

Dept. of Theriogenology,  
Fac. of Vet. Med., Assiut University, Assiut Egypt

## EFFECT OF SEASONS ON OVARIAN MORPHOLOGY AND OOCYTES QUALITY IN SLAUGHTERED BUFFALOES (With 8 Tables)

By

**A.M. OSMAN and S.H. SHEHATA**

(Received at 5/3/2005)

تأثير المواسم على وصف المبيض ونوع البويضات في الجاموس المذبوح

احمد ممدوح حامد عثمان ، شحاته حسن محمد شحاته

تم فحص وتشريح الاعضاء التناسليه لعدد ٢٩٣ جاموسه سليمه وغير عشار مذبوحه في السلخانه وتم الفحص بعد الذبح مباشرة في المعمل لمدة عام كامل -وبعد استبعاد الاعضاء التناسليه الغير طبيعيه تركزت الدراسه على ٢٤٢ جهاز تناسلى سليم حيث تم فحص المبايض لكل جاموسه لمعرفة الحاله التناسليه وبعد ذلك تم قياس ابعاد كل مبيض وكل جسم اصفر وتم تقدير الوزن. تم عد الحويصلات الموجوده على سطح المبيض لكل جاموسه مع قياس قطر كل حويصله بالمسماك. بواسطه مضخه شفط كهربائيه وحقنه طبيه سعة ١٨ - متصله بقنينه زجاجيه محكمة الغطاء تم سحب محتويات الحويصلات. ومن خلال الاستريوميكروسكوب والميكروسكوب المعملى تم التعرف على البويضات الموجوده حيث تم عدھا وفحصھا لدراسة تأثير المواسم على جودتها. بينت النتائج ان المبايض التي تحمل جسم اصفر كانت اكبر من المبايض الاخرى بفروق معنويه ( $P > 0.05$ ) يمثل الجسم الاصفر ٨١ .٠ ± ٣٠,١% من وزن المبيض. لم يكن هناك تأثير معنوى للمواسم على مقاسات وأوزان المبيض. تبين ان عدد الحويصلات الكلية بكل مبيض كان عاليا خلال موسم الربيع عن باقى المواسم (٢,١٥ مقابل ١,٩ فى الصيف، ١,٨ فى الخريف، ١,٥ فى الشتاء) اما عدد الحويصلات الكبيره (أكبر من ٠,٩ سم) بكل مبيض كان قليلا جدا فى موسم الصيف عن باقى المواسم (٠,٢٧ مقابل ٠,٤٣ فى الربيع، ٠,٤٢ فى الخريف، ٠,٣٧ فى الشتاء). ادت الحويصلات الصغيره (اصغر من ٠,٤ سم) فى المبيض خلال الصيف عنه فى باقى المواسم وكان الفرق معنويا بين الصيف والشتاء ( $P > 0.05$ ). وكانت النسبة ٥٧,٦% فى الصيف مقابل ٣٢,٥% فى الخريف و ٣١,٨% فى الشتاء و ٤٢,٩% فى الربيع. بلغ العدد الاجمالي للبويضات المعرفه ٥٦٣ بويضه منهم ٤٧٧ بويضه سليمه (جيده وعليها تراكم من ٣ طبقات) وكانوا اكثر انتشارا فى الحويصلات الكبيره عن ٠,٣ سم - بلغت نسبة استخلاص البويضات الجيده ٩٠,٦% فى الحويصلات المشفطه التى كانت اكبر من ٠,٣ سم مقابل ٦٢,٥% فى الحويصلات الأصغر من ذلك. وكان تأثير المواسم على هذه النتائج يرجع الى توزيع الحويصلات الصغيره فى المبايض خلال المواسم حيث كان مرتفعا خلال

الصيف. بالنسبة للحويصلات الكبيرة فكانت أكثر انتشارا في الربيع. كانت النسبة الكلية لاستخلاص البويضات هي ٢, ٩٠ % من اجمالي ٦٢٤ حويصلة مشفطة. يستنتج من هذه الدراسة أن موسم الصيف ليس بالموسم المناسب لمثل هذه الدراسة نظرا لقلّة عدد الحويصلات المناسبة في الحجم والتي تعطى بويضات جيدة سليمة تؤدي الى نجاح أى دراسة لاحقة. كما أن حرارة الجو المرتفع وسوء معدلات التغذية لها تأثير سلبي على الوظائف الفسيولوجية للمبايض خلال هذا الموسم في الجاموس لانتاج حوصلات متوسطة وكبيرة ذات بويضات سليمة صالحة لأى تجارب لاحقة.

