African Journal of Agricultural Research Vol. 6(22), pp. 5040-5046, 12 October, 2011
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR10.1084
ISSN 1991-637X ©2011 Academic Journals

Full Length Research Paper

The effect of immunization against Xinjiang fine wool sheep inhibin α on the reproductive traits of rats

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Accepted 26 September, 2011

The purpose of this study was to investigate the effects of the Xinjiang fine wool sheep inhibin α subunit on the reproductive traits. Rats were immunized with the Xinjiang fine wool sheep inhibin α subunit recombinant protein, using Freund's adjuvant as the adjuvant. Compared to the control group, the antibody titers increased steadily in the 1 mg inhibin-immunized group, and the antibody titers in 1 mg inhibin-immunized rats were highest among all groups on day 28 and day 38. Moreover, the level of follicle stimulating hormone (FSH), luteinising hormone (LH) and progesterone in all groups showed an impulse pattern throughout estrous cycle, and peak values of FSH and progesterone secretion in 1 or 2 mg inhibin-immunized groups were significantly higher than that in the other groups, however, the level of LH in the all inhibin-immunized groups during the estrous cycle was similar to the control group. Besides, the number of ovarian follicles (≥ 0.8 mm) in 1.0 mg inhibin-immunized group was significantly higher than that in other groups. In addition, the correlation analysis showed that the level of antibody was the positive correlation with the number of ovarian follicles. Together, the data suggested that immunization with the Xinjiang fine wool sheep recombinant inhibin could facilitate the ovarian follicular development via an inhibin α-dose dependent way.

Key words: Inhibin α subunit, Sprague-Dawley rat, antibody, hormone, progesterone.

INTRODUCTION

Xinjiang fine wool sheep with the characters of the high-yield meat and wool was bred, in Yili, Xingjiang in 1954 (Wang, 2004). It is the oldest and most of fine wool sheep breed in China, with many advantages such as strong adaptability, good resistance and so on. But, Xingjiang fine wool sheep is also a kind of lower prolific breed, because it's ovulation rate and fecundity are lower than other breeds. So, many researchers tried to improve the prolificacy of Xinjiang fine wool sheep via the crossbreeding system and within-breed selections, however, these processes are very slow and complicated (Zhang, 2000). Thus, it is very essential to find a new strategy of increasing the prolificity of Xinjiang fine wool sheep. Inhibin (INH), consisting of an α and α subunit, is an heterodimeric glycoprotein hormone belonging to the transforming growth factor α (TGF-α) superfamily (Kingsley, 1994). The inhibin plays an important role in the regulation effect of the hypothalamo-pituitary-gonadal axis. the negative correlation between follicle-stimulating hormone (FSH) and levels of INH in humans have been reported (Jensen et al., 1997; Anawalt et al., 1996). INH α can suppress FSH levels in castrated rams and monkeys, but not influence LH secretion (Tilbrook et al., 1993; Ramaswamy et al., 1998). The active immunization against INH can neutralize endogenous INH, increase the ovulation rate in several mammalian species, such as

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sheep (Wrathall et al., 1990), gilt (Brown et al., 1990; King, 1990), and heifer (Scanlon et al., 1993), and up-regulate the levels of FSH in ewes (Montgomery et al., 1987), goats (Medan et al., 2003a), and heifers (Glencross et al., 1992).

In this study, we sought to evaluate effect of the Xinjiang fine wool sheep inhibin α on the reproductive traits of rats. Our results demonstrated for the first time that the Xinjiang fine wool sheep inhibin α could influence the ovarian follicular development of rats via inducing the higher antibody, and stimulating the hormone secretion on the dose dependent way.

MATERIALS AND METHODS

Animals, kits and adjuvant

The adult female Sprague-Dawley rats, eight weeks of age, were purchased from the Experiment Animal's Center of Sichuan University(Sichuan, China), and cared for under a 12 h light cycle, and fed with pathogen-free food and water condition. The radio immunoassay (RIA) kits for testing the concentration of FSH, LH and progesterone in serum were obtained from Jiuding Medical and Depu Biotechnology Company (Tianjin, China). Freund's adjuvant was bought from the Sigma Company (Sigma, St. Louis, IL)

Vaccine

The Xinjiang fine wool sheep inhibin α subunit fusion protein was prepared as previously described (Zhang, 2009). Briefly, the recombinant plasmid pET-32a-INH,(INHαgene was constructed into the prokaryotic expression vector pET-32a) was transformed into the prokaryotic expression bacterial strain *Escherichia coli* BL21(DE3), and the *E. coli* BL21(DE3) containing PET-32a-INH was induce with the isopropyl beta-D-thiogalactopyranoside (Sigma, St. Louis, IL) for 5 h at 37℃. The Xinjiang fine wool sheep inhibin αsubunit fusion protein was purified by the osmotic shock method for the preparation of the vaccine, and the recombinant protein was dissolved in PBS solution and emulsified with an equal volume of Freund's adjuvant.

