Jurnal Veteriner
pISSN: 1411-8327; eISSN: 2477-5665
Terakreditasi Nasional, Dirjen Penguatan Riset dan Pengembangan, Kemenristek Dikti RI S.K. No. 36a/E/KPT/2016

September 2023 Vol. 24 No. 3 : 271 - 278
DOI: 10.19087/jveteriner.2023.24.3.271
online pada http://ojs.unud.ac.id/index.php/jvet

Ovarian Dinamics of Simmental *Crossbreed* Diagnosed *Prolonged Oestrus* Cows Treated With a Intravenous of *Human Chorionic Gonadotrophin* (hCG)

(DINAMIKA OVARIUM PADA SAPI PERANAKAN SIMMENTAL MENGALAMI ESTRUS BERKEPANJANGAN YANG DITERAPI DENGAN *HUMAN CHORIONIC GONADOTROPHIN* (hCG) SECARA INTRAVENA)

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ABSTRACT

This study was aimed to identify ovarian dynamics using ultrasonography (USG) in simmental crossbreed cows diagnosed prolonged oestrus treatment with human chorionic gonadotrophin (hCG). A total of four Simmental crossbreed cows diagnosed with prolonged oestrous were used in this study. The first oestrous observations in all four cows were a control group, where as the next oestrous cycle observation to the same four cows treated with 1500 IU hCG in jugular vein refer to treated group. The observation of ovarian dynamics was started from the first oestrous day until ovulation occurs in the second oestrus cycle using ultrasonography (USG) with a linear rectal probe. At the same time, follicle and *corpus luteum* (CL) images were recorded every day. Data were analyzed using paired data comparison test (pair sample t-test). The observation results ovarian dynamics on prolonged oestrus cows showed that the long of oestrous cycle was 22.5 ± 0.5 days, and there are three follicular wave patterns in oestrous cycle. The first wave of dominant follicle was firstly identified on day 3.3 ± 0.5, reached maximum diameter 12.0 ± 0.3 cm. The second and third dominant follicle appeared on day 10.8 ± 0.5 and 15.8 ± 0.5 cm reached maximum diameter 1.2 ± 0.3 cm dan 1.5 ± 0.3 cm. Therapy with hCG does not significantly affect the dominant follicle's diameter during oestrous ($p>0.05$). The conclusion of this study showed that 1500 IU of hCG therapy does not affect the development of dominant follicle, however, it can shorten the duration of oestrous and ovulation time.

Keywords*:* Simmental *crossbreed; prolonged oestrous*; ultrasonography; hCG.

ABSTRAK

Penelitian ini bertujuan untuk mengidentifikasi gambaran dinamika ovarium dengan metode ultrasonografi (USG) pada sapi peranakan simmental (PS) yang didiagnosis birahi/ estrus berkepanjangan yang diterapi dengan *human chorionic gonadotrophin* (hCG). Sebanyak empat ekor sapi persilangan simmental yang didiagnosis birahi berkepanjangan digunakan dalam penelitian ini. Pengamatan estrus pertama pada keempat ekor sapi merupakan kelompok kontrol, sedangkan pengamatan siklus estrus berikutnya pada empat ekor sapi yang sama yang diberi hCG 1500 IU melalui vena jugularis merupakan kelompok perlakuan. Pengamatan dinamika ovarium dimulai dari hari pertama terlihatnya tanda-tanda estrus sampai ovulasi pada siklus estrus berikutnya dengan menggunakan ultrasonografi (USG) dengan pemindai/*probe* linier secara rektal. Pada saat yang sama, pengambilan gambar folikel dan *corpus luteum* (CL) dilakukan setiap hari sekali. Data dianalisis menggunakan uji banding data berpasangan *(pair sampel t test)*. Hasil pengamatan dinamika ovarium pada sapi yang didiagnosis estrus berkepanjangan memperlihatkan bahwa rata-rata panjang siklus estrus 22,5 ± 0,5 hari dan terdapat tiga pola gelombang folikel dalam satu siklus estrus. Pertumbuhan gelombang folikel pertama dimulai hari ke-3,3 ± 0,5 dengan ukuran folikel dominanya 1,2 ± 0,3 cm. Pertumbuhan gelombang folikel kedua dan ke tiga terjadi hari ke-10,8 ± 0,5 dan 15,8 ± 0,5 dengan ukuran folikel dominanya 1,2 ± 0,3 cm dan 1,5 ± 0,3 cm. Pemberian hCG tidak memberikan pengaruh yang nyata terhadap diameter folikel dominan pada saat estrus ($p>0,05$). Simpulan penelitian ini menunjukkan bahwa terapi hCG 1500 IU tidak memengaruhi perkembangan folikel dominan saat estrus. Namun, dapat memperpendek masa estrus dan waktu ovulasi.

