Effect of active immunization with recombinant-derived goose INH-α, AMH, and PRL fusion protein on broodiness onset and egg production in geese (*Anser cygnoides*)

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ABSTRACT This study was conducted to investigate the potential effects of active immunization against recombinant-derived goose inhibin-α (**INH-α**), anti-Müllerian hormone (**AMH**), and prolactin (**PRL**) fusion protein on broodiness onset and egg production in geese. The purified fusion proteins (INH-α, AMH, and PRL) were prepared using a prokaryotic expression system. Female Zhedong geese (10 mo old) were randomly assigned to one of 4 treatments and raised in separate pens. The geese were actively immunized with the recombinant goose INH-α, AMH, or PRL, respectively, and phosphate-buffered saline as control. The results showed the corresponding antibodies were produced when the geese were immune INH-α, AMH-, and PRL-recombinant proteins. The significantly higher luteinizing hormone contents were observed in the INH-α, AMH, and PRL recombinant protein-immunized geese, while the lower AMH hormone content only in PRL-immunized birds. AMH recombinant protein immunized geese had more large yellow follicles of ovary, while the INHα-treated birds with more other follicles compared with control geese. In addition, the geese receiving INH-α recombinant protein, the broodiness onset was about 6 d, which significantly shorter than did PBS immunization (16 d). The INHα- and PRL-immunization also resulted in 12.5 and 8.5 d shorter broody duration intervals compared to the control birds. Moreover, the lower new broodiness rate was observed in three recombinant proteins treated birds. Finally, the PRL recombinant protein-immunization resulted in an average increase of 1.34 eggs during a 40-d observation. Collectively, the data demonstrated that active immunization against recombinant proteins INH-α or AMH could promote LH hormone secretion, regulate follicle development and decrease the broodiness rate. Also, active immunization with a recombinant-derived goose PRL protein might improve egg laying performance.

Key words: goose, active immunity, egg production performance

INTRODUCTION

China produces the most geese in the world (FAOSTAT, 2020), but low egg-laying performance has always been a key bottleneck restricting goose industry development (Liu et al., 2018). Numerous studies have shown that broody behavior in domestic geese reduces egg production and causes ovary and oviduct degradation (Kovacs et al., 1992; Xu et al., 2013; Yu et al., 2016). Broodiness is a behavior when geese protect the eggs in the nest for a period of time after laying, thus halting laying, eventually leading to decreased egg production (Romanov et al., 2002). Inhibiting broodiness, shortening the broodiness stage, or eliminating broodiness and prolonging the laying period can contribute to increased goose egg production.

In the hormone immunoneutralization technique, a hormone or factor that can inhibit reproductive performance is fused with an exogenetic protein to enhance its immunogenicity, and animals are immunized with the syncretic protein to induce the body to produce an antibody that inhibits or attenuates a physiological function. The use of active immunization of recombinant hormones has been recognized as a successful approach in the modern production enhanced production performance of a wide range of animal species, including cattle (Bo and Mapletoft, 2014), sheep (Rutigliano et al., 2014), pigs (Swanchara, et al., 1999), and chickens (Satterlee et al., 2002). A recent study showed immunization against AMH accelerated ovarian follicular

development and increased clutch sizes by 1 to 2 eggs in 2 consecutive laying incubation cycles in geese (Chen et al., 2020), but there is a lack of studies on whether active immunization can regulate broodiness and egg production in geese.

Inhibin-α (**INH-α**) can regulate follicle development by responding to increasing levels of follicle-stimulating hormone (**FSH**) in the gonads that triggers negative feedback to the anterior pituitary, lowering FSH circulating levels (McAsey et al., 1995). Anti-Müllerian hormone (**AMH**) is an important factor related to avian broodiness, and it is highly expressed in the broodiness stage and associated with enhancing ovarian granulosa cell proliferation and follicle development selection (Johnson et al., 2009). Prolactin (**PRL**) plays a key role in initiating and maintaining broody behaviors; it is regarded as the most direct hormone contributing to avian broodiness (Yao et al., 2019). In this study, the Zhedong white goose was selected, which was characterized by the low-yielding and strong-brooding compared to the other breeds of geese, and the 3 recombinant proteins (INH-α, AMH, and PRL) were produced using prokaryotic expression technology that were used as the immunogen to actively immunize geese and induce production of antibodies for immune neutralization. Then we investigated the effects of active immunization against recombinant-derived goose INH-α, AMH, and PRL fusion protein on broodiness onset, follicle development and egg production in Zhedong Geese.

MATERIALS AND METHODS

Ethics Statements

All experimental animals in this study were approved by the Institutional Animal Care and Use Committee of Yangzhou University (Jiangsu, China) and were strictly implemented according to the regulations for experimental animals. A standard housing facility was used and was consistent with the national standard, Laboratory Animal Requirements of Environment and Housing Facilities (GB 14925-2001). Laboratory animal care and animal experiment protocols and conditions conformed to the Jiangsu Administration Rule for Laboratory Animal Use.

