PMSG in ewes: A Practical and Efficient Step for Superovulation

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Abstract. Ewes have a prolific nature, but in reality, the average number of lambs (litter size) is still around 120%. The application of the PMSG (Pregnant Mare Serum Gonadotrophin) hormone aims as a superovulation agent for obtaining twins in one birth. PMSG is a non-pituitary gonadotrophin hormone that has the same function as FSH (Follicle Stimulating Hormone) and LH (Luteinizing Hormone) which stimulates follicular and ovulation growth. Various methods of applying the PMSG hormone to the ewes were quite diverse and produced different responses. Which method is considered the most practical and efficient is the main topic in this paper. Details of the discussion in this paper include the nature and biochemical characteristics of PMSG, the performance of PMSG as a superovulation agent, the comparison of PMSG with other reproductive hormones to the superovulation response in ruminants, and the impact of PMSG on superovulation in ewes.

1. Introduction

Ewes have a prolific nature or have the potential to produce more than one lamb, but the results of the study show that the average number of lambs born (litter size) is less than 200% [1]–[5]. One of the causes of the low litter size of sheep was the high number of embryo or fetal deaths during pregnancy which could reach 19.9% [6]. The number of embryos formed greatly influenced their life endurance. A single embryo has a survival rate of 95%, while the number of embryos formed by two and three each has the ability to survive as much as 85 and 70% [7]. The graph decreases according to the number of embryos produced.

The main causes of loss of embryo or fetus during pregnancy are the type of ewes and the amount of the progesterone hormone concentration in the blood of the ewes [6]. The type of ewes referred to is the type of black-faced sheep and white-faced sheep sheeps in the world are grouped based on the color of the fur on the head. Black-faced sheep have a tendency of low progesterone concentration thus the percentage of embryo loss is quite high. This condition caused black-faced sheep to have a short mating period and the anestrus phase is longer than white-faced or mottled sheep[8].

Various efforts to increase the number of offspring after birth had been pursued, one of which was through the application of reproductive hormones. PMSG (Pregnant Mare Serum Gonadotrophin)
hormone induction is an effort for superovulation. There have been enough studies that use the PMSG hormone as a superovulation agent in various ruminants. The method of application was quite diverse and produced different responses. Which method is the most practical and effective to do with ewes that are being taken care of in Indonesia is the main study in this paper. This paper also discusses the origins of PMSG hormones, the nature and biochemical characteristics of PMSG, PMSG performance as a superovulation agent, comparison of PMSG with other reproductive hormones to the superovulation response in ruminants, and the impact of PMSG on superovulation in ewes.

2. Method

2.1. Biopotency

PMSG is a glycoprotein hormone that is secreted in high concentrations in the blood of young pregnant mares (40-130 days) [9]. Interestingly, in one gonadotrophin hormone molecule, there are biological activities of the FSH (Follicle Stimulating Hormone) hormone and LH (Luteinizing Hormone) [10]. FSH functions to stimulate follicular maturation, produce estrogen hormone, and control the sexual characteristics of males or females. The impact of the FSH hormone’s performance is the onset of estrus symptoms. While the LH plays a role in triggering the development of follicles with FSH and triggers ovulation. As a result of LH's performance was the secretion of the hormone progesterone and egg maturation [11]. The following is an Estrous ration of PMSG hormone concentrations taken from pregnant pony mares.

![Figure 1. PMSG Concentration in the Serum of Pony Mares](image)

Figure 1 shows that PMSG was only found in gestation mares species and secreted by endometrial cups. PMSG became active between 38-40 days of gestation. The endometrial cup grew rapidly reaching a maximum dimension of 2-10 cm until the age of 70 days of gestation. Furthermore, it will decrease slowly until the fetus attaches [12]. Endometrial cup tissue produced PMSG in very large numbers, could reach 1,000,000 IU/g of new tissue. The tissue will be released at gestation age 130-180 days (Clegg et al. 1954). PMSG hormone content increased rapidly at 37 and 40 days of gestation and reached a peak at 55-75 days of gestation. PMSG content decreased slowly until it was no longer detected between 120-150 days of gestational age [12]. Based on the results of a randomized study of the pregnant mare, obtained the diversity of PMSG content, the lowest was 10 IU / ml blood serum and the highest was 250 IU / ml blood serum [12]. The amount of PMSG content was also affected by the type of horse breed and the number of endometrial cups in the uterus. Pregnant mare with twins have two sets of endometrial cups, thus the PMSG content in their blood was doubled [14]. Increasing age and parity also influenced the production of
PMSG hormone, the older and the more often the mare giving birth, the more the production of the PMSG hormone decreased [15].

Papkoff [16] stated that PMSG is a molecule whose biological activities such as FSH and LH. In addition, PMSG is also a single molecule that is similar to the pituitary gonadotropin hormone and other placental gonadotropin hormones and human chorionic gonadotrophin (HCG) which have $\alpha$ and $\beta$ subunits. Cole et al [17] stated that PMSG has a longer half-life than pituitary hormones and gonadotropin placental hormones. PMSG which contains FSH and LH, is ready to be used for the superovulation induction in various types of livestock.

