Effects of Kamdhenu Ark and Active Immunization by Gonadotropin Releasing Hormone Conjugate (GnRH-BSA) on Gonadosomatic Indices (GSI) and Sperm Parameters in Male Mus musculus

Javid Ahmad Ganaie, Varsha Gautam, Vinoy Kumar Shrivastava*

- Endocrinology Laboratory, Department of Biosciences, Barkatullah University, Bhopal, India

Abstract

Background: Active immunization against GnRH decreases the secretion of gonadotropins and causes cessation of gonadal function, thereby, inducing infertility. Based on the immunoenhancing activity of Kamdhenu ark (distilled cow urine), this study was performed to evaluate its effects on the gonadosomatic indices (GSI) and sperm parameters in male mice receiving a GnRH contraceptive vaccine.

Methods: Sixty adult male mice of Parke’s strain were divided into three groups of twenty. Group I served as the controls, while group II was immunized by GnRH-BSA conjugate (50/0.2/35 µg/ml/g BW) by four intraperitoneal injections at different intervals on days 1, 30, 60 and 90. However, group III was supplemented daily by oral Kamdhenu ark (100 ppm) along with GnRH-BSA immunizations. The animals were sacrificed after 30, 60, 90 and 120 days and their testis and epididymis were dissected out weighed and semen analysis was performed.

Results: GSI values, sperm motility, sperm count and sperm morphology in male Mus musculus were decreased significantly in all the experimental groups as compared to the control group (p<0.01). Kamdhenu ark significantly enhanced the effect of GnRH vaccine on the aforesaid parameters especially in 90 and 120 days treated groups (p<0.05).

Conclusion: The changes witnessed in sperm parameters suggested that the GnRH-BSA immunization suppressed the activities of gonadotropins and testosterone directly through hypothalamo-hypophysial-gonadal axis and indirectly by acting on the testes which may modulate the sperm morphology, sperm count and motility. However, Kamdhenu ark seems to have enhanced these effects because of its immune-modulatory properties too.

Keywords: GnRH-BSA, Gonadosomatic indices (GSI), Immunization, Kamdhenu ark, Mus musculus, Sperm parameters.

To cite this article: Ganaie JA, Gautam V, Shrivastava VK. Effects of Kamdhenu Ark and Active Immunization by Gonadotropin Releasing Hormone Conjugate (GnRH-BSA) on Gonadosomatic Indices (GSI) and Sperm Parameters in Male Mus musculus. J Reprod Infertil. 2011;12(1):3-7.

Background

Gonadotropin-releasing hormone (GnRH) controls the production of gonadotropins, thereby having an orchestrating effect on the reproductive hormone cascade and spermatogenesis (1). Active immunization against GnRH has successfully suppressed the secretion of gonadotropins and decreased sperm production, follicular development, ovulation and conception in male and female mammals (2, 3). Vaccination against GnRH blocks the hypothalamic-pituitary-gonadal axis. Therefore, it can be used as an alternative for castration and fertility control in farm animals, companion animals and wildlife species (4-6). Application of GnRH vaccination in humans has been suggested for controlling fertility-related endocrine disorders and gonadal steroid-dependent diseases (7). Active immunization of adult animals against GnRH causes the loss of synthesis and secretion of gonadotropins and cessation of gonadal function as long as the antibody titers...
remain elevated (8).

It has been reported that cow urine contains all beneficial elements such as chemical properties, potentialities and constituents that are capable of removing all the ill effects and imbalances of body caused by various infectious agents and toxicants. In this way, it ensures a protection against various ailments including the most dreaded diseases like cancer, diabetes, hepatitis etc. (9). Kamdhenu ark (distilled cow urine) has been reported as a strong immunomodulator and bioenhancer by various researchers (10, 11). Experimental studies of Rangasamy and Kaliapan revealed the protective effects of cow urine on haematological, serum biochemical parameters and immune status of broilers (12).

The present study attempts to evaluate the effects of GnRH-BSA immunization on gonadosomatic indices (GSI) and sperm parameters in male mice and to examine the modulatory role of Kamdhenu ark following the immunization.

