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IMMUNIZATION AGAIST LHRH IN EWES FOR SUPPRESSING REPRODUCTIVE FUNCTIONS AND POSSIBILITIES OF USING PMSG OR LHRH IN RESTORING REPRODUCTIVE FUNCTIONS IN LHRH IMMUNIZED EWES[1]

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Abstract: In this study, the purposes were to determine the effectiveness of recombinant LHRH fusion proteins (Ovalbumin-LHRH-7 and Thioredoxin-LHRH-7) in suppressing reproductive functions in ewes, and investigate the possibilities of using PMSG or LHRH in restoring reproductive functions in LHRH immunized ewes. Thirtythree nulliparous Kıvırcık ewes were randomly assigned into control (n=11) and immunization (n=22) groups. Ewes immunized against LHRH by injecting with a cocktail of ovalbumin-LHRH-7 and thioredoxin-LHRH-7 fusion proteins generated by recombinant DNA technology. PMSG or LHRH analogue was used to induce ovulations. No estrus activities observed in immunized ewes. Two more attempts were failed to induce ovulation in this group as well. Serum LHRH antibodies were present in animals of the immunized group beginning the second week after the first immunization and maintained thorough out study. These results indicate that recombinant LHRH fusion proteins are effective in immunocastration in ewes. Additionally, this immunization generated a permanent like immunocastration effect as observed that neither PMSG nor LHRH injection was able to restore reproductive function in immunized animals and immunization effect lasted more than a year.

Keywords: LHRH, fusion proteins, immunization, ewes

Introduction

Immunization against luteinizing hormone releasing hormone (LHRH) has been described as one of the effective means to reduce reproductive functions in farm animals and a possible alternative to surgical castration. (*Reeves et al., 1989; Bonneau and Enright, 1995; Thompson, 2000*). Two recombinant fusion proteins, ovalbumin-LHRH-7 and thioredoxin-LHRH-7, were developed to be used as a sterilization vaccine (*Zhang et al., 1999; Quesnell et al., 2000*). The effectiveness of these recombinant proteins in suppressing reproductive functions was demonstrated in heifers (*Sosa et al., 2000)*, bulls (*Aissat et al, 2002*) and ram lambs (*Ülker et al., 2001; 2005*). The effectiveness of these recombinant proteins in suppressing reproductive functions in ewes has not been studied.

Many researchers report that active immunization against LHRH induces only a temporary suppression of reproductive functions after which animals return to normal fertility (*Reeves et al., 1989; D'Occhio, 1993; Bonneau and Enright, 1995; Thompson, 2000)*. Alternatively, reproductive functions in LHRH immunized animals could be restored by using LHRH agonist (*Adams and Adams, 1986, Herman and Adams, 1990, Sakurai et al., 1992*) or PMSG (*Oatley et al., 2005*), nevertheless, single injection of LHRH to restore reproductive functions after active immunization done in early life failed to do so (*Brown et al., 1994; 1995; Clarke et al., 1998*). Immunizing farm animals against LHRH allows animal owners keep male and female animals together as long as immunization effect lasts, i.e, higher levels of anti-LHRH antibodies to neutralize LHRH are present. Restoring reproductive functions in immunized animals without waiting the decline in circulating anti-LHRH antibodies below a threshold required to neutralize LHRH would be management easiness.

The purposes of this study were, 1. to determine the effectiveness of recombinant LHRH fusion proteins in suppressing reproductive functions in ewes, and, 2. investigate the possibilities of using PMSG or LHRH analogue in restoring reproductive functions in LHRH immunized ewes.

