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# Time Lapse Changes in the Ovarian Activity of Dairy Cows During the Postpartum Period Guided by Ultrasonography: A Pilot Study

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**Abstract**

This study aimed to characterize the postpartum (pp) follicular development and the process of resumption of ovarian activity in dairy cows. Six multiparous lactating Holstein cows were kept and examined twice weekly using ultrasound from day 11-19 PP until the appearance of a large mature corpus luteum (CL). The results showed that four of six cows (66.66%) exhibited dominant follicles (DF >10 mm) within 2 weeks PP (day 11, 15, 15, 16) and one cow (16.6%) needed 3 weeks (day 19) to develop DF. While only one cow (16.6%) displayed a delay (30 days pp) in the appearance of DF. The shortest interval between calving to the beginning of 1st luteal formation was 15 days recorded for one cow and the longest interval was 57 days and recorded also for one cow. While, in the other 3 cows, 29, 33, 43 days were recorded as the interval from calving to formation of first luteal tissue. The development of cysts following anovulated follicles with the size of 19-20mm occurred in two cows (33.3%). The persistence of cysts was longer in larger size cysts compared with the smaller one. Spontaneous recovery of the smaller cyst was faster than the larger cyst (10 vs. 23 days). Altogether, results showed that of the ultrasound scanning can be used as a guide to follow up the ovarian activity during PP period and thereby, identifying the optimal therapeutical and managemental interference to solve any reproductive disorder and maintain standard fertility parameters within a dairy herd.

**Keywords:** Ultrasound; Cow; Postpartum; Ovary; Follicle; Corpus luteum

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## 1.Introduction

In the dairy industry, it is a prerequisite to reduce the period between 2 successive calving (calving interval), into 12 to 13 months (Genzebu et al., 2016). In order to achieve this, cows should get pregnant Within 90 days of calving. This requires the resumption of normal ovarian function in the earlier weeks after calving. According to previous studies, in the postpartum period, delayed return of ovarian activity was the main factor that negatively impacted dairy cows' reproduction (Shrestha et al 2004 and Kawashima et al 2006).

Reist et al (2000) concluded 30 days postpartum as an optimum resumption of estrous cycle under practical conditions. Some studies used 3 weeks (Kawashima et al 2006) or 4 weeks postpartum (Huszenicza al 2005) as a threshold to categorize cows into delayed or non-delayed ovulating groups. While others have considered that the resumption of ovarian activity is normal even if the first luteal activity occurred as late as 56 days (Petersson et al 2006), 60 d (Garbarino et al, 2004), or 65 d postpartum (Santos et al 2009). First ovulation described as a delayed if occurred after 35 days postpartum (incidence, 35%), whereas the first ovulation occurring within 35 days postpartum, but the absence of luteal activity 14 days later was defined as delayed ovarian resumption (Gautam et al 2010). However, most other studies considered ovulation beyond 45 days postpartum as delayed resumption of ovarian activity (Opsomer et al 2000; Shrestha et al 2004, Shrestha et al 2005).

Ultrasound imaging technique has been applied to investigate follicular growth, ovulation, and luteal tissue formation in cattle. In relation to the resumption of postpartum ovarian activity, authors have interested mainly in dynamics and hormonal profiles of the first follicular wave (Kawashima et al., 2007), time of the first ovulation (Galvao et al., 2010), the first appearance of luteal activity (Hayashi et al., 2008). Most postpartum follicular growth occurs in a wave-like pattern in normal cycling cattle (Rajamahendran and Taylor, 1990; Savio et al., 1990a). It is not a lack of follicular development that causes postpartum anovulatory anestrus in dairy cows; rather, it is the failure of a dominant follicle (DF) to ovulate (Roche et al., 2000). Preovulatory follicles in high-yielding cows' range in size from 16 to 20 mm, according to ultrasonography (Savio et al., 1990b; Hamilton et al., 1995; Roth et al., 2001; Lopez et al., 2004; Bleach et al., 2004; Wolfenson et al., 2004).