Stewart et al [18] reported the results of their study of six pregnant ponies that the hormone ratio of FSH: LH (PMSG) in their blood serum was an average of $1.45 \pm 0.03$. More details of FSH: LH ratio in the Pony’s PMSG hormone is presented in Table 1 below.

**Table 1. FSH: LH Ratio in the Six Pregnant Ponies’ Serum**

| Mare | Gestational Age (Weeks) | Average |
|------|-------------------------|---------|
|     | 6          | 7       | 8       | 9       | 10      | 11      | 12      | 13      |
| A   | 1.37       | 1.34    | 1.36    | 1.57    | 1.34    | 1.50    | 1.27    | 1.39    |
| B   | 1.45       | 1.25    | 1.53    | -       | -       | -       | -       | 1.41    |
| C   | 1.30       | 1.16    | 1.57    | 1.62    | 1.42    | -       | -       | 1.41    |
| D   | 1.69       | 1.29    | 1.50    | 1.66    | 1.46    | 1.82    | -       | 1.57    |
| E   | 1.75       | 1.60    | 1.16    | 1.71    | -       | -       | -       | 1.56    |
| F   | -          | 1.92    | 1.73    | 1.31    | 1.24    | 1.47    | 1.13    | 1.11    | 1.42    |
|     | 1.69       | 1.51    | 1.43    | 1.43    | 1.52    | 1.51    | 1.32    | 1.19    | 1.45    |

Note: The analysis of variance shows that there is no significant difference ($P>0.05$) of the content of FSH: LH in the pregnant ponies’ blood serum every week for the first 13 weeks of pregnancy.

Schams et al [19] tested the FSH: LH content in PMSG commercial products (Folligon, Intervet Laboratories, Bar Hils, Cambridge), the results show that there was no significant difference ($P>0.05$) of biological activity from each package of the PMSG hormone products. In another study, Allen [19] reported that the PMSG with Folligon brand which was injected with two groups of ewes at a dose of 1,000 IU each did not show any significant effect on the average ovulation of each ewe.

The gonadotrophin hormone content in the blood of pregnant horses was first discovered by Cole and Hart [20]. A few years later, this hormone was known to have a fairly long half-life (6 days) in the body of a horse or any other animal given the experiment, and the PMSG hormone is known based on various experiments that have a biological activity such as FSH and LH from the pituitary gland [21]. PMSG can be evaluated biologically by measuring total gonadotropin levels (FSH and LH activity) or through testing specifically for FSH and LH content. Pure PMSG preparations have specific activities with 10,500-18,000 tu/mg serum contents [10], [19].

### 2.2. Biochemical Characteristics

The biochemical characteristics of all known pituitary gonadotrops, as well as HCG, are glycoproteins. PMSG hormone is no exception, although this hormone stands alone with its high carbohydrate content (41-45g /100g glycoprotein), compared to FSH and LH the total carbohydrate content is only 24.2 and 25.4 g /100g glycoprotein, respectively. The highest amino acid composition of PMSG is threonine and proline, which is as much as 8.6% and 12.5%, while histidine, methionine, and tyrosine are relatively lower massing by 2.5%, 1.7%, and 2.4 % [21] (Table 3).

Details of carbohydrate and amino acid content of PMSG, LH, FSH and their subunits are presented in Table 2, Table 3, and Table 4:
Table 2. Carbohydrate content * PMSG, LH, and FSH and its subunits **

| Hormones and Sub-units | Neutral Sugar | Hexosamine | Sialic Acid | Total  |
|------------------------|---------------|------------|-------------|--------|
| PMSG                   | 13.0          | 17.6       | 10.8        | 41.4   |
| LH                     | 8.9           | 8.6        | 7.9         | 25.4   |
| FSH                    | 9.7           | 7.7        | 6.8         | 24.2   |
| PMSG-α                 | 8.4           | 5.7        | 4.5         | 18.6   |
| LH-α                   | 7.5           | 7.3        | 5.0         | 19.8   |
| FSH-α                  | 7.4           | 6.7        | 5.6         | 19.7   |
| PMSG-β                 | 15.8          | 18.2       | 21.3        | 55.3   |
| LH-β                   | 6.9           | 6.5        | 9.5         | 22.9   |
| FSH-β                  | 9.8           | 7.8        | 7.4         | 25.0   |

Note: *g/100 g glycoprotein; ** [21]

LH and FSH hormones from mares (Table 2) contain about 25% carbohydrates, and the amount of sugar is lower compared to sugar or PMSG carbohydrates. The sugar level of sialic acid in LH is relatively high (7.9%), and so far in other species the levels are minimal or nonexistent. Sialic acid referred to here is possible as a determinant of the half-life of glycoprotein hormones [16]. The highest sialic acid content was found in PMSG, which caused PMSG to have a longer half-life compared to LH and FSH.

