Original article

Model testing of induction of multiple follicular growth and atresia in goats

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Abstract To test a model of induction of multiple follicular growth and atresia using FSH treatment and subsequent withdrawal. Sixteen female Thai-native goats were randomly assigned into four groups: control (no hormone), a day FSH + two days withdrawal (1 d FSH + 2 d W), two days FSH + a day withdrawal (2 d FSH + 1 d W), and three days FSH (3 d FSH). 3 d FSH group received twice daily injections FSH beginning on days 17, 18, and 19 in decreasing doses (24 mg total), whereas 2 d FSH + 1 d W group received on days 17, and 18 (18 mg total) followed by normal saline injection on day 19. 1 d FSH + 2 d W group received FSH on day 17 (10 mg total) followed by normal saline injection on days 18 and 19. All goats underwent ovariectomized by laparotomy on day 20. The number of all follicles was determined and classified as small (1-3 mm), medium (4-6 mm), or large > 6 mm) follicles. After oocyte collection, oocytes were determined as good, fair or poor quality. The 3 d FSH group showed a significantly greater average total number of follicles than 2 d FSH + 1 d W, 1 d FSH + 2 d W, and control groups. No significant differences were observed between FSH treatments and control group in oocyte recovery rates. Additionally, the proportions of good oocyte quality were greatest (50%) in goats receiving 3 d FSH and were significantly greater than that of 1 d FSH + 2 d W group (10%). These result indicated the 3 d FSH induces follicular growth whereas 1 d FSH + 2 d W induces follicular atresia. Thus, this model was validated and should be useful for induction of follicular growth and atresia in goats. **Chiang Mai Veterinary Journal 2013;11(2): 121-130**

Introduction

The small ruminants (sheep and goat) represent a valuable model system for the elucidation of endocrine and local mechanisms that control both the early and final stages of follicle development in monovulatory species. (Saraiva et al., 2012) In humans, limited availability of suitable ovarian tissue is a major constraint to research in this area, and monovulatory domestic ruminants represent a physiologically relevant model to elucidate basic mechanisms before moving on to more focused clinical investigations. (Campbell et al., 2003) However, to address common causes of infertility and to devise innovative strategies to increase the efficiency of assisted reproduction technologies, it is necessary to understand the basic physiology underlying the complex process of follicular growth and development. (Quirk, Cowan, Harman, Hu, & Porter, 2004) Among the main substances that regulate the complex mechanisms of follicular growth and development are the gonadotropins FSH and LH, key hormones that regulate folliculogenesis in the ovary. (Matos et al., 2007)

Wavelike patterns have been demonstrated with signs of dominance during the final growth of the ovulatory follicles in goats. (Ginther & Kot, 1994; Gonzalez-Bulnes et al., 2005) During ovarian follicular development, most ovarian follicles at some stage of their development undergo a degenerative process called atresia, and only few follicles reach to the ovulatory stage. (Evans, 2003) The preovulatory phase of the estrous cycle in goat is characterized by selection of one to several dominant follicles and atresia of subordinate follicles coincident with decreasing FSH concentrations in peripheral plasma. (Rubianes & Menchaca, 2003) During the early preovulatory phase, concentrations of FS Hin peripheral plasma have been shown to be directly correlated with growth of small antral follicles. (Medan et al.,

2003) Furthermore, FSH treatment promoted an increase in the population of small follicles. (Menchaca, Pinczak, & Rubianes, 2002) Previous studies showed that FSH could prevent follicular atresia, and in fact, early atretic follicles can be rescued by the administration of exogenous FSH. (Kumar, Osborn, Cameron, & Trounson, 1992) FSH enhanced development of goat follicles by suppressing the apoptosis of granulosa cells, and this effect was best displayed in antral follicles. (Yu et al., 2003)

It is recognized that apoptotic cell death is an underlying mechanism of cell loss during follicular atresia. (Yu et al., 2003; Yu et al., 2004) The effect of follicular cell apoptosis on oocyte developmental competence should be best studied by individual culture that demonstrates a functional relationship between follicular origin and quality of oocytes. However, studies in this field are few and results are discrepant. (Behl & Pandey, 2002) Previous studies reported a model for gonadotropin induced follicular growth and atresia in ewes by administration of FSH-P during the follicular phase of the estrous cycle. (Jablonka-Shariff, Fricke, Grazul-Bilska, Reynolds, & Redmer, 1994) However, only a few studies have dealt with the effects of gonadotropins on follicular development and oocyte quality during the estrous cycle of goat. (Behl & Pandey, 2002) Thus, the use of FSH-treated model can help explain the effects of gonadotropins on follicular growth and atresia in ruminants.

