

The Effect of Using CIDR and Various Doses of PMSG as Well as Genistein on the Reproductive Characteristics of Palu Fat-tailed Sheep

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Abstract

The objective of this study was to observe the reproductive characteristics of Palu fat-tailed sheep affected by animal parities given pregnant mare serum gonadotropin (PMSG) and genistein (GEN) as a synthetic phytoestrogen. This study employed 32 ewes arranged in a completely randomized experimental design. The first factor was parity (P2 and P3) and the second factor was a hormonal treatment as follows; H0 (14 d-controlled internal drug release or CIDR implant as control), H1 (CIDR+PMSG at 15 IU/kg BW, im injection), H2 (CIDR+GEN 0.1 mg/kg BW, iv injection); H3 (CIDR+PMSG at 15 IU/kg BW and GEN at 0.1 mg/kg BW, iv injection). Results indicated that all animals exhibited estrous and the onset for H1 was significantly earlier than for H2 and H0. The duration of estrous for P3 was significantly longer than for P2, while that for H1 was significantly longer than for H2 and H0. The Gestation rate was 93.75%, the birth rate was 83.33% and length of gestation was 147.76±1.54 d. Birth weight for P3 was significantly higher than for P2. It is concluded that CIDR, PMSG, and genistein enhanced the estrous characteristics in Palu fat-tailed sheep but did not affect litter size and birth weight.

Keywords: Estrous cycle, ewe, exogenous hormone, genistein, gestation period

Respon Pemberian Hormon CIDR, PMSG dan Genistein Sintetik terhadap Karakteristik Reproduksi Domba Ekor Gemuk Palu

Abstrak

Penelitian ini bertujuan untuk mengetahui karakteristik reproduksi domba ekor gemuk Palu yang dipengaruhi oleh paritas dan pemberian pregnant mare serum gonadotropin (PMSG) dan genistein (GEN) sebagai fitoestrogen sintetik. Penelitian ini menggunakan 32 ekor domba betina dewasa dengan rancangan percobaan acak lengkap. Faktor pertama adalah paritas (P2 dan P3) dan faktor kedua adalah perlakuan hormonal sebagai berikut; H0 (implan controlled internal drug release atau CIDR selama 14 hari sebagai kontrol), H1 (CIDR+PMSG pada 15 IU/kg BB, injeksi im), H2 (CIDR+GEN 0,1 mg/kg BB, injeksi iv); H3 (CIDR+PMSG pada 15 IU/kg BB dan GEN pada 0,1 mg/kg BB, injeksi iv). Hasil menunjukkan bahwa semua hewan mengalami estrus dan onset untuk H1 secara signifikan lebih awal daripada H2 dan H0. Durasi estrus untuk P3 secara signifikan lebih lama daripada P2, sedangkan H1 secara signifikan lebih lama daripada H2 dan H0. Angka kebuntingan, angka kelahiran, dan lama kebuntingan masing-masing adalah 93,75%, 83,33% dan 147,76±1,54 hari. Berat lahir untuk P3 secara signifikan lebih tinggi daripada P2. Pemberian CIDR, PMSG, dan genistein disimpulkan dapat meningkatkan karakteristik estrus pada domba ekor gemuk Palu tetapi tidak mempengaruhi jumlah anak yang lahir (litter size) dan bobot lahir.

Kata kunci: Fitoestrogen sintetik, kebuntingan, paritas, siklus estrus

Introduction

Sheep in Central Sulawesi has been identified as fat-tailed sheep and designated as one of Indonesia's local livestock genetic resources (Kementerian Pertanian Republik Indonesia, 2013). This determination should be followed by an increase in these animals' reproductive performance. The performance is indicated by the ability of the animal to represent estrous, become pregnant, and produce offspring, but the reproductive characteristics in Palu fat-tailed sheep have been less-documented so far.

Naturally, the ovulation rate of sheep is between 1 and 4, depending on the level of fertility (Jainudeen & Hafez, 1987a). Previous research found a low litter size in Palu fat-tailed sheep, which may indicate the presence of reproductive problems in these animals. Malewa (2014) found that the birth rate of twins in Palu fat-tailed sheep was only 4%, while in Garahan fat-tailed sheep in East Java, the incidence of twins reached 25%. This is probably one of the causes of the low population increase in Palu fat-tailed sheep. In 2015, the sheep population in Palu increased by

1%, while the national population increase by 2.5% (Direktorat Jenderal Peternakan dan Kesehatan Hewan, 2016).

