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ULTRASOUND IMAGING OF POSTPARTUM UTERINE INVOLUTION AND OVARIUM DYNAMIC IN ONGOLE CROSSBREED COWS

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ABSTRACT

This study aimed to determine ultrasound of uterine involution and postpartum ovarian dynamics on Ongole Crossbreed Heifer (PO) associated with postpartum estrus signs. This study used 6 PO cows which were divided into primiparous group and pluripara group. The observation of uterine involution and ovarian dynamics was started from the first day postpartum using ultrasonography (USG) with a linear probe rectally, while the reproductive organs images were recorded every 2 days. The results showed that the time needed for all reproductive organs of 3 primiparous groups to complete the uterine involution after parturition was 37.33±1.15 days when the diameter of the vagina, cervix uterine, corpus uterine, left cornua, and right cornua uterine were 3.14±0.00 cm, 2.86±0.00 cm, 3.20±0.06 cm, 4.66±0.01 cm, and 4.66±0.01 cm, respectively. The time needed for all reproductive organs of 3 pluripara groups to complete uterine involution postpartum was 38.67±1.15 days with the diameter of the vagina, cervix uterine, corpus uterine, left cornua, and right cornua uterine 3.18±0.00 cm, 2.70±0.02 cm, 3.08±0.02 cm, 4.42±0.01 cm, and 4.42±0.01 cm, respectively. The average times of the first and second ovulation of primiparous cattle were 27.67±1.15 and 47.67±1.15 days postpartum, whereas in pluripara group was 28.33±1.15 and 48.33±1.15 days postpartum. At first ovulation all cows were not accompanied by signs of estrus, while at the second ovulation 2 primiparous cows and 1 pluripara cow showed less obvious signs of estrus, 1 primiparous cow and 1 pluripara cow showed signs of medium estrus, and 1 pluripara cow showed clear estrus signs.

Key words: ongole crossbreed heifer, ovarian dynamics, postpartum, uterine involution

ABSTRAK

Penelitian ini bertujuan mengetahui gambaran ultrasound involusi uteri dan dinamika ovari postpartus pada sapi Peranakan Ongole (PO) yang berkaitan dengan tanda-tanda estrus postpartus. Dalam penelitian ini digunakan 6 ekor sapi PO yang dibagi menjadi kelompok primipara dan kelompok pluripara. Pengamatan involusi uteri dan dinamika ovari dimulai pada hari pertama postpartus menggunakan ultrasonografi dengan menempatkan linear probe secara per rektal dan gambaran organ reproduksi direkam setiap 2 hari sekali. Hasil penelitian menunjukkan bahwa waktu yang dibutuhkan semua organ reproduksi selesai mengalami involusi uteri postpartus dari 3 ekor sapi primipara adalah 37,33±1,15 hari ketika diameter vagina, serviks, korpus, kornua kiri dan kornua kanan berturut-turut yaitu 3,14±0,00 cm, 2,86±0,00 cm, 3,20±0,06 cm, 4,66±0,01 cm dan 4,66±0,01 cm, sedangkan waktu yang dibutuhkan semua organ reproduksi selesai mengalami involusi uteri postpartus dari 3 ekor sapi pluripara adalah 38,67±1,15 hari dengan diameter vagina, serviks, korpus, kornua kiri, dan kornua kanan berturut-turut yaitu 3,18±0,00 cm, 2,70±0,02 cm, 3,08±0,02 cm, 4,42±0,01 cm dan 4,42±0,01 cm. Rataan waktu ovulasi pertama dan kedua dari sapi primipara adalah 27,67±1,15 dan 47,67±1,15 hari postpartus, sedangkan rataan waktu ovulasi pertama dan kedua dari pluripara adalah 28,33±1,15, dan 48,33±1,15 hari postpartus. Pada ovulasi pertama semua sapi tidak disertai dengan tanda-tanda estrus, sedangkan pada ovulasi kedua 2 ekor sapi primipara dan 1 ekor sapi pluripara menunjukkan tanda-tanda estrus kurang jelas, 1 ekor sapi primipara dan 1 ekor sapi pluripara menunjukkan tanda-tanda estrus sedang, dan 1 ekor sapi pluripara menunjukkan tanda-tanda estrus jelas.

