

The effect of active immunization with gonadotropin releasing hormone conjugate (GnRH-BSA) on gonadosomatic indices (GSI) and sperm parameters in mice

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Abstract:

Background: Gonadotropin releasing hormone conjugate (GnRH-BSA) raises antibodies against biologically active gonadotropin releasing hormone (GnRH) which affects body weight, gonadosomatic indices (GSI) and sperm quality in male mice.

Objective: The objective of this experimental investigation is to develop an effective and reliable hormonal immunocontraceptive vaccine to suppress spermatogenesis by using GnRH-BSA conjugate.

Materials and Methods: Forty sexually mature mice, *Mus musculus* were divided into two groups of twenty each. Group 1, served as control, while group 2 were immunized at monthly intervals for four times against GnRH with a GnRH-BSA conjugate (50 µg) with aqueous adjuvant (Freund's adjuvant). After 30 days of each immunization, body weight, GSI and sperm quality were observed in the immunized groups and compared with the control group.

Results: Body weight showed alterations in immunized animals as compared to control. However, GSI, sperm motility, sperm count and sperm morphology were significantly decreased in immunized animals throughout the experimental investigation and these effects were more prominent and significant in the later part of the experiment.

Conclusion: These results suggested that the active immunization against GnRH produced bioeffective antibodies as indicated by significant reduction in GSI level and sperm quality and induced infertility in male mice.

Key words: GnRH, Active immunization, Sperm quality, GSI, Infertility, *Mus musculus*.

Introduction

Gonadotropin releasing hormone (GnRH) is produced and secreted from the hypothalamus. This hypothalamic peptide hormone controls the synthesis and secretion of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) from anterior pituitary, which in turn regulates the synthesis and secretion of sex steroid hormones (testosterone in males and estradiol and progesterone in females) and thus controls the whole reproductive function (1).

A technique has been developed to cause a mammal to generate antibodies against its own hormone i.e., GnRH. In general the technique involves the injection of a synthetic GnRH conjugated to a non-native protein (Bovine Serum Albumin, Human Serum Albumin, Tetanus toxoid etc.). The conjugated GnRH when enters the circulation, raises the antibodies against it. Antibodies to the hypothalamic hormone reduce the circulating level of biologically active GnRH, thereby reducing the level of subsequent reproductive hormones and inducing the infertility. Active immunization of various mammals to GnRH, has been shown to lead in the case of males to testicular regression, reduction of testosterone secretion and cessation of spermatogenesis and in the case of females to loss of cycling and ovarian

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regression (2-5). A novel GnRH analogue conjugated to mycobacterium hsp70 has been reported to elicit a relatively higher level of antibodies that neutralize the activity of native GnRH (6). Immunization against GnRH may also be useful for treating sex steroid-dependent abnormal growth of mammary glands and prostate (7). Recently a recombinant anti-GnRH vaccine has been designed with relatively higher immunogenicity for controlling the fertility (8). GnRH vaccine has been employed with good results for control of wild animal populations (9-10). In connection to this, present experimental investigation is aimed to evaluate the infertility effects induced through GnRH immunization in male mice.

Materials and methods

For the present study, forty adult male mice, *Mus musculus* (P) weighing 30 ± 5 gms were used. The animals were divided into two groups of twenty each. Group-1 served as control, while group 2, were immunized with 50 µg GnRH-BSA conjugate (Sigma Aldrich) dissolved in 100 µl phosphate buffered solution (0.01N) and emulsified with an equal volume i.e., 100 µl Freund's adjuvant for 30, 60, 90, and 120 days respectively. After above durations, five animals were weighed and then sacrificed and their organs testes and epididymides were dissected out quickly. Testes were weighed for observing gonadosomatic indices, while epididymides were cut to release sperms in normal saline (100mg tissue/ 2ml) for sperm suspension. For sperm motility, a drop of sperm suspension was placed on the Neubauer's chamber which was focused on the four squares (WBC squares) and the motile spermatozoa were counted along with the total number of spermatozoa in each small sub square. A total of minimum 10-12 separate fields were scored and the percent motility was calculated. For sperm count (millions/ml), sperm suspension was sucked upto 0.5 mark in the WBC pipette and then diluted upto the 11 mark with 5% sodium bicarbonate solution. A drop of this fluid was then

placed on the Neubauer's chamber and the total number of spermatozoa was counted in 64 small WBC squares with the help of microscope. While, sperm count (millions/ml) was done by adding sodium bicarbonate (spermicide) to a part of sperm suspension and counted on Neubauer's chamber under microscope. For sperm morphology, one to two drops of sperm suspension were put on a glass slide, spread with other slide to form a smear, air dried and finally stained with Leishman's stain and observed under microscope at a magnification of 400X for observing normal and abnormal sperm morphological forms (11). Results of the experiments were expressed as mean and standard error of different groups.