The low litter size in Palu fat-tailed sheep on smallholder farms is thought to be due to low pasture quality. Salmin *et al.* (2003) found the pasture to contain 3.18% crude protein and 58.46% crude fiber, and this may indirectly affects the fertility due to disruption of hormonal balance (Scaramuzzi *et al.*, 2006). Administration of flushing followed by an estrous induction with prostaglandin F2 α (PGF2 α) was only able to produce a conception rate of 52.50% (Duma *et al.*, 2001). In contrast to the Java fat-tailed sheep, *Gliricidia sepium* gave an ovulation rate at the second parity of 4 and an average litter size of 2.38 individuals (Supriyati *et al.*, 1999). This research was conducted in an intensive rearing system and the application of pregnant mare serum gonadotropin (PMSG) hormones and synthetic phytoestrogens to the Palu fat-tailed sheep. This method was aimed at controlling the estrous cycle, increasing follicle growth, ovulation, gestation, birth, litter size, gonadotropin, and steroid hormones (Akoz *et al.*, 2006; Amiruddin *et al.*, 2013). Hormone manipulation improving reproductive performance has been widely carried out in cattle (Depison, 2009), goats (Semiadi *et al.*, 2003), and sheep (Salmin *et al.*, 2003).

Control of the estrous cycle using the hormones progesterone and estrogen is intended to have a positive effect on the hypothalamus, thereby releasing GnRH (Reeves, 1987). Therefore, many workers combine the administration of progesterone and estrogen with gonadotropin hormones (FSH, LH, PMSG, and hCG). PMSG has biological effects similar to FSH and a little LH. The FSH and preovulatory LH release waves occur very close to the time of ovulation, but the preovulatory LH waves can only be initiated if there is a sufficiently high increase in plasma estrogen levels. Estrogen exerts a positive effect on the hypothalamus to release GnRH. High levels of estrogen can trigger a preovulatory LH surge, causing rupture of the follicle wall and thus ovulation (Hafez, 1987). LH surge is a time when the LH concentration increases 5 times above the basal level (Siregar & Armansyah, 2010).

Provision of PMSG can increase follicle development, ovulation, and the number of CL, causing an increase in gestation hormones.

PMSG treatment for ovulation induction following progesterone treatment indicates desired results in sheep and goats. Increased gestation hormones can improve prenatal growth and increase both fetal and birth weights (Adriani *et al.*, 2007; Andriyanto & Manalu, 2012).

Fluorogestone acetate (progesterone) implants as much as 30 mg and 40 mg gave the same and significantly better birth rates in sheep injected with PMSG 700 IU after releasing progesterone compared to PMSG 300 IU and 500 IU (Akoz *et al.*, 2006). Provision of PMSG from 400 IU to 500 IU, was able to increase litter size in sheep from 1.33 to 1.49 (Ince & Karaca, 2009). PMSG level at 15 IU/kg body weight (BW) in goats significantly increased the number of CL compared to without PMSG (Adriani *et al.*, 2007) and can increase estrogen (67%) and progesterone (42%) compared to control treatment. Low doses of PMSG (200 IU/head) increased litter size to 1.74 compared to control (1.38) and increased birth weight by 25.7% (Andriyanto & Manalu, 2012).

The use of exogenous hormones, which are relatively inexpensive and have similar effects to endogenous hormones should be attempted. Phytoestrogens are compounds in plants (found mostly in soybeans) that have estrogenic properties such as the flavone, isoflavone, and coumestant groups (Tanu, 2005). Isoflavones are a group of phytoestrogens that have a chemical structure and activity similar to estrogen and therefore can replace endogenous estrogen's function. Estrogen receptors can bond with components that have similar chemical structures to estrogen, such as genistein (GEN) (Biben, 2012). Therefore, GEN is the most widely used isoflavone compound to determine the effect of phytoestrogens on reproduction. Administration of GEN in adult female mice with an intact hypothalamic-pituitary-ovarian axis, shows a non-functional feedback mechanism (Medigović *et al.*, 2012). In contrast to female OVX rats, GEN administered intravenous (0.01 and 10 mg/kg BW) and subcutaneous (0.8 or 8 mg/kg BW) significantly decreased GnRH and LH, but the 0.1 mg/kg BW dose could increase GnRH and LH and did not differ from controls (1 mg/kg BW) (Hughes, 1988; Hughes *et al.*, 1991). Injection of GEN subcutaneous 50 mg/kg BW in dimethylsulfoxide (DMSO) resulted in

