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Effect of high doses of equine chorionic gonadotrophin (eCG) treatments on follicular developments, ovulation and pregnancy rate in boer goats

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The aim of this study was to determine the effects of the superovulatory technique using equine chorionic gonadothrophin (eCG) on follicle response, ovulation and pregnancy rate in Boer goats. Twenty nine (29) does were divided into three groups, G1 (n = 11), G2 (n = 8) and G3 (control, n = 10). All groups had their estrus synchronized by the use of controlled internal drug release (CIDR) containing 0.3 g progesterone for 18 days. Twenty-four hours prior to CIDR removal, all animals were intramuscularly injected with different eCG doses: does in G1, G2 and G3 received 600, 800 and 1000 IU eCG, respectively. Follicular activity was determined once a day for four consecutive days by ultrasonographic monitoring starting at eCG treatment (day 17) in all groups. The number of corpora lutea were assessed on day seven after estrus to calculate ovulation rate, whereas the pregnancy diagnosis was detected on 30 days post mating. Follicles response resulted in significant differences (P < 0.05) only under small size follicles but not significant difference (*P* > 0.05) on number of follicles under medium and large size follicles among treatments. Ovulation rate recorded a significant difference (*P* < 0.05) among treatments after seven days post estrus with the highest rate at 2.3 ± 0.3, 1.6 ± 0.2 and 1.4 ± 0.1 for G2, G3 and G1, respectively. Meanwhile, pregnancy rate that showed the highest recorded was 50, 45.5 and 12.5% for G3, G1 and G2, respectively. The results concluded that there was no significant difference on follicle number recorded among treatments except for small size follicle numbers on days 19 and 20. Meanwhile, we concluded that 800 IU eCG was the best treatment resulting in the highest ovulation rate. Different doses of eCG however did not influence the pregnancy rate in superovulated does.

Key words: Equine chorionic gonadotrophin, follicular, ovulation, pregnancy, estrus synchronization, goat.

INTRODUCTION

The optimization of reproductive performance is one of the main facts that assure high productivity on goat farms. This requires that the management practices take into account the physiology and behavior of the animals since environmental, managerial and sanitary aspects interfere with fertility and can impair it. Indeed, reproduction could be considered a “luxury” function and the female appears able to feel whether the conditions are too severe and risky for a successful reproductive cycle (Fringgens, 2003). Multiple ovulation and embryo transfer (MOET) is widely used to increase genetically superior offspring produced from selected females (Greyling, 2002). Ovarian superstimulation in domestic animals may thus be used to increase the number of developmentally competent

oocytes for *in vivo* or *vitro* embryo production (Malhi et al., 2008). This variation may be due to both extrinsic equine chorionic gonadotrophin (eCG) treatment and follicle stimulating hormone (FSH) preparation, mode of administration or the dosage regimens) and/or intrinsic (ovarian status, genetic variation) factors (Cognie et al., 2003; Gonzales-Bulnes et al., 2004; Shipley et al., 2007).

Superovulatory procedures using eCG commonly used high dose of eCG after synchronizing their estrus cycles with controlled internal drug release (CIDR). This procedure agreed with a previous research (Holtz, 2005) which they reported that, in goats, superovulatory treat-ment typically consists of a combination of estrous cycle control (usually involving application of progestagen implants), with an elevated dose of gonadotropin, to induce the ovary to release more than the typical number of oocytes. The eCG traditionally termed "pregnant mare serum gonadotropin" (PMSG) is an exogenous gonado-tropin which can stimulate follicular growth and subse-quently the number of ovulation (ewe on day 12 of the cycle) (Cumming, 1975). PMSG resembles pituitary FSH and luteinizing hormone (LH) in that it is a glycoprotein. It can be said to be the complete gonadotrophin, since it is able to induce follicle growth, estrogen production, ovula-tion, luteinization and progesterone synthecic (Cahill, 1982). PMSG is administered as a single subcutaneous or intramuscular injection given one day prior to the last synchronization treatment. In goat, 1000 IU PMSG is given on day 17 of the oestrous cycle (Bondurant, 1986; Drost, 1986) or 2 days before progesterone sponge removal or cessation of daily (12 mg/day) progesterone injection. Several reports claim good results using doses of eCG that ranged from 200 to 600 IU Greyling and Van Niekerk, 1990; Ritar et al., 1994; Menegatos et al., 1995; Freitas et al., 1996; Selvaraju and Kathiresan, 1997).

