—Research Note—

Passive Immunoneutralization of Endogenous Inhibin Increases Ovulation Rate in Miniature Shiba Goats

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Abstract. We reported previously that passive immunization against inhibin enhances follicular growth and increases the ovulation rate. However, the ovulation rate was not comparable to the number of follicles. Therefore, the aim of this study was to attempt to increase the ovulation rate by increasing the interval between inhibin immunization and PGF_{2α} injection. Five miniature Shiba goats were treated with 10 ml inhibin antiserum (inhibin-AS) developed against [Tyro³⁰]-inhibin α (1–30). A control group (n=5) was treated with normal goat serum. All animals were injected intramuscularly with 125 μ g PGF_{2α} 72 h after treatment to induce estrus and ovulation. Blood samples were collected for hormonal assay and the ovulation rate was determined by laparotomy. In contrast to the control group, there was a significant increase in plasma concentrations of FSH in the immunized group. After luteolysis, plasma concentrations of estradiol-17 β increased markedly to a preovulatory peak about 2 folds higher ($P<0.01$) than that of controls. In addition, the ovulation rate was greater in the immunized group (14.4 ± 2.2) than in the control group (2.2 ± 0.6), and the mean number of follicles ≥ 4 mm in diameter was 10.0 ± 0.8 in the inhibin-AS group compared with 2.4 ± 0.3 in control group. The present results demonstrate that immunoneutralization of endogenous inhibin increased FSH secretions in miniature shiba goats. The increased FSH secretion enhanced follicular growth and increased the ovulation rate. Additionally, increasing the interval between inhibin-AS and PGF_{2α} injections (to 72 h) resulted in a greater ovulation rate compared with the previous protocol (48 h). Therefore, inhibin-AS treatment proved to be an effective alternative to exogenous gonadotropin methods for induction of superovulation in goats.

Key words: Inhibin, FSH, Ovulation rate, Miniature Shiba goats

(J. Reprod. Dev. 50: 705–710, 2004)

Immunoneutralization of endogenous inhibin selectively increases FSH secretion, providing evidence that inhibin is an important factor in the inhibitory regulation of FSH secretion in goats [1, 2]. Multiple ovulations have been induced successfully by passive immunoneutralization of

endogenous inhibin in several species such as rats [3, 4], ewes [5–7], hamsters [8], cows [9, 10], mares [11], guinea pigs [12], mice [13, 14], and goats [2].

In previous studies, a combination of equine chorionic gonadotrophin (eCG) and human chorionic gonadotrophin (hCG) has been widely used to induce superovulation in goats. However, the long half-life of eCG interferes with normal

fertilization and embryo development [15,16], and repeated eCG treatments induced anti-eCG antibodies that clearly had negative effects on reproduction in goats [17]. This led us to establish a new protocol for superovulation in goats, and we succeeded in inducing superovulation in goats by using passive [2] and active [18] immunization against inhibin. However, in our previous passive immunization protocol for goats, the ovulation rate (4.2 ± 0.5) was not comparable to the greater number of follicles (13.5 ± 0.8) observed in the ovary at ovulation. Therefore, in the present study, we injected PGF_{2α} 72 h after treatment with inhibin-AS (24 h later than the previous protocol) in an attempt to increase the ovulation rate. The present study aimed to improve the passive immunization protocol for induction of superovulation in goats.

Materials and Methods

Experimental animals

Ten mature miniature Shiba goats (*Capra hircus*), 1.5 to 3 years of age and weighing 28.0 ± 1.5 kg, housed under natural day-light and fed hay *ad libitum* were used in this study. Estrous cycles were synchronized with 2 injections of 125 µg of a synthetic analogue of prostaglandin F_{2α} (Estrumate, Schering Plough Animal Health, New Jersey, USA) 11 days apart. Estrous behaviour was observed and checked with mature bucks. On day 10 of the estrous cycle animals were allocated to two groups: (1) control, treated with i.v. injection of 10 ml normal goat serum (n=5); and (2) inhibin-immunized, treated with i.v. injection of 10 ml inhibin antiserum (n=5). The time of injection of serum was defined as 0 h. At 72 h, all animals were injected intramuscularly with 125 µg PGF_{2α} to induce estrus and ovulation. Blood samples were collected at 6-h intervals from 24 h before until 144 h after treatment. For determination of LH surge, blood samples were collected every 2 h from 48 to 72 h after PGF_{2α} injection. Blood samples were collected from jugular venipuncture into heparinized Vacutainer tubes (Terumo Venoject II, Tokyo, Japan), and centrifuged at 1200 g for 15 min at 4 °C. The plasma was separated and stored at -20 °C until assayed for hormones.

