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Comparison of Superovulatory Effect of Equine Chorionic Gonadotrophin and Follicle Stimulating Hormone on Embryo Production in Crossbred (Boer × Katjang) Goats

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Abstract.- An experiment was carried out using 25 crossbred does to evaluate the efficacy of gonadotrophin sources on ovarian responses during superovulation. Oestrus was synchronised by inserting CIDR for 14 days and 125 µg of PGF2α was injected intramuscularly on Day 11. All the donor does were divided into 2 groups, namely Groups 1 and 2 with the respective treatments of 1500 IU of equine chorionic gonadotrophin (eCG) through a single intramuscular injection and 200 mg of follicle stimulating hormone (FSH) through multiple intramuscular injections. Gonadotrophin treatments were initiated from 1 day before the CIDR removal. For ovulation synchronisation, 1000 IU of human chorionic gonadotrophin (hCG) were injected intramuscularly through two equal dosages on Days 15 and 16. Ovarian responses of both treatments were evaluated on Day 7 after CIDR removal. In both treatments, all the does showed sign of oestrus and responded to the treatments by ovulating at least 4 follicles. After signs of oestrus were detected, the doe was placed in a pen with a buck male for natural mating. Average number of embryo recovery, corpus luteum, anovulatory follicles and unfertilised oocytes for Groups 1 and 2 were 0.53 vs. 2.0, 6.73 vs. 6.40, 13.00 vs. 13.70 and 1.53 vs. 1.80, respectively. No significant ($p > 0.05$) differences were observed between groups. In conclusion, superovulatory treatment using eCG was comparable with that of FSH in producing embryos in the crossbred goats; however, further studies are needed to conclude about the effectiveness of gonadotrophin source for goat superovulation under local condition.

Key words: Superovulation, FSH, eCG, embryo production, ovarian response, goat.

INTRODUCTION

Superovulation is the main conventional approach to utilise the female genetic potential effectively and to rapidly produce higher number of quality embryos from valuable goat breeds. However, the outcomes of superovulation programme are variable and inconsistent ranging from complete failure to total success without any changes in the standard operating procedure (Baldassarre and Karatzas, 2004). This variation is the major drawback for the success of multiple ovulation and embryo transfer (MOET) technology (Cognie, 1999). The success of a MOET programme is determined by many intrinsic and extrinsic factors, including season, breed, age, nutrition, management, stress, quality and source of gonadotrophin and treatment protocols (Gonzalez-Bulnes *et al*., 2004).

Goat superovulation protocol consists of long progesterone priming followed by administration of gonadotrophin hormone during the latter part of protocol. Gonadotrophins used in superovulation are either equine chorionic gonadotrophin (eCG) (Goel and Agrawal, 2005) or combination of hormones such as P.G. 600 (it is a mixture of 2 gonadotrophins; 400 IU of eCG and 200 IU of hCG) (Rowe and East, 1996) or follicle stimulating hormone (FSH) (Abdullah *et al*., 2012; Lehloenya, 2013). The eCG has some advantages like single administration, low cost and easily available (Armstrong *et al*., 1983a,b; Pendleton *et al*., 1992), while it has some disadvantages such as long biological half-life, large number of unovulated follicles, early regression of corpus luteum (CL), reduced fertilisation and embryo quality (Armstrong *et al*., 1983b; Pintado *et al*., 1998). On the other hand, FSH resulted in higher ovarian responses (ovulation and embryo recovery rates) than eCG (Goel and Agrawal, 2005). However, FSH is usually injected twice a day, over a period of 3 to 4 days due to short half-life (Demoustier *et al*., 1988;

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Menchaca *et al*., 2007) which gives more stress to animal and also increase the labour cost.

