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Ultrasound scanning of ovarian activity in postpartum Iraqi buffaloes

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Abstract

Endometritis is one of the most common infections in buffaloes and occurs several weeks postpartum, causes severe economic losses, including increased open days and calving intervals. This study was aimed to demonstrate the ovarian activity in postpartum buffaloes two months after calving. Moreover, this study was aimed to demonstrate the incidence of endometritis during 21–24 days postpartum in buffaloes.

A total of 72 multiparous buffaloes, 3-10 years and three weeks post-birth were involved in the current research. All the buffaloes were examined weekly from week 3 after birth by routine rectal palpation, ultrasound scanning, and vaginal discharge checking. A 4-value point (0 = bright mucus, 1 = discharge with little pus, 2 = discharge with less than 50% pus discharges, and 3 = with > 50% pus discharges) was depended to classify vaginal discharges of these buffaloes. Cytobrush specimens were dependent to get endometrial samples from these animals. Ten from seventy-two buffaloes (13.8%) suffered from abnormal vaginal discharges grade (1-3) and pointed to acute clinical endometritis (CE), and about (16.6%) 12 of 72 clinically healthy buffaloes had subclinical endometritis (SCE) (≥ 8 % neutrophils). The diameter of the large follicle was significantly ($p < 0.05$) greater in healthy buffaloes compared with the animals that suffered from clinical and subclinical endometritis in the postpartum period. The size of the corpus luteum was significantly ($p < 0.05$) lower (7 mm) in clinical endometritis postpartum buffaloes than the healthy and subclinical endometritis buffaloes. In conclusion the ultrasound technique is a precise method to evaluate ovarian activity in postpartum buffaloes.

Keywords: Postpartum disorders, ovarian activity, uterine infections, ultrasound technique.

المسح بالموجات فوق الصوتية للنشاط المبيضي في الجاموس العراقي خلال فترة النفاس

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الخلاصة

لتهاب بطانة الرحم تعتبر واحدة من المشاكل الشائعة بعد الولادة في الجاموس العراقي مسببة خسائر اقتصادية للمربين. هدفت الدراسة الحالية لمعرفة النشاط الجريبي خلال الشهرين بعد الولادة بالاضافة الى معرفة نسبة التهاب بطانة الرحم في الجاموس بعد اربعة اسابيع من فترة النفاس. شملت الدراسة ٧٢ جاموسة متعددة الولادات اعمارها بين ٣-١٠ سنوات وفي الثلاث اسابيع من فترة النفاس. تم فحص حيوانات الدراسة اسبوعيا الدراسة منذ الاسبوع الثالث بعد الولادة بواسطة الفحص المستقيمي الدوري وباستخدام جهاز الموجات فوق الصوتية بالاضافة الى تقيم السوائل المهبلية وتقسيمها الى اربعة درجات ابتداء من افرازات سليمة صافية، الى افرازات قيحية وبدرجات متفاوتة. اخذت عينات رحمية بواسطة مسحات cytobrush لتقييم وتشخيص حالات التهاب بطانة الرحم. اظهرت الدراسة ان عشرة حيوانات ١٣,٨٪ مصابة بالتهاب بطانة الرحم الحاد باعتمادا الافرازات الغير طبيعية المهبلية والقيحية بينما ١٢جاموسة اكثر من 16% كانت تعاني من التهاب بطانة الرحم المزمن اعتمادا ع وجود نسبة ٨٪ او اكثر من كريات الدم البيضاء النتروفيل في المسحة الرحمية . اثبت الدراسة الحالية ايضا ان النمو الجريبي في الحيوانات السليمة اكثر وبفارق احصائي ٠،٠٥ عن الحيوانات التي تعاني التهاب بطانة الرحم الحاد والمزمن وايضا بينت الدارسة ان حجم الجسم الاصفر في الجاموس المصاب