Immunization

For vaccination, Sprague-Dawley rats were randomly divided into four groups (n = 6 each) , and subcutaneously injected with 0.5, 1.0 and 2.0 mg inhibin α recombinant protein in 1 ml vaccine on day 0, 14, and 28, respectively. PBS was used as the sham control.

Blood samples collection

For examination of inhibin specific antibody titer, Sprague-Dawley rats were bled by tail vein on day 0, 14, 28, 38 after the primary immunization. However, blood samples were collected every 6 h for 4 days (from day 34 to 37) to detect concentration of the hormone (FSH, LH, progesterone). The serum samples were prepared by centrifugation at 3000 rpm at 4℃ for 20 min, and stored at -20℃.

Detection of specific antibody by ELISA

Antibody against inhibin in sera was determined by an enzyme linked immunosorbent assay (ELISA) (Wang et al., 2008). Ninety-six well microtiter plates were coated with 2 αg/ml of recombinant inhibin protein in per well 100 α l of 0.05 M bicarbonate buffer (pH 9.6) at 4℃ overnight. Each well was blocked with 3% of BSA-PBST at 37℃ for 2 h, and the rat serum with a dilution at 1:800 were incubated with each well. A secondary goat anti-rat antibody of IgG conjugated with horseradish peroxidase (Sigma, St. Louis, IL) at 1:1000 was added into each well and incubated at 37℃ for 1 h. A 10 mg of TMB tablet (Sigma, St. Louis, IL) was dissolved in 0.025 M phosphate-citrate buffer and added each well for the color development. Addition of 2 M of H_2SO_4 stopped the reaction, and the plates were read with a plate reader (Magellan, Tecan Austria GmbH) at 450 nm. Antibody responses against the inhibin in the sera were reported as optical density (OD) values.

Hormone analysis

Levels of FSH, LH and progesterone in sera, were measured by the radio immunoassay (RIA) kits, according to the manufacturer' instruction. The concentration of FSH, LH and progesterone in samples was calculated according to the standard curve plotted using standard FSH, LH and progesterone from the kits.

Determination of ovarian response

The population of ovarian follicles in each group were examined on day 10 after the second booster immunization as described previously (Mao et al., 2003). Rats were executed and took out the bilateral ovaries without fat from the estrus. Ovary was measured in length and width with Vernier caliper. Follicles ≥ 0.8 mm in diameter were counted.

Statistical analysis

All experiments were performed at least three times, and the results of the representative experiment are presented. Data were analyzed using the SPSS 13.0 Data Editor. Differences were considered to be statistically significant at $p < 0.05$.

RESULTS

To evaluate whether the Xinjiang fine wool sheep inhibin recombinant protein can induce the antigen-specific antibody responses, OD values of antibody in the sera were analyzed by ELISA on day 0, 14, 28 and 38. As shown in Figure 1, OD values of anti-inhibin antibody increased steadily after the primary immunization, and OD values of anti-inhibin antibody in inhibin-immunized groups were significantly higher ($p < 0.05$) than that in the control group on day 28 and 38. The result suggested that the Xinjiang fine wool sheep inhibin α recombinant protein could induce the antibody responses against the inhibin.