Besides hormone, breed has also been identified as a major factor that affects MOET program. All the breeds do not respond same way to the superovulation treatment. A higher number of embryos (10.1) were recovered in Alpine does compared to Angora does (7.5) (Baril *et al*., 1989). Breed effect has been associated with the prolificacy of breed where a high prolific breed has been reported to respond better to exogenous gonadotrophins (Bindon *et al*., 1986). Moreover, different breeds produced different results using different gonadotrophin. For this reason, a lot of comparisons among different gonadotrophin in different breeds have been made to know the interaction between the breed and the gonadotrophin source. According to the findings of earlier researchers, FSH produces more embryos than eCG such as 8.80 and 3.00 in Jamunapari (Goel and Agrawal, 1990); 9.20 and 3.10 in Boer goats (Nowshari *et al*., 1995); 8.00 and 5.80 in Jakhrana goats (Goel and Agrawal, 2005); 9.30 and 2.30 in indigenous dairy goats (Pampukidou *et al*., 2011), respectively.

Reproduction of small ruminant is influenced by environment (Qureshi *et al*., 2010). In many countries, crossbreeding programme is applied to improve the local stocks with exotic breed for the production of environmentally adapted high performing stocks. For example, Malaysia imported Boer and other goat breeds for upgrading the local stock by crossbreeding. These crossbred goats are more adaptive to the local condition. However, these crossbred goats are rarely used in studies for further development. Some researchers conducted research on growth performance (Vadiveloo, 1988; Hirooka *et al*., 1997) and heterosis (Tsukahara *et al*., 2008) using crossbred goats. Although goats in Malaysia did not show any strong seasonality in responding to gonadotrophins, more does respond during the rainy season compared with dry season (Rosnina *et al*., 1992). The superovulation and embryo production performance of pure breeds are well known in the world, but the information of crossbred goat performance in different ecosystem, for example in the non-conducive and adverse tropical region, is limited. Therefore, the present experiment was conducted to evaluate the superovulation responses and embryo production efficacy in crossbred goats using two exogenous gonadotrophins sources (eCG vs. FSH).

MATERIALS AND METHODS

Location of experiment

This experiment was carried out at the Institute of Biological Sciences Mini Farm (at an altitude of 2º30´ N, 112 º 30´ E), University of Malaya, Malaysia. This location is 60 m above sea level and has annual rainfall of 2600 ml.

Experimental animal management

Twenty five mature crossbred (Boer × Katjang) does of 16.5 to 35.0 kg body weight and with 4 to 8 permanent incisor teeth or 2 to 4 years old were used for this experiment. The experimental does were reared under intensive management system and received pellet at a rate of 400 g/head/d. The pellet fed contained 15% crude protein, 15% crude fibre, 0.5% phosphorus and 0.8-1.5% calcium. The pellet was offered to the animals once in the morning, while the Napier grass was offered in the afternoon. All animals had free access to water and salt lick. The study was conducted between December 2011 and May 2012. All animals used in this experiment were in accordance to the guidelines of Institute of Biological Sciences, Faculty of Science, University of Malaya.

Ultrasonography of experimental animal

All the donor does were scanned to identify the ovarian status by using ultrasound machine (ALOKA SSD 500, Japan) equipped with 7.5 MHz transrectal probe. The tip of the probe was lubricated with carboxyethyl cellulose contact gel and was gently inserted until the urinary bladder was identifiable. The probe was moved gently forward and backward and rotated at 90° clockwise and 180° counter-clockwise to identify both ovaries. Only non-pregnant does were used for this experiment.

Oestrus synchronisation and superovulation treatments

Oestrus was synchronised by inserting controlled internal drug release dispenser (CIDR

0.33 g natural progesterone hormone; EAZI-BREED CIDR, Pharmacia & Upjohn Limited, NZ) for 14 days and supplemented with a single injection of 125 µg of PGF2α (Estrumate®; Intervet International B.V. Netherlands) intramuscularly on day 11. Subsequently, all the donor does were divided into 2 groups, namely groups 1 and 2. Groups 1 and 2 were administered with 1500 IU of eCG (Folligon®; Intervet International B.V. Netherlands) through a single intramuscular injection and 200 mg of FSH (Folltropin®-V; Bioniche Animal Health, Canada) through multiple intramuscular injections, respectively. FSH treatment was divided into six decreasing dosages given twice daily (2.5 ml, 50 mg; 2.5 ml, 50 mg; 1.5 ml, 30 mg; 1.5 ml, 30 mg; 1.0 ml, 20 mg; and 1.0 ml, 20 mg). Gonadotrophin treatments were initiated from 2 days before the CIDR removal. For ovulation synchronisation, 1000 IU of hCG (Ovidrel®; PreFilled Syringe, Industria Farmaceutica Serono, S.P.A., Bari, Italy) was injected intramuscularly through two equal dosages on Days 15 and 16. Ovarian responses of both treatments were evaluated during laparotomy session on Day 7 after CIDR removal (Fig. 1).

