Induction of Follicular Development and Ovulation in Thai-native Goat Using Decreasing Dose of FSH with hCG

การเหนี่ยวนำพัฒนาการของฟอลลิเคิลและการตกไข่ในแพะพื้นเมืองไทยโดยใช้ฮอร์โมน FSH แบบลด ร่วมกับ hCG

Jaruk Nutthakornkul[1*] and Chainarong Navanukraw[2]

จารึก ณัฏฐากรกุล[1*] และ ไชยณรงค์ นาวานุเคราะห์[2]

[1]Program in Animal Production Technology, Faculty of Agro-Industrial Technology, Kalasin University, Kalasin 46000, Thailand

[1]สาขาวิชาเทคโนโลยีการผลิตสัตว์ คณะเทคโนโลยีอุตสาหกรรมเกษตร มหาวิทยาลัยกาฬสินธุ์ จ. กาฬสินธุ์ 46000

[2]Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002, Thailand

[2]ภาควิชาสัตวศาสตร์ คณะเกษตรศาสตร์ มหาวิทยาลัยขอนแก่น จ. ขอนแก่น 40002

**Corresponding author: Email: jnuttha@gmail.com*

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บทคัดย่อ: วัตถุประสงค์ในการวิจัยเพื่อศึกษาผลของฮอร์โมน FSH แบบลดจำนวนเหลือ 2 หรือ 3 วันร่วมกับ hCG ต่อพัฒนาการของฟอลลิเคิลและการตกไข่ในแพะพื้นเมืองไทย โดยทดลองในแพะที่ไม่เคยตั้งท้อง (จำนวน 20 ตัว อายุเฉลี่ย 14 เดือน) แบ่งเป็น 2 กลุ่ม คือ ให้ FSH 18 mg แบบ 2 วัน (2D-FSH) และ ให้ FSH 24 mg แบบ 3 วัน (3D-FSH) ฉีดเข้ากล้ามเนื้อวันละ 2 ครั้ง เช้า-เย็น กลุ่มที่ฉีด 2 วัน เริ่มวันที่ 18, 19 และฉีด hCG (300 IU) เข็มเดียวในวันที่ 20 ของวงรอบการเป็นสัด กลุ่มที่ฉีด FSH 3 วัน เริ่มวันที่ 17, 18, 19 และ hCG วันที่ 20 หลังจากนั้น 24 ชั่วโมง เปิดช่องท้องของแพะเพื่อตรวจนับ corpora hemorrhagica (CH) และที่ 72 ชั่วโมงเปิดช่องท้องอีกครั้งเพื่อนับจำนวน corpora lutea (CL) เก็บตัวอย่างเลือดเพื่อนำไปวิเคราะห์หาฮอร์โมนโปรเจสเทอโรน (P4) ในวันที่ 17, 18, 20, 21 และวันที่ 23 ผลการทดลองพบว่าอัตราการตกไข่ ที่ 24 ชั่วโมงในกลุ่มของ 3D-FSH เท่ากับ 66.66 เปอร์เซ็นต์ดีกว่ากลุ่ม 2D-FSH ที่มีอัตราการตกไข่ 56.09 เปอร์เซ็นต์ (P<0.05) จำนวนของฟอลลิเคิลขนาด 1-3, 4-6 และ ≥7 มม. ที่นับได้เมื่อ 24 ชั่วโมง ในกลุ่ม 2D-FSH และ กลุ่ม 3D-FSH ไม่แตกต่างกัน (P>0.05) แต่ที่เวลา 72 ชั่วโมง พบว่าจำนวนฟอลลิเคิลขนาด ≥7 มม. ในกลุ่ม 3D-FSH มีมากกว่ากลุ่ม 2D-FSH (4.10 ± 1.52 กับ 2.80 ± 1.47; P<0.05) ส่วนจำนวนฟอลลิเคิลขนาด 1-3 และ 4-6 มม. ไม่แตกต่างกัน (P>0.05) ระดับของ P4 มีระดับสูงในวันที่ 17 หรือ 18 และต่ำลงในวันที่ 20, 21 และ 23 อย่างไรก็ตามระดับความเข้มข้นของ P4 ในแพะทั้ง 2 กลุ่มไม่แตกต่างกัน จากผลการทดลองครั้งนี้ชี้ให้เห็นว่าการใช้ฮอร์โมน FSH แบบ 3 วัน กับ hCG สามารถใช้เป็นโปรแกรมในการเหนี่ยวนำการพัฒนาการของฟอลลิเคิลและการตกไข่ในแพะพื้นเมืองได้ และช่วยเพิ่มประสิทธิภาพทางด้านการสืบพันธุ์ในสัตว์เคี้ยวเอื้องขนาดเล็ก

