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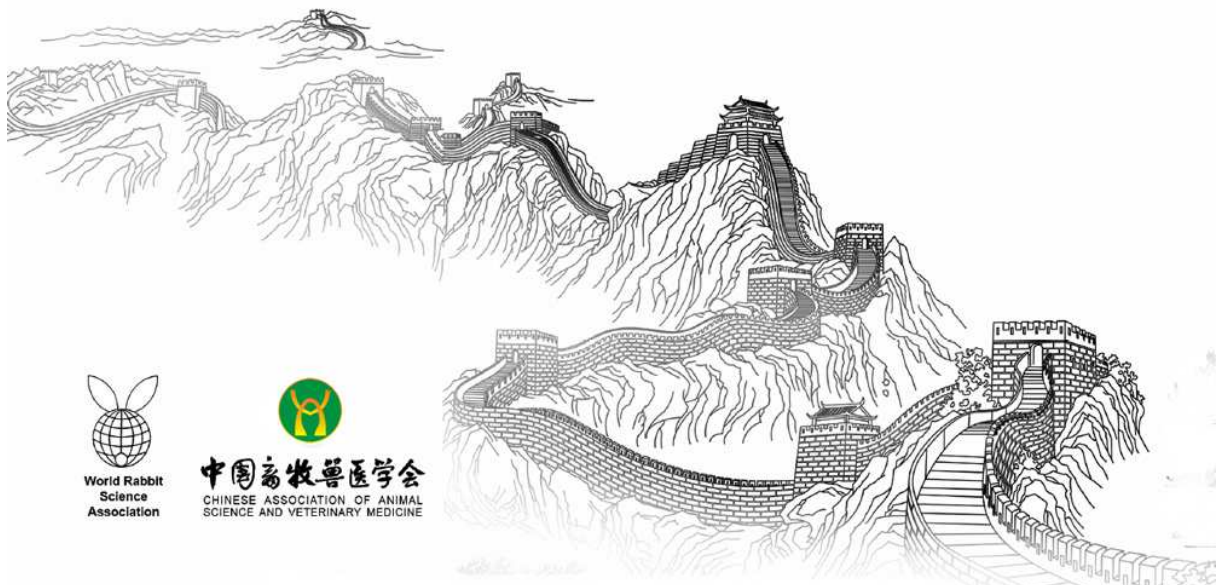
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## EFFECTS OF IMMUNIZATION AGAINST INHIBIN ON THE SEMEN QUALITY IN REX RABBITS IN SUMMER

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### ABSTRACT

High temperature is a serious threat to male rabbit fertility. In this study, we investigated the effect of immunization against inhibin on rabbit semen quality in summer. Eighteen male Rex Rabbits were injected subcutaneously with recombinant porcine inhibin  $\alpha$  subunit (INH) at 0 (control), 0.05 and 0.125 mg/kg respectively. All immunizations were conducted 3 times for each rabbit. Semen was collected every 4-5 days to assess sperm concentration and motility by Computer Assisted Sperm Analysis (CASA); blood was collected every 10 days to determine inhibin antibody levels. Results showed that antibody levels increased significantly from the second immunization ( $P < 0.05$ ). Semen quality was improved by immunization against inhibin, considering the fact that both sperm concentration and motility were higher in 0.05 and 0.125 mg/kg injection groups compared to control group ( $P < 0.05$ ).