بالتهاب بطانة الرحم الحاد هو اصغر ٧ ملم وبفارق احصائي ٠،٠٥ عن الحيوانات السليمة والمصابة بالتهاب بطانة الرحم المزمن. خلصت الدراسة ان استخدام الموجات فوق الصوتية هي طريقة مفيدة ودقيقة لمتابعة النشاط الجريبي في فترة النفاس في الجاموس
الكلمات الدالة: الاضطرابات النفاسية، النشاط المبيضي، الالتهابات الرحمية، تقنية الموجات فوق الصوتية

Introduction

Uterine infection and anestrous after calving were the most common postpartum disorders that happen in buffaloes and had negative impacts on reproductive performance with the serious economic loss (**1**). In Iraq, Poor reproductive performance, delay conception rate, and increased calving interval were the common properties of buffaloes (**2**).

Post calving disorders like endometritis increased days open, the period to first heating, repeat breeding and negative impact on conception rate which increased chance to culling rate (3, 4). There is a strong relationship between postpartum uterine diseases and delay resumption of ovarian activity that subsequently prolonged the period between two calving (5, 6). Anyway, delay resumption of ovarian activity is more common in buffaloes than cows (7), and this can be explained due to the high rate of postpartum uterine diseases in buffaloes compared with cows (2) and this issue has a negative impact on reproductive performance for these animals in future (8). The drop of immune response before calving considers the common reason for the progress of postpartum uterine diseases in cattle (9). Calf suckling considers the essential cause that had a negative effect on the resumption of ovarian activity in postpartum buffaloes (10), and restricted the calf suckling support recovery ovarian function and shorten anestrus after birth (11).

The resumption of ovarian activity post-calving basically depended on increasing FSH hormone which enhances ovarian follicles growth and developed dominant follicles (12). Moreover, many other factors had a negative impact on the resumption of ovarian activity in buffaloes like uterine infections, dietary supplementation, season, and light–darkness alteration during months both with hot or wintry climatic conditions (13). The reproductive performance was low in primiparous buffaloes and aging old especially when postpartum interval concurs with increment daylight period (13).

The high blood level of ovarian and placenta steroids before calving affects negatively on ovarian resumption activity after calving due to their negative impact on the hypothalamus-pituitary axis (14). A previous study to both ovaries in slaughterhouses demonstrated a small size of corpus luteum 3mm in a day of calving without the significant size of growing follicles (15). Moreover, another study by Jainudeen (16) confirmed small CL, size less than 3mm in diameter on the ovary after ten days after birth depended on the transrectal palpation method. There is no agreement about a day of CL regression after calving, one study for (17) reported the complete regression after three weeks postpartum period while another previous study confirmed the destruction of CL during the first week after birth (18). The number of small and medium-size follicles during the first week postpartum was between 5-8 and 1-2 follicles respectively (19). Another previous study confirmed that most buffaloes resume ovarian activity after three weeks postpartum and most buffaloes had follicles more than 8.5 mm in diameter which considered premature follicles and most animals ovulated during two months after calving. Presicce (20) reported that the numbers of the small follicle is less than 3mm in diameter decline gradually during two months postpartum while the follicle more than 3 mm begins to grow on the ovary after 2-3 weeks postpartum. Many previous studies showed that the life span of CL in 26-86% of postpartum buffaloes that resume the ovarian activity and first ovulation was short and about 6-13 days and associated with low serum progesterone levels (21, 22). Moreover, a previous study demonstrated that about 10% of multiparous and 30% primiparous of buffaloes that included in the study remained anovulatory after two months postpartum period (20). There are few studies about the resumption of ovarian activity in healthy and endometritis buffaloes using ultrasound technique, so the current study focused on demonstrating ovarian activity during two months in postpartum buffaloes.

Materials and methods

Animals

A total of 72 Iraqi buffaloes at 20 days to 50 days post-calving period were obtained from three farms between April 2019 and December 2020. These farms are located in Diyala province where the average temperature is 28 °C, and relative humidity is about 20%. The buffaloes of the current study were between 3 and 10 years old, weighing between 350–450 kg, and managed under a semi-intensive system of management. The animals were also fed with concentrated feed which was composed of corn silage, beet pulp, cottonseed, soybean, corn, and barley. Individual animal data on calving history, lactation, breed, and parity were all recorded. The three farms practiced natural mating.