The present study aimed to compare the lower dosages of eCG at 600 and 800 IU, compared to 1000 IU which eventually improve reproductive efficiency as well as reducing cost of superovulatory treatment in the goats.

MATERIALS AND METHODS

The experiment was conducted in a total of 29 healthy pluriparous Boer does, aged from three to five years and body weight between 45 and 65 kg maintained indoors at the experimental farm of Kampung Kuala Pah Breeding Goat Center, owned by Department of Veterinary and Services (DVS), Malaysia. The animals were randomly divided into three groups. Group 1 consisted of 11 does, group 2 with eight does and group 3 (control) with 10 does. Animals were housed in an animal shed (6.1 × 6.1 m) built approximately 3 m above the ground level. The goat house was located at the hill top surrounded by open paddock. All does were kept indoors and fed twice daily. In the morning, animals were fed with *Bracharia humidicola* (Rendle) and *Panicum maximum* (Guinea). In the evening, commercial concentrates (Biopalma®) (Crude protein ≤ 14.9%, crude fiber ≤ 26.1%, crude fat ≤ 5.1%, calcium ≤ 0.72%, phosphorus ≤ 0.36 % and metobolisable energy ≤ 9.06 MJ/kg) were given to the animals at 450 g/doe/day. Mineral blocks and water were provided *ad libitum* to the animals.

Does in the three experimental groups were followed superovulation protocol by synchronizing with controlled internal drug release (CIDR®) device which contained 0.3 g of progesterone and left intravaginally for 18 days. The day of CIDR insertion was considered as day 0. Twenty four hours prior to CIDR removal, superovulatory treatments were given using eCG. Does in groups 1, 2 and 3 received a single intramuscular injection of 600, 800 and 1000 IU eCG (Foligon®, Intervet, The Netherlands), respectively.

Ovarian images were obtained with a B-mode ultrasound scanner (ALOKA SSD-500®, Tokyo, Japan) equipped with a 5 MHz linear array transducer. During scanning, goats were restrained in a wooden chute in a standing position. Before scanning, feces were removed as much as possible by hand and some carboxymethylcellulose gel placed on the transrectal probe. Then, the lubricated transrectal probe was inserted into the rectum. When the urinary bladder was surpassed and the uterine horns were located, the probe was rotated laterally clockwise for 90° and counterclockwise for 180° to evaluate both ovaries and their structures as described (Ginther and Kot, 1994). The ovaries were scanned in several planes to identify all visible follicles that are >1 mm in diameter. Follicles that were more than 3 mm were counted and grouped into one of the following classes: small (3 to < 4 mm), medium (4 to < 5 mm) and large (≥ 5 mm) follicles, following the previous study (Menchaca and Rubianes, 2002). Follicular development was observed once a day for four consecutive days starting on the day of eCG treatment. Twenty-four hours after CIDR removal, a buck was mixed with the females in each group. The information pertaining to the mating time such was recorded.

The ovarian response in terms of number corpora lutea (CLs) was assessed by 5 MHz linear probe attached to an ultrasound scanner (ALOKA SSD-500, Tokyo, Japan) seven days after the onset of estrus. We recorded the number of CL's with the antral follicles ≥ 3 mm in diameter. Meanwhile, pregnancy rate was determined by pregnancy diagnosis on 30 days post mating.

Statistical analysis was performed using Predictive Analytics SoftWare (PASW®) version 17.0. All follicles were grouped as described earlier. The effects of treatment on number of folicles and ovulation rate were analyzed by analysis of variance (one way ANOVA). Meanwhile, percentage calculation and Chi-square analysis were conducted to measure the effect of treatments on pregnancy rate. Data were expressed as mean ± standard error of mean (S.E.M) and differences were considered to be statistically significant at $P < 0.05$.