## SUMMARY

Normal genitalia (242 out of 293) from healthy, non-pregnant slaughtered buffaloes were taken freshly and dissected. Ovaries were examined to determine the reproductive status of animals. Dimensions and weight of each ovary and Corpus Luteum (CL) were measured. Number of follicles per animal was counted and their diameter was measured, and then follicular fluid was aspirated using an 18-gauge needle, fitted with stopper glass bottle and electric suction pump. Pooled samples were examined under a stereomicroscope to identify and evaluate the oocytes. The results showed that ovaries with CL were significantly larger ( $P < 0.01$ ). CL represents  $30.1 \pm 0.81\%$  of ovarian weight. Seasons have no significant effect on both criteria. Total number of follicles per ovary was higher during spring than other seasons (2.15 versus 1.9 summer, 1.8 autumn and 1.5 winter). Numbers of follicles ( $> 0.9$  cm) per ovary were much lower in summer than other seasons (0.27 versus 0.43 spring, 0.42 autumn and 0.37 winter). Total number of recovered oocytes was 563, and among them 477 was intact (good with comulus 3-4 layers). The incidence of intact oocytes was higher in medium and large follicles than smaller ones (92.3 % , 88.9 % and 71.8 % respectively). The average recovery rate was 90.2% from 624 aspirated follicles without marked variations between seasons. It was suggested that summer seemed to be unfavorable for similar studies due to the lower number of medium and large follicles having high incidence of intact oocytes leading to further successful studies.

**Key words:** Buffalo, season, ovary, oocytes, follicles

## INTRODUCTION

The technology of in vitro fertilization still facing a lot of difficulties in buffaloes. The origin of the oocytes is of critical value in

the progress of such technique. Badr *et al* (2003) concluded that in order to achieve a reasonable recovery rates for in vitro maturation (IVM) and fertilization (IVF) the ovaries might be stored not more than 4 hours post-slaughter in worm saline. In cows and buffaloes, Ectors *et al* (1995) and Yousaf and Chohan (2003) cited that oocytes from abattoir ovaries displayed a size-dependant ability to undergo maturation and oocytes larger than 4 mm can give better results for IVM and IVF. In addition, Neglia *et al* mentioned that aspirated cattle oocytes had significant higher cleavage rate than buffalo ( 83,9 % versus 64.8% )

Abdoon and Kandil (2001) found that slicing of ovary produced higher oocytes recovery rate than aspiration but percentages of fair, poor and denuded oocytes were also higher. Sharma and Taneja (2000) recorded that individual follicle isolation yielded the highest number of oocytes per ovary when compared with other methods. Moreover, the presence of corpora lutea significantly raised the number of follicles and number of good quality oocytes. However, good quality oocytes were recovered from ovaries of cycling buffaloes at days 1-3 and 10-16 of the estrous cycle (Abdoon and Kandil, 2001).

Suzuki *et al* (1992) and Abdoon *et al* (2001) reported that buffalo oocytes surrounded by multi-layers of cumulus cells had a significant higher rates for IVM and IVF than partially surrounded or denuded oocytes. The cumulus cells surrounding the oocytes are essential to facilitate the transport of nutrients and signals into and out of the oocytes (Anderson 1991).

In order to optimize the IVF technology in buffalo, Ravindranatha *et al* (2003) suggested that storage of ovaries at 4 C° for 12 or 24 hour significantly reduced the developmental potential of oocytes. Kumar *et al* (1997) established a biphasic relationship of growth between oocytes diameter and follicular size in ovaries from slaughtered buffaloes. It was concluded by Raghu *et al* (2002) that the larger the size of the follicles the greater the in vitro development and competence of buffalo oocytes.

The body condition score (3-5 degrees) of buffalo was recorded by Ahmed *et al* (1999) to improve the oocytes quality. Nandi *et al* (2001) found that more oocytes were recovered per ovary during the cool period of the year than during the hot period in Indian buffaloes. Reproductive efficiency in buffalo cows is hampered by many factors including the season. Osman (1984), El Naggat *et al* (1985), Abdoon *et al* (1992), Srivastava and Pant (1999) and Sing *et al* (2000) gave an

ample information about the domination of ovarian sub-function in water buffalo during the dry hot period of the year.

The current investigation was carried out to emphasize the effect of seasons on ovarian morphology as implicated to the quality of oocytes in slaughtered buffaloes.

## **MATERIALS and METHODS**

### **Collection of genitalia:**

From a local slaughterhouse, 293 genitalia were taken freshly from adult healthy buffalo cows and immediately brought to the lab within an hour (20 km distance). The materials were collected during a whole year. Abnormal, pathologically affected genitalia were excluded and the study included finally 242 pair ovaries out of them 3 contained large dermoid cysts. Further 10 ovaries with corpora lutea were taken from pregnant genitalia (less than 2 months) for comparative studies.

