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EFFECTS OF INHIBIN-α AND ANTI-INHIBIN-α IMMUNIZATION ON THE REPRODUCTIVE HORMONES IN KAZAKH SHEEP

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ABSTRACT

The Kazakh sheep is native to China. The breed has many advantages, including its size, hardiness, and good meat production, it has the economic disadvantage of having a low reproductive rate. Inhibin-α (INHα) regulates the synthesis and secretion of follicle-stimulating hormone (FSH) and could feasibly be used to boost reproduction in the Kazakh sheep. However, the current methods of INHα preparation are both costly and time-consuming. Here, we investigated the effects of INHα on Kazakh sheep reproductive performance by immunization with INHα and analyzing the subsequent changes in reproductive hormone levels and blood biochemical indices. An anti-INHα polyclonal antibody was raised in camel. It and a recombinant INHα protein were used to immunize groups of adult Kazakh sheep in anestrus. Reproductive hormones (FSH, luteinizing hormone [LH], progesterone [P_4], and estradiol [E_2]) were measured by ELISA, together with the measurement of changes in INH levels and blood physiological and biochemical indicators. The blood levels of LH and P_4 in the sheep immunized with the camel anti-INHα polyclonal antibody (group A) did not differ significantly from those in the recombinant INHα protein (group B) and the control group (group C) ($P > 0.05$). FSH and E_2 levels in group A were significantly higher than the controls ($P < 0.05$) and the INH concentrations were significantly lower than those in group C ($P < 0.05$). There were no abnormalities in the blood biochemical indices in groups A, B, and C.In conclusion, immune INHα preparations significantly affected the blood reproductive hormone levels of Kazakh sheep. This technique has potential application for improving the reproductive performance in these sheep and is also relevant for future research into the development of an INHα vaccine.

Key words: Inhibinα, Immune, Kazakh sheep, Polyclonal antibody, Reproductive hormones

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INTRODUCTION

Sheep, one of the most common livestock ruminants, are raised all over the world, providing a variety of meat, fat, milk, skin, and wool products (Pierson and Ginther, 1988). China's sheep stock and meat production rank first in the world but still require additional mutton imports to meet the market demand. The main reason for this phenomenon is the low lambing rate and poor reproductive performance of China's sheep. In addition, most sheep are seasonal estrous animals and only breed in the autumn and winter. Seasonal estrus also contributes to the long lambing interval and low reproductive rate (Ortavant *et al*., 1985). The improvement of reproductive performance is, therefore, a concern in sheep breeding. Kazakh sheep are one of the local breeds in China, farmed mainly in Xinjiang. The sheep are concentrated in the Hami region and the edge of Junggar basin and are also distributed in the border regions of Xinjiang, Gansu, and Qinghai. Kazakh sheep generally reach sexual maturity at the age of 4-6 months. They have the unique advantages of large size, strong physique, resistance to poor conditions and low temperatures, fast fattening resulting in more meat production, tender meat, and light smell. The Kazakh sheep is a typical seasonal estrus sheep. Estrus usually occurs in the autumn, with the remainder of the year termed anestrus (Ortavant *et al*., 1985). The complete estrous cycle lasts about 18 days (Zhai, *et al*., 2018), and the average estrous interval is 16.5-17.5 days (Di *et al*., 2014). The gestational period is about 150 days, and the lambing rate is 102% (Wang *et al*., 2015).

Inhibin (INH) is a glycoprotein hormone derived from the Sertoli cells in the testis and the granulosa cells of the ovary. It belongs to the member of transforming growth factor beta (TGFP) superfamily (Meldi *et al*., 2012). The protein is a hetero-dimer composed of α and β

subunits linked by disulfide bonds (Medan *et al*., 2012). Its main function is to inhibit the synthesis and secretion of follicle-stimulating hormone (FSH) thus affecting the development and maturation of follicles (Huang *et al*., 2019). The inhibin α subunit, encoded by the *INHA* gene, has been found to selectively regulate FSH secretion and synthesis (Hennies *et al*., 2010; Rateb *et al*., 2015; Yan *et al*., 2015). Numerous studies both in China and abroad have shown that *INHA* gene immunization can not only improve the animal's FSH level but also improves the ovulation rate and litter size (Burkart *et al*., 2006; Sasaki *et al*., 2006; Mao *et al*., 2016; Haney *et al*., 2018). However, the preparation of the inhibin immunogen has challenges, such as high cost and time-consuming production, which present difficulties to the popularization and application of inhibin immuno-technology. Therefore, it is particularly important to investigate a low-cost preparation method that provides antigens of high titer. In this study, a high purity recombinant INHα was prepared using an INHα expression strain, followed by isolation and purification of the protein. The recombinant INHα was used as an antigen to immunize a Xinjiang Bactrian camel to prepare a polyclonal antibody. The two immune preparations were injected into Kazakh sheep, and the effects on the reproductive hormone concentrations in the blood of the sheep were compared. These results provide reference data for further study on the mechanism of INH α in the regulation of the reproductive function in Kazakh sheep.

