Effect of supplementation of hCG or GnRH on ovulation and subsequent embryo production of eCG superovulated goats

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ABSTRACT
The aim of this study was to enhance the ovulation and subsequent embryo production of equine chorionic gonadotrophin (eCG) superovulated goats by supplementing with human chorionic gonadotrophin (hCG) or gonadotrophin releasing hormone (GnRH). Thirty crossbred donor does were oestrus synchronized using controlled internal drug release (CIDR) device and superovulated using 1500 IU eCG. Then all the donor does were randomly divided into 3 groups. Group 1 were administered 500 IU hCG each at 2 successive days starting from 24 hours after CIDR removal; Group 2 received a single dosage of 20 µg GnRH and Group 3 was control. Ovulation and embryo production responses were evaluated during laparotomy session on Day 7 after CIDR removal. Does in Group 1 showed higher number of CL (10.90) than the Group 2 (1.90) or Group 3 (0.90). The number of ovarian stimulation and number of anovulatory follicles had no significant (P>0.05) differences among the treatment groups. Average number of structure recovered was significantly (P<0.05) higher in Group 1 (3.10) than Group 2 (0.70) or Group 3 (0.00). Moreover, average number of embryo production was higher in Group 1 (0.90) than Group 2 (0.50) and Group 3 (0.00), but no significant difference was observed between Group 1 and Group 2. Results indicated that using hCG hormone would be an effective means for increasing ovulation in eCG superovulated crossbred goats, although it was unable to enhance the embryo number due to increase number of unfertilised ovum.

Key words: Equine chorionic gonadotrophin (eCG), Goat, Gonadotrophin releasing hormone (GnRH), Human chorionic gonadotrophin (hCG), Superovulation.

INTRODUCTION
Equine chorionic gonadotrophin (eCG) is the first gonadotrophin used for goats superovulation and it has some advantages like single administration, low cost and easily available while it has some disadvantages like long biological half-life, heterogeneous follicular growth, large number of anovulated follicles, early regression of corpus luteum (CL), reduced fertilisation and embryo quality, and finally impaired embryo production outcome (Cognie, 1999). Equine chorionic gonadotrophin stimulates follicular growth and recruits immature follicles in ovaries, while leutinising hormone (LH) is generally required for successful superovulation (Howles, 2000). In oestrus synchronised goat the level of progesterone on the day of insemination might be a crucial factor for deciding pregnancy (Sneha et al., 2015). Numerous efforts have been made to minimise the disadvantageous effect of eCG by reducing premature CL regression by Ovsynch protocol (Holtz et al., 2008) and using a simplified FSH/eCG treatment (Forcada et al., 2011) in a superovulation protocol.

Human chorionic gonadotrophin (hCG) has structural similarity with LH and it showed luteotrophic activity in eCG superovulated goats (Saharrea et al., 1998). The hCG administration at different stages of oestrous cycle in goats induced additional CL formation and increased circulating progesterone levels (Fonseca et al., 2005, 2006). It also increased the total weight of the CL in ewes and cattle (Schmitt et al., 1996). The administration of hCG during the early luteal stage might be induced supplementary CL formation (Rajamahendran and Sianangama, 1992).

On the other hand, administration of GnRH at the time of mating improved luteal function as a result of the release of FSH and LH from the anterior pituitary (Hafez and Hafez, 2000). The endocrine effect of GnRH administration is a temporary increase in plasma progesterone and oestradiol concentrations, followed by a prolonged decrease in oestradiol concentrations.

Some researchers used hCG for ovulation induction, evaluating follicular and luteal characteristics and serum progesterone concentrations in eCG-superovulated does.
(Kelidari et al., 2010) and pharmacokinetics after i.m. administration (Saleh et al., 2012). Many researchers used hCG and GnRH for improving the synchrony of timing of the LH surge and ovulation (Pierson et al., 2003), pregnancy rate and litter size (Riaz et al., 2012), homogeneity in development of the embryos collected (Baldassarre et al., 2004) and pre-treatment for improving superovulation response and embryo recovery (Heidari et al., 2010). Some researchers used hCG and GnRH for evaluating premature luteal regression in eCG superovulated does (Saharrea et al., 1998), for improving ovarian function, conceptus growth and development (Khan et al., 2007) and pregnancy and lambing in ewes (Kaya et al., 2013). Currently, there is little information available on the effect of hCG or GnRH supplementation on the ovulation and subsequent in vivo embryo production in eCG superovulated goats in an embryo production programme. Therefore, this study was carried out to determine the effects of hCG or GnRH supplementation on the ovarian function, and their subsequent effects on in vivo embryo production of eCG superovulated goats.

