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IMMUNIZING NORDUZ GOATS AGAINST INHIBIN**

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Abstract: The objective of this study was to investigate the effect of active inhibin immunization on follicular development and prolificacy in Norduz goats. Two times inhibin α- subunit 1-32 porcine was used for preparation of vaccine. It was observed that goats were successfully immunized against inhibin, following ultrasonographic inspection and antibody binding test. High number of follicles were developed in immunized goats. But , active immunization does not improve prolificacy in Norduz goats.

Key words: Active, Inhibin, Immunization, Goat

Introduction

Norduz goat is one of the indigenous goat breeds of East-Anatolia. This breed is distributed onto 23 villages of Gurpinar district located in Van province. Norduz goat is very appreciated by farmers due to its milk yield, meat and fibre production, as well as its adaptation capability in harsh environment (*Daskiran and Cedden* 2005). However, Norduz goat is not a prolific breed. Twin birth appears scarcely, while high prolificacy is essential for increasing meat production from goats. There are some methods which are commonly recognized for increasing ovulation rate in farm animals, such as flushing and exogenous gonadotropin utilization by administering hormones with FSH-like activity. But, there is also another method composed of removing the inhibitory effect of ovarian hormones on gonadotropin release by the hypothalamus-pituitary axis. Principal ovarian peptides which modulate FSH secretion are inhibin, activin and molestation (*Ying* 1988). Among them, inhibin shows synergistic action with oestradiol resulting in negative feedback effect on FSH at the anterior pituitary

(*Findley and Clarke* 1997*; Taya* 1993*; Taya and Watanabe* 1999). The feedback effect of inhibin has been studied in small ruminants by injecting a recombinant inhibin fragment alone (*Mizumachi et al* 1990*; Dietrich et al* 1995). Some researches showed that passive immunization against inhibin may increase plasma FSH level resulting in increased number of oocytes to ovulate (*Nambo et al* 1998*; Shi et al* 2000). However, active immunization against inhibin has different effects on FSH secretion (*Medan et al* 2003[a]). The objective of this study is to determine the effect of active immunization against inhibin on ovulation rate and prolificacy in Norduz goats.

Material and Method

Animals and treatment

Twelve primiparious Norduz goats were randonmly chosen from the flock of experimental farm in Faculty of Agriculture of Yuzuncuyil University. The goats were housed under natural light and fed 500 g/animal of concentrate, beside daily grazing near the pasture of farm until winter. Water was freely avaible. Each goats were received two times 125 µg of Estrumate which is a synthetic analogue of prostaglandin F_2 α (Dalmazin Vetas 10 ml) with 11 days interval on mid-october which is normal breeding season for Norduz goats. Goats were allocated to two groups. Each one of immunized goat group (n=6) received sc 0,1 mg inhibin vaccine (α- subunit Fragment 1-32 Porcine SIGMA-Germany) emulsified in 0,5 ml Freund's complete adjuvant containing 1 mg/ml heat killed and dried Mycobacterium tuberculosis (F 5881 10 ml SIGMA-Germany) and 0,5 PBS (phosphate Buffered Solution) into three different sites of body (neck, thigh and forleg) followed by one booster injections at 3 days interval. The group of controle (n=6) received serum phsiologique (1 ml) in stead of vaccine. The booster was prepared with Freund's incomplete adjuvant (F 5506 SIGMA-Germany). After the booster injection to immunized group, all goats received first PGF_2 α injection for oestrous synchronization. Second PGF_2 α administration was made 11 days apart from first injection. Two bucks were put into the pens for each group and oestrous signs were detected from 36 hours following 2nd PGF_2 α. Bucks were kept with goats until disappearance of oestrous signs.

Inhibin antibody titers

Blood samples were collected by jugular vene puncture before

introducing bucks for mating. Ordinary vacutainer tubes were used for blood sampling. Blood sera were obtained from samples kept 24 hours at 5 ºC in refrigirator. Standard ELISA procedure was used to determine antibody titers. Briefly, high-binding 96-well plates (Greiner, Germany) were coated with α-subunit (fragment 1-32) of inhibin at 20 ng/well in 100 µl PBS (phosphate-buffered) saline for 1 hours at room temperature. Plates were then blocked for 2 hours 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 hour at room temperature. After washing the plates three times, a horseradish peroxidase-conjugated rabbit anti-goat antibody was added to the wells (at 1:5000 dilution in PBS). Following 2 hours of incubation with secondary antibody, plates were washed three times and an ECL substrate (Amersham, UK) was added and luminescence was counted immediately in a Wallac counter in photon counting mode (Microbeta TriLux) for 1 sec/well.