**Key words:** immunization against inhibin, semen quality, rabbit, summer

### INTRODUCTION

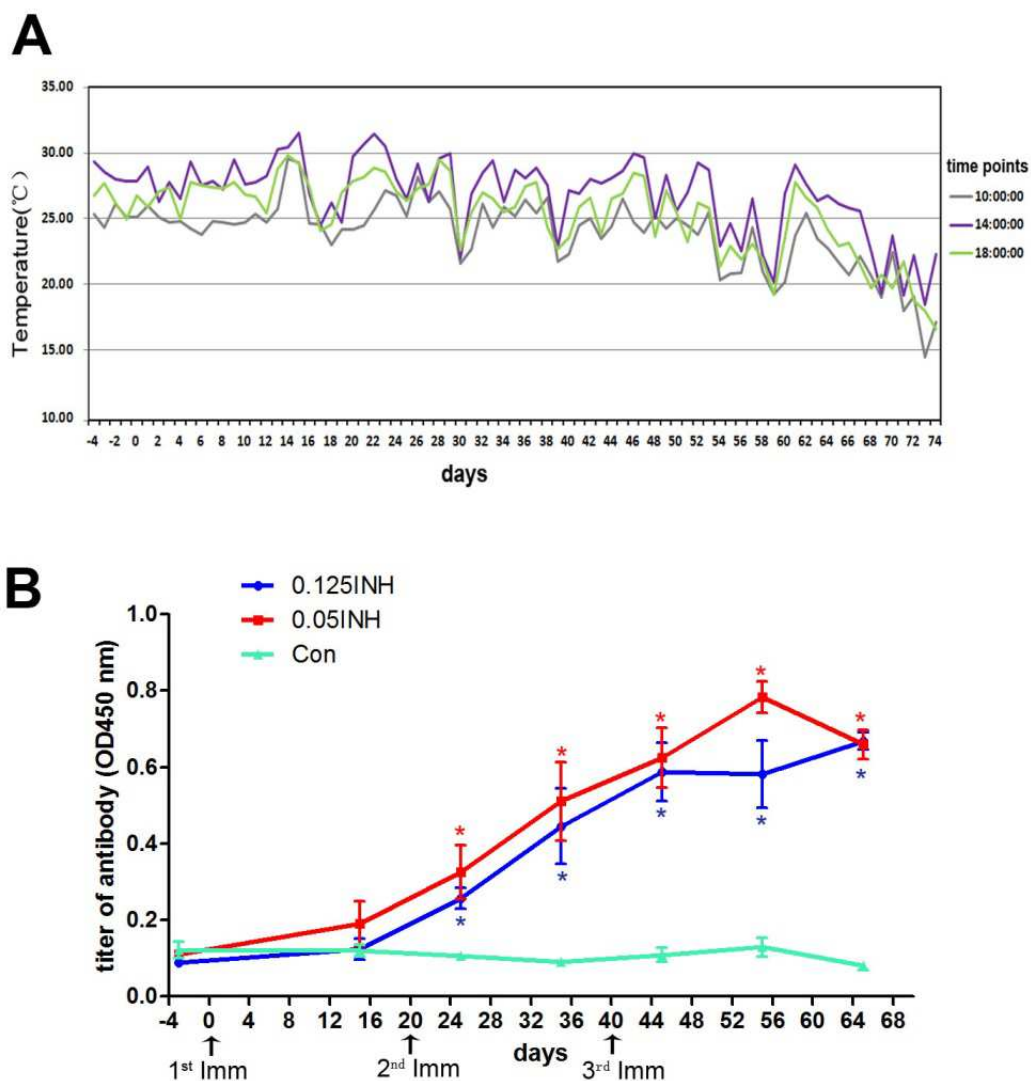
Our previous studies have shown that high temperature enforces a serious impact on reproductive performance of male rabbits (Pei *et al.*, 2012a; Pei *et al.*, 2012b). Heat stress decreased FSH and testosterone secretions, damaged the structure of testicular tissue, and depressed sexual desire, which finally compromised fertility.

Inhibin is known to inhibit FSH secretion. Immunization against inhibin by injection of inhibin peptides is identified to be an effective measure to increase fertility. For example, it enhanced both embryo quantity and quality in Holstein heifers (Li *et al.*, 2009; Liu *et al.*, 2013) and benefited the sperm output in bulls (Bame *et al.*, 1999) and bucks (Medan *et al.*, 2006). However, whether immunization against inhibin could improve the reproduction performance in rabbit has not been investigated. The aim of this study is to identify the effect of immunization against inhibin on the semen quality in Rex rabbit in summer. The results could provide a practical and effective method for alleviating summer- subfertility of rabbit.

