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EFFECT OF SEASONS ON OVARIAN MORPHOLOGY AND OOCYTES QUALITY IN SLAUGHTERED BUFFALOES

(With 8 Tables)

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تأثيرالمواسم على وصف المبيض ونوع البويضات فى الجاموس المذبوح

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تــم فحــص وتشريح الاعضاء التناسليه لعدد ٢٩٣ جاموسه سليمه وغير عشار مذبوحه فى السـلخانه وتم الفحص بعد الذبح مباشرة فى المعمل لمدة عام كامل –وبعد استبعاد الاعضاء التناسـليه الغـير طبيعـيه تركـزت الدراسه على ٢٤٢ جهاز تناسلى سليم حيث تم فحص المبايض لكل جاموسه لمعرفة الحاله التناسليه وبعد ذلك تم قياس ابعاد كل مبيض وكل جسم اصـفر وتم تقدير الوزن. تم عد الحويصلات الموجوده على سطح المبيض لكل جاموسه مع قـياس قطـر كل حويصله بالمسماك. بواسطة مضخه شفط كهربائيه وحقنه طبيه سعة ١٨ – متصـله بقنيـنه زجاجـيه محكمـة الغطاء تم سحب محتويات الحويصلات. ومن خلال الاستريوميكروسكوب والميكروسكوب المعملى تم التعرف على البويضات الموجوده حيث تم عدها وفحصها لدراسة تأثير المواسم على جودتها. بينت النتائج ان المبايض التى تحمل جسم اصفر كانت اكبر من المبايض الاخرى بفروق معنويه ($P > 0.05$) يمثل الجسم الاصفر ٨١ ، . ± ٣٠،١% مـن وزن المبيض. لم يكن هناك تأثير معنوى للمواسم علىمقاسات وأوزان المبـيض. تبيـن ان عدد الحويصلات الكلية بكل مبيض كان عاليا خلال موسم الربيع عن بـاقى المواسـم (٢,١٥ مقابل ١,٩ فى الصيف، ١,٨فى الخريف، ١,٥ فى الشتاء) اما عدد الحويصـلات الكبـيره (أكبر من ٠,٩سم) بكل مبيض كان قليلا جدا فى موسم الصيف عن بـاقى المواسـم (٠,٢٧مقـابل ٠,٤٣فى الربيع ،٠,٤٢فى الخريف ،٠,٣٧فى الشتاء). ادت الحويصلات الصغيره(أصغر 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 مقـابل ٣٢,٥ % فـى الخريف و ٣١,٨ % فى الشتاء و ٤٢,٩ % فى الربيع. بلغ العدد الاجمالى للبويضات المعرفه ٥٦٣ بويضه منهم ٤٧٧ بويضه سليمه (جيده وعليها تراكم من ٣ طـبقات) وكـانوا اكـثر انتشـارا فـى الحويصلات الكبيره عن ٠,٣ سم – بلغت نسبة استخلاص البويضات الجيدة ٩٠,٦ % فى الحويصلات المشفطه ألتى كانت أكبر من ٠,٣ سم مقابل ٦٢,٥ % فى الحويصلات الأصغر من ذلك. وكان تأثير المواسم على هذه النتائج يـرجع الى توزيع الحويضلات الصغيرة فى المبايض خلال المواسم حيث كان مرتفعا خلال

الصـــيف. بالنســـبة للحويصلات الكبيرة فكانت أكثر انتشارا فى الربيع. كانت النسبة الكلية لاســـتخلاص البويضات هى ٢, ٩٠ % من اجمالي ٦٢٤ حويصلة مشفطة. يستنتج من هذه الدراســـة أن موســـم الصـــيف لـــيس بالموســـم المناسب لمثل هذة الدراسه نظرا لقلة عدد الحويصـــلات المناســـبة فـــى الحجم والتى تعطى بويضات جيده سليمه تؤدى الى نجاح أى دراســـة لاحقـــة. كمـــا أن حرارة الجو المرتفع وسوء معدلات التغذية لها تأثير سلبى على الوظـــائف الفسيولوجية للمبايض خلال هذا الموسم فى الجاموس لانتاج حوصلات متوسطه 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 Naggar *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-orcine 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 lulea 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 ± 0.04, n= 158, versus 2.12 ± 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 o.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 grvidatis 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.