Statistical analysis

The statistical analysis in between control Vs experimental groups were done by Student's 't' test. The values for $p < 0.01$ were considered significant while the values $p < 0.001$ were considered as highly significant.

Results

The body weight and GSI levels were lowered significantly in the later part of the experiment after GnRH-BSA immunizations when compared to control group (Table I). Besides this, the sperm motility, sperm count and sperm morphology were also significantly decreased in the immunized animals throughout the experiment and the effects were more prominent in later part of the experiment i.e., 90 and 120 days (Table II). However, morphological studies denote that more than 70 percent of the sperms were abnormal in shape characterized by pin head, large head, tapering head, oval head, small head, headless double head, bend neck, looped mid piece, coiled tail and double tail sperms in GnRH - BSA immunized animals of all groups i.e., 30, 60, 90 and 120 days when we compared with control groups (figures 1-5). The percentage of morphological abnormalities was more prominent and highly significant in the later part of the experiment i.e., 90 and 120 days.

Table I. Body weight and Gonadosomatic Indices (GSI) in GnRH-BSA immunized and control male mice.

Duration (Days)	Body weight (gms)		GSI (gm/100gm b.w.)	
	Control	GnRH-BSA immunized	Control	GnRH-BSA immunized
0	23.00±0.88	25.00±1.19		
30	26.80±1.03	28.22±1.55	0.40 ± 0.02	0.32 ± 0.06
60	31.00±0.57	29.42±0.79	0.43 ± 0.05	0.23 ± 0.02 ***
90	36.20±1.55	26.81±0.96 ***	0.50 ± 0.01	0.16 ± 0.00 ***
120	40.80±1.51	27.00±1.41 ***	0.54 ± 0.04	0.09 ± 0.00 ***

± SEM of five animals.

*** = Highly significant ($p < 0.001$) from the control Vs experimental group by Student's 't' test.

Table II. Sperm motility and sperm count in GnRH-BSA immunized and control mice.

Duration		30 days	60 days	90 days	120 days
Sperm motility (%)	Control	49.20±1.06	53.76±1.77	59.05±2.31	62.60±1.19
	GnRH-BSA immunized	38.30±2.44**	21.56±1.36***	12.00±2.11***	8.40±1.14***
Sperm count (millions/ ml)	Control	62.00±3.18	68.60±2.10	71.50±1.76	82.00±4.11
	GnRH-BSA immunized	43.10±2.65**	38.22±3.21***	26.00±2.20***	22.50±1.78***
Sperm morphology (%)	Control	58.44±2.12	64.00±1.41	67.10±0.74	72.00±0.70
	GnRH-BSA immunized	27.77±0.78***	23.20±0.60***	14.24±0.48***	10.00±0.70***

± SEM of five animals.

** = Significantly different ($p < 0.01$) from the control Vs experimental group by Student's 't' test.

*** = Highly significant ($p < 0.001$) from the control Vs experimental group by Student's 't' test.

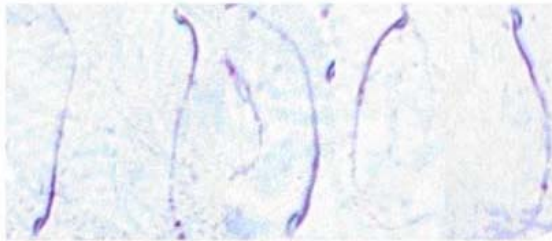


Figure 1. Normal sperm forms with normal heads, mid pieces and tails in control mice (Leishman x400).

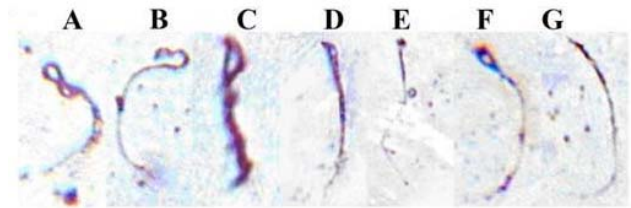


Figure 4. High percentage of abnormal structure of sperms after 90 days of GnRH-BSA immunization ; A- Looping mid piece, B- Bend neck, C- Tail less and large head, D- Tapering head, E- Pin head, F- Oval head, G- Head less (Leishman x400).