RNA Isolation, cDNA Synthesis, and Sequencing Analysis

Total RNAs were extracted from the ovaries of Zhedong geese bred in the Jiangsu Waterfowl Conservation Farm (Taizhou, Jiangsu, China) at the broodiness stage using RNAiso Plus (TaKaRa, Kyoto, Japan) according to the manufacturer's instructions. Briefly, 100 mg tissue was put in 1 mL TRIzol and homogenized, then centrifuged at 12,000 rpm for 10 min at 4°C. Next, 0.2 mL chloroform was added, the sample was mixed by vigorous shaking for 15 s, then centrifuged at 12,000 rpm for 10 min at 4°C. The resulting pellet was washed with 75% ethanol, then centrifuged at 12,000 rpm for 5 min at 4°C, air dried, and dissolved in 50 μL diethylpyrocarbonate-treated sterile water. To avoid contamination with genomic DNA, total RNA samples were treated with RNase-Free DNase (TaKaRa). Primers were designed based on the AMH, INH-α, and PRL of *Anser cygnoides* domesticus according to the reported coding sequences (**CDSs**) in the NCBI database (Table 1). The polymerase chain reaction (**PCR**) products were cloned into the pMD18-T vector (TaKaRa) to confirm amplification, followed by sequencing at Sangon Biotech Company (Guangzhou, China). The signal peptides of the *Anser cygnoide*s domesticus AMH, INH-α, and PRL

Table 1. Sequences of primers used for RT-PCR.

Gene name	Primer Sequence (from 5'-3')	Product length	GenBank accession
INH-α	F:TGTTTAACTTTAAGAAGGAGA-TATACATATGCACCACCATCAC-CATCATCATTTTGGCCTGTG CCTGCATCACGGTCAAG R:TAGCAGCCGGATCTCAGTGG TGGTGGTGGTGGTGCTCGAGTG CGGCCGCAAGCTTTTCATTAA CCTTGCCCCA	1074	XM_013171136
AMH	F:GTTTAACTTTAAGAAGGAGATA-TACATATGTTACCGCGTAAAGG-TAGTCCGGGCGAACGCGT-TAGTCCGGCAGGTACCTTAG R:CAGTGGTGGTGGTGGTGGTGC TCGAGTGCGGCCGCAAGCTTT-CATTAATGGTGATGATGA TGACGACAACCGCACTCTTCT	1989	XM_013196337
PRL	F:TGTTTAACTTTAAGAAGGAGA TATACATATGCACCACCATCAT-CATCATCTGCCGATTTGCCC-GAACGGTAGC R:TAGCAGCCGGATCTCAGTGGT GGTGGTGGTGGTGCTCGAGTG CGGCCGCAAGCTTTCATTAGCA GTTGCTATC	633	XM_013184821

were analyzed using the SignalP 4.1 server (https://www.cbs.dtu.dk/Services/signalP) (Figure S1). We queried the CDS with SignalP v4.1 to identify N-terminal signal peptides, setting the default D-cutoff for SignalP-noTM networks at 0.45 and the D-cutoff for SignalP-TM networks at 0.5 (Petersen et al., 2011).

Recombinant Protein Expression and Purification

The cDNA fragments that encode *Anser cygnoides domesticus* AMH, INH-α, and PRL were amplified by PCR from the plasmids described above with restriction enzymes *Nde*I and *Hind*III and inserted into the PET-30a(+) vector (Promega, Madison, WI). Then the recombinant plasmid was transformed into competent *Escherichia coli* BL21 (DE3) cells and induced with 0.2 mM isopropyl β-D-1-thiogalactopyranoside (**IPTG**) at 15°C for 16 h when the optical density (**OD**) at 600 nm was 0.6 to 0.8. The bacteria cells were harvested by centrifugation at 4°C and then resuspended in ice-cold lysis buffer (50 mM Tris-HCl, 300 mM NaCl, 20 mM imidazole, 1% Triton X-100, 1 mM dithiothreitol [**DTT**], pH 8.0) and lysed using an ultrasonic cell disrupter. Inclusion bodies were washed with wash buffer (50 mM Tris-HCl, 300 mM NaCl, 1% Triton X-100, 2 mM ethylenediaminetetraacetic acid, 5 mM DTT, pH 8.0) and then dissolved in elution buffer (50 mM Tris-HCl, 300 mM NaCl, 20 mM imidazole, 8 M Urea, pH 8.0). The lysate mixtures were purified and protein refolding was carried out using Ni-IDA kits (BioTsz, San Francisco, CA) and elution buffer with different concentrations of imidazole according to the manufacturer's instructions. The fusion proteins were resolved by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (**SDS-PAGE**) at 90 V, using the Miniprotean III system (Bio-Rad, Hercules, CA) and stained with Coomassie brilliant blue R250. The higher elution purity protein was added to the treated dialysis bag and renaturation was performed in renaturing phosphate-buffered saline (**PBS**; 4 mM glutathione, 0.4 mM glutathione disulfide, 0.4 M L-arginine, 1 M urea, pH 8.0) at 4°C. After renaturation, the protein was finally dialyzed against a PBS storage solution (pH 8.0) for 6 to 8 h. After dialysis refolding, the supernatant was filtered using a 0.22-μm filter and stored at −80°C. The concentrations of the purified proteins were determined by the Bradford method using bovine serum albumin (**BSA**) as the standard.