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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 ^{ab}	2.4± 0.08	2.5± 0.07	2.4 ± 0.06 ^{a**}	2.2± 0.12	2.4± 0.05	2.3± 0.04^{b}	2.04±0.09	2.1± 0.1	2.05 ± 0.07^{c}
Breadth (cm)	1.5±0.06	1.7±0.12	1.6±0.09 ^{b}	1.8±0.06	1.9±0.07	1.87±0.05 ^{a**}	1.6±0.09	1.6±0.05	1.6± 0.03^{b}	1.4 ±0.06	1.3± 0.07	1.4 ± 0.05^{b}
Thickn-ess (cm)	1.3±0.03	1.4±0.08	1.4±0.06 ^{b}	1.5±0.04	1.5±0.10	1.53±0.05^{a**}	1.4±0.07	1.3±0.04	1.4± 0.03^{b}	1.1 ±0.06	1.2± 0.09	1.2 ± 0.05^{b}
Weight (g)	4.2±0.2	4.6±0.4	4.5±0.32 ^{b}	5.5±0.35	5.5±0.40	5.5 ±0.3^{a**}	3.9±0.5	3.9±0.2	3.9± 0.2^{b}	2.8 ±0.28	2.6 ± 0.3	2.75±0.19^{b}
n (Total = 130)	3	10	13	23	13	36	24	30	54	16	11	27
Incide-nce 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.

OVARIES	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^{a}	2.5 ± 0.07	2.6 ± 0.15	$2.5 \pm 0.08^{a**}$	2.1 ± 0.6	2.6 ± 0.1	2.2 ± 0.07^{b}	2.1 ± 0.15	2.2 ± 0.1	2.1 ± 0.09^{b}
Breadth (cm)	1.8 ± 0.03	1.68 ± 0.06	1.7 ± 0.08^{a}	1.8 ± 0.11	1.7 ± 0.06	$1.8 \pm0.07^{a**}$	1.5 ± 0.05	1.5 ± 0.08	1.5 ± 0.04^{b}	1.2 ± 0.07	1.2 ± 0.08	1.1 ± 0.05^{c}
Thickness (cm)	1.5 ± 0.08	1.51 ± 0.08	1.5 ± 0.06^{a}	1.6 ± 0.05	1.6 ± 0.08	$1.6 \pm0.05^{a**}$	1.5 ± 0.06	1.4 ± 0.07	1.4 ± 0.04^{b}	1.2 ± 0.12	1.1 ± 0.06	1.2 ± 0.07^{c}
Weight (g)	6.0 ± 1.9	4.8 ± 0.28	5.1 ± 0.4^{a}	5.67 ± 0.4	5.9 ± 0.29	$5.46 \pm0.9^{a**}$	3.4 ± 0.17	4.6 ± 0.5	3.9 ± 0.2^{c}	3.03 ± 0.4	2.7 ± 0.26	2.8 ± 0.2^{b}
n (Total = 83)	4	10	14	9	8	17	21	12	33	9	11	19
Incidence 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 ^{b}	2.5 ±0.05	2.5± 0.04	2.5 ± 0.04^{a**}	2.3± 0.1	2.2± 0.06	2.2± 0.05^{b}	1.9±0.1	2.1± 0.19	1.97 ± 0.1^{b}
Breadth (cm)	1.7±0.1	1.6 ±0.05	1.65±0.05^{a}	1.7 ±0.06	1.7±0.06	1.7 ±0.04^{a**}	1.5±0.05	1.4±0.05	1.4± 0.03^{b}	1.1 ±0.05	1.2± 0.08	1.3 ± 0.06^{b}
Thickn-ess (cm)	1.5±0.08	1.4 ±0.05	1.4±0.05^{b}	1.5 ±0.06	1.6±0.04	1.6 ±0.04^{a**}	1.5±0.04	1.4±0.04	1.4± 0.03^{b}	1.1 ±0.04	1.2± 0.06	1.18 ± 0.04^{c}
Weight (g)	4.5±0.23	5.04±0.29	4.8±0.20^{b}	5.7±0.09	5.6±0.20	5.6 ±0.20^{a**}	4.1±0.3	3.8±0.2	4.0± 0.2^{c}	2.5 ±0.2	2.7 ± 0.21	2.6 ± 0.14^{d}
n (Total = 157)	12	14	26	18	23	41	39	34	73	9	8	17
Incide-nce 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.

