Archiv Tierzucht 55 (2012) 2, 179-183, ISSN 0003-9438

Inhibin immunization in Norduz sheep

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Abstract

The objective of this study was to investigate the possibility of increasing ovulation rate in Norduz sheep by immunization against inhibin-based peptide immunogens. A total of 18 primiparious Norduz ewes were used for this experiment. Eight ewes have received two times inhibin α-subunit 1-32 porcine vaccine with 3 weeks interval. The rest was kept as control. Antibody binding test by standard ELISA method did not provide reliable information. However, ultrasonographic inspection showed that significantly high number of follicle with 5 mm and larger diameter ($P<0.01$) has developed in immunized ewes. Moreover, 3 of immunized ewes have lambed twin. However, twin birth was not observed in control group.

Keywords: inhibin, immunization, Norduz sheep

Introduction

Norduz sheep is one of native sheep breeds originated from the Eastern part of Anatolia, Turkey. Principal characteristics of Norduz sheep are high pre-weaning viability for lambs and adaptation capacity in high lands of East-Anatolia (Bingol 1998). Main characteristics of male Norduz goat have robust, long and upward horns and principle colour is black. Also white, black-white, grey, roan and brown colour can be found (Daskiran *et al.* 2004). However, twin birth is not usual for Norduz sheep. Average twinning rate does not exceed 11 % (Ulker *et al.* 2003). Inhibin is a non steroidal substance which is capable of inhibiting pituitary cell activity. It was identified in testicular extracts in 1932 (Schneyer 2004). Inhibins are heterodimeric glycoproteins composed of an α-subunit and one of several forms of β-subunits, resulting in active forms termed inhibin A and B (Ying 1988). Inhibins are produced by granulosa cells during follicular development in mammals (Rokukawa *et al.* 1986, Piquette *et al.* 1990, Engelhardt *et al.* 1993, Nagamine *et al.* 1998). In the ewe, either active or passive immunization techniques may be used in order to contribute the negative feedback regulation of follicle stimulating hormone (FSH) release from pituitary gland (Terqui *et al.* 1995). Inhibin immunization was applied in several species for improving prolificacy such as goat (Medan *et al.* 2003, Cedden *et al.* 2007) ewe (Wheaton *et al.* 1992, Naqvi *et al.* 2009) and cattle (Wood *et al.* 1993, Konishi *et al.* 1996). Active immunization against inhibin-based peptide immunogens can be effective in increasing the ovulation rate in non-prolific sheep breeds (Naqvi *et al.* 2009). Recombinant inhibin use at early age of lambs provides increased inhibin antibodies in the plasma of immunized lambs (O'Shea *et al.* 1993). Moreover, some researchers reported that inhibin

immunized ewe shows more mature follicles, greater ovulation rate and increased litter sizes (O'Shea *et al.* 1993, Han *et al.* 2007). The objective of this study was to observe the effect of active immunization against inhibin-based peptides on follicular development.

Material and methods

Animal and treatment

A total of 18 primiparious Norduz ewes were randomly chosen from small ruminant flock of experimental farm of Yuzuncu Yil University, Turkey. Flock management was applied as an usual manner of sheep rearing for East-Anatolia. Pasture-based feeding on spring, summer and early period of fall with 500 g/animal of concentrate daily and freely available water regime was practiced. The flock was kept in pen during winter. The study was conducted in normal breeding season which starts from mid-September and finishes on November.

Among all, 8 ewes were randomly separated as trial group. The rest was kept as control. Each one of trial group (n=10) received subcutaneous 0.1 mg inhibin vaccine (α-subunit Fragment 1-32 Porcine, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) emulsified in 0.5 ml Freund's complete adjuvant containing 1 mg/ml heat killed and dried *Mycobacterium tuberculosis* (F 5 881 10 ml, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and 0.5 PBS (phosphate buffered solution) into three different sites of body (neck, thigh and forleg). Three weeks later, all animals of both trial and control groups received two times 125 µg of cloprostenol (a PGF2 α analogue, Dalmazin-Vetas 10 ml) with 9 days interval for each animal . But all animals of trial group received the booster which was prepared with Freund's incomplete adjuvant (F 5 506, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) at the time of first cloprostenol injection. Three rams were put into the pens for each group and oestrous signs were detected from 36 h following 2nd PGF2 α. Rams were kept with ewes until disappearance of oestrous signs.