Animal Immunizations and Ovary Sample Collection

A total of 200 Zhedong geese were raised at the Jiangsu Waterfowl Conservation Farm (Taizhou, Jiangsu, China). At 10-mo-old, 60 geese were selected and four multi-male parent families (3 males and 12 females) were established in their laying period. In the initial immunization, the geese were intramuscularly inoculated with 0.8 mg AMH, INH-α, or PRL protein dissolved in 0.5 mL PBS and emulsified with an equal volume of Complete Freund's adjuvant (Sigma, St. Louis, MO). The second immunizations occurred 10 d later when the geese were injected with 0.8 mg protein dissolved in 1 ml PBS and emulsified with an equal volume of Freund's incomplete adjuvant. In the control group, PBS instead of recombinant protein was injected using the same dose of Freund's adjuvant. All geese were provided with the same diet (crude protein: 17%, metabolizable energy: 10.45 MJ/kg), which was combined with coarse and concentrated material and fed ad libitum. The geese were kept in pens (1.5 individuals/m^2) that included a playground and pool. Daylight hours were from 6:00 a.m. to 9:00 p.m.

Three geese from each group were selected using the abdominal palpation technique in the same clutch, and slaughtered after anesthetization with sodium pentobarbital. Ovarian samples comprising the ovarian stroma, small and large yellow follicles, were obtained rapidly and weighed. The follicles were immediately dissected and placed on a filter paper moistened with physiological saline. The follicles were distinguished according to their type and diameter (Yang et al., 2019).

Serum Detection of Antibody Titer Indices and Reproductive Hormone AMH, LH, and PRL Concentrations

Blood samples were collected from the brachial vein in heparinized containers every 10 d after immunization, and the serum was separated. Antibody titers (anti-INH-α, anti-AMH, anti-PRL) and hormone levels (AMH, luteinizing hormone [**LH**], PRL) were measured at an OD value at 450 nm using enzyme-linked immunosorbent assays (ELISAs; Goose AMH Ab ELISA Kit, Goose INH-A Ab ELISA Kit, Goose PRL Ab ELISA Kit, Goose AMH ELISA Kit, Goose LH ELISA Kit, and Goose PRL ELISA Kit; BioTsz) according to the manufacturer's instructions.

Western Blot Analysis

Proteins were separated by 12% SDS-PAGE and transferred onto polyvinylidene fluoride (**PVDF**) membranes. The membranes were blocked with 3% BSA in 0.5% PBS-Tween 20 (pH 7.4) for 2 h. Subsequently, serum from the immunized goose was diluted (1:10,000) and incubated with the membranes for 1.5 h at room temperature (**RT**). Next, the membranes were washed thrice and incubated with horseradish peroxidase (**HRP**)-conjugated rabbit anti-goose immunoglobulin G (IgG; 1:5,000; Renova Life, College Park, MD) for 1 h at RT. Finally, the membranes were washed, and bands were visualized with enhanced chemiluminescence solution from Bio-Rad and exposed to films obtained from Agfa (Mortsel, Belgium).
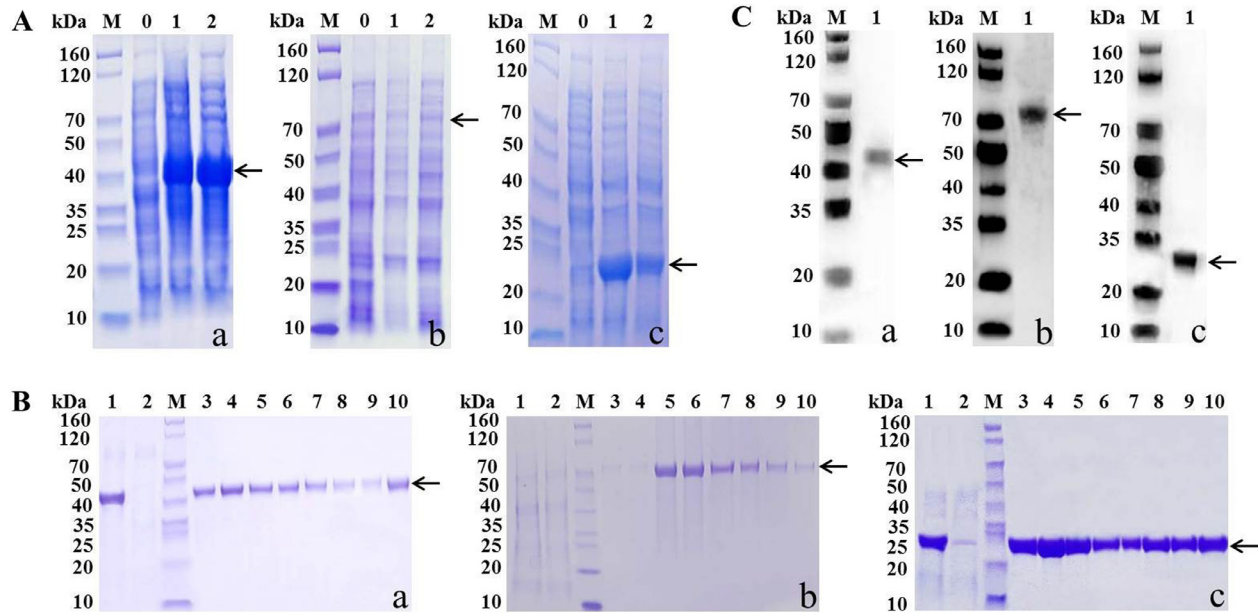
Figure. 1. Expression, purification and identification of recombinant INH-α, AMH, and PRL. (A) Analysis of recombinant bacteria PET-INHα, PET-AMH, and PET-PRL induced by IPTG at different temperatures. Lane 0: Control, Lane 1: IPTG induction at 15°C for 16 h, Lane 2: IPTG induction at 37°C for 16 h. (B) Protein purification in the inclusion body of INH-α, AMH, and PRL of the geese. Line 1: The supernatant of inclusion bodies after dissolution and centrifugation, Line 2: The effluent of supernatant after incubation with Ni-IDA. (a) Line 3-9: Elution with 50 mM imidazole, Line 10: Elution with 100 mM imidazole. (b) Line 3-4: Elution with 50 mM imidazole, Line 5-10: Elution with 500 mM imidazole. (c) Line 3-7: Elution with 50 mM imidazole, Line 8-10: Elution with 300 mM imidazole. (C) Western blot of the purification INH-α, AMH, and PRL of the geese. Line 1: Recombinant fusion protein. a: INH-α, b: AMH, c: PRL. Abbreviations: AMH, anti-Müllerian hormone; INH-α, immunization against recombinant-derived goose inhibin-α; IPTG, isopropyl β-D-1-thiogalactopyranoside; PRL, prolactin.