Kata kunci: sapi PO, dinamika ovari, postpartus, involusi uteri

INTRODUCTION

Ongole crossbreed cows (PO) is one of the local cows that plays an important role in supplying meat in Indonesia (Putri *et al*., 2014). However, a problem that is often encountered in PO business is repeated marriage with >2 service per conception (S/C) and low pregnancy rate (<60%) causing prolonged calving interval becomes (> 18 months) (Matondang and Rudiana, 2014). Birth is the success of reproductive process which starts from estrus, ovulation, fertilization, pregnancy until delivery. A one-time birth cycle per year can only be achieved if the cow has high fertility and postpartum conception rate, accurate estrus detection so that days open (DO) can be shortened to 85 days (Pryce *et al*., 2004; Wahyudi *et al*., 2013). A good DO is normally 40-60 days or in the range of 80-85 days for efficient reproduction after delivery (Stevenson, 2001; Putri *et al*., 2014).

The postpartum period is declared successful if it is marked by complete uterine involution and the return of ovarian function (Pryce *et al*., 2004, Prasdini *et al*., 2015). Saut *et al*. (2011) and Wathes *et al*. (2011) stated that the duration required for a complete uterine involution process is around 21-35 days. Meanwhile, Hajurka *et al*. (2005) also reported a duration uterine involution of 23 days postpartum in primiparous cows and 27 days in pluripara cows. Murphy *et al*. (1990), Beam and Butler (1999), and Crowe (2008) stated that cow's ovarian activity returned to function several days postpartum, whereas the first follicular wave appeared 7-10 days postpartum and followed by ovulation of the dominant follicle 21-30 days postpartum if the estradiol concentration is sufficient to trigger a spike in luteinizing hormone (LH). The process of uterine involution and the return of postpartum ovarian activity are strongly influenced by the hormonal system (Ball and Peters, 2004; Crowe, 2008).

MATERIALS AND METHODS

Experimental Animals

This study used 6 PO cows divided into primiparous cows (n= 3) and pluripara cows (n= 3). All cows recently gave birth normally, with a body condition score 2.5-3.0 on a scale of 1-5, body weight 250-280 kg, aged 2.5-6.0 years old and physically fit.

The cows were kept inside cages, given feed in the form of forage and concentrate twice per head per day; drinking water was given ad libitum.

Ultrasonography (USG) Observation of Uterine Involution and Ovarian Dynamics

Observation of uterine involution and postpartum ovarian dynamics was carried out using a real-time, transrectal, B-mode ultrasonography (USG) (ALOKA SSD-500, Aloka Co. Ltd, Japan) by placing probes (linear probe 7.5 MHz, Japan) rectally. Reproductive organ USG images were printed with a printer (SONY UP-895 MD, Japan). Observation of uterine involution and ovarian dynamics began shortly after delivery by the same operator. Cows are placed in a stable pen, then observed with USG every two days.

Observation of uterine involution was done by measuring the diameter of the vagina, cervix, uterine body (in the middle) and uterine horn (measured at the base of cross sections of the longest axis) (Okano and Tomizuka, 1987). Observation of ovarian dynamics was carried out according to Fricke method (2002) with repeated scanning of ovarian surface to view the follicle and luteal body. The parameters observed include corpus luteum (CL) diameter and the number and size of follicles, which were then classified into small follicles (class I) with a diameter of 0.3-0.5 cm, medium follicles (class II) with a diameter of 0.6-0.9 cm and large follicles (class III) with a diameter of ≥1 cm. The diameter of each follicle and CL in a cow's ovary was measured using internal calipers in USG such as the distance between the two axis points based on the longest axis in cm (Lucy *et al*., 1992).

