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STUDIES ON THE INFLUENCE OF IMMUNIZATION AGAINST HUMAN CHORIONIC GONADOTROPHIN (hCG) ON REPRODUCTIVE FUNCTION OF BARKI RAMS

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SUMMARY

Active immunization against hormones is considered one of the best methods to investigate their effects. Six Barki rams were immunized against LH hormone (hCG, Pregnyl- Organon) which was emulsified with complete Fruend's adjuvant. Reproductive function of rams, testicular measurements, Semen parameters and testosterone level, were estimated before and for eight weeks after immunization. The results revealed that there is no significant effect on semen volume, minor and major sperm abnormalities. On the other hand, Duncan's Multiple Range test revealed differences in mass motility individual motility, sperm concentration and live dead ratio. It was found that there was a gradual decrease in sperm concentration, live /sperm percent after immunization while total testicular circumference decreased gradually from the 1st to 5th week after immunization and then returned back to the normal circumferences during the 8th week after immunization.

INTRODUCTION

Immunization and antibodies have been raised against gonadotrophic hormones for various purposes including use in assays, investigations of the molecular structure of hormones and suppression of the biological activity. Active immunization against gonadotrophins has also been considered of potential value in investigating their effects in both female and male animals (Hassanin, 1990).

In male animals, LH and FSH play an improtant role in controlling testicular functions (Chowen et

al., 1989). Chronic treatment with GnRH, which is a potent releaser of FSH and LH from the pituitary, results in reduced production of testosterone and disrupted spermatogenesis (Vale et al., 1977 and Sandow et al., 1980). Repeated immunization of adult male and female animals against GnRH has been reported to prevent the production of goandotrophins which resulted in cessation of reproductive function (Jeffcoate et al., 1982. Robertson et al., 1982 and Schanbacher, 1982).

It is well established that administration of LH and hCG increased testosterone value in plasma and interstitial fluid of hypophysectomized animals to values similar to those of intact animals (Valence and Negro-Vilar, 1986). Although LH-RH has been used successfully as antigen for the induction of immunocastration, the efficiency of LH and/or FSH for this purpose is less clear (Arimura et al., 1973; Keeling and Crighton, 1984). Rams actively immunized against GnRH were used as a hypogonadotropic model for studies of significance of the pulsatility of LH secretion in determining the trophic actions of the hormones on testicular steroidogenesis (Chase et al., 1988). Thus, the aim of the present study was to investigate the effects of ram immunization against LH (hCG) on testicular dimensions (Biometry), some semen parameters and testosterone level.

MATERIAL AND METHODS

Experimental animals:

Six sexually mature Barki rams, about 3 years of age, were used in this study. The rams were clinically normal and kept on the standard system of management in the experimental farm of Animal Reproduction Research Institute. They were immunized once weekly for 4 weeks against LH hormone. The injected dose was 1250 IU (0.5ml) of hCG (Pregynl-Organon) which was emulsified in an equal amount (0.5 ml) of complete Freand's adjuvent (Behring). The emulsified antigen was injected intradermally at several site along the dorsal midline of the rams.

Experimental procedure:

Testicular measurements:

Testicular dimensions (length, width and thickness) were measured using a pair of calipers. Along the course of study, the total circumference of both testes were determined using a flexible plastic tape.

Semen collection and evaluation:

Semen samples were collected from each ram twice weekly, 4 weeks before and for 8 weeks after immunization by means of the artificial vagina. Immediately after collection, the ejaculates were transferred to the laboratory and were placed

in a warm incubator at 37°C. Ejaculate volume, sperm mass motility, individual motility, live sperm percent, sperm concentration (x 10^7), minor and major abnormalities were examined.

Blood samples:

Blood samples were collected from the jugular vein using vacutainer tubes, two weeks before immunization as a control samples and then weekly for 8 weeks after immunization. Sera were separated and kept frozen at -20°C till testosterone assay.

Testosterone assay:

Testosterone levels were assayed in the serum using RadioImmuno Assay according to Kogan and Glick (1982).