คำสำคัญ: การเหนี่ยวนำ การตกไข่ FSH hCG แพะพื้นเมืองไทย

Abstract: The objective of this study was to evaluate induction of multiple follicular growths and ovulation in Thai-native goats treated with decreasing dose 2 or 3 days of follicle stimulating hormone (FSH) and human chorionic gonadotrophin (hCG). Thai-native non pregnant goats (n = 20; 14 months of age) received two treatments: 2 days FSH (2D-FSH) and 3 days FSH (3D-FSH). Group of 2D-FSH was intramuscularly injected with twice daily FSH for 2 days (18 mg), starting on day 18, 19 and with 300 IU hCG on day 20 of estrous cycle. Group of 3D-FSH was injected with twice daily FSH for 3 days (24 mg), starting on day 17, 18, 19 and with hCG on day 20. Goats underwent laparotomy at 24 h after injected hCG to count corpora hemorrhagica (CH) and at 72 h to count corpora lutea (CL). Blood samples were taken to determine plasma progesterone (P4) concentrations on day 17, 18, 20, 21 and 23. Ovulation rates at 24 h of 3D-FSH were as 66.66 % greater than 2D-FSH as 56.09% (P<0.05). Numbers of follicles in class 1-3, 4-6 and ≥7 mm at 24 h were not statistically different between 2D-FSH and 3D-FSH groups (P>0.05). However, the number of follicles in class ≥7 mm of 3D-FSH group was greater than 2D-FSH group (4.10 ± 1.52 vs 2.80 ± 1.47; P <0.05) but were not differ in class 1-3 and 4-6 mm at 72 h (P>0.05). Plasma P4 concentrations were high on day 17 and 18 then decreased on day 20, 21 and 23. However, The plasma P4 were not statistically different between groups (P>0.05). These results indicate that superovulation with decreasing dose FSH (3D-FSH) and 300 IU hCG can be an effective protocol in goats for inducing multiple follicular development and ovulation in order to achieve successful development of reproductive technologies in small ruminants.

Keywords: Induction, ovulation, FSH, hCG, Thai-native goat

Introduction

More than 80% of the goat population in Thailand are in the southern region. They are primarily raised for meat by small holders as a secondary enterprise to crop production. Thai native goats are similar to the Katjang breed of Malaysia (Saithanoo and Milton, 1988). The major colors of goats were brown, (49-60%) followed by cream, black and the combination of brown, black and white (Saithanoo and Milton, 1988). Estimates on the average body weights of adult indigenous goats also vary. Suthiwanich (1983) reported that female goats (1-3 years old) average 21-25 kg and males, 26-29 kg. Goats in Thailand are not seasonal breeders and showing estrus with ovulation in all months of the year. Indigenous does first come into estrus as early as 3-4 months of age. Does can be used for breeding when they reach 8-10 months of age or 20-25 kg in body weight. The estrous cycle in general ranges from 18-22 days (Pralomkarn *et al*., 1996). Although goat is potentially a highly prolific animal which has relatively short gestation period compared to other livestock species, little producer is known about reproductive management due to lack of information on the reproductive management i.e., method of mating, time of first mating, pre-partum and post-partum management. In addition, lack of knowledge in management application and assortments of reproductive technology for Thai-native goat is another major concern. These limitations therefore will mostly affect the efficiency and capacity of goat production.