increase of FSH (19.7%) and LH (20%) compared to controls. This indicated that GEN acts as an estrogenic agonist and stimulates gonadotropin cells, decreasing primordial and secondary follicles, but increasing secondary follicles with atresia 5-fold and tertiary follicles with atresia by 35.64%. GEN has an inhibitory effect in the early stages of folliculogenesis but acts as an agonist by stimulating the follicular transition from preantral to antral stage and supports the formation of ovarian stroma (Medigović *et al.*, 2012). Realizing the low reproductive performance of Palu fat-tailed sheep, this study was conducted to determine the reproductive characteristics of the animals at different parities given PMSG and synthetic phytoestrogen.

Materials and Methods

Experimental animals

This study was conducted for 8 months in the experimental station of the Faculty of Animal Husbandry and Fisheries, Tadulako University, Palu. The study employed 32 ewes (P2 dan P3) with age range of 2 – 4 years and four dams (aged 3-4 years) which were healthy and had a normal libido to be used as breeders for natural breeding. The animals were confined in individual cages, and they were acclimatized for one month to adapt to the environment and treatment feeds. Feeds offered were maize forage (*ad libitum*) and a concentrate (1% BW). Drinking water was freely available to the animals. The maize forage included stalks, leaves, and fruit of young corn harvested at the age of 50 - 65 d. Its nutritional contents were: 23.35% dry matter; 7.23% crude protein, 37.18% crude fiber, 1.78% crude fat, and 49.26% total digestible nutrients. The concentrated feed consisted of rice bran (60%), milled corn (30%), and fish meal (10%), with a dry matter content of 90.16%, crude protein of 14.28%, crude fiber of 19.71%, crude fat of 4.99%, and total digestible nutrients of 68.72%.

Treatments

This research was designed with a 2×4 factorial completely randomized design. The first factor was parity (P2 and P3) and the second factor was hormonal treatments (H0: CIDR implants for 14 days (Gunawan *et al.*, 2012) as control; H1: 14 days CIDR implants

and PMSG (Intervet Ltd, Cambridge, UK) at 15 IU/kg body weight intramuscularly shortly after CIDR release (Medigović *et al.*, 2012); H2: 14 days CIDR implant and GEN (CAS 446-72-0, LC Labs, Woburn, MA, USA) at 0.1 mg/kg body weight administered intravenously (Hughes *et al.*, 1991); and H3: 14 days CIDR implant, PMSG 15 IU/kg body weight, and GEN 0.1 mg/kg BW administered intravenously.

Estrous synchronization, estrous and pregnancy detections

Estrous synchronization was done using CIDR-S (EAZI-BREEDTM CIDR®, Pharmacia & Upjohn, New Zealand) containing 0.3 g of progesterone equipped with an applicator, which was implanted into the vagina for 14 days. The administration of PMSG and GEN according to the treatment was carried out immediately after the removal of the CIDR implant, then the estrous was observed three times a day every 8 hours until day 5. Mating was done naturally, then pregnancy detection was carried out with the non-return rate or NRR method, in which the ewes who did not indicate estrous signs in the next estrous cycle period were declared pregnant. The examination was started from day 14 after CIDR release until day 19, then it was repeated for the next estrous cycle (days 28 to 33). The research variables observed were (1) the percentage of estrous, i.e., the ratio between the number of estrous animals and the total number of animals in the treatment group, (2) the onset of estrous (hours), i.e., the time from the release of CIDR until the first signs of estrous were seen (when the ewes were silent and showed willingness to accept the dams); (3) The duration of estrous (hours), i.e., the time from the ewe accepted the dam until the ewe no longer accepted the dam, (4) gestation rate (%), (5) birth rate (%), (6) litter size (head), and (7) birth weight (kg).

