Vet.Med.J.,Giza. Vol.49, No.1. (2001): 17-25.

FACTORS AFFECTING NUMBER OF OVARIAN FOLLICLES AND OOCYTES YIELD AND QUALITY IN EGYPTIAN BUFFALOES

A. S. S. ABDOON and OMAIMA M. KANDIL

Dept. Animal Reproduction and A.I, National Research Centre
Dokki, 12622 Giza, Egypt.

Received: 11.5.2000
Accepted :10.8.2000

SUMMARY

Three experiments were conducted. In Experiment 1, ovaries (n=126) were collected in pairs from slaughtered anoestrus, early pregnant and cyclic buffaloes. Ovarian follicles (1-3, 4-9 and (≥10 mm diameter) were counted, aspirated and oocytes were recovered and evaluated. In Experiment 2, ovaries were divided into 2 groups. Group 1, ovaries bearing a CL (n-74) and Group 2 non-bearing CL (n= 74), ovarian follicles (2-8 mm) were counted, aspirated and oocytes evaluated. Experiment 3, oocytes were recovered using aspiration or slicing methods. In all experiments, oocytes were classified into good, fair, poor and denuded. Results showed that the development of small and total ovarian follicles are continuous and independent in early pregnant or cyclic buffalo cows, however, it significantly decreased ($P<0.01$) in the ovaries from anoestrus buffaloes. Number of medium and large sized follicles significantly increased ($P<0.01$) in cyclic buffaloes on Days 10-16 and 17-22 of the oestrous cycle, while large follicles significantly decreased ($P<0.01$) in the ovaries of pregnant buffaloes. A significantly higher ($P<0.01$) percentage of poor and denuded oocytes were recovered from anoestrus and pregnant buffalo ovaries. While, the highest ($P<0.01$) percentage of good quality oocytes were recovered from ovaries of cyclic buffaloes on Days 1-3 and 10-16 of the oestrous cycle, eliciting that the stage of oestrous cycle is affecting the quality of buffalo oocytes. In addition, the presence of CL stimulates the development of a significantly higher ($P< 0.01$) number ovarian follicles which produced a significantly ($P<0.05$) higher number of good quality oocytes. Slicing of buffa-

produced a significantly higher number of fair, poor and denuded oocytes. In conclusion, number of ovarian follicles and yield and quality of oocytes were affected by the reproductive status, stage of the oestrous cycle, presence of a CL or the methods of oocytes retrieval.

Key words: reproductive status, follicles, oocytes quality, , oocyte yield CL.

INTRODUCTION

A better understanding of ovarian folliculogenesis is essential to deal with reproductive performance of buffalo. Counts of follicles on the ovarian surface and their classification into size categories based on diameter can be used to obtain a reasonable indication of actual population of follicles in the ovary (Fitzpatrick and Enywistle, 1997). In this respect, Matton *et al.* (1981) found that small follicles decreased in number gradually from day -3 to-18, while, medium sized follicles were more numerous on Day 13 than at other stages of oestrous cycle in cows. In the meantime, pregnant cows showed fewer medium and large follicles than cyclic cows, whereas, development of small follicles is not impeded during pregnancy (Dominguez, 1995). Also, Dufour *et al.* (1972) found that the ovary that contained the corpus luteum had greater follicular development than the ovary that contained no corpus luteum in sheep. However, the number of antral follicles in swamp buffalo is 20% of those observed in cattle under similar conditions and the number of non atretic follicles (>1.7mm) average 2.9 for buffalo and 22.1 for cattle (Ty *et al.*, 1989)

The collection of high quality oocytes capable of maturing and fertilized *in vitrio* is an important limiting factor particularly in species like buffalo (Totey *et al.*, 1992). A higher proportion of oocytes developed into blastocysts, faster when oocytes were isolated at the end of the luteal phase of oestrous cycle (Machatkova *et al.*, 1996). Other records found that the stage of oestrous cycle has no effect on the oocyte developmental potential (Leibfried and First, 1979). In addition, sever al researchers recorded that the presence of a CL stimulates follicular development, so that the CL bearing ovary contains more follicle (savio *et al.*, 1988), and affect oocyte maturation and their ability to develop *in vitro* (Boediono *et al.*, 1995).

