—Full Paper—

Immunization of Goats against Inhibin Increased Follicular Development and Ovulation Rate

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Abstract. In the present study, two experiments were conducted to induce superovulation in goats using passive and active immunization against inhibin. In the first experiment, two groups of goats were given an intravenous injection of either 10 ml normal goat serum (control; n=6) or inhibin antiserum developed against [Tyro³⁰]-inhibin α (1–30) (passively immunized; n=6) 48 h before treatment with PGF₂ α . In the second experiment, two groups of goats were immunized with inhibin vaccine (actively immunized; n=5) or Freund's adjuvant (control; n=5) followed by three booster immunizations at 4 week intervals. Blood samples were collected for determination of FSH, LH, estradiol-17 β , and progesterone. Ultrasonography was used to determine ovarian activity at PGF₂ α injection and ovulation rate one week after estrus. In both experiments, there was a significant increase in plasma FSH concentration compared with the controls. However, the pattern of the FSH levels was different between the passively and actively immunized goats. The numbers of follicles in passively and actively immunized goats (22.4 ± 2.3 and 18.6 ± 2.1 , respectively) were significantly greater than those in the controls (2.6 ± 0.4 and 2.3 ± 0.4 , respectively). In addition, the ovulation rate was greater in the immunized animals compared with the controls. Therefore, either passive or active immunization against inhibin could be used to induce superovulation in goats.

Key words: Goats, Inhibin, Ovulation rate, Passive and active immunization

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Inhibin is a heterodimeric glycoprotein hormone that selectively inhibits secretion of follicle stimulating hormone (FSH) from the pituitary gland [1]. A negative relationship between plasma concentrations of FSH and inhibin has been established in several mammalian species [2, 3]. The number of follicles that develop to ovulatory size depends on both the amount of FSH and the

time of exposure to FSH [4, 5]. The release of FSH by the pituitary is in turn controlled by synergistic action of two of the major products of ovulatory follicles, inhibin and estradiol [6, 7]. Previous findings [8–10] that immunoneutralization of endogenous inhibin produced a significant elevation of peripheral FSH offer evidence that inhibin is an important factor in the inhibitory regulation of FSH secretion in domestic animals as well as laboratory animals [11–13]. On the other hand, ultrasonographic observation of the ovary

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correlated with hormonal profiles demonstrated that an increase in plasma FSH preceded emergence of each follicular wave [14–17] and a decrease in FSH was coincident with functional selection of follicles [15, 18], suggesting that the fluctuation in peripheral FSH levels is a trigger for growth, selection, and atresia of follicles. Multiple ovulations have been induced successfully by passive immunization against endogenous inhibin in several species, such as rats [1], ewes [19, 20], hamsters [12], cows [21, 22], mares [10], guinea pigs [13], mice [23], and goats [24, 25]. Also, active immunization against inhibin has been used in cows [26–28], goats [29], ewes [30], and mares [31]. Thus, these initial results indicate that immunization of animals against endogenous inhibin to induce superovulation through increased endogenous FSH secretion is an alternative method to the current exogenous gonadotropin protocols.

In most studies, the use of a combination of equine chorionic gonadotrophin (eCG) and human chorionic gonadotropin (hCG) is the most common method for induction of superovulation in goats. However, a disadvantage of these protocols is the long half-life of eCG, which interferes with normal fertilization and embryo development [32–34], and repeated eCG treatments induced anti-eCG antibodies that clearly have negative effects on the reproduction of goats [35]. There is a few previous studies regarding immunization against inhibin and further investigation is needed. Therefore, the aim of the present study was to determine the effect of passive and active immunization against inhibin on FSH secretion, follicular development, and ovulation rate in goats.