### MATERIALS AND METHODS

#### Animals and immune procedure

About thirty Rex Rabbits aged 6-9 months old were bred in Beijing Fangshan Rex rabbit Test Station. Eighteen rabbit were chosen out according to their similar weight, sperm concentration and motility, and were randomly divided into 3 groups (six rabbit for each group): control, 0.05INH and 0.125INH, considering the subcutaneous injection dose (0, 0.05 and 0.125 mg/kg, respectively) of antigen. The antigen was recombinant porcine inhibin  $\alpha$  subunit (Li *et al.*, 2009), and was emulsified on homogenizer with Freund's adjuvant (Sigma Aldrich). Each rabbit was challenged 3 times with the antigen. The first time was conducted on 07/05/2014, which was defined as experimental time D0. Second and third immunizations were conducted after 20 and 40 days (D20 and D40, respectively). The experiments lasted a whole summer. The variations of temperature were recorded during the experiment (Figure 1A).



**Figure 1 :**Temperature records during the experiment and antibody titer changes. (A) Temperature during the whole experiment. The grey, purple and green lines mean the measured temperatures at 10:00, 14:00, and 18:00 o'clock for each day, respectively. (B) Antibody titers were determined by Elisa. Con, 0.05INH, 0.125INH indicate the subcutaneous injection doses of different groups (0, 0.05 and 0.125 mg/kg, respectively) with porcine inhibin  $\alpha$  subunit peptides. Arrows show the dates when immunizations were conducted. Data is shown as OD450 values. Six animals in each group were assayed. Data in (B) is shown as means  $\pm$  SEM. \*  $P < 0.05$  compared to the Con group.

### Sample collection and determination

Semen was collected every 4-5 days using artificial vagina. Blood was collected every 10 days using vacuum tubes. Serum was obtained by centrifugation after coagulation. To determine the sperm motility, semen was diluted 10 times in TCG dilution (Tris 3.8%, W/V; citric acid 2%, W/V; sucrose 0.6%, W/V; penicillin and streptomycin 0.3IU/L), and the percentage of the straight-line movement sperm was measured by Computer Assisted Sperm Analysis (CASA, Minitube International Inc.) and defined as sperm motility. The sperm density was determined using hemacytometer. Elisa was used to determine the antibody titer as previously described (Liu *et al.*, 2013). Briefly, a 96-well plate was incubated with porcine inhibin  $\alpha$  antigen. After washing and blocking, the plate was incubated with diluted sera (1:8000) at 37°C for 1 h, and then with labeled secondary antibody (horse radish peroxidase) for 45 min. Optical Densities at 450 nm were read under ELIASA (TECAN Inc.).

### Statistical analysis

All data were analyzed using one-way analysis of variance (ANOVA), followed by Student's *t* test. All values are expressed as means  $\pm$  SEM. *P* value of  $< 0.05$  was considered significant.