Table 3. Composition of Amino Acids * PMSG Compared to Mares’ LH and FSH

| Amino Acids | PMSG | LH** | FSH** |
|-------------|------|------|-------|
| Lysine      | 5.4  | 5.2  | 7.0   |
| Histidine   | 2.5  | 1.9  | 2.5   |
| Arginine    | 6.1  | 4.5  | 3.8   |
| Aspartic    | 5.5  | 5.3  | 7.3   |
| Threonine   | 8.6  | 8.4  | 9.7   |
| Serine      | 8.0  | 9.6  | 6.8   |
| Glutamic    | 8.4  | 7.8  | 7.9   |
| Proline     | 12.5 | 10.4 | 7.8   |
| Glycine     | 5.1  | 6.7  | 6.7   |
| Alanine     | 7.5  | 8.4  | 6.7   |
| Half-Cystine| 7.5  | 7.9  | 6.7   |
| Valine      | 4.6  | 5.6  | 5.8   |
| Methionine  | 1.7  | 1.6  | 1.1   |
| Isoleucine  | 4.8  | 4.8  | 4.5   |
| Leucine     | 6.3  | 6.3  | 5.3   |
| Tyrosine    | 2.4  | 1.8  | 4.2   |
| Phenylalanine| 4.0 | 4.2  | 4.1   |

Note: *calculated based on residues / 100 analyzed residues
**Data from research [21] (data processed)

Table 4. Composition of Amino Acids * of α and β PMSG, LH, and FSH

| Amino Acids | PMSG-α | LH**-α | FSH**-α | PMSG-β | LH**-β | FSH**-β |
|-------------|--------|--------|---------|--------|--------|---------|
| Lysine      | 9.4    | 6.3    | 8.4     | 3.1    | 2.7    | 5.9     |
| Histidine   | 3.0    | 2.7    | 3.2     | 1.6    | 1.3    | 2.7     |
| Arginine    | 4.9    | 4.0    | 4.2     | 6.4    | 4.8    | 2.5     |
| Aspartic    | 6.5    | 6.2    | 6.8     | 4.8    | 4.2    | 9.6     |
| Threonine   | 9.9    | 9.4    | 9.3     | 7.7    | 8.1    | 11.1    |
| Serine      | 5.0    | 6.9    | 4.7     | 10.0   | 9.7    | 7.2     |
| Glutamic    | 8.7    | 9.1    | 9.2     | 7.2    | 6.6    | 7.2     |
It is known that the PMSG hormone has α and β subunits, although the detection and separation of the two subunits were not easy, which was different from the LH and FSH hormones. When compared between carbohydrate content (Table 1) PMSG subunits with LH subunits and FSH subunits, there were two interesting things: 1) there was a significant difference between carbohydrate content of PMSG-α subunits and PMSG-β (18.6% vs 55.3%); and 2) it is clear that PMSG-α has a sugar content that is very similar to LH-α and FSH-α. However, PMSG-β has 2.2-3.0 times more sugar than alpha or beta subunits, total carbohydrate PMSG-β (55.3%), sialic acid is present in the largest amount (21.3%)[22].

Table 4 shows the number and amino acid comparison of PMSG alpha and beta with LH and FSH amino acid subunits. The three alpha subunits on PMSG, LH, and FSH have very similar compositions. The observation results of the subunit beta composition show very clearly that PMSG-β is similar to LH-β rather than FSH-β, although the absolute identity does not appear to be possible[22].

3. Result and Discussion

3.1. PMSG’s Performance as a Superovulation Agent

As explained in the previous sub-chapter, the biological activity of PMSG hormones such as FSH and LH. FSH and LH are hormones that regulate reproduction[23]. The function of FSH and LH is to stimulate follicle and ovulation growth[11] and corpus luteum formation[24]. PMSG is a serum of pregnant mare containing gonadotropin which if given as an exogenous hormone will strengthen the process of folliculogenesis and ovulation thus that superovulation will occur[25].

The difference between the performance of the PMSG hormone with FSH and LH is the long half-life of the hormone. The half-life of PMSG is longer than that of FSH and LH, which is 118-123 hours[26], whereas FSH and LH has half-life of 3-5 hours and even 30 minutes[27], [28]. The advantage of PMSG as a superovulation agent is that it can be used in a single dose[11], while FSH must be given twice a day to get good results[29], [30].

Several ways of PMSG performance as a superovulation agent include 1) accelerating the growth of small follicles into growing follicles; 2) increase the number of preantral follicles; 3) the decrease of antral follicles proportion with atresia as a result of increased growth of large or small antral follicles; and 4) rescuing atresia follicles (mild stage) to be able to grow and ovulate[26]. Exogenous use of PMSG can increase ovulation in cows, ewes, and sows intended to be taken and transferred the embryos[12].

