8$
Recovered oocytes/ovary	$2.79^{a}\pm 2.0$	$3.22^{b}\pm 3.1$	$3.80^{b}\pm 1.9$	$5.75^{c}\pm 2.4$

Values in Means±SD. CL: corpus luteum, DF: dominant follicle, nDS: no dominant structure (no DF or CL). Values with different superscript letters in the same row differ significantly ($p<0.05$).

Table 2: Effect of ovarian structures on the quality of the recovered oocytes

Different criteria	Ovarian Structures			
	DF	CL	CL + DF	nDS
Number of ovaries	14	9	5	12
Number of the recovered oocytes	39	29	19	69
Quality of the oocytes				
I. According to the number of cumulus layers:				
A. Denuded oocytes or those with < 3 layers (%)	46.2^{a}	48.2^{a}	36.8^{a}	31.1^{a}
B. Oocytes with 3-5 layers (%)	48.7^{a}	41.7^{a}	53.0^{a}	40.0^{a}
C. Oocytes with > 5 layers (%)	5.1^{a}	10.1^{a}	10.2^{a}	28.9^{b}
II. According to the quality of cumulus layer:				
A. Compact cumulus (%)	46.2^{b}	24.1^{a}	52.6^{b}	53.3^{b}
B. Expanded cumulus (%)	7.6^{b}	27.7^{a}	11.6^{b}	15.6^{a}
III. According to the quality of the ooplasm:				
A. Homogeneous ooplasm	64.1^{a}	60.7^{a}	63.2^{a}	75.6^{a}
B. Heterogeneous ooplasm	35.9^{a}	39.3^{a}	36.8^{a}	24.4^{a}

CL: corpus luteum, DF: dominant follicle, nDS: no dominant structure (no DF or CL)
Percentages with different superscript letters in the same row differ significantly ($p<0.05$).

Table 3: Effect of the recovery methods on the number of the recovered oocytes.

Different criteria	Recovery Methods	
	Aspiration	slicing
Number of ovaries	40	28
Observed follicles/ovary	$5.20^{a}\pm 3.4$	$5.42^{a}\pm 3.3$
Recovered oocytes/ovary	$3.90^{a}\pm 2.4$	$5.00^{b}\pm 2.3$

Values in Mean±SD. Means with different superscript letters in the same row differ significantly ($p<0.05$).

Table 4: Effect of the recovery method on the quality of the recovered oocytes

Different criteria	Recovery Method	
	Aspiration	Slicing
Number of ovaries	40	28
Total number of the recovered oocytes	156	140
Quality of the oocytes		
ling to the number of cumulus layers:		
a. Denuded oocytes or those with < 3 layers (%)	40.2^{a}	17.1^{b}
b. Oocytes with 3-5 layers (%)	44.5^{a}	20.7^{b}
c. Oocytes with > 5 layers (%)	15.3^{a}	62.1^{b}
II. According to the quality of cumulus layers:		
a. Compact cumulus (%)	44.7^{a}	74.3^{b}
b. Expanded cumulus (%)	15.1^{a}	8.6^{a}
III. According to the quality of the ooplasm:		
a. Homogeneous ooplasm (%)	67.2^{a}	75.3^{a}
b. Heterogeneous ooplasm (%)	32.8^{a}	34.7^{a}

Percentages with different superscript letters in the same row differ significantly ($p<0.05$).

DISCUSSION

Although non-surgical method of embryo recovery from superovulated donor buffaloes has been available for some years, such method suffer from the problem of low superovulatory response (Madan, 1990, Misra *et al.,* 1990). With the appropriate IVF system, ovaries from slaughterhouse may provide an abundant and easily accessible source of buffalo embryos for breed improvement.

The mammalian oocyte grows and gains developmental

competence within a complex of follicular and ovarian environment. As the oocyte grows within the follicles a number of factors might influence its health and developmental competence (Hageman, 1999). Little is known about the effect of DF on the oocytes of the subordinate follicles in buffaloes. This study was planned to investigate the influence of DF on the quantity and quality of the subordinate follicles in buffalo-cows ovaries.