Determination of ovarian response

The follicle populations and ovulation rate

(ovulation rate was calculated from the number of corpora lutea) were determined one week after estrus by mid-ventral laparotomy. All animal procedures were completed in accordance with institutional animal care and use guidelines.

Antisera

The antiserum against inhibin used in this study was raised in ovariectomized goats to a synthetic peptide of the 1–30 amino acid sequence of the N-terminal of the α-chain of porcine inhibin conjugated to rabbit serum albumins as described previously [1].

Hormone analysis

Plasma concentrations of FSH were measured by radioimmunoassay (RIA) system as described previously [1] by using NIDDK-antiovine FSH-1 (AFP-C5288113), NIDDK-FSH-I-1 (AFP5679C) for radioiodination, and NIDDK-oFSH-RP-1 as a reference standard. Plasma concentrations of LH were measured by RIA as described previously [19] by using antiovine LH (YM No. 18), NIDDK-oLH-I-3 for radioiodination, and NIDDK-oLH-RP-24 as a reference standard. The intra- and inter-assay coefficients of variation were 9.8% and 12.2% for FSH and 5.5% and 6.3% for LH, respectively. Plasma concentrations of estradiol-17β and progesterone were determined by a double antibody RIA system using ¹²⁵I-labeled radioligands as described previously [20]. 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 inter-assay coefficients of variation were 5.9% and 7.2% for estradiol-17β and 8.0% and 9.4% for progesterone, respectively.

Statistical analysis

Data are presented as means (± SEM). Student's *t*-test was used to compare between the two groups using the SAS computer package [21]. Values were considered statistically different when *P* < 0.05.

Results

Plasma concentrations of FSH and LH

In the control group, plasma concentrations of FSH did not significantly change after injection of

Fig. 1. Changes in plasma concentrations of FSH (A) and LH (B) in miniature shiba goats after treatment with inhibin-AS (closed circle) or control serum (open circle). Black arrows indicate time of serum injection and white arrows indicate time of PG injection. Values are mean $\pm$ SEM of five animals. * ($P<0.05$) versus control values.

normal goat serum during the period before FSH surge. In contrast, treatment with the inhibin antiserum resulted in a significant ($P<0.05$) increase in plasma concentrations of FSH compared with control values within 6 h after treatment (Fig. 1A). In addition, the preovulatory FSH peak in the inhibin immunized group was significantly higher than in the control group. Moreover, the time interval between the PGF_{2 α} injection and the preovulatory gonadotropin surge tended to be shorter ($P=0.07$) in the immunized group than in the control group (Table 1). On the other hand, there was no significant difference between the immunized and control groups in LH levels (Fig. 1B).

Plasma concentrations of estradiol-17 β and progesterone

The plasma concentration of estradiol-17 β is shown in Fig. 2A. In control animals, plasma concentrations of estradiol-17 β increased after the PGF_{2 α} injection and reached a peak (51.3 ± 12.3 pg/ml) level within 48 h. However, treatment with inhibin antiserum induced a rapid and marked increase in plasma estradiol-17 β which reached a peak level (96.5 ± 24.5 pg/ml) earlier than the control animals. On the other hand, passive immunization against inhibin had no effect on plasma progesterone concentrations and injection of PGF_{2 α} 72 h after inhibin antiserum or normal goat serum treatment resulted in a sharp decline in progesterone concentrations in both immunized

Table 1. Effect of immunoneutralization of endogenous inhibin on estrus and ovarian response in miniature Shiba goats

Item	Control group	Immunized group
Number of treated goats	5	5
Number of goats detected in estrus ^a	5	5
Number of goats ovulating	5	5
Interval to estrus ^b (h)	57.6 $\pm$ 4.5	48.0 $\pm$ 3.8
Interval to preovulatory gonadotropin surge ^c (h)	65.6 $\pm$ 5.0	53.6 $\pm$ 2.8
Ovulation rate ^d	2.2 $\pm$ 0.6	14.4 $\pm$ 2.2**
Mean number of follicles $\geq$ 4 mm in diameter ^e	2.4 $\pm$ 0.3	10.0 $\pm$ 0.8**
Ovarian activity ^f	4.6 $\pm$ 0.8	24.4 $\pm$ 2.9**

** $P<0.01$, significantly different from the control value in the same row. ^aDetected by estrous observation and mature bucks. ^bHours from PGF_{2 α} injection to onset of estrus. ^cHours from PGF_{2 α} injection to preovulatory FSH and LH surges. ^dBy direct observation and counting corpora lutea at laparotomy. ^eBy direct observation and counting follicles at laparotomy. ^fNumber of follicles and corpora lutea.

and control groups (Fig. 2 B).