Ovarian follicular cyst formation is one of the major disorders affecting fertility in dairy cows. Retained Follicles with a diameter >20 mm (Vanholder et al., 2005; Ortega et al., 2008) or 22 mm (Braw-Tal et al., 2009) have been defined as ovarian follicular cysts. according to many ultrasonographic investigations, about 21 to 27% of cows have a cyst-like structure in the postpartum period (Savio et al., 1990a; Vanholder et al., 2005) and approximately 50% of this cyst spontaneously regress prior to time of first insemination (Peter, 2000; López-Gatius et al., 2002). The precise process behind the postpartum development of ovarian follicular cysts is still not well understood (Peter, 2004). The objective of our study was to investigate the postpartum follicular growth and the resumption of ovarian function (1st ovulation and subsequent CL formation) in postpartum dairy cows. Moreover, detection of abnormal ovarian structures as ovarian cyst and persistent follicles was explored.

## 2.Material and Methods

### *2.1. Ethics statement*

Animal experiments described in this article were conducted in accordance with the Guiding Principles for the Care and Use of Research Animals Promulgated by Faculty of Veterinary Medicine, Damanhour University, Egypt. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Faculty of Veterinary Medicine, Damanhour University (Permit number: DMU/VetMed-2023/022).

### *2.2. Animals*

The investigation was done in a commercial dairy farm in Al-Beheira governorate, Egypt. The experiment was performed on 6 lactating multiparous Holstein cows, average 2-6 years of age, with an average milk yield between 6000-8000 liters per season. Cows were fed on Total Mixed Ration (TMR) according to the stage of lactation, with a postpartum diet fed for the 1st 21 days in milk (DIM) and a lactating diet for the remainder of lactation. Cows were fed twice daily, and TMR were based on alfalfa hay, corn silage, steam-rolled corn, soybean meal, whole cottonseed, protein supplement, calcium salts of palm oil, a mineral and vitamin. The diets were refer to the National Research Council (NRC) requirements for Holstein cows weighing 500 kg and producing 35 kg of milk/day, containing 3.5% fat (National Research Council, 2001). Cows had free access to water. All the used cows were confirmed to experience normal birth without any history of dystocia, retained fetal membranes or uterine infection.

### *2.3. Monitoring ovaries dynamics*

Six Holstein cows were monitored by ultrasound imaging starting from day fifteen post calving twice per week for 3 weeks then once per week until appearance of luteal structure and continued until mature CL formation, with recording any physiological events during this period. Detection of the first dominant follicle, 1$^{st}$ PP ovulation, diameter of ovulatory follicle, 2$^{nd}$ PP ovulation, interval between 1$^{st}$, 2$^{nd}$, and 3$^{rd}$ PP ovulation, post ovulation ovarian activity and CL volume were recorded.

### *2.4. Ultrasound scanning*

Ultrasonography was performed using a real time B mode scanner (Sonoscape A5, China) equipped with 5L vet linear array auto adapted frequency transducer range between 6 to 9 MHZ. Ultrasonic gel was used as a coupling agent between transducer and animal tissues to produce good quality images. Before transrectal scanning, animals were adequately restrained in head locks and the scanning unit was carried by an assistant on opposite side to the inserted arm. The scanning was done in a low lighted animal house for good observation. The rectum was evacuated from all feces before the introduction of the transducer. The scan head pressed firmly against the rectal mucosa to prevent air interface. For orientation, the transducer was first moved along the dorsal surface of the uterine horns and body then moved laterally to examine ovaries. The image was retained on screen and saved.

## 3. Results

Results of ovarian ultrasound scanning of cow #1 revealed that 2 large follicles (16 mm in diameter) appeared at day 11 PP and 4 days later (at day 15 PP), 12 mm in diameter CL was observed with one medium follicle and 3 small follicles. The formed CL continued to increase in size from 15 mm at 19 to reach 25 mm in diameter at day 32 PP **(Figure 1)**. No large follicles were observed at day 15 to 29 PP. On day 32, a large follicle was found. The existence of a large follicle with mature CL indicated that the cow has entered the follicular phase of the next cycle. Ovarian structures indicated that the cow was in the luteal phase from day 15 to day 32. Altogether, the observed ovarian structures suggest an early resumption of ovarian activity with induction of ovulation, and CL formation in this cow compared with other scanned cows (**Table 1**).