Protocols for multiple ovulation are widely used to improve number of offspring from selected female goats (Baril and Saumande, 2000), as in other ruminant species. However, similar to cows (Moonmanee *et al*., 2016) or sheep, the high variability in the number of corpora lutea and embryos obtained in response to superovulation, between treatments and between individual animals in the same treatment group, is a major limiting factor in goat embryo transfer programs. This variability, due to both extrinsic factors-source, purity of gonadotropins and protocol of administration and intrinsic factors-breed, age and reproductive status (Holtz, 2005). In goats, superovulatory treatment typically consists of a combination of estrous cycle control (usually involving application of progestagen implants) with an elevated dose of a gonadotropin, to induce the ovary to release more than the typical number of oocytes. The use of equine chorionic gonadotrophin (eCG) with or without a follow-up with eCG antibodies, in many cases did not deliver the anticipated response (Pintado *et al*., 1998). This might be associated with the rapid degradation of eCG in goats; its half-life being only 10-15 hour, which is several times shorter than in cows. Follicle stimulating hormone, usually of porcine origin (pFSH), proved to be more efficacious than eCG (Nowshari *et al*., 1992), provided it contains an appropriate admixture of luteinizing hormone (LH). Since the half-life of pFSH in goats is only 5 h (Demoustier *et al*., 1988), FSH is administered twice daily for 3-4 days, usually in decreasing dosage, beginning between 1 and 3 days before the end of the progestagen treatment. On average 8–16 ovulations are generated, although individual variability is immense (Baril and Saumande, 2000). Several attempts have been made to devise less labor-intensive treatment regimes without compromising embryo yield. One such attempt was to inject FSH at 24 instead of 12 h intervals while doubling the dose. This resulted in an average ovulation rate of 8.9 as compared to 10.8 in a control group submitted to conventional treatment. This treatment saves on labor and expense and has been adopted by a number of commercial embryo transfer operations.

The objectives of the present study was to evaluate that the induction of multiple follicular growths and ovulation in Thai-native goats treated with FSH and hCG (FSH decreasing dose, 2 or 3 days protocols) can be an effective protocol in goats for Thai-native goat production and expecting that these appropriate technologies and management regimens could be used to enhance the efficiency of goat production.

Materials and Methods

Animals and design

Experiment protocols were approved by the animal ethics committee of Rajamangala University of Technology Isan, Kalasin Campus. The study was conducted during the rainy season at the experimental farm of the University. The University (Kalasin Campus) is located at 102 degrees east longitude and 16 degrees north latitude with a tropical climate. The experiment was carried out at the experimental farm, the small ruminant unit, Department of Animal Production Technology, Faculty of Agro-Industrial Technology, Kalasin Campus during June to October 2014. Twenty nulliparous Thai-native does with the average age and body weight of 10 months and 17 ± 2.15 kg, respectively. Animals were routinely assessed for estrous activity by exposing all does to a vasectomized buck. The estrus was detected and designed as day 0. Animals were then randomly assigned to one of two treatments goats: FSH treatment for two days (2D-FSH) (FSH-P; Folltropin-v®, Bioniche Animal Health, Canada), or FSH treatment for three days (3D-FSH). Group of 2D-FSH was intramuscularly injected with twice daily FSH for 2 days (5, 4 units per injections; 18 mg), starting on day 18, 19 and with 300 IU hCG (Chorulon®, Intervet, UK) on the morning day 20 of estrous cycle. Group of 3D-FSH was injected with twice daily FSH for 3 days (5, 4, 3 units per injections; 24 mg), starting on day 17, 18, 19 and with hCG on day 20 of the estrous cycle. Animals were fed with roughage, clean water and mineral block were provided for animals *ad libitum*. The concentrate (16% CP) was fed 1% of body weight.