MATERIALS AND METHODS

Experimental animals: Three three-year-old Xinjiang Bactrian camels were purchased from Xinjiang Shawan County Wushi Bulake Township camel farm. Forty-five healthy adult Kazakh sheep were selected from the second company of the 151 Regiment of the eighth division of the Xinjiang Production and Construction Corps. The experimental sheep were injected with vaccines and anthelmintics regularly and were raised in the spring and autumn pasture of the 151 Regiment by natural free-range.

Materials: The INHA expression strain, His-tag Ni column, mouse anti-His-tag antibody, and horseradish peroxidase (HRP)-labeled rabbit anti-mouse IgG were purchased from Solebao Biotechnology Co., Ltd (Beijing, China).; heparin sodium blood collection tubes, HRP-labeled rabbit anti-camel IgG, and saturated sulfuric acid ammonium solution were purchased from Tiangen Biochemical Technology Co., Ltd. (Beijing, China); Freund's complete adjuvant and Freund's incomplete adjuvant were purchased from Sigma (St Louis, MO, USA); sheep follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone (P_4), estradiol (E_2), and inhibin (INH) enzyme-linked immunoassay kits were purchased from Jingmei Biotechnology (Jiangsu, China).

Induced expression of INHα protein: The BL21 expression strain containing the INHα recombinant plasmid was inoculated into 20 mL LB liquid medium containing ampicillin (100μg/mL) and then shaken at 37 ℃ and 160 rpm for overnight culture. The shaken bacterial solution was transferred to fresh LB liquid medium with the same ampicillin concentration. The shaking culture was continued until it reached an OD_{600} of 0.4-0.6. One milliliter of culture was taken as the control. Isopropyl ß-D-1-thiogalactopyranoside (IPTG) was added to the remaining bacterial solution to a final concentration of 1 mmol/L and incubated with the bacteria at 37 ℃ with shaking at 180 rpm for 4 h. After induction, samples were removed for SDS-PAGE analysis. After 4 h of this induction culture, samples were taken for SDS-PAGE analysis.

Recombinant INHα protein expression: One hundred milliliters of the IPTG-induced bacterial culture were used for the expansion culture. This was centrifuged at 5000 rpm at 4°C for 20 min, the supernatant discarded, and the pellet was resuspended in 10 mL of phosphate-buffered saline (PBS) and left to stand for 30 min at room temperature. The bacterial suspension was then subjected to repeated freezing and thawing cycles for 3 times, together with ultrasonication, until the suspension was clear. At this point, the ultrasonication was halted and the material was centrifuged at 12 000 rpm for 10 min at 4°C. Both the supernatant and precipitate were retained for SDS-PAGE analysis.

Purification and identification of inclusion body proteins: The inclusion bodies were dissolved in 8 mol/L urea and filtered through a 0.45 μm filter. Sodium chloride (0.5%) was used to remove impurities from the His-tag-Ni column at the rate of 2 drops/s, and the Ni column was cleaned with deionized water. The column was then equilibrated with solution A (3.12 g $NaH_2PO_4.2H_2O$, 29.24 g NaCI, and 240 g urea dissolved in ddH_2O and diluted to 500 ml). 56 g $NaH_2PO_4.2H_2O$, 14. 62 g NaCI, 13. 6 g imidazole, and 240 g urea were dissolved in ddH_2O, and the volume was made up to 500 ml. The purified inclusion body protein was dialyzed and renatured with successive buffer changes containing 6, 4, 2, and 1 mmol/L urea. Finally, 1 × PBS buffer solution was used for dialysis to remove hetero-proteins for 6-8 h, and sucrose was used for concentration. The protein concentration was determined by the BCA method. The immunogenicity of the purified protein was determined by Western blotting. The purified proteins were stored at -80℃.