We hypothesized that continuous administration of FSH to female goats would result in both multiple follicular growth and oocyte development. To test this hypothesis, we performed FSH treatment and subsequent withdrawal in goat ovarian model. Therefore, our aim was to test a model of induction of multiple follicular growth and atresia using FSH treatment and subsequent withdrawal.

Materials and methods

All experimental goats and procedures were managed according to the guidelines approved by the Animal Ethic Committee of Khon Kaen University (Record No. AEKKU 53/255 and Reference No. 0514.1.12.2/54).

Animals and treatments

Nulliparous (n = 16) Thai-native goats (10-11 months of age and 23-25 kg of body weight) that had exhibited at least one estrous cycle of normal duration (20-21 days) were used for this study. Day 0 of the estrous cycle (standing estrus) was determined by using vasectomized buck. Beginning on day 17 after estrus, goats received no hormone or twice daily (morning and evening) intramuscular injections of FSH-P (a pituitary extration; potency of one Armour Unit/mg; Sioux Biochemical, Inc., IA, USA) and subsequent withdrawal (W; intramuscular injection of normal saline). For ovarian stimulation, goats were allocated randomly to four treatment groups as follows: control (no hormone), a day FSH-P + two days withdrawal (1 d FSH + 2 d W), two days FSH-P + a day withdrawal (2 d FSH + 1 d W), and three days FSH-P (3 d FSH).

The control group received twice daily injections of normal saline (1 ml) beginning on days 17, 18, and 19. The 3 d FSH group received twice daily injections of FSH-P beginning on days 17, 18, and 19 in decreasing doses (24 mg total), whereas the 2 d FSH + 1 d W group received on days 17 and 18 (18 mg total) followed by normal saline (1 ml) injection on day 19. The 1 d FSH + 2 d W group received FSH-P on day 17 (10 mg total) followed by normal saline (1 ml) injection on days 18 and 19.

Ovariectomy and classification of ovarian follicles

All goats underwent ovariectomized by laparotomy on day 20. Goats were treated with 0.075 mg xylazine (Rompun®; L.B.S. Laboratory,

Thailand) and 100 mg ketamine hydrochloride (Ketaset®; Wyeth Animal Health, Canada) as previously described for ewes. (Luther et al., 2007) Ovariectomy was performed to determine numbers of follicle as previously described. (Samaké, Amoah, Mobini, Gazal, & Gelaye, 1999) Ovaries were collected and swiftly transported to the laboratory. All visible follicles were then classified by diameter into large (> 6 mm), medium (4-6 mm), or small (1-3 mm) as described. (González et al., 2001)

Oocyte collection and grading

Cumulus oocyte complexes (COCs) recovered from surface visible follicles using 22 G needle attached to a disposable 5 ml syringe containing 1 ml of oocyte handling medium (TCM-199; Sigma, St. Louis, M.O., USA). (Rahman, Abdullah, & Wan-Khadijah, 2008) The COCs were isolated under a stereomicroscope and qualitatively graded as good (Fig. 1A and B), fair (Fig. 1C) or poor (Fig. 1D). (Rahman, Abdullah, & Wan-Khadijah, 2008)

Statistical analysis

The data were expressed as means ± SEM per goats. Data on number of visible follicles were analyzed using the general linear model (GLM) procedure of SAS (SAS Inst. Inc., Cary, NC). The Chi-square test was used to compare the recovery rates of oocytes among groups. A 4 × 3 contingency table was constructed to determine the proportions of good, fair and poor oocytes. (Stokes, 2000) Differences between means were evaluated by Duncan's New Multiple Range Test. (Steel, 1994) Means were considered significantly different if $P<0.05$, unless otherwise stated.