3.2. PMSG and Other Gonadotrophin Hormones to Superovulation Response in Ruminants

There have been many reports of superovulation trials using various types of gonadotrophin hormones on large ruminants and showing good results. The superovulation process in large ruminants aims to carry out embryo transfer. While the treatment of small ruminants aims to increase the number of births and the quality of the newborn, as small ruminants already had a prolific nature.

Reproductive hormones commonly used in the superovulation process include FSH (Folikel Stimulating Hormone), eCG (equin Corionic Gonadotrophin), hCG (human Corionic Gonadotrophin),
hMG (human Menopausal Gonadotrophin) and PMSG (Pregnant Mare Serum Gonadotrophin). The hormones that were often used in the superovulation process are FSH and PMSG. FSH has a higher success rate, while PMSG has a better economic value because it is sufficient to be used in a single dose [31].

[32] have compared the success rate of superovulation by using three hormone treatments, namely FSH, eCG + FSH, and hMG to Dromedary Camel. The results of these experiments showed that there were no significant differences (P> 0.05) on the number of dominant follicles, number of corpus luteum, number and size of embryos. The results of these experiments are presented in Table 5 below.

Table 5. Superovulation Responses to Dromedary Camels Through the Hormones Treatment FSH, eCG + FSH, and hMG

| Treatment    | Qty. Dominant Follicles (>6mm) | Qty. CL on the 7th day after mating | Total | Embryo Diameter (µm) |
|--------------|---------------------------------|-------------------------------------|-------|----------------------|
| FSH          | 15.2 ± 2.20\(^a\)              | 13.8 ± 2.65\(^a\)                  | 5.7 ± 2.32\(^a\) | 738.1 ± 37.02\(^a\) |
| eCG-FSH      | 19.8 ± 2.81\(^a\)              | 15 ± 2.60\(^a\)                    | 8.8 ± 2.10\(^a\) | 793.9 ± 23.65\(^a\) |
| hMG          | 19.8 ± 2.81\(^a\)              | 10.8 ± 2.30\(^a\)                  | 5.8 ± 2.40\(^a\) | 886.2 ± 57.97\(^a\) |

Note: Superscript in the same column shows no significant difference (P> 0.05)

The data in Table 5 shows that the eCG-FSH treatment obtained a higher value than the other treatments although the difference in value was not significantly different (P> 0.05). This was probably due to the longer half-life of the eCG hormone compared to the FSH and hMG hormones, so that the resulting follicles become more numerous [33]. However, superovulation using a combination of eCG-FSH results is lower than a single eCG (1500-6000 IU) treatment which produced up to 19 embryos [34]. The combination of eCG-FSH treatment with a longer half-life resulted in the process of forming follicles continuously without ovulation, thus the embryo formation was not more than a single eCG treatment [33].

The use of FSH and FSH-hCG combination to the superovulation of beef cattle has been carried out by Kaiin and Tappa [35]. The results showed that the combination treatment of FSH-hCG obtained 91.7% of beef cattle that responded to superovulation. The results were significantly different (P<0.05) with a single FSH treatment that received a 68% superovulation response. The number of embryos produced by a single FSH treatment was 3.17 ± 4.02, while the combination treatment for FSH-hCG produced an embryo of 5.44 ± 4.49. Better results in the combination treatment of FSH-hCG were suspected because the biological activity of hCG was the same as LH (Luteinizing Hormone) which was used to ovulate the egg. The number of ovulated eggs allowed more embryo formation [36].

Another reproductive hormone that was often used as a superovulation agent in large ruminants is PMSG. Gonzales et al. [39] conducted a study of beef cattle from crossing result by injecting various doses of PMSG intra-muscular (IM) and followed by prostaglandins injection (PGF2α) 48 hours later. PMSG doses given were 1,200; 2,400; and 3,600 IU. The results show that the higher the PMSG dose given, the more corpus luteum (CL) produced. However, the number of embryos that could be transferred was significantly more (P<0.05) in the administration of PMSG at a dose of 2,400 IU compared to other treatments, which was 3.4 (62%) of the total average number of embryos of 5.4.

Another use of PMSG was carried out by Fu et al. [40] to Chinese Holstein dairy cows with a dose variance of 2; 2.5; 3; 3.5; and 4 IU / kg body weight and Estrous synchronized with PGF2α 48 hours after IMSC injection in IM. The results showed that there was no significant difference (P> 0.05) on
estrous response, estrus duration, and broodstock number. However, for the average number of pregnant cows, the high dose treatment (4 IU / kg body weight) was significantly lower than the low dose (2 and 2.5 IU / kg body weight), namely 66.7% compared to 71.4%. The number of twins was more common and significantly different (P <0.05) in high dose treatment (4 IU / kg body weight), which was 50%. Based on the results of this study it can be stated that the Chinese Holstein dairy cows induction by using PMSG can improve the quality of estrus and ovum fertility.