Materials and Methods

Experimental animals and treatments

The goats (*Capra hircus*) used in this study were housed under natural day lighting and fed a maintenance diet of 700 g/animal daily of hay cubes. Estrous cycles were synchronized with 2 injections of 125 µg of a synthetic analogue of prostaglandin F_{2α} (PGF_{2α}) (Estrumate, Schering-Plough Animal Health, NJ, USA) 11 days apart. Estrous behavior was checked every 6 h with an aproned mature buck throughout the experimental period. On day 10 of the estrous cycle (day 0=the first day showing estrous behavior), the goats were

passively or actively immunized against inhibin. In passively immunized goats, animals were treated with i.v. injection of 10 ml normal goat serum (control; n=6) or 10 ml inhibin antiserum raised in ovariectomized goats as described by Araki *et al.* [36] (passively immunized; n=6). Two days later (0 h), all animals were injected with 125 µg PGF_{2α} to induce estrus and ovulation. Blood samples were collected at 6-h intervals from 24 h before until 72 h after treatment with PGF_{2α}. In actively immunized goats, animals were treated with subcutaneous injection of 1 ml inhibin vaccine emulsified in 1 ml Freund's complete adjuvant into 4 different sites followed by 3 booster injections at 4 weeks intervals (actively immunized; n=5) or were treated with s/c injection of 1 ml saline emulsified in 1 ml Freund's complete adjuvant (control; n=5). After the 3rd booster immunization, PGF_{2α} was used to induce estrus and ovulation. Blood samples were collected at 6-h intervals from 24 h before until 72 h after PGF_{2α} injection. Blood samples were collected into heparinized Vacutainer tubes (Terumo Venoject II, Tokyo, Japan), centrifuged at 1700 g for 15 minutes, and the plasma was separated and stored at –20 °C until assayed for hormones.

Ultrasound scanning and determination of ovarian response

The number of follicles ≥4 mm in diameter and corpora lutea were determined at the time of PGF_{2α} injection, and the ovulation rate was determined one week after induction of estrus using a B-mode scanner (ECHOPAL ultrasound scanner, Hitachi Medical Corporation, Tokyo, Japan) equipped with a 7.5 MHz transrectal transducer. The ovulation rate was determined by counting the number of corpora lutea.

Hormone analysis

Plasma concentrations of FSH and LH were measured by a radioimmunoassay (RIA) system as described by Araki *et al.* [36] using anti-ovine FSH (AFP-c5288113), NIDDK-FSH-I-1 for radioiodination, and NIDDK-oFSH-RP-1 as a reference standard. Plasma concentrations of LH were measured by RIA as described by Mori and Kano [37] using anti-ovine LH (YM No.18), NIDDK-oLH-1-3 for radioiodination, and NIDDK-oLH-RP-24 as a reference standard. The intra- and interassay coefficients of variation were 9.8% and 12.6% for FSH and 5.9% and 6.5% for LH,

Fig. 1. Changes in plasma concentrations of FSH in goats after treatment with inhibin-AS (●) or normal goat serum (○) (A) and after the 3rd booster immunization against inhibin (■) or Freund's adjuvant (□) (B). The arrows indicate the time of PGF₂α injection. Values are means ± SEM of six (A) or five (B) animals. *P<0.05: significance of differences between the immunized and control groups.

Fig. 2. Changes in plasma concentrations of LH in goats after treatment with inhibin-AS (●) or normal goat serum (○) (A) and after the 3rd booster immunization against inhibin (■) or Freund's adjuvant (□) (B). The arrows indicate the time of PGF₂α injection. Values are means ± SEM of six (A) or five (B) animals. *P<0.05: significance of differences between the immunized and control groups.

respectively. Plasma concentrations of estradiol-17β and progesterone were determined by a double antibody RIA system using ¹²⁵I-labeled radioligands as described previously [38]. Antisera against estradiol-17β (GDN 244) and progesterone (GDN 337) were kindly provided by Dr. G. D. Niswender (Animal Production and Biotechnology, Colorado State University, Fort Collins, CO, USA). The intra- and interassay coefficients of variation were 5.8% and 7.2% for estradiol-17β and 8.0% and 8.9% for progesterone, respectively.