Immunization of Xinjiang Bactrian camels and serum collection: After feeding the Xinjiang Bactrian camels for one week, they were immunized with INHα protein as

antigen. Before the first immunization, 10 ml of blood was collected from the jugular vein of the camels as a negative control. The first dose was 5 mL (1 mg/mL), and the second dose was half of the first. The camels were immunized every 14 days for a total of five immunizations. At the end of the immunization period, 50 mL of blood was collected from the camels' jugular veins. After standing for 24 h, the blood was centrifuged for 10 min at 4℃ and 4000 rpm under aseptic conditions. The serum was collected and aliquoted in 1.5 ml EP tubes. Equal volumes of 100% glycerol (400-800 µl per tube) were added and stored in a - 20℃ freezer.

Anti-INHα polyclonal antibody titer determination (indirect ELISA method): The anti-INHα antibody titer was measured in serum collected on the 10th day after the last immunization, with the serum collected before immunization used as the negative control. Recombinant INHα protein was diluted to 40 µg/ml with PBS, added to 96-well plates, and stored overnight at 4 ℃. The next day, the plates were washed four times with PBST (pH = 7.4), blocked at room temperature for 2 h, and washed again four times. The serum samples were initially diluted 2000-fold with the blocking solution, and serial dilutions were added to the plate and incubated at 37 ℃. After two hours of reaction, the plate was washed and dried to ensure that the unconjugated serum was washed away. Horseradish peroxidase (HRP) was diluted 1:5000 with PBS and used to label the rabbit anti-camel secondary antibody. The labeled antibody was incubated in the plate at 37 ℃ for 2 h, and the plate was again washed four times. TMB (3,3',5,5'-Tetramethylbenzidine) chromogenic substrate was added at 25 ℃ in dark. After 30 min, the reaction was terminated and the absorbances at 450 nm were measured.

Purification of anti- INHα polyclonal antibody: The INHα polyclonal antibody was purified by salting out with saturated ammonium sulfate solution (SAS). The serum was centrifuged at 4 ℃ for 20 min at 4000 rpm and the supernatant was removed. The same volume of PBS (0.01 mol/L, pH = 7.2) was added, and twice the volume of SAS solution was added dropwise under magnetic stirring, stirring at low speed for 30 min. After standing at 4 ℃ for 3 h and centrifugation at 12 000 rpm for 20 min, the supernatant was discarded, and PBS was added to twice the volume of the sample to dissolve the precipitate. An equal volume of SAS solution was added dropwise with stirring to achieve 33% saturation. The sample was then stirred at low speed for 30 min and left standing overnight at 4℃, before centrifugation at 12 000 rpm for 20 min at 4℃. The supernatant and was carefully discarded the precipitate dissolved in PBS. The dissolved precipitate was then dialyzed against PBS at 4 ℃ for 48 h with 6-8 changes of buffer. The antibody-containing solution was removed from the dialysis bag and centrifuged at 12 000 rpm for 20 min at 4 ℃. Eighty microliters of the supernatant were removed for Western blotting, and the remainder was stored at -80℃.

INHα preparation for Kazakh sheep immunization

Animal immunization program: Forty-five healthy adult Kazakh sheep with relatively consistent estrus times (the difference in estrus time does not exceed 3 ~ 5 days) in the inter-estrous period were selected and randomly divided into three groups of 15 animals per group. Group A was immunized with the camel anti-INHα polyclonal antibody, group B with the recombinant INHα protein, and group C was the control group. After the first immunization, the test group was immunized once every 10 days, for a total of three immunizations. Animals were injected subcutaneously in the neck. The injection volume was 3 mL of INHα polyclonal antibody (dilution ratio 1:1000), INHα protein 3 mL (1 mg/mL), and 3 mL of normal saline for the control group, respectively.

Serum collection and hormone determination: Sheep sera were collected to determine the levels of reproductive hormones (FSH, luteinizing hormone [LH], progesterone [P_4], estradiol [E_2], and INH) by enzyme-linked immunosorbent assay (ELISA). The method was modified from the kit instructions to make the precision range of the standard curve consistent with that of normal animal hormones.

Detection of blood biochemical indices in Kazakh sheep: To detect the safety of INHα protein and the anti-INHα polyclonal antibody immunization on Kazakh sheep, blood samples were sent to the Affiliated Hospital of Shihezi University to detect blood biochemical indices including alanine transaminase (ALT), total protein (TP), albumin (ALB), globulin (glo), creatinine (crea), and urea.