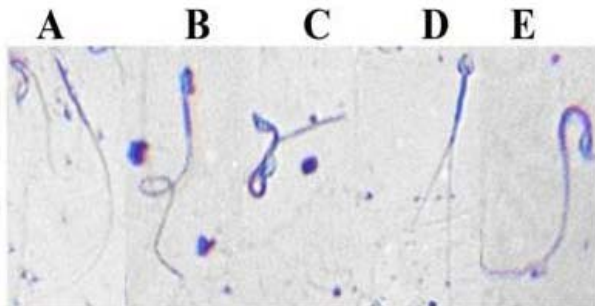


Figure 2. Abnormal structure of sperms; A- Head less, B- Coiled tail, C- Looping mid piece, D- Double tail and large head, E- Bend neck after GnRH-BSA immunization for 30 days (Leishman x400).

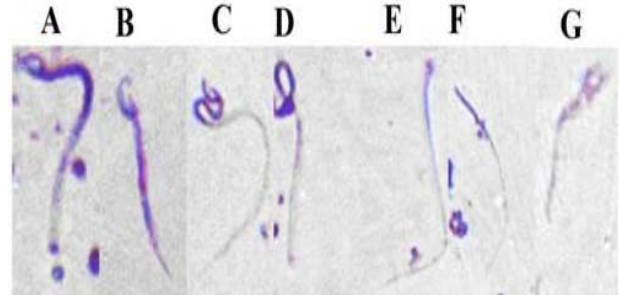


Figure 5. More severe structural sperm abnormalities after 120 days of GnRH-BSA immunization ; A- Bend neck, B- Tail less, C- Folded mid piece, D- Looping mid piece, E- Small head, F- Head less, G- Oval head (Leishman x400).

Discussion

In the pituitary, GnRH binds to the GnRH receptors on the gonadotropic cells to stimulate the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) to the circulation. The pulsatile secretion pattern of GnRH induces the cyclic release of LH and to a lesser extent of FSH. In male mammals, LH stimulates the secretion of androgens (e.g. testosterone) from the leydig cells in the testis. Production of antiserum to gonadotropin releasing hormone was found to be associated with gonadal atrophy in mammals after GnRH immunization (12-14). Antifertility effects

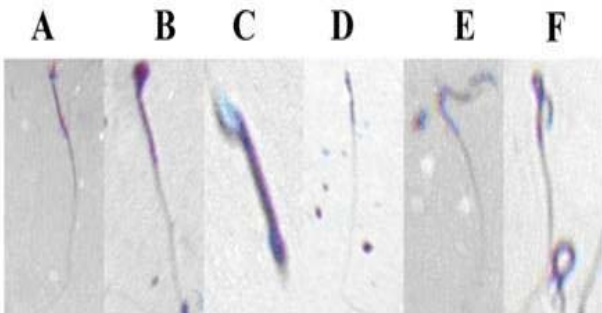


Figure 3. GnRH-BSA immunization after 60 days showing more abnormal structure of sperms; A- Small head, B- Oval head, C- Tail less, D- Head less, E- Bend neck, F- Looping mid piece (Leishman x400).

of immunization against GnRH in male rats and rabbits were reported by Kumar *et al* (2000) (15). Malmgren *et al* (2001) also reported the changes in sexual behavior, androgen deprivation and testicular atrophy in male Stallions after GnRH immunization (16). Immunization with a GnRH analogue conjugated to mycobacterium hsp70 has induced changes in the reproductive system of male mice (17). Talwar *et al* (2004) reported the ability of a multimer recombinant anti-LHRH vaccine to cause decline of testosterone to castration level and atrophy of prostate of rats (18). In addition Turkstra (2005) revealed active immunization against GnRH as an active tool to block the fertility axis in mammals (19). More recent reports on contraception have pointed out that the immunization against GnRH could be an alternative to castration of male and female animals (20-21). Our results also denote that after immunization with GnRH-BSA conjugate at different intervals significantly lowered the body weight, GSI, sperm motility, sperm count and modulated sperm morphology in male mice. These significant effects were more prominent in later part of the experiment. These results suggested that active immunization against GnRH produced antibodies which reduced the level of biologically active GnRH by hypothalamohypophysial gonadal axis which might inhibit androgen level in circulation resulting inhibition of spermatogenesis in mice. Alterations in GnRH level induced changes in reproductive function of male mice, which are duration dependent.

Conclusion

We conclude that the immunization with GnRH-BSA at different intervals induced antifertility effects in male mice. Thus the hormonal conjugate may be used as a hormonal immunocontraceptive to control fertility.