Ovulation rate and number of follicles

In the immunized group, the number of corpora lutea was significantly ($P < 0.01$) higher than in the control group as determined by direct observation one week after estrus. Moreover, the number of unovulated follicles ≥ 4 mm in diameter was significantly ($P < 0.01$) greater in the immunized group. The ovarian activity, number of follicles and corpora lutea, of the immunized group was found to be 5 times greater than that of the control group (Table 1).

Discussion

Immunoneutralization of endogenous inhibin in cyclic miniature Shiba goats resulted in an increase in plasma concentrations of FSH and ovulation rate. The preovulatory gonadotropin surge occurred earlier than in the control group. This may be attributable to the earlier peak in estradiol- 17β in the immunized goats which in turn induced GnRH release from the hypothalamus and release of LH and FSH. The FSH-mediated mechanism of action of inhibin immunization on the ovulation rate in the present study is in agreement with our previous results [2]. Furthermore, a reciprocal relationship between circulating FSH and inhibin levels during the estrous cycle has been reported in goats [22], suggesting that inhibin secreted from the ovary plays an important role as a regulator of FSH secretion. The rise in FSH concentration seen following immunization stimulated multiple growth of follicles and increased the ovulation rate. However, the ovulation rate in the present study was greater than that which we previously reported in goats [2]. The greater ovulation rate seen in the present study might be attributable to the injection of PGF $_{2\alpha}$ 72 h after inhibin-AS treatment (24 h later than the previous protocol) allowing more time for follicular growth and differentiation.

Previous results obtained from active immunization [23] or repeated injections of inhibin antiserum [24, 25] suggested the possibility that ovarian response to inhibin immunization is not only mediated by the rise in circulating FSH levels but also by the direct effect of immunization on follicular growth. Hennies *et al.* [26] reported an

Fig. 2. Changes in plasma concentrations of estradiol- 17β (A) and progesterone (B) in miniature Shiba goats after treatment with inhibin-AS (closed circle) or control serum (open circle). Black arrows indicate time of serum injection and white arrows indicate time of PG injection. Values are mean $\pm$ SEM of five animals. * ($P < 0.05$) versus control values.

increase in ovulation rate in goats actively immunized against inhibin without a marked increase in FSH. In goats treated with inhibin antiserum, there was a marked rise in estradiol- 17β . This rise in estradiol- 17β may reflect an increased number of growing follicles. Therefore, immunoneutralization of endogenous inhibin enhances follicular development and in turn secretion of estradiol- 17β from the ovarian follicles. Similar results were recorded in cattle [10, 27].

It is well known that repeated use of eCG for inducing superovulation in goats results in diverse effects because of the formation of anti-eCG antibodies [17]. Moreover, a higher incidence of premature corpus luteum regression in eCG-stimulated versus FSH-superovulated goats has been recorded [15, 28, 29]. Therefore, passive immunization against inhibin which selectively increases FSH secretion resulting in a dramatic increase in the ovulation rate may be a suitable and

simple alternative to eCG for inducing superovulation in goats.

In conclusion, the present study demonstrated that passive immunization against inhibin increased FSH secretions. In addition, injection of PGF_{2α} 72 h after inhibin immunization improved the superovulation response resulting in a greater ovulation rate. Therefore, neutralization of inhibin bioactivity followed by PGF_{2α} injection can be used as an alternative method for inducing superovulation in goats.

Acknowledgements

We are grateful to Dr AF Parlow (National Institute of Diabetes and Digestive and Kidney

Diseases, Beltsville, MD, U.S.A.) for providing RIA materials for ovine FSH and LH, Dr G D Niswender (Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, CO, U.S.A.) for providing antisera to estradiol-17β (GDN 244) and progesterone (GDN 337) and Dr Y Mori (University of Tokyo, Bunkyo-ku, Tokyo, Japan) for antiserum to ovine LH (YM-18). This work was supported in part by the Ito Foundation and the Japan Livestock Technology Association, a Grant-in-Aid for Scientific Research (The 21st Century Center of Excellence Program, E-1) from the Ministry of Education, Culture, Sports, Science and Technology of Japan and a Grant-in-Aid for Scientific Research (No. 03338) from the Japan Society for the Promotion of Science.

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