Thorough morphological examinations were done to classify the reproductive condition of the animal into: proestrus, estrus, metestrus and diestrus according to the ovarian structures. Intact ovaries were taken, washed with water and kept in a dish with normal saline. Dimensions and weights of each ovary and corpus lutea were performed. Numbers of follicles were counted per each ovary and per animal. Their diameters were measured to the nearest 1/10 cm using a caliber.

### **Follicular fluid aspiration:**

Ovaries with follicles were plotted with filter paper. An electrical suction pump, fitted with a sterile stopper glass bottle and a receiving sterile conical flask connected to a rubber tube with 18 gauge needle were used to puncture and aspirate the follicular fluid. At the end of each collection, few ml of saline were drawn to avoid any oocytes losses. Pool samples in the flask were transferred to a sterile test tube and kept vertically for 20 minutes before microscopic examination. Attention was paid to collect the pool samples according to the size of the follicles in many cases.

### **Oocyte-Cumulus Complexes (OCCs) recovery and evaluation:**

Using a lab water pump the supernatant fluid in the test tube was removed. The rest portion containing the oocytes was transferred to small petri dishes and examined under a Stereomicroscope to identify, count and evaluate the oocytes according to Yong and Lu (1990). Some

oocytes were aspirated with a mouth pipette, fixed on slide and stained with aceto-orcein and Gemsa stains for further investigations.

The results were recorded as:

**Intact good quality oocytes:**

With cumulus > 2 layers and homogenous cytoplasm

**Denuded bad quality oocytes:**

With cumulus < 3 layers.

**Statistical analysis:**

All data were subjected for statistical analysis according to Snedecor and Cochran (1980) and the Costat Computer Program (1986).

## RESULTS

The biometrical studies of the ovaries from slaughtered buffaloes as related to seasons are presented in Tables 1-4. It is evident from these findings that no significant differences could be traced in the dimensions and weights of the ovaries between seasons as well as between the right and left side. The 3 ovaries with dermoid cysts were much larger in size and varied in weight between 8-12 gm and excluded from the data.

On the other hand significant differences were proved between the different conditions of the ovaries within seasons and as well among all organs (Tables 5). Ovaries carrying corpora lutea and follicles were the largest while static ovaries were the smallest. Ovaries with corpora lutea alone or follicles alone lies in between with regard to dimensions (Length, breadth and thickness) and weight.

Table 6 summarized the weights of corpora lutea as related to their ovaries. Significant differences between seasons were not evident and the average corpus luteum weight represents about 1/3 of the intact ovary. Moreover, the corpus luteum periodicum was significantly ( $P < 0.05$ ) smaller than that of pregnancy ( $1.59 \pm 0.04$ ,  $n = 158$ , versus  $2.12 \pm 0.2$  gm,  $n = 10$ ).

As far as the numbers of follicles per ovaries are concerned, table 7 showed their results according to their diameters in different seasons.

There is no marked difference between the right and left ovaries. However, during summer the percentages of large follicles ( $> 0.9$  cm) represented only 16% of the total follicles while during autumn, winter and spring the percentages were 31%, 32% and 26% respectively. Per

ovary the average number of large follicles ( $> 0.9$  cm) was low in summer 0.27, versus 0.44 spring 0.42 autumn and 0.37 winter.

The number of small follicles ( $< 0.4$  cm) per ovary was higher in summer than any other seasons (0.9 summer versus 0.32 autumn, 0.24 winter and 0.6 spring). It is evident from these findings that during summer the incidence of large follicles per ovary decreased while that of small follicles increased. Moreover, the incidence of ovaries without CL and or follicles (static ovaries) was relatively higher in summer than other seasons (22.9 % summer, versus 10.8 % autumn, 20.8 spring and 18.9 % winter.

The total number of follicles per ovary (Table 8) was higher during spring than any other seasons. The relatively high incidence for the number of follicles per ovary during summer (1.94) was attributed to the high incidence of small dormant follicles ( $< 0.4$  cm) per ovary in that season (57.6 % versus 32.5% autumn, 31.8% winter and 42.9% spring).