Potency of antisera:

The potency of the antisera was determined after the end of immunization by their addition in serial dilution in WHO plates for microagglutination technique (Hassanin, 1990). The antisera to be tested were previously incubated with tanned sheep cells for one hour at room temperature to remove non-specific agglutinins. These sera were diluted successively by transferring 0.025 ml PBS (pH 7.2) to the wells, after mixing, a drop of PBS was added. An additional drop of the prepared suspension of sheep red cells sensitized with the hormone was added to each well. The plates were covered by adhesive tape to prevent evaporation. The plates were left for three hours. When the red cells agglutinated a uniform mat covering the entire bottom of the well was formed. At the point where agglutination did not take place, the red cells formed a closed button in the center of the well. The end point was taken as the average titre of the succesive two wells showing beginning of inhibition (open ring) and inhibition (closed ring). This average indicated the potency of antisera to agglutinate the specific sensitized sheep red cells. The minimal dilution of the selected rabbit antiserum leading to agglutination of the specific sensitized sheep red cells were recorded. Moreover, agar gel precipitation test was used to test the collected antisera and to determine the disappearance time of antibodies after end of the immunization course.

Statistical analysis:

Data obtained from the experiment were subjected to statistical analysis using Duncan's Multiple Range test, Costat computer program Version 3.03 copyright 1986. Cotlert Software.

RESULTS

Effect of immunization on testicular measurements:

Immunization of rams against LH hormone (hCG) did not reveal any significant difference in testicular measurement (length, width, thickness and circumference). However, the total testicular circumference decreased gradually from 1st to 5th week post-immunization then returned back' gradually to its normal size at 8th week after th end of immunization ocurse (Table 1).

Effect of immunization on some semen parameters:

Effects of immunization against hCG are summarized in Table 2. It is clear that semen volume, minor and major sperm abnormalities were not signifiantly affected by immunization. On the other hand, mass motility, individual motility, live sperm percent decreased significantly after immunization and returned back to normal values on the 7th week after the end of immunization course. Sperm concentration decreased gradully but significantly from th 1st week through the 8th week after immunization.

Immunological evaluation of antisera:

a- Agar-gel precipitaiton test:

Immunization of rams against LH resulted in the development of Ag-Ab precipitation lines in the Agar-gel. The precipitation lines were observed only in case of using the antisera collected after 1st , 2nd and 3rd weeks post-immunization while no precipitaiton was noticed on using the antisera collected after 4th week indicating disappearance of antibodies.

b- Immunoassay:

The minimal dilution of antisera prepared against LH (during the 1st week after immunization) leading to agglutination of the specific sheep red cells sensitized with LH was 1/4640.

Testesterone assay:

The concentrations of testosterone were estimated before and after immunization against LH (Fig. 1). A significant decrease in the testosterone level occurred during the 1st 5 weeks post-immunization followed by a gradual increase to the normal level at the 8th week.

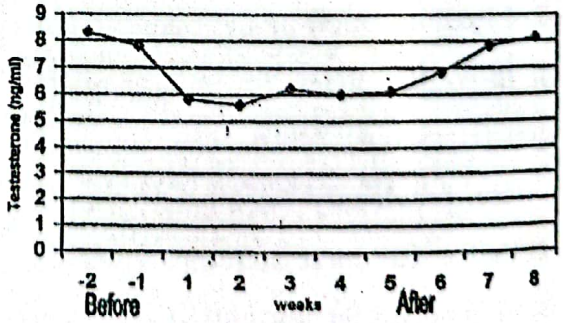

Fig. 1: Testosterone levels before and after immunization against LH.

Table 1: Effect of ram immunization against LH hormone on testicular measurement (cm, Means +SD).

Time of immunization	Right testis			Left testis			Total
	Length	Width	Thickness	Length	Width	Thickness	Circumference
Pre-immunization	8.43±1.87	5.48±1.36	4.68±1.61	9.05±1.99	5.78±0.76	5.65±0.31^{bc}	30.0±3.24^{a}
Post-immunization							
Week: 1	8.43±1.31	5.05±0.82	4.60±1.13	9.10±0.92	5.08±0.87	4.75±0.65^{a}	27.50±1.29^{ab}
2	7.80±2.00	4.47±0.94	4.57±0.95	9.10±1.21	5.18±0.88	4.88±0.82	27.00±1.41^{ab}
3	8.13±2.31	4.38±0.30	4.10±1.07	8.58±1.11	5.30±0.83	5.00±0.73	26.88±2.02^{b}
4	7.95±1.58	4.45±1.07	4.65±0.87	8.38±1.51	5.75±0.87	5.33±1.60	26.38±1.89^{b}
5	7.70±0.70	4.73±1.12	5.03±0.51	8.00±0.87	5.70±0.70	5.60±1.83	27.00±1.00^{b}
6	8.13±0.32	5.03±0.96	5.10±0.50	8.33±0.76	5.83±0.76	6.43±0.75	27.33±0.58^{ab}
7	8.27±0.80	5.26±0.81	5.10±0.50	8.40±0.75	5.83±0.58	7.27±0.55	27.67±0.58^{ab}
8		5.43±6.86	5.27±0.50	8.30±0.70	5.77±0.55	7.17±0.55	27.77±0.25^{ab}

Means with different superscripts within columns are significantly different at P<0.05.

