USE OF A GnRH AGONIST TO DELAY THE OVULATION AND INJECTION OF hCG TO INDUCE OVULATION IN GOATS SUPERSTIMULATED WITH FSH

PENGUNGAAN AGONIS GnRH UNTUK MENUNDATA OVULASI DAN PEMBERIAN hCG UNTUK INDUKSI OVULASI PADA KAMING YANG DISUPERSTIMULASI DENGAN FSH

Aris Jusadi 1 and Scott Thomas Norman 2

1Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta 55281, Indonesia.
2University of Queensland, PO Box 128 St Lucia, 4000, Qld, Australia

ABSTRACT

The aim of this study was to evaluate the effect of delaying ovulation subsequent to superstimulation with FSH in goats on the ovarian responses. Ten mature does of mixed breeding were used in this study. All does were implanted subcutaneously with GnRH agonist deslorelin for 15 days. Each doe received a total of 18 mg FSH in six injections of decreasing doses 12 hours apart for 4 days, starting at day 7 after deslorelin insertion. On day 9 each doe was treated with 5 mg dienogest trometamime (PGF2α, Lutalyse®). Ovulation was induced by injection of 500 I.U. hCG (Chorulon®, Intervet Australia) on Day 11 and does were ran with the buck to assist with monitoring of estrus behaviour. The ovaries of all the does were examined laparoscopically 7 days after the hCG was administered. In each case the CL’s were counted and classified as either normal or as regressing. The results from laparoscopic examination showed that the number of CL, involuted follicle and CL albuscens were as follows 2.5 ± 1.62, 2.8 ± 1.29 and 0.8 ± 0.62, respectively. The onset of estrus and the duration of behavioural estrus following prostaglandin injections were 37.6 ± 3.48 and 35.8 ± 3.87, respectively. It was concluded that delaying ovulation using GnRH agonist implant and inducing ovulation using hCG in superstimulated goats did not improve the number of ovulations as indicated by the total number of CL.

Key words: GnRH agonist, superovulation, FSH, hCG

ABSTRAK

Tujuan dari penelitian ini adalah untuk mengevaluasi pengaruh penundaan ovulasi pada kambing yang disuperstimulasi dengan FSH pada respon ovarium. Sepuluh ekor kambing dewasa dari rasi campuran digunakan dalam penelitian ini. Seluruh kambing betina diberi implant secara subcutan dengan agens GnRH deslorelin selama 19 hari. Setiap kambing disuperstimulasi dengan 18 mg FSH yang diberikan secara bersamaan selama 12 jam selama 4 hari, dimulai pada hari ke 7 setelah implantasi deslorelin. Pada hari ke 9 setiap kambing diberi 5 mg dienogest trometamime (PGF2α, Lutalyse®). Induksi ovulasi dengan injeksi 500 I.U. hCG (Chorulon®, Intervet Australia) pada hari ke 11 dan kambing dipasangkan dengan pejantan untuk melihat dan memonitor tingkah laku estrus. Ovarium dari seluruh kambing diperiksa dengan laparoskopi 7 hari sesudah pemberian hCG. Pada setiap kasus korpus koeum (CL) dihitung dan di klasifikasikan sebagai normal atau regresi. Hasil dari penelitian laparoskopi memunjukkan bahwa CL folikel yang tidak ovulasi dan CL albuscens secara bersamaan adalah sebagai berikut 2.5 ± 1.62, 2.8 ± 1.29 dan 0.8 ± 0.62. Periode estrus dan durasi estrus setelah pemberian prostaglandin adalah 37.6 ± 3.48 dan 35.8 ± 3.87. Dikesimpulkan bahwa penundaan ovulasi menggunakan agens GnRH dan induksi ovulasi menggunakan hCG pada kambing yang disuperstimulasi tidak menambah jumlah ovulasi seperti ditunjukkan dari jumlah total CL.

Kata kunci: GnRH agonis, superovulasi, FSH, hCG
INTRODUCTION

The variability in ovarian response to the superovulatory treatment is a major problem in commercial multiple ovulation and embryo transfer (MOET) programs in sheep and goats (Bienias et al., 1992; Smith, 1986). The standard of superovulation programs with consistent of ovulatory responses in goats has not been established yet (Greyling et al., 2002) and need to be investigated (Nowakari et al., 1995).

