

Original article

Serum Progesterone profile of pregnant Ettawa crossbred does at day-0, -21, -42, -63 and -84

Profil Progesterone serum kambing peranakan Ettawa bunting pada hari ke-0, -21, -42, -63 dan -84

Ayu Aprodhita Cintya Bellani¹, Widya Paramita Lokapirnasari²,
Mas'ud Hariadi¹, Ismudiono Ismudiono^{1*}

¹ Division of Veterinary Reproduction, ² Division of Animal Husbandry
Faculty of Veterinary Medicine, Universitas Airlangga

* Corresponding author, e-mail: ismudiono@fkh.unair.ac.id

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ABSTRACT

This study aimed to determine the progesterone concentrations of Ettawa crossbred does at day-0, -21, -42, -63, and -84 after mating. Five Ettawa crossbred does were injected with PGF₂α twice with an interval of 11 days for estrous synchronization followed by natural mating on day-3 after the second PGF₂α injection. Blood was collected through the jugular vein for the measurement of serum progesterone concentrations using ELISA, whereas pregnancy was diagnosed at 90 days after mating by abdominal palpation. The results showed that all does were on estrus on the third day after the second PGF₂α, three of them diagnosed to be pregnant following mating. The progesterone concentration at day-0 was 1.52 ± 0.33 ng/mL, increased (p <0.05) to 7.08 ± 1.02 and 5.50 ± 2.82 ng/mL at day-21 and day-42, and increased (p <0.05) again to 12.01 ± 5.30 and 12.04 ± 0.30 ng/mL at day 63 and day 84, respectively. There was no significant difference in progesterone concentrations between day 21 and day 42 and between day 63 and day 84. It could be concluded that there was a biphasic increase of progesterone concentrations of the pregnant Ettawa crossbred does, i.e. from day-0 to day-21/day-42 and from day-21/day-42 to day-63/day-84.

Keywords: Ettawa crossbred, estrus synchronization, post-mating, progesterone concentration

INTRODUCTION

Ettawa goat is a popular name in Indonesia for Jamnapari (or Jamunapari), a breed of goat originating from the Indian subcontinent (Susilowati *et al.*, 2019). The Ettawa crossbred goat is a crossbreed between Ettawa and Kacang goats (Indonesian local goat) (Winaya *et al.*, 2017). This breed is widely spread throughout Indonesia and has a similar appearance to an Ettawa goat with a smaller size. Ettawa crossbred goat is potential as one of the sources of animal protein obtained through both meat and milk. Ettawa crossbred

goats could be categorized as superior dairy goats because they could produce milk on average 2 to 3 liters per day (Triswanto *et al.*, 2019). Therefore, Ettawa crossbred goats are interesting to raise.

In the development of the livestock industry, reproductive management is vital to improve productivity and maintenance management, and good breeding. The reproductive cycle of livestock relates to various phenomena, including puberty and reproduction maturity, breeding season, estrous cycle, and postpartum reproduction activity (Hafez and Hafez, 2000). Progesterone

concentration in puberty showed cyclic ovarian activity before the first estrus. Therefore, puberty could be interpreted as the progesterone concentration in blood samples exceeding one ng/mL (Estill, 2015). The estrous cycle is the rhythm of certain physiological functions of the gonadal system in animals after puberty (Lestari and Ismudiono, 2014) that could be divided into four periods, i.e. proestrus, estrus, metestrus, and diestrus. The reproductive system begins to prepare for ovulation in the proestrus phase (Dávila *et al.*, 2017). The metestrus phase is showed by the sudden cessation of the estrus phase, and the diestrus phase, shows the development of corpus luteum and progesterone production (Ismudiono *et al.*, 2010). During pregnancy, uterine growth and development are affected by increasing progesterone and estradiol concentrations (Anderson, 2003). Progesterone concentration could be measured in biological fluids such as blood and milk. A Low concentration of progesterone was found in non-pregnant animals, while pregnant animals showed higher concentration (Nepal *et al.*, 2019). Progesterone is primarily produced by granulosa-lutein cells in the corpus luteum (Cable *et al.*, 2021). Progesterone concentration in serum/plasma or milk could be used for determining the specific stage in the reproductive cycle of observed animals (Antanaitis *et al.*, 2020).

Therefore, this study aims to determine the profile of serum progesterone, which can be useful information in increasing the reproductive efficiency of Ettawa crossbred does.