Animals physical checking

All buffaloes were exanimated rectally between 18- 30 days after parturition to estimate the degree of the involution of the uterus, position, and also the symmetry of the uterine horns. Vaginal discharges were evaluated by hands coated with plastic transrectal palpation sleeve at 21 days to 28 days postpartum to know CE condition. A 4-value point (0 = bright mucus, 1 = discharge with little pus, 2 = discharge with less than 50% pus discharges, and 3 = with > 50% pus discharges) was depended to classify vaginal discharges of these buffaloes (23).

Endometrium cytology sample

Endometrial cytological specimens were got from buffaloes by using clean cytobrush, [(Medical, Germany (Fig. 1)] was modified for use in cattle [24]. The spindle of the cytobrush is shortened to 3 cm to be inserted into a stainless rod (70 cm × 3 mm). Both the steel rod and cytobrush were then introduced into a plastic sheath (IMV Technologies Sanitaire Chemise, France) to flee contamination by the vagina of the animal, and the device was inserted into the vagina. The sleeved arm was inserted in the rectum to help passage the device through the vagina and os cervix. When the instrument has passed during the cervix, the Cytobrush was attached and turned (270-360º) to obtain cellular samples from the endometrium. Collected specimens were then rolled 2-4 times on a sterile glass slide (70×20mm) and saved in a transport broth medium for bacterial tests. The slide was fixed with using methanol for 40 minutes, transferred to the laboratory office within 5h, was stained with 10% Giemsa stain for 2 minutes, and then dried. Each slide was estimated by counting about 300 endometrial cells under 400× magnification to demonstrate the proportion of PMN (Figure 2). Endometrial cut-off ≥ 8% was dependent (24; 25) to determine the cytological endometritis incidence in these herds 21 - 30 days after calving.

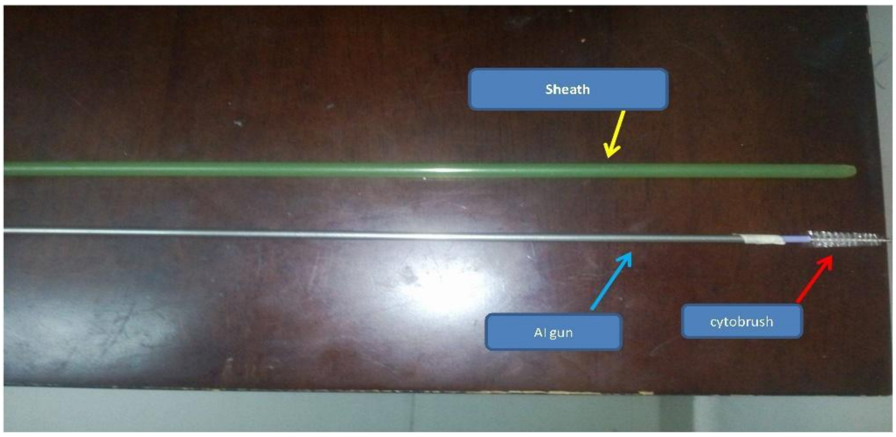


Figure 1: Cytobrush, handle (AI gun), and sheath that used to collect endometrial cells.

Ultrasound examination

All buffaloes were checked weekly by transrectal palpation and transrectal ultrasound technique from week 2 until week 9 after calving to evaluate ovarian activity.

Ovaries were scanned once weekly using B-mode ultrasound attached with a linear probe of 7.5 MHz frequency (Sonosite VET 180 Plus, Bothell, WA, USA) from week 2 postpartum until week 9 or when the ovulation was confirmed. The follicular diameter was measured as the mean of the two measurements. Follicles ≥ 5 mm in diameter were registered as the diameter of the largest dominant follicle at the first examination. Intervals from calving to largest dominant (10 mm) follicle and ovulation were also registered (26). Anovulation was proved when a large preovulatory follicle that was existing at the last examination disappeared, leaving behind a big hole (12-15 mm) and a corpus luteum in the same location on the ovary during the following examination (27). Delayed ovulation (>60 days postpartum) was used as an indicator of ovarian cessation (28).

