

## THE EFFECTS OF IMMUNIZATION AGAINST LHRH USING RECOMBINANT LHRH FUSION PROTEIN, OVALBUMIN-LHRH-7, ON DEVELOPMENT, HISTOLOGIC AND ULTRASONOGRAPHIC APPEARANCE OF RAM LAMB TESTIS<sup>1</sup>

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**Abstract:** The purpose of this study is to evaluate the effectiveness of recombinant ovalbumin-LHRH-7 (OL) fusion protein on reproductive traits in ram lambs. Twenty ram lambs at 19 wk of age were divided into control (C, n = 10) and immunization (I, n = 10) groups. Immunized animals received OL protein generated by recombinant DNA technology. Ultrasonographic and histological examination of the testes was performed. At 41 wk of age animals were slaughtered. Semen and epididymis were evaluated for the presence of sperm cells. Testicular development and sperm production were suppressed in the immunized animals. Seminiferous tubule diameters decreased, basal membrane of the tubule was thickened and hyalinized in immunized ram lambs. Immunization affected ultrasonographic appearance of the testes drastically. While testes of control animals gained their normal ultrasonographic appearance as the age increased, immunized animals had uniform hypoechoic testicular structure as observed at 19 wk of age until slaughter. In conclusion, these results indicate that recombinant ovalbumin-LHRH-7 fusion protein is effective in immunocastration in ram lambs and has a potential to be used as an alternative to physical castration. Testicular development and sperm production in immunized ram lambs were suppressed. The effect of immunization affected testicular histology and ultrasonographic appearance of testis.

**Keywords:** LHRH, fusion proteins, immunization, ewes

### Introduction

Livestock species have been castrated for centuries because of management difficulties associated with rearing gonad-intact male and female animals, to reduce aggressive and sexual behaviors, to improve meat and carcass quality. Immunizing farm animals against luteinizing hormone releasing hormone (LHRH) has been studied as an alternative sterilization technique (immunocastration) for surgical castration (Reeves *et al.*, 1989; Bonneau and Enright, 1995; Thompson, 2000).

Recombinant LHRH fusion protein (ovalbumin-LHRH-7 (OL)) was tested in mice for antigenicity and biological effects, and, has been found that it elicited higher LHRH antibody production and decreased uterine-ovarian weight (Zhang *et al.*, 1999). Also, ovalbumin-LHRH-7 was evaluated for its effectiveness in suppression of estrus in heifers (Sosa *et al.*, 2000; Stevens *et al.* 2005) as well. Nevertheless, the effectiveness of ovalbumin-LHRH-7 in suppressing reproductive functions in ram lambs has not been determined.

Ultrasonography is a non-invasive technique allows studying the changes in reproductive organs. Several studies to determine the structure of the testis using ultrasound imaging (Ahmad *et al.*, 1991; Evans *et al.*, 1996; Aravindakshan *et al.*, 2000; Gouletsou *et al.*, 2003) have been reported. Recently, ultrasonographic appearance of testis in LHRH immunized ram lambs with a comparative approach using histological structure has been reported (Ülker *et al.*, 2005), nevertheless, there is no report on determining the effects of immunocastration on ultrasonographic appearance of the testis in relation to time after immunization.

Thus, the first purpose of this study is to evaluate the effectiveness of recombinant ovalbumin-LHRH-7 (OL) fusion protein, a new LHRH sterilization vaccine, on reproductive traits in ram lambs. The second purpose of this study is to determine the changes in ultrasonographic appearance of testis in LHRH immunized ram lambs with a comparative approach using histological structure in relation to time after immunization.