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Material and Methods

33 nulliparous Kivircik ewes at 3-8 age and weighed average 39.48 kg were randomly assigned into control (n=11) and immunization (n=22) groups. Animals in control group were not treated. Ewes immunized against LHRH by injecting with a cocktail of ovalbumin-LHRH-7 and thioredoxin-LHRH-7 proteins generated by recombinant DNA technology (*Zhang et al., 1999; Quesnell et al., 2000*). Immunization schedule was initiated to obtain high antibody levels at proposed mating time. First immunization was followed by a booster immunization 1 month later. 95 days after the first immunization all animals were subjected to a typical estrus synchronization program: Progestagen impregnated sponges were inserted for 14 days. At sponge withdrawal control animals and 11 animals in immunization group were injected with 400 IU PMSG. 11 animals in immunization group were injected with 0.9 ml/animal LHRH agonist. Two days after PMSG or LHRH injections rams were introduced. Behavioral estrus and mating activities were observed. Since no estrus activities observed in immunized ewes 10 days after sponge removal all animals in this group were injected with 400 IU PMSG to induce ovulation. All animals were examined for pregnancy using 6 MHz linear rectal probe 35 days after sponge withdrawal. Since no animals were pregnant in immunization group a 'long term ovarian stimulating program with, multiple PMSG injections' was initiated. For this purpose 14 animals in immunization group were injected with 400 IU PMSG once and then 280 IU PMSG three times at six days intervals and once three days after forth one. Progestagen containing sponges were inserted at the third PMSG injection and removed at the last injection. At sponge removal fertile rams were introduced and estrus behaviors were monitored. Rams remained with ewes all time. A radioactive binding assay was used to evaluate the percentage of ^{125}I-LHRH that would bind to the anti-LHRH antibody present in the serum at a 1:1.00 dilution. None of the ewes in this group were pregnant even though they were kept with fertile rams more than two months in which breeding season ended. Furthermore, these animals were kept with fertile rams during the breeding season next year but they did not get pregnant.

Results and Discussion

All ewes in control group showed estrus behavior 1-2 days after sponge removal and were mated with fertile rams. None of the ewes in immunization group exhibited estrus behavior after sponge withdrawal. All ewes in control group were diagnosed as pregnant at 35 days after sponge withdrawal and lambed in term (Table 1). These results indicated that recombinant fusion proteins are effective in suppressing reproductive functions in ewes and a single dose of PMSG or LHRH is not effective in restoration of reproductive functions in immunized ewes using these proteins.

Table 1. Reproductive traits in control and immunized ewes after a typical estrus synchronization program.

	Ewes exhibited estrus behavior	Pregnant ewes at 35 days	Lambed ewes	Lamb numbers per ewe
Control + PMSG (n=11)	11	11	11	1.3
Immunization + PMSG (n=11)	0	0	0	0
Immunization + LHRH (n=11)	0	0	0	0

Several studies related to restoration of pituitary and ovarian functions via hormone treatment after immunocastration have been reported (Adams and Adams, 1986; Herman and Adams, 1990; Sakurai *et al*., 1992). In the present study neither PMSG nor LHRH injections combined with FGA treatment were able to induce estrus in immunized (anestrus) ewes (Table 1 and 2). Brown *et al*., (1995) reported that LHRH injections to the non-cyclic immunized ewes in early ages did not cause a marked increase in LH concentrations. Nevertheless, in 35% of sexually mature bulls immunized against LHRH the testes continued to decrease in size for 4 months and did not show any re-initiation of growth for 1 year after immunization (D'Occhio *et al*., 2001). It was concluded, therefore, that active immunization against LHRH could induce a long-term, possibly permanent, suppression of reproductive function in bulls. Thus, a permanent castration like effect could be speculated for protein combination used in the present study as well.

Table 2. Reproductive traits in immunized ewes subjected to single or multiple PMSG injections.

	Treatment			
	Single PMSG injection[1]		Multiple PMSG injection + FGA treatment[2]	
	Ewes exhibited estrus behavior	Pregnant ewes	Ewes exhibited estrus behavior	Pregnant ewes
Immunized ewes (n=14)	0	0	1	0

[1] 400 IU PMSG injection 10 days after the first synchronization attempt

[2] Total five PMSG injections (400 IU PMSG once and then three times 280 IU PMSG at six days intervals, and one 280 IU PMSG three days after forth one)

The mechanism for sustained suppression of reproductive functions after immunization against LHRH is poorly understood. Preliminary findings in sheep and swine suggest that basal hypothalamus-median eminence is a primary target site for anti-LHRH antibodies, and immunization may lead to a disruption of the integrity of basal hypothalamus-median eminence (Molenaar *et al*., 1993; Clarke *et al*., 1998). Often time in such immunizations LHRH Ab concentrations decrease by the time depending on the antigenicity of the immunogen. In the present study 14 months after the first immunization a considerable high LHRH concentrations were observed Table 3). Nevertheless, it is still not clear whether the high Ab concentrations or a disruption in the hypothalamus is the main cause for not restoring reproductive functions in immunized ewes.