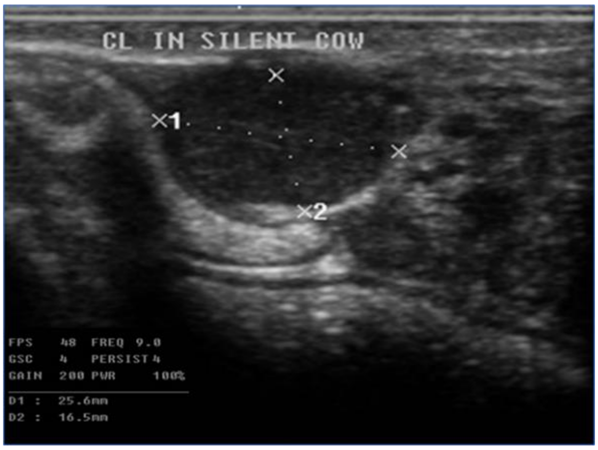



**Figure 1.** Representative image of mature CL

In the case of cow #2, all appeared follicles in the scanning at days 19,22, and 26 PP were ≤ 5mm in diameter. While follicles with size of 10, 12,13, 15,18 mm were found at day 30,33,36,43 and 50 PP respectively **(Figure 2)**. CL with 15 mm in diameter was detected at day 57 PP and was grown to become 25 mm in diameter at day 64 PP (**Table 2**).

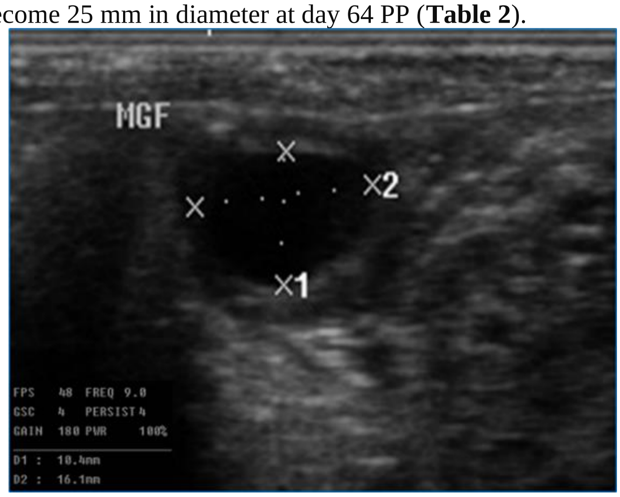



**Figure 2.** Representative image of mature follicle

In cow # 3, a dominant follicle (15 mm in diameter) was detected at day 15 and reached 20 mm in diameter at day 23 PP. On day 26 PP, the ovarian follicle continually increased and became larger than the average diameter of the preovulatory follicle (25mm in diameter) and this fluid-filled structure reached its maximum (33 mm) at day 35 PP and persisted until day 49 PP with a slight reduction in size. The size of this fluid-filled structure and its persistence for 23 days with the absence of luteal structure indicate the structure was a follicular cyst (**Figure 3**). On day 56, the ovarian cyst disappeared, and two new dominant follicles were noted. The diameter of the largest one was 20 mm at day 63PP then gradually decreased to 10 mm in diameter at day 77 PP. Results of ovarian scanning suggest that although ovarian cyst in this cow was spontaneously recovered, it resulted in a delay in the resume of normal ovarian activity (**Table 3**).