Statistical Analysis

Data on estrous percentage and litter size were analyzed descriptively while those on estrous onset, duration of estrous, and birth weight were analyzed with an analysis of variance. Any significant differences between treatments were identified with the Honestly Significant Difference test. Data on gestation and birth rates were analyzed using the Chi-squared test (Steel & Torrie, 1997).

Results and Discussion

Estrous synchronization, onset, and duration

Results indicated that all the 32 Palu fat-tailed sheep ewes used in this study exhibited estrous at almost the same time, which may indicate that parity and hormonal treatment did have a significant effect on the percentage of estrous. This result is in agreement with that obtained by Rizal (2005), who found that intravaginal CIDR implantation in sheep for 13 days resulted in a 100% estrous rate in sheep after the release of CIDR, while under smallholder farm conditions the estrous rate was found to be 92.87% (Adiati *et al.*, 2007). The working principle of CIDR is to extend the functional life of corpus luteum due to the high progesterone concentration during implantation. Progesterone suppresses follicular growth and triggers the start of the next wave of follicles. The lifespan of exogenous progesterone is only about 8-10 minutes, so upon discontinuation of progesterone administration, GnRH is immediately secreted from the hypothalamus. The GnRH stimulates the anterior pituitary to immediately secrete FSH and LH, resulting in growth and maturation of follicles, an increase in the hormone estrogen accompanied by estrous and ovulation (Wurlina, 2005).

It was found that the onset of estrous in Palu fat-tailed sheep occurred at 22.40 to 48.20 hours (mean 33.90 ± 3.25 hours) after the release of CIDR (Table 1). The difference in parity did not affect the onset time of estrous, while the H1 treatment provided the fastest onset of estrous, and it was significantly different ($p < 0.01$) from H2 and H0 (control). Likewise, H3 treatment showed a very significant difference ($p < 0.01$) from H0. These results are similar to those obtained by Rizal (2005). CIDR-G implants in sheep caused estrous onset to occur at 28-37 hours (mean 33.47 hours) after CIDR-G removal. Suharto *et al.* (2008) found that the onset of estrous was 26.59 ± 0.98 hours after the release of CIDR in Ettawah cross goat which was faster than that found in this study. In contrast, Adiati *et al.* (2007) found a much longer onset of estrous in Garut sheep following fluorogeston acetate (progesterone) sponge implant (61.25-85.25 hours).

The onset of estrous is influenced by the presence of estrogen in the blood circulation, which then lead to estrous (Herdis, 2005).

PMSG is a hormone that has biological activities similar to FSH and LH functions in the process of folliculogenesis, estrogen synthesis and secretion, and ovulatory stimulation (Satiti *et al.*, 2014). The onset of estrous that was achieved at earlier time for the H1 and H3 treatments was thought to be due to the growth of follicles and more of them that reached the De Graaf follicle, causing an increase in the concentration of estrogen in the blood. According to Hastono *et al.* (2000), administering gonadotropin hormones (FSH and PMSG) can accelerate the onset of estrous. The use of PMSG (10 IU/kg BW) caused the onset of estrous more quickly (37.07 ± 11.21 h) than the control (40.37 ± 6.66 h), or 3 hours earlier than the control. The onset of estrous in goats treated with progesterone and PMSG occurred 14-43 hours after sponge extraction and those receiving PMSG treatment occurred 16-21 hours, faster than controls (Artiningsih *et al.*, 1996), while (Adiati *et al.*, 1998) reported that PMSG injection at 15 IU/kg BW caused the onset of estrous 10 hours earlier than control. Duration of estrous in Palu fat-tailed sheep which lasted for 24.00-46.10 h (mean 33.16 ± 2.98 h). This duration was longer than that obtained by Herdis (2005) and (Adiati *et al.*, 1998) which were 24-31 h and 18-36 h, respectively. The duration of estrous for P3 (35.38 ± 2.56 h) was significantly ($P < 0.01$) longer than for P2 (30.93 ± 0.96 h). The duration of estrous for H1 (38.35 ± 4.79 h) and H3 (35.92 ± 4.11 h) was longer and significantly different ($P < 0.05$) from that for H0 (28.81 ± 3.75 h) and H2 (29.56 ± 4.74 h).