The intact good quality oocytes of buffaloes were surrounded by variable thickness of cumulus, more than 3 layers, within a condense homogenous cytoplasm. Oocytes surrounded by less layers or dispersed granulosa cells were considered denuded and of bad quality. Degenerated or abnormal shaped oocytes were among this group. The average recovery rate for all oocytes (Table 8) was 90.4 % from 624 aspirated follicles (77.1 % for intact and 13.3 % for denuded oocytes). Moreover, the average number of oocytes per ovary was 1.68. The recovered total oocytes per ovary was higher in spring followed by summer, autumn and winter but the differences were not significant.

Regarding the effect of follicular size on oocytes quality, 63 intact oocytes were recovered from 76 follicles with variable size. The incidence of good quality oocytes was 92.3 % in medium size follicle (0.4-0.9 cm ) versus 88.9 % in larger and 71.8 % in smaller follicles.

## DISCUSSION

The low efficiency of superovulation and embryo transfer program in buffalo lead to an increasing interest for in vitro embryo production technologies to faster the propagation of superior germ plasm in this species (Gasparrini, 2003). The available literature reviewing an ample evidence about the factors influencing the quality of oocytes obtained from abattoir for such purposes. The integral effect of season

on in vitro oocytes maturation and fertilization appeared lacking and needs certain elucidation.

The obtained results showed variations between seasons in the biometrical data of the ovaries but not at a significant levels. The prevalence of ovaries without CL and F in summer coincides with the recent investigation of Ahmed (1997). In harmony with these findings, Hemieda (1988) and Eltohamy (1994) cited that ovarian inactivity during the dry season of the year (June-November) was high. However, the significant differences observed in weight between the different cyclic conditions of the ovaries, support the findings of Farrag (1978) and Osman (1984) that ovaries with CL and F were larger than ovaries without any of both. The significant difference between CL gravidatis and CL periodicum weights might reflect the huge tasks for progesterone production during early pregnancy in buffaloes. Between seasons, no valuable differences could be traced in CL weight as related to the ovarian weight. However, Abdoon *et al* (1992) recorded higher levels of progesterone during the luteal phase of the cycle in summer, autumn and winter than spring.

It is concluded from the biometrical investigations on buffalo ovaries that seasons have no significant effects on their size or weight.

In concern with the number and diameter of follicles per ovary, the obtained results showed marked reduction in the incidence of large follicles ( $> 0.9$  cm) during summer (0.27 versus 0.38 in other seasons). In addition, the increased number of small follicles ( $< 0.4$  cm) per ovary in summer than other seasons (0.9 versus 0.38) leads us to the persuasion that inactive ovaries were dominated in such dry hot season in Egypt. Singh *et al* (2000) cited that reproductive efficiency is hampered in Indian buffalo by poor estrus expression in summer.

Ahmed (1997) reported that ovarian inactivity was prevailed during the dry season of the year and in animals with poor body condition score compared to green season and those with good body condition score.

With regard to the incidence of intact good quality oocytes per ovary, our results showed no significant effects for the seasons on such criteria. On the other hand, the incidence of intact oocytes was higher (92.3 %) in the medium sized follicles (0.4-0.9 cm) than either the larger (88.9 %) or smaller follicles (71.8 %). Ectors *et al* (1995) observed that better in vitro development occurred with oocytes collected from follicles larger than 4 mm. Comparable results were recorded by Kumar

*et al* (1997), Yousaf and Chohan (2003) who emphasized a size dependent ability between follicles and oocytes to obtain better results for IVF.

As a major conclusion from the present study, it seems possible that summer can adversely affect the functional activity of the ovaries to produce medium and large size follicles suitable to give high incidence of good quality oocytes favorable for further successful IVF processing. Thus, dry summer season might be not suitable for such technique of breeding in buffalo.

## 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, 1, 71-77.
- Abdoon, A.S.; Ahmed, W.M. and Hassan, S.G. (1992):* Seasonal variations in blood biochemistry in normal and anoestrus buffalo cows. *Egypt. J. Vet. Sci.* 29, 35-46.
- Abdoon, A.S.; Kandil, O.M.; Otoi, T. and Suzuki, T. ((2001):* Influence of oocytes quality, culture media and gonadotropins on cleavage rate and development of in vitro fertilized buffalo embryos. *Anim. Reprod. Sci.* 65, 215-223.
- Ahmed, W.M. (1997):* Investigations on ovarian inactivity in Egyptian buffaloes. *Zag.Vet. J.* 25, 3, 94-102.
- Ahmed, W.M.; Abdoon, A.S.; Shalaby, S.I. and Kandil, O.M. (1999):* effects of reproductive status and body condition on ovarian follicles and oocytes quality in buffalo cows. *Buffalo Journal*, 15, 3, 333-343.
- Badr, M.R.; Darwish, G.M.; Sosa, G.A.; Agag, M.A. and El Azab, A.I. (2003):* Factors affecting the technology of in vitro fertilization and And embryo production in buffaloes. *Proc. 15<sup>th</sup> Ann. Congr. E.S.A.R.F., Quena and Luxor, 26-30 January, 93-105.*
- Costat Computer Program (1986):* Version 3.03 Copyright C.C.P. Software.
- Ectors, F.J.; Koulischer, L.; Jamar, M.; Herens, C.; Verloes, A.; Remy, B. and Beckers, J.F. (1995):* Cytogenetic study of bovine oocytes matured in vitro. *Theriogenology*, 44, 445-400.
- El-Nagggar, M.A.; Osman, A.M.; Farrag, A.A. and Shehata, S.H.M. (1985):* Ovarian inactivity among Egyptian cows and buffaloes .A: seasonal variations. *Assiut Vet. Med. J.* 14, 28, 212-216.