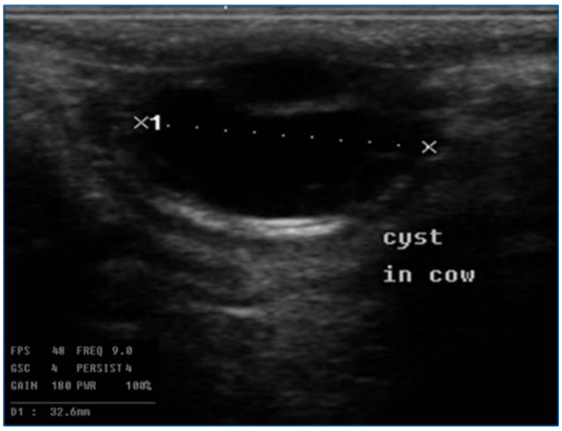



**Figure 3.** Representative image of follicular cyst

In cow # 5, one medium and one small follicle were observed at day 12PP. Dominant follicles with sizes of 11, 13, 15,17 mm were found on days 16,19,23, and 26 PP respectively. 9,17, and 23mm CL were detected at day 29,36 and 43 PP respectively with the absence of large follicles. The beginning of formation of luteal tissue was distinguished at day 29 days PP (**Table 5**).

In cow # 4, a large dominant follicle (17 mm) with 3 small follicles were observed at day 19 and day 23 PP followed by three and one medium size (14 mm) at day 26 and 30 respectively. Next, a 10 mm and 13mm CL with 3 small follicles and one medium were observed at day 33 and 36 PP respectively. Large mature CL (25 mm in diameter) in addition to 3 medium follicles and one large follicle were detected during ovarian scanning at day 43 PP. Growing of CL from 10 mm at day 3 3PP to 25mm at day 43 PP revealed the beginning of ovarian cyclicity in this cow (**Table 4**).

In cow # 6, dominant follicles with sizes 15,18, and 18 mm were found at days 15,19, and 23 PP respectively. Scanning at days 26, 30, 33, and 36 PP showed the presence of a cyst with sizes of 27,26, and 25 mm at days 26,30, and 33 PP respectively. A large follicle (22 mm) was observed at day 36 PP followed by 13 mm CL 43 PP. This CL grown and reached 20 mm at day 50 PP and 25 mm at day 57 PP. Beginning of formation of luteal tissue was noticed at day 43 PP (**Table 6**). Based on ultrasound ovarian scanning, the time of the beginning of appearance of ovarian follicle and the interval from calving till the beginning of CL formation in all tested cows were summarized in **Table 7**.

## 4. Discussion

Despite the fact that follicular dynamics in heifers and cows during the estrous cycle have been thoroughly investigated (Sartori et al., 2004; Šichtař et al., 2010), the early postpartum period's follicular growth is not well-understood in detail, particularly in high-lactating dairy cows. Early monitoring of ovarian structures by ultrasound illustrates whether the ovarian activity is normal or abnormal. This allows for the early interference to treat or manage any reproductive disorder and maintain standard fertility parameters within a dairy herd. It is generally accepted that the onset of normal ovarian cyclicity after parturition is a significant event for dairy cows to maintain maximum breeding capacity. The two most common abnormal ovarian activities in lactating dairy cows have been observed to be prolonged luteal phase and delayed ovulation. (Taylor et al. 2003; Shrestha et al.2005).

The largest follicle that reached a diameter of more than 10 mm and was at least 2 mm larger than other follicles was considered a dominant follicle (Sirois and Fortune, 1990). In the present work, the presence of >10mm follicles (DF) started at day 11,15,15,16,19 and 30pp. Four of six cows (66.66%) had dominant follicles (>9mm) 2 weeks PP. Only one cow (16.6%) showed delayed (30dayspp) appearance of the dominant follicle. in high yielding cows, the preovulatory follicles range in size from 16 to 20 millimeters. (Savio et al., 1990b; Hamilton et al., 1995; Roth et al., 2001; Lopez et al., 2004; Bleach et al., 2004; Wolfenson et al., 2004). In the current study, the development of ovulatory follicles (OF ≥15mm) was observed around day 15 PP in 50% of cows and at day 21 in 33% of cows. Development of dominant follicle to ovulatory follicle was delayed to day 43 postpartum in one cow. These results agreed with previous results of Savio et al., (1990a). They stated that selection of DF (> 9 mm) occurs around day 10 postpartum. Similarly, according to Butler (2003) The first follicles wave development starts soon after delivery.