Oestrus detection and natural mating

After CIDR withdrawal, oestrus was observed 3 times in a day (morning at 0800-0900 h, afternoon at 1300-1400 h and evening 1900-2000 h) until exhibition of overt oestrus by placing a buck of proven libido. After signs of oestrus were detected, the doe was placed in a pen with a male for natural mating.

Surgical procedure for ovarian responses assessment

On Day 7 after CIDR removal, ovarian responses were evaluated and embryos were collected during laparotomy session. The donor does were off-fed and water for 18 h before surgery. For goat anaesthesia, xylazine hydrochloride (Ilium Xylazil – 100, Troy Laboratories, Australia) was mixed with ketamine hydrochloride (Ketamil Injection, Troy Laboratories, Australia) at a ratio of 1:50 and administered through intramuscular injection (11 mg/kg body weight). After anaesthesia, the reproductive tract was exteriorised through a mid-ventral incision and the superovulatory responses were assessed by counting the number of CL and anovulatory follicles of both ovaries. Both the Fallopian tubes were flushed by using a flushing medium consisted of phosphate buffered saline (PBS Dullbecco A, Oxoid, England) supplemented with streptomycin (Streptomycin sulfate salt, Sigma-Aldrich,USA), penicillin (Penicillin G sodium salt, Sigma-Aldrich,USA) and polyvinyl-pyrrolidone (Polyvinylpyrrolidone-360,Sigma-Aldrich,USA). A two-way Foley catheter was used for embryo flushing. The recovered structures (embryos plus unfertilised oocytes) in the flushing medium were evaluated and classified accordingly under a stereomicroscope (SZH10; Olympus Optical Co. Ltd, Japan).

Statistical analysis

The effects of different gonadotrophin sources (eCG and FSH) on parameters (numbers of CL, embryo, anovulated follicle and unfertilised oocyte) were analysed by using Student's *t* test.

RESULTS

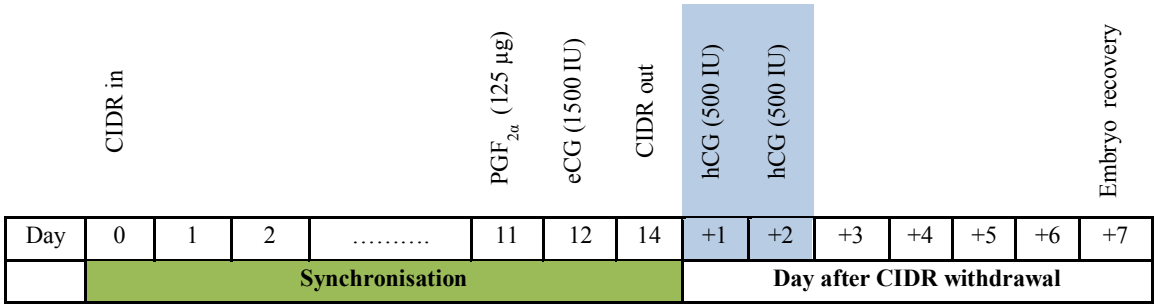
Superovulatory responses of crossbred goats treated with eCG and FSH are presented in Tables I and II. All the goats in eCG and FSH treatment

Table I.- Superovulatory response of crossbred goats treated with eCG and FSH.