Results

Number of visible follicles

Average numbers of small follicles of control group were significantly greater ($P<0.05$) than
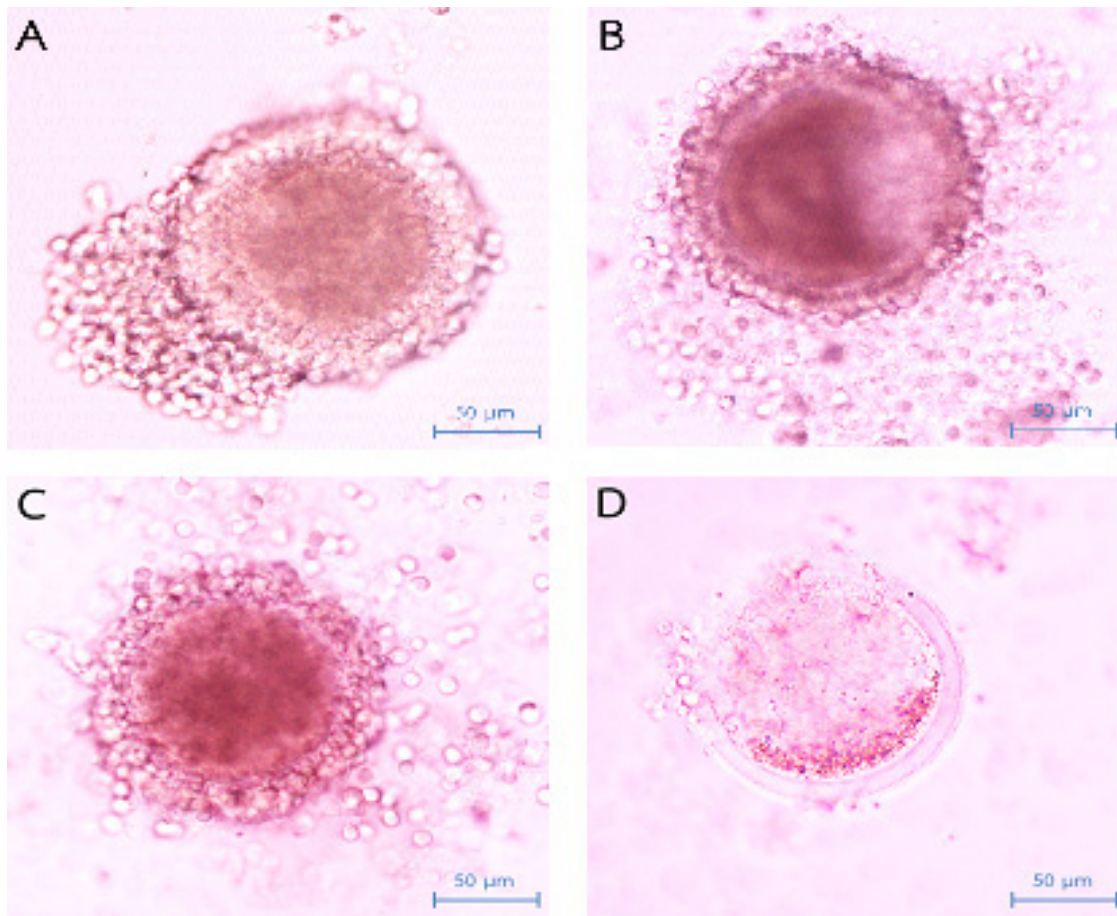
Figure 1. Goat oocytes grading according to cumulus cell investment and morphology of the oocyte. Good quality: oocyte completely surrounded by cumulus cells (A and B), fair quality: oocyte partially surrounded by cumulus cells (C), and poor quality: oocyte completely denuded (D).