MATERIALS AND METHODS

Experimental animal and location: This study was carried out with 30 mature crossbred (Boer × Katjang) does at the Institute of Biological Sciences Farm (2°30’ N, 112°30’ E), University of Malaya, Malaysia. The location is 60 m above sea level and has annual rainfall of 2600 mm. All animal used in this study were in accordance to the guidelines of University of Malaya. Average body weight (BW) and age of does used in this study were 25 kg and 4 years, respectively. The experimental does were reared under intensive management system and received fresh soya waste (20% DM) at a rate of 1 kg/head/d. The soya waste contained 27.9% crude protein, 30.5% neutral detergent fibre and 5.3% ash. Soya waste which was offered to the animal once in the morning, while Napier grass (Pennisetum purpureum) was offered in the morning and afternoon. All animals had free access to water and salt lick.

Oestrus synchronisation, detection and superovulation treatments: All the donor does were synchronised for oestrus by inserting a CIDR (0.33 g natural progesterone hormone; EAZI-BREED CIDR, Pharmacia & Upjohn Limited, New Zealand) for 14 days followed by a intramuscular injections of 125 µg of PGF2α (Estrumate®; Intervet International B.V. Netherlands) on 2 days before the CIDR removal. Superovulation was carried out by administering 1500 IU eCG (Folligon®; Intervet International B.V. Netherlands) on 2 days before the CIDR removal. After CIDR removal, oestrus was observed 3 times in a day (morning, afternoon and evening) until exhibition of overt oestrus by placing a buck of proven libido and mating was done. Subsequently, all the donor does were divided equally into 3 treatment groups and animal received with or without hCG (Ovidrel®; PreFilled Syringe, Industria Farmaceutica Serono, S.P.A., Bari, Italy) or GnRH (Receptal®; Intervet International B.V. EU) after CIDR removal. The 3 treatment groups were (i) administered with 2 dosages of 500 IU hCG each at 2 successive days (Group 1) (ii) administered with single dosage of 20 µg GnRH (Group 2) (iii) without hCG or GnRH (Group 3).

Surgical procedure for ovarian responses assessment: Ovarian responses evaluation and embryos collection were carried out during laparotomy session on Day 7 after CIDR removal. The donor goats were off-feed and -water for 16 to 20 hour before surgery. For goat anaesthesia, a mixture of xylazine hydrochloride and ketamine hydrochloride (1:50) was administered through intramuscular injection at a rate of 11 mg/kg BW. After anaesthesia, the reproductive tract was exteriorised through a mid-ventral incision and the superovulatory responses were assessed by counting the CL and anovulatory follicles of both ovaries. Both the fallopian tubes were flushed using a flushing medium consisted of phosphate buffered saline (PBS) supplemented with streptomycin, penicillin and polyvinylpyrrolidone (PVP). A two-way Foley catheter was used for embryo flushing and all embryos and unfertilised oocytes recovered, evaluated and classified according to the stage of embryonic development (embryo or unfertilised oocytes) accordingly under a stereomicroscope (SZH10, Olympus Optical Co. Ltd., Japan).

Statistical analysis: The effects of hormones on ovulation and subsequent embryo production were examined by one-way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) test using SPSS software (version 16.0, Statistical Package for the Social Sciences software, Chicago, IL, USA). Results were expressed as mean ± SEM (standard error of mean), and differences were considered as significant when P<0.05.

RESULTS AND DISCUSSION

Ovarian stimulation of crossbred goats as affected by hCG or GnRH is presented in Table 1. All the donor does (100%) of three treatment groups showed signs of standing oestrus within 24 hour upon CIDR removal. Among the treatments, the higher number of does in Group 1 (10) had CL than the Group 2 (5) or Group 3 (4). Does in Group 1 showed significantly higher number of CL (10.90) than the Groups 2 (1.90) and 3 (0.90). Similarly, ovulation percentage also followed same trends and it was 61.77 for Group 1, 11.40 for Group 2 and 10.24 for Group 3. However, the number of ovarian stimulation and number of anovulatory follicles had no significant (P>0.05) differences among the treatment groups.