3.3. PMSG and Superovulation in Ewes

Livestock reproduction performance can be assessed as good if all reproduction aspects have a value according to the standard for each animal commodity that has been set. The average number of lambs born is still below 200%, with the potential is twinning. Economically, the ewes that give birth to twins is more profitable than giving birth to a single lamb. Environmental factors are the main cause of the difficulty of achieving the ewes to give birth to twins, such as the low quality of feed and livestock maintenance systems carried out. The feed quality was very influential on the performance of endogenous reproductive hormones. The efforts to improve the reproductive performance of ewes in the field which inclined to the quality of unstable feed, the help of exogenous reproductive hormone application is one solution.

[41] compared the two treatments between the use of fluorogestone acetate (FGA) sponges and prostaglandins (PGF2α) followed by injection of 500 IU PMSG in ewes. The note in the technical treatment is for FGA (FGA for 14 days followed by PMSG injection at FGA revocation) and PGF2α (FGA-PMSG followed by PGF2α-PMSG on the 12th day). The measured variables were reproductive performance (fertility, proliferation, and fecundity). The results obtained indicated that fertility in the FGA and PGF2α treatments was 66% and 60%, respectively. Next, for the proliferation of FGA and PGF2α treatment respectively 3.2 and 2.2, and for the value of fecundity are 170% and 107%. The reproductive performance value of the ewes given FGA treatment was significantly better than PGF2α.

Four years later, Ainsworth and Shrestha [42] conducted a similar follow-up study using different doses of PMSG administration. The treatment given was the use of FGA-PMSG 250 IU (FGA 1) and FGA-PMSG 500 IU (FGA 2). The technical administration of FGA-PMSG was the same as that done by [41]. The results obtained showed that the fertility, proliferation, and fecundity of the ewes in the FGA 1 and FGA 2 treatments were 88.6% and 90.9%, respectively; 2.6 and 2.8; and 236% and 258%. It appeared that the FGA 2 treatment displayed better reproductive performance.

The reproductive performance comparison of ewes using various doses of PMSG with no PMSG administration as a control had been carried out by [43]. In this study, the Estrous synchronizer used are still the same as previous studies, namely FGA sponges. FGA sponges were planted in the reproductive tract for 10 days and then followed by PMSG injection at the time of FGA sponge extraction. PMSG doses given were 100, 200, and 400 IU and without PMSG administration as a control. The results were reported that the fertility of the ewes increased according to the dose given. The fertility of ewes with doses of 0, 100, 200, and 400 IU PMSG as many as 41.7%, 45.4%, 61%, and 81.8%, respectively. However, the reproduction performance trend was not followed on the proliferation and fecundity value variables, each of which has a value of 2; 1.8; 1.9; 2.2 and 79%, 68%, 113%, 177%. Overall, the best reproductive performance data is shown at a dose of PMSG of 400 IU.

The application of Estrous synchronization which was considered quite simple was by PGF2α injection IM. The Estrous synchronizer was given twice, namely day 0 and day 11. Furthermore, the PMSG injections were carried out simultaneously at the second PGF2α injection. The doses of PMSG given were 0 (control) and 200 IU. The results obtained were the fertility value of the ewes in both treatments at 100%, and the litter size or the proliferation level of the ewes as much as 1.38 and 1.74. The results of the variance analysis showed no significant difference (P> 0.05) in the two treatments [31]. The result details of the above studies are presented in Table 6 below:
Table 6. Reproduction Performance of Ewes Based on Estrous Synchronizer and PMSG Doses.

| Source                  | Synchronizer | Long Synchronization (day) | Application Time PMSG (hour) | Dose PMSG (IU) | Observed Variables |
|-------------------------|--------------|----------------------------|-----------------------------|----------------|--------------------|
|                         |              |                            |                             |                | Fertility (%)       |
|                         |              |                            |                             |                | Proliferation (%)   |
|                         |              |                            |                             |                | Fecundity (%)       |
| Hackett et al., [41]    | FGA          | 14                         | 0                           | 500            | 66                 |
|                         | PMSG         | 12                         | 0                           | 500            | 60                 |
|                         |              |                            |                             |                | 2                  |
| Ainsworth & Shrestha,   | FGA          | 14                         | 0                           | 250            | 88.6               |
| [44]                    | FGA          | 14                         | 0                           | 500            | 90.9               |
|                         |              |                            |                             |                | 2.8                |
| Elisea et al., [45]     | FGA          | 10                         | 0                           | 0              | 41.7               |
|                         | FGA          | 10                         | 0                           | 100            | 45.4               |
|                         | FGA          | 10                         | 0                           | 200            | 61                 |
|                         | FGA          | 10                         | 0                           | 400            | 81.8               |
|                         |              |                            |                             |                | 2.2                |
| Andriyanto & Manalu,    | PFG2α        | 11                         | 0                           | 0              | 100                |
| [31]                    | PFG2α        | 11                         | 0                           | 200            | 100                |

Notes: FGA : Fluorogestone acetat; PGF2α : prostaglandin; PMSG : Pregnant Mare Serum Gonadotrophine; IU : International Unit.