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### Materials and Methods

Twenty native Karakaş ram lambs at 19 wk of age were divided into control (C, n = 10) and immunization (I, n = 10) groups. Animals in control group were not treated. Animals in immunization group were immunized against LHRH using OL protein generated by recombinant DNA technology (Zhang *et al.*, 1999) as a primary and a booster injection at 19 and 21 wk of age, respectively. Scrotal circumference measurements were collected every 2 wk for each animal. Testes were scanned bi-weekly to determine the changes in ultrasonographic appearance as the age increased. Testes of ram lambs in C (n=4) and I (n=4) groups were biopsied under local anesthesia to attain testicular tissue for histology of the testes at 19 and 29 wk ages. Semen was collected using electro ejaculator at 37 wk age. At 41 wk of age animals were slaughtered. Biopsied testicular tissues taken at 19, 29 and 41 wk age were evaluated histologically. After slaughter epididymides were evaluated for the presence of sperm cells.

### Results and Discussion

Scrotal circumference in control group increased steadily as the age increased whereas it stayed as almost the same size as measured at the beginning of the study in immunized animals (Table 1). The suppression in testicular development was reported as the first indication for immunocastration by several researchers using various LHRH antigens (Brown *et al.*, 1994; Kiyama *et al.*, 2000; D'Occhio *et al.*, 2001; Aissat *et al.*, 2002; Ülker *et al.*, 2005).

Table 1. Scrotal circumference (cm) in control and immunized ram lambs

	19 wk age	29 wk age	41 wk age
Control	15.90	22.56	25.44
Immunization	15.80	14.92	15.39

Mature spermatozoa were found in ejaculates collected at 37 wk age or epididymis after slaughter (41 wk age) in control group, whereas no spermatozoa were obtained from immunized group (Table 2). These data indicate that OL protein is effective in suppressing testicular development and sperm production in ram lambs. This is the first report showing that immunization against LHRH using recombinant OL protein suppressed testicular development and sperm production in ram lambs.

Table 2. Spermatozoa in ejaculate or epididymis in control and immunized ram lambs

	Spermatozoa in ejaculate <sup>1</sup>	Spermatozoa in epididymis <sup>2</sup>
Control	Present	Present
Immunization	None	None

<sup>1</sup>Collected via electroejaculator at 37 wk age

<sup>2</sup>Obtained from epididymis after slaughter at 41 wk age

Semiferous tubule diameter increased in control animals whereas this measurement decreased in immunized ram lambs (Table 3). Basal membrane of the tubule was thickened and hyalinized; there was an increase in peritubular connective tissue in immunized animals. Reduction in semiferous tubule diameter and histological changes in immunized ram lambs were reported previously (Kiyama *et al.*, 2000; Ülker *et al.*, 2001; 2005).

Ultrasonographic appearance of the testes of 19 wk old ram lambs happened to be homogeneously hypoechogenic. As the age increased, the testes gained its normal echogenic ultrasound appearance with a coarse medium echo-pattern at 29 wk age. Testes of control animals at slaughter were slightly hyperechoic (Table 4). Immunization affected ultrasonographic appearance of the testes drastically. Immunized animals had uniform hypoechogenic testicular structure as observed at 10 wk of age and this echogenicity did not increase until slaughter. Similar finding was reported previously (Ülker *et al.*, 2005). In fact, it was expected that there would be an increase in the echogenicity of the testis before immunization effect begins, but

apparently immunization effect took place shortly after immunization and therefore there was not an increase in echogenicity of the testis making unable the immunization effect on echogenicity regarding to immunization time to be determined.

Table 3. Seminiferous tubule diameter (micron) in control and immunized ram lambs

	19 wk age	29 wk age	41 wk age
Control	95.75	89.17	106.78
Immunization	94.81	71.25	44.53

Contrary to the ultrasonographic findings in pathological conditions (Gouletsu *et al.*, 2004) the testes of immunized animals generated homogenous echogenic pattern. Simultaneous histological and ultrasonographic evaluations indicated that the changes in testicular histology could partly be monitored via ultrasonographic imaging; nevertheless, it is difficult to claim that ultrasonographic image reflects the exact changes in such instances.