Embryo production performances of eCG superovulated does supplemented with hCG or GnRH are presented in Table 2. No structure (embryo plus unfertilised oocyte) was recovered from Group 3. Average number of structure recovered was significantly (P<0.05) higher in
Table 1: Effect of hCG or GnRH on ovulation.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 (Mean±SEM)</th>
<th>Group 2 (Mean±SEM)</th>
<th>Group 3 (Mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of doe</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Number of doe showed oestrus</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Average number of CL/doe</td>
<td>10.90±1.73b</td>
<td>1.90±0.91a</td>
<td>0.90±0.50a</td>
</tr>
<tr>
<td>Average number of anovulatory follicle (AF)/doe</td>
<td>9.80±4.10a</td>
<td>14.20±4.18a</td>
<td>13.80±3.50a</td>
</tr>
<tr>
<td>Average number of ovarian stimulation (CL + AF)/doe</td>
<td>20.70±4.42a</td>
<td>16.10±4.19a</td>
<td>14.70±3.41a</td>
</tr>
<tr>
<td>Ovulation percentage*</td>
<td>61.77±10.13b</td>
<td>11.40±5.69a</td>
<td>10.24±7.05a</td>
</tr>
</tbody>
</table>

*a,b Mean values with different superscripts within a row were significantly different (P<0.05). SEM= standard error of mean. *Ovulation percentage was calculated for each donor and from that the average was calculated.

Table 2: Effect of hCG and GnRH on embryo production characteristics

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 (Mean±SEM)</th>
<th>Group 2 (Mean±SEM)</th>
<th>Group 3 (Mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of doe flushed</td>
<td>10</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Average number of embryos/doe</td>
<td>0.90±0.35a</td>
<td>0.50±0.40a</td>
<td>0</td>
</tr>
<tr>
<td>Average number of transferable embryos/doe</td>
<td>0.40±0.22a</td>
<td>0.30±0.21a</td>
<td>0</td>
</tr>
<tr>
<td>Average number of degenerated embryos/doe</td>
<td>0.50±0.27a</td>
<td>0.20±0.20a</td>
<td>0</td>
</tr>
<tr>
<td>Average number of structures recovered/doe</td>
<td>3.10±1.02b</td>
<td>0.70±0.42a</td>
<td>0</td>
</tr>
<tr>
<td>Recovery rate (%)</td>
<td>25.89±7.57a</td>
<td>21.11±11.53a</td>
<td>0</td>
</tr>
<tr>
<td>Average number of unfertilised oocytes/doe</td>
<td>2.20±0.81b</td>
<td>0.20±0.13a</td>
<td>0</td>
</tr>
</tbody>
</table>

*a,b Mean values with different superscripts within a row were significantly different (P<0.05). SEM, standard error of mean.

Group 1 (3.10) than Group 2 (0.70), while recovery rate was not showed any significant difference between Group 1 (25.89) and Group 2 (21.11). Although there was no significant difference for average number of embryo production, Group 1 (0.90) produced numerically higher number of embryo than Group 2 (0.50). There was no significant difference on transferable and degenerated embryos between the treatment groups. Although, the number of structure recovered was higher in Group 1 than Group 2, it was negated by the production of significantly higher number of unfertilised oocytes in Group 1 (2.20) than Group 2 (0.20).

Oestrus sign in does is a very important physiological reproductive behaviour typical in mammalian species particularly signifying ovulation during the oestrous cycle to facilitate successful natural mating and application of numerous reproductive techniques such as superovulation, artificial insemination and embryo transfer. In this study, regardless the hormonal treatment, 100% of the donor does showed sign of oestrus which was similar with the findings of earlier researchers who reported 100% of oestrus by using different dosages of eCG with CIDR (Motlomelo et al., 2002). On the other hand, other researchers reported lower values which were 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. Our present research data also confirmed that CIDR could be an effective means of oestrus synchronisation of Boer crossbred does.

Equine chorionic gonadotrophin superovulatory responses on number of ovulation and ovulation percentage in this study were significantly higher in hCG-supplemented group compared to GnRH-supplemented and control groups. This might be due to the similarity of the time of LH surge of hCG-supplemented doe with the non-superovulated normal cyclic doe. The time interval between onset of oestrus and LH surge was 11 to 13 hour for normal cyclic goats (Greyling and van Niekerk, 1990). However, this aspect was not carried out in this study. Saleh (2011) reported that the time interval between GnRH- or hCG-supplementation and LH surge were 1 and 12 hour, respectively. From pharmacokinetics point of view, the faster absorption (12 hr) and slower elimination (70 hr) of hCG resulted prolonged bioavailability in the circulation, maintaining the function of CL (Saleh et al., 2012). In addition, hCG had been reported to have long half-life (40 hr) compared with the endogenous LH in doe (Saleh et al., 2012).