Data collection

Animals were subjected to a laparotomy to determine the number of corpora hemorrhagica (CH), and corpora lutea (CL). Ovulation rate was then calculated for 24 and 72 h by expressing the number of CH observed at each time point as a percentage of the number of CL at the time of laparotomy, with the assumption that the numbers of CL represent the total number of follicle ovulated. Does wich had 4 or more CL were considered as responding to the FSH-treatment as previously described (Stenbak *et al*., 2003). Follicular growth was determined by number and size of follicle and classified as 1-3, 4-6 and ≥7 mm, according to Gonzalez-Bulnes *et al.* (2003). Blood samples were taken to determine plasma progesterone (P4) concentration on day 17 (group of 3D-FSH) or 18 (group of 2D-FSH), 20 (before inject hCG) 21 (laparotomy at 24 h) and day 23 (laparotomy at 72 h). Samples were collected in cooled test tubes containing 100 µl EDTA kept on ice. Immediately after collection, blood samples were centrifuged at 1500 × g for 15 min. The plasma was aspirated into vials and then stored at -20 °C until assayed for P4. In addition, other characteristics such as body weight (BW), body condition score (BCS). Body condition scores were assessed by the herd manner at laparotomy to determine the effect of BCS on response variables. Score was assigned to each goat using a quarter-point scale from 1 to 5, where 1 = emaciated and 5 = obese (Ferguson *et al*., 1994).

Hormone assays

Plasma P4 concentrations were determined by competitive ELISA (Crane *et al*., 2006). Goat anti-mouse IgG (H+L) was made in mouse by using a P4-horse radish peroxidase conjugate. Intraassay coefficients of variation was 2.65%, and assay sensitivity was 0.025 ng/ml.

Data analysis

The statistical model included treatment, the ovulation rate (OR) at 24 h and BCS. The effect of OR and BCS were converted to categorical variables by grouping goats by ovulation rate at 24 h and BCS at initial trial (SAS, 2000). Treatment interactions the OR, and BCS were then reanalyzed using Chi-square analysis (Cochran-Mantel-Haenszel statistic) of SAS. Continuous data (CH, CL, Follicle and P4) were analyzed using procedure GLM of SAS. Plasma P4 concentrations were analyzed with a nested analysis of variance with treatment, animal (treatment), and day included in the model, and differences between specific means were evaluated by using the student *t*-tests (SAS, 2000).

Results

Superovulatory response

Characteristics of BCS in goats no differ between 2D-FSH and 3D-FSH groups in this study (2.97 ± 0.05 vs 2.83 ± 0.08 respectively, $P>0.05$). Ovarian follicular development of 2D-FSH were evaluated from numbers of CH by laparotomy at 24 h as 2.30 ± 1.15 and numbers of CL at 72 h as 4.10 ± 1.66, ovulation rate by laparotomy at 24 h were as 56.09% (Table 1). In 3D-FSH group, number of CH, CL at 24 and 72 h were as 4.00 ± 1.15 vs 6.00 ± 1.41 respectively. Moreover, ovulation rate was as 66.66% during at 24 h (Table 1).

Number of CH of goat receiving 2D-FSH was less than goat receiving 3D-FSH (2.30 ± 1.15 vs 4.00 ± 1.15 respectively; $P<0.05$). However, the number of CL in 3D-FSH greater than 2D-FSH group at 72 h (6.00 ± 1.41 vs 4.10 ± 1.66 respectively; $P<0.05$) and ovulation rate of 3D-FSH group were same too. In addition, numbers of CH, CL and percentage of ovulation at 24 and 72 h after inducing by FSH show in Table 1. Ovulation rate of all groups at 72 h were as 100% because of found only CL on ovaries, but there were not expressed of CH.

In this study, the goat of 2D-FSH group was distinctly response on FSH less than 3D-FSH group, such as number of CH at 24 h, number of CL at 72 h and ovulation rate in 2D-FSH group. BCS were assessed by the herd manner (BCS = 2.5-3.0) to determine the effect of BCS on response ovulation rate, no effect on present study.

Effect of FSH and hCG on follicular growth

Numbers of follicles in class 1-3, 4-6 and ≥7 mm at 24 h were not significantly different between 2D-FSH and 3D-FSH group. However, the numbers of follicles in class 1-3, 4-6 mm at 72 h were not significantly different between 2D-FSH and 3D-FSH group but in class ≥7 mm of 3D-FSH were greater than the 2D-FSH group ($P<0.05$); presented in Table 2.