- Eltohamy, M.; Ahmed, W.M. and Abdoon, A.S. (1994):* Studies of reproduction in farm animals under the conditions of new reclaimed areas-ovarian activity in relation to blood composition Proc. 4<sup>th</sup> Wld. Buffalo Congr., Sao Paulo, Brazil. 3, 612-614.
- Farrag, A.A. (1978):* Ovarian syndromes in cattle and buffaloes with special reference to certain infertility problems. Ph.D. Thesis, Fac.Vet. Med., Assiut Univ.
- Gasparrini, B. (2002):* In vitro embryo production in buffalo species: State of the art. Theriogenology,57,1,237-256.
- Hemieda, N.A. (1988):* Reproductive problems in buffalo in the world. Proc. 2<sup>nd</sup> Wld. Buffalo Congr., New Delhi, India, 3, 197-205.
- Kumar, A.; Solanki, V.S.; Jindal, S.K.; Tripathi, V.N. and Jain, G.C. (1997):* Oocyte retrieval and histological studies of follicular population in buffalo ovaries. Anim. Reprod. Sci. 47, 3, 189-195.
- Madison, V.; Avery, B. and Greve, T. (1992):* Selection of immature bovine oocytes for developmental potential in vitro. Animal Reprod. Science 27: 1-11.
- Nandi, S.; Chauhan, M.S. and Palta, P. (2001):* Effect of environmental temperature on quality and developmental competence in vitro of buffalo oocytes. Vet. Rec. 148, 28, 279.
- Osman, A.M. (1984):* Ovarian inactivity and repeat breeder syndromes in buffalo with possible treatment. J. Egypt. Vet. Med. Assoc., 44, 2, 85-98.
- Raghu, H.M.; Nandi, S. and Reddy, S.M. (2003):* Follicle size and oocytes diameter in relation to development competence of buffalo oocytes in vitro. Reprod. Fertil. Dev., 14, 1-2, 56-61.
- Ravindranatha, B.M.; Nandi, S.; Raghu, H.M. and Reddy, S.M. (2003):* In vitro maturation and fertilization of buffalo oocytes-effect of storage of ovaries, IVM temperatures, storage of processed sperm and fertilization media. Reprod. Domest. Anim., 38, 1, 21-26.
- 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. Ind. 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. Science, 2, 60, 593-604.

- Snedecor, G.W. and Cochran, W.C. (1980):* Statistical Methods. 7<sup>th</sup> Ed., Iowa Univ. Press, Ames, USA.
- Srivastava, S.K. and Pant, H.C. (1999):* Inhibitory activity in buffalo follicular fluid and its elution pattern during different seasons. *Ind. J. Exp. Biol.*, 77, 4, 370-373.
- Suzuki, T.; Singla, S.K.; Sujata, J. and Madan, M.L. (1992):* In vitro fertilization of water buffalo follicular oocytes and their ability to cleave in vitro. *Theriogenology*, 30, 1187-1194.
- Yong, Y.B. and Lu, K.H. (1990):* The influence of bovine Oocyte type on the in vitro fertilization and subsequent in vitro development. *Theriogenology*, 33: 355.
- Yousaf, M.R. and Chohan, K.R. (2003):* Nuclear morphology, diameter and meiotic competence of buffalo oocytes relative to follicular size. *Reprod. Fertil. Dev.*, 13,