However, in buffalo the number of follicles and yield of oocytes per ovary were less from ovaries bearing a CL (Das *et al.*, 1996; Kumar *et al.*, 1997). Furthermore, methods of oocyte retrieval are directly affecting the yield and quality of oocytes. Slicing of ovary yielded a significantly more oocytes per ovary in cows (Hamano and Kuwayama, 1993) and buffaloes (Kumar *et al.*, 1997).

A very few studies have dealt with ovarian follicular development in relation to the reproductive status in buffalo; also, selection for stage of the reproductive status that yielding a higher proportion of buffalo oocytes with optimum characteristics for appearance and greater probability of maturation *in vitro* were not identified in buffalo. The present investigation was undertaken to examine: (1) The effect of reproductive status and the presence of a corpus luteum (CL) on number of ovarian follicles, oocyte yields and quality in buffalo ovaries; (2) effect of methods of oocyte retrieval on yields and quality of oocytes from buffalo ovary.

MATERIALS AND METHODS

Experiment 1

This study aimed to examine the effect of reproductive status on the number of surface ovarian follicles and its relation to oocyte quality. After slaughtering the genitalia, 126 buffaloes were examined carefully to determine the reproductive status either true anoestrus (n=17); smooth ovaries without any specific stuctures (follicles or CL), early pregnant (n=22, 1-2 month) or cyclic (n=87). The ovaries were collected in pairs, placed in plastic bags containing phosphate buffered saline (PBS) and stored in thermos containing PBS at 32-36°C. In the laboratory, identification of the stage of oestrous cycle was determined using the characteristics of the ovaries based on corpus luteum color and shape according to the method adopted by Jainudeen (1986). Briefly, this combination of criteria allowed classification of pairs of ovaries into, early luteal (Day 1-3) ovulation point red and presence of red spot or small red elevation; developed CL (Days 4-9) with dark red soft protruded CL; mature CL (Days 10-16) with a sharply demarcated red to orange protrusion or embedded CL, regressing CL (Days 17-22), hard yellow to white small protrusion. Follicular diameter was measured on the ovarian surface using a caliper and follicles were classified into 3 categories: small (1 to 3 mm), medium (4 to 9 mm) and large (10 mm) according to Dominguez (1995). The content of the visible ovarian follicles was aspirated from each pair using 10-ml syringe and 18 - gauge needle. Follicular contents were transferred into 6-cm glass Perti dish and oocytes were collected and evaluated under stereomicroscope (28-30x).

Experiment 2

This experiment was designed to evaluate the effect of presence or absence of a CL on the number of ovarian follicles and oocytes yield and quality. A total of 148 ovaries collected from cyclic or pregnant buffalo cows. In the laboratory, ovaries were divided into: Group 1 (n= 74) ovaries bearing a CL; Group 2 (n= 74), with ovaries non-bearing CL. The number of ovarian follicles (2-8 mm diameter) was counted. Follicular contents were aspirated and oocytes were recovered and evaluated under stereomicroscope.

Experiment 3

This experiment was carried out to investigate the effect of methods of oocyte retrieval using aspiration or slicing on yields and quality of oocytes from buffalo ovaries. A total of 61 ovaries was collected from buffalo cows of unknown reproductive history. All visible follicles (2-8 mm diameter) on the ovarian surface were aspirated using a 10-ml syringe and an 18-gauge needle. Another, 67 ovaries were placed into a 10 cm glass petri dish containing 15 ml PBS and were chopped into small pieces with surgical blade. The sliced stromal tissues were discarded. For the 2 methods (aspiration or slicing) each ovary was processed and examined separately to assess the total oocyte recovery and quality.