Kata- kata kunci: Simmental *crossbreed; Prolonged oestrus*; ultrasonografi; hCG

INTRODUCTION

Increasing beef cows productivity through crossbreeding programs between *Bos taurus* and local cows has long been done through artificial insemination mating system. One type of crossbreed cows commonly developed in Indonesia is the simmental crossbreed. Simmental crossbreed cows are cows from crosses between Simmental with local cows such as Ongole (*Bos indicus*) crossbreed and Bali cattle (*Bos sondaicus*). The primary purpose of crossing is to combine two or more superior heterozygosity traits (advantages in individual) that were found initially in two livestock nations into one cruciferous nation (Hardjosubroto, 1994). Simmental crossbreed cows have good daily body weight gain in bulls of 0.72 kg and 0.66 kg in cows (Suliani *et al.,* 2017). Even though the daily body weight gain is good, Simmental crossbreed cow have reproductive problems, one of which is prolonged oestrous (Arun *et al.,* 2020).

Prolonged oestrous is an occurrence of reproductive disorders in cows with a normal oestrous cycle but a longed oestrous duration of more than 36 hours, even though ovulation is followed afterwards. Prolonged oestrous causes a decrease in reproductive efficiency, resulting from longed oestrous, causing a mismatch between the onset of oestrous and the time of ovulation. So that if artificial insemination is carried out following standard procedures, in these cows, ovulation has not occurred (delayed ovulation), which results in pregnancy failure (Duchens *et al.,* 1995; Dadarwal *et al.,* 2005). According to Dadarwal *et al.* (2005), prolonged oestrous results in repeat breeding, high service per conception, and long calving interval. The incidence of prolonged oestrous in crossbreed cows reached 25.86%, of which 30.29% occurred in cows, and 16.66% occurred in heifers. Based on the age group 2-4 years 28.60%, 5-8 years 22.73%, and 8-12 years 22.97% (Arun *et al.,* 2020).

One of the observation methods to identify reproductive status in cows is by observing ovarian dynamics using ultrasound (Melia *et al.,* 2014; Sukareksi *et al.,* 2019). The dynamics of the ovaries in cows occur in the form of follicular waves. Follicular surge involves the simultaneous growth of a group of follicles, one of which will become 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). Ultrasound examination by the transrectal method shows cows have 2-3

follicular waves in one oestrous cycle (Ginther *et al.,* 1995; Adams *et al.,* 2008; Melia *et al.,* 2014; Sukareksi *et al.,* 2019). However, the information of observation oestrous cycle in Simmental crossbreed cows that diagnosed prolonged estrus related to ovarian dynamics has never been continuously observed using ultrasound. In contrast, understanding ovarian dynamics in cows that diagnosed prolonged oestrus is crucial in supporting success and increasing reproductive efficiency. This study was aimed to identify ovarian dynamics and analyze the effectiveness of hCG hormone therapy in simmental crossbreed cows diagnosed prolonged oestrous.

RESEARCH METHODS

Ethical Approval

This research was approved by the Ethics Committee for Animal Experiments of the National Research and Innovation Agency (BRIN) with certificate number 032/KE.02/SK/8/2022.

Research Animal

This study used four Simmental crossbreed cows diagnosed prolonged oestrous from East Ogan Komering Ulu District, South Sumatra Province. The first oestrous observations in all four cows were a control group, where as the next oestrous cycle observation to the same four cows treated with 1500 IU hCG in jugular vein refer to treated group. All cows showed oestrous duration more than 36 hours with ovulation, 3-5 years old, 1-2 parities, and body condition score (BCS) 2.5-3.0 on a scale of 1-5. The results were obtained through interviews with the farmers, and reported by the ATR officer, followed by visual and ultrasonographic observation.