Parameters	Gonadotrophin sources (Mean±SEM)	
	eCG	FSH
No. of does	15	10
Does in oestrus (No.; %)	15 (100%)	10 (100%)
Duration between CIDR out and onset of oestrus (h)	36.00±6.93	27.00±3.00
Duration of oestrus (h)	36.00±4.90	33.00±3.00
CL/doe (No.)	6.73±1.70	6.40±1.55
Anovulated follicles/doe (No.)	13.00±3.01	13.70±2.17
Unfertilised oocytes/doe (No.)	1.53±0.59	1.80±1.00
Embryos collected/doe (No.)	0.53±0.27	2.00±1.03
Structure recovered (embryos plus unfertilised oocytes)/doe (No.)	1.79±0.74	3.80±1.42

No significant ($p > 0.05$) differences among the gonadotrophin sources for all the parameters
*SEM= Standard Error of Mean.

a)



b)

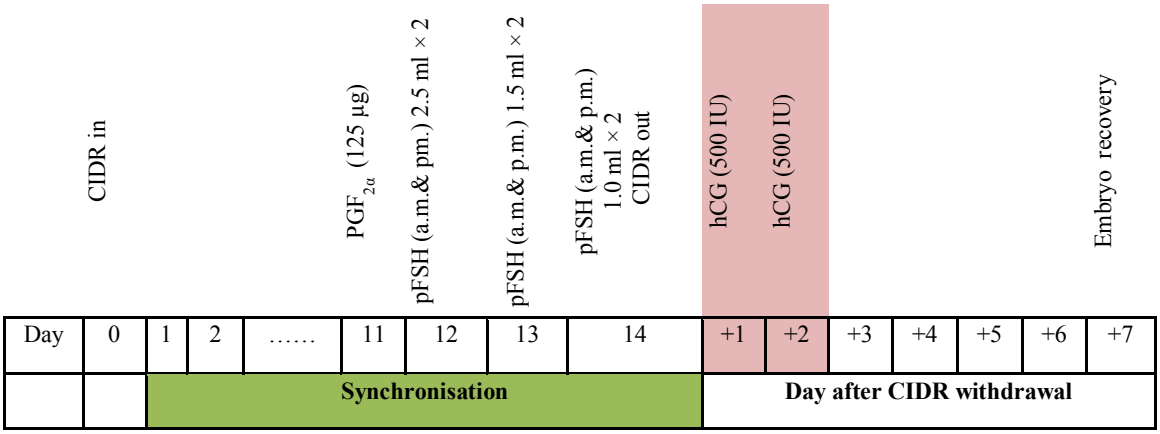


Fig. 1. Schematic representation of the superovulation protocol. a) eCG, b) pFSH.

groups showed signs of standing oestrus following CIDR treatment (Table I). The duration between CIDR removal and onset of oestrus in eCG group (36.00±6.93 h) was comparable with FSH group (27.00±3.00 h). Similarly, the duration of oestrus in eCG group (36.00±4.90 h) was also similar with FSH group (33.00±3.00 h). Although the average number of CL for both eCG (6.73±1.70) and FSH (6.40±1.30) treated groups were similar but the number of structures recovered (embryos plus unfertilised oocytes) per doe was numerically higher in FSH group (3.80±1.42) than eCG group (1.79±0.68). However, the differences were not significantly different ($p > 0.05$).

The ovulatory responses of left and right ovaries (Table II). Right ovary of the goats superovulated using eCG and FSH produced similar number of CLs (3.20±0.92 and 3.80±0.90, respectively) with left ovary (3.53±0.94 and 2.60±0.65, respectively). In case of anovulated follicles, left ovary possessed similar numbers of follicles for eCG and FSH (7.40±1.73, 7.90±1.46, respectively) with the right ovary (5.60±1.42 and 5.8±0.80, respectively). Average embryo recovery for left and right ovaries of eCG treated does (0.27±0.18 and 0.26±0.12, respectively) were numerically lower than FSH treated does (0.70±0.40 and 1.30±0.80, respectively). However, the differences were not significant ($p > 0.05$) between the groups for the left and right ovaries. All the embryos recovered in this experiment were morula (Fig. 2).