Data analysis: SPSS version 25.0 (IBM Corp., Armonk, NY, USA) statistical analysis software was used to analyze the data. The experimental results were expressed as mean ± standard deviation with ($P < 0.05$) representing significance. The comparison between the experimental group and the control group was determined by random one-way ANOVA.

RESULTS

Inducible expression of INHα: Expression of INHα was induced by addition of IPTG to a final concentration of 1 mmol/L. One milliliter of the bacterial solution was taken before and after IPTG addition for SDS-PAGE analysis. The results showed a clear band at about 36 kDa which was consistent with the expected protein size (Fig. 1).

Optimization of INHα expression conditions: The INHα expression showed no significant change in relation to IPTG concentrations (Fig. 2 and Fig. 3). The optimal concentration of IPTG was found to be 0.6

mmol/L. INHα expression increased in relation to the induction time, being highest at 4 h after induction, after which there was no significant change. Therefore, the optimal induction time was 4 h.

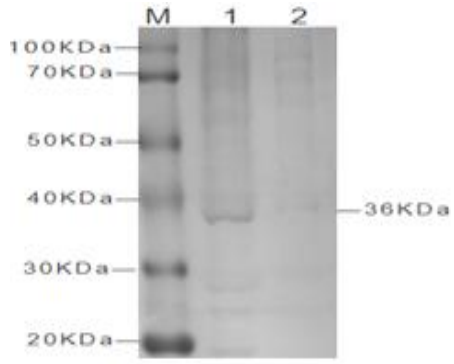

Fig.1 Results of SDS-PAGE electrophoresis of INHα protein expression. M, protein markers; 1, IPTG-induces INHα BL21 bacteria; 2, uninduced INHα BL21 bacteria.

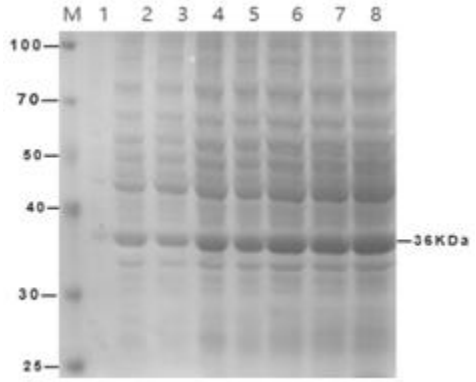

Fig.2 Induced expression of INHα by different IPTG concentrations. M, protein markers; 1-8, IPTG concentrations of0, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mmol/L, respectively.

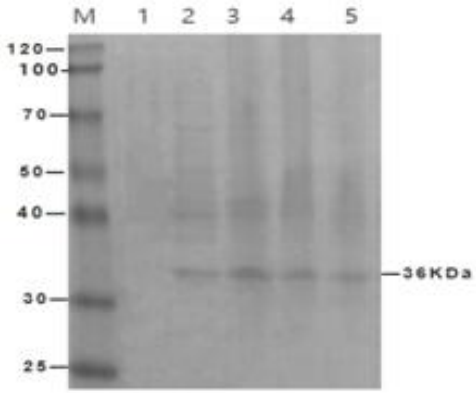

Fig.3 INHα expression at different induction times. M, protein markers; 1-5, INHα expression after 0, 2, 4, 6, and 8 h, respectively.

Identification of expressed recombinant INHα protein: The expression products were collected, sonicated, and analyzed by SDS-PAGE. It was found that most of the recombinant INHα was present in inclusion bodies, with a molecular weight of 35 kDa, with no expression observed in the supernatant. Therefore, the recombinant INHα was expressed in inclusion bodies, as shown in Fig. 4.

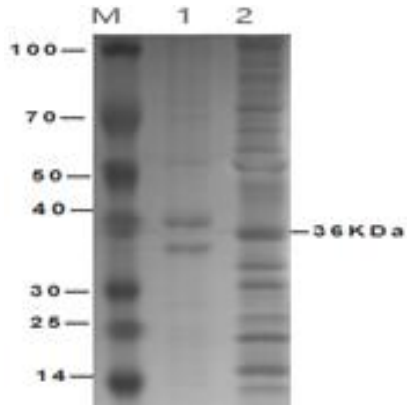

Fig.4 INHα expression in the supernatant and precipitate. M, protein markers; 1, supernatant; 2, precipitate.

Purification of recombinant INHα: The crude recombinant INHα protein was obtained by washing the sonicated inclusion bodies with urea and then filtering through a 0.45 μm membrane. A single target band of 36 kDa was obtained after Ni column affinity chromatography adsorption and elution. This showed that the purified INHα protein was pure and without contaminants, as shown in Figure 5.