To determine whether the Xinjiang fine wool sheep inhibin immunization could influence the hormone secretion such as FSH, LH and progesterone, the levels of the hormone in sera were determined by the RIA kits. As depicted in Figure 2A, FSH secretion showed an impulse pattern during throughout the estrous cycle and a highest peak value of serum FSH was observed at 24 h of pro-oestrus. Besides, the FSH concentration of the inhibin immunized groups at each time point was higher than

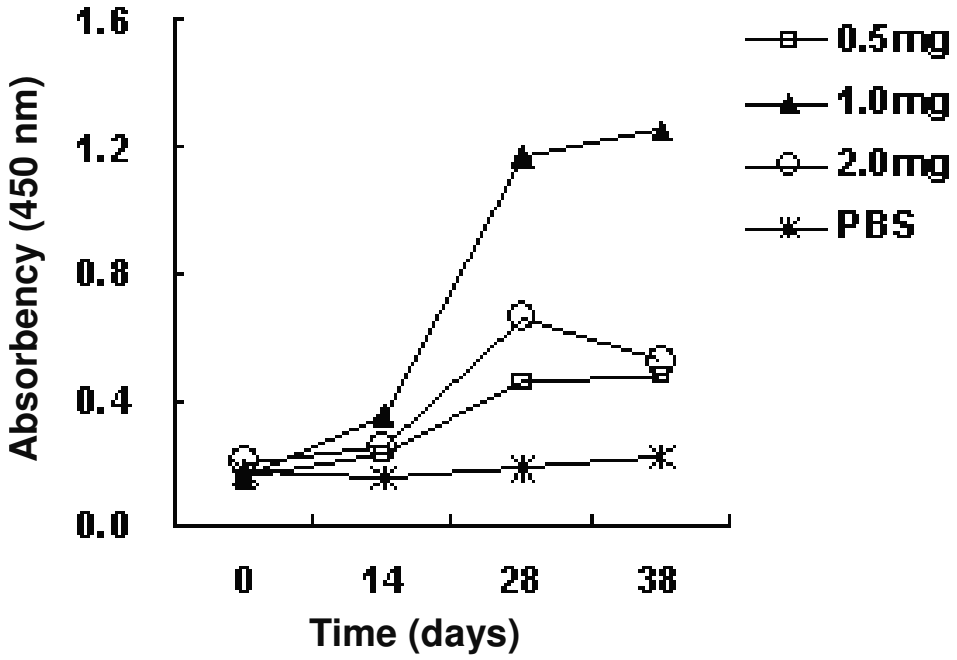

Figure 1. Antibody titer. The rats were immunized with the inhibin α on day 0, 14, 28, and 38, sera samples were collected on day 0, 14, 28 and 38. Antibody titers in the sera samples were tested by ELISA as described in the materials and methods section. The mean titers were expressed as average OD values of six rats, measured in triplicate. Means without a common letter differ, *p < 0.05.*

control groups, and the FSH concentration at the highest peak value (28.25 ± 0.83 mIU/ml) was significantly higher than the control groups (12.47 ± 2.71 mIU/ml) ($p < 0.05$). The average concentration of FSH during the estrous cycle was 13.83±0.79 mIU/ml, and was significant higher than the control group (5.36 ± 0.57 mIU/ml) ($p < 0.05$). But, the serum LH concentration of the inhibin-immunized groups during the oestrus cycle was similar to the control groups ($p > 0.05$) (Figure 2B). The progesterone concentration at the highest peak value in the inhibin-immunized groups (51.34 ± 9.30 ng/ml) was significantly higher than that in the control group (30.86 ± 0.92 ng/ml) ($p < 0.05$) during the oestrus cycle (Figure 2C).

To examine whether the immunization of inhibin α could influence ovarian follicular development, we investigated the population of ovarian follicles and the ovarian morphology in each group on day 10 after the second booster immunization. The results indicated that the number of follicles ≥ 0.8 mm in 1.0 mg inhibin-immunized group (31.0 ± 2.65) were significantly higher than that in other groups (20.4 ± 2.31, 22.25 ± 3.42 and 15.33 ± 0.67) ($p < 0.05$) (Figure 3A). Moreover, the ovaries developed well, and the number of antral follicles increased in the groups immunized with inhibin (Figure 3B). There was more antral follicles than other groups, and the oviduct was thin and long in the 1.0 mg inhibin-immunized group (Figure 3B). To explore further the correlation between the antibody response and the follicular development, the correlation between OD value of the antibody response on day 38 and follicular numbers, ovarian length, and ovarian width was analyzed by ANOVA method, the result showed that a remarkable positive correlation was observed between the antibody response and follicular numbers ($r = 0.73$, $p < 0.01$), but, there was the negative relationship between antibody and weight of ovaries (Table 1).

