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# Optimum dose and time of pregnant mare serum gonadotropin injections in Kacang goats to increase endogenous secretion of estrogen and progesterone without superovulation response

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ABSTRACT

The present study was conducted to determine what the optimum dose and time of pregnant mare serum gonadotropin (PMSG) injections in Kacang does to increase endogenous secretion of estrogen and progesterone without increasing the number of ovulating follicles above the normal range. Forty-eight Kacang does were assigned to a 3 × 4factorial randomized experimental design with 4 replications. The first factor was the dose of PMSG injection, and it consisted of three levels: 0, 7.5, and 15 IU/kg body weight (BW). The second factor was the time of PMSG injection at the four stages of follicular development: the pre-luteolytic, luteolytic, pre-antral, and antral stages. The experimental goats were injected twice with prostaglandin to synchronize their estrous cycles. The parameters measured included the number and diameter of the dominant follicles and serum estrogen concentrations during the pre-ovulatory period and the number and diameter of corpora lutea and serum progesterone concentrations during the post-ovulatory period. The diameter of each dominant follicle and corpus luteum was also calculated. The results showed that an increased dose of PMSG injection significantly increased the numbers, total diameters, and diameter of each dominant follicle and corpus luteum, as well as serum estradiol and progesterone concentrations ($P < 0.05$). The time of PMSG injection significantly affected all parameters ($P < 0.05$), except for the diameter of each dominant follicle and corpus luteum. There was a significant effect of the dose and time of injection of PMSG on all parameters, except on the diameter of each dominant follicle and corpus luteum. Average pre-ovulatory serum estrogen concentrations and post-ovulatory serum progesterone concentrations in does injected with 7.5 IU/kg BW at the luteolytic stage increased by 133.68% and 545.11%, respectively. Injection of PMSG during the pre-antral and antral stages also increased estradiol and progesterone secretion to a lesser degree without increasing the number of ovulating follicles and corpora lutea. In conclusion, the results of the present study indicate that injection of 7.5 IU PMSG/kg BW at the luteolytic stage could be used to increase the secretion of pregnancy hormones without increasing the number of ovulating follicles and corpora lutea above the normal ranges.


## 1. Introduction

The main hormones that control and regulate reproduction are estrogen, produced by the developing follicles prior to ovulation, and progesterone, produced by the corpus luteum and placenta during pregnancy. An increase in progesterone concentration in maternal circulation improves the chance of a successful pregnancy (Forde et al., 2009) through improved blastocyst growth and elongation (Satterfield et al., 2009) and of embryonic survival (Jindal et al., 1997). Improved growth and development of the uterus and placenta will improve the flow and availability of nutrients to the developing embryo and fetus (Fowden et al., 2006; Sferruzzi-Perri et al., 2013), which eventually will improve birth weight and pre-weaning growth to maturity.

Superovulation as a result of increased gonadotropin leads to an increase in the number of growing and ovulating follicles and corpora lutea (Armstrong et al., 1983a,b), which increases progesterone secretion during pregnancy (Manalu et al., 1998). However, dose amounts of 15–20 IU/kg body weight (BW) of gonadotropin have been found to stimulate superovulation (Goel and Agrawal, 2005; Quintero-Elisea et al., 2011) and thereby increase the number of ovulating follicles and eventually increase the number of

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fertilized ovum and the litter size. This increase in litter size above the normal range (three or more) causes limitations in the growth and development of the embryo and fetus, decreases prenatal growth and birth weight, and decreases the survival of the offspring (Andriyanto and Manalu, 2011).

The synthetic capacities of each follicle and corpus luteum to produce estrogen and progesterone, respectively, are dependent on the number and functionalities of the cells synthesizing the hormones. The number of luteal cells in the corpus luteum and their activities are affected by the size of the pre-ovulatory follicle (Perez-Marin, 2009). Luteal cells of the corpus luteum consist of large and small luteal cells, which are developed from the granulosa and theca cells of pre-ovulatory dominant follicles (Niswender et al., 2000). Therefore, the synthesis and secretion of estrogen and progesterone can be increased by increasing the number of cells synthesizing each hormone without increasing the total number of recruited and ovulating follicles to form the corpus luteum.

The dose and time of gonadotropin administration could be modified to modulate the number of recruited and selected follicles to ovulate (González-Reyna et al., 1999; Menchaca et al., 2002; Veiga-Lopez et al., 2008). The present study was designed to investigate the optimum dose and time of administration of pregnant mare serum gonadotropin (PMSG) injections to increase endogenous secretions of estrogen and progesterone without causing the number of recruited follicles to ovulate above the normal range. The expected results were increased pre-ovulatory endogenous secretion of estradiol and post-ovulatory secretion of progesterone without increasing the number of the dominant follicles and corpora lutea above the normal range.