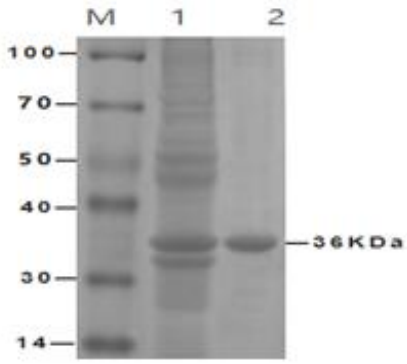

Fig. 5 Purity of INHα protein. M, protein markers (14-100 kDa);1, before application to the column;2, after elution from the column.

Identification of recombinant INHα by Western blotting: The purified INHα protein was analyzed by Western blotting which showed that the purified protein was specifically recognized by the mouse anti-sheep INHα antibody (Fig. 6).

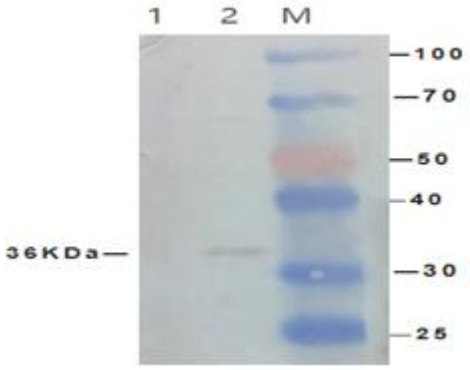

Fig.6 Western blotting of INHα protein M, protein markers;1, negative control; 2, INHα antiserum.

Measurement of the anti-INHα polyclonal antibody titer: Antibody titers were determined by indirect ELISA. The results are shown in Figure 7. No anti-INHα antibody was detected in the camel serum before immunization. The antibody titer detected in serum after immunization was 1:512 000.

Specificity of antisera as detected by Western blotting: Western blotting results showed in Figure 8,that the polyclonal antibody in the serum of the INHα-immunized camel could bind to the purified INHα protein, while no binding was observed for the control serum. The results showed that the anti-INHα polyclonal antibody was successfully prepared and had good affinity for the INHα protein.

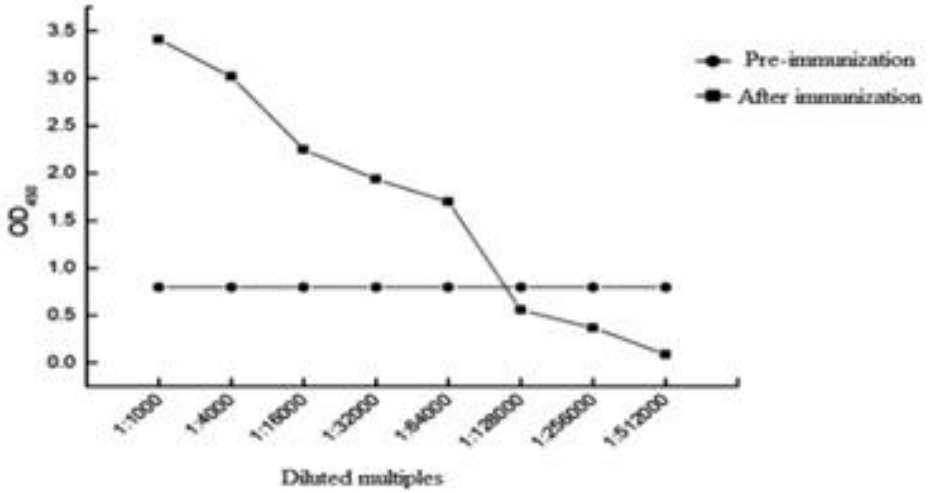

Fig.7 Antiserum titers as shown by ELISA

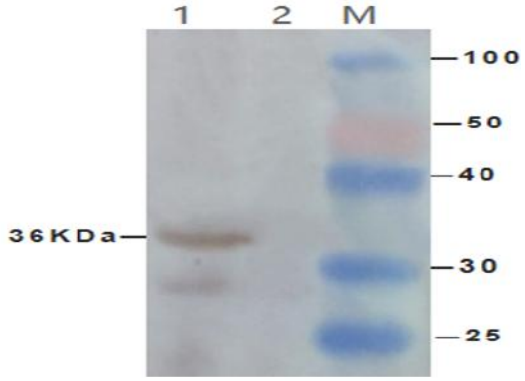

Figure.8 Specificity of antiserum detected by Western blotting.