Animal age and body weight have a significant effect on estrous duration (Hastono & Bintang, 2008). The animals with parity 3 (P3) are ewes that have given birth 3 times and were about 3-4 years old, while P2 were those that have given birth twice and were around 2 - 3 years old. Animals in P3 had a higher body weight than P2, and this might have been the cause for differences in estrous duration between the two parities. Livestock treated with PMSG experienced estrous 1.6-4.8 hours longer than the control (Artiningsih *et al.*, 1996). The duration of estrous in sheep increased by 6 hours due to PMSG administration, associated with increased number of follicles developing and reaching ovulation, and thus high estrogen in the blood circulation (Sugiyatno *et al.*, 2001).

Table 1. Estrous characteristics in Palu fat-tailed ewes at different parity given PMSG and genistein

| Traits | Parity group | Hormone treatment | | | | Mean |
|-------------------------|--------------|-------------------------|-------------------------|--------------------------|--------------------------|-------------------------|
| | | H0 | H1 | H2 | H3 | |
| n=32 | | | | | | |
| Estrous percentage | P2 | 8(100%) | 8(100%) | 8(100%) | 8(100%) | 8(100%) |
| | P3 | 8(100%) | 8(100%) | 8(100%) | 8(100%) | 8(100%) |
| Onset of estrous (h) | P2 | 41.90±7.13 | 26.08±4.27 | 37.71±7.10 | 31.74±1.14 | 34.36±3.38 |
| | P3 | 39.29±5.53 | 25.73±4.24 | 37.55±7.73 | 31.70±0.48 | 33.57±3.61 |
| | Mean | 40.59±6.07 ^a | 25.68±4.10 ^c | 37.63±6.87 ^{ab} | 31.72±0.81 ^{bc} | 33.90±3.25 |
| Duration of estrous (h) | P2 | 27.96±4.09 | 35.69±4.77 | 27.91±4.02 | 32.18±0.78 | 30.93±0.96 ^y |
| | P3 | 29.65±3.76 | 41.01±3.47 | 31.20±5.39 | 39.66±1.20 | 35.38±2.56 ^x |
| | Mean | 28.81±3.75 ^b | 38.35±4.79 ^a | 29.56±4.74 ^b | 35.92±4.11 ^a | 33.16±2.98 |

Notes: ^{abc}The mean followed by a superscript with different letters on the same line indicates very significant differences ($p<0.01$); ^{xy} The mean followed by a superscript with different letters in the same column shows a very significant difference ($p<0.01$)

PMSG is very effective as a superovulatory hormone because it has a long half-life of 123 hours (Sumaryadi & Manalu, 1995). However, the opposite was found by (Bradford *et al.*, 1986) who found stated that there had no effect on the number of ovum ovulated due to increased duration of estrous in sheep.

The slower onset and short duration of estrous for H2 were thought to be due to that genistein as a source of phytoestrogens w only functioned as a luteolytic agent rather than increasing the concentration of endogenous estrogens. In sheep and cattle, estrogen also has luteolytic action, and this may be due to the subsequent action induced by PGF2 α (Reeves, 1987). This mechanism is thought to be due to increased secretion of oxytocin by neurohypophysis in the endometrium that has previously received progesterone enabling it to increase the synthesis of PGF2 α (Hadley, 1992). According to Hafez (1987), studies with increasing estradiol during the luteal phase led to a PGF2 α surge when the endometrium was primed with progesterone.

Gestation and birth rates

The gestation rate obtained in this study was 93.75% (30 ewes did not return to estrous (NRR) in the two periods of estrous cycle after mating, while the other 2 (6.25%) animals were not pregnant as they exhibited estrous in the second estrous cycle. There were no differences in gestation rates due to parity and hormonal treatments. The gestation rate for P3 and H3 was 100%, while for P2, H0, and H3 was

87.50%. These lower gestation rates were probably due to the shorter duration of estrous in animals receiving P2, H0, and H3, leading to inappropriate mating time. It might have also be due to non-ovulation or incomplete follicular development. Artiningsih *et al.* (1996) found that 10% of younger experimental goats did not ovulate. The development of follicles that are not perfect can also be a factor in the failure of ovulation, even though animals may show signs of normal estrous.

