Ovarian responses after superovulation with different dosages of oFSH in goats

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Abstract

A total of 35 crossbred goats (*Capra hircus)* were used to evaluate the effect of oFSH dosages on ovarian responses during superovulation. A controlled internal drug release (CIDR) was inserted for 14 days in each goat. The animals were divided into 3 groups. Groups M_1 and M_2 received 8.8 mg and 14.08 mg, respectively, of oFSH through multiple intramuscular (i.m.) injections starting from 2 days before the CIDR removal and Group S received 8.8 mg of oFSH through a single i.m. injection upon CIDR removal. Ovarian responses of all treatments were evaluated during laparotomy session on Day 3 or 7 after CIDR removal. All of the does (100%, 22/22) of Groups M_1 and M_2 responded positively to the treatments by ovulating at least 1 tertiary follicle. On the other hand, only 69% (9/13) of the does of Group S responded correspondingly to the treatment. The total ovarian responses (CL plus anovulatory follicles) among the treatments were significantly higher ($P<0.05$) in Groups M_1 and M_2 compared with Group S. There were no significant differences between multiple-administration groups for CL per doe (high dosages vs. low dosages: 16.9±3.9 vs. 11.6±1.0, respectively). The percentage of anovulatory follicles was significantly higher ($P<0.05$) in Group S followed by Groups M_1 and M_2. The structure (embryo plus oocyte) recovery rate per doe was significantly the highest ($P<0.05$) in Group M_2 followed by Group M_1 and Group S (5.4±2.4, 3.2±1.2 and 0.5±0.5, respectively). The numbers of embryos per doe were 0.1, 2.4 and 2.0, for Groups S, M_1 and M_2, respectively. It is concluded that multiple injection at high dosage (14.0 mg) of oFSH in goat superovulation protocol gives better structure recovery than multiple injection at low dosage (8.8 mg) as well as single injection (8.8 mg) of oFSH. However, more detailed research is needed to optimize the dosage of oFSH for viable embryo recovery during superovulation of Malaysian crossbred goats.

Keywords: Superovulation, oFSH dosage, ovarian response, multiple-single injection, goats.

Introduction

Continuous supply of viable embryos is the key factor to ensure the success of any multiple ovulation and embryo transfer (MOET) programme in goats. Specifically, superovulation is the most fundamental step in obtaining multiple embryos from a donor animal. In any superovulation protocol, the ovarian response is controlled by the administration of different preparations of exogenous gonadotrophins, resulting in follicular recruitment, maturation and finally ovulation of a large number of responded follicles. Superovulation protocol in goats normally consists of oestrus synchronisation by inserting 11-17 days of controlled internal drug release (CIDR) into the vagina of goats and superovulation treatment by administration of gonadotrophins starting at 48-72 hours before CIDR removal (McNatty *et al.*, 1989; Selgrath *et al.*, 1990; Gonzalez-

Bulnes *et al*., 2003). Superovulation responses vary, depending on extrinsic (origin and purity of gonadotrophin and superovulation protocol, e.g. dosages, single or multiple administration of gonadotrophin and nutrition) and intrinsic factors such as breed, age and breeding stage (Gonzalez-Bulnes *et al*., 2004). Superovulation protocols were developed for different goat breeds in the temperate region, however, there is a scarcity of information regarding the superovulation protocol for goats in the tropical environment including Malaysia. Therefore, the present study was carried out to evaluate the effect of oFSH dosages on ovarian responses during superovulation in goats.

Materials and Methods

Animals and location of research

Thirty five mature mixed Malaysian crossbred (Boer X Katjang) does (1 to 4 years old) of 18 to 45 kg body weight were randomly selected and reared under an intensive management system at the Institute of Biological Sciences Mini Farm, University of Malaya, Kuala Lumpur, Malaysia. Does were fed daily with pellets containing 15% crude protein, 15% crude fibre, 0.5% phosphorus and 0.8-1.5% calcium in the morning at a rate of 400 g/head and Napier grass in the evening. Clean water and salt lick were supplied *ad libitum*. The study was conducted between December 2009 and July 2010.