Recording of Behaviors and Egg Production

Colored paint was used to mark the necks and backs of the geese for identification during observations of courtship and mating behaviors. A video camera (720 p, Jindun, Nanjing, China) was set up to record the goose broody behavior at 9:00, 11:00, 13:00, and 15:00 every day. Goose eggs were collected every day at 9:00 am throughout the 40-d experimental period and calculate the total number of eggs. Broodiness was assessed as the time spent lying on the ground over 24 h, and pin bone spacing was measured using fingers (width less than 2 fingers). The broody geese spent lying on the ground during 4 observations within 24 h.

Statistical Analysis

Statistical analyses were performed with one-way analysis of variance and Tukey multiple range tests using IBM SPSS Statistics 22.0 software (Armonk, NY). The data are presented as mean $\pm$ SEM (n = 3). $P <$ 0.05 was considered significant.

RESULTS

Recombinant Protein Expression and Purification

The CDS regions of goose *INH-α, AMH,* and *PRL* genes were cloned, and the prokaryotic expression vectors PET-INHα, PET-AMH, and PET-PRL were

constructed. PET-INHα, PET-AMH, and PET-PRL fusion proteins were successfully expressed following induction by 0.2 mM IPTG at 37°C and 15°C for 16 h, and PET-PRL expression was higher at 15°C (Figure 1A). PET-INHα, PET-AMH, and PET-PRL recombinant proteins were observed as inclusion bodies on SDS-PAGE. The inclusion bodies were lysed and centrifuged, and after NI-IDA filtration and adsorption, the corresponding protein bands were obtained using elution buffer with different concentrations of imidazole. INH-α and PRL fusion proteins were eluted with 50 mM imidazole, and AMH fusion proteins were eluted with 500 mM imidazole; the proteins were approximately 42 kDa, 72 kDa, and 30 kDa, respectively, which corresponded to the speculated weight (Figure 1B). To test whether the denatured and renatured fusion proteins were immunogenic and reactive, the INH-α, AMH, and PRL proteins eluted through NI-IDA columns were transferred to PVDF membranes and incubated with serum from the immunized goose as the primary antibody and the HRP-conjugated rabbit anti-goose IgG as the secondary labeled antibody. Each of the three proteins appeared as a specific band (Figure 1C).

Antibody Responses and Reproductive Hormone Measurement After Active Immunization