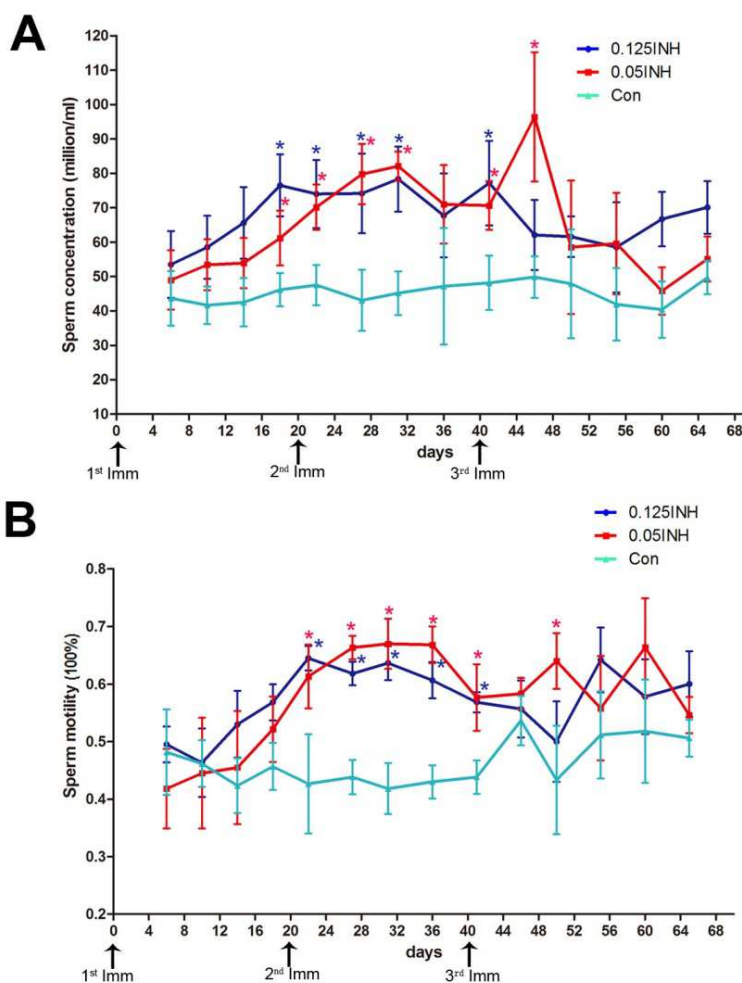
## RESULTS AND DISCUSSION

In order to know whether the injection of INH induces functional immunization against inhibin, we determined the titer of inhibin antibody in the serum. Results showed that the antibody levels in rabbits challenged with 0.05 and 0.125 mg/kg INH were notably higher than the control group 5 days after the second immunization (D25), and remained in that condition until the end of experiment (D65) (Figure 1B). Surprisingly, the 0.05mg/kg INH dose seemed to induce a greater antibody production in comparison to 0.125mg/kg INH at D55. These results confirmed that the injection of both 0.05 mg/kg and 0.125 mg/kg INH are successful inducers of immune response against inhibin in rabbit.

Rex rabbit usually produces 100-300 million/ml sperm. However, it was lower in control group during our experiment (40-60 million/ml). This is probably because our study lasted from July to September, when the temperature was higher (Figure 1A), and it is considered that heat stress could decrease the quality of semen (Pei *et al.*, 2012). The sperm concentrations were obviously raised in the 0.125INH and 0.05INH groups after the second immunization and maintained at a higher level (70 million/ml) than control group until the third immunization (Figure 2 A). The sperm motility values were between 40-50% in the control group throughout the study. These values notably increased to 60% in both 0.125INH and 0.05INH, similarly to sperm concentration (Figure 2B). These results also agree with previous reports on bull and bucks (Bame *et al.*, 1999; Medan *et al.*, 2006).

However, no differences were observed in semen quality between groups after D50. The inaction of INH at later stages is probably due to the recovery of semen quality in control group

caused by a lower environmental temperature (Figure 1A), or maybe it reveals the short-term function of INH. A stable environmental study is needed to illustrate the reason in the future.



**Figure 2.** Sperm concentration and motility were increased by INH injection at some time points. Con, 0.05INH, 0.125INH indicate the subcutaneous injection doses of different groups (0, 0.05 and 0.125 mg/kg, respectively) with porcine inhibin  $\alpha$  subunit peptides. Six animals in each group were assayed. Arrows show the dates when immunizations were conducted. Data is shown as means  $\pm$  SEM. \*  $P < 0.05$  compared to the Con group.

## CONCLUSIONS

Immunization of inhibin has been reported to promote sperm production. However, whether it can rescue sperm decrease induced by heat stress had not been studied yet. Here we proved that the injection of inhibin antigen could partially relieve bad effect of heat stress on sperm concentration and motility.

## ACKNOWLEDGEMENTS

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