In the current study more follicles were aspirated from ovaries without DF than that from those with DF. Consequently, a higher number of oocytes were obtained from ovaries without DF than that with DF. This indicated a negative effect of the DF on the quantity of the subordinate follicles of the same wave. The same observation was noticed in cattle using ultrasound examination, where the number of medium sized follicles (5–8 *mm* in diameter) was lower on ovaries with DF than on the contralateral one (Fortune, 1994, Kastelic, 1994 and Ali, 2000). Daily ultrasound examination revealed that, at the beginning of estrous cycle (day of ovulation) a number of small follicles (< 3 mm) recurred. These follicles developed together up to day 4 or 5 of the cycle, where thereafter only one follicle continued to develop (DF), while the other follicles (subordinate follicles) stop growth and reduced in number (Singh *et al,* 2000 Ali, 2000, Ginther *et al.,* 1996, Ireland *et al.,* 1979)

The presence of DF not only affect the growth of subordinate follicles but the study revealed the tremendous effect on the oocyte morphology, where higher number of oocytes with more than 5 cumulus layers was collected from ovaries with nDF than from those with DF. This indicates a negative effect of DF on the quality of the oocyte from the subordinate follicles. In cattle, some authors reported a negative effect (Matton *et al.,* 1981 and Wolfsdorf *et al.,* 1997), others could not find any adverse influence (Smith *et al.,* 1996).

The question raised now, how the DF adversely affects the oocyte from the subordinate follicles. There are two possibilities. The first one is through the production of estrogen and inhibin hormones from the DF. Estrogen and inhibin hormones suppress the release of FSH from the anterior pituitary. Because, FSH is essential for the development of the subordinate follicles, so suppression of FSH results in atresia and degeneration of the subordinate follicles (Danell, 1987). Draincourt (1991) named this possibility as the negative or systemic way of follicular suppression. There are some observations, which could support this hypothesis. Destruction or removal of the DF in cattle resulted in increase blood level of FSH and LH one day after DF

removal (Ginther *et al.*, 1998, Adams *et al.*, 1992 Villa-Godoy *et al.*, 1985). A new follicular growth started 2 days after destruction of the DF (Staigmiller and England, 1985, Ko *et al.*, 1991, Bergfelt *et al.*, 1991, Adams *et al.*, 1992). Immunization against Inhibin hormone increased the ovulation rate in heifers (Morris, *et al.*, 1993). The second possibility of the effect of DF on the subordinate ones is the direct or active way, where the DF releases certain substances (e.g. IGF, EGE), which affected locally and directly (paracrine effect) on the subordinate follicles. Such substances decrease the sensitivity of the subordinate follicles for the FSH (Draincourt, 1991).

In contrast to Abdoon and Kandil (2001), who recorded that the presence of CL stimulates the development of a significantly higher number ovarian follicles which produced a significantly higher number of good quality oocytes, the present study revealed that, neither CL nor DF improve the number and quality of the obtained oocyte. This agree with the result of El Sherry (2003). While absence of the two structures improve the quantity and quality of the harvested oocytes. A similar results recorded by De Wit *et al.*, (2000), that recorded the stage of the cycle had no effect on the distribution of the COC.

The effects of method of harvesting of oocyte were studied and it was clear that slicing the ovaries produced more oocytes than the aspiration technique. This can be explained by: aspiration harvest the oocyte population from only the visible surface follicles. However slicing the ovaries make collection of oocytes possible from surface as well as cortical follicles. When the number of oocytes from both peripheral and cortical follicles combined, the yield of oocytes was approximately doubled (Arlotto *et al.*, 1996 and Sharma and Taneja, 2000).

The study also revealed that, oocytes harvested by slicing posses the culturable and competing quality than that collected by aspiration. Where the slicing technique produces less number of denuded oocytes and more with >5 comulus layers which is also more compact than that collected by aspiration. This is in agreement with the result of Das et al., (1996), Kumar *et al.*, (1997), and Datta and Goswami, (1998). This may be due to the effect of needle diameter or the aspiration pressure (Hashimoto *et al.*, 1999). But this disagreed with the finding of Abddoon and Kandil (2001).