MATERIALS AND METHODS

This study was approved ethically by Animal Care and Use Committee of the Faculty of Veterinary Medicine, Universitas Airlangga, with ethical clearance No.2.KE.143. 07.2019. The does used in this study were reared at the Technical Implementation Unit of Livestock Breeding and Forage, Singosari, Malang. Five, 4-5-year-old healthy Ettawa crossbred does weighing 20-30 kg, body condition score (BCS) 2-3 (scale 1-5), and a minimum parity of one, had their estrus synchronized using 7.5 mg double PGF₂α (Enzaprost T®, the Netherland)

intramuscular injection with 11 days interval (Siregar *et al.*, 2013). Estrus was detected visually, followed by natural mating to an Ettawa crossbred buck 12-18 hours after the onset of the observed second estrus. Blood samples were collected through the jugular vein five times with 21 days intervals, i.e., day 0 (estrus), day 21, 42, 63, and 84 after mating.

The measurement of serum progesterone concentration was conducted using Enzyme-Linked Immunosorbent Assay (ELISA) (He, 2013; Gan and Patel, 2013) with Progesterone ELISA Kit of Fine Test (China) at the Institute of Tropical Disease, Universitas Airlangga. Pregnancy diagnosis was conducted using abdominal palpation 90 days post-mating. Progesterone concentrations at days 0, 21, 42, 63, and 84 of Ettawa crossbred does were analyzed with General model repeated measure at 95% level of significance using SPSS v.23 (Singh *et al.*, 2013).

RESULTS

The five does which had their estrus synchronized showed a 100% (5/5) estrus rate, three days onset of estrus, and 60% (3/5) pregnancy rate after natural mating (Tabel 1). Serum progesterone concentrations of the pregnant does at 0, 21, 42, 63, and 84 days after mating is presented in Figure 1. There were two phases of significant increases ($p < 0.05$) of progesterone concentration from day 0 to day-21/day-42 and from day-21/day-42 to day-63/day-84.

Table 1 The results of estrus synchronization and pregnancy diagnosis of Ettawa crossbred does

does #	estrus observed	onset of estrus	pregnancy diagnosis
318	+	3	-
105	+	3	+
125	+	3	+
G010	+	3	-
324	+	3	+

Onset of estrus was counted from the second PGF₂α injection; pregnancy was diagnosed by abdominal palpation 90 days after mating.

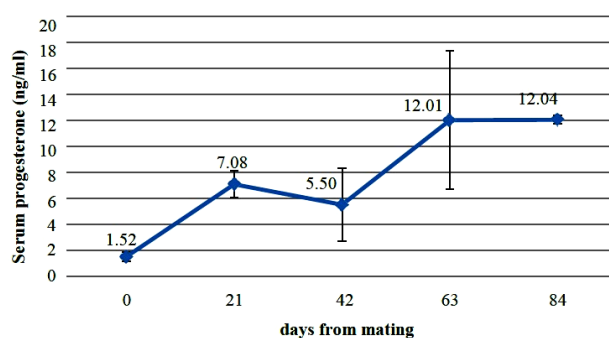


Figure 1 Serum progesterone concentrations (ng/mL) of pregnant Ettawa crossbred does at day-0, -21, -42, -63, and -84 days (from mating).

DISCUSSION

Three days after the second PGF₂ α injection, does was observed showing signs of estrus such as the redness and swelling around vulva, tail wagging, and mounted the other goats, followed by a thick and clear discharge from the vulva (Triagil *et al.*, 2020). All of the estrus synchronized does were observed for their estrus after twice PGF₂ α injection. This result was better than twice PGF₂ α injection on Kacang that resulting in an 85-95% estrus rate (Susilowati *et al.*, 2020).

Hormonal treatments for synchronization of estrus were essential for improving the goats' reproductive efficiency and genetic progress (Luo *et al.*, 2019). Synchronization of estrus is useful for insemination on the same day (Sen and Onder, 2016) and control the estrus behavior and lambing rate (Almadaly *et al.*, 2016). Prostaglandin (PG) F₂ α injection was used for regressing corpus luteum (CL). Double PGF₂ α injections are commonly used for estrus synchronization in ewes (Hashemi and Safdarian, 2017). Prostaglandins are mainly administered intramuscularly (Simões *et al.*, 2015). Double injection of prostaglandin, administered 11 days apart, resulted in higher estrus (Omontese *et al.*, 2016).