Table 2: Effect of ram immunization against LH hormone on some semen parameters (Means +SD).

Time of immunization	Semen volume	Motility		Sperm concentration ($x10^7$)	Live sperm (%)	Abnormalities %	
		Mass (+)	Individual			Major	Minor
Pre- immunization	0.88±0.25	4.13±0.46^{ab}	86.25±2.50^{a}	278.0±32.92^{a}	86.50±2.38^{a}	4.50±0.90	3.50±0.57
Post-immunization							
Week: 1	0.68±0.24	3.87±0.47^{ab}	83.75±2.50^{ab}	263.5±25.57^{ab}	86.00±1.41^{ab}	5.00±0.10	3.75±0.26
2	0.81±0.24	3.36±1.18^{ab}	77.50±3.23^{ab}	204.0±36.59^{bc}	86.25±2.75^{ab}	5.75±0.50	4.75±0.50
3	0.88±0.14	4.13±0.25^{ab}	78.75±2.50^{ab}	160.5±31.39^{cd}	85.50±3.00^{abc}	4.75±0.50	4.50±0.65
4	0.69±0.13	3.75±0.65^{ab}	80.00±4.10^{ab}	130.3±20.54^{d}	80.50±7.05^{c}	4.50±0.26	4.50±0.29
5	0.73±0.25	3.50±0.01^{b}	81.67±2.89^{ab}	118.0±14.11^{d}	87.00±2.00^{ab}	4.67±0.15	4.33±0.58
6	1.06±0.46	4.00±1.00^{ab}	81.67±2.89^{ab}	114.0±31.32^{d}	87.00±2.00^{ab}	4.33±0.15	4.33±1.86
7	0.92±0.52	4.50±0.10^{ab}	86.67±2.29^{a}	93.60±37.74^{d}	88.33±1.53^{a}	5.33±0.05	4.00±1.46
8	0.83±0.38	4.67±0.28^{a}	86.33±4.04^{a}	93.03±26.87^{d}	88.33±2.89^{a}	4.67±0.53	3.67±0.57

Means with different superscripts within columns are significantly different at P<0.05.

DISCUSSION

Immunization of adult animals against GnRH appears to produce only temporary suppression of gonadal function and sexual behavior as reproductive function usually resumes fairly rapidly after decline of antibody titre without any apparent detrimental effects occurring (Robertson et al., 1982 and Keeling and Crighton, 1984). The temporary suppression of gonadal function appeared to be due to reduction of LH release. In the present study immunization of rams against LH hormone (hCG) resulted in a significant decrease in total testicular circumference which decreased gradually from 1st to 5th week of immunization and returned back to the normal on the 8th week of immunizaiton. This result is coinciding with that of Brown et al., (1994), who found that GnRH immunization of rams is rapidly reversible.

Our study indicated that there was a significant decrease in the testosterone level during the 1st 5 weeks post-immunization. Testosterone began to rise again afterward to the normal levels. Endogenous LHRH action was not required for increased steroidogenic activity in bulls treated with LHRH agonist. However, circulating LH was necessary for increasing plasma testosterone in bulls implanted with deslorelin (Aspden et al., 1997). According to Htzel et al., 1997, the concentration of LH, FSH and testosterone were significantly lower in rams immunized against GnRH than in rams immunized against BSA.

Total testicular circumference was significantly affected by immunization which decreased gradually from 1st to 5th week post-immunization then returned back gradually after immunization to its normal size on the 8th week after the end of immunization. Tilbrook et al. (1993) found that administration of GnRH against to prepubertal rams for 16 weeks immediately prior to puberty inhibited the development of sexual behavior, reduced plasma concentrations of testosterone, retarded testicular and epididymal development. The effects on sexual behavior were clearly reversible in treated rams 8 weeks after the end of treatment. These results indicated that the reproductive function of rams is sensitive to gonadotropins and testicular hormones immediately prior to puberty.

GnRH immunized rams had suppressed reproductive function and hormones, where, after peripubertal immunization, the mass of testes declined and remained regressed (Brown et al., 1994). Chase et al., (1988) reported that high frequency, low amplitude pattern of LH secretion characteristic of reproductively active animals has trophic actions on the testes, increasing their responsiveness to gonadotropic stimulation.

