

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

### PENGGUNAAN AGONIS GnRH UNTUK MENUNDA OVULASI DAN PEMBERIAN hCG UNTUK INDUKSI OVULASI PADA KAMBING YANG DISUPERSTIMULASI DENGAN FSH

Aris Junaidi<sup>1</sup> and Scott Thomas Norman<sup>2</sup>

<sup>1</sup>Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta 55281, Indonesia.

<sup>2</sup>University of Queensland, PO Box 125 Kenmore, 4069, 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 19 days. Each doe received a total of 18 mg FSH in serial 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 dinoprost trometamine (PGF<sub>2</sub> $\alpha$ , Lutalyse®). Ovulation was induced by injection of 500 I.U. hCG (Chorulon®, Intervet Australia) on Day 11 and does were run 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, unovulated follicle and CL albicans were as follows  $2.5 \pm 1.62$ ,  $2.8 \pm 1.29$  and  $0.8 \pm 0.62$ , respectively. The onset of estrus and the duration of behavioural oestrus following prostaglandin injections were  $37.6 \pm 3.48$  and  $35.8 \pm 3.87$ , 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 ras campuran digunakan dalam penelitian ini. Seluruh kambing betina diberi implan secara subcutan dengan agonis GnRH deslorelin selama 19 hari. Setiap kambing disuperstimulasi dengan 18 mg FSH yang diberikan secara berseri selang 12 jam selama 4 hari, dimulai pada hari ke 7 setelah implantasi deslorelin. Pada hari ke 9 setiap kambing diberi 5 mg dinoprost trometamine ((PGF<sub>2</sub> $\alpha$ , Lutalyse®). Induksi ovulasi dengan injeksi 500 I.U. hCG (Chorulon®, Intervet Australia) pada hari ke 11 dan kambing dilepas dengan pejantan untuk menilai dan memonitor tingkah laku estrus. Ovarium dari seluruh kambing diperiksa dengan laparoskopi 7 hari sesudah pemberian hCG. Pada setiap kasus korpus luteum (CL) dihitung dan di klasifikasikan sebagai normal atau regresi. Hasil dari pemeriksaan laparoskopi menunjukkan bahwa CL folikel yang tidak ovulasi dan CL albikan secara berurutan adalah sebagai berikut  $2.5 \pm 1.62$ ,  $2.8 \pm 1.29$  and  $0.8 \pm 0.62$ . Permulaan estrus dan durasi estrus setelah pemberian prostaglandin adalah  $37.6 \pm 3.48$  dan  $35.8 \pm 3.87$ . Disimpulkan bahwa penundaan ovulasi menggunakan agonis 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 (Brebion *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 (Nowshari *et al.*, 1995).

Many attempts have been made to improve the number of ovulations and to reduce the variability of response to pFSH between animals, the new one is using GnRH agonist (D'Occhio *et al.*, 1999). A pretreatment over 2 weeks with a GnRH agonist buserelin (Hoechst) could increase the number of ovulations (McNeilly 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 desensitized pituitary GnRH in heifers, and the ovulation was induced by injecting the pLH. The researchers found that a delay in occurrence of the LH surge can increase the number of ovulations (D'Occhio *et al.*, 1999). Baril *et al.*, (1996) 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 pFSH 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  $141.2 \pm 2.4$  day. The goats was in good body condition (mean 3.5 on a 5 point scoring system) and was 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 were 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 trometamine (PGF $2\alpha$ , 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 mucus 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 rejection 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 food for 24 hours and water for 12 hours, the doe was sedated with 0.25 ml xylazil-20 (Ilium Xylazil-20, Provect Veterinary Supplies) administered intravenously and restrained on a laparoscopic

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

**Table 1. Mean ( $\pm$  S.E) estrus responses, duration of estrus and ovarian responses in superovulation in goats.**

Parameters	Animals
Animal treated	10
Onset of estrus after injection of prostaglandin (h)	$37.6 \pm 3.48$
Duration of estrus (h)	$35.8 \pm 3.87$
Animal responding (>3CL) to superovulation treatment (n)	3
Ovulation rate, CL/animals	$2.5 \pm 1.62$
Unovulated large follicle (UF)/animals	$2.8 \pm 1.28$
Total ovarians response	$5.3 \pm 1.03$
CL regression	$0.8 \pm 0.61$

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, Ilium 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 hindquarters 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 7 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

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 albicans. Following ovarian examination, the skin incisions was closed with a single absorbable suture and sprayed with fly repellent.

**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  $37.6 \pm 3.48$  h and  $35.8 \pm 3.87$  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 prostaglandin injections. These findings suggest that the used of prostaglandin for synchronization estrus in does was efficient and in agreement with those reported by Godfrey *et al.*, (1999). The overall mean interval to onset of estrus following prostaglandin injections was  $37.6 \pm 3.48$  hours, and the mean duration of the induced estrus  $35.8 \pm 3.87$  hours obtained in this study is comparable to the previous finding  $33.40 \pm 5.70$  hours and  $36.6 \pm 3.36$  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 ovarian responses may due to their relatively small size when the FSH treatment is initiated (D'Occhio *et al.*, 1999), or relatively small size follicle at the start of FSH treatment do not have enough time to complete normal maturation (Liu and Sirois, 1998; Xu *et al.*, 1995) prior to the LH surge (Bever and Dieleman, 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.

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