Detection of reproductive hormones in the sera of experimental animals: FSH, LH, P_4, E_2, and INH in the sera of sheep in groups A, B, and C were detected by ELISA. As shown in Table 1, there were no significant differences in the levels of the five hormones among the three groups before immunization. After immunization, the FSH levels in group A were significantly higher than those in the control group C ($P < 0.05$). While the FSH levels in group B were higher than those in group C, the difference was not significant ($P > 0.05$) (Fig. 9-1). The levels of LH in groups A and B were lower than that in group C, but the difference was not significant ($P > 0.05$) (Fig. 9-2). The P_4 levels in groups A and B were also higher than those in group C, but again the difference was

not significant ($P > 0.05$) (Fig. 9-3). The level of estradiol (E_2) in group A was significantly higher than that in group C ($P < 0.05$) and, while the mean E_2 level in group B was higher than that in group C, the difference was not significant ($P > 0.05$) (Fig. 9-4). While the INHα level in group A was significantly lower than that in group C ($P < 0.05$) and the INHα level in group B was lower than that in group C, the latter difference was not significant ($P > 0.05$) (Fig. 9-5). These results indicate that passive immunization with INHα affects the levels of several reproductive hormones (FSH, E_2, and INH) in Kazakh sheep, while the relationships between the changes in LH and P_4 levels require further investigation.

Table1 Effects of INH active and passive immunity on reproductive hormone levels（ng/L).

Hormone categories	INHα polyclonal antibody immunized group	INHα recombinant protein-immunized group	Control group
FSH	43.383±1.22[a]	39.206±4.47[b]	37.018±5.1[b]
LH	22.929±2.93[a]	23.234±3.31[a]	23.713±3.56[a]
P_4	338.755±72.39[a]	337.283±22.65[a]	336.144±70.71[a]
E_2	75.886±11.08[b]	70.678±7.68[a]	64.089±7.83[a]
INH	1066.19±98.66[b]	1144.193±122.43[a]	1172.493±82.55[a]

Note: different lowercase letters in superscript indicate significant differences (P＜0.05), while the same letters indicate non-significant differences (P＞0.05).