those of FSH-treatment groups (Table 1). Average numbers of medium follicles were greatest (P<0.05) in the 1 d FSH + 2 d W group compared with the control and 3 d FSH groups but were not different when compared with the 2 d FSH + 1 d W group (Table 1). Average numbers of large follicles were greatest (P<0.05) in goats receiving 3 d FSH and were significantly than that of other groups (Table 1). Additionally, the 3 d FSH group showed a significantly greater (P<0.05) average total number of follicles than that of the 2 d FSH + 1 d W, 1 d FSH + 2 d W, and control groups (Table 1).
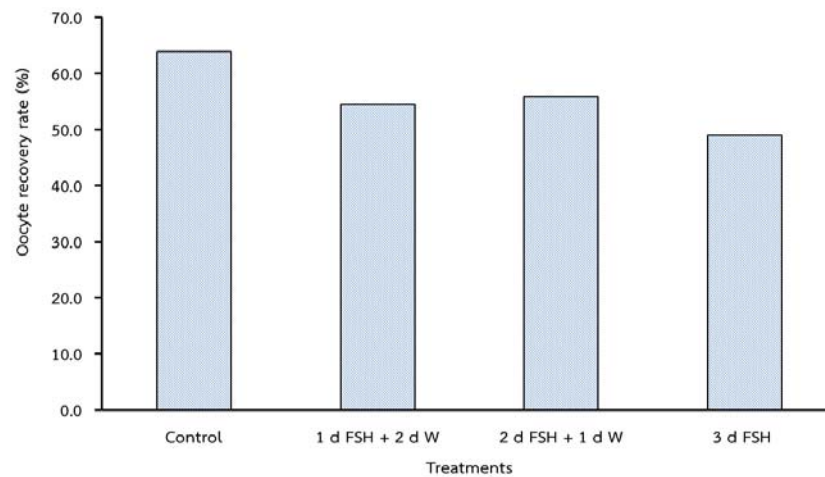
Oocyte recovery and oocyte quality

No significant differences were observed between FSH treatments and control group in oocyte recovery rates (P>0.05; Fig. 2). The proportions of good oocyte quality were greatest (P<0.05) in goats receiving 3 d FSH and were significantly greater than that of the 1 d FSH + 2 d W group (Table 2). The proportions of fair oocyte quality were greatest (P<0.05) in the 2 d FSH + 1 d W group compared with the control group but were not different when compared with the 1 d FSH + 2 d W and 3 d FSH groups (Table 2). Additionally, the proportions of poor oocyte quality were least (P<0.05) in the 3 d FSH group and were significantly lower than that of the 1 d FSH + 2 d W group (Table 2).

Table 1. Mean number of small, medium, large, and total follicles in goats received FSH or withdrawal.

Size of follicle	Treatments			
	Control	1 d FSH + 2 d W	2 d FSH + 1 d W	3 d FSH
Small follicle 1-3 mm (n)	9.0 ± 1.0^a	3.3 ± 1.1^b	3.3 ± 0.7^b	3.5 ± 0.5^b
Medium follicle 4-6 mm (n)	2.7 ± 0.7^a	8.5 ± 0.5^b	$6.7 \pm 0.3b^c$	6.3 ± 0.8^c
Large follicle > 6 mm (n)	0.7 ± 0.3^a	2.3 ± 1.1^b	10.0 ± 0.6^c	14.7 ± 1.5^d
Total number of follicles (n)	12.3 ± 1.5^a	12.8 ± 0.2^a	20.0 ± 0.6^b	24.3 ± 0.5^c

[1]Means ± SEM are expressed per goat.

Different letters (a, b, c, d) in the same row are different ($P<0.05$).

Figure 2. Oocytes recovery rate (%) in goats received
FSH or withdrawal.

Table 2. The proportions of oocyte quality in goats received FSH or withdrawal.

Oocyte quality	Treatments			
	Control	1 d FSH + 2 d W	2 d FSH + 1 d W	3 d FSH
Good oocyte (%)[1]	47.6^{ab}	10.0^a	28.6^{ab}	50.0^b
Fair oocyte (%)[2]	9.5^a	20.0^{ab}	32.1^b	20.8^{ab}
Poor oocyte (%)[3]	42.9^{ab}	70.0^a	39.3^{ab}	29.2^b

[1]Number of good oocytes expressed as a percentage of total number of oocytes per goat.

[2]Number of fair oocytes expressed as a percentage of total number of oocytes per goat.

[3]Number of poor oocytes expressed as a percentage of total number of oocytes per goat.

Different letters (a, b) in the same row are different ($P<0.05$).