The average birth rate was found to be 83.33% (Table 2). Parity and hormone treatments did affect the birth rates. The 10.42% decrease in the birth rate compared to the gestation rates might have been due to the occurrence of death in the ovum and embryonic stages. The incidence of death generally occurs in the first 3-4 weeks of gestation, with a range of 10-40% (Jainudeen & Hafez, 1987a). Artiningsih *et al.* (1996) stated that PMSG can induce a lot of ovulations but there was a tendency for failure of fertilization and an increase in embryo mortality. Provision of PMSG to sheep can increase the number of fetuses twice compared to control, but the incidence of prenatal mortality was 33.33% (Andriyanto & Manalu, 2012). The finding of a low percentage of births for P2 and H3 (50%) and H1 (75%) in this study were thought to be due to the condition of the uterine environment of the young ewes that was not supportive enough for the growth and development process during gestation, which causes implantation failure or imperfect placentation.

Table 2. Gestation and birth traits in Palu fat-tailed ewes at different parity given PMSG and genistein

| Traits | Parity group | Hormone treatment | | | | Mean |
|--------------------------|--------------|-------------------|---------------|---------------|-------------|------------------------|
| | | H0 | H1 | H2 | H3 | |
| Conception rate (head/%) | P2 | n=4(3/75) | n=4(4/100) | n=4(3/75) | n=4(4/100) | n=16(14/87.50) |
| | P3 | n=4(4/100) | n=4(4/100) | n=4(4/100) | n=4(4/100) | n=16(16/100) |
| | Mean | n=8(7/87.50) | n=8(8/100) | n=8(7/87.50) | n=8(8/100) | n=32(30/93.75) |
| Litter size (head/%) | P2 | n=3 (3/100) | n=4 (3/75) | n=3 (3/100) | n=4 (2/50) | n=14 (11/78.57) |
| | P3 | n=4 (3/75) | n=4 (4/100) | n=4 (3/75) | n=4 (4/100) | n=16 (14/87.50) |
| | Mean | n=7 (6/85.71) | n=8 (7/87.50) | n=7 (6/85.71) | n=8 (6/75) | n=30 (25/83.33) |
| Gestation Length (d) | P2 | 146.67±2.08 | 147.67±1.73 | 148.00±1.73 | 147.00±1.41 | 147.18±1.60 |
| | P3 | 148.33±2.08 | 149.00±1.15 | 148.00±1.73 | 147.50±0.58 | 148.21±1.37 |
| | Mean | 147.50±2.07 | 148.00±1.68 | 148.00±1.55 | 147.25±0.82 | 147.76±1.54 |
| Birth weight (kg) | P2 | 2.28±0.19 | 2.35±0.15 | 2.33±0.12 | 2.33±0.18 | 2.33±0.13 ^y |
| | P3 | 2.45±0.18 | 2.59±0.17 | 2.48±0.18 | 2.43±0.10 | 2.49±0.15 ^x |
| | Mean | 2.37±0.19 | 2.47±0.19 | 2.41±0.16 | 2.38±0.12 | 2.42±0.17 |

Notes: ^{xy} The mean followed by a superscript with different letters in the same column shows a very significant difference ($p < 0.01$)

The level of litter size is determined by the number of ovum being ovulated, the number of fertilized ova, and the ability to implant. According to Geisert & Schmitt (2002), the most critical time in the livestock reproductive cycle is the maternal recognition of gestation phase, which is the ability of the mother to receive signals sent by the conceptus to prevent luteolysis and to maintain gestation. These are conditions that affect the percentage of births and litter size.

The litter size obtained in this study was entirely 1.0, which is in contrast to previous researchers who found litter size of 1.77 in sheep (Inounu *et al.*, 1999) 1.98 (Tiesnamurti, 2002); and 1.36 in tropical sheep (Gatenby, 1986). (Inounu *et al.*, 1999) stated that a sheep population can be classified as prolific sheep if it has an average litter size of ≥ 1.75 . There were no multiple births found and this was thought to be due to genetic factors. Owens *et al.* (1985) stated that the rate ovulation of Booroola Merino sheep is influenced by a single FecB gene. According to (Jainudeen & Hafez, 1987b), the average ovulation rate in Merino Sheep is around 1.2. It was probable that the single gene carrier genotype caused low prolific traits in Palu fat-tailed sheep resulting in the absence of twin births. A previous study on Palu fat-tailed sheep found that the animals have impure genotypes due to cross mating with Merbas, so that Palu fat-tailed sheep and Palu fat-tailed sheep x Merbas cross have 99% genetic similarities (Duma *et al.*, 2000).