DISCUSSION

In this study, all the donor does showed signs of oestrus after 14 days CIDR treatment. This value was similar to the findings of Lehloenya *et al*. (2008) and Lehloenya and Greyling, (2009) who recorded 100% oestrus for Boer goat using 17 days CIDR and superovulated with FSH. On the other hand, present result was superior to some

Table II.- Comparison of reproductive performances among the ovaries of goats treated with eCG and FSH.

Parameters	Gonadotrophin sources (Mean±SEM)	
	eCG	FSH
CL on left ovary/doe (No.)	3.53±0.94	2.60±0.65
CL on right ovary/doe (No.)	3.20±0.92	3.80±0.90
Anovulated follicles on left ovary/doe (No.)	7.40±1.73	7.90±1.46
Anovulated follicles on right ovary/doe (No.)	5.60±1.42	5.80±0.80
Embryos collected from left ovary/doe (No.)	0.27±0.18	0.70±0.40
Embryos collected from right ovary/doe (No.)	0.26±0.12	1.30±0.80
Structure recovered (embryos plus unfertilised oocytes) from left ovary/doe (No.)	0.93±0.37	1.40±0.58
Structure recovered (embryos plus unfertilised oocytes) from right ovary/goat (No.)	0.86±0.48	2.40±0.92

No significant ($p > 0.05$) differences among the gonadotrophin sources and ovaries for all the parameters

researchers, who described 87% and 85% of does showed oestrus using different amount of eCG with intravaginal sponges (Kelidari *et al*., 2010) and CIDR (Xiao *et al*., 2013), respectively. This data indicated that CIDR was an effective means of oestrus synchronisation of crossbred does at Malaysia. The average time interval from CIDR removal to oestrus was 36.00±6.93 h for eCG and 27.00±3.00 h for FSH group, which was shorter than the findings of Goel and Agrawal, (2005) who reported 38.4±8.2 h and 36.0±8.2 h, respectively, for the Jakhrana goat. The mean duration of oestrus was 36.00±4.90 h for eCG and 33.00±3.00 h for FSH group, which were longer than other breeds such as 25 h in Alpine (Fonseca *et al*., 2008), 31 h in Boer (Greyling and Van der Nest, 2000) and 32 h in Toggenburg (Zambrini, 2006).

In this study, numbers of anovulated follicles per doe were 13.00±3.01 and 13.70±2.17 for eCG and FSH groups, respectively. The reason of that unovulated follicle has not been identified. It might be a combination of several factors, like the goats used in this study were from unknown genetic background, improper nutrition (no flushing diet was provided), FSH preparation and protocol of

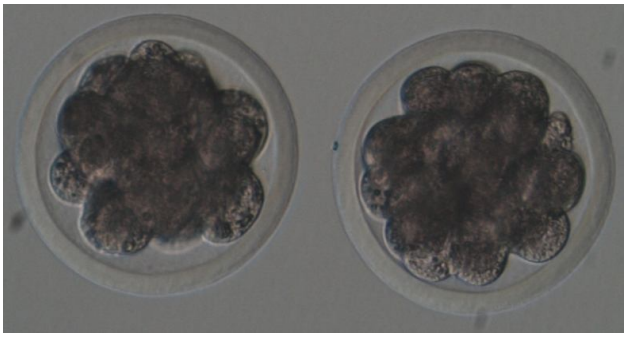


Fig. 2. Recovered goat morula at 20X magnification.