Inhibin antibody titers

Blood samples were collected by jugular vein puncture before introducing rams for mating. Blood sampling is made with usual vacutainer tubes. Blood sera were obtained from blood samples kept 24 h at 5 °C in refrigerator. Antibody titers are determined by using Standard ELISA procedure. High-binding 96-well plates (Greiner Bio-One GmbH, Solingen, Germany) were coated with α-subunit (fragment 1-32) of inhibin at 20 ng/well in 100 µl PBS (phosphate-buffered) saline for 1 h at room temperature. Plates were then blocked for 2 h by using 1 % (w/v) of bovine serum albumin in PBS. Twelve serial dilutions of each serum samples (starting from 1:100 with ½ serial dilutions) (including negative control) were distributed to the plates in 100 µl and incubated for 1 h at room temperature. The plates were washed three times, and then a horseradish peroxidase-conjugated rabbit anti-sheep antibody was added to the wells (at 1:5 000 dilution in PBS). Following 2 h of incubation with secondary antibody, plates were washed three times and an ECL substrate (Amersham plc, Amersham, UK) was added and luminescence was counted immediately in a Wallac counter in photon counting mode (Microbeta TriLux, PerkinElmer, Inc. Waltham, MA, USA) for 1 s/well.

Ultrasound examination and follicle classification

The ovaries were inspected by using B-mode scanner ultrasound (HS-1 500 Vet, Honda Electronics Co., Ltd., Japan) equipped with a 50 mm, 7.5 MHz transducer (1:HLV-375M) and applied trans-rectally after 48 h following 2nd PGF2 α administration. Number of follicles larger than 3 mm were counted and their diameter were measured for both ovaries of all ewes. Follicles were classified in three groups according to measured diameters as smaller than 3 mm, 3-5 mm and larger than 5 mm (England *et al.* 1981).

Statistical analysis

The comparison of follicle rate by diameter between trial and control groups was performed by using Z-test. Minitab 14 package program (Minitab Inc., State College, PA, USA) was used for calculations. Probability thresholds for significance were defined at the level $P<0.01$ or $P<0.05$.

Results

Inhibin antibody titer

Standard ELISA test used for antibody titers did not provide reliable information from sera of Norduz ewes. The result obtained from antibody titers was not correlated in accordance with dilution rate in order to distinguish correctly trial and control groups from each other.

Follicle development

Active immunization against inhibin-based vaccine increased follicle development in the ovary. Compared to the controls, overall follicle number developed in the ovaries was higher in the immunized group (Table 1). Both left and right ovaries of immunized ewes had significantly higher follicle rates with 5 mm and larger diameter ($P<0.05$). Total number of follicle with 5 mm and larger diameter was also significantly higher in the immunized ewes ($P<0.01$).

However, number of follicle with 3-5 mm and smaller than 3 mm of diameter did not vary significantly by groups. Number of follicle with 5 mm and larger of diameter was found 3 fold more in inhibin-immunized Norduz ewes.

Table 1
Distribution of follicle rates by diameter in immunized and control groups

	Number of follicle by diameter					
	3> mm		3-5 mm		5≤ mm	
	n	%	n	%	n	%
Trial group, n=10						
Left	14	43.75	12	37.50	6	18.75[b]
Right	10	40[a]	9	36	6	24[b]
Total	24	42.11	21	36.84	12	21.43[d]
Control group, n=8						
Left	3	17.65	12	70.59	2	11.76[a]
Right	18	72.00[b]	5	20.00	2	8.00[a]
Total	21	50.00	17	40.48	4	9.52[c]

[a,b]$P<0.05$, [c,d]$P<0.01$

Lambing

Among inhibin immunized ewes, only 3 ewes have given twin birth. But, no twin birth was observed in control group.

Discussion

The result obtained from this study was found in accordance with indications of other earlier researches based on active inhibin immunization in prolific (Wrathall *et al.* 1990, McLeod *et al.* 1992) and non-prolific Naqvi *et al.* 2009) sheep. Recent studies clearly pointed out that inhibin immunization increases number of follicle which may result in higher ovulation rate. Immunoneutralization of endogenous inhibin was thought to result in decreased negative feedback on the pituitary gland resulting in elevated FSH release, then increased follicular development and also increased ovulation rate (Medan *et al.* 2003). Thus, the percentage of follicle having 5 mm and larger size was found about 2.5 fold higher, compared to control group. However, Anderson *et al.* (1998) reported 4 fold more large-sized follicle rate in Merino ewes. Naqvi *et al.* (2009) reported that it is possible to increase large-sized follicle rate up to 5 fold and to maintain same response during 3 years in non-prolific Malpura ewes, if three subsequent inhibin vaccines were applied. Type of inhibin-based peptide, number of booster and as well as sheep breed are major factors which affect immunological response of inhibin immunization. Inhibin immunization can be used as an alternative to multi ovulation instead of using conventional exogenous hormone administration. But, further studies are needed for improving ovulation rate and prolificacy in Norduz sheep.

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Received 14 June 2011, accepted 10 January 2012.

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