Table 1. Numbers of CH, CL and percentage of ovulation at different times responding FSH and hCG treatments

Items	2D-FSH	3D-FSH	*P*-value
Number of CH at 24 h	2.30 ± 1.15[a]	4.00 ± 1.15[b]	0.03
Number of CL at 72 h	4.10 ± 1.66[a]	6.00 ± 1.41[b]	0.04
Ovulation rate at 24 h (%)	56.09[c]	66.66[d]	0.02

[a,b] Means ± SE differ within row, $P<0.05$

[c,d] Percentages of ovulation differ within row, $P<0.05$

Table 2. Numbers of 1-3, 4-6, and ≥7 mm follicles in FSH treated goats

Size of follicle	2D-FSH	3D-FSH	P-value
1-3 mm	1.60 ± 0.96	2.00 ± 0.81	0.08
4-6 mm	2.30 ± 0.94	1.90 ± 0.99	0.08
≥7 mm	2.80 ± 1.47 [a]	4.10 ± 1.52 [b]	0.03
Mean ± S.E.M.	6.90 ± 1.19	8.00 ± 2.16	0.07

[a,b] Percentages of ovulation differ within row, $P<0.05$

Progesterone profile

In the present study, the plasma P4 concentrations of all groups were high on day 17, 18 (Mean ± S.E.M., 3.24 ± 0.30 and 3.49 ± 0.12 ng/ml respectively) then decreased on day 20, after that to lowest on day 21, there were not differ between groups (P>0.05). However, there were different plasma P4 concentrations within groups before injected FSH and after on day 20, 21 and 23 (P<0.05; Figure 1).

Discussion

Protocols for induction of multiple follicular growth and superovulation currently used in the animal industry are not fully optimized (Gordon, 1997). The goat semi-industry has been reluctant to commercialize embryo transfer and other reproductive technologies because of the inconsistency of ovarian response to the superovulatory treatments (Cognie, 1999). It is known that ovarian stimulation with gonadotropins has an effect on oocyte maturation and competence. The FSH is the gonadotropins most frequently used, providing the best results in small ruminants. Both in sheep and in goat, FSH treatment results in high ovulation rate and better quality embryos per donor than treatment with PMSG. Currently, FSH is administered at 12 h intervals in decreased or constant doses for 3-4 days starting two days before sponge withdrawal or implant removal, and around the time at which prostaglandin $F_{2\alpha}$ is administered. The dose of total FSH administered varies from 16 to 20 mg depending on type of FSH preparation and genetic features of the sheep and goats donors (Baril and Saumande, 2000). However, this research was to optimize the FSH decreasing dose, 2 or 3 days can be effective protocols for goats expecting that these appropriate technologies could be used to enhance the efficiency of goat production.

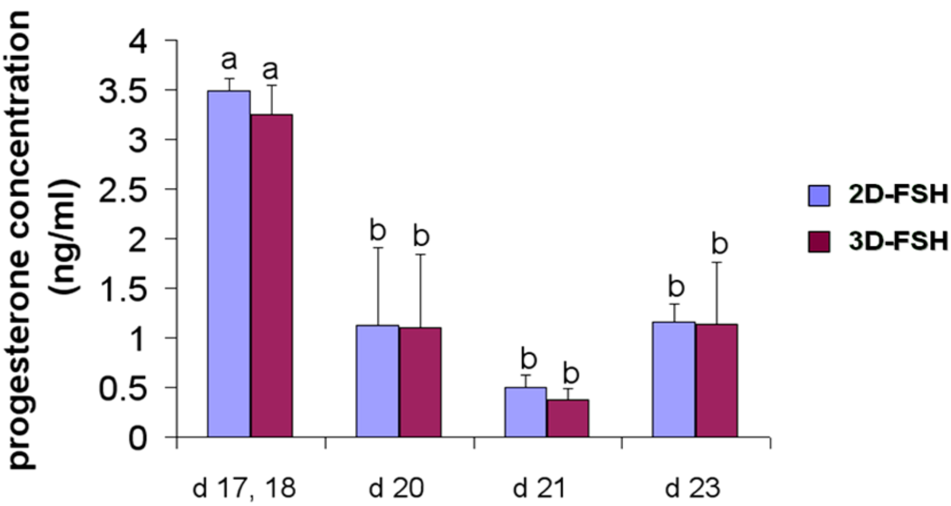

Figure 1. Means (± SEM) plasma P4 concentrations (ng/ml) of goats on days prior injected 2D-FSH (d18) 3D-FSH (d17), hCG (d 20), laparotomy at 24 (d 21) and 72 h (d 23)