Statistical analysis

All the statistical analyses were performed by using SPSS software (version 18.0, IBM SPSS Inc., Chicago: USA). All values were expressed as mean ±SEM of the mean. The Shapiro–Wilk test was used to confirm the normal distribution of the traits examined. The results of the control (healthy buffaloes) and endometritis groups were calculated using one-way ANOVA as well as Tukey and Duncan post hoc tests at the probability threshold, $p < 0.05$. Since the data on resumption of ovarian activity was non-parametric data, we used the Mann-Whitney test was used to analyze the results among endometritis, healthy, buffaloes' groups.

Results

All buffaloes were sampled by using cytobrush, 12 buffaloes registered SCE (16.6%) by depending ≥ 8% PMN as cut off and with vaginal secretion score = 0.

Vaginal evaluation for postpartum buffaloes 21-24 days after calving, showed 10 (13.8%) buffaloes had abnormal vaginal discharges. Four buffaloes had vaginal discharges grade 1, four buffaloes were scored 2, and two buffaloes had grade 3.

The current study showed that out of 50 healthy buffaloes, 48% had cessation of ovarian cyclicity during the first 60 days postpartum period without significant difference with SCE buffaloes (Table 1). Moreover, eight (80%) from ten buffaloes that suffered from CE, the ovarian activity had delayed with significant difference ($p < 0.05$) compared with healthy and SCE animals. Table 2 showed that the size of small and large follicle diameter significantly lower ($p < 0.05$) in CE buffaloes than in healthy and SCE buffaloes. Anyway, the size of CL in healthy buffaloes greater (14mm) with a significant difference ($p < 0.05$) compared with SCE and CE buffaloes.

Table (1): Delay the resumption of ovarian activity in healthy, SCE and CE buffaloes.

Groups	Numbers of animals	Delay ovarian resumption	Normal cycling
Healthy	50	24 (48%) ^{a}	26 (52%) ^{a}
SCE	12	7(58.3%) ^{a}	5(41.7%) ^{a}
CE	10	8 (80%) ^{b}	2 (20%) ^{b}

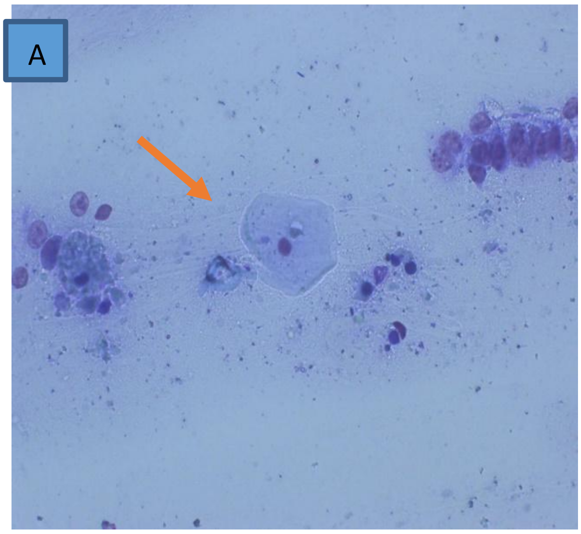
$^{a\,b}$ Mean Values within different superscripts within a column indicates significantly at P<0.05.

This study also reported decreasing the interval period to first estrus and ovulation in healthy buffaloes compared with others SCE and CE animals and with significant differences ($p < 0.05$). The resumption of ovarian activity was delayed in the buffaloes that suffered from uterine infections like SEC and CE.