The application of PMSG as a superovulation agent also influenced the quality of pre-reproduction such as the Estrous response and the ewes pregnancy percentage (Table 7). The Estrous Synchronization shown in Tables 6 and 7 shows that Fluorogestone acetate (FGA) is the most widely used, and gives Estrous response between 83-98.8% without the addition of PMSG applications. Whereas PFG2α had an impact on the Estrous response of 89%. The average number of gestation from FGA and PFG2α applications without the addition of PMSG applications were 68.6-90% and 89%, respectively. The impact on the proliferation level or the average number of lamb births produced by FGA was 2 lambs, while PFG2α was 1.38 lambs.

The application of FGA required special skills and tools, FGA was stored in the spongy reproductive tract (cervix) which aimed to inhibit ovulation. After 10-14 days of FGA planting, the livestock will experience Estrous simultaneously. Unlike the case with PFG2α, its application was more practical by IM injecting twice. The second injection was done on the 11th day after the first, a few days later the cattle will experience Estrous.

Table 7. Quality of Pre-production of Ewes by Type of Estrous Synchronizer and PMSG Doses

| Source                  | Synchronizer | Long Synchronization (day) | Application Time PMSG (hour) | Dose PMSG (IU) | Observed Variables |
|-------------------------|--------------|----------------------------|-----------------------------|----------------|--------------------|
|                         |              |                            |                             |                | Estrous Responses (%) | Onset Estrous (hour) | Pregnancy Rate ( %) |
| Eppleston et al., [46]  | FGA          | 13                         | 0                           | 400            | 75                 |
|                         | FGA          | 13                         | -24                         | 400            | 71                 |
|                         | FGA          | 12                         | 0                           | 0              | 83                 |
|                         | FGA          | 12                         | 0                           | 200            | 100                |
| Mutigai & Mukasa-Mugerwa, [47] | FGA      | 12                         | 0                           | 300            | 75                 |
|                         | PFG2α        | 12                         | 0                           | 0              | 75                 |
|                         | PFG2α        | 12                         | 0                           | 200            | 92                 |
|                         | PFG2α        | 12                         | 0                           | 300            | 75                 |
| Koyuncu and Alticekik [48] | FGA          | 14                         | -24                         | 500            | 96.7               |
|                         | FGA          | 14                         | 0                           | 500            | 95.9               |
|                         | FGA          | 14                         | 24                          | 500            | 96.9               |
| Nosrati et al., [49]    | CIDR         | 14                         | 0                           | 300            | 100                |
|                         | CIDR         | 14                         | 0                           | 400            | 100                |
4. Conclusion

PMSG biological activities such as FSH and LH can be used as a single dose superovulation agent. The Estrous Synchronizer can be applied simultaneously with PMSG and has a low level of difficulty thus the one that can be used by traditional farmers in Indonesia is PGF2α. The combination of PGF2α application as an Estrous synchronizer and PMSG as a superovulation agent is a practice and efficient technique to improve the reproductive performance of ewes.

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References

[1] L. Khotijah, K. G. Wiryawan, M. A. Setiadi, and D. A. Astuti, “Reproductive performance, cholesterol and progesterone status of garut ewes fed ration containing different levels of sunflower oil,” Pakistan J. Nutr., vol. 14, no. 7, pp. 388–391, 2015.
[2] R. M. Gatenby, “Conduction of heat from sheep to ground,” Agric. Meteorol., 1977.
[3] I. Inounu, B. Tiesnamurti, Subandrio, and H. Martojo, “Produksi Anak pada Domba Prolifik,” JIV, vol. 4, no. 3, pp. 148–160, 1999.
[4] B. Tiesnamurti, I. Inounu, and Subandrio, “Kapasitas Produksi Susu Domba Priangan Peridi: I. Performans Anak Prasaph,” JIV, vol. 7, no. 4, pp. 227–236, 2002.
[5] R. Somanjaya, D. Heriyadi, and I. Hernaman, “Performa Domba Lokal Betina Dewasa pada Berbagai Variasi Lamanya Penggembalaan di Daerah Irigasi Rentang Kabupaten Majalengka,” J. Ilmu Ternak, vol. 15, no. 1, pp. 41–49, 2015.
[6] A. B. Dixon et al., “Patterns of late embryonic and fetal mortality and association with several factors in sheep,” J. Anim. Sci., vol. 85, pp. 1274–1284, 2007.
[7] P. A. Geisler, J. E. Newton, and A. E. Mohan, “A mathematical model of fertilization failure and early embryonic mortality in sheep,” J. agric. Sci., Camb, vol. 89, no. 2, pp. 309–317, 1977.
[8] R. C. Debaca, A. C. War_Nick, G. H. Hitchcock, and R. Bogart, “actors Associated With the Onset of Estrus in Ewes Agricultural Experiment Station Oregon State College-Corvallis,” 1954.
[9] H. H. Cole and G. H. Hart, “The Potency of Blood Serum of Mares in Progressive Stages of Pregnancy in Effecting the Sexual Maturity of the Immature Rat,” Am. J. Physiol. Content, vol. 93, no. 1, pp. 57–68, 1930.
[10] D. Gospodarowicz, “Purification and Physicochemical Properties of the Pregnant Mare Serum Gonadotropin (PMSG),” Endocrinology, vol. 91, no. 1, pp. 101–106, 1972.
[11] Andriyanto, Amrozi, M. Rahminiwati, A. Boediono, and W. Manalu, “Korelasi Folikel Dominan Akiat Penyuntikan Hormon Pregnant Mare Serum Gonadotropin (PMSG) dengan Peningkatan Respons Berahi pada Kambing Kacang,” J. Keput. Hewan, vol. 9, no. 1, pp. 20–23, 2015.
[12] W. R. Allen, “The Immunological Measurement of Pregnant Mare Serum Gonadotrophyn,” J. Endocrinol., vol. 43, no. 4, pp. 593–598, 1969.
[13] M. T. CLEGG, J. M. BODA, and H. H. COLE, “The endometrial cups and allantochorionic pouches in the mare with emphasis on the source of equine gonadotrophin,.” Endocrinology, vol. 54, no. 4, pp. 448–463, 1954.
[14] I. W. Rowlands, “Serum Gonadotrophin and Ovarian Activity in the Pregnant Mare,” J. Endocrinol., vol. 6, no. 2, 1949.
[15] F. T. Day and I. W. Rowlands, “The Time and Rate of Appearance of Gonadotrophin in the Serum of Pregnant Mares,” *J. Endocrinol.*, vol. 2, no. 2, pp. 255–261, 2008.