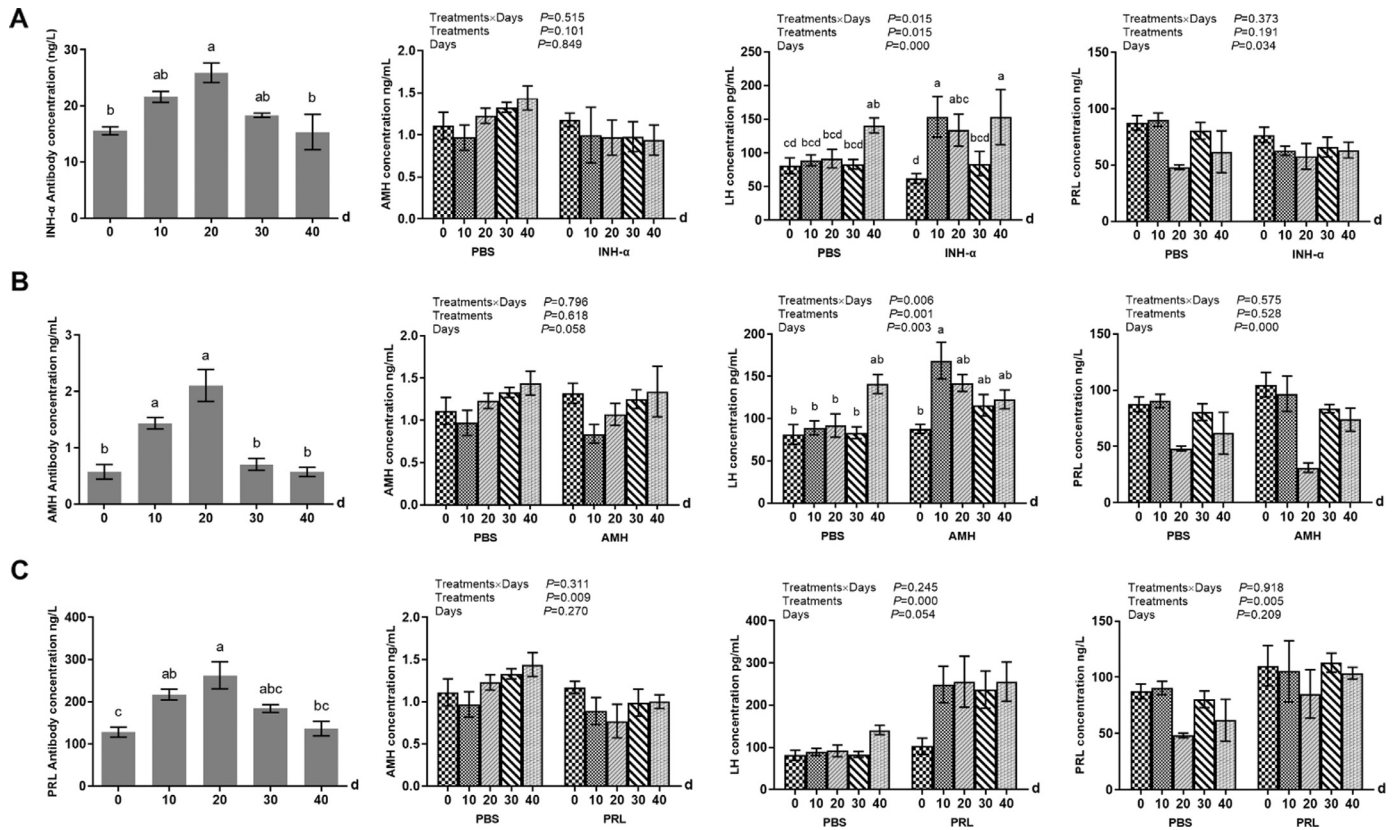The changes of anti-INHα, anti-AMH, anti-PRL antibody titers and AMH, LH, and PRL hormone levels in

Figure. 2. The antibody titer and related reproductive hormone level after active immunization against INH-α, AMH, and PRL. (A) The anti-INH-α titer and the levels of AMH, LH, and PRL after immunization against INH-α. (B) The anti-AMH titer and the levels of AMH, LH, and PRL after immunization against AMH. (C) The anti-PRL titer and the levels of AMH, LH, and PRL after immunization against PRL. Vertical bars represent the mean ± SEM (n = 6). [a,b,c] Different superscripts within columns indicate means are significantly different. The labels 0, 10, 20, 30, and 40 d indicated the days of the experiment, the labels PBS, INH-α, AMH, and PRL indicated the different treatment of the geese. Abbreviations: AMH, anti-Müllerian hormone; INH-α, immunization against recombinant-derived goose inhibin-α; LH, luteinizing hormone; PBS, phosphate-buffered saline; PRL, prolactin.

serum were detected by ELISA. Anti-INH-α, anti-AMH, and anti-PRL antibody titers increased gradually and peaked at 25.9 ng/L, 2.1 ng/mL, and 263 ng/L 20 d after immunization, respectively ($P < 0.05$). At 40 d, they had decreased to 15.4 ng/L, 0.57 ng/mL, and 136.4 ng/L, respectively. For hormone levels, compared to the control group, immunization with INH-α and AMH fusion proteins increased LH level of serum, when with PRL fusion proteins reduced AMH level and increased LH and PRL levels ($P < 0.05$; Figure 2).

Effect of Active Immunization on Follicular Development

Except for the AMH group, there were no significant differences in hierarchical and large yellow follicles (the total number is 5−10) of the other groups. There was a highly significant difference in the number of the pre-hierarchical follicles except for large yellow follicles (>1 mm in diameter). In the INH-α immunized group, the number of follicles reached a maximum of 195, which was 2.5 times that of the control group ($P < 0.05$). The number of follicles in the immunized PRL fusion protein

group was 140, which were 1.6 times that of the control group (Figure 3).

Effect of Active Immunization on Egg Production

Goose eggs were collected every day to evaluate egg output. We found that animals in the AMH and PRL injected groups had higher egg production 12 d after immunization, but the INH-α fusion protein group showed an inversion on d 14. The egg productions of the immunized AMH and INH-α groups did not increase from 16 to 29 d but had secondary increases at 31 d compared with the control group (Figure 4A). Overall, during the 40-d observation period, the numbers of egg produced by geese immunized with PRL fusion proteins increased by 1.34 per goose ($P < 0.05$; Figure 4B).