The serum progesterone concentration on day 0 was 1.52 ± 0.33 that was lower than 2.1 ± 0.5 ng/mL, the basal concentration during estrus in goats (Socheh *et al.*, 2019; Susilowati *et al.*, 2020).

The progesterone concentration of Ettawa crossbred goat at day-21 was 7.08 ± 1.02 ng/mL that was lower than the progesterone concentration of Kacang goats on day-22 (15.45 ± 3.94 to 16.58 ± 2.85 ng/mL) (Susilowati *et*

al., 2020). Meanwhile, progesterone concentrations on day-42 was in accordance with Devalal *et al.* (2018) who reported that progesterone concentrations at day 45 post-service of the goat was 6.27 ± 0.45 ng/mL. The progesterone concentrations at day-63 and day-84 were 12.01 ± 5.30 and 12.04 ± 0.30 ng/mL ($p > 0.05$). Singh *et al.* (2019) reported that during mid-pregnancy (day-50 to day-100), progesterone concentrations were relatively stable in around 12-14 ng/mL. Progesterone concentration in ewes was significantly influenced by the number of corpora lutea found in the ovaries (Hennebold, 2018). The pattern of progesterone concentrations during pregnancy was increased; it is low from mating and increases noticeably by the 3rd week and remains high until the 19th week (Hussain, 2015). Progesterone concentration during pregnancy in single kid bearing does was lower than those in twin kid bearing does (Madan *et al.*, 2020).

Pregnancy after artificial insemination is the best indicator of the reproductive potential of sires (Moura and Memili, 2016). Serum progesterone is indicative of pregnancy after an estrus cycle of insemination. Blood plasma concentrations >1 ng/mL between days 18–22 in parous goats indicate pregnancy. The accuracy for the diagnosis of pregnancy is 75-86%, while the accuracy for the diagnosis of non-pregnancy is 90-100%. The non-return to oestrus in a cycle is a very early pregnancy diagnosis after the mating (Karadaev, 2015). Islam *et al.* (2014) reported progesterone concentrations of 1.5 ng/mL, 6.6-9.3 ng/mL, and 14-15.5 ng/mL respectively on 25-30, 60-70, and 90-92 days of pregnancy. However, a high concentration of progesterone has not proven pregnancy because it could not differentiate between mid-cycle, true or false pregnancy (Devalal *et al.*, 2018). On the other hand, the doe may not be diagnosed as pregnant for having low progesterone concentration (1ng/mL) around day-20 to day-24 after mating. Previous research in Kamori goats reported that pregnancy reliably could be diagnosed at day-21 after breeding using ELISA, and the concentration was 2.71 ± 1.06 ng/mL (Kunbhar *et al.*, 2019).

There are some possible causes for the increased and decreased progesterone concentration, such as pseudopregnancy, nutrition during pregnancy, BCS, and the number of corpus luteum. Progesterone concentrations in pseudopregnancy decline over time, eventually resulting in the spontaneous evacuation of the uterus and similar to those observed during normal pregnancy (serum concentrations greater than one ng/mL) (Menzies, 2019). Nutrition during pregnancy also affects embryo survival and fetal programming of adult performance (Herring *et al.*, 2018). Underfeeding is thought to act on progesterone delivery to the uterus, which is essential for developing the placenta and fetus (Cable *et al.*, 2021).

Body Condition Scoring is an essential tool for livestock managers, feeding programs, reproduction, and welfare of the animals. The scoring was based on the amount of muscle and fat around the vertebrae. Scoring is performed in goats using a BCS ranging from 1.0 to 5.0 (Widiyono *et al.*, 2020). Ideally, the BCS of goats was 2.56 ± 0.13 for a higher pregnancy rate (Nawito *et al.*, 2015). BCS directly affects hypothalamic activity and GnRH secretion, but not pituitary sensitivity to GnRH (Sejian *et al.*, 2010). These effects on reproductive performance are also mediated through changes in ovarian hormones or hypothalamic-pituitary sensitivity to ovarian hormones (Marques *et al.*, 2018).

CONCLUSION

There was a two phase (biphasic) increase in progesterone concentrations of pregnant Ettawa crossbred does, from day-0 to day-21/day-42 and from day-21/day42 to day-63/day-84.

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