Assessment of oocyte quality:

Evaluation of oocyte was carried out according to Hamano and Kuwayama (1993). Good oocytes were surrounded by more than 6 layers of cumulus cells adhering to the zona pellucida. 3-5 and 1-2 layers of cumulus cells surrounded fair and poor oocytes respectively. Necked oocytes were assigned as denuded.

Statistical analysis:

The analysis of data was carried out using analysis of variance ANOVA (Table 1) , Chi- square (Table 2) and Student " t" test (Table 3 and 4) according to Snedecor and Cochran (1980).

RESULTS

The mean (±SEM) count of ovarian follicles in small (1-3), medium (4-9) and large (10 mm) categories and total ovarian follicles are presented in Table 1. Results illustrated that ovaries from anoestrous buffaloes possess a significantly low ($P<0.01$) number of small and total ovarian follicles compared with pregnant or cyclic ones. While, difference in small and total ovarian follicles lack the significance between pregnant and cyclic buffaloes. Moreover, a significant increased ($P<0.01$) in number of medium and large follicles was observed in ovaries of cyclic buffalo on Day 17-22 and 10-16 of the oestrous

Table (1): Follicular population in relation to reproductive status in buffaloes (Mean ± SEM)

Size / Status	1-3mm	4-9 mm	≥10mm	Total no.follicle
Anoestrus (n=17)	5.167±0.757^{b}	1.286±0.338^{a}	0.372±0.078^{b}	6.825±0.916^{b}
Pregnant (n=22)	10.320±1.713^{a}	0.558±0.043^{b}	0.050±0.039^{b}	10.929±1.643^{a}
Day 1-3	10.543±2.565^{a}	0.838±0.201^{b}	0.167±0.153^{b}	11.548±2.765^{a}
Day 4-9(n=22)	11.001±1.594^{a}	0.833±0.251^{b}	0.250±0.112^{b}	12.083±1.765^{a}
Day 10-16(n=35	10.688±0.939^{a}	1.693±0.176^{a}	0.672±0.125^{a}	13.053±1.078^{a}
Day 17-22(n=16)	0.167±1.167^{a}	1.937±0.258^{a}	1.134±0.224^{a}	112.238±1.353^{a}

a,b: Difference within column ($P<0.01$)

Table (2): Percentage of oocyte quality in relation to reproductive status of buffalo ovaries.

Status \ Type	Oocyte quality (%)			
	Good	Fair	Poor	Denuded
Anoestrus (n=17)	11.1(7/63)^{c}	34.9 (22/63)^{c}	33.3 (21/63)^{c}	20.6 (13/63)^{b}
Pregnant (n=22)	31.3(52/166) ^{b}	25.3(42/66)^{c}	18.7(31/166)^{a}	24.7 (41/166)^{c}
Days 1-3	39.6 (40/101)a	23.8(24/01)^{c}	17.8(18/101)^{a}	18.8 (19/101)^{b}
Days4-9(n=22)	14.3 (24/168)c	57.1(96/68)^{a}	21.4(36/168)^{a}	7.1 (12/168)^{a}
Days10-16(n=35	37.9(108/285)a	32.3(92/85)^{a}	17.9 (51/285)^{a}	11.9 (34/285)^{a}
Days17-22(n=16)	30.4 (39/128) ^{b}	45.3(58/128)^{b}	14.8(19/128)^{a}	9.4(12/128)^{a}

a, b difference within column (P<0.05) a, c difference within column (P<0.01)

cycle, respectively. While, ovaries of pregnant buffaloes had the lowest number of large follicles.