Effect of Active Immunization on Broody Behavior

To assess the effects of active immunization with INH-α, AMH, and PRL fusion proteins on the reproductive performance, we conducted behavioral observation
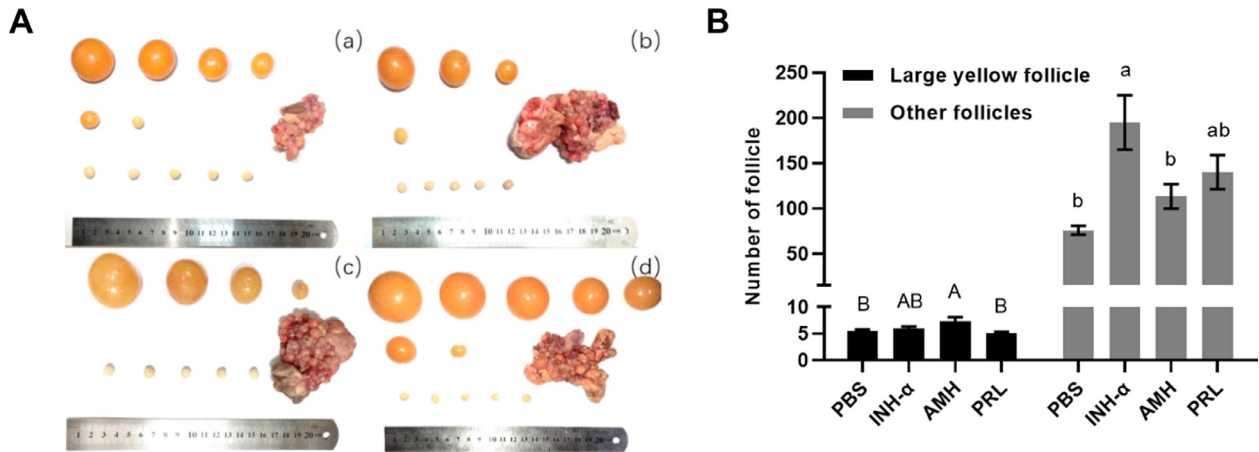
Figure. 3. Effect of follicle development of geese after immunized against INH-α, AMH, and PRL fusion proteins. Isolation of follicles and ovaries from laying geese immunized with INH-α, AMH, and PRL fusion proteins. (A) The developmental morphology of follicles and ovaries of geese after immunized against INH-α, AMH, and PRL fusion proteins. The first and second row is the hierarchical follicles, the bottom row is the large yellow follicle (prehierarchical follicles), and the lower right corner is the ovary and other follicles, (a) PBS, (b) INH-α, (c) AMH, (d) PRL. (B) The number of follicles of the INHα-, AMH-, and PRL-immunized geese. Vertical bars represent the mean $\pm$ SEM (n = 6). Different superscripts within columns indicate means are significantly different. Abbreviations: AMH, anti-Müllerian hormone; INH-α, immunization against recombinant-derived goose inhibin-α; PBS, phosphate-buffered saline; PRL, prolactin.

4 times a day to quantify broody behavior. Compared with the control group, the broodiness period of experimental geese occurred earlier and had a shorter duration. For control geese, the broodiness period lasted 20.5 d and happened 16 d after immunization. Compared with the control, the onset of broodiness of the INH-α fusion protein immunized geese was 12.5 earlier and the duration was also shorter by 10 d ($P < 0.05$). The onset of broodiness of the immunized AMH geese and the duration of the broodiness period of immunized PRL geese were less 4 d and 8.5 d compared with the control, respectively. During the entire 40-d observation period, the new broodiness rates (ratio of newly broody geese in the total female population) of the INH-α, AMH, and PRL groups were decreased to 8.33, 11.11, and 8.33% compared with the control group (25%), respectively ($P < 0.05$; Figure 5).