Many attempts have been made to improve the number of ovulations and to reduce the variability of response to PMSG between animals, the new one is using GnRH agonist (D'Occhio et al., 1999). A pre-treatment over 2 weeks with a GnRH agonist based on (Hoechst) could increase the number of ovulations (McNeill and Fraser, 1987). The used of GnRH antagonist to delay the ovulation and inducing ovulation with hCG has been reported by (Rieger et al., 1990). They found a higher proportion of transferable embryos when the LH surge was delayed for 72 hours. The GnRH agonist-LH protocol was developed to desensitize pituitary GnRH in heifers, and the ovulation was induced by injecting the pLG. The researchers found that a delay in occurrence of the LH surge can increase the number of ovulations (D'Occhio et al., 1999). Buri et al., (1990) developed a new superovulatory treatment based on the control of the occurrence of the endogenous LH peak with a GnRH antagonist in goats, they found that this technique was very efficient for controlling the time of ovulation. Nogueira et al., (2002) comparing the used of GnRH agonist (deslorelin) to delay ovulation to increase embryo recovery with the conventional superovulation programme (using CIDR-B) to induce superovulation in beef cow, they found that embryos obtained for both treatment were similar. Therefore, the present investigation was designed to study the effect of implantation using GnRH agonist deslorelin on ovarian responses in goats superovulated with PMSG and inducing ovulation at the onset of estrus using hCG.

MATERIALS AND METHODS

Experimental animals:

A total of 10 mixed breed goats ranging in age from 3 to 4 years, weighing 35 – 45 kg were studied, together with one buck of proven fertility. All the animals were multiparous and the average duration from last kidding was 141.2 ± 2.4 day. The goats was in good body condition (mean 3.5 on a 5 point scoring system) and fed lucerne hay, with free access to water. They were housed in one groups in the experimental animal feedlot maintained by the Department of Animal Studies, University of Queensland, Gatton College, Gatton, Queensland. All procedures involving animals was in compliance with the Animal Code of Ethics.

Experimental design:

All does were implanted with a Gonadotropin Releasing Hormone agonist, deslorelin (Peptech Animal Health, Sydney Australia) for 19 days. Each doe received serial injections of FSH in decreasing doses 12 hours apart for 4 days, starting 7 days after deslorelin insertion. On Day 9, all does were injected intramuscularly with 5 mg dinoprost trometamol (PGF2A, Lutalyse®). On Day 11, each doe was injected intramuscularly with 500 I.U. hCG (Chorulon®, Intervet Australia, Pty. Ltd).

Oestrus detection:

Observation began immediately after prostaglandin injection and was performed four times daily for a period of two hours. Does were considered to be in oestrus when they showed outward signs including reddening of the vulva, vaginal discharge of mucous and homosexual behaviour. The first observation of one of the signs was recorded as onset of oestrus. The duration of oestrus was recorded as a time from showed a sign of oestrus on the doe appeared and injection of the doe mounted by the buck. All the animals detected in oestrus were bred by natural service with a fertile buck at approximately 12 hour intervals until the end of oestrus.
Ovarian response.

The ovarian response was determined by laparoscopic examination 7 days after the last mating. Does were considered to be responding of the treatment when the total number of CL in both ovaries was more than 3. After withholding feed for 24 hours and water for 12 hours, the doe was sedated with 0.25 ml xylazine-20 (Ilum Xylazil-20, Provst Veterinary Supplies) administered intravenously and restrained on a laparoscopic cradle. The hair was removed from an area enclosed by the anterior border of the udder to approximately 10 cm cranial to the udder and approximately 10 cm either side of the midline. The skin was surgically prepared using soap, alcohol and 1 % povidone iodine solution in that order. Three ml of local anaesthetic (2 % lignocaine, Ilum Veterinary Products, Sydney, Australia) was injected subcutaneously at each laparoscopic insertion site, approximately three cm anterior to the udder, and 3 to 4 cm on each side of the ventral midline. The cradle was then tilted at an angle of approximately 60° so that the head-quarters of the doe were elevated. Two small skin incisions were made on each side approximately three to five cm from the midline and three cm cranial to the anterior border of the udder. A 5 mm trocar and cannula was stabbed through the right skin incision and abdominal wall. Medical grade carbon dioxide gas was then insufflated into the abdomen to facilitate laparoscopic viewing of the abdominal organs. A 5 mm trocar and cannula was stabbed through the left skin incision to provide a portal for manipulation of the ovary. Both trocars were removed and the viewing telescope (Storz model 26031 B Storz, West Germany), connected to an endoscopic light source was inserted into the 7 mm cannula. The ovaries were gently manipulated to view the number of corpora lutea (CL). Corpora lutea were considered functional if they were red in colour and larger than 5 mm, as gauged by comparison to the 5 mm cannula tip. Structures less than 5 mm in diameter and pale pink to white in colour were classified as corpora albicantia. Following ovum examination, the skin incisions was closed with a single absorbable suture and sprayed with fly repellent.