**Table 1:** Biometry of the ovaries in slaughtered buffaloes during Spring.

OVAR-IES	With Corpora Lutea only			With Corpora Lutea and Follicles			With Follicles only			Without Corpora Lutea or Follicles (Static)		
	Right	Left	Total (Average)	Right	Left	Total (Average)	Right	Left	Total (Average)	Right	Left	Total (Average)
Length (cm)	2.3±0.05	2.4 ± 0.08	2.3± 0.06 <sup>ab</sup>	2.4± 0.08	2.5± 0.07	2.4 ± 0.06 <sup>***</sup>	2.2± 0.12	2.4± 0.05	2.3± 0.04 <sup>b</sup>	2.04±0.09	2.1 ± 0.1	2.05 ± 0.07 <sup>c</sup>
Breadth (cm)	1.5±0.06	1.7±0.12	1.6±0.09 <sup>b</sup>	1.8±0.06	1.9±0.07	1.87±0.05 <sup>***</sup>	1.6±0.09	1.6±0.05	1.6± 0.03 <sup>b</sup>	1.4 ±0.06	1.3± 0.07	1.4 ± 0.05 <sup>b</sup>
Thickness (cm)	1.3±0.03	1.4±0.08	1.4±0.06 <sup>b</sup>	1.5±0.04	1.5±0.10	1.53±0.05 <sup>**</sup>	1.4±0.07	1.3±0.04	1.4± 0.03 <sup>b</sup>	1.1 ±0.06	1.2± 0.09	1.2 ± 0.05 <sup>b</sup>
Weight (g)	4.2±0.2	4.6±0.4	4.5±0.32 <sup>b</sup>	5.5±0.35	5.5±0.40	5.5 ±0.3 <sup>***</sup>	3.9±0.5	3.9±0.2	3.9± 0.2 <sup>b</sup>	2.8 ±0.28	2.6 ± 0.3	2.75±0.19 <sup>b</sup>
n (Total = 130)	3	10	13	23	13	36	24	30	54	16	11	27
Incidence among total ovaries	10.0%			27.7%			41.5%			20.8%		

\*\* P<0.05

Values with different superscript for the same character differ significantly.

**Table 2: Biometry of the ovaries in slaughtered buffaloes during Summer.**

OVAR-IES	With Corpora Lutea only			With Corpora Lutea and Follicles			With Follicles only			Without Corpora Lutea or Follicles (Static)		
	Right	Left	Total (Average)	Right	Left	Total (Average)	Right	Left	Total (Average)	Right	Left	Total (Average)
Length (cm)	2.4±0.03	2.3 ± 0.09	2.4±0.1 <sup>a</sup>	2.5±0.07	2.6±0.15	2.5 ± 0.08 <sup>a**</sup>	2.1±0.6	2.6±0.1	2.2±0.07 <sup>b</sup>	2.1±0.15	2.2±0.1	2.1 ± 0.09 <sup>b</sup>
Breadth (cm)	1.8±0.03	1.68±0.06	1.7±0.08 <sup>a</sup>	1.8 ± 0.11	1.7±0.06	1.8 ± 0.07 <sup>a**</sup>	1.5±0.05	1.5±0.08	1.5±0.04 <sup>b</sup>	1.2 ± 0.07	1.2±0.08	1.1 ± 0.05 <sup>c</sup>
Thickness (cm)	1.5±0.08	1.51±0.08	1.5±0.06 <sup>a</sup>	1.6 ± 0.05	1.6±0.08	1.6 ± 0.05 <sup>a**</sup>	1.5±0.06	1.4±0.07	1.4±0.04 <sup>b</sup>	1.2 ± 0.12	1.1±0.06	1.2 ± 0.07 <sup>c</sup>
Weight (g)	6.0±1.9	4.8±0.28	5.1±0.4 <sup>a</sup>	5.67±0.4	5.9±0.29	5.46 ± 0.9 <sup>a**</sup>	3.4±0.17	4.6±0.5	3.9±0.2 <sup>a</sup>	3.03 ± 0.4	2.7 ± 0.26	2.8 ± 0.2 <sup>b</sup>
n (Total = 83)	4	10	14	9	8	17	21	12	33	9	11	19
Incid-ence among total ovaries	16.9%			20.5%			39.7%			22.9%		

\*\* P<0.05  
Values with different superscript for the same character differ significantly.