Estrus Observation

Estrus observation was conducted visually twice a day in the morning and evening after delivery until the two estrus periods thereafter. The focus of attention was when the first signs of estrus appear and estrus intensity, including physical changes that occurring in the reproductive organs outside the cow. Estrus intensity was observed based on the Kune and Solihati (2007) method by scoring based on signs of estrus that appeared in cows: changes in vulva (color, swelling, and temperature), mucus secretion (abundance, characteristics, and concentration), changes in behavior in one cycle. The estrus score which is measured based on the appearance of the estrus signs is then scored to find out the apparent cumulative value of the estrus. A score of 1 is given if the estrus sign is unclear, 2 if the estrus sign is moderate and 3 if the estrus sign is clear.

Data Analysis

The data collected were analyzed quantitatively by calculating the average and standard deviations, while the qualitative data were analyzed descriptively (Steel and Torrie, 1999). Statistical data analysis was conducted using MS Office Excel 2016 software and IBM SPSS Statistics ver. 23.

RESULTS AND DISCUSSION

Reproductive efficiency is considered good if a cow can give birth to one calf in one year (Ball and Peters, 2004). The process of childbirth is the success of a long reproductive process starting from estrus, ovulation, and pregnancy until delivery. Fertility of postpartum mother cows is largely determined by two main factors, uterine involution and the start of postpartum ovarian activity. The results showed that the time required for all reproductive organs to undergo involuntary postpartum involution in 3 the primiparous cows was 37.33±1.15 days when the diameter of the vagina, cervix, uterine body, left uterine horn and right uterine horn of the cows were 3.14±0.00 cm, 2.86±0.00 cm, 3.20±0.06 cm, 4.66±0.01 cm and 4.66±0.01 cm, while the time required for all reproductive organs to undergo uterine involution in the 3 pluripara cows was 38.67±1.15 days with the diameter of vagina, cervix, uterine body, left and right uterine horn of 3.18±0.00 cm, 2.70±0.02 cm, 3.08±0.02 cm, 4.42±0.01 cm and 4.42±0.01 cm respectively (Figure 1).

There was no significant difference in mean postpartum involution time between pluripara cows and primiparous cows (Table 1). However, the time for uterine involution in the study was longer than reported

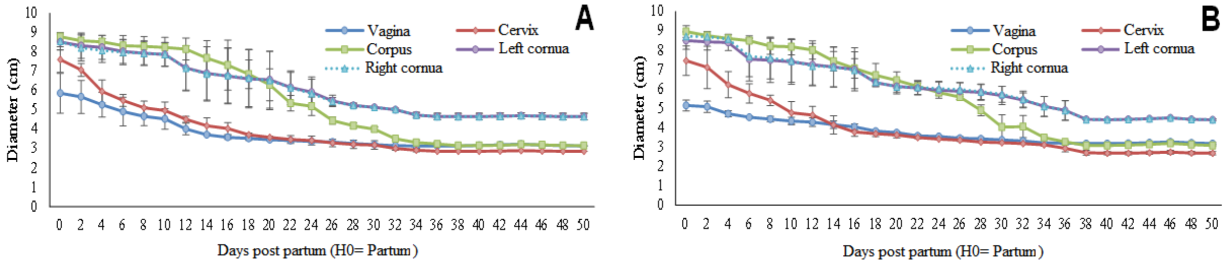

Figure 1. Graph of uterine diameter measurement. A= Primiparous cows, B= Pluripara cows. Changes in the diameter of vaginal lumen, cervix, uterine body, left and right uterine horn in postpartum primiparous cows and pluripara cows shortly after delivery until maximum uterine involution. The vagina and cervix underwent involution more quickly compared to the body and horns of the uterus. The uterine horn involution in primiparous cows and pluripara cows was completed at the 6th week postpartum

Table 1. Average time for uterine involution after parturition in primiparous and pluripara cows

No.	Parent parity	Uterine involution (Days)
1.	Primiparous cows	37.33±1.15[a]
2.	Pluripara cows	38.67±1.15[a]

[a]The same superscript within the same column indicate no significant difference (P>0.05)

by Bastidas *et al*. (1984), Saut *et al*. (2011) and Wathes *et al*. (2011), namely the length of time required for a complete uterine involution was 21-35 days. Likewise, Hajurka *et al*. (2005) stated that the time for uterine involution was 23 days in primiparous cows and 27 days in pluripara cows.