RESULTS

The follicle response of Boer does following estrus synchronization is shown in Table 1. The patterns of follicular

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Abbreviations: CIDR, Controlled internal drug release; **CL,** corpus luteum; **DVS,** department of veterinary and services; **eCG,** equine chorionic gonadotrophin; **FSH,** follicle stimulating hormone; **g,** gram; **LH,** luteinizing hormone; **mg,** miligram; **MHz,** Megahertz; **MOET,** multiple ovulation and embryo transfer; **PASW®,** predictive analytics software; **PMSG,** pregnant mare serum gonadotropin.

Table 1. Follicular development of Boer does following estrus synchronization (Mean ± S.E.M).

Day	Size	G1 (n = 11)		G2 (n = 8)		G3 (n = 10)	
		LO	RO	LO	RO	LO	RO
Day 17	S	1.3 ± 0.3	4.0 ± 0.5	2.1 ± 0.5	2.0 ± 0.0	2.3 ± 0.2	2.0 ± 0.5
	M	1.8 ± 0.3	1.2 ± 0.2	1.5 ± 0.2	1.0 ± 0.0	1.5 ± 0.2	1.4 ± 0.2
	L	1.0 ± 0.0	1.2 ± 0.2	5.0 ± 0.0	1.5 ± 0.5	1.4 ± 0.4	2.3 ± 0.3
Day 18	S	2.0 ± 0.7	1.7 ± 0.4	1.0 ± 0.0	2.0 ± 0.0	2.6 ± 0.3	3.0 ± 2.0
	M	1.5 ± 0.2	1.6 ± 0.3	1.2 ± 0.2	1.0 ± 0.0	3.4 ± 1.2	4.3 ± 1.0
	L	2.2 ± 0.4	1.5 ± 0.5	1.7 ± 0.7	2.0 ± 0.3	3.0 ± 0.7	2.2 ± 0.9
Day 19	S	1.2 ± 0.1[a]	3.1 ± 0.3	1.7 ± 0.2[a,b]	1.8 ± 0.3	2.5 ± 0.6[b]	3.5 ± 1.5
	M	1.8 ± 0.3	1.6 ± 0.2	2.0 ± 0.6	1.0 ± 0.0	2.4 ± 0.6	2.0 ± 0.4
	L	1.4 ± 0.2	2.0 ± 0.3	2.1 ± 0.4	1.6 ± 0.6	3.0 ± 0.7	1.8 ± 0.4
Day 20	S	1.4 ± 0.2	1.0 ± 0.0[x]	2.0 ± 1.5	1.0[x] ± 0.0	1.7 ± 0.2	2.5 ± 0.2[y]
	M	1.2 ± 0.1	1.1 ± 0.1	2.0 ± 0.6	1.5 ± 0.5	2.1 ± 0.5	1.4 ± 0.2
	L	2.0 ± 0.0	1.5 ± 0.3	1.6 ± 0.4	2.1 ± 0.4	1.2 ± 0.2	2.4 ± 0.5

[a, b]Values with different superscript in the same row differ significantly at $P < 0.05$. [x, y]Values with different superscript in the same row differ significantly at $P < 0.05$. S, Small size follicle; M, medium size follicle; L, large size follicle; LO, left ovary; RO, right ovary.

growth within different size categories indicate the duration of exogenous gonadotrophin stimulus. The shift from the numerical superiority of the smaller follicle category to the superiority of the next higher one at the attainment of the next phase of the estrus period (pre-estrus to estrus, or estrus to post estrus) represents the end of recruitment, and therefore the end of the exogenous gonadotrophin action. Regarding the experiment, after an hour super-ovulatory treatment given, the highest mean number of follicles found was 4.0 ± 0.5, under small size category in right side ovary of G1. However, mean number of follicles for small, medium and large size category at left and right side ovary was not significant ($P > 0.05$) among group treatments on day 17.