**Table 3: Biometry of the ovaries in slaughtered buffaloes during Autumn.**

OVAR-IES	With Corpora Lutea only			With Corpora Lutea and Follicles			With Follicles only			Without Corpora Lutea or Follicles (Static)		
	Right	Left	Total (Average)	Right	Left	Total (Average)	Right	Left	Total (Average)	Right	Left	Total (Average)
Length (cm)	2.3±0.07	2.3 ± 0.10	2.29± 0.07 <sup>b</sup>	2.5±0.05	2.5± 0.04	2.5 ± 0.04 <sup>***</sup>	2.3± 0.1	2.2± 0.06	2.2± 0.05 <sup>b</sup>	1.9±0.1	2.1± 0.19	1.97 ± 0.1 <sup>b</sup>
Breadth (cm)	1.7±0.1	1.6 ± 0.05	1.65±0.05 <sup>a</sup>	1.7±0.06	1.7±0.06	1.7 ± 0.04 <sup>***</sup>	1.5±0.05	1.4±0.05	1.4± 0.03 <sup>b</sup>	1.1 ± 0.05	1.2± 0.08	1.3 ± 0.06 <sup>b</sup>
Thickness (cm)	1.5±0.08	1.4 ± 0.05	1.4±0.05 <sup>b</sup>	1.5±0.06	1.6±0.04	1.6 ± 0.04 <sup>***</sup>	1.5±0.04	1.4±0.04	1.4± 0.03 <sup>b</sup>	1.1 ± 0.04	1.2± 0.06	1.18 ± 0.04 <sup>c</sup>
Weight (g)	4.5±0.23	5.04±0.29	4.8±0.20 <sup>b</sup>	5.7±0.09	5.6±0.20	5.6 ± 0.20 <sup>***</sup>	4.1±0.3	3.8±0.2	4.0± 0.2 <sup>c</sup>	2.5 ± 0.2	2.7 ± 0.21	2.6 ± 0.14 <sup>d</sup>
n (Total = 157)	12	14	26	18	23	41	39	34	73	9	8	17
Incidence among total ovaries	16.6%			26.1%			39.7%			10.8%		

\*\* P<0.05

Values with different superscript for the same character differ significantly.

**Table 4: Biometry of the ovaries in slaughtered buffaloes during Winter.**

OVAR-IES	With Corpora Lutea only			With Corpora Lutea and Follicles			With Follicles only			Without Corpora Lutea or Follicles (Static)		
	Right	Left	Total (Average)	Right	Left	Total (Average)	Right	Left	Total (Average)	Right	Left	Total (Average)
Length (cm)	2.26±0.08	2.4 ± 0.07	2.3± 0.06 <sup>a</sup>	2.45 ± 0.10	2.3± 0.08	2.39 ± 0.06 <sup>***</sup>	2.2± 0.07	2.19± 0.1	2.2± 0.05 <sup>a</sup>	1.9± 0.1	1.95± 0.08	1.94 ± 0.07 <sup>b</sup>
Breadth (cm)	1.56±0.06	1.9 ± 0.12	1.7± 0.07 <sup>a</sup>	1.8 ± 0.08	1.9 ± 0.07	1.83 ± 0.05 <sup>**</sup>	1.6± 0.06	1.5± 0.09	1.5± 0.05 <sup>b</sup>	1.07 ± 0.09	1.2± 0.07	1.19 ± 0.06 <sup>c</sup>
Thickness (cm)	1.36±0.09	1.5 ± 0.07	1.4± 0.06 <sup>b</sup>	1.6 ± 0.05	1.56± 0.06	1.56 ± 0.04 <sup>a</sup>	1.4± 0.04	1.37± 0.08	1.4± 0.04 <sup>b</sup>	1.2 ± 0.09	1.1± 0.4	1.1 ± 0.04 <sup>c</sup>
Weight (g)	4.6± 0.2	5.3 ± 0.25	4.8± 0.15 <sup>b</sup>	5.5± 0.5	5.7± 0.3	5.6 ± 0.28 <sup>***</sup>	3.9± 0.3	3.8± 0.4	3.9± 0.2 <sup>c</sup>	2.0 ± 0.3	2.8 ± 0.25	2.5 ± 0.2 <sup>d</sup>
n (Total = 111)	10	7	17	16	17	33	26	14	40	4	17	21
Incidence among total ovaries	15.3%			26.7%			36.1%			18.9%		

\* P<0.01

\*\* P<0.05

Values with different superscript for the same character differ significantly.

**Table 5:** Biometry of the ovaries in slaughtered buffaloes as related to their conditions among all samples.

Biometry of the ovaries	Conditions of the ovaries			
	With corpora lutea	With corpora lutea and follicles	With follicles only	Without corpora lutea or follicles (Static)
Length (cm)	2.33 ± 0.024 <sup>ab</sup>	2.45 ± 0.034 <sup>a*</sup>	2.22 ± 0.066 <sup>ab</sup>	2.015 ± 0.105 <sup>b*</sup>
Breadth(cm)	1.68 ± 0.026 <sup>***</sup>	1.80 ± 0.038 <sup>***</sup>	1.50 ± 0.041 <sup>b**</sup>	1.25 ± 0.0599 <sup>b**</sup>
Thickness(cm)	1.43 ± 0.026 <sup>***</sup>	1.75 ± 0.041 <sup>***</sup>	1.40 ± 0.029 <sup>b**</sup>	1.17 ± 0.020 <sup>b**</sup>
Weight (g)	4.80 ± 0.122 <sup>d**</sup>	5.54 ± 0.041 <sup>c**</sup>	3.93 ± 0.029 <sup>a**</sup>	2.66 ± 0.084 <sup>b*</sup>
Total n (481)	70	127	200	84