Our data revealed that a normal PP ovarian activity pattern was attained in 4 of 6 tested cows. According to previous reports, the normal ovarian pattern started with the development of dominant ovulatory follicles followed by ovulation and the formation of small size CL grew to mature large CL with a normal luteal length. Results of our scanning were like the normal pattern reported in the previous studies.

Our results revealed that the development of cysts following unovulated follicles with the size of 19-20 mm occurred in two cows (33.3%). The definition of the ovarian cyst varied among authors. Previously, the term "ovarian follicular cyst" refers to a big, follicle-like structure that has a diameter of at least 25 mm and has been present on the ovary for at least 10 days without an active corpus luteum. (Garverick, 1997; Opsomer et al., 1999; López-Gatius et al., 2002; Van-holder et al., 2006). Recently, Follicles with a diameter >20 mm (Vanholder et al., 2005; Ortega et al., 2008) or 22 mm (Braw-Tal et al., 2009) have also been defined as ovarian follicular cysts according to the same conditions. Ovarian cysts were defined as a persistent large follicular structures (19 to 32 mm in diameters), with abnormal growth rate (>1 mm/day) sometimes, persistent follicles had the same growth rate and diameter as the dominant follicles, but remained for ≥10 days (Roth et al, 2012).

Particularly during the postpartum period, the mechanisms of cyst development are still poorly known. Many researchers agree that preovulatory follicles that fail to ovulate remain in the ovary and develop cysts, which impair the normal function of the ovary (Vanholder et al., 2006). The current data revealed that the persistence of cysts was longer in large size cysts compared with the small ones. Spontaneous recovery of the smaller cyst was faster than the larger cyst (10 vs. 23 days). The size of the cyst may determine its persistence. A large cyst may be difficult and take a long time for spontaneous correction. Further investigation with a greater number of animals is needed. Obviously, suppression of the development of follicles during the persistence of ovarian cyst in both affected cows agreed with Rajmon et al (2012). They claimed that follicle development was markedly inhibited beyond but not before the developing of the cyst, and so that stopping the formation of the cyst accelerated the development of ovarian follicles (Rajmon et al., 2012).

**Table 1.** Ultrasound scanning results of Cow # 1

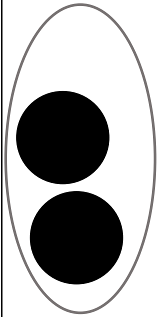
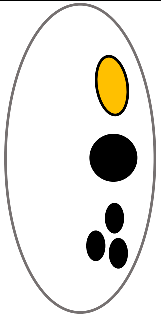
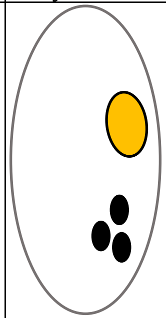
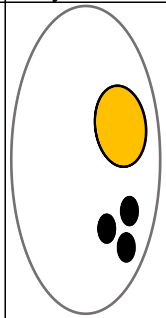
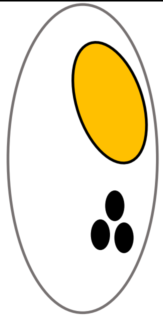
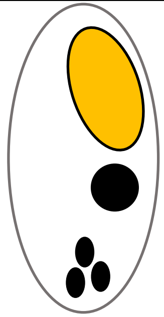
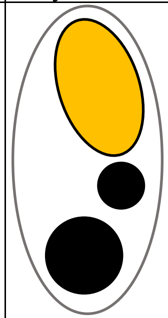
| **Time** | **Day-11** | **Day-15** | **Day-19** | **Day-22** | **Day-26** | **Day-29** | **Day-32** |
|---|---|---|---|---|---|---|---|
| Ovarian structures |  |  |  |  |  |  |  |
| Diameter (mm) | LF = 16 | MCL = 12 | MCL = 15 | MCL = 18 | LCL = 20 | LCL = 21 | LCL =25 |