Table (2): Mean diameter of the different follicles and interval among groups and first identification of small follicle, large follicle, and corpus luteum in buffaloes

Parameters	Healthy (50) (Mean±SD)	SCE (12) (Mean±SD)	CE (10) (Mean±SD)
Small follicle (MM)	4.7±0.3 ^{a}	3.8±0.4 ^{a}	2.2±0.1 ^{b}
Large follicle (MM)	11±0.4 ^{a}	9.3±0.3 ^{a}	5.1±0.1 ^{b}
CL (MM)	14±0.2 ^{a}	9±0.4 ^{b}	7±0.2 ^{b}
The interval from calving to the first large follicle (Days)	22±6.5 ^{a}	43±5.5 ^{b}	52±6.3 ^{b}
The interval from calving to first ovulation (days)	25±4.3 ^{a}	55±6.8 ^{b}	78±8.6 ^{c}
The interval from calving to first CL (days)	29±8.4 ^{a}	59±9.1^{b}	82±7.4 ^{c}

$^{a\,b}$ Mean Values within different superscripts within a row indicates significantly at P<0.05.



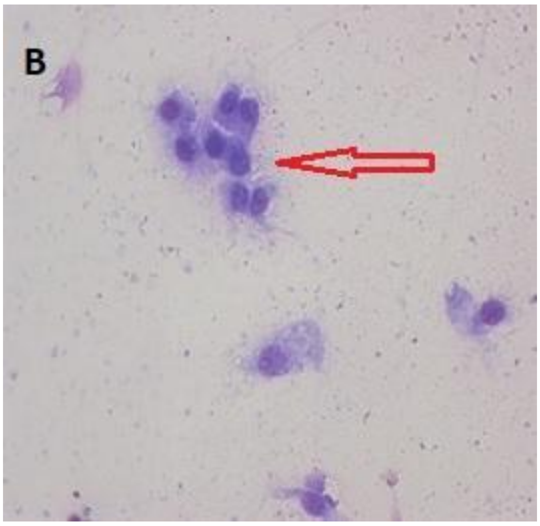


Figure 2: Cytology smears from uterine healthy buffaloes by cytobrush, stained by Giemsa. The red arrows show endometrial cells (400x).

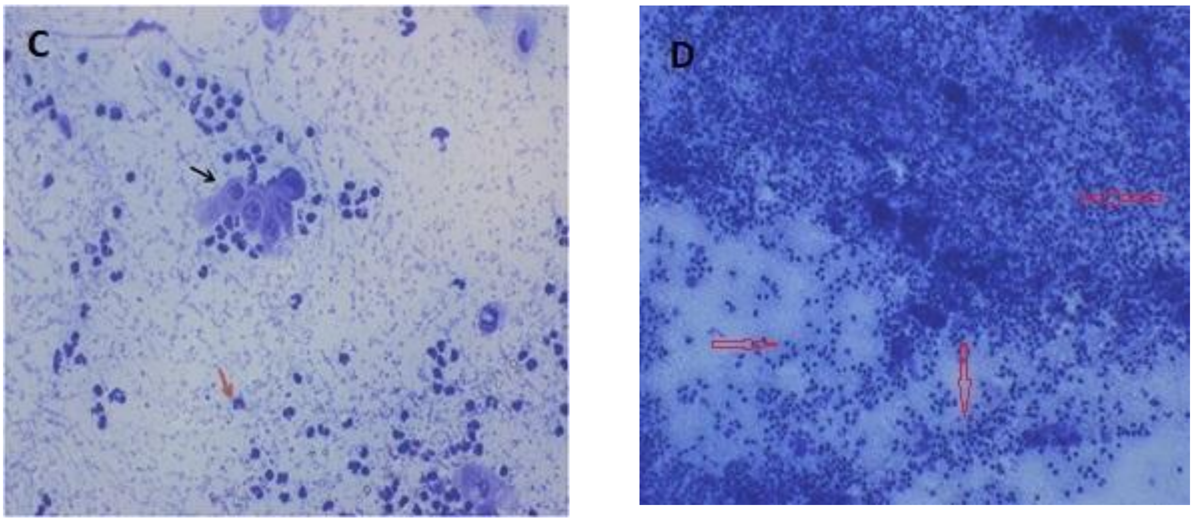


Figure 3: Cytology smears from SEC buffaloes(C) and (D) CE buffaloes by cytobrush, stained by Giemsa. The black arrow shows endometrial cells, red arrows show neutrophils (400x).