Research Procedure

Ultrasonography Observation of Ovarian Dynamics

The observation of ovarian dynamics was carried out using a real-time, transrectal, ultrasonography (Sono-Vet portable®. Meditech Equipment, Shandong, China) by rectally placing a probe (6.5 MHz linear probe). Observation of ovarian dynamics was started from the first oestrous day until ovulation occurs in the second oestrus cycle by the same operator. The onset of oestrus day is determined based on the observation signs of oestrus and the time of ovulation is determined based on the disappearance of the anechoic picture of the dominant follicle with diameters under 1 cm (Ginther *et al.,* 1995; Rubianes *et al.,* 1997; Flynn *et al.,* 2000). Cows are placed in a stable pen, then observed by ultrasound once a day. Observation of ovarian dynamics was carried out according to a method of Fricke (2002) with repeated scanning of ovarian surface to view the follicle and luteal body. The parameters observed include *corpus luteum* (CL) diameter and diameter of follicles size. The diameter of each follicle and CL in a cow's ovary was measured using internal callipers in USG such as the distance between the two axis points based on the longest axis in cm (Amrozi *et al.,* 2004).

Treatment hCG Hormone

Hormones were administered using the Human Chorionic Gonadotropin (hCG) hormone (Chorullon®, MSD Animal Health/PT Intervet Indonesia, Jakarta, Indonesia) via jugular vein with a dose of 1500 IU/cow, which was given 12 hours from the start of the cow showing symptoms of oestrous or immediate after implementation of artificial insemination (Mathew *et al*., 2016).

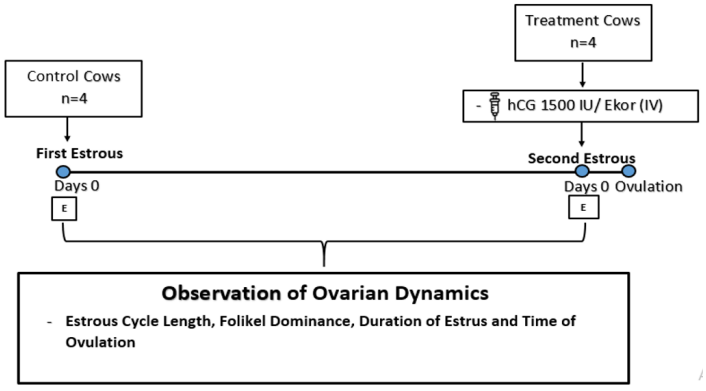

Figure 3. Diagram of observation procedures in the farm: E = period estrous

Statistical Analysis

The data of ovarian dynamics were shown as the mean ± SD (Melia *et al.,* 2014). The data were analyzed using paired data comparison test *(paired sample t-test)*. was performed to determine if there was any significant difference among the treatments at P<0.05. Statistical analysis was conducted using IBM SPSS® Statistics version 23.0 (IBM Corp., Armonk, NY, US).

RESULTS AND DISCUSSION

Observation of Ovarian Dynamics

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; Sukareksi *et al.,* 2019). 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 pre pubertal period (Melvin *et al.* 1999), during the oestrous cycle (Roche *et al.,* 1999), during the pregnancy (Taylor and Rajamahendran, 1991) and after delivery (Murphy *et al.,* 1990). Based on daily observations of ovarian dynamics during oestrous cycle in Simmental crossbreed cows diagnosed prolonged oestrous using ultrasonography, there were three follicular wave patterns that occurred during one oestrous cycle (Figure 1). 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); Sukareksi *et al.* (2019) also reported three follicular wave patterns in six Ongole crossbreed cows observed. The growth of the first follicular wave in Simmental crossbreed cows diagnosed prolonged oestrous starts from the day 3.3 ± 0.5, the second follicular wave occurred on day 10.8 ± 0.5 and the third wave occurred on day 1.5 ± 0.3 after the onset of oestrous (Figure 1). 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 three waves pattern while the majority of pluripara cows (83%) showed two waves pattern. Satheshkumar *et al.* (2015) reported that three wave patterns occurred more in winter than two wave patterns, and vice versa in summer. Follicular waves occur in response to increased FSH plasma concentrations (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).