DISCUSSION

In this study, we have demonstrated that active immunization with the Xinjiang fine wool sheep inhibin α-subunit recombinant fusion protein vaccine could up-regulate the circulating inhibin antibody titer (Figure 1) and concentrations of average serum FSH and progesterone (Figure 2A and C), but not influence the LH secretion (Figure 2B).

Besides, α inhibin α could improve the follicular development (Figure 3A and B).

Previous studies had demonstrated that the fusion protein could induce the strong humoral response. For instance, Satterlee et al. (2002) had reported that the immunization with the inhibin fusion protein consisted with

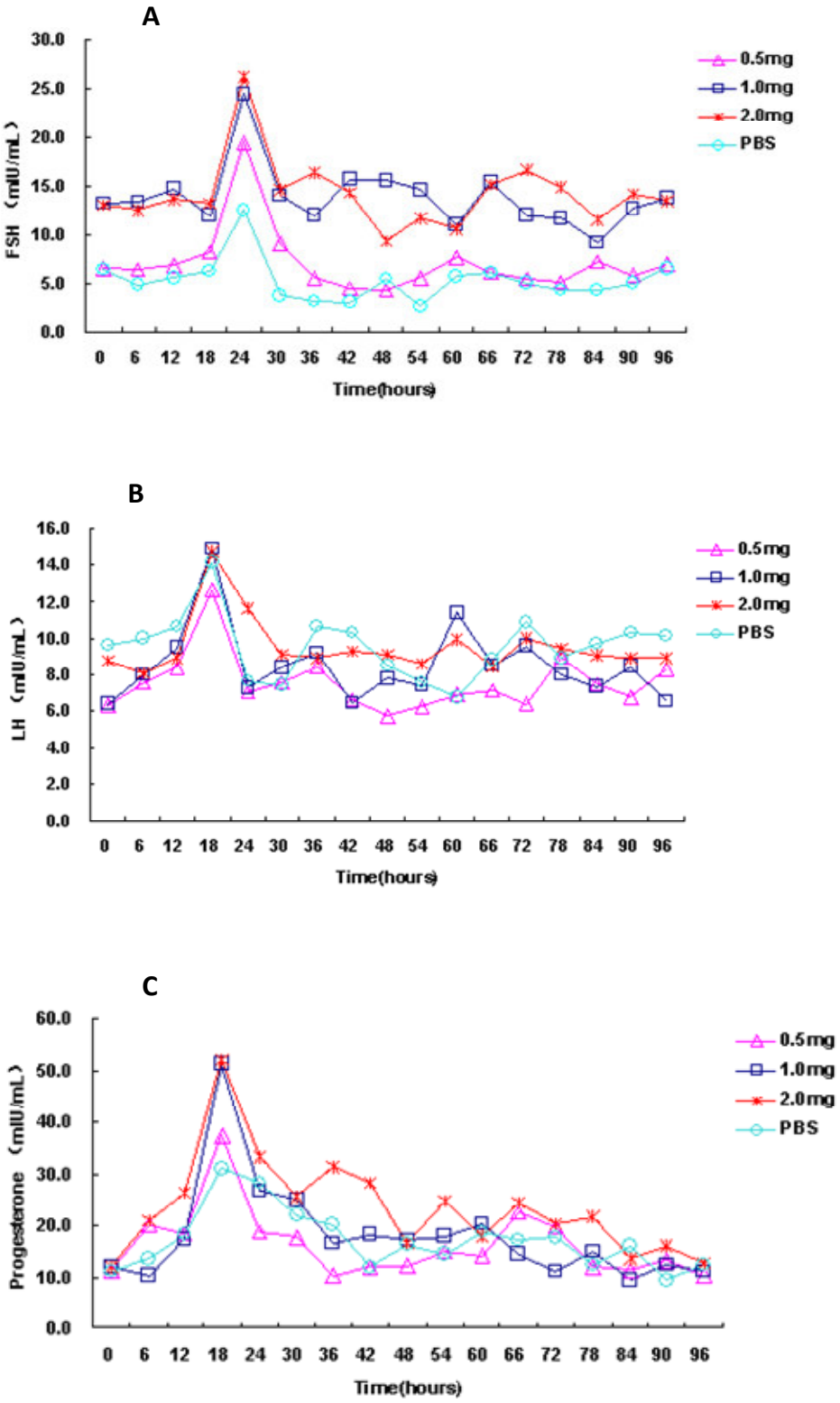

Figure 2. Analysis of the hormone secretion in the sera. The sera samples were collected every 6 h for 4 days during estrous cycle. The hormone in the sera was detected by RIA kits as described in the *materials and methods section.* The mean concentration was expressed in milli-international units per milliliter, and represented the average values of six rats, measured in triplicate. (A) FSH secretion during the estrous cycle; (B) LH secretion during the estrous cycle; (C) Progesterone secretion during the estrous cycle. Means without a common letter differ, $p < 0.05$.