Ultrasound examination

The inspection of ovaries was made by using B-mode scanner ultrasound (Honda Electronics HS-1500 Vet) equipped with a 50 mm, 7,5 MHz transducer (1:HLV-375M) and performed trans-rectally every 24 h after 48 hours folowing 2nd PGF_2 α administration during 3 days. Number of follicles larger than 3 mm were counted and their diameter were measured for both ovaries of all goats. Ovulated follicles were also observed.

Statistical analysis

Two-sample t test was used in order to compare two independent groups. The coefficients of correlation was calculated for determining the relationship between two traits (Minitab 1993). As the data were obtained from counting, square root transformation [$\sqrt{(x+3/8}$] was performed before analysis.

Results and Discussion

Inhibin binding activity

Dilution curves were fitted by the nonlinear regression of a four-

parameter logistic equation and titers were given as the dilution that gives a binding signal at an arbitrary cutoff value. Cutoff was 10% of the maximum binding signal (obtained at 1:100 dilution of the most reactive serum). Average binding capacity was found almost 10 times higher in inhibin-immunized goats (See Figure 1).

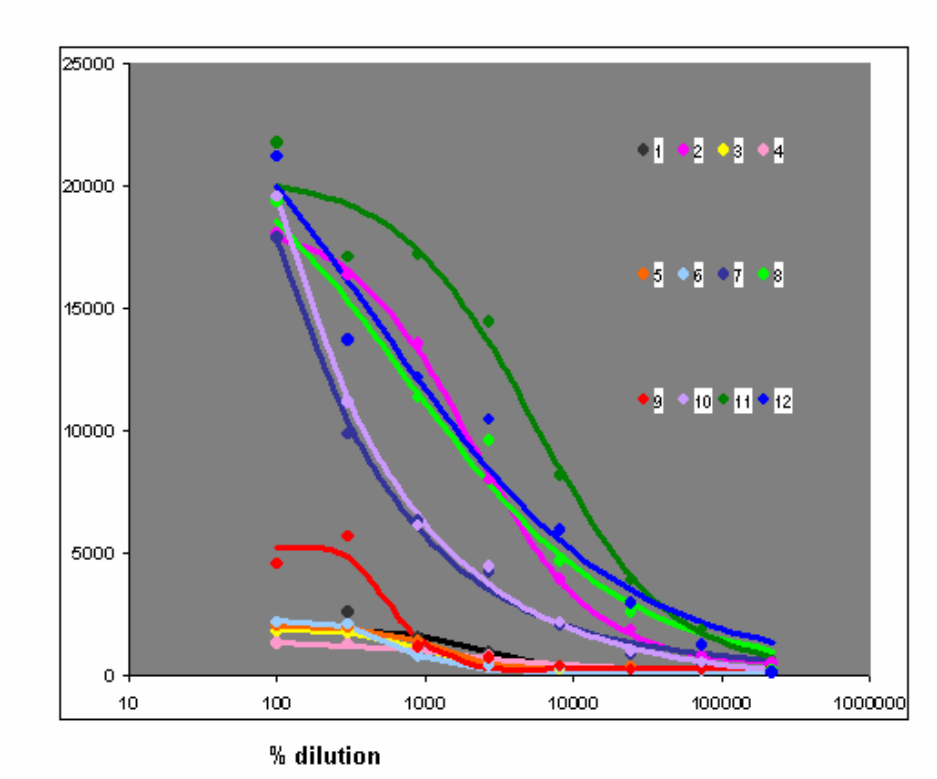

Figure 1. Antibody binding capacity at a dilution from 1:100 in immunized and control group

Ovarian activity

All goats showed oestrous signs within 48-72 hours after second $PGF_2\alpha$ administration. The comparison of immunized and control groups regarding total follicle numbers was based on the diameter. The follicles were classified as smaller than 3 mm, between 3-5 mm and larger than 5 mm. After the immunization, goats had significantly higher number of follicle sized larger than 3 mm. The goats of control group had 17 follicles <3 mm

of diameter, whereas immunized goats had only 10 in total. But, total number of follicle larger than 3 mm was significantly higher in immunized goats, when comparing to goats of control group (47 vs 13) (P<0,01). Also, right ovaries developed higher number of follicle >3 mm. in immunized goats (P<0,01). However, both immunized and control group had no significantly different follicle number >3 mm, regarding left ovaries. Following three successive ultrasonographic examination, total number of corpus luteum was found as 8 in immunized goat. This value was found as 4 in control group. The correlation coefficiant between number of follicle >3mm and antibody binding value was found significant and positive (r=796) (P<0,01).