Twenty four hours after superovulatory treatment, the highest mean number of follicles recorded was from medium size categories at 4.3 ± 1.0 from G3 on right side ovary, followed by G1 (2.2 ± 0.4) and G2 (2.0 ± 0.3), on left and right ovary, respectively. Only G1 resulted in an increasing number of follicle on large size categories in both side ovary compared to others group. On day 18, no significant difference ($P > 0.05$) was found on number of follicles between group treatments at left and right side ovary on all size categories.

Twenty four hours after CIDR removal, the highest mean number of follicles recorded on day 19 was in G3 (3.5 ± 1.5) followed by G1 (3.1 ± 0.3) both under small size category and G2 (2.1 ± 0.4) under large size category. The mean number of follicles are significantly ($P < 0.05$) higher among group treatments in left ovary on small size categories at 2.5 ± 0.6, 1.7 ± 0.2 and 1.2 ± 0.1 for G3, G2 and G1, respectively. However, no significant difference ($P > 0.05$) on number of follicles was recorded in right ovary under small size categories at the same time. We also recorded no significant difference ($P > 0.05$) under medium and large size categories on both sides of the ovary on day 19.

On day 20, 48 h after CIDR removal, a highly signifi cant difference ($P < 0.05$) in number of follicles was recorded with G3 at 2.5 ± 0.2 follicles, followed by G2 (2.0 ± 1.5) and G1 (1.4 ± 0.2) under small size categories. The highest number of follicles recorded from G1 and G2 on day 20 was 1.5 ± 0.3 and 2.1 ± 0.4, respectively both under large size follicles. Table 2 shows the ovulation rate of does administered with CIDR + eCG. In this study, 11 (100%) out of 11 does in G1, eight (100%) out of eight does in G2 and 10 (100%) out of 10 does in G3 ovulated during estrus. The highest mean numbers of CL at the left side ovary was from G2, which is 1.3 ± 0.1. However, the mean numbers of CL on the left ovary was not significantly different ($P > 0.05$) compared with G1 (1.11 ± 0.11) and G3 (1.00). Meanwhile, the highest mean numbers of CL found on the right ovary was from G2 at 1.3 ± 0.2, followed by G3 and G1 at 1.3 ± 0.1 and 1.0, respectively. This result shows that the ovulation rate from right ovary was not significantly different ($P > 0.05$) among group treatments. The highest numbers of CL recorded in the right side ovary was two from G2 and G3; meanwhile the lowest CL recorded was 1 from G1.

Table 3 shows the result of does that conceived by natural mating. Out of 21 estrus does, 11 (52.4%) does were pregnant. The highest pregnancy rate in this study was from G3 at 71.4% followed by G1 (55.5%) and G2 (20%). Only G2 and G1 resulted more than 50% pregnancy rate. In contrast, G1 and G2 recorded the highest non pregnant rate, with four does in each group,

Table 2. Ovulation rate after following 18 days CIDR treatment with eCG injection on day 17.

Parameter	G1	G2	G3	Total
Number of females synchronized	11	8	10	29
Number of does ovulated	11 (100%)	8 (100%)	10 (100%)	29 (100%)
Mean number of CL on left ovary (range)	1.1 ± 0.1 (1-2)	1.3 ± 0.1 (1-2)	1.0 ± 0.0	
Mean number of CL on right ovary (range)	1.0 ± 0.0	1.3 ± 0.2 (1-2)	1.3 ± 0.1 (1-2)	
Mean number of CL per doe (range)	1.4 ± 0.1[a] (1-2)	2.3 ± 0.3[b] (1-4)	1.6 ± 0.2[a,b] (1-3)	

[a, b]Values with different superscript in the same row differ significantly at P<0.05. Mean ± S.E.M.

Table 3. Pregnancy rate following 18 days CIDR treatment with eCG injection on day 17.