OVARIES	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^{a}	2.45 ± 0.10	2.3 ± 0.08	$2.39\pm0.06^{a**}$	2.2 ± 0.07	2.19 ± 0.1	2.2 ± 0.05^{a}	1.9 ± 0.1	1.95 ± 0.08	1.94 ± 0.07^{b}
Breadth (cm)	1.56 ± 0.06	1.9 ± 0.12	1.7 ± 0.07^{a}	1.8 ± 0.08	1.9 ± 0.07	$1.83\pm0.05^{a**}$	1.6 ± 0.06	1.5 ± 0.09	1.5 ± 0.05^{b}	1.07 ± 0.09	1.2 ± 0.07	1.19 ± 0.06^{c}
Thickness (cm)	1.36 ± 0.09	1.5 ± 0.07	1.4 ± 0.06^{b}	1.6 ± 0.05	1.56 ± 0.06	$1.56\pm0.04^{a*}$	1.4 ± 0.04	1.37 ± 0.08	1.4 ± 0.04^{b}	1.2 ± 0.09	1.1 ± 0.4	1.1 ± 0.04^{c}
Weight (g)	4.6 ± 0.2	5.3 ± 0.25	4.8 ± 0.15^{b}	5.5 ± 0.5	5.7 ± 0.3	$5.6\pm0.28^{a**}$	3.9 ± 0.3	3.8 ± 0.4	3.9 ± 0.2^{c}	2.0 ± 0.3	2.8 ± 0.25	2.5 ± 0.2^{d}
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^{ab}	$2.45 \pm 0.034^{a*}$	2.22 ± 0.066^{ab}	$2.015 \pm 0.105^{b*}$
Breadth(cm)	$1.68 \pm 0.026^{a**}$	$1.80 \pm 0.038^{a**}$	$1.50 \pm 0.041^{b**}$	$1.25 \pm 0.0599^{b**}$
Thickness(cm)	$1.43 \pm 0.026^{a**}$	$1.75 \pm 0.041^{a**}$	$1.40 \pm 0.029^{a**}$	$1.17 \pm 0.020^{b**}$
Weight (g)	$4.80 \pm 0.122^{d**}$	$5.54 \pm 0.041^{c**}$	$3.93 \pm 0.029^{a**}$	$2.66 \pm 0.084^{b*}$
Total n (481)	70	127	200	84

* $P< 0.05$
**$P< 0.01$
Values with different superscript for the same character differ signifcantly

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) 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	Total
	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
Summer	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%**
	R 59	3	10	12	23	6	-	6	7	11	4	2	1	3	2	3	2	95
Autumn	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%**
	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
Winter	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%**
	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
Spring	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	Recovary rate of oocytes (%) (From aspirated follicles)		
				Intact	Denuded	Total		Intact	Denuded	Total
Summer	51	99	1.94	79	10	89	1.75	79.8	10.1	89.8
Autumn	118	212	1.79	164	31	195	1.65	77.4	14.6	91.9
Winter	70	107	1.53	84	15	99	1.41	78.5	14.01	92.5
Spring	96	206	2.15	150	30	180	1.88	72.8	14.6	87.4
Total	335	624	1.86	477	86	563	1.68	77.1	13.3	90.4