DISCUSSION

Hormone immunization stimulates animals to produce specific antibodies and neutralizes the corresponding endogenous hormones in the body, which regulates reproductive function (Moreau et al., 1998; El-Halawani et al., 2000; Kingsbury et al., 2015). In this study, we investigated the effect of active immunization with recombinant-derived goose INH-α, AMH, and PRL fusion protein on broodiness onset and egg production in geese. Two decade ago, the investigator has detected active immunization of the INH-α subunit increased FSH concentrations in chicken (Knight, 1996). In addition, active immunization against AMH also upregulated pituitary FSH synthesis and secretion in geese (Chen et al., 2020). In this study, we detected high LH levels in recombinant INH-α and AMH protein-
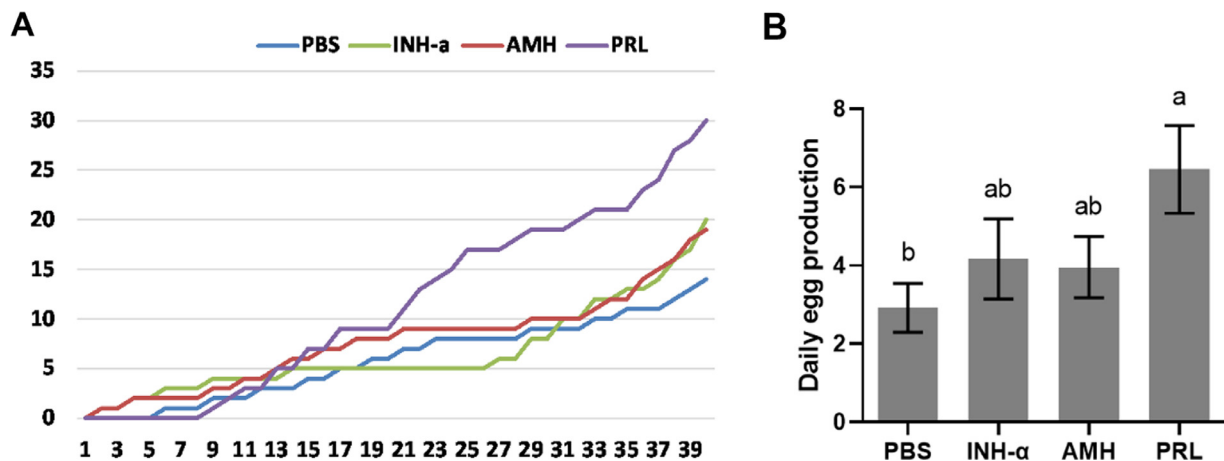

Figure. 4. Effect of egg production of geese after immunized against INH-α, AMH, and PRL fusion proteins. (A) Egg production of the different groups of geese after immunized against INH-α, AMH, and PRL. (B) The daily egg production of the different groups of geese after immunized against INH-α, AMH, and PRL. Vertical bars represent the mean $\pm$ SEM (n = 40). [a,b,c] Different superscripts within columns indicate means are significantly different. Abbreviations: AMH, anti-Müllerian hormone; INH-α, immunization against recombinant-derived goose inhibin-α; I; PRL, prolactin.
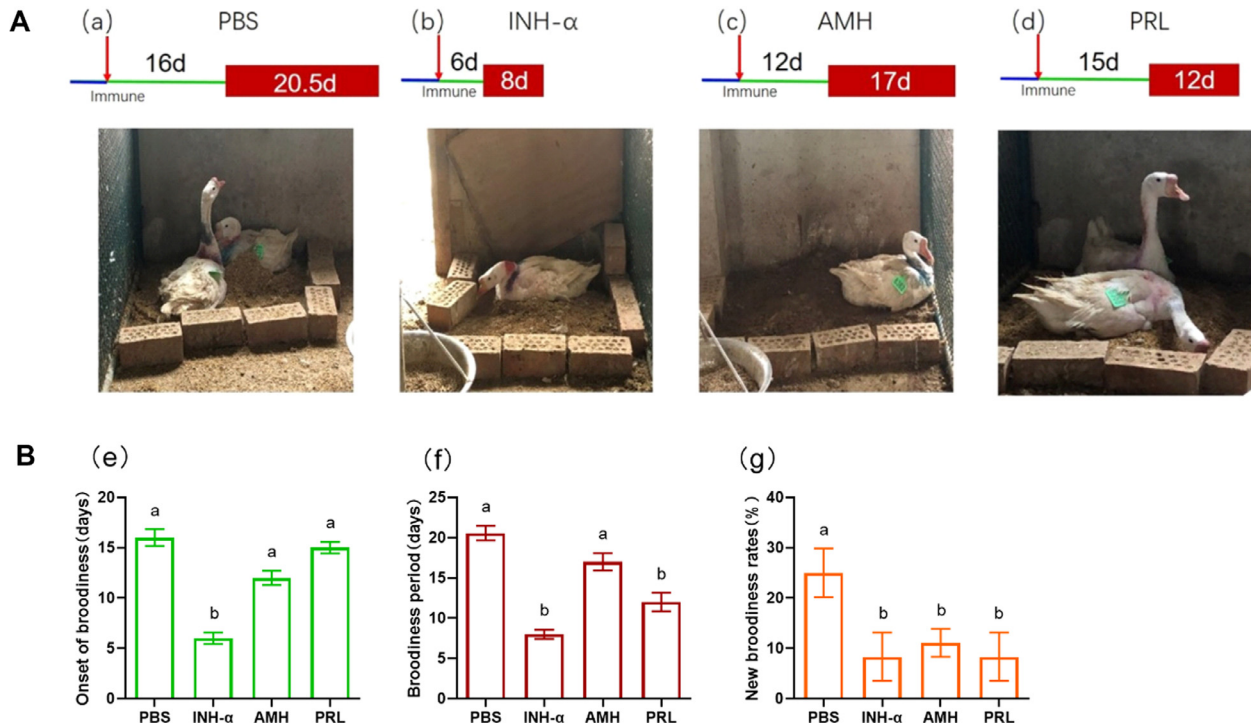
Figure. 5. Effect of broody behavior of geese after immunized against recombinant INH-α, AMH, and PRL proteins. (A) Video recording of broody behavior of geese of PBS-, INHα-, AMH-, and PRL-immunized group. Red arrows indicated the immunized. The green horizontal lines indicated the onset of broodiness of the geese after immunization. Red boxes indicated the broodiness period of the geese after immunization. (a) PBS, (b) INH-α, (c) AMH, (d) PRL. (B) Data analysis details of broody behavior of geese. (e) The onset of broodiness, (f) broodiness period, (g) new broodiness rates. Abbreviations: AMH, anti-Müllerian hormone; INH-α, immunization against recombinant-derived goose inhibin-α; I; PBS, phosphate-buffered saline; PRL, prolactin.