Table 4. Ultrasonographic appearance of the testes in control and immunized ram lambs

	19 wk age	29 wk age	41 wk age
Control	Hypoechoic	Moderately echoic	Hyperechoic
Immunization	Hypoechoic	Hypoechoic	Hypoechoic

#### Conclusion

In conclusion, these results indicate that recombinant ovalbumin-LHRH-7 fusion protein is effective in immunocastration in ram lambs and has a potential to be used as an alternative to physical castration. Testicular development and sperm production in immunized ram lambs were suppressed. Immunization affected testicular histology and ultrasonographic appearance of testis.

## UTICAJ IMUNIZACIJE PROTIV LHRH KORIŠĆENJEM REKOMBINANTNOG LHRH FUZIONOG PROTEINA, OVALBUMIN-LHRH-7, NA RAZVOJ, HISTOLOŠKI I ULTRAZVUČNI IZGLED TESTISA KOD MUŠKE JAGNJADI

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#### Rezime

Cilj ovog istraživanja je bio otkriti učinkovitost rekombinantnog ovalbumin-LHRH-7 (OL) fuzionog proteina, nove sterilizacije LHRH na reproduktivne osobine muške jagnjadi – mladih ovnova. Dvadeset domaćih/autohtonih mladih rase Karakaş u uzrastu od 19 nedelja je podeljeno u dve grupe – kontrolnu (C, n = 10) i grupu koja je imunizovana (I, n = 10). Životinje u kontrolnoj grupi nisu bile tretirane. Životinje u imunizovanoj grupi su tretirane protiv LHRH korišćenjem OL proteina koji stvara rekombinantna DNK tehnologija kao primarni i "booster" injekciju u uzrastu od 19 i 21 nedelje, respektivno. Mere obima mošnica su uzimane svake dve nedelje od svake životinje. Testisi su skenirani dva puta nedeljno kako bi se utvrdile promene u ultrazvučnom izgledu sa povećanjem starosti životinja. Testisi mladih ovnova u C (n=4) i I (n=4) grupama su biopsirani pod lokalnom anestezijom kako bi se dobilo testikularno tkivo za histologiju testisa u uzrastu od 19 i 29 nedelja. U uzrastu od 41 nedelje životinje su zaklane. Biopsirano testikularno tkivo uzeto u 19, 29 i 41 nedelji starosti je histološki ocenjivano. Nakon klanja epididimus je ocenjivan u pogledu prisustva spermatozoida.

Obim mošnica u kontrolnoj grupi se postepeno povećavao sa uzrastom dok je skoro ostao isti kao na početku oglada kod imunizovanih životinja. Prečnik cevčice u kojoj se formira sperma se povećavao kod kontrolnih životinja dok se kod imunizovanih smanjivao. Zreli spermatozoidi su nađeni u ejakulatu

sakupljenom u uzrastu od 37 nedelja ili epididimusu nakon klanja (41 nedelja) kod životinja kontrolne grupe, dok kod životinja iz imunizovane grupe nisu nađeni spermatozoidi. Bazalna membrane cevčice je bila zadebljana, nije bilo povećanja peritubularnog konektivnog tkiva kod imunizovanih životinja. Ultrazvučni izgled testisa kod ovnova u uzrastu od 19 nedelja je bio homogeno hipoekoičan. Sa povećanjem uzrasta testisi su dobili normalni ekogenični izgled sa grubim srednjim eco-obrascima u uzrastu od 29 nedelja. Testisi kontrolnih životinja nakon klanja su bili neznatno hiperekoični. Imunizacija je uticala na ultrazvučni izgled testisa drastično. Imunizovane životinje su imale uniformnu hipoekogeničnu strukturu testisa kao što je registrovano u uzrastu od 10 nedelja do uzrasta pri klanju.

Kao zaključak, može se reći da ovi rezultati pokazuju da je rekombinantni ovalbumin-LHRH-7 fuzioni protein efikasan u imunokastraciji mladih ovnova ima potencijal za upotrebu kao alternative fizičkoj kastraciji. Testikularni razvoj proizvodnja sperme kod imunizovanih ovnova je bila potisnuta. Imunizacija je uticala na testikularnu histologiju i ultrazvučni izgled testisa.

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