It can finally be concluded that, presence of dominant follicle on the buffalo ovary affect the number and quality of harvested oocytes. Slicing of ovaries is a preferable method for harvesting more oocytes

capable to compete during in vitro maturation and fertilization.

REFERENCES

Abdoon, A.S. and Kandil, O.M. (2001): Factors affecting number of surface ovarian follicles and oocytes yield and quality in Egyptian buffaloes. Reprod. Nutr. Dev. 41: 71-77.

Adams, G.P.; Matteri, R.L.; Kastelic, J.P.; Ko, J.C.H. and Ginther, O.J. (1992): Association between surges of follicle stimulating hormone and the emergence of follicular waves in heifers. J. Reprod. Fertil. 94:177-188.

Ali, A. (2000): Zur Charakterisierung des dominanten Follikels der ersten Follikelwelle unter Berucksichtigung seines Einflusses auf den Erfolg der Superovulation im Rahmen des Embryotransfers beim Rind. Ph. D. Thesis Berlin, Germany.

Ali, A.; Lange, A.; Gilles, M. and Glatzel, P.S. (1999): The relationship between hormonal activity of the bovine dominant follicle on day 10 of estrous cycle and in vivo follicular maturation after PMSG-stimulation program. Reprod. Domes. Anim. 34:28-32.

Ali, A. and Abdel-razek, A. Kh. (2001): Comparison of number and quality of oocytes in the Egyptian buffaloes *(Bubalus bubalis),* cows *(Bos taurus)* and Camel *(Camelus dromedarius)*. Assiut Veterinary Medical Journal, 45:317-325.

Ali, A.; Abdel-Razek, A. Kh.; Abd-Elghaffar, S. Kh. and Glatzel, P. S. (2003): Ovarian follicular dynamics in buffalo (Bubalus bubalis). Reprod. Dom. Anim. 38:1-5.

Arlotto, T.; Schwartz, J.L.; First, N.L. and Liebfried-Rutledge, M.L. (1996): Aspects of follicle and oocyte stage that affect in vitro maturation and development of bovine oocytes. Theriogenology, 45: 943-956.

Bergfelt, D.R.; Kastelic, J.P. and Ginther, O.J. (1991): Continued periodic emergence of follicular waves in non-bred progesterone-treated heifers. Anim. Reprod. Sci. 24:193-204.

Danell, B.(1987): Oestrous behaviour, ovarian morphology and cyclical variation in follicular system and endocrine system in water buffalo heifers. Ph. D. Thesis, Uppsala, Sweden.

Das, S.K.; Jain, G.C.; Solanki, V.S. and Tripathi, V. (1996): Efficacy of various collection methods for oocyte retrieval in buffalo. Theriogenology 46:1403-1411.

Datta, T.K. and Goswami, S.L. (1998): Feasibility of harvesting oocytes from buffalo (Bubalus bubalis) ovaries by different methods. Buffalo J. 2: 277-284.

De Wit, A.A.; Wurth, Y.A. and Kruip, T.A. (2000): Effect of ovarian phase and follicle quality on morphology and developmental capacity of the bovine cumulus-oocyte complex. J Anim.Sci.78: 1277-1283.

Draincourt, M.A. (1991): Follicular dynamics in sheep and cattle. Theriogenology, 35:55-79.

El-Sherry, T.M. (2003): Follicular dynamics and ovarian changes in cyclic and superovulated buffaloes. M. Sc. Thesis, Assiut University.

Fortune, J.E. (1994): Ovarian follicular growth and development in mammals. Bio. Reprod. 50:225-232.

Ginther, O.J.; Bergefelt, D.R.; Kulick, L.J. and Kot, K. (1998): Pulsatility of systemic FSH and LH concentrations early in a follicular wave. J Reprod. Fertil. 109:181-186.

Ginther, O.J.; Wiltbank, M.C.; Frike, P.M., Gibbons J.R. and Kot, K. (1996): Selection of dominant follicle in cattle. Biol. Reprod. 55:1187-1195.

Gordon, I. (1997): Introduction to controlled reproduction in buffaloes. In: Controlled reproduction in cattle and buffaloes. CAB international, pp 423-448.