Table 2 summarizes the results of oocyte quality in relation to reproductive status in buffaloes. The analysis of data revealed that the percentage of good quality oocytes was significantly low (P<0.01) and poor quality oocytes was high (P<0.01) when oocytes were recovered from ovaries of anoestrous buffaloes. In addition, ovaries from pregnant and anoestrus buffaloes produced significantly high (P<0.01 and P<0.05, respectively) percentages of denuded oocytes. The highest percentage of good oocytes was recovered from ovaries of cyclic buffaloes on Day 1-3 and 10-16 of the oestrous cycle. Also, a significantly high (P<0.01) percentage

Table (3): Effect of presence or absence of CL on number of ovarian follicles and oocytes quality in buffalo (Mean ±SEM)

Groups	No.follicles/ ovary	Oocyte quality			
		Good	Fair	Poor	Denuded
With a CL	5.832±0.298**	1.74 ± 0.22 *	0.98 ± 0.09	0.54 ± 0.11	0.34 ± 0.01
Without CL	4.318 ± 0.180	1.15 ± 0.32	0.78 ± 0.04	0.51 ± 0.08	0.44 ± 0.08*

P<0.05

Table (4): Effect of methods of retrieval on oocytes yield and quality in buffalo ovaries (mean ± SEM

Groups	No.follicles/ ovary	Oocyte quality			
		Good	Fair	Poor	Denuded
Aspiration	3.34 ± 0.16	1.98 ± 0.14	0.76 ± 0.13	0.31 ± 0.08	0.18 ± 0.09
Slicing	8.08 ± 0.42**	2.15 ± 0.16	1.24 ± 0.18*	1.44 ± 0.11**	3.43±0.32**

P<0.05 **P<0.01

of fair oocytes was collected from ovaries of cyclic buffaloes on Day4-9 and 17- 22 of the oestrous cycle.

In this experiment, data showed that buffalo ovaries bearing a CL had a significantly higher ($P<0.05$) number of ovarian follicles and produced significant higher ($P<0.05$) number of good quality oocytes than ovaries non-bearing CL (Table 3).

The analysis of data (Table 4) indicated that slicing of buffalo ovaries produced a significantly higher ($P<0.01$) number of oocytes per ovary compared with aspiration of follicles. In the mean time, a significantly highernumber of fair and poor ($P<0.05$) or denuded ($P<0.01$) oocytes were recovered by using slicing than aspiration method.

DISCUSSION

Results obtained in this study indicate that reproductive status of buffalo cows significantly affect the number of ovarian follicles. In experiment 1, number of small and total ovarian follicle significantly decreases in ovaries from anoestrus compared with cyclic or pregnant buffalo. Similar results were previously reported by (Das *et al.*, 1996) who found poor reserves of follicles in non-cyclic buffalo. The condition was attributed to the presence of less number of primordial follicles in ovaries of non-cyclic buffalo (Danell, 1987). In addition, the present work showed that the number of small and total follicles was lack of significance between ovaries of pregnant and cyclic buffalo, indicating that the development of small ovarian follicles is continue during pregnancy and independent of the stage of oestrous cycle. These results are consistent with previous observation (Roche *et al.*, 1991) in pregnant cows, (Dufour *et al.*, 1972) and cyclic cows. In contrary, Gutierrez et al (1997) reported that the number of small size follicles increased significantly on Days 2 and 14 of oestrous cycle in cows. This discrepancy may be attributed to species difference. Concurrently, in the present study the development of medium and large sized follicle was significantly increased on Days 10-16 and 17-22 of the oestrous cycle. This result concordant with previous results in cattle (Matton *et al.*, 1981). Moreover, in the present study these was a decrease in the number of large follicles in ovaries of early pregnant buffaloes. The developing follicles reach only a smaller size and are less likely to continue into later stage (Knickerbocker *et al.*, 1986).

Prediction of morphologically normal oocytes would facilitate studies, which assess factors influencing in vitro maturation, fertilization and culture of buffalo oocytes. In the present study the percentage of good quality oocytes was significantly low, while, poor and denuded oocytes were significantly higher when oocytes were recovered from ovaries of anoestrus buffalo cow, indicating that most of the ovarian follicles in anoestrus buffalo may be atretic. Similar results