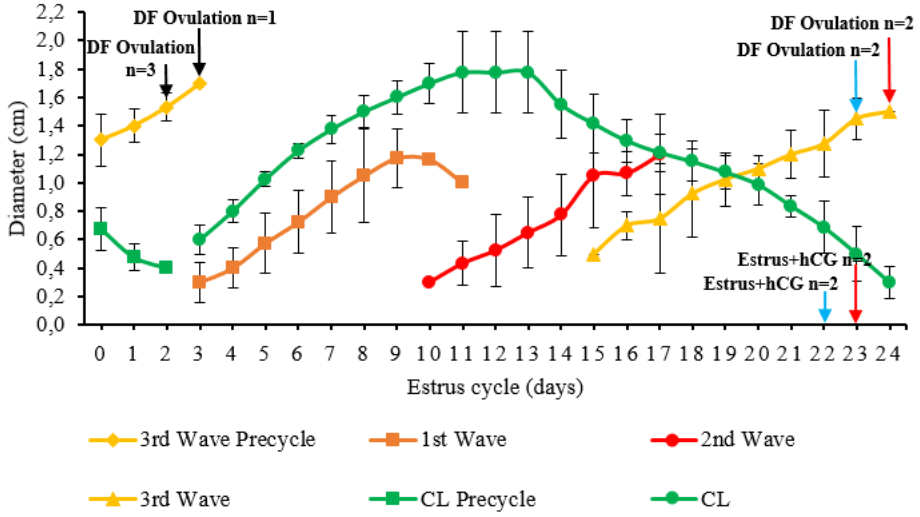

Figure 1. Overview of the ovarian dynamics of simmental crossbreed cows that diagnosed prolonged oestrous.

Table 1. Diameter of ovulation dominant follicles in Simmental crossbreed cows diagnosed prolonged oestrus.

Identity of Cows	Folikel Dominance (cm)	
	Control	Treatment hCG
1	1,7	1,4
2	1,5	1,5
3	1,5	1,5
4	1,6	1,5
Mean±SD	1,6±0,1[a]	1,5±0,1[a]

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

The ovulation of follicle dominance in Simmental crossbreed cows that diagnosed prolonged oestrous in the control group occurred on day 2.3 ± 0.5 with a dominant follicle size of 1.6 ± 0.1 cm whereas, in the group treated with hCG it occurred on day 22.5 ± 0.5 or one day after the hCG injection with a diameter of 1.5 ± 0.1 cm dominated by follicles (Table 1).

Based on a statistical test using a paired sample t-test, it did not show a significant difference, which means that the administration of the hormone hCG does not affect the diameter of the dominant follicle but causes an exogenous increase in LH levels so that the dominant follicle can ovulate more quickly. Canfield and Butler (1990) suggested that the factors that cause ovulation of follicular domination accompanied by signs of oestrous are highly dependent on the level of adequacy of the concentration of the hormone estradiol to trigger the distribution of LH hormones so that signs of oestrous can appear and domination of follicles can ovulate at the right time. The diameter of this follicle dominance is larger than Wicaksono *et al.* (2020), who stated that the dominant ovulation size in crossbreed between Simmental cattle with-Ongole crosbreed cattle was 1.4 ± 0.5 cm, in line with what Keskin *et al.* (2016) that the 1.2-1.8 cm follicle diameter has a high pregnancy success rate, but in cows in heat, the success rate is low. This condition is caused in cows with prolonged oestrus duration more than 36 hours, which results in one of the triggers for the exact time between the implementation of insemination and the time of ovulation (Singh *et al.,* 2012; Dadarwal *et al.,* 2005).