Hormonal treatments

Oestrus was synchronized by using 14-day controlled internal drug release (CIDR: 0.33 g natural hormone progesterone; EAZI-BREED CIDR, Pharmacia & Upjohn Limited, NZ) insertion. The donors were randomly divided into three groups and the treatments were randomly assigned. The superovulatory treatments were as follows: Group M_1 (n=13) received a total dosage of 8.8 mg oFSH (OVAGEN™, ICPbio Limited, New Zealand) through 8 equal (1.1 mg x 8) i.m. injections, Group M_2 (n=9) received a total dosage of 14.08 mg oFSH in 8 decreasing dosages of 2.64, 2.64, 1.76, 1.76, 1.76, 1.76 and 0.88, 0.88 mg, respectively, i.m. injection and Group S (n=13) received a single injection of 8.8 mg oFSH. Multiple injections were given twice daily at 12-h intervals (morning and evening) and started from 2 days before the CIDR removal and single treatment was given at the time of CIDR withdrawal. A single i.m. injection of 250 IU hCG (Ovidrel®, PreFilled Syringe, Industria Farmaceutica Serono, S.P.A., Bari, Italy) was administered 36 h after CIDR removal in Groups M_1, and M_2 and 2 h after CIDR removal in Group S. After CIDR withdrawal oestrus detection was performed with fertile adult bucks at 24, 36 and 48 h and observed for symptoms of oestrus such as tail wagging, frequent urination, allowing male and other female to mount on. During standing oestrus all of the does were naturally bred using 3 bucks with proven fertility.

Ovarian responses assessment

Ovarian responses for all the treatments were evaluated during laparotomy sessions on Day 3 or 7 after CIDR removal. In other words, embryos from all groups were recovered on Day 3 for oviduct flushing or Day 7 for uterus flushing. The donor goats were off-feed and water for 15 h before surgery. Goats were anaesthetized with Xylazine hydrochloride (0.22 mg/kg body weight) and Ketamine hydrochloride (1.1 mg/kg body weight). After anaesthesia, the reproductive tract of the goat was exteriorized through a mid-ventral incision

and flushed with a flushing medium consisted of phosphate buffered saline (PBS) supplemented with Gentamycin, heparin and bovine serum albumen. A 2-way foley catheter (Imex®) was used for embryo flushing. The recovered structures (embryos plus unfertilized ova) in the flushing medium were evaluated and classified as embryo and unfertilized ova by using a stereomicroscope (SZH10; Olympus Optical Co. Ltd, Japan). Number of anovulatory follicles and number of viable embryos were analyzed by using analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT) of SPSS (Statistical Packages for Social Sciences) for windows, version 14, SPSS Inc, USA.

Results and Discussion

Ovarian responses of three hormonal treatment groups are presented in Table 1. All does (100%, 22/22) of Groups M_1 and M_2 responded positively to the treatments by ovulating at least 1 tertiary follicle. On the other hand, only 69% (9/13) of the does of Group S responded to the corresponding treatment. The total ovarian responses (CL plus anovulatory follicles) among the treatments were significantly higher ($P<0.05$) in multiple administration groups (Groups M_1 and M_2) compared with the single administration group (Group S).

Although no significant ($P>0.05$) difference was found between multiple administration groups for percent ovulation and CL per doe, higher dosage of oFSH gave higher results (72.4%±7.6 vs. 58.8%±3.9 and 16.9±3.9 vs. 11.6±1.0, respectively). The anovulatory percentage of follicles was significantly ($P<0.05$) higher in Group S followed by Groups M_1 and M_2. The structures (embryo plus unfertilized oocyte) recovered per doe was significantly ($P<0.05$) higher in Group M_2 followed by Group M_1 and in Group S (5.4±2.4, 3.2±1.2 and 0.5±0.5, respectively).