**Table 2.** Ultrasound scanning results of Cow # 2

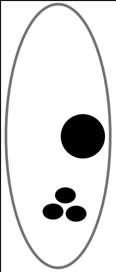
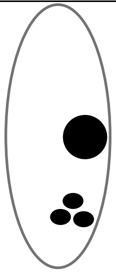
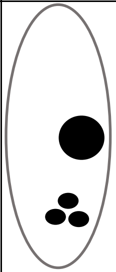
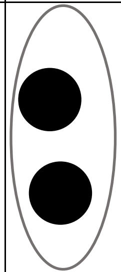
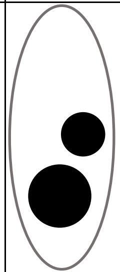
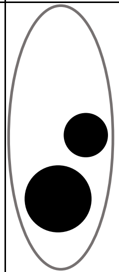
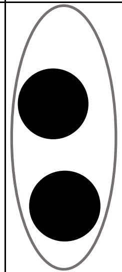
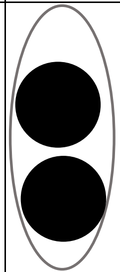
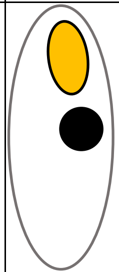
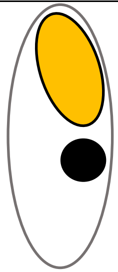
| **Time** | **Day-19** | **Day-23** | **Day-26** | **Day-30** | **Day-33** | **Day-36** | **Day-43** | **Day-50** | **Day-57** | **Day-64** |
|---|---|---|---|---|---|---|---|---|---|---|
| Ovarian structures |  |  |  |  |  |  |  |  |  |  |
| Diameter (mm) | MF=5 | MF=5 | MF=5 | LF=10 | LF=12 | LF=13 | LF=15 | LF=18 | MCL=15 | LCL=25 |

**Table 3.** Ultrasound scanning results of Cow # 3

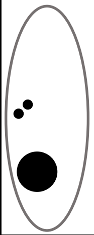
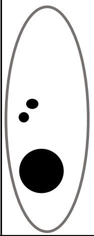
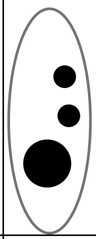
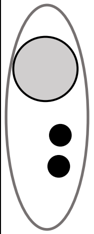
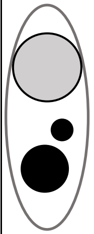
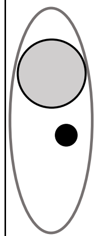
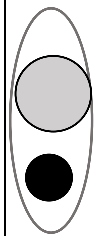
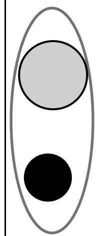
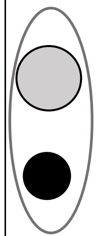
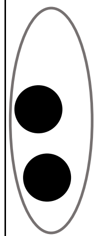
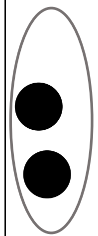
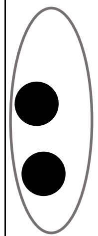
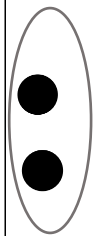
| **Time** | **Day-15** | **Day-19** | **Day-23** | **Day-26** | **Day-30** | **Day-33** | **Day-35** | **Day-42** | **Day-49** | **Day-56** | **Day-63** | **Day-70** | **Day-77** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ovarian structures |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Diameter (mm) | LF=15 | LF=17 | LF=20 | Cyst=25 | Cyst=28 | Cyst=30 | Cyst=33 | Cyst=27 | Cyst=25 | LF=20 | LF=20 | LF=15 | LF=10 |