were recorded by Jain *et al.* (1995) in buffalo. In addition, the highest percentage of good quality oocytes was recovered from ovaries of cyclic buffalo cows on Days 1-3 and 10-16 of the oestrous cycle, eliciting that the stage of oestrous cycle is influencing the quality of oocytes regardless the number of ovarian follicles. These results are similar to that previously reported in cattle (Matchatkova et al., 1996; Hagemenn *et al.*, 1997). Those authors found that oocyte competence is influenced by the stage of the oestrous cycle, oocytes collected during Days 2 and 10 produced significantly more high quality oocytes for in vitro fertilization. On the other hand, Arlotto *et al.* (1997) observed that the stage of oestrous cycle is not influencing the quality of oocytes. Moreover, the present work demonstrated that high percentages of denuded oocytes were recovered from ovaries of pregnant buffalo. While, Domingues (1995) reported that pregnancy did not seem to affect oocyte quality and the proportion of normal oocytes was similar in cyclic and pregnant cows. This discrepancy may by attributed to the stage of pregnancy or species difference.

The results obtained in Experiment 2 indicated that in buffaloes, ovaries bearing a CL had a significantly higher number of follicles and yielded a significantly higher number of good quality oocytes compared with ovaries non-bearing CL. This finding is consistent with the observation of Savio *et al.* (1988) in cattle. However, Das *et al.* (1996) recorded that the presence of a CL significantly reduced the number of ovarian follicles as well as the quality of the oocytes. The reason for such difference may be attributed to breed or genotyping difference in ovarian function between the Mediterranean and swamp buffaloes.

Mean while, the present study showed that the number of oocytes recovered per buffalo ovary was significantly higher by using slicing of ovaries than aspiration of follicles. Similar observation has been reported in cattle (Martino *et al.*, 1992) and buffalo (Das *et al.*, 1996; Kumar *et al.*, 1997). Slicing of ovary release oocytes from surface follicles and these in the deeper cortical stroma. However, the present results illustrated that slicing of ovaries produced significantly higher number of fair, poor and denuded oocytes. Martino *et al.* (1992) attributed the condition to the fact that this technique recovers heterogeneous population of oocytes from all kinds of follicles distributed throughout the ovarian stroma, and many of these oocytes are not fully grown. In contrary, Hamano and Kuwayama (1993) recorded that slicing of ovaries produced significantly higher good quality oocytes. This difference may be due to the method of slicing and apparatus used or species difference.

In conclusion, number of surface ovarian follicles is continue to develop during pregnancy and independent of the stage of oestrous cycle, but it significantly reduced in ovaries from anoestrus buffalo. Growth and dominance of follicles oc-

curs on Days 10-16 and 17-22 in Egyptian buffalo. Higher percentage of good quality oocytes was recovered from ovaries of cyclic buffalo on Days 1-3 and 10-16 of the oestrous cycle, eliciting that stage of oestrous cycle affect the quality of oocytes recovered. Higher percentage of poor and denuded oocytes was recovered from ovaries of anoestrus or early pregnant buffalo cows. Ovary bearing CL had a significantly higher number of follicles and yielded higher number of good quality oocytes. Slicing of buffalo ovaries produced a significantly higher number of fair, poor and denuded oocytes.

ACKNOWLEDGEMENT

This work was financially supported by a Grant (2/1/2/2/5/B) from the National Research Centre of Egypt.

REFERENCES

Arlotto T, Schwartz JL., First NL. and Leibfried ñ Rutledge ML. (1996): Aspects of follicle and oocyte stage that affect in vitro maturation and development of bovine oocytes. Theriogenology 45: 943 – 956.

Boediono A, Rajamahendran R, Saha S, Sumantri C and Suzuki T. (1995): Effect of the presence of a CL in the ovary on oocyte number, cleavage rate and blastocyst production in vitro in cattle. Theriogenology 43: 169 (Abst).

Carolan C, Monaghan P, Mehmood A, Lonergam P, Gallagher M and Gordon I.. (1992): Slicing of bovine ovaries as a means of oocyte recovery. J. Reprod. Fert. 9: 51 (Abst).

Danell B., (1987): Oestrous behavior, ovarian morphology and cyclical variation in the follicular system and endocrine pattern in water buffalo heifers. Ph.D. Dissertation, Swedish University of Agricultural Science, Upsala.