Table 3: Percentage LHRH Ab binding in control and immunized ewes

	Months							
	0	2	4	6	8	10	12	14
Control	0.21	0.70	0.58	0.18	NA	NA	NA	NA
Immunization	0.37	40.94	51.16	47.98	50.32	44.12	46.90	43.89

NA: Not Assayed

Conclusion

Data showed that recombinant LHRH fusion proteins (Ovalbumin-LHRH-7 and Thioredoxin-LHRH-7) were effective in suppressing reproductive functions in ewes. It seemed that this immunization led to a permanent disruption of the integrity of basal hypothalamus-median eminence causing this immunization be irreversible.

IMUNIZACIJA PROTIV LHRH KOD OVACA RADI SUZBIJANJA REPRODUKTIVNIH FUNKCIJA I MOGUĆNOST KORIŠĆENJA PMSG ILI LHRH U USPOSTAVLJANJU REPRODUKTIVNIH FUNKCIJA KOD LHRH IMUNIZOVANIH OVACA

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Rezime

U ovom istraživanju cilj je bio određivanje efikasnosti rekombinantnih LHRH fuzionih proteina (Ovalbumin-LHRH-7 i Thioredoxin-LHRH-7) u suzbijanju reproduktivnih funkcija kod ovaca i ispitivanje mogućnosti korišćenja PMSG ili LHRH u uspostavljanju reproduktivnih funkcija kod LHRH imunizovanih ovaca. Tridesetri ovce rase Kıvırcık koje se nisu jagnjile su metodom slučajnog odabira podeljene u kontrolnu (n=11) i imunizacionu (n=22) grupu. Životinje u kontrolnoj grupi nisu tretirane. Ovce koje su imunizovane protiv LHRH I to pomoću koktel injekcije ovalbumin-LHRH-7 i thioredoxin-LHRH-7 proteina stvorenih pomoću tehnologije rekombinantne DNK. Imunizacioni program je započeo 111 dana pre predloženog vremena/termina parenja. Ekvimolarne količine svakok LHRH fuzionog proteina su korišćene za prvu i

"booster" injekciju koja je data mesec dana nakon prve imunizacije. Devedesetpet dana nakon prve imunizacije sve životinje su podvrgnute tipičnom programu sinhronizacije estrusa: sunđeri natopljeni Progestagenom su unošeni tokom 14 dana. Pri vađenju sunđera kontrolne životinje I 11 životinja iz imuniyovane grupe su odbile injekciju sa 400 IU PMSG. Preostalih 11 životinja iz imuniyacuine grupe je dobilo injekciju sa 0.9 ml/životinji LHRH analogno. Pošto nikakvi znaci estrusa nisu primećeni kod imunizovanih ovaca 10 dana nakon uklanjanja sunđera sve životinje iz ove grupe su primile injekciju sa 400 IU PMSG kako bi se izazvala ovulacija. Ova injekcija sa PMSG nije mogla da izazove estrus kod imunizovanih ovaca. Sve ovce u kontrolnoj grupi su bile trudne 35 dana nakon uklanjanja sunđera iI ojagnjile se u terminu. Pošto među oglednim životinjama nije bilo steonih započet je program stimulisanja jajnika pomoću višestrukih PMSG injekcija. Nije primećen estrus kod ovaca iz imuniyacione grupe, osim kod jedne na kraju programa stimulacije jajnika sa višestrukim injekcijama PMSG. Nijedna ovca iz ove grupe nije bila trudan iako su držane zajedno sa plodnim ovovima više od dva meseca do kraja sezone parenja. Takođe, ove životinje su držane sa plodnim ovnovima tokom sezone parenja I naredne godine ali nisu ostajale trudne. Ovi rezultati ukazuju da rekombinantni LHRL fuzioni proteini predstavlaju efikasno sredstvo u imunokastraciji ovaca. Takođe, ovakva imunizacija je stvorila efekat imunokastracije sličan permanentnom jer ni sa PMSG ni LHRH injekcijama nije bilo moguće ponovno uspostavljanje reproduktvinih funkcija kod imunizoavnih životinja. Efekat imunizacije je trajao više od godinu dana. Izgleda da je imunizacija dovela do permanentnog poremećaja integriteta bazalne hipotalamus-median eminencije izazivajući iriverzibilnost ovakve imunizacije. Uticaj doze takođe ima značaj na dobijene rezultate. Postoji mogućnost da niža doza navedenih proteina može izazvati samo kratkotrajnu imunoneutralizaciju LHRH preko LHRH anti tela I na taj način omogući naknadno uspostavljanje reproduktivnih funkcija.

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