USG of postpartum cows showed continuous shortening of the diameters of vagina, cervix, uterine body, left and right uterine horn until a normal size was achieved when uterine involution was completed (Figure 2). In the USG, vagina of both postpartum pluripara and primiparous cows appears as a hypoechoic (gray) to hyperechoic (white) mass. Significant change in vaginal size occured during the first to the 14th day postpartum.

Cervix appears as a hypoechoic to hyperechoic mass with a white line in the middle; between the cervical ring gap, there is anechoic (black) area which is the cervical lumen (Figure 3). Significant changes in cervical size occured during the first to the 16th day postpartum.

Uterine body can be seen as hypoechoic to hyperechoic mass in the longitudinal axis; uterine lumen appeared anechoic and parallel to endometrial lining. There was no clear distinction between the different layers in the uterine wall (Figure 4). Significant changes in uterine body size occured during the first to the 32nd day postpartum.

Cross-sectional images of left and right uterine horns showed a hypoechoic to hyperechoic mass that is

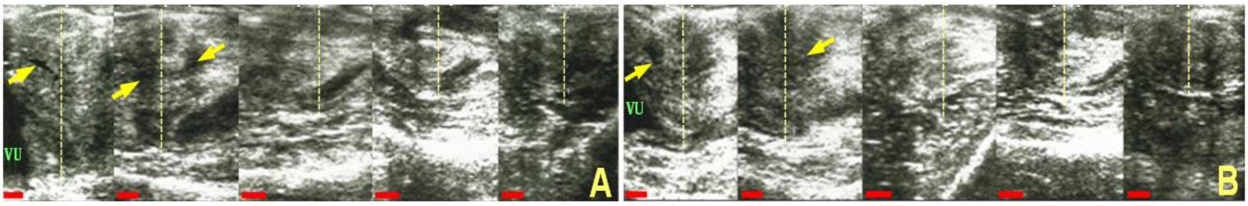

Figure 2. Longitudinal section USG showing postpartum changes in vaginal diameter. A= In primiparous cows, B= In pluripara cows, ➚ = Lochia, VU= Bladder. Bar scale: 1 cm

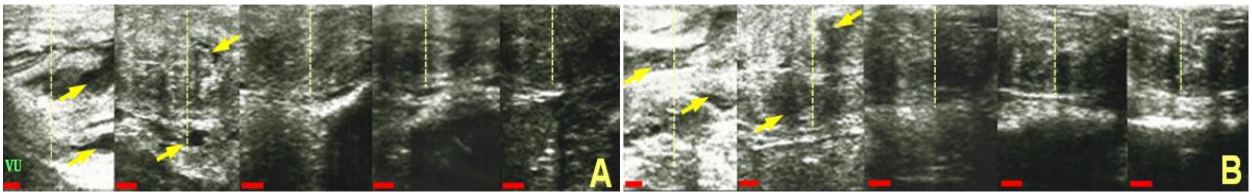

Figure 3. Longitudinal section USG showing postpartum changes in cervix. A= Primiparous cows, B= Pluripara cow. Ultrasound images of the cervical lumen showed lochia from day 0 postpartum to day 10 postpartum. (➚ = Lochia). Bar scale: 1 cm