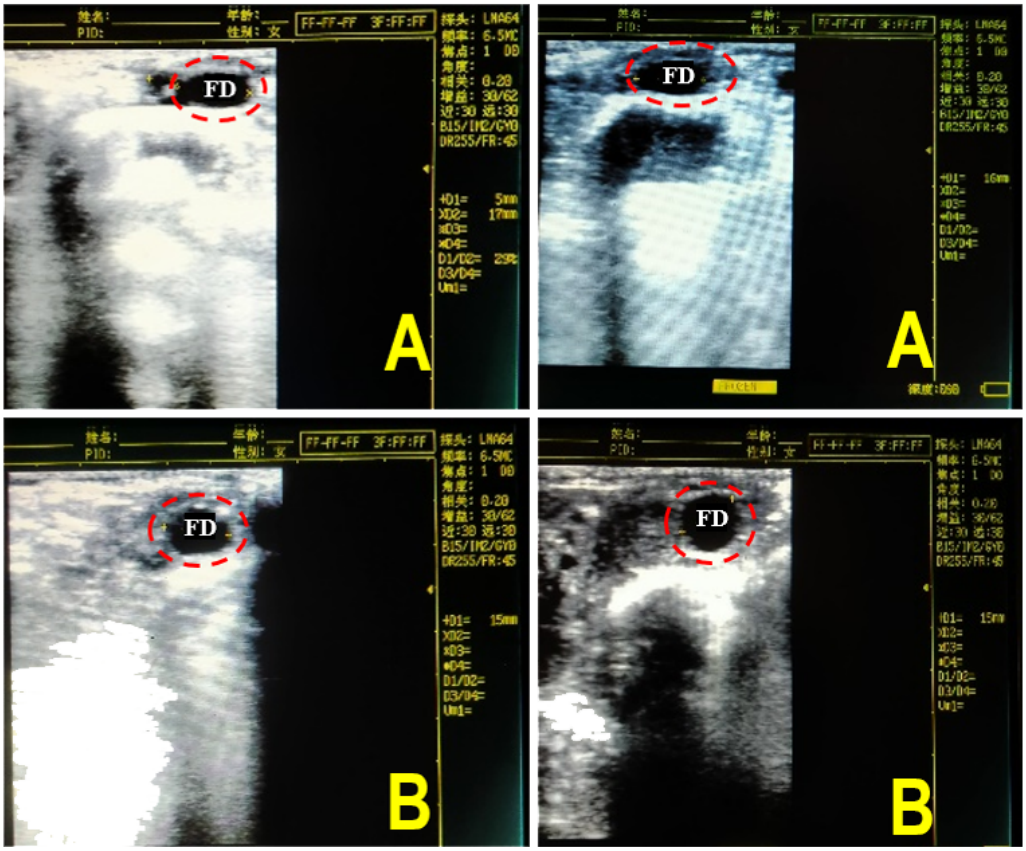

Figure 2. Ultrasound image of the dominant follicle (FD) in Simmental crossbreed cows diagnosed prolonged oestrus: A. Dominant follicle in control cows, B. Dominant follicle in hCG-treated cows. (◌=FD)

This is related to the life span of the inseminated sperm; if the sperm dies before ovulation occurs, it will result in planning failure. According to Hawk (1987) the life span of sperm in the reproductive tract ranges from 12-24 hours. Increasing the duration of oestrous can also cause follicular domination to last for a long time, resulting in oocytes becoming old and decreasing oocyte quality, and in the end, it also affects the decrease in pregnancy rates (Mihm *et al.,* 1994).

The diameter of CL during the oestrous cycle shows that the new CL formed results from ovulation of the dominant follicle. The CL ultrasound picture of the control group on four cows showed that there was still CL during oestrous with a diameter of 0.7 ± 0.2 cm, while the group given hCG had a CL diameter of 0.6 ± 0.1 cm. At the time of CL ovulation, it is still difficult to distinguish the follicle from the picture because it is still in the luteal tissue formation phase (corpus hemorrhagic or corpus rubrum stage). In line with Ghuman *et al.* (2014) in prolonged oestrous cows, when oestrous is detected, there is still CL with a size of 0.82-0.89 cm. The presence of CL during oestrous is thought to result in high progesterone concentrations or at suprabasal levels resulting in prolonged oestrous as evidenced by the undetected CL ovulation. In accordance with Duchens *et al.* (1995); Honparkhe *et al.* (2010), suprabasal progesterone arises as a result of incomplete luteolysis originating from the previous cycle due to suppression of PGF2α synthesis or can originate from extra gonads.