immunized geese. Because both FSH and LH are glycoprotein hormones secreted by adenohypophysial gonadotropin cells, and they have a synergistic effect (H. Liu et al., 2018), high LH content in hormone -immunized geese was expected. Also, higher LH contents were observed in the PRL recombinant protein-immunized geese. PRL was thought to be a hormone that negative regulated the LH (Huang et al., 2008). Hence, immunoneutralization of PRL bioactivity enhances LH secretion.

Hormone immunity can not only regulate hormone levels in the body, but also regulate the reproductive activities of animals. Studies have demonstrated that INH-, AMH-, and PRL-immunization were involved in follicular growth and development, as well broodiness behavior (van Rooij et al., 2002; Jiang et al., 2005; Yan et al., 2015). In chicken, active immunization with a recombinant chicken inhibin fusion protein stimulated the follicle development and enhanced egg laying performance (Satterlee et al., 2002; Mao et al., 2016). In goose, Akhtar et al. (2019) found active immunization against inhibin a-subunit affected spermatogenesis and testicular development. In this study, the recruited more follicles in INHα-immunized geese were observed, which meant immunization against INH-α rescued some recruited follicles from atresia. Accordingly, broodiness behavior was shortened in the INHα-immunized geese by 12.5 d. AMH, known as the Müllerian inhibiting substance or Müllerian inhibiting factor, plays a vital role in regulating ovarian follicular development in mammals

(Durlinger et al., 2002; Cimino et al., 2016). In this study, AMH recombinant protein immunized geese had more large yellow follicles of ovary. Results of the present study were in agreement with the findings of Chen et al. (2020), who reported that the immunoneutralization of AMH bioactivity enhanced ovarian follicular development. PRL is the most direct hormone that regulates broodiness in poultry (Li et al., 2011). To develop techniques for improving reproductive efficiency in broody prone fowls, studies have been performed to immunize against PRL to inhibit development of broodiness, and subsequently to extend egg-laying duration (Sharp, 2007).

Active immunization against prolactin resulted in the prevention of incubation behavior expression in turkey hens (Crisóstomo et al., 1998). The broodiness was also inhibited by PRL immunization in laying Yuehuang hens (Li et al., 2011), the rise of egg laying was also inhibited. When Magang geese were similarly immunized against PRL, the development of incubation behavior was slowed down and subsequently increased the final number of clutch eggs (Liu et al., 2009). In the present study, we get similar results of Magang geese, as the immunization against PRL in Zhedong geese reduced broodiness duration and increased the clutch size by average 1.34 eggs. However, only decreasing broodiness was observed in Wanxi goose of active immunization with PRL, the rate of egg production did not increase (March et al., 1994). Geese breeds can be classified into 3 types according to their breeding season:

Type 1 and Type 2 belong to long-day breeding geese, and inhabit higher latitude or mid-latitude (30−40° N) temperate zones, respectively. Type 3 is the short-day breeding geese, located in subtropical areas (Shi et al., 2008). From this, Magang goose and Zhedong goose are short-day breeds, while Wanxi goose is long-day breed. The hormones in the body are regulated by a variety of internal and external factors, and it is possible to immunize the same birds with different hormones or even with the same hormones to produce different effects (Huang et al., 2008; Li et al., 2011). It was reported that the effect of egg laying after immunization against PRL often occurred under a short photoperiod (Li et al., 2011). As a result, it is hardly surprising that the more eggs were laid in Magang goose and Zhedong goose of PRL immunization. Strangely, the PRL content in the serum increased after PRL immunization. This phenomenon might be caused by the different physiological state of the experimental geese. Although from all appearances, the geese were in the laying period, did not exclude that some geese have been in the later stage of laying and were about to enter the broodiness.

CONCLUSIONS

In summary, this study demonstrated that active immunization against INH-α, AMH, and PRL in egg-laying geese could affect the broodiness onset and egg production in geese by immunoneutralization. Immunization with recombinant proteins INH-α or AMH could promote LH hormone secretion regulating follicle development. While active immunization against recombinant PRL could increase LH and PRL levels, decreased AMH level. Besides, the geese receiving INH-α recombinant protein, the broodiness onset was significantly 10 d shorter compared to the control geese. And the 12.5 and 8.5 d shorter broody duration intervals were observed in INHα- and PRL-immunization geese. Moreover, the PRL recombinant protein-immunization resulted in an average increase of 1.34 eggs during a 40-d observation. Our study suggests that active immunization against recombinant proteins INH-α or AMH could promote LH hormone secretion, regulate follicle development, and decrease the broodiness rate. Also, active immunization with a recombinant-derived goose PRL protein might improve egg laying performance.

ACKNOWLEDGMENTS

DISCLOSURES

The authors have no conflicts of interest to report

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2021.101452.

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