Table 1. Mean (± S.E.) estrus responses, duration of estrus and ovarian responses in superovulation in goats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal treated</td>
<td>10</td>
</tr>
<tr>
<td>Onset of estrus after injection of prostaglandin (h)</td>
<td>37.6 ± 3.48</td>
</tr>
<tr>
<td>Duration of estrus (h)</td>
<td>35.8 ± 3.87</td>
</tr>
<tr>
<td>Animal responding (&gt;3CL) to superovulation treatment (n)</td>
<td>3</td>
</tr>
<tr>
<td>Ovulation rate, CL/animals</td>
<td>2.5 ± 1.62</td>
</tr>
<tr>
<td>Unovulated large follicle (UF)/animals</td>
<td>2.8 ± 1.28</td>
</tr>
<tr>
<td>Total oviums response</td>
<td>5.3 ± 1.03</td>
</tr>
<tr>
<td>CL regression</td>
<td>0.8 ± 0.61</td>
</tr>
</tbody>
</table>

Statistics.

The data was analyzed using means, standard error of the means and analysis of variance using the statistics software package Statistica Version 5.1.

RESULTS AND DISCUSSION

The onset and the duration of estrous were 37.6 ± 3.48 h and 35.8 ± 3.87 h after the injection of prostaglandin, respectively (Table 1).

The effect of treatment on ovarian response is presented in Table 1. The number of animals responding to superovulation treatment was 30%.
In the present study, all the goats (100%) exhibited estrus within 30 hours of progesterin injection. These findings suggest that the use of progesterin for synchronization estrus is not as efficient and is in agreement with those reported by Godfrey et al. (1997). The overall mean interval to onset of estrus following progesterin injections was 37.6 ± 3.48 hours, and the mean duration of the induced estrus 35.8 ± 3.87 hours obtained in this study is comparable to the previous finding 33.48 ± 5.70 hours and 36.6 ± 5.36 hours respectively (Selvaraju et al., 2003).

The implantation of GnRH agonist deslorelin and the administration of hCG at the time of the onset of estrus in goats superovulated with FSH in this study unable to increase the number of ovulations. This may due to the lack of response of some follicles to the LH secreted at that time. Furthermore, the failure of ovum response may due to their relatively small size when the FSH treatment is initiated (D’Occhio et al., 1999), or relatively small size follicles at the start of FSH treatment do not have enough time to complete normal maturation (Lis and Sirois, 1998; Xu et al., 1995) prior to the LH surge (Bevers and Dielemann, 1987).

The timing of the treatment may be important to cause ovulatory responses. Armstrong et al. (1982) found that premature luteal regression was happened when administration of hCG at the onset of estrus, it may be too early to allow these follicles to reach normal maturation.

It is concluded that the implantation of GnRH agonist deslorelin and the administration of hCG on the onset of estrus in goats superstimulated with FSH were unable to increase ovulation response. Additional research is needed to determine the timing of administration hCG which could be used to achieve higher ovulatory responses in goats implanted with GnRH agonist deslorelin.

ACKNOWLEDGMENTS

This work was funded by SEAMEO-SEARCA grant No. RL.V02-1145. We would like to thank Dr. Peter Murray, School of Animal Studies, University of Otagon, Oatton campus for the use of his goats, Ms Nancy Phillips for her valuable technical assistance and Dr. Tim Trigg (Peptech Animal Health, Sydney Australia) for the generous supply of GnRH agonist buseriplan.

REFERENCES