[a, b] Proportion differ on days (P<0.05)

FSH was shown to induce development of multiple follicles on each ovary when injected into goat or sheep for two or more days at regular intervals during the normal breeding season and non-breeding season (Gordon, 1997; Stenbak *et al*., 2001). Previous studies have shown that exposure of oocytes to various hormones *in vivo* causes maturational changes that are necessary for proper development to occur (Stenbak *et al*., 2001). Optimal levels of exogenous gonadotropins should be used to promote proper oocyte development and depending on the regime of gonadotropin treatment, positive or negative effects on oocyte maturation and fertilization have been observed (Stenbak *et al*., 2001).

The results from this study indicate that is ovulation rate of all groups during at 72 h were as 100% because of found only CL on ovaries, but there were not present of CH. Moreover, in this study using hCG was similar as LH to manipulated of limit timing in ovulation within 72 h. Similarly with Walker *et al*. (1986) using FSH or PMSG with GnRH induced of multiple follicular development and ovulation in Marino ewes, found that ovulation rate were as 79% within during at 54-66 h in ewes.

In this study, the goat of 3D-FSH group was distinctly response on FSH greater than 2D-FSH group, such as number of CH at 24 h, number of CL at 72 h and ovulation rate in 3D-FSH group. Moreover, the Thai-native goats adequate responded to FSH treatments by exhibiting greater than or equal to four CL (2D-FSH = 5.0 ± 0.70, 3D-FSH = 8.50±1.04 CL) but less than to four CL were not responding on FSH (Stenbak *et al*., 2003). However, Cognie (1999) reported that about 20% of ewes do not respond to superovulatory procedures.

The ovulation rate was positively affected by a high number of follicles with a diameter of 1–6 mm at the onset of the treatment and negatively affected by a high number of follicles with a diameter ≥7 mm. Ovulatory follicles in superovulated does are derived from smaller antral follicles present at the onset of the FSH treatment which are able to respond of exogenous FSH. On the other hand, the initiation of the FSH treatment after the selection of the large dominant follicle could result in a lower ovulatory response, as in cows (Guilbault *et al*., 1991) or ewes (Stenbak *et al*., 2003).

The response of goats, like other mammals, to treatments for the induction of superovulation and embryo production is strongly influenced by the structures (follicles and corpora lutea) present in the ovaries at the beginning of the exogenous gonadotropin regimen. Thus, ovulation rate at the end of the superovulatory treatment is related positively to the number of small gonadotropin-responsive follicles (2-3 mm in size) at first FSH dose (Gonzalez-Bulnes *et al.,* 2003). On the other hand, the ovulation rate is affected negatively by the presence of a large dominant follicle (7 mm in size) in superovulatory protocols with a single dose of eCG or FSH (Lopez-Sebastian *et al*., 1999).

Early findings in goats showed that follicle waves developing under continuous and high progesterone concentrations have smaller follicles than those developing when progesterone concentration are low (Rubianes and Menchaca, 2003). In the present study, the plasma P4 concentrations all groups were high on day 17, 18 then decreased on day 20, after that to lowest on day 21 confirmed this relationship between progesterone concentrations and the number of follicles development. Moreover, previous study of FSH surges necessarily precede the emergence of a follicular wave in heifers (Adams *et al*., 1992), and a similar association of both events has been reported in ewes (Lopez-Sebastian *et al*., 1999) but we did not measure FSH levels in the present study. According to Baird *et al*. (1991) FSH secretion was not affected directly by progesterone but was regulated by estradiol and inhibin, which was produced mainly by the largest follicles that developed during the cycle.

Conclusion

In summary, the majority of ovulation in Thai-native goat using FSH and hCG occurred between 24 and 72 h. The results indicate that superovulation with decreasing dose FSH (3D-FSH) and 300 IU hCG can be an effective than FSH (2D-FSH) protocol for Thai-native goat superovulation.

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