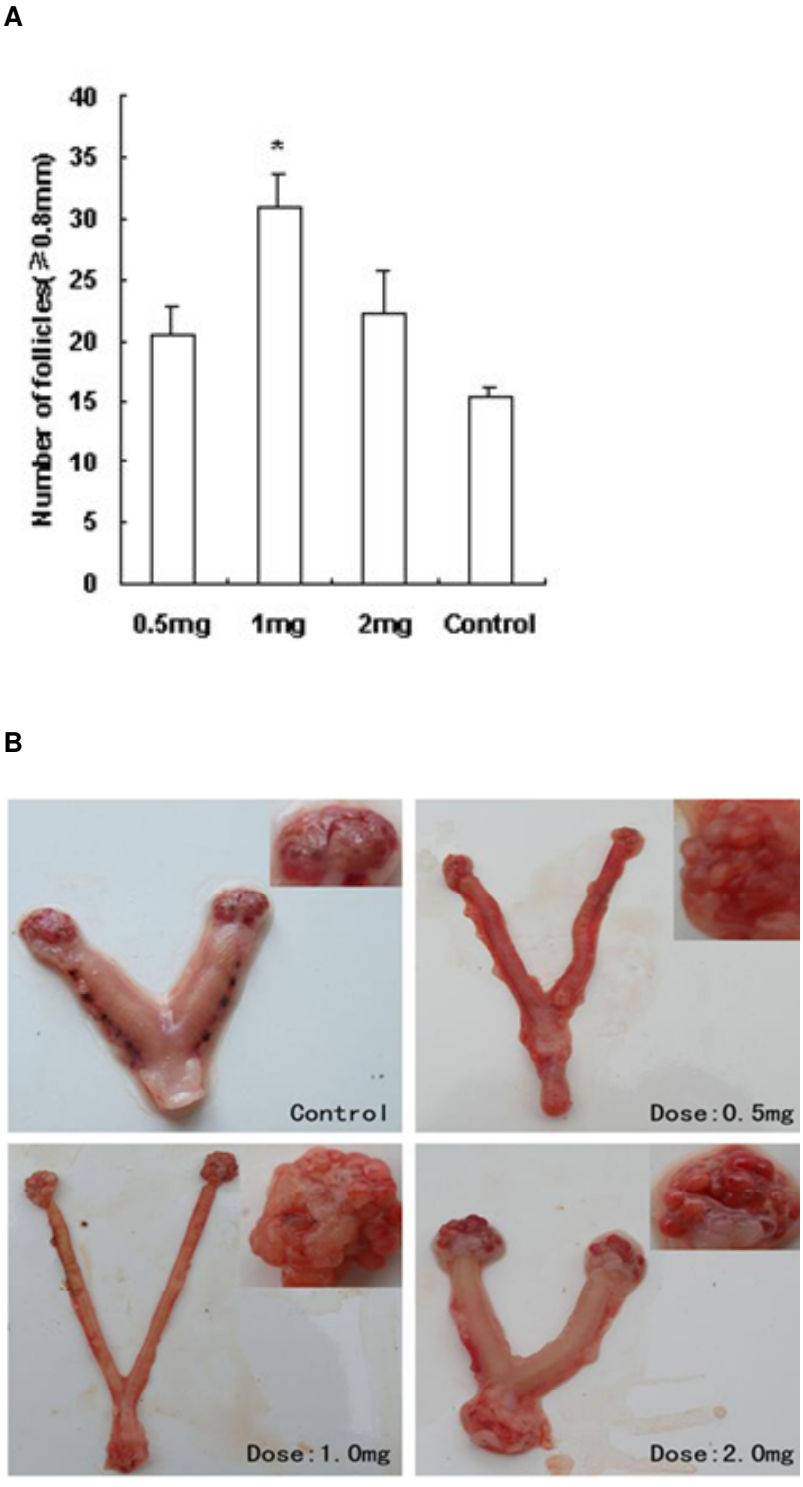

Figure 3. The ovarian follicular development. Rats were sacrificed on days 10 after final immunization, and the ovaries were excised. The follicles (≥0.80) in diameter were counted. A) the number of follicles, B) the photograph of the ovaries. Means without a common letter differ, $p < 0.05$.