Concerning semen parameters, there was no significant effect on semen volume, minor and major sperm abnormalities. On the other hand, there was significant decrease in sperm mass activity, individual motility, sperm concentration, and live sperm percent. This result could be explained by

Mclachlan et al. (1994) who reported that GnRh immunization suppress the release of LH which disturbs spermatogenesis. Brown et al., (1994) reported that in rams immunized against GnRH, semen did not differ neither in the percent of live sperm nor in the sperm abnormalities. It has been shown that the suppressive effects of Anti-GnRH immunizaiton of adult sheep and cattle (Keeling and Crighton, 1984) on reproductive function is reversible with time.

REFERENCES

Arimura, A.; Sato, H.; Hkumasoka, T.;Worobec, R.; Debeljuk, L.; Dunn, J. and Shally, A. (1973): Production of antisera to LH-RH associated with gonadal atrophy in rabbits. Endocrinology 93 : 1092-1103.

Aspden, W.J; Van Reenen, N; Whyte, T. R; Maclellan, L. J; Scott, P. T.; Trigg, T. E; Walsh, J. and Docchio, M. J. (1997): Increased testosterone secretion in bulls treated with a luteinizating hormone releasing hormone (LHRH) agonist requres endogenous LH but not LHRH. Domest. Anim. Endocrinol., 1997, 14:6, 421-8.

Brown, B. W.; Mattner, P. E.; Carroll, P. A.; Holland, E. J.; Paull, D. R.; Hoskinson, R. M. and Rigby, R. D. (1994): Immunization of sheep against GnRH early in life: effects on reproductive function and hormones in rams. J. Reprod. Fertil. 101: 1, 15-21.

Chase D. J.; Schanbacher B. D. and Lunstra D. D. (1988): Effects of pulsatile and continous luteinzing hormone (LH) infusions on testosterone response to LH rams actively immunized against gonadotropin-releasing hormone. Endocrinol. 123: 2, 876-26.

Chowen, J.; Fraser, H.; Vician, L.; Damasse,D.; Clifton, D. and Steinerm, R. (1989): Testosterone regulation of Pro-opimelanocortin messenger Ribonuclic acid in the arcuate nucleus of the male rat. Endocrinology 124 :1697-1702.

Hassanin, K.D. (1990): Immunological studies on pituitary gonadotrophins. M.V.Sc. thesis (Physiology), Faculty of Vet. Med.- Cairo University.

Htzel M. J.; Caraty, A. and Martin, G. B. (1997): Effects of nutrition on testicular growth in mature Merino rams actively immunized against GnRH. J. Reprod. Fertil. 110: 2, 307-13.

Jeffcoate, I.; Lucas, J. and Crighton, D. (1982): Effect of active immunization of ram lambs and bull calves against synthetic LHRH. Theriogenology 18 :65-77.

Keeling, B.J. and Crighton, D.B. (1984): Reversibility of the effects of active Immunization against LHRH. In immunological Aspects of Reproduction in Mammals. Ed. D.B. Crighton, Butterworth-London.

Kogan, A. and Glick, S. (1982): Methods of hormone radioimmunoassay Eds.B. M. Jaffe.

McLachlan, R. I.; Wreford, N. G; Tsonis, C, De Kretser, D. M and Robertson, D. M. (1994): Testosterone effects on spermatogenesis in the gonadotropin-releasing hormone-immunized rat. Biol. Reprod. 50: 2, 271-80.

Robertson, I.; Fraser, H.; Innes,G. and Jones,A. (1982): Efect of immunological castration on sexual and production characteristics in male cattle. Veterinary Record 111 :529-531.

Sandow, J.; Rechenberg, W.; Jerjabek, G.; Engelberg, K.; Khul, II. and Fraser, H. (1980): Hypothalamic pituitary-testicular functions in rats after supraphysiological doses of a highly active LHRHa (buserelin). Acta Endocrinol.(Copenh.), 94 :489-495.

Schanbacher, B.D. (1982): Responses of ram lambs to active immunization against testesterone and LHRH). American J of Phsiology, 242 :201-205.

Tilbrook, A.J.; Galloway, D.B.; Williams, A. H. and Clarke, I.J. (1993): Treatment of young rams with an agonist of GnRH delays reproductive development. Horm. Behav. 27:1, 5-28.

Vale, W. Rivier, C.; Rivier, J. and Brown, M. (1977): Hypothalamic regulatory peptides. In Mathien J. (ed.) Medicinal Chemistry, Vol. 5- Elsevier Amsterdam.

Valence, M. and Negro-Vilar A. (1986): Proopimelanocortin derived in testicular interstitial fluid: Characterization and changes in secretion after hCG or LHRH analogue treatment. Endocrinology 118 :32-37.