## 2. Materials and methods

The experimental study was conducted in the Reproduction and Rehabilitation Unit, the Reproduction and Obstetric Division, Department of Clinic, Reproduction, and Pathology, Faculty of Veterinary Medicine, Bogor Agricultural University, Bogor, Indonesia. The experiment was conducted in accordance with the National Institute of Health's guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

Forty-eight adult Kacang goats, aged between 2–3 years old with live body weights ranging from 15 to 20 kg and normal reproductive cycles and performances, were used in the experiment. Before the treatment, the experimental does were acclimatized for one month to the experimental cage and management methods. During this acclimatization period, the experimental does were given vitamin B complex and anthelmintics. The experimental does were fed 0.2 kg concentrates and 2 kg forage/does/day and drinking water was available ad libitum. After acclimatization, the experimental does were injected twice with 0.7 mg/kg BW prostaglandin ($PGF_{2\alpha}$, Noroprost® 0.5%, Norobrook, UK), with an eleven-day interval between the injections to synchronize their estrous cycles.

The does were assigned to a randomized experimental design with a $3 \times 4$ factorial arrangement and 4 replications. The first factor was the dose of PMSG injection (Gonaser®, Hipra, Spain) and consisted of three levels: 0, 7.5, and 15.0 IU/kg BW. The second factor was the time of the PMSG injection and consisted of four time periods: one day before the second $PGF_{2\alpha}$ injection (−1 PG or pre-luteolytic stage), day zero or at the same time as the second $PGF_{2\alpha}$ injection (0 PG or luteolytic stage), one day after the second $PGF_{2\alpha}$ injection (1 PG or pre-antral stage), and two days after the second $PGF_{2\alpha}$ injection (2 PG or antral stage).

The parameters measured in this study included serum estradiol concentrations and the number and diameter of the dominant follicles during the pre-ovulatory period, and serum progesterone concentrations and the number and diameter of corpora lutea during the post-ovulatory period. Serum estradiol concentrations and the number and diameter of the dominant follicles were measured one day after the second $PGF_{2\alpha}$ injection (pro-estrus) and two days after the second $PGF_{2\alpha}$ injection (estrus) during the period of follicle development prior to ovulation. Serum progesterone concentrations and the number and diameter of corpora lutea during the post-ovulatory period were measured at 4, 8, 12, 16, and 20 days after the second $PGF_{2\alpha}$ injection during the periods of metestrus, early diestrus, early peak of diestrus, late peak of diestrus, and end of diestrus, respectively.

Blood samples were drawn from the jugular vein using a 10 mL syringe. The blood samples were stored for 24 h in the refrigerator (4 °C) and then centrifuged at 2500 rpm for 10 min to obtain serum. The serum was separated and stored in plastic tubes in the freezer (−20 °C) until estrogen and progesterone were measured. Serum pre-ovulatory estradiol concentrations were measured by enzyme link immune sorbent assay method (ELISA, EIA1561 DRG International Inc. Marburg, Germany), while serum post-ovulatory progesterone concentrations were measured by radioimmunoassay (Izotop progesterone [$I^{125}$] RIA kit Institute of Isotope Ltd., Budapest, Rumania). Inter- and intra-assay variations of coefficients were 9.0% and 4.0%, respectively. The number and diameter of the dominant follicles during the pre-ovulatory period and the number and diameter of corpora lutea during the post-ovulatory period were determined using ultrasound sonography (Aloka SSD-500 model, Aloka Co. LTD, Tokyo, Japan), a linear probe 7.5 MHz (Aloka Co. LTD, Tokyo, Japan), and a SONY UP-895 MD thermal video printer (Video Graphic Printer, Japan). For each doe, the diameter of each individual dominant follicle at the time of measurement was calculated by dividing the total diameter of the dominant follicles by the total number of the dominant follicles. Similar measurements were calculated for the corpus luteum.

For each experimental doe, serum estradiol concentrations, the number of the dominant follicles, the total diameter of the dominant follicles, and the diameter of each individual dominant follicle measured one and two days after the second $PGF_{2\alpha}$ injection were averaged, and the averages were used for statistical analyses. Serum progesterone concentrations, the number of corpora lutea, the total diameter of corpus luteum, and the diameter of each corpus luteum measured at 4, 8, 12, 16, and 20 days after the second $PGF_{2\alpha}$ injection were averaged, and the averages were used for statistical analyses. The data obtained in this study were analyzed using the General Linear Model in the Minitab 16 software.

## 3. Results

### 3.1. Pre-ovulatory serum estradiol concentration

Generally, the dose and time, as well as the interaction of these two factors, of PMSG injection had significant effects on pre-ovulatory serum estradiol concentrations ($P < 0.01$) (Table 1). The control goats without PMSG injection had normal, low pre-ovulatory serum estradiol concentrations. PMSG injection increased the pre-ovulatory serum estradiol concentrations, and the dose of 15 IU gave the highest increase. Regardless of the dose of PMSG injection, the injections at the pre-luteolytic and luteolytic stages gave the highest pre-ovulatory serum estradiol concentrations. The pattern of pre-ovulatory serum estradiol concentrations decreased with the time of PMSG injection from the pre-luteolytic, luteolytic, pre-antral, and antral stages.

### 3.2. Post-ovulatory serum progesterone concentrations

Similar to the pattern observed in the pre-ovulatory serum estradiol concentrations, the dose and time, as well as the inter-