Lambing and twinning rate

Al goats in immunized and control group kidded. Only one twin birth was observed in immunized group, wheras no twinning in control group.

Table 1. Comparison of ovarian response after inhibin immunization (x ± SE)

Traits	Immunized Group	Control group
Number of treated goats	6	6
Average number of follicles < 3 mm	1,67±0,39	2,83±0,40
Average number of follicles >3 mm	7,83**±1,87	1,83±0,48
Average number of follicles > 3mm in right ovaries	5,00**±1,03	0,67±0,33
Number of follicles > 3mm in left ovaries	2,83±1,08	1,17±0,48
Average number of CL	1,83	0,83

** P<0,01

The results showed that Norduz goats can be immunized with two times practiced inhibin vaccine. Increased follicular development was observed in inhibin-immunized goats. The number of corpus luteum was also doubled in immunized goats. However, twin birth did not appear in accordance with follicular development and corpus luteum number. Although the number of follicles larger than 3 mm was higher especially, these larger than 5 mm was found as 22 in immunized goats, high twinning rate did not appear as expected. This was probably related in inadequate release of LH. Some authors reported lower LH level of plasma in inhibin-immunized goats, because of increased level of oestradiol secreted by large number of

developing follicles. Low number of ovulated follicle, despite more developing ones, may be attributed to this effect in immunized goats (*Medan et al* 2003[b]). Same results were observed in hamsters treated with inhibin antiserum (*Kishi* 1996) and also in other species. Inhibin immunization improves markedly follicle development and oestradiol secreted by follicles. However, LH release induced by GnRH does not reach sufficient level for ovulation in ewe (*Wrathal et al* 1990) and cattle (*Takedomi et al* 1997). Right ovaries were more active in our study. A recent research showed similar observation in single-ovulating ewes kept in harsh environment. Also, high embryo loss was observed in ewes following twin ovulations on the right ovary (*Regessa et al* 2007).

Conclusion

In conclusion, the use of inhibin immunization along does not improve prolificacy in indigenious single-bearing goats. But, it would be a potential method for enhancing multiple follicular development resulting in superovulation, in order to provide embryos for transfer technology. However, further studies are suggested for improving applicability of inhibin immunization in goats.

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IMUNIZACIJA KOZA RASE NORDUZ PROTIV INHIBINA

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Rezime

Istraživanje je sprovedeno radi utvrđivanja uticaja imunizacije aktivnim

inhibinom koza rase norduz korišćenjem inhibin vakcine. Dve grupe koza koje su jare po prvi put su primile bilo fiziološki rastvor kao kontrolu (n=6), ili dva puta 0,01 mg inhibina α- podjedinica 1-32 svinjski razređen sa PBS kao imunizovana grupa (n=6). Sve koze su dobile prostaglandin F_2 α analog za sinhronizaciju estrusa u intervalu od 11 dana tokom normalne sezone parenja. Jarčevi su uključeni radi parenja i znaci estrusa su registrovani 48-72 sati nakon druge PG F_2 α injekcije. Uzorci krvi su uzimani od koza pre parenja i testirana je sposobnost vezivanja antitela u serumima korišćenjem običnog ELISA metoda. Ultrasonografsko ispitivanje je urađeno pomoću B-mode skenera i 7,5 MHz sonde, svaka 24 sata tokom 72 sata, 36 sati nakon druge PG F_2α injekcije. Utvrđeno je vezivanje antiteta 10 puta veće nego kod imunizovanih koza. Prosečni broj folikula >3 mm je bio signifikantno veći u grupi imunizovanih koza (7,83 prema 1,83) ($P<0,01$). Veliki broj folikula se razvio u desnim jajnicima u poređenju sa levim (5,00 vs 0,67) ($P<0,01$). Utvrđena je pozitivna i signifikantna korelacija između broja folikula >3mm i vrednosti za vezivanje antitela ($r=796$) ($P<0,01$). Broj žutih tela je takođe bio veći kod imunizovanih koza. Prosečan broj žutih tela je bio 1,83 prema 0,83. Sve koze iz imunizovane i kontrolne grupe su se ojarile. Ali samo u grupi imunizovanih koza je registrovan jedan slučaj dvojki. Prema tome, imunizacija aktivnim inhibinom može stimulisati razvoj višestrukih folikula. Međutim, ne poboljšava plodnost koza rase Norduz.

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