**Table 4.** Ultrasound scanning results of Cow # 4

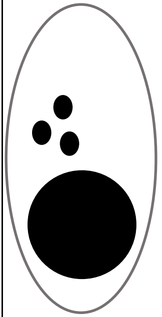
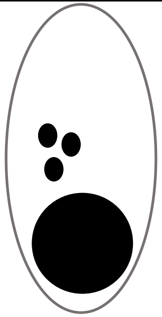
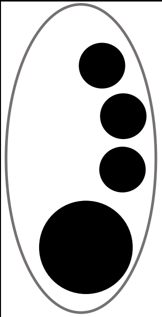
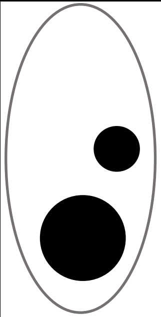
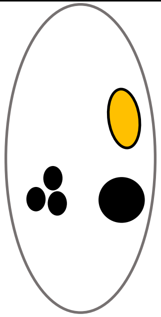
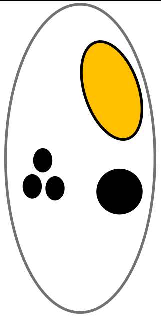
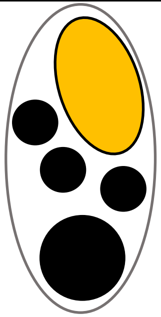
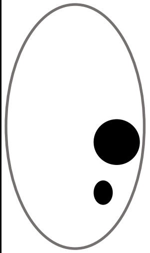
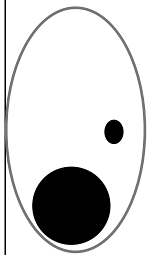
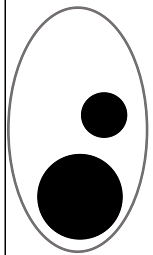
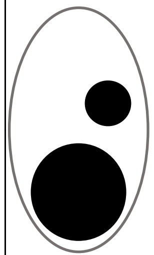
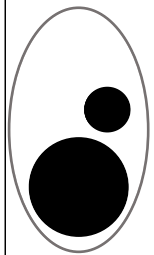
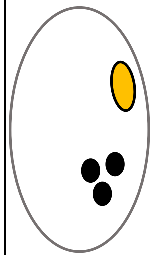
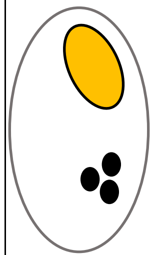
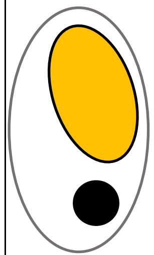
| **Time** | **Day-19** | **Day-23** | **Day-26** | **Day-30** | **Day-33** | **Day-36** | **Day-43** |
|---|---|---|---|---|---|---|---|
| Ovarian structures |  |  |  |  |  |  |  |
| Diameter (mm) | LF=17 | LF=15 | LF=14 | LF=13 | MCL=10 | MCL=16 | LCL=25 |

**Table 5.** Ultrasound scanning results of Cow # 5

| **Time** | **Day-12** | **Day-16** | **Day-19** | **Day-23** | **Day-26** | **Day-29** | **Day-36** | **Day-43** |
|---|---|---|---|---|---|---|---|---|
| Ovarian structures |  |  |  |  |  |  |  |  |
| Diameter (mm) | MF=9 | LF=11 | LF=13 | LF=15 | LF=17 | SCL=9 | MCL=17 | LCL=23 |

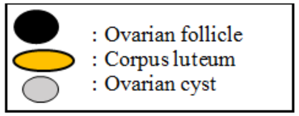
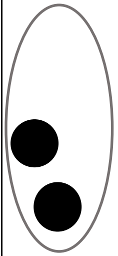
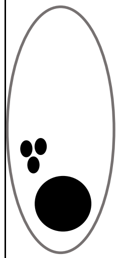
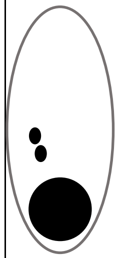
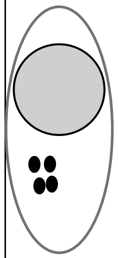
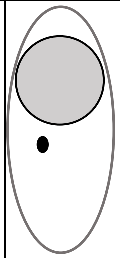
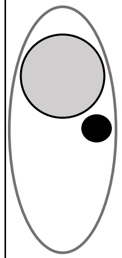
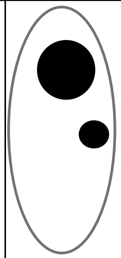
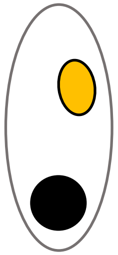
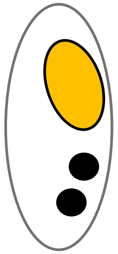
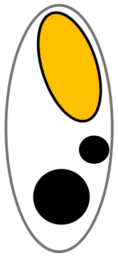