Hagemann, L.J.(1999): Influence of the dominant follicle on oocytes from subordinate follicles. Theriogenology 51: 449-459.

Hagemann, L.J.; Beaumont, S.F.; Berg, M.; Donnison, M.J.; Ledgard, A.; Peterson, A.J.; Schurmann, A. and Tervit, H.R. (1999): Development during single IVP of bovine oocytes from dissected follicles: Interactive effects of estrous cycle stage, follicular size and atresia. Mol. Reprod. Dev. 53: 451-8.

Hashimoto S.; Takakura R.; Kishi M.; Sudo T; Minami N. and Yamada M. (1999): Ultrasound-guided follicle aspiration: the collection of bovine cumulus-oocyte complexes from ovaries of slaughtered or live cows. Theriogenology 51:757-65.

Ireland, J.J.; Coulson, P.B. and Murphree, R.L. (1979): Follicular development during four stages of the estrous cycle of beef cattle. J. Anim. Sci. 49:1261-1269.

Kastelic, J.P.(1994): Understanding ovarian follicular development in cattle. Vet. Med. J., 89: 64-71.

Ko, J.C.H.; Kastelic, J.P.; Del Campo, M.R. and Ginther, O.J. (1991): Effects of dominant follicle on ovarian follicular dynamics during the estrus cycle in heifers. J. Reprod. Fertil. 91: 511-519.

Kruel, W. (1991): Water buffaloes-exotic animals with a future? Tierzuchter 43: 518-519.

Kumar, A.; Solanki, V.S.; Jindal, S.K.; Tripathi, V.N. and Jain, G.C. (1997): Oocyte retrieval and histological studies of testicular population in buffalo ovaries. Anim. Reprod. Sci.. 47: 189-195.

Madan, M.L. (1990): Conservation of germplasm through embryo transfer in buffaloes. Proceeding of 23rd Inter. Dairy Cong. (Montreal) 1: 301-314.

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

Misra, A.K.; Joshi, B.V.; Agrawala, P.L.; Kasiraj, R.; Sivaiah, S.: Rangareddi, N.S. and Siddiqui, M.U. (1990): Multiple ovulation and embryo transfer in Indian buffaloes (Bubalus bubalis) Theriogenology 33:1131-1141.

Morris, D.G.; McDermott, M.G.; Diskin, M.G.; Morrison, C.A.; Swift, P.J. and Sreenan, J.M. (1993): Effect of immunization against synthetic peptide sequences of bovine inhibin x-subunit on ovulation rate and twin calving rate in heifers. J. Reprod. Fertil. 97: 255-261.

SAS (1992): SAS/STAT Guide for personal computers, SAS Inst., Cary. N.C. USA.

Sharma, D. and Taneja, V.K. (2000): Number and quality of buffalo oocytes recovered relative to method of harvest, stage of estrous cycle and corpus luteum. Indian J. Anim. Sci. 70: 684-687.

*Singh, J.; Nanda, A.S. and Adams, G. P. (2000):*The reproductive pattern and efficiency of female buffaloes. Anim. Reprod. Sci. 60-61: 593-604.

Smith, L.C.; Olivera-Angel, M.; Groome, N.P.; Bhatia, B. and Price, C.A. (1996): Oocyte quality in small antral follicles in the presence or absence of a large dominant follicle in cattle. J. Reprod. Fertil. 106: 193-9.

Staigmiller, R.B. and England, B.G.(1985): Folliculogensis in the bovine. Theriogenology 17:43-52.

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

Villa-Godoy, A.; Ireland, J.J.; Wortman, J.A.; Ames, N.K.; Hughes, T.L. and Fogwell R.L. (1985): Effect of ovarian follicles on luteal regression in heifers. J. Anim. Sci. 60: 519-27.

Wolfsdorf, K.E.; Diaz, T.; Schmitt, E.J.R.; Thatcher, M.J.; Drost, M. and Thatcher, W.W. (1997): The dominant follicle exerts an intra ovarian inhibition of FSH- induced follicular development. Theriogenology, 48: 435-447.