Parameter	G1 (n=11)	G2 (n=8)	G3 (n=10)	Total
Number of does in estrus	9	5	7	21
Number of does that did not display oestrus	2	3	3	8
Number of does pregnant	5 (55.5%)	1 (20%)	5 (71.4%)	11 (52.4%)
Number of does aborted	0	0	2	2
Gestation period (days)	144.4 ± 7.5	145.0	145.3 ± 6.5	

Mean ± S.E.M.

after estrus. Only two does from G3 failed to become pregnant in this study. However, we observed two pregnant does from G3 has aborted on midway stage pregnancy. According to the mean gestation period recorded, G3 showed the longest period with 145.3 ± 6.5 days. Meanwhile, G2 and G1 recorded 145 and 144.4 ± 7.5 days gestation period, respectively. Thus, the proportion of gestation period was not influenced by eCG doses.

DISCUSSION

On the first day of eCG treatment, the highest mean number of follicles was from G1 at 4.0 ± 0.5 in small size category. This finding was in line with previous study (Francicco Carlos de Sousa et al., 2011) which found 7.1 ± 0.9 follicles in 3 to 4 mm size at the ovarian stimulation day. Another study (Riesenberg et al., 2001) also concurred to our study which reported 8 h after eCG treatment given, mean number of follicles that range 0.3 and 0.4 cm was 2.7. The highest mean number of follicles found on day two after eCG treatments in the current study was 4.3 ± 1.0 from G3 under medium size category. Our finding is higher than previous study (Kermani et al., 2012) which recorded 1.6 ± 0.4 follicles (≥ 4 mm) at the same time after receiving 850 IU eCG. However, the same findings are more similar with the present study after comparing the highest mean number of follicles (4 ≤ 5 mm) found in G2 (800 IU) at 1.2 ± 0.2 at the same period.

The present study shows the highest mean number of5 follicles found on day three after eCG treatment was 3.5 ± 1.5 from G3 in small size category. This is comparible with previous findings by Kermani et al. (2012) which recorded 3.5 ± 0.6 mean number of follicles at the same size category. Four days after eCG treatments, our study recorded the highest mean number of follicle at 2.5 ± 0.2 from small size category from G3. This finding agrees with previous study by Ali (2007) who found the highest follicles on day four after eCG treatment was 2.5 follicles. These findings are also close to the previous study by Salehi et al. (2010) who showed that the administration of eCG and FSH caused growth and development of the small follicles present in the ovaries. Subsequently, the large follicles during estrus will ovulate and transform to mature CL's.

The ovulation rate in our study showed that all (100%) does ovulated with at least 1 CL observed by transrectal ultrasonography on day 7 after mating. The present finding is higher than previous study (Gonzalez de Bulnes et al., 1999a) which only recorded 89.3% ovulation rate. These treatments have met our goal to induce multiple ovulation by giving the minimum of 600 IU eCG dosage. Some of the does from G1 (2 does), G2 (3 does) and G3 (3 does) failed to display oestrus within 48 h after CIDR removal. This might be due to either inadequate oestradiol secretion, or oestrus was displayed silently without any overt signs of oestrus (Romano and Wheaton, 1998; Cardwell et al., 1998). Besides that, the absence of oestrus and ovulation may be due to insufficient gonado-trophic hormone released by the pituitary, leading to poor response by the ovary to the exogenous eCG.

Previous studies had reported that the lower pregnancy rate was recorded after a long-term (12 days) progestagen treatment and related to a slower follicular turnover, promoting the ovulation of persistent dominant follicles. In

agreement with that, our findings showed that only 52.4% out of 21 estrus does became pregnant after undergoing 18 days CIDR treatment with eCG treatment on day 17. Our findings were also supported by previous findings of Barrett et al. (2004) who reported that the administration of 500 IU eCG, 12 days after progestagen treatment, had limited effects on the dynamics of ovarian follicular wave development. The short-term progestagen treatment (six days) on the other hand resulted in a higher pregnancy rate, probably due to the ovulation of newly recruited growing follicles (Vinoles et al., 2001).

Conflict of Interests

The author(s) have not declared any conflict of interests.

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