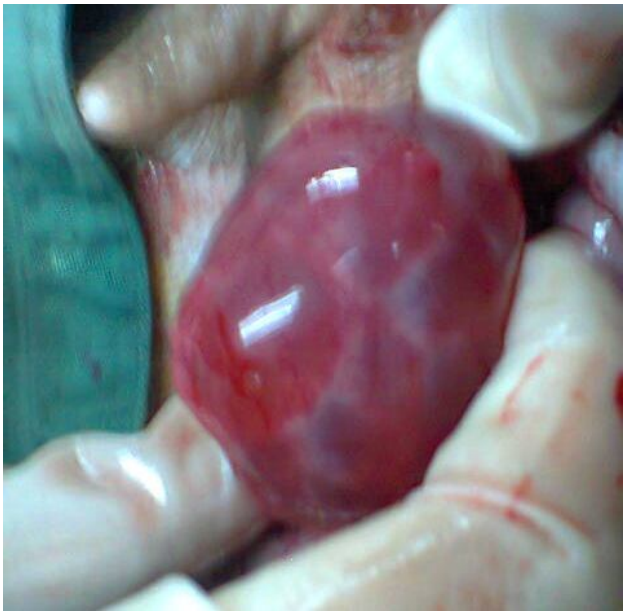


Fig. 3. Superovulated ovary with anovulated follicles.

administration, dosages of exogenous gonadotrophin. Immature or early atretic follicles promoted to grow by the gonadotrophin treatment might be one of the causes for anovulation. Most of the follicles secreted low levels of oestrogens and did not affect the viability of oocytes/embryos from ovulated follicles (Veiga-Lopez *et al*., 2006).

Average number of CL was similar for both groups. In eCG treated group, the number of CL was 6.73±1.70 and for FSH was 6.40±1.30; both the values were lower than the findings of Goel and Agrawal (2005) who reported 8.4±2.3 and 11.8±2.9, respectively, for the Jakhrana goats. In this experiment, the superovulatory responses were very

low and this result may be due to the influence of breed, weight and nutrition. Bindon *et al*. (1986) reported that prolific breed respond better to exogenous gonadotrophin. Mani *et al*. (1992) reported that low level of nutrition reduced ovulation rate and Abecia *et al*. (2006) and Scaramuzzi *et al*. (2006) reported that animal feeding with flushing diets increased folliculogenesis and ovulation rates. In this study, FSH-treated groups produced numerically more embryos than eCG treated group and this result was similar with Goel and Agrawal (2005) who observed in Jakhrana goats. Other researchers also observed higher numbers of total and transferable embryos in FSH than in eCG treated does and they reported significant differences in embryo recovery (Armstrong *et al*., 1983a; Goel and Agrawal, 1990; Mahmood *et al*., 1991; Pendelton *et al*., 1992). eCG treatment resulted in the production of lower number of embryo. The reason has not been identified, but, it was suggested that the high embryo loss after synchronisation and eCG treatment was caused by a functionally poor CL bringing low doses of progesterone (Chao *et al*., 2008).

In the present study, the embryo production using eCG was 0.20, which was less than other breeds like, 3.00 in Jamnapari (Goel and Agrawal, 1990); 2.50 in Pashmina (Mahmood *et al*., 1991); 1.75 in Assam local (Rajkhowa *et al*., 1992); 7.90 in Angora (Armstrong *et al*., 1983a); and using FSH treatments was 2.00, which was similar to 2.07 in Jamunapari (Goel *et al*., 1993) and lower than 5.11 in Jamnapari (Goel and Agrawal, 1990); 4.72 in Pashmina (Mahmood *et al*., 1991); 8.70 in Alpine and Nubian (Nuti *et al*., 1987); 6.0 in Korean Native goats (Lee *et al*., 2000). Ovarian hyperstimulation (more than 15 observable CL per doe) was observed in two does treated with eCG. As a result of eCG administration in these does, ovarian size increased many fold and ovarian surfaces had multiple unovulated follicles, giving a blister-like appearance (Fig. 3). One doe of each group had no stimulation and one doe of FSH treated group had no ovulation. The entire embryos recovered from this experiment were morula, which was similar with the result of Sasada *et al*. (2001). Although no significant difference among the left and right ovaries, but right ovary showed comparatively more response than the left ovary. This findings coincided with the findings in sheep (Moghaddama and Gooraninejad, 2007), in goat (Al-Baggal *et al*., 1993) and in human (Lan *et al*., 2010).

In conclusions, oestrus of crossbred goats can be synchronised effectively by using 14 days CIDR and superovulatory treatment using eCG was comparable with that of FSH in producing embryos. However, further studies are needed with large sample size to conclude about the effectiveness of gonadotrophin source for goat superovulation.

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