CONCLUSION

Based on the results of this study, it can be concluded that Simmental crossbreed cows diagnosed with *prolonged oestrus* shows that the oestrous cycle length is 22.8 ± 0.4 days and

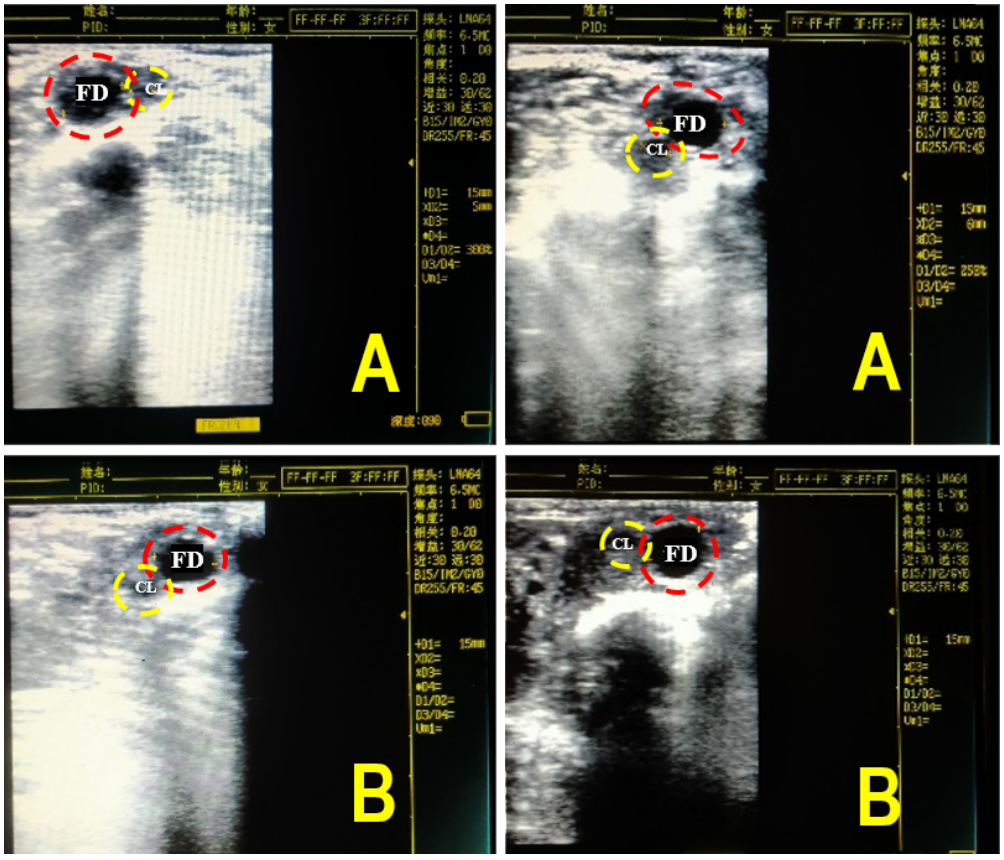

Figure 3. Ultrasound imaging of dominant follicle (FD) and *corpus luteum* (CL) in simmental crossbreed cowsthat diagnosed prolonged oestrous during estrus. The dominant follicle in primiparous cows with clear follicular black liquor (anechoic) and luteal body in primiparous cows with gray lutein tissue appears gray (hypoechoic): A. control group, B. group given hCG. (◯ = FD; ◯ = CL).

has three follicular wave patterns in one oestrous cycle. The diameter of the dominant follicle, duration of oestrous and time of ovulation in the control group was 1.6 ± 0.1 cm, 3.3 ± 0.3 days and 2.3 ± 0.3 days, while the group was therapy with hCG was 1.5 ± 0.1 cm, 2.0 ± 0.0 days and 1.0 ± 0.0 days after oestrous. The injection of 1500 IU hCG hormone immediate after implementation of artificial insemination showed shortening the duration of oestrous and time of ovulation, however, did not the dominant follicles diameter.

SUGGESTION

In Simmental crossbreed cows diagnosed with prolonged oestr*o*us*,* it is better to injection of the hCG hormone because it has been proven to shorten the duration of oestrous and time of ovulation.

ACKNOWLEDGEMENT

The writers would like to thank the District Animal Husbandry and Fisheries Service. of East Ogan Komering Ulu, South Sumatra. which provides the facilities and locations to conduct the study and postgraduate veterinary medicine programme of IPB university.

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