\* P < 0.05

\*\*P < 0.01

Values with different superscript for the same character differ significantly

**Table 6:** Weights of Corpora Lutea as related to their ovaries among different seasons in slaughtered buffaloes.

Seasons	n	Weights (g)		C.L. Ovary (%)
		Ovaries	Corpara Lutea (C.L. Periodicum)	
Autumn	60	5.4 ± 0.16	1.56 ± 0.16	29.69 ± 0.06
Spring	38	5.4 ± 0.24	1.68 ± 0.09	32.11 ± 1.85
Summer	24	5.2 ± 0.29	1.61 ± 0.06	31.08 ± 1.52
Winter	36	5.6 ± 0.25	1.49 ± 0.08	27.65 ± 1.49
Total	158	4.8 ± 0.16	1.59 ± 0.04	30.05 ± 0.81

N.B.: Corpus Luteum gravidatis weighed (g) 2.12 ± 0.2 (n = 10)

Table 7: Number of follicles according to their diameters (cm) among different seasons in slaughtered buffaloes

Seasons	Side (n)	Follicles Diameters (cm)																Total
		0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	
Summer	R.25	11	17	6	4	2	2	1	7	1	1	1	-	1	1	1	-	56
	L.26	5	13	5	4	3	2	1	2	1	5	1	-	-	1	-	-	43
	Tot.51	16	30	11	8	5	4	2	9	2	6	2	-	1	2	1	-	99
	%	16.2	30.3	11.1	8.1	5.1	4.04	2.02	9.1	2.02	6.1	2.02	-	1.01	2.02	1.01	-	100%
Autumn	R.59	3	10	12	23	6	-	6	7	11	4	2	1	3	2	3	2	95
	L.59	4	21	19	16	16	5	7	7	14	2	2	1	2	1	-	-	117
	Tot.118	7	31	31	39	22	5	13	14	25	6	4	2	5	3	3	2	212
	%	3.3	14.6	14.6	18.4	10.4	2.4	6.1	6.6	11.8	2.8	1.9	0.9	2.4	1.4	1.4	0.9	100%
Winter	R.35	-	7	10	6	8	4	7	1	6	3	3	1	-	1	-	1	58
	L.35	3	7	7	9	5	4	2	1	6	-	-	1	2	2	-	-	49
	Tot.70	3	14	17	15	13	8	9	2	12	3	3	2	2	3	-	1	107
	%	2.8	13.1	15.9	14.0	12.1	7.5	8.4	1.9	11.2	2.8	2.8	1.9	1.9	2.8	-	0.9	100%
Spring	R.48	19	12	9	9	10	7	5	6	5	9	4	-	1	2	1	1	100
	L.48	21	8	20	11	16	4	6	1	5	7	1	1	1	3	-	1	106
	Tot.96	40	20	29	20	26	11	11	7	10	16	5	1	2	5	1	2	206
	%	19.2	9.7	14	9.7	12.6	5.3	5.3	3.4	4.9	7.8	2.4	0.5	0.97	2.4	0.5	0.97	100%
Total number percent		66	95	88	82	66	28	35	32	49	31	14	5	10	13	5	5	624
		10.6	15.2	14.1	13.1	10.6	4.5	5.6	5.1	7.9	5.0	2.2	0.8	1.6	2.1	0.8	0.8	100%



**Table 8 : Recovered oocytes in relation to aspirated follicles at different seasons in slaughtered buffaloes**

Season	No. of Ovaries with Follicles	No. of Follicles (Aspirated)	No. of Follicles per ovary	Recovered oocytes		No. of oocytes per ovary	Recovery rate of oocytes (%) (From aspirated follicles)		
				Intact	Denuded		Total	Intact	Denuded
Summer	51	99	1.94	79	10	89	79.8	10.1	89.8
Autumn	118	212	1.79	164	31	195	77.4	14.6	91.9
Winter	70	107	1.53	84	15	99	78.5	14.01	92.5
Spring	96	206	2.15	150	30	180	72.8	14.6	87.4
Total	335	624	1.86	477	86	563	77.1	13.3	90.4