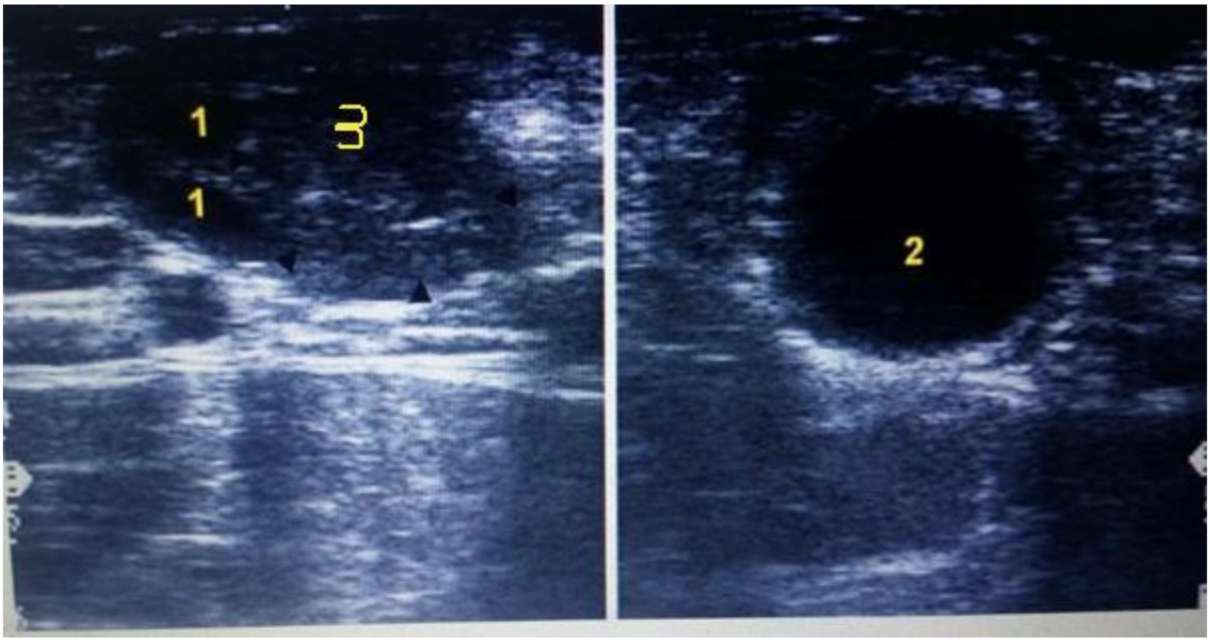
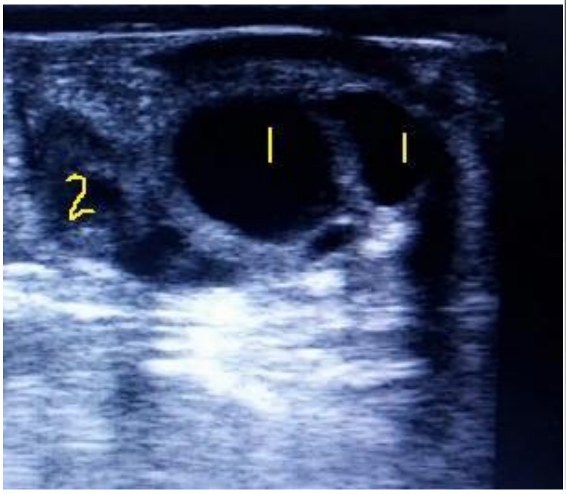


Figure-4: Ultrasound images in B - mode in healthy buffalo (probe 7.5 MHz; depth 6cm). Small follicles (1), the large follicle (2), and CL (3).