**Table 6.** Ultrasound scanning results of Cow # 6

| **Time** | **Day-15** | **Day-19** | **Day-23** | **Day-26** | **Day-30** | **Day-33** | **Day-36** | **Day-43** | **Day-50** | **Day-57** |
|---|---|---|---|---|---|---|---|---|---|---|
| Ovarian structures |  |  |  |  |  |  |  |  |  |  |
| Diameter(mm) | LF=15 | LF=18 | LF=19 | Cyst=27 | Cyst=26 | Cyst=25 | LF=22 | MCL=13 | LCL=20 | LCL=25 |

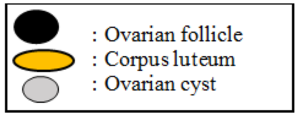



**Table 7.** Summary of the **days** Postpartum for the beginning of follicular and luteal tissue formation in all tested cows

| **Cow #** | **Dominant follicle formation (15 mm or more)** *Days postpartum* | **Luteal tissue formation** *Days postpartum* | **Development of cyst** |
|---|---|---|---|
| **1** | 11 | 15 | No |
| **2** | 43 | 57 | No |
| **3** | 15 | No Cl until day 77 | Cysts persist for 23 days |
| **4** | 19 | 33 | No |
| **5** | 23 | 29 | No |
| **6** | 15 | 43 | Small cyst for 8-10 days |

Results of the present work indicated that ovulation followed by CL formation which started by medium-size CL then became mature and large occurred in five of six cows (83%). One of them was observed after spontaneous recovery of the cyst. The periods from calving to 1st PP ovulation and luteal tissue formation varied among animals. The shortest interval from calving to the first luteal formation was 15 days recorded for one cow and the longest interval was 57 days and recorded also for one cow. 29, 33, and 43 days were estimated as the interval from calving to first formation of luteal tissue for the other 3 cows. The indication of the return of postpartum ovarian activity is the 1st ovulation followed by regular, 18–24-day ovarian cycles The obtained results after the exclusion of cows that had the persistent ovarian cyst for more than 20 days agreed with Shrestha et al., 2005. They noted a typical return of ovarian activity, with ovulation taking place 45 days or less after calving and regular ovarian cycles lasting 18 to 24 days. The interval from calving to ovulation and subsequent CL formation in most of our experimental animals was longer than that reported by Rajmon et al (2012) considered as better for fertility (ovulation within 20 days PP). In the same direction of our data, Crowe, (2008) reported that dominant follicles of the first follicular growth wave ovulate in 30–80% by day 20 and Peter et al., 2009 added that in the case of successful ovulation, CL forms and subsequent luteolysis can result in the regularity of cyclical ovarian activity.

Additionally, it was clear from our data that cows of the ultrasound experiment have not been seen in estrus during the experiment and this revealed that 1st PP ovulations were silent. Silent 1st PP was reported in many previous studies. Shipka (2000) observed a 94.7% of first ovulation postpartum were silent according to visual estrus detection, which was confirmed by P4 profiles instead, however, Ranasinghe et al (2010) noted that, 55.2% of postpartum cows kept in a free-stall system experienced silent first ovulation.

Altogether, our data revealed the Appearance of >10mm follicles (DF) started within 2-3 weeks PP. Additionally, the periods from calving to 1st PP ovulation and luteal tissue formation were ≤ 45 day while the development of ovarian cyst extends this period to 75 day. Development of cysts following anovulated follicles with the size of 19-20mm occurred in two cows (33.3%). One cyst persisted for 10 days and the other for 23 days. Both cysts were spontaneously recovered.

**Conflict of interests:** There are no conflicts of interest stated by the authors.

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