[16] H. Papkoff, “Chemical and Biological Properties of The Subunits OF Pregnant Mare Serum Gonadotropin,” *Biochem. Biophys. Res. Commun.*, vol. 58, no. 2, pp. 397–404, 1974.

[17] H. H. Cole, M. Bigelow, J. Finkel, and G. R. Rupp, “Biological Half-life of Endogenous PMS Following Hysterectomy and Studies on Losses in Urine and Milk,” *Endocrinology*, vol. 27, no. 5, pp. 927–930, 1963.

[18] F. Stewart, W. R. Allen, and R. M. Moor, “Pregnant Mare Serum Gonadotrophin: Ratio of Follicle Stimulating Hormone and Luteinizing Hormone Activities Measured by Radioreceptor Assay,” *J. Endocrinol.*, vol. 71, no. 3, pp. 371–382, 1976.

[19] D. Schams, C. Menzer, E. Schallenberger, B. Hoffmann, J. Hahn, and R. Hahn, “Some Studies on Pregnant Mare Serum Gonadotropin (PMSG) and on Endocrine Responses after Application for Superovulation in Cattle,” in *Control of Reproduction in the Cow*, Springer Netherlands, 1978, pp. 122–143.

[20] H. R. Catchpole, H. H. Cole, and P. B. Pearson, “Studies on The Rate of Disappearance and Fate of Mare Gonadotropic Hormone Following Intravenous Injection,” *Am. J. Physiol. Content*, vol. 112, no. 1, pp. 21–26, 1935.

[21] T. D. Landefeld and W. H. McShan, “Isolation and Characterization of Subunits from Equine Pituitary Follicle-stimulating Hormone* Downloaded from,” *J. Biol. Chem.*, vol. 249, no. 11, pp. 3527–3530, 1974.

[22] H. Papkoff, “Relationship of PMSG to the Pituitary Gonadotrophins,” in *Control of Reproduction in the Cow*, Springer Netherlands, 1978, pp. 73–86.

[23] J. Aizen, M. Kobayashi, I. Selicharova, Y. C. Sohn, G. Yoshizaki, and B. Levavi-Sivan, “Steroidogenic response of carp ovaries to piscine FSH and LH depends on the reproductive phase,” *Gen. Comp. Endocrinol.*, vol. 178, pp. 28–36, 2012.

[24] D. B. Laster, “Disappearance and Uptake of FSH in the Rat, Rabbit, Ewe, and Cow,,” *J. Reprod. Fertil.*, vol. 30, no. 3, pp. 407–415, 1972.

[25] D. Monniaux, D. Chupin, and J. Saumande, “Superovulatory responses of cattle,” *Theriogenology*, 1983.

[26] C. R. Looney, B. W. Boutte, L. F. Archbald, and R. A. Godke, “Comparison of once daily and twice daily FSH injections for superovulating beef cattle,” *Theriogenology*, 1981.

[27] Andriyanto and W. Manalu, “Peningkatan Produktivitas Domba pada Skala Peternakan Rakyat Melalui Pemberian Hormon Pregnant Mare Serum Gonadotrophin,” *J. Vet.*, vol. 13, no. 3, pp. 235–241, 2012.

[28] T. Ararooti, ; Niasari-Naslaji, ; Razavi, and F. Panahi, “Comparing three superovulation protocols in dromedary camels: FSH, eCG-FSH and hMG,” *J. Vet. Res.*, vol. 18, no. 4, pp. 249–252, 2017.