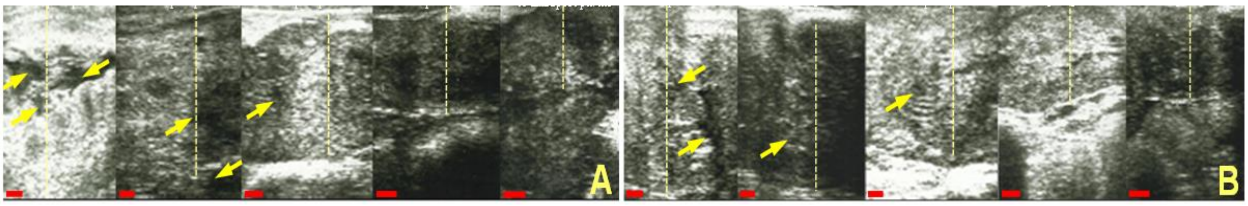

Figure 4. Longitudinal section USG showing postpartum changes in uterine body. A= Primiparous cows, B= Pluripara cows. Ultrasound images of the lumen showed lochia from day 0 to day 20 postpartum. (➚ = Lochia). Bar scale: 1 cm

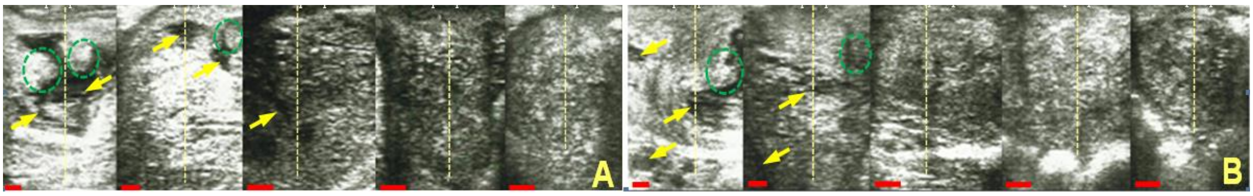

Figure 5. Cross section USG showing postpartum changes in left uterine horn. A= Postpartum primiparous, B= Pluripara cow. Ultrasound images on the left horn lumen showed lochia from day 0 postpartum to day 20 postpartum. (➚ = Lochia, ◌ = Placentom). Bar scale: 1 cm

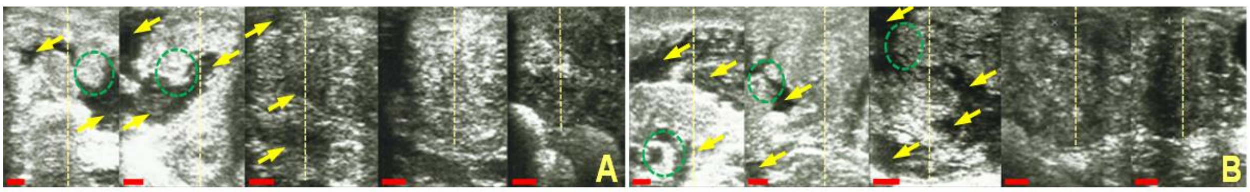

Figure 6. Cross section USG showing postpartum changes in right uterine horn. A= Primiparous, B= Pluripara cows. Ultrasound images on the right horn lumen showed lochia from day 0 to day 20 postpartum. (➚ = Lochia, ◌ = Placentom). Bar scale: 1 cm

comprised of a hypoechoic outer layer, surrounded by a black ring of protective vascular and longitudinal, circular and obligate layers of the myometrium. Longitudinal sections showed an anechoic lumen that is parallel to endometrial lining and muscular layer (Figures 5 and 6). Significant change in the size uterine horns occurred from the first to the 34th day postpartum.

Determination of reproductive status is an important factor in cow breeding. Various techniques can be performed to determine the reproductive status of cows including observation of ovarian dynamics using ultrasonography (Fricke, 2002; Amrozi *et al*., 2004; Melia *et al*., 2014). Ovarian dynamics in cows occur in the form of follicular waves. A follicular wave includes the simultaneous growth of a group of follicles, one of which will be the dominant follicle, reaching the largest size, and will suppress the development of other smaller follicles (Pierson *et al*., 1988; Adams *et al*., 2008; Melia *et al*., 2014). This pattern of follicular wave growth can be observed in the prepubertal period (Melvin *et al*., 1999), during the estrus cycle (Roche *et al*. 1999), during the pregnancy (Taylor and Rajamahendran, 1991) and after delivery (Murphy *et al*., 1990).