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References

1. Garner LL, Campbell GT, Blake CA. Luteinizing

- hormone (LH)-releasing hormone; Chronic effects on LH and Follicle Stimulating Hormone cells and secretion in adult male rats. *Endocrinology* 1990; 126: 992-1000.
2. Ladd A, Tsong YY, Prabhu G, Thau R. Effects of long - term immunization against GnRH and androgen treatment on gonadal function. *J Reprod Immunol* 1989; 15: 85-101.
3. Ladd A, Tsong YY, Walfield AM, Thau R. Development of antifertility vaccine for pets based on active immunization against luteinizing hormone - releasing hormone. *Biol Reprod* 1994; 51: 1076-1083.
4. Prevendiville DJ. Immunization of Heifers against gonadotropin releasing hormone: Antibody titers, Ovarian function, Body growth and Carcass characteristics. *J Anim Sci* 1995; 73: 2382-2389.
5. Upadhyay SN, Alam A, Talwar GP. Functional morphology of testis and its excurrent ducts in rats immunized with synthetic luteinizing hormone-releasing hormone conjugated to tetanus toxoid. *J Reprod Immunol* 1989; 16: 151-163.
6. Fromme B, ftekhari P, Van Regenmortel M, Hoebeke J, Katz A, Millar R. A novel retro-inverso gonadotropin - releasing hormone (GnRH) immunogen elicits antibodies that neutralize the activity of native GnRH. *Endocrinology* 2003; 144: 3262-3269.
7. Parkinson RJ, Simms MS, Broome P, Humphreys JE, Bishop MC. A vaccination strategy for the long - term suppression of androgens in advanced prostate cancer. *Eur Urol* 2004; 45:171-175.
8. Jinshu X, Jingjing L, Duan P, Zheng Z, Ding M, Jie W, et al. The immunogenicity of recombinant and dimeric gonadotrophin- releasing hormone vaccines incorporating a T-helper epitope and GnRH or repeated GnRH units. *J Immunol Methods* 2004; 289: 111 - 122.
9. Miller LA, Johns BE, Killian GJ. Immunocontraception of white-tailed deer with GnRH vaccine. *Am J Reprod Immunol* 2000; 44: 266-274.
10. Curtis PD, Pooler RL, Richmond ME, Miller LA, Mattfeld GF, Quimby FW. Comparative effects of GnRH and porcine zona pellucida (PZP) immunocontraceptive vaccines for controlling reproduction in white-tailed deer (*Odocoileus virginianus*). *Reproduction* 2002; 60:131 - 141.
11. Highland HN, Rao MV, Chinoy NJ, Shah VC. Analysis of the functional and nuclear integrity of human spermatozoa. *Int J Fertil* 1991; 36: 43-47.
12. Arimura A, Sato H, Kumasaka T, Wordrobe RB, Debeljuk L, Dunn J, et al. Production of antiserum to LH-releasing hormone (LHRH) associated with gonadal atrophy in rabbits; Development of radioimmunoassay for LHRH. *Endocrinology* 1973; 93: 1092 - 1103.
13. Fraser HM, Gunn A, Jeffcoate SL, Holland DT. Effect of active immunization to luteinizing hormone releasing hormone on serum and pituitary gonadotropins, testes and accessory sex organs in the male rat. *J Endocrino* 1974; 63: 399 - 406.
14. Giri DK, Chaudhuri MK, Jayashankar R, Neelaram GS, Jayaraman S, Talwar GP. Histopathological changes in reproductive organs of male Wistar rats following active immunization against GnRH. *Exp Mol Pathol* 1990; 52:54 -62.
15. Kumar N, Sarage T, Dejesus W, Tsong YY, Didolkar A, Sundara K. Chronic Toxicity and reversibility of antifertility effect of immunization against gonadotropin releasing hormone in male rats and rabbits. *Toxicological Sciences* 2000; 53: 92-109.
16. Malmgren L, Andersen O, Dalin AM. Effect of GnRH

- immunization on hormonal levels, Sexual behavior, semen quality and testicular morphology in mature stallions. *Equine Vet J* 2001; 33: 75-83.
17. Hannesdottir SG, Han X, Lund T, Singh M, Van Der Zee R, Roitt IM, et al. Changes in the reproductive system of male mice immunized with a GnRH-analogue conjugated to mycobacterial hsp70. *Reproduction* 2004; 128: 365-371.
 18. Talwar GP, Raina K, Gupta JC, Ray R, Wadhwa S, Ali MM. A recombinant luteinizing – hormone - releasing – hormone immunogen bioeffective in causing prostatic atrophy. *Vaccine* 2004; 22: 3713-3721.
 19. Turkstra JA. Active immunization against gonadotropin releasing hormone, an active tool to block the fertility in mammals. PhD thesis, Utrecht University, Netherlands 2005; 13- 50.
 20. Benagiano G, Bastianelli C, Farris M. Contraception today. *Ann N Y Acad Sci* 2006; 1092: 1-32.
 21. Page ST, Amory JK, Bremner WJ. Advances in male contraception. *Endocr Rev* 2008; 29:465-493.