inhibin α and maltose-binding protein could elicit antibody response against the inhibin α in the chicken (Satterlee et al., 2002). In our study, rats were immunized with 1 mg of the inhibin α fusion protein contained thioredoxin, which

Table 1. Correlation analysis. Correlation of antibody and follicular development was measured by ANOVA method.

Parameter	Number of rats follicles	Length of ovaries	width of ovaries	Weight of ovaries
Antibody titer	0.73**	0.36	0.23	-0.43
Number of rats follicles		0.53	0.40	-0.08
Length of ovaries			0.61	0.59
Width of ovaries				-0.34

$^{**}p < 0.01$.

induced higher antibody titer. Thus, the inhibin α fusion protein has the good immunogenicity.

The mechanism of the inhibin immunization regulating the levels of hormone is very complicated. Medan et al. (2003) had demonstrated that after inhibin immunization, the antibody against inhibin could neutralize endogenous inhibin, lower the biological activity of inhibin and weaken the suppression effect on FSH secretion, but not impact on the secretion of LH (Medan et al., 2003b). However, Sasaki et al. (2006) had reported that the inhibin immunization increased concentration of the FSH, progesterone and LH in the goats (Sasaki et al., 2006). In this study, 1 mg of the inhibin immunization has improved the secretion of FSH and progesterone, but did not influence the concentration of LH in the rats, suggesting inhibin immunization may play an important role in influencing level of reproductive hormone. We inferred that the different animal species might get different results of secretion of LH after the inhibin immunization.

It has been known that development of follicles in the ovary is very important for increasing the animal's prolificacy. Derar et al. (2004) have demonstrated that inhibin α was a chemical signal of the number of growing follicles in the ovary, and a key hormone in determining species-specific ovulation rates (Derar, 2004). Scanlon et al. (1993) had shown that ovulation rates and fecundity were increased in the heifers using a synthetic fragment of bovine inhibin α (Scanlon, 1993) In this study, 1 mg of inhibin α could significantly improve the number of follicles (0.8 mm in diameter), and the number of follicles was significantly positive correlation with the antibody titer, suggesting that inhibin α up-regulated the development of follicle by higher antibody titer against the inhibin α But, why is it the negative correlation between antibody titer and the ovarian, we presumed that there are perhaps some special mechanisms involved in the ovarian development.

In summary, the data we presented in this study demonstrated that the Xinjiang fine wool sheep inhibin α subunit recombinant fusion protein could induced the strong humoral immune response, enhance the levels of FSH and progesterone, and meliorate reproductive trait. In addition, this strategy has an import advantage over other traditional way since the inhibin immunization is very simple and cost-effective. However, the further research is necessary to understand the relationship between the reproductive performance and the inhibin immunization.

ACKNOWLEDGEMENTS

This work was supported by program for Changing Scholars and Innovative Research Team in University (IRT0848), and Sichuan Education Commission (Project No. 09ZA072).

REFERENCES

Anawalt BD, Bebb RA, Matsumoto AM, Groome NP, Illingworth PJ, McNeilly AS, Bremner WJ(1996). Serum inhibin B levels reflect Sertoli cell function in normal men and men with testicular dysfunction. J. Clin. Endocrinol. Metab., 81: 3341-3345.

Brown RW, Hungerford JW, Greenwood PE, Bloor RJ, Evans DF, Tsonis CG, Forage RG (1990). Immunization against recombinant bovine inhibin {alpha} subunit causes increased ovulation rates in gilts. J. Reprod. Fertil., 90: 199-205.

Chengyun Z, Xianyin Z (2009). The cDNA cloning, sequencing analysisi and expression of Xinjiang fine-wool sheep inhibin subunit. Acta Vet. Zootech. Sin., 40: 15-19.

Derar RI, Maeda Y (2004). Effect of active immunization of pony mares agaist recombinant porcine inhibin α subunit on ovarian follicular development and plasma steroids and gonadotropins. Theriogenology 66: 31-35.