Based on daily observations of the postpartum ovarian dynamics in primiparous cows and pluripara cows using ultrasonography, there were 3 follicular wave patterns that occured during one estrus cycle (Figure 7). This is in line with Adams *et al*. (2008) who stated that the majority of cows show 2-3 follicular wave patterns in one etrous cycle. Melia *et al*. (2014) also reported 3 follicular wave patternss in 6 PO cows observed. The growth of the first follicular wave in primiparous and pluripara cows began on the first day postpartum, the second follicular wave occurred on day 12.67±1.15 postpartum and the third wave occurred on day 19.67±1.15 postpartum in primiparous cows while on day 20.67±1.15 postpartum in pluripara cows (Figure 7).

The first postpartum ovulation in a cow reflects the complete and perfect development of follicles in the

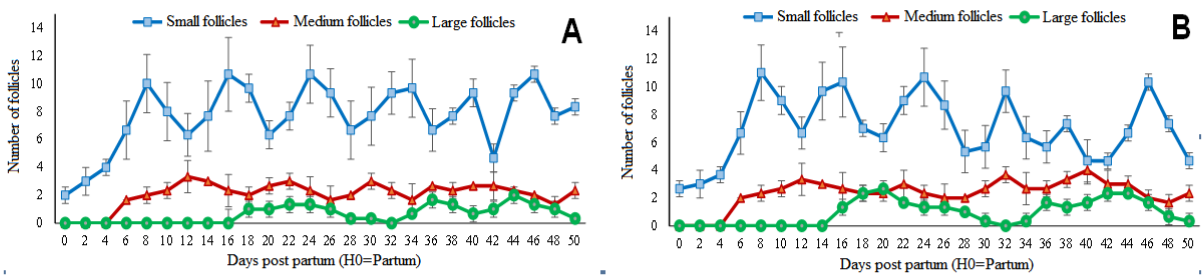

Figure 7. Classification of follicular size. A= In primiparous cattle, B= Pluripara cattle. An increase in the number of small follicles occurs 3 times in one estrus cycle, medium follicles 2 times and large follicles 1 time. An increase in the number of small follicles begins at day 0 postpartum, medium follicles day 6 postpartum and large follicles day 16 postpartum

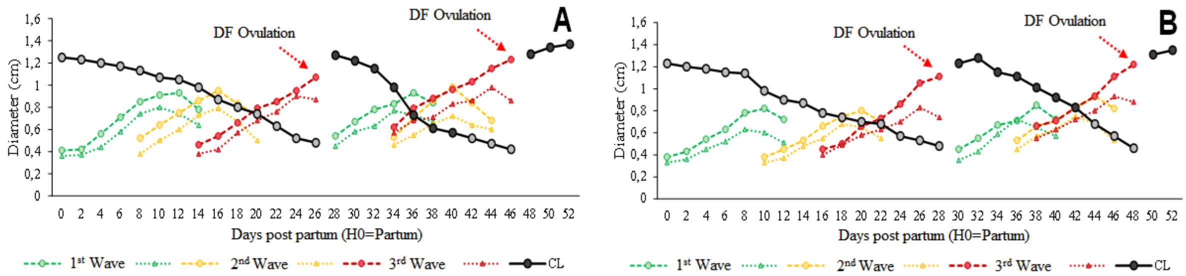

Figure 8. Postpartum ovarian dynamics. A= In primiparous cows, B= Pluripara cows. There were 3 follicular wave patterns that occurred in one estrus cycle. The first follicular wave started at day 0 postpartum, the second and third waves started at day 7 and 13 postpartum. The first and second ovulation of the dominant follicles in primiparous cow occurred on day 27 and 47 postpartum, while in pluripara cows it occurred on day 28 and 48 postpartum