Table 1. Ovarian response (mean±SEM) in superovulated donor goats

Parameter	Treatment group[1]		
	S	M_1	M_2
Number of animals (n)	13	13	9
Percent responded does (n)	69 (9)	100 (13)	100 (9)
Ovarian response CL & AF/doe	8.5±1.4[a]	20.0±1.5[b]	23.7±4.4[b]
Percent ovulation	35.5±10[a]	58.8±3.9[b]	72.4±7.6[b]
CL/doe	2.7±0.7[a]	11.6±1.0[b]	16.9±3.9[b]
Percent anovulatory follicles	60.7±11.[b]	41.2±3.9[ab]	28.2±7.8[a]
No. of anovulatory follicles (AF/doe)	6.2±1.5[a]	8.4±1.1[a]	7.0±1.7[a]
Structures of embryos & ova recovered/doe	0.5±0.5[a]	3.2±1.2[ab]	5.4±2.4[b]
Unfertilized and degenerated ova/doe	0.5±0.5[a]	0.8±0.5[a]	3.4±2.0[a]
Viable embryos/doe	0.1±0.1[a]	2.4±1.0[a]	2.0±1.4[a]

[1]S - single administration of 8.8 mg oFSH, M_1 - multiple administrations of 8.8 mg oFSH and M_2 - multiple administration of 14.08 mg oFSH

[abc] Means with different superscripts within a row differed significantly ($P<0.05$)

Table 2 shows the number of CL and anovulatory follicles of superovulated donors. Does of Groups M_1 and M_2 showed good response of 62 and 56%, respectively, by ovulating more than10 CL, however 46% does of Group S showed poor response by ovulating <5 CL and none of them showed good response. Both ovaries showed similar trends of number of CL per doe with the values of 8.8 to 9.6, 5.6 to 6.3 and 1.0 to 1.6 for M_2, M_1 and S, respectively. Percentage of does without anovulatory follicle was higher in Group M_2 followed by S and M_1 ($P>0.05$).

There were 0.1, 2.4 and 2.0 viable embryos per doe for Groups S, M_1 and M_2, respectively. The number of recovered viable embryos per doe by using FSH was reported to be 6.6 in Jakhrana (Goel and Agrawal, 2005), 2.07 in Jamnapari (Goel *et al.*, 1993), 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) and 6.0 in Korean Native goats (Lee *et al.*, 2000).

Among the three oFSH treatments used in the current study, ovarian responses, specifically in terms of percentage of responded does, percent ovulation, CL/doe, anovulatory percentages showed better results in multiple oFSH administration groups compared to the oFSH single administration. It is concluded that multiple injection at high dosage of oFSH in goat superovulation protocol gives better ovarian responses than multiple injection at low dosage as well as single injection of oFSH. However, more detailed research is needed to optimize the amount of oFSH for superovulation of Malaysian crossbred goats.

Table 2. No. of CL and anovulatory follicles in superovulated donor goats

Parameter	Treatment group[1]		
	S	M_1	M_2
CL/doe	2.7±0.7[a]	11.6±1.0[b]	16.9±3.9[b]
Percent of good responder does (>10 CL) (n)	0 (0)	62 (8)	56 (5)
Percent of moderate responder does (6-10 CL) (n)	23 (3)	38 (5)	33 (3)
Percent of poor responder does (1-5 CL) (n)	46 (6)	0 (0)	11 (1)
Number of CL on left ovary/doe	1.0±0.5[a]	6.3±0.7[b]	8.8±1.8[b]
Number of CL on right ovary/doe	1.6±0.5[a]	5.3±0.6[b]	9.6±1.9[c]
Percent does without anovulatory follicles (n)	15(2)	8(1)	23(2)
Number of anovulatory follicles on left ovary/doe	3.2±0.8[a]	3.3±0.6[a]	4.3±1.3[a]
Number of anovulatory follicles on right ovary/doe	3.1±1.1[a]	5.1±0.9[a]	4.1±1.0[a]

[1]S - single administration of 8.8 mg oFSH, M_1 - multiple administrations of 8.8 mg oFSH and M_2 - multiple administration of 14.08 mg oFSH

[abc] Means with different superscripts within a row differed significantly ($P<0.05$)

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