In spite of the above hypothesis, administration of GnRH at the time of mating improved luteal function as a result of the release of FSH and LH from the anterior pituitary (Hafez and Hafez, 2000). Additionally, improved ovulation synchronisation in does by reducing the variation of timing between sponge removal and LH surge from 40 to 26 hour (Pierson et al., 2003), increased the fertility in ewe (McMillan et al., 1986) and cow (Sheldon and Dobson, 1993). On the contrary, supplementation of GnRH at the time of mating did not improve the pregnancy rate in doe which might be due to an endogenous LH peak before administration of GnRH (Riaz et al., 2012). The result of present research was similar with the findings of above mentioned research.

In this study, the average numbers of recovered and transferable embryos were higher in hCG-supplemented group than GnRH-supplemented and control groups, which
was in agreement with the findings of Saleh (2011). The reason of this result might be due to premature luteal regression after superovulation. According to Saharrea et al. (1998), 100% of hCG-supplemented does had functional CL, while 57% of the control does and 38% of the GnRH-supplemented does had undergone premature luteal regression. This luteal malfunction might be due to untimely PGF2α secretion (Saharrea et al., 1998; Taponen et al., 2003). Saharrea et al. (1998) reported average progesterone concentration in the hCG-supplemented does (11 ng/ml) at Day 6 was higher than GnRH-supplemented does (4 ng/ml). In addition, Kaya et al. (2013) did not observe any increase in plasma progesterone level due to supplementing GnRH in ewe. Aslan et al. (2011) reported that hCG-supplemented cow resulted in an increase in the CL blood flow significantly than GnRH supplementation.

The numbers of anovulated follicles per doe in this study were varied from 10 to 14, which was similar among the supplemented does. The result was contradicted with the finding of Saleh (2011) who reported higher number of anovulated follicle in hCG-supplemented does than GnRH-supplemented does. The reason for having anovulated follicle has not been identified. It might be a combination of several factors, like source and purity of the gonadotrophin (Lindsell et al., 1986), alterations in endocrine patterns, such as lowered endogenous LH secretion (Kendall et al., 2004) or defective or mistimed LH surges (Noel et al., 1994; D’Occhio et al., 1999), dominant follicles present in the ovaries at the onset of the superovulatory treatment (Veiga-Lopez et al., 2006), atretic follicles rescued by superovulatory treatments (Monniaux et al., 1983), defective maturation of follicles (Veiga-Lopez et al., 2006) and desensitisation of the LH receptors of the follicles (Garcia-Garcia, 2003).

Superovulatory responses using eCG under present study were not up to expectation, which might be due to the influence of different factors or their combinations such as breed, age and reproductive status. Moreover, different goat breeds produced variable results by using eCG for superovulation such as 3 embryos in Jamunapari (Goel and Agrawal, 1990), 3 embryos in Boer goats (Nowshari et al., 1995); 6 embryos in Jakhrana goats (Goel and Agrawal, 2005); 2 embryos in indigenous dairy goats (Pampukidou et al., 2011) and 1 embryo in crossbred does (Xiao et al., 2013).

Although, both hCG and GnRH were supplemented for ovulation synchronisation, hCG supplementation produced better results. Human chorionic gonadotrophin supplementation produced a significantly higher number of structures but due to higher number of unfertilised oocyte total recovered and transferable embryos were not increased significantly. LH had a significant effect on the embryo production performance of eCG-supervoluted does. In contrast to GnRH, present results indicated that hCG-supplementation was suitable for ovulation induction in the eCG-supervoluted Boer crossbred goat.

It is concluded that hCG stimulated higher number of ovulation as well as higher number of recovered and transferrable embryos than GnRH and control groups. However, this increased ovulation was negated by a large number of unfertilised oocytes. Further studies are needed to conduct for increasing the fertilisation rate of ovulated oocytes in eCG superovulated goats which will help to establish a eCG based superovulation protocol for ET programme.

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