Das GK, Jain GC, Solanki VS. and Tripathi VN., (1996). Efficacy of various collection methods for oocyte retrieval in buffalo. Theriogenology 46: 1403 – 1411.

Dominguez MM. (1995): Effect of body condition, reproductive status and breed on follicular population and oocyte quality in cows. Theriogenology 25: 800 – 805.

Dufour J, Whitemore HL, Ginther OJ. and Casida LE., (1972): Identification of the ovulating follicle by its size on different days of the estrous cycle in heifers. J. Anim. Sci. 34: 85 – 87.

Fitzpatrick LA. and Entwistle KW. (1997): A comparison of dissected follicle numbers and follicle counts on the ovarian surface for the evaluation of ovarian follicular population in Bos indicus cows. Anim. Reprod. Sci., 46: 179 – 186.

Gutierrez CG, Oldman J, Bramle TA, Gong JG, Campbell B K. and WebbR. (1997): The recruitment of ovarian follicles is enhanced by increased dietary intake in heifers. J. Anim. Sci., 75: 1876 – 1884.

Hagemann LJ, Peterso AJ, Beaumont S, Berg M, Donnison MJ, Ledgard A, Schurmann A. and Tervit HR. (1997): Stage of estrous cycle affects overall development of cattle oocytes to blastocyst in vitro. Theriogenology 48: 27 (Abst).

Hamano S. and Kuwayma M. (1993): In vitro fertilization and development of bovine oocytes recovered from the ovaries of individual donors: A comparison between the cutting and aspiration method. Theriogenology 39:

703 - 712.

Jain GC, Das GK, Solanki VS. and Tripthai VN.(1995): Comparative efficiency of three different collection techniques for oocyte retrieval in buffaloes. Theriogenology 43: 240 (Abst).

Jainudeen MR., (1986): Reproduction in the water buffalo. In: Morrow DA (ed.). Current Therapy in Theriogenology. WB Saunders Company Philadelphia, USA.

Knickerbocker JJ, Drost M, Thatcher WW., (1986): Endocrine patterns during the initiation of puberty, the estrous cycle, pregnancy and parturition in cattle In: Morrow DA (ed.), Current Therapy in Theriogenology. WB Saunders Company Philadelphia, 117– 125.

Kumar A, Solanki V S, Jindal SK, Tripathi VN. and Jain GG. (1997): Oocyte retrieval and histological studies of follicular population in buffalo ovaries. Anim. Reprod. Sci., 47: 189 – 195.

Leibfried L. and First NL. (1979): Characterization of bovine follicular oocytes and their ability to mature in vitro. J. Anim. Sci., 48: 76 - 86.

Machatkova M, Jokesova E, Petelikova J. and Dvoracek V. (1996): Developmental competence of bovine embryos derived from oocytes collected at various stages of the estrous cycle Theriogenology 45: 801 – 810.

Martino A, Mogas T, Palomo MJ. and Paramio MT. (1992): Effect of recovery on the IVM of prepubertal goat oocytes. J. Reprod. Fert., 9: 53 .

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

Roche JF. and Boland MP., .Roche JF. and Boland MP. (1991): Turnover of dominant follicles in cattle of different reproductive states. Theriogenology 35: 81 – 90.

Savio, J.D., Keenan, L., Boland. M.P and. Roche, J.F. (1988): Pattern of growth of dominant follicles during the oestrous cycle of heifers. J.Reprod. Fert., 83: 663 – 671.

Snedecor GW. and Cochran WG. (1980):. Statistical Methods. Iowa State University Press.

Totey SM, Singh G, Taneja M, Pourste CH. and Talwar GP. (1992): In vitro maturation, fertilization and development of follicular oocytes from buffalo (Bubalus bubalis). J. Reprod. Fert. 95: 597 –607.

Ty LV, Chupin D. and Driancourt DA., (1989): Ovarian follicular population in buffaloes and cows. Anim. Reprod. Sci., 19: 171- 178.