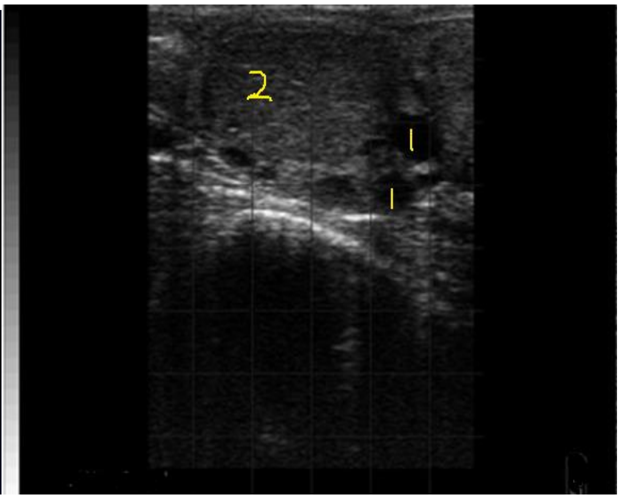


Figure-5: Ultrasound images in B - mode in healthy buffalo (probe 7.5 MHz; depth 6cm). Follicles (1), and CL (2).

Discussion

The current study showed that incidences of SCE and CE were 16.6% and 13.8% in postpartum buffaloes respectively, and these results are lower compared with previous studies.

In Iraq, a previous study by El-Dossokey and Juma, (29) had reported about 45.3% of uterine diseases in postpartum buffaloes. Moreover, both previous studies registered about 43% of subclinical endometritis in postpartum buffaloes in Basera (30, 31). In Baghdad, another previous study registered about 47.9 % of endometritis buffaloes (32). A recent study for Elsayed et al. (33) reported a high prevalence of subclinical endometritis 69.2% in postpartum buffaloes in Egypt and this may be too low hygienic status and wallowing habits for these animals (2). While another study recorded 22.4 % of endometritis in Egyptian postpartum buffaloes (34).

The low SCE prevalence may be attributed to differences in geographic area, environment, and the number of endometrial cells counted among the studies. A total of 300 cells were counted per slide in the present study, whereas 100 cells were counted in previous studies (35).

The anestrus after birth has an essential impact on reproductive performance (36). Opsomer *et al*. (37) reported that increasing the prevalence of anestrus cases in high-production animals. Maybe increase in demand for energy to milk production can delay in resumption of ovarian function post-calving. However, there are many factors such as postpartum disorder, low body energy reserve, and restricted energy intake that could also cause postpartum anestrus. Maintaining the health of cows during the periparturient period is the best way to reduce anestrus cases in dairy farms (38).

Most of the uterine infections buffaloes suffered from the delayed resumption of ovarian activity and this result agrees with previous studies by Senosy *et al*. (39) and also Burke *et al*. (40) who reported a relationship between uterine infection and ovarian activity during the postpartum period, and impact of endometritis on anestrus incidence within two months postpartum period. This agrees with previous studies that confirmed the effect of uterine infection on ovarian activity (41). Also, about 48% of the healthy buffaloes failed to restore the ovarian activity during the first two months after calving, and this may be due to the effect of prolonged suckling in buffaloes and season impact on the breeding season in these animals (10, 13), and restricted calf suckling enhance recovery ovarian activity and shorten anestrus after calving (42). A previous study demonstrated that 10- 30% of postpartum buffaloes during two months had anestrus due to anovulatory follicles (20).

The current study showed that the interval period for large follicles and ovulation was shorter in healthy buffaloes compared with infected buffaloes. It may be due to lysis of pregnancy CL and decreasing of progesterone level, the last drop of progesterone level led to increasing GnRH, FSH, and LH and resumption of ovarian activity after calving (43).

Only approximately 50% of healthy dairy cows ovulate the first dominant follicle within 3 weeks after calving (44).
The absence of the small and medium follicles in many animals of the current study during 21 days after calving, may be due to decreasing sensitivity of the pituitary gland to GnRH and gradually resumption of its sensitivity from 2-35 postpartum period [45], and also a negative effect of the season (46) Also, the ultrasound scanning of ovaries of postpartum buffaloes showed the different small size of CL especially in buffaloes that suffered from endometritis. This may be to ovulation for medium follicles with size of 8mm (47).

Conclusion: The ultrasound technique is a precise method to evaluate ovarian activity in postpartum buffaloes

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