Fengling Z, Wang W (2000). Discussion on the continuable development of the Xingjiang fine wool sheep breeding. XJ. Agr. Reclam. Econ., 2: 41-43.

Glencross RG, Bleach ECL, McLeod BJ, Beard AJ, Knight PG (1992).Effect of active immunization of heifers against inhibin on plasma FSH concentrations, ovarian follicular development and ovulation rate. J. Endocrinol., 134(1): 11-18.

Jensen TK, Andersson AM, Hjollund NHI, Scheike T, Kolstad H, Giwercman A, Henriksen TB, Ernst E, Bonde JP, Olsen J, McNeilly A, Groome NP, Skakkebak NE (1997). Inhibin B as a Serum Marker of Spermatogenesis: Correlation to Differences in Sperm Concentration and Follicle-Stimulating Hormone Levels. A Study of 349 Danish Men. J. Clin. Endocrinol. Metab., 82: 4059-4063.

King BF, Sesti JH, Britt KL (1990). Enhancement of ovulation rate by immunization of gilts against inhibin. Program for the 82nd Annual Meeting of the American Society of Animal Science: ASAS, Savoy, IL.

Kingsley DM (1994). The TGF-beta superfamily: new members, new receptors, and new genetic tests of function in different organisms. Gene. Dev., 8: 133-146.

Mao DG, Ying LG (2003). Effect of inhibinα(1-32) gene immunization on the follicular development and reproductive hormones in rats. Agr. Sci. China, 3: 337-343.

Medan MS, Watanabe G., Sasaki K, Nagura Y, Sakaime H, Fujita M, Sharawy S, Taya K (2003). Effects of passive immunization of goats against inhibin on follicular development, hormone profile and ovulation rate. Reproduction, 125: 751-757.

Medan MS, Watanabe G, Sasaki K, Nagura Y, Sakaime H, Fujita M, Sharawy S, Taya K (2003). Ovarian and hormonal response of female goats to active immunization against inhibin. J. Endocrinol., 177: 287-294.

Montgomery GW, Martin GB, Blanc MR, Pelletier J (1987). Season influences FSH concentration in ovariectomized Ile-de-France ewes. J. Reprod. Fertil., 80: 271-277.

Ramaswamy S, Pohl CR, McNeilly AS, Winters SJ, Plant TM(1998). The Time Course of Follicle-Stimulating Hormone Suppression by Recombinant Human Inhibin A in the Adult Male Rhesus Monkey (Macaca mulatta). Endocrinology, 139: 3409-3415.

Sasaki K, Medan MS, Watanabe G., Sharawy S, Taya K (2006). Immunization of goats against inhibin increased follicular development and ovulation rate. J. Reprod. Dev., 52(4): 543-550.

Satterlee DG, Cadd GG, Fioretti WC (2002). Active immunization of broiler breeder hens with a recombinant chicken inhibin fusion protein enhances egg lay. Poult. Sci., 81: 519-528.

Scanlon AR, Sunderland SJ, Martin TL, Goulding D, O'Callaghan D, Williams DH, Headon DR, Boland MP, Ireland JJ, Roche JF (1993). Active immunization of heifers against a synthetic fragment of bovine inhibin. J. Reprod. Fertil., 97: 213-222.

Scanlon AR, Sunderland SJ (1993). Active immunization of heifers against a synthetic fragment of bovine inhibin. J. Reprod. Fertil., 97: 213-222.

Tilbrook AJ, Kretser DM, Clarke IJ (1993). Human recombinant inhibin A suppresses plasma follicle-stimulating hormone to intact levels but has no effect on luteinizing hormone in castrated rams. Biol. Reprod., 49: 779-788.

Wang X, Zhang X, Kang Y, Jin H, Du X, Zhao G., Yu Y, Li J, Su B, Huang C, Wang B (2008). Interleukin-15 enhance DNA vaccine elicited mucosal and systemic immunity against foot and mouth disease virus. Vaccine, 26: 5135-5144.

Wang Y (2004). The guidance of introduced good sheep strain. Beijing, Golden Press, pp. 63-66.

Wrathall JHM, McLeod BJ, Glencross RG, Beard AJ, Knight PG (1990). Inhibin immunoneutralization by antibodies raised against synthetic peptide sequences of inhibin {alpha} subunit: effects on gonadotrophin concentrations and ovulation rate in sheep. J. Endocrinol., 124: 167-176.