Discussion

The major purpose of this study was to establish a model for the study of FSH-induced multiple follicular growth and atresia as well as oocyte development in goats. In the present model, we found that FSH treatment (3 d FSH) commenced on day 17 to day 19 increased the number of large follicles and total number of follicles, while the number of small follicles decreased slightly. The increase in the number of large follicles of the 3 d FSH treatment was probably due to the continued effect of FSH to stimulate growth of small and medium follicles, respectively, into medium and large follicles. (Greenwald, & Terranova, 1986.; Jablonka-Shariff, Reynolds, & Redmer, 1996) Additionally, FSH withdrawal (1 d FSH + 2 d W treatment) decreased the number of large follicles compared with continuous 3 d FSH treatment. The decrease in the number of large follicles after FSH withdrawal may have been due to the loss of gonadotropic support necessary to stimulate or maintain the growth of small and medium follicles, as evidenced by the decrease in granulosa and (or) thecal cell proliferation. (Jablonka-Shariff et al., 1996) It has been hypothesized that larger antral follicles require gonadotropic support to sustain their growth and prevent follicular atresia. (Rubianes & Menchaca, 2003; Jablonka-Shariff et al., 1994; Jablonka-Shariff et al., 1996) The decrease of mitotic activity in granulosa cells during follicular development, therefore, is associated with granulosa cell differentiation and illustrates the inverse relationship between granulosa cell growth and differentiated function. (Jablonka-Shariff et al., 1994 ; Jablonka-Shariff et al., 1996)

Previous studies reported in goat that FSH enhanced development of follicles by suppressing the apoptosis of granulosa cells. FSH inhibited granulosa cell apoptosis by increasing production of steroids and insulin-like growth factor-1 (IGF-1). (Behl & Pandey, 2002; Chaves et al., 2012) Recent studies demonstrated that FSH acts synergistically with IGF-1 to increase cell number and expression of steroidogenic enzymes in granulosa cells. (Mani et al., 2010) Follicular atresia in cattle, sheep and goat was also characterized by a loss of cytochrome P450 aromatase (P450arom) in granulosa cells and a decrease in levels of cytochrome P450 17α-hydroxylase (P450c17) in the theca interna. (Xu et al., 1995; Huet, Monget, Pisselet, & Monniaux, 1997; Yuan et al., 2008) Furthermore, the reversal of atresia by administration of gonadotropins has been reported for many domestic livestock species including ewes, pigs, and cows. (Jablonka-Shariff et al., 1994; Gutherie, & Bolt, 1989; Fricke et al., 1997)

Since oocyte developmental competence increased with follicular sizes, an increased expression of these enzymes may be associated with the selection of dominant follicles and oocytes that are more competent. (Yuan et al., 2008) Previous studies conclude that the morphological quality of COCs appears to be the most important factor that influences the efficiency of in vitro embryo production in goats. (Katska-Ksiazkiewicz, Opiela, & Rynska, 2007) In the recent studies indicate a high correlation between folliclequality or granulosa cells apoptosis and distribution of COCs with different morphology. (Han et al., 2006) In most studies reported an overall positive effect of FSH treatment on the developmental competence of COCs. (Gonzalez-Bulnes et al., 2003; Lopez-Alonso et al., 2005 ; Pereira et al., 2012) In present study, the proportions of good oocyte collected from 3 d FSH group were higher than 1 d FSH + 2 d W. Likewise, the proportions of poor oocyte in 3 d FSH group were lower than 1 d FSH + 2 d W. These data imply that, ovarian stimulation with FSH positively affectsquality of oocytes. It has been well established in

several domestic and model species that the proportion of competent oocytes (or good oocyte quality) decreases with follicular atresia. (Mermillod et al., 2008) With respect to the occurrence of good oocyte quality in atretic follicles, previous study demonstrated that follicles with a low degree of atresia contained a relatively high percentage of good oocyte quality, and follicles with a higher degree of atresia contained higher percentages of poor oocyte quality. (De Wit, Wurth, & Kruip, 2000)

Conclusion

In the present study, continuous FSH treatment resulted in increased number of large follicles and an increased the proportions of good oocyte quality. On the other hand, FSH treatment and subsequent withdrawal resulted in decreased numbers of medium and large follicles, decreased the proportions of good oocyte quality, and an increased the proportions of poor oocyte quality. These result indicated the 3 d FSH induces follicular growth whereas 1 d FSH + 2 d W induces follicular atresia. Thus, this FSH-P-treated model was validated for the induction of multiple follicular growth and oocyte developmentin the ovaries. This model should prove useful for studying the effects of FSH treatment and subsequent withdrawal on follicular growth and atresia as well as oocyte development in goats.

Acknowledgement

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