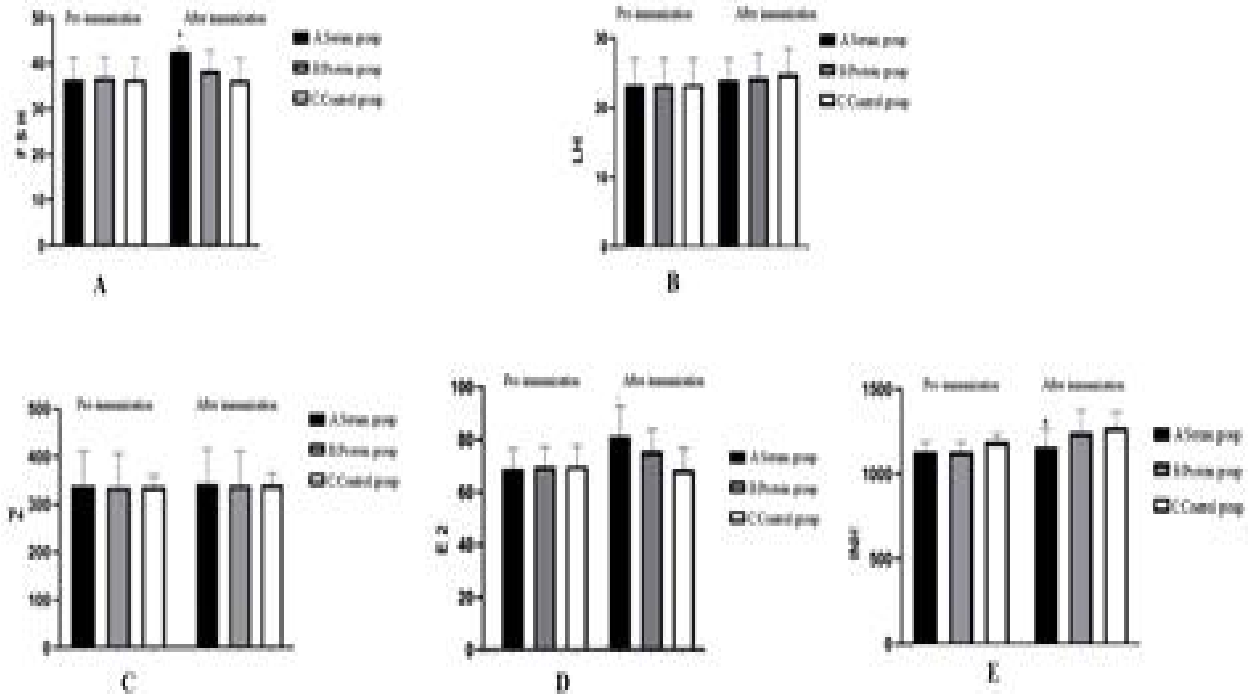

Fig.9 Detection of reproductive hormones in the sera of experimental animals. A: Effect of INHα on FSH; B: Effect of INHα on LH; C: Effect of INHα on P_4; D: Effect of INHα on E_2; E: Effect of INHα

Safety of the INHα immune preparation

General clinical observations: The diet, general activity, and mental state of the Kazakh sheep immunized during the experiment were good, and no discomfort or death occurred.

Test results of blood biochemical indexes: To ensure the safe application of the antigen, six biochemical indices over different time periods were analyzed and compared. The results are shown in Table 2. There was no significant difference in serum biochemical indices (ALT, serum total protein, albumin, urea, creatinine, and globulin) between the second and third blood sampling and the first blood sampling ($P > 0.05$). The results showed that both the INHα protein and INHα antiserum had no significant effects on the functioning of the heart, liver, and other organs of the sheep, and had no toxic effects.

Table. 2: Effects of INHα on blood biochemical indices of Kazakh sheep.

Index		ALT(g/L)	ATP(g/L)	ALB(g/L)	UREA (mmol/L)	CREA (μmol/L)	GLO(g/L)
First blood collection	A	24.014±1.02	67.582±1.58	41.44±0.76	7.33±0.53	40.768±2.64	35.54±1.61
	B	23.472±0.87	67.84±1.25	42.05±1.16	7.39±0.51	40.422±1.71	35.012±1.97
	C	23.55±1.12	68.154±1.31	41.762±0.92	7.186±0.35	40.982±1.61	35.88±1.43
Second blood collection	A	26.664±1.63	76.436±0.69	45.33±1.32	6.89±0.34	48.96±2.46	37.412±1.15
	B	25.032±1.14	72.3±4.45	44.342±0.64	7.042±0.24	47.07±1.68	36.946±0.74
	C	24.996±2.02	71.1±2.65	43.526±3.82	7.3±0.36	46.658±1.88	36.496±1.12
Third blood collection	A	24.95±1.23	69.916±0.95	42.568±0.95	7.432±0.68	42.8±1.34	36.33±1.01
	B	24.142±0.90	68.774±1.18	41.88±0.91	7.436±0.47	41.476±2.16	37.134±0.91
	C	24.382±1.07	68.852±1.42	42.75±0.98	7.42±0.55	41.31±1.63	36.928±0.97

DISCUSSION

Induced expression of the target protein: The prokaryotic expression system has the advantages of simple preparation, low cost, and high levels of expression. We, therefore, used prokaryotic expression to produce the recombinant INHα protein at relatively low cost and in sufficient quantities for subsequent experiments; this required optimization and expansion of the expression conditions. The expression level of a target protein is affected not only by the selected vector and expression conditions but also by the induction time and IPTG concentration used. Here, we optimized the induction time and IPTG concentration (Bandaranayake *et al*, 2011). IPTG is expensive and is also toxic to bacteria. Therefore, to reduce the toxicity of IPTG on bacterial growth and reduce the cost of the experiment, the optimal concentrations and incubation times of IPTG were explored. The results showed that the expression level of the INHα protein was highest at the final concentration of 0.1 mmol/L and an incubation time of 4 h but that the expression level did not increase with prolongation of incubation time, which may be due to aging caused by over-culture and the gradual inactivation of the ampicillin in the culture media.

Purification strategy of the target protein: The protein was expressed in the form of inclusion bodies and was conducive to isolation and purification. However, the conformational structures of proteins in inclusion bodies do not necessarily conform to the native structures and, therefore, may have no biological activity (Zheng, *et al*., 2013). To obtain the target protein in its native conformation, the protein in the inclusion bodies should be refolded and further purified. At present, Ni column affinity chromatography is one of the most effective methods for protein purification. Therefore, Ni column affinity chromatography was used to purify the inducible INHα protein in this experiment as the procedure can both remove inclusion body impurities as far as possible and assist the correct folding of the protein in subsequent renaturation (Chai *et al*., 2019). The purified INHα protein was analyzed by SDS-PAGE and a single band of 36 kDa with a concentration of 1.5 mg/ml was obtained.