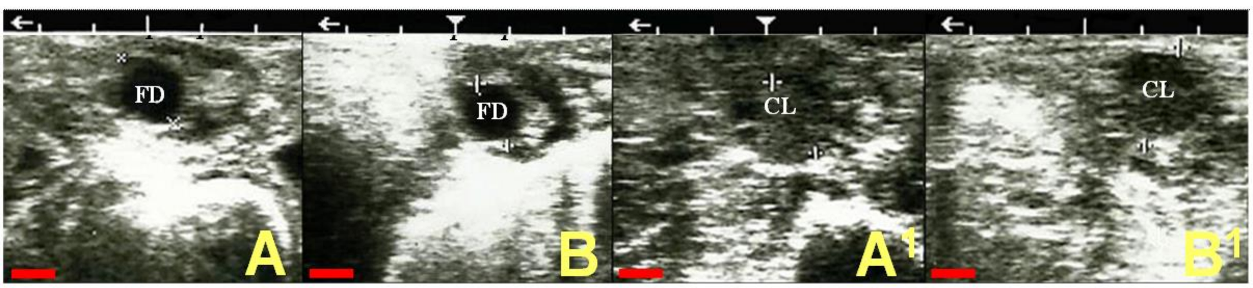

Figure 9. Ultrasound imaging of dominant follicles (FD) and luteal body (CL) post-partum in ongol crossbred cattle. A= Dominant follicles in primiparous cows with clear follicular black liquor (anechoic) and slightly gray debris in the ventral section, B= Large follicles in pluripara cattle, A1= The luteal body in primiparous cows with lutein tissue appears gray (hypoechoic), B1= The luteal body in pluripara cattle. Bar scale: 1 cm

ovary. This process subsequently determine the cow's fertility. Various physiological processes are needed so that the cow can restart the postpartum estrus cycle (Pryce *et al*., 2004). Recovery of postpartum reproductive organs is needed so that follicular growth can develop perfectly followed by ovulation and enough follicle stimulating hormone (FSH) and LH are secreted to stimulate follicular development and trigger ovulation from dominant follicles (Butler, 2005). Ovulation of the first dominant follicle in primiparous cows occurred on day 27.67±1.15 postpartum while in pluripara cows occurred on day 28.33±1.15 postpartum (Figure 8).

The growth of the first follicular wave occurred on day 0.33±0.58 after the first postpartum ovulation in primiparous cows while it occurred on day 0.67±0.58 before the first postpartum ovulation in pluripara cows. The growth of second and third follicular waves occurred on days 7.33±1.15 and 13.67±0.58 after the first postpartum ovulation in primiparous cows, while they occurred on day 6.00±0.00 and 13.33±0.58 after the first postpartum ovulation in pluripara cows. The second ovulation occurred on day 47.67±1.15 postpartum in primiparous cows while it occurred on day 48.33±1.15 postpartum in pluripara cows (Table 2).

Wolfenson *et al*. (2004) reported no significant differences in the proportion of follicular wave patterns between young and adult cows; however, in another study, Adams *et al*. (2008) stated that the majority of primiparous cows (65%) showed a 3 waves pattern while the majority of pluripara cows (83%) showed a 2 waves pattern. Satheshkumar *et al*. (2015) reported that 3 wave patterns occurred more in winter than 2 wave patterns, and vice versa in summer. Follicular waves occur in response to increased FSH plasma concentrations which peaks on day 4-5 postpartum (Sheldon, 2004). The presence of follicular waves immediately after birth indicates that the blood FSH concentration is sufficient. Thus it is estimated that the real limiting factors for ovulation resumption are the adequacy LH secretion in stimulating late follicular maturity and the ovulation process of dominant follicles (Canfield and Butler, 1990). This implies that postpartum anestrus in cows might be caused more by the failure of the follicle to ovulate, rather than the tendency for follicular underdevelopment (Figure 9).