The length of gestation in Palu fat-tailed sheep varied up to 5 days, with a range of between 145-150 days (mean 147.76 ± 1.54 days). There was no difference in the length of gestation between parital and hormonal treatments. The length of gestation in sheep ranges from 140 to 159 days (mean 149 days) (Jainudeen & Hafez, 1987a); 148.00 ± 3.22 days (Handarini *et al.*, 2016); and 147 ± 4.45 days (Mathius *et al.*, 2002). The length of gestation differs between breeds and individuals within breeds can also vary up to 13 days (Mathius *et al.*, 2002). The shorter length of gestation in this study was thought to be due to differences in breeds, which affect both body weight and birth weight. Ewes having higher body weight have a longer gestation period (Handarini *et al.*, 2016), while younger animals have a shorter gestation period (Jainudeen & Hafez, 1987a). The length of gestation is also influenced by the age of the ewes, birth weight and sex of the offsprings.

Birth weight of Palu fat-tailed sheep obtained in this study ranged from 2.15 kg to 2.80 kg (mean 2.42 ± 0.17 kg), which is similar to that reported for for local sheep of 2430 g (Mathius *et al.*, 2002) and Jember fat-tailed sheep of 2.45 ± 0.58 kg (Sumadi *et al.*, 2014), but slightly higher than the Sumatran composite sheep with birth weight of 2.19 ± 0.41 kg (Adiati & Subandriyo, 2014) and Lombok fat-tailed sheep an average birth weight of 2.23 ± 0.37 kg (Ashari *et al.*, 2015). The mean birth weight for P3 (2.49 ± 0.15 kg) was significantly higher ($p < 0.05$) than for P2 (2.32 ± 0.14 kg), while

hormonal treatment showed no difference. The results indicate that the lambs' birth weight was strongly influenced by the age and body weight of the ewes. The P3 sheep were about 3-4 years old in age and with body weight higher than P2 animals. Ashari *et al.* (2015) found that the birth weight of old ewes (2.44 kg) was significantly higher than that of young ones (2.19 kg). Differences in birth weight are due to differences in breed, age/parity of ewes, sex of offspring, endometrial development before placentation, and placental size (Iniguez *et al.*, 1991).

Body weight and age of the ewes were positively correlated with prenatal growth. Young animals were still experiencing growth, and the food they consume are still needed for the growth of the ewes and fetus, while the older ewes have a well-developed uterus and placenta, so they might have sufficient the fetus' capacity and nutrient supply. According to Hafez (1969), an animal with a small body size limits the size of the fetus so that the birth process becomes easy, while on the other hand, a larger animal can increase birth weight. This indicates that the size of the ewe's body is a limiting factor for birth weight. Ewes parity can also affect parental maturity in raising offsprings. The reproductive appearance of the animals increases from parity 1 to 4 and then decreases, primiparous animals produce lower birth weight than pluriparous (Inounu *et al.*, 1999).

Provision of PMSG resulted in higher birth weight (2.47 ± 0.19 kg) compared to other treatments, although it did not differ statistically. The PMSG can increase endogenous secretion of gestation hormones, embryo, and fetal growth, increase birth and weaning weights, growth, and development of mammary glands (Manalu *et al.*, 1999). According to Lopian (2014), the PMSG significantly increases birth weight due to high differentiation and fetal development during gestation. PMSG also increases the concentration of estrogen and progesterone and stimulates the growth of uterine tissue in preparing preparation for implantation and placentation (McDonald, 1980), so as to ensure the availability of nutrients for the fetus. (Adriani *et al.*, 2007) found that improved birth weight is a consequence of increased endogenous secretion of gestation hormones (estrogen and progesterone). In addition, superovulation causes a better microuterine

environment so that fetal growth and development in the womb is optimal (Mege *et al.*, 2007). This increased endogenous secretion of gestation hormones and microuterine that will improve the quality of the resulting offsprings (Adriani *et al.*, 2007; Mege *et al.*, 2007).

Conclusion

Provision of CIDR, PMSG, genistein, and their combination can increase estrous traits in Palu fat-tailed ewes but not their litter size and birth weight.

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