[29] A. G. Morell, G. Gregoridis, I. H. Scheinberg, J. Hickman, and G. Ashwells, “The Role of Sialic Acid in Determining the Survival of Glycoproteins in the Circulation*,” *J. Biol. Chem.*, vol. 246, no. 5, pp. 1461–1467, 1971.

[30] S. Vyas, A. K. Singh, P. Goswami, U. K. Bissa, A. K. Rai, and N. D. Khanna, “Superovulation and Non-Surgical Embryo Flushing in Indian Camel (Camelus dromedarius),” *Int. J. Anim. Sci.*, vol. 13, pp. 147–148, 1998.

[31] E. M. Kainin and B. Tappa, “Induksi Superovulasi dengan Kombinasi CIDR, Hormon FSH dan hCG pada Induk Sapi Potong,” *Media Peternak.*, vol. 29, no. 3, pp. 141–146, 2006.

[32] M. Nishigai, A. Takamura, H. Kamomae, T. Tanaka, and Y. Kaneda, “The Effect of Human
Chorionic Gonadotropin on the Development and Function of Bovine Corpus Luteum,” 2001.

[37] C. M. Barros et al., “Improvement of A Superstimulatory Protocol in Nelore Cows: Replacing The Last Two Doses Of pFSH by eCG,” Reprod. Fertil. Dev., vol. 20, no. 1, p. 152, 2008.

[38] R. L. Davis, A. Arteaga, and J. F. Hasler, “Addition of Equine Chorionic Gonadotropin to A Traditional Follicle Stimulating Hormone Protocol for Superovulation of Bos taurus Beef Cows,” Reprod. Fertil. Dev., vol. 24, no. 1, 2012.

[39] A. Gonzalez, H. Wang, T. D. Carruthers, B. D. Murphy, and R. J. Mapleton, “Superovulation in the cow with pregnant mare serum gonadotrophin: Effects of dose and antipregnant mare serum gonadotrophin serum,” Can. Vet. J., vol. 35, no. 3, pp. 158–162, 1994.

[40] S. Fu, “Effects of different doses of PMSG on reproductive performance in Chinese holstein dairy cows,” Pak. Vet. J., vol. 33, no. 2, pp. 209–212, 2013.

[41] A. J. Hackett, H. A. Robertson, and M. S. Wolynetz, “Effects of Pgf2α and Pregnant Mares’ Serum Gonadotropin (Pmsg) on The Reproductive Performance of Fluorogestone Acetate-Pmsg-Treated Ewes,” J. Anim. Sci., vol. 53, no. 1, pp. 154–159, 1981.

[42] L. Ainsworth and J. N. B. Shrestha, “Effect of PMSG Dosage on the Reproductive Performance of Adult Ewes and Ewe Lambs Bred at a Progestagen PMSG Synchronized Estrus,” Theriogenology, vol. 24, no. 5, pp. 479–487, 1985.

[43] J. A. Quintero-Elisea et al., “The effects of time and dose of pregnant mare serum gonadotropin (PMSG) on reproductive efficiency in hair sheep ewes,” Trop. Anim. Health Prod., vol. 43, pp. 1567–1573, 2011.

[44] L. Ainsworth and J. N. B. Shrestha, “Effect of PMSG Dosage on Tre Reproductive Performance Of Adult Ewes and ewe Lambs Bred At A Progestagen-PMSG Sincronized Estrus,” Theriogenology, vol. 24, no. 5, pp. 479–487, 1985.

[45] J. A. Quintero-Elisea et al., “The effects of time and dose of pregnant mare serum gonadotropin (PMSG) on reproductive efficiency in hair sheep ewes,” Trop. Anim. Health Prod., vol. 43, pp. 1567–1573, 2011.

[46] J. Eppleston, G. Evans, and E. . Roberts, “Effect of time of PMSG and GnRH on the time of ovulation, LH secretion and reproductive performance after intrauterine insemination with frozen ram semen,” Anim. Re, vol. 26, no. 3–4, pp. 227–237, 1991.

[47] E. R. Mutigai and E. Mukasa-Mugerwa, “Effect of the Method Of Estrus Synchronization and Pmsg Dosage on Estrus and Twinning In Ethiopian Menze Sheep,” Theriogenology, vol. 38, no. 4, pp. 727–734, 1992.

[48] M. Koyuncu and S. O. Alticekic, “Effects of Progestagen and Pmsg on Estrous Synchronization and Fertility In Kivircik Ewes during Natural Breeding Season,” Asian-Australian J. Anim. Sci., vol. 23, no. 3, pp. 308–311, 2010.

[49] M. Nosrati, M. Tahmoorespoor, M. Vatandoost, and M. Behgar, “Effects of PMSG Doses on Reproductive Performance of Kurdi Ewes Artificially Inseminated during Breeding Season,” Iran. J. Appl. Anim. Sci., vol. 1, no. 2, pp. 125–129, 2010.