Statistical analysis

Mean values (± SEM) were calculated and analyzed using 2-way ANOVA. Duncan's multiple-range test was used for detection of significant differences using the SAS computer package [39]. A value of P<0.05 was considered to be statistically significant.

Results

Plasma concentrations of FSH and LH

The plasma concentrations of FSH and LH are shown in Figs. 1 and 2. Treatment of the goats with inhibin antiserum (passively immunized group) or inhibin vaccine (actively immunized group) resulted in a marked increase (P<0.05) in the plasma concentrations of FSH from the basal levels compared with the controls. In addition, the preovulatory FSH peak in the inhibin-immunized groups was higher than in the control groups. However, the pattern of FSH secretion was different between the passively and actively immunized goats. Plasma concentrations of FSH gradually declined from the time of PGF₂α injection (0 h) to 36 h after PGF₂α injection in the passively immunized group, whereas it gradually increased from 12 h to 54 h after PGF₂α injection in the

Fig. 3. Changes in plasma concentrations of estradiol-17 β in goats after treatment with inhibin-AS (●) or normal goat serum (○) (A) and after the 3rd booster immunization against inhibin (■) or Freund's adjuvant (□) (B). The arrows indicate the time of PGF₂ α injection. Values are means $\pm$ SEM of six (A) or five (B) animals. * P <0.05: significance of differences between the immunized and control groups.

actively immunized group. On the other hand, there were no significant changes in the basal levels of LH in all groups (Fig. 2 A and B). The peak of the LH surge occurred 12 h earlier in the passively immunized group compared with the control group, although the peak level was not different.

Estradiol-17 β and progesterone

Plasma concentrations of estradiol-17 β and progesterone were measured every 24 h throughout the experiment. In both groups, plasma concentrations of estradiol-17 β rose after PG-induced luteolysis and reached a peak value that was significantly (P <0.05) higher in the immunized groups than in the controls (Fig. 3 A and B). Thereafter, estradiol-17 β declined rapidly in the passively immunized group compared with the control group, whereas the high level remained at 72 h after PGF₂ α injection in the actively

Fig. 4. Changes in plasma concentrations of progesterone in goats after treatment with inhibin-AS (●) or normal goat serum (○) (A) and after the 3rd booster immunization against inhibin (■) or Freund's adjuvant (□) (B). The arrows indicate the time of PGF₂ α injection. Values are mean $\pm$ SEM of six (A) or five (B) animals. * P <0.05: significance of differences between the immunized and control groups.

immunized group. On the other hand, plasma concentrations of progesterone suddenly decreased after PGF₂ α injection in the passively and actively immunized groups (Fig. 4 A and B). At the time of PGF₂ α injection, the progesterone level was significantly (P <0.05) higher compared with the control value in the actively immunized group, indicating more ovarian activity (higher number of corpora lutea, Table 1).

The effect of immunization against inhibin on ovarian response

As determined by ultrasound scanning, the number of corpora lutea was significantly higher in the actively immunized group than the other groups (Table 1). Thereafter, there was a significant (P <0.01) rise in the total number of follicles (≥ 4 mm in diameter) in animals immunized against inhibin either passively or actively compared with the

Table 1. Effect of passive and active immunization against inhibin on ovarian response in goats

Group	Ovarian response		
	Number of corpora lutea ^(a)	Number of follicles ≥4 mm in diameter ^(a)	Ovulation rate ^(b)
Goats passively immunized against inhibin (n=6)	1.8 ± 0.4	22.4 ± 2.3**	9.4 ± 1.4**
Goats injected with normal goat serum (n=6)	2.0 ± 0.4	2.6 ± 0.4	2.2 ± 0.6
Goats actively immunized against inhibin (n=5)	6.6 ± 0.8**	18.6 ± 2.1**	8.6 ± 1.3**
Goats injected with Freund's adjuvant (n=5)	1.8 ± 0.4	2.3 ± 0.4	1.8 ± 0.3

***P* < 0.01, significantly different compared with the other values in the same column.

^(a)By using ultrasound scanning at the time of PGF₂α injection.