Preparation of the polyclonal antibody: At present, polyclonal antibodies are mainly prepared by immunizing rabbits, pigs, chickens, and other animals with antigens (Nakazawa *et al*, 2011). However, while these antibodies are able to detect the specificity of the antigen, it is difficult to obtain large quantities. Large animals such as the alpaca and camel can produce large quantities of antibodies, and their bodies are rich in connective tissue, so their susceptibility to foreign proteins is much higher than other animals. Therefore, camels were selected as the experimental animals in this experiment, and the INHα protein after induced expression and purification was used as immunogen to immunize Xinjiang Bactrian camels. Nevertheless, the ideal production of the antibody requires enhancement through multiple immunizations. This requires the optimization of the immunization numbers and intervals. We immunized the camels with the INHα protein as the immunogen once every 14 days, a total of 5 times, and finally obtained a high titer antibody, which provided a high titer immunogen for the follow-up study of INHα on the reproductive function of the Kazakh sheep.

Purification of the antibody: Protein purification with saturated ammonium sulfate uses ammonium sulfate solutions of different concentrations to repeatedly salt out the antibody. The process removes contaminating proteins and impurities, resulting in a relatively pure antibody. The method is simple, effective in terms of time and cost, and the purified antibody can be used to immunize animals directly. In this experiment, the camel antiserum was purified by this method, resulting in a purified antibody with a titer of 1:512 000, which met the requirements of the subsequent experiments.

Effect of INHα immunization on reproductive hormone levels in sheep: In this experiment, passive immunization with the INHα protein significantly increased the FSH, E_2, and INH levels in sheep compared with the control group. This shows that the antibody produced after the INHα immunization neutralizes the inhibin in the body, thereby reducing the content of endogenous INH which, in turn, weakens the inhibitory

effect of inhibin on FSH, resulting in more available FSH for follicle stimulation, allowing greater secretion of E_2. These results are essentially consistent with previously reported results on the passive immunization of goats with a goat INHα antibody (Kandiel *et al*., 2008; Bahareldin-Ali, *et al*., 2015). Although the levels of LH and P_4 in the immune group were higher than those of the control group, the difference between the two groups was not significant ($P>0.05$), which is consistent with the conclusion that inhibin immunization has no direct effect on the secretion of LH and P_4 (Puentes *et al*., 2016; Seigo *et al*., 2021). This may be because inhibin immunity hinders the positive feedback effect of E_2 on LH secretion (Han *et al*., 2007) or because the sensitivity of LH and P_4 to inhibin in the luteal phase differs from that of FSH and E_2 (Voge and Wheaton 2007; Bingol *et al*., 2012; Liu *et al*., 2013; Cai *et al*., 2015).

Effect of INHα immunization on blood biochemical indices of sheep: The biochemical indices of blood can reflect the functional state of various organs. Six biochemical blood indices were measured in this experiment. ALT and ALB can reflect the function of the liver, skeletal muscle, and heart in animals. The concentrations of ALT and ALB are usually low in the blood but increase when the liver or myocardium is damaged. Creatinine and urea can reflect the renal function of animals. When the kidney receives adverse stimulation, the levels of these indicators will decrease. TP levels can directly reflect the body's metabolism and immune level. Glo can reflect the body's immunity. When damage occurs to the animal body, ALB decreases, followed by significant decreases in the levels of TP and glo (Wang *et al*., 2014; M'BAYE *et al*., 2015). Therefore, this experiment compared the data of six blood biochemical indices in the Kazakh sheep and found that the indices in the experimental and control groups had an upward and downward trend, respectively but that the changes were within the normal ranges. The results showed that INHα had no significant effects on the functions of the liver, kidney, heart, and other organs of the Kazakh sheep.

Conclusion: The INHα recombinant protein and anti-INHα polyclonal antibody were used to immunize Kazakh sheep both actively and passively, and the reproductive hormone levels and biochemical indices of the sheep were measured. It was found that passive immunization with the INHα polyclonal antibody could significantly promote the secretion of both FSH and E_2 in the sheep with optimal immune effect.

Competing interests: The authors declare there are no competing interests.

Ethics statement: All animal care and experimental procedures were approved by the Animal Protection and Use Committee of Shihezi University. All research was carried out in strict accordance with Shihezi University experimental animal welfare and ethical guidelines (SHZUAC20203309).

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