Nuryadi and Wahjuningsih (2011) reported that detection of postpartum estrus in PO cows was not easy due to vague estrus signs in cows. Nevertheless, according to Sakaguchi *et al*. (2004) the signs of estrus postpartum has been shown to be very closely related to the age and parity of cows; the older the cows, the clearer the estrus signs. Zang *et al*. (2010) also stated that the older the cows, the more obvious the estrus signs become. This is because of the larger ovarian size of older cows. The ovaries produce estrogen which plays an important role in estrus intensity.

A high estrus performance score indicates good estrus quality. Clearer signs of estrus makes estrus identification more accurate and more precise artificial insemination. Estrus scores can be assessed based on the cumulative value of the appearance of vulva changes, mucus production, and cows' behavior during estrus (Kune and Solihati, 2007; Putri *et al*., 2014). Estrus scores can be assessed in scores of 1 to 3, where a score 1 stands for unclear estrus signs, 2 for sufficiently obvious estrus signs (moderate), and 3 for clear estrus signs (Kune and Solihati, 2007).

Out of all cows observed, the first ovulation in both primiparous and pluripara cows was not accompanied by signs of estrus (silent heat), whereas in the second ovulation there were 2 primiparous cows and 1 pluripara cow showing vague signs of estrus, 1 primiparous cows and 1 pluripara cow showing signs of moderate estrus and only 1 pluripara cow from 6 PO cows observed showed clear signs of estrus (Table 3).

The difference in time of first and second ovulation in primiparous cows which is shorter than that of pluripara cows; it might be caused by larger milk production (peak lactation) in pluripara cows compared to primiparous cows. This is in line with the opinion of Butler (2001), whereby during the early postpartum period, cows in peak period of lactation are usually in a condition of negative energy balance (negative energy balance) because of their inability to consume sufficient amount of energy in the ration. Stevenson

Table 2. Time of postpartum ovulation in primiparous cattle and pluripara cattle

No.	Parent parity	Ovulation time (Days)	
		First	Second
1.	Primiparous cows	27.67±1.15[a]	47.67±1.15[a]
2.	Pluripara cows	28.33±1.15[a]	48.33±1.15[a]

[a]The same superscript within the same column indicate no significant difference (P>0.05)

Table 3. Estrus time, ovulation time and signs of postpartum estrus in primiparous cows and pluripara cows

No.	Cows	Estrus time (Days)		Sign of estrus (Score)		Ovulation time (Days)	
		First	Second	First	Second	First	Second
1	Primipara I	26	46	0	1	27	47
2	Primipara II	26	46	0	1	27	47
3	Primipara III	28	48	0	2	29	49
Mean±SD		26.67±1.15[a]	46.67±1.15[a]	0.00±0.00[a]	1.33±0.58[a]	27.67±1.15[a]	47.67±1.15[a]
1	Primipara I	26	46	0	2	27	47
2	Primipara II	28	48	0	1	29	49
3	Primipara III	28	48	0	3	29	49
Mean±SD		27.33±1.15[a]	47.33±1.15[a]	0.00±0.00[a]	2.00±1.00[a]	28.33±1.15[a]	48.33±1.15[a]

[a]The same superscript within the same column indicate no significant difference (P>0.05)

(2001) also stated that the ovulation time of the dominant follicle is known to be related to the rate in which positive energy balance is achieved. Negative energy balance results in decreased postpartum FSH and LH secretion, slowing the return of ovarian activity, which causes prolonged ovulation interval and delayed first postpartum estrus. Therefore, if the cow's energy reserves are low, postpartum anestrus will be prolonged.

CONCLUSION

Based on the results of this study, it can be concluded that uterine involution was completed on day 37.33±1.15 postpartum in primiparous cows and day 38.67±1.15 postpartum in pluripara cows. The average time of first and second ovulation for the 3 primiparous cows was 27.67±1.15 and 47.67±1.15 days postpartum, while for the 3 pluripara cows it was 28.33±1.15 and 48.33±1.15 days postpartum. The first ovulation postpartum in primiparous and pluripara cows were not accompanied by signs of estrus while the second ovulation was accompanied by signs of estrus.

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