^(b)By counting corpora lutea by ultrasound scanning one week after the induced estrus.

control values. In the group immunized with inhibin antiserum, the number of follicles was 22.4 ± 2.3 , while it was 2.6 ± 0.4 in the control group. In addition, the number of follicles was 18.6 ± 2.1 in goats actively immunized against inhibin compared with the control group (2.3 ± 0.4). The ovulation rate was 9.4 ± 1.4 and 8.6 ± 1.3 , respectively, in the passively and actively immunized goats compared with the control groups (2.2 ± 0.6 and 1.8 ± 0.3).

Discussion

The present study clearly demonstrated that immunoneutralization of endogenous inhibin in cyclic goats either passively or actively caused an increase in plasma concentrations of FSH which, in turn, led to stimulation of ovarian follicular development and an increased ovulation rate. The magnitude and quantitative nature of the FSH secretory profiles induced by passive immunization support an endocrine role for inhibin in regulating FSH secretion in goats. Thus, inhibin, through negative feedback regulation of FSH secretion, appears to be an important factor in regulation of follicular development in the goat. In previous studies, a significant increase in plasma concentrations of FSH has been shown by passive immunization against inhibin during the estrous cycle in cows [9, 21, 22], sheep [19, 40–42], rats [43], mice [44], hamsters [12, 45], and guinea pigs [13], indicating the important role of inhibin in regulating FSH secretion during the estrous cycle in

these species. The rise in FSH concentration seen following immunization resulted in marked stimulation of follicular growth and improved ovarian response. The observation that the rise in plasma FSH preceded the emergence of new follicles leads to the conclusion that hypersecretion of FSH stimulates multiple growth of follicles. This action, timed to coincide with PGF₂α injection in the present study, enlarged the pool of recruitable follicles, and this gave rise to a greater ovulation rate in the immunized goats. Previous results obtained from active immunization [47] or repeated injections of inhibin antiserum [8, 48] have indicated a possibility that ovarian response to inhibin immunization is not only mediated by the rise in circulating FSH levels but is also mediated by the direct effect of immunization on follicular growth probably through local effects of inhibins in the ovary. Regarding LH levels, there was no marked change in the basal LH concentrations between the immunized and control goats. Plasma concentrations of estradiol-17β rose after PGF₂α injection in all goats. The peak levels of the plasma concentrations of estradiol-17β in the immunized groups were significantly higher than in the controls. This was similar to previous results recorded in goats [49]. The increase in the plasma concentrations of estradiol-17β was probably due to increased numbers of estrogenic follicles destined to ovulate. The high level of plasma estradiol-17β induced the LH surge 12 h earlier in the passively immunized group, and this is similar to the results reported previously by Campbell and Scaramuzzi [50]. In addition, estradiol-17β remained relatively

high in the actively immunized group, suggesting that large follicles capable of secreting estradiol-17 β still remained in the ovaries of the actively immunized group. This probably depends on circulating levels of FSH. At the time of PGF $_2\alpha$ injection, the progesterone level was significantly higher in the actively immunized group than in the controls. This might reflect increased numbers of corpora lutea.

It is well known that repeated use of eCG to induce superovulation in goats results in diverse effects because of the formation of anti-eCG antibodies [35]. Therefore, use of passive or active immunization against inhibin is a suitable, simple, and practical alternative to eCG for induction of superovulation in goats. Moreover, active immunization against inhibin improves superovulatory response to exogenous FSH in cattle [27]. In the present study, the ovulation rate was not comparable to the follicular population. Therefore, further studies are needed to improve ovulation rate in inhibin-immunized animals.

In conclusion, the present study demonstrated that inhibin is an important factor as a regulator of FSH secretion in goats. The present results also demonstrated that passive and active immunization against inhibin induced a marked

increase in FSH, ovarian follicular population, and ovulation rate. Therefore, neutralization of inhibin bioactivity through passive or active immunization is a practical and potential method for inducing follicular development and increasing ovulation rate in goats.

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