Methods
Sixty adult male mice, Mus musculus, of Parke’s strain (P), weighing 30±5 g were used in the study. The animals were divided into three groups of twenty. The mice in Group I served as the controls, receiving intraperitoneal Phosphate Buffer Saline (PBS) injections (100 µl) on the 1st, 30th, 60th and 90th days, while the mice in group II were immunized by GnRH-BSA conjugate (50/0.2/35 µg/ml/g BW) (Sigma-Aldrich, USA) dissolved in 100 µl of phosphate buffered solution (0.01 N) emulsified with an equal volume (100 µl) of Freund’s adjuvant (Sigma Aldrich, USA). GnRH-BSA injections were given intraperitoneally at different intervals, i.e. on days 1st, 30th, 60th and 90th. However, the mice in group III were supplemented with daily Kamdhenu ark (100 ppm) (Gaytri Shakti Peeth, India) orally along with the intraperitoneal injections of GnRH-BSA. Five animals from each group were sacrificed in monthly intervals, i.e. on days 30, 60, 90 and 120 and their testes and epididymides were quickly dissected. The testes were weighed for observing gonadosomatic indices [gonad weight/100 g BW], while the epididymides were processed for semen analysis, i.e. sperm motility, sperm count and morphology by Prasad method (13). Cauda epididymides were dissected out to release sperms in normal saline (100 mg tissue/2 ml N.S.) for sperm suspension. For studying sperm morphology, Leishman’s stain was used and the slides were finally observed at 400× magnification (14).

Statistical Analysis: The collected data were analyzed through one way ANOVA and post-hoc methods using EZANOVA software. P-values <0.05 or <0.01 were considered significant while values <0.001 were considered as highly significant.

Results
GSI values decreased in all the experimental groups compared to the control group. However, more significant decrease in GSI was observed in the group treated by Kamdhenu ark along with GnRH-BSA, especially in the later part of the experiment (p<0.01) (Table 1). Moreover, sperm motility and sperm count significantly decreased throughout the investigation in all the treated groups compared to the control group (p<0.01) (Table 2). However, some mice immunized by GnRH-BSA + Kamdhenu ark also showed decreased values for sperm motility and count than the GnRH-BSA immunized groups (p<0.05). The

Table 1. Gonadosomatic indices (GSI) in the experimental and control groups of male mice, Mus musculus, after different intervals

| Group                  | GSI (gonad weight/100 g BW) | Duration |
|------------------------|----------------------------|----------|
|                        | 30 days        | 60 days        | 90 days        | 120 days       |
| Control                | 0.40 ± 0.02    | 0.43 ± 0.05    | 0.50 ± 0.01    | 0.54 ± 0.04    |
| GnRH-BSA               | 0.36 ± 0.06    | 0.30 ± 0.02 a* | 0.23 ± 0.00 a  | 0.16 ± 0.00 a  |
| GnRH-BSA + KA          | 0.32±0.03 a    | 0.26±0.01 a    | 0.19±0.01 ab   | 0.12±0.00 ab   |

Mean ± SEM of five animals (Accuracy of calculation up to two decimal digits)

*a = Significant difference with the controls in the same column (p<0.01)

*b = Significant difference with GnRH-BSA groups in the same column (p<0.01)

* = Significant differences (p< 0.05)
The percentage of morphologically normal sperm decreased significantly with increased percentage of abnormal forms of sperms, i.e. pin head, large head, oval head, double head, head less, bent neck, looping mid piece, coiled-tail, double-tailed, tailless in all the experimental groups as compared to the control group ($p<0.01$) (Table 3, Figure 1). Moreover, some significant alterations in normal sperm morphology, such as large head, headless and pin head sperm were also observed in GnRH-BSA + Kamdhenu ark treated group when compared with GnRH-BSA, especially in the later part of the experiment ($p<0.01$).

**Discussion**

The endocrine effects of active immunization against GnRH have been studied in a variety of young adult male and female animals (15-17). Experimental studies have demonstrated decreases in gonadotropins, sperm production, follicular development, ovulation and conception after immunization against GnRH, chemically conjugated to a carrier protein. GnRH immunization affected sperm motility and sperm counts in ram lambs, boars and colts (18, 19). Several other experimental studies have revealed the deleterious effects of immunization against GnRH on different sperm parameters in rats, bulls, stallions, cats and dogs (20-24).

Cow urine has been tested for its immunomodulatory properties that enhance both cellular and humoral immune responses (25, 26). Kamdhenu ark (distilled cow urine) has been reported to increase the humoral immunity in rats (27). Chauhan et al., (2004) observed that Kamdhenu ark may modulate the immune responses because it increases the secretion of interleukin-1 and 2.

**Table 2.** Sperm motility and sperm count in the experimental and control groups of male mice, *Mus musculus*, after different intervals.

| Parameters          | Group               | Duration       | 30 days | 60 days | 90 days | 120 days |
|---------------------|---------------------|----------------|---------|---------|---------|----------|
| Sperm Motility (%)  | Control             | 59.00±4.33     | 63.76±2.77 | 65.05±2.31 | 69.60±3.19 |
|                     | GnRH-BSA            | 39.40±3.81     | 21.56±1.36 | 13.00±2.11 | 9.40±1.14 |
|                     | GnRH-BSA + KA       | 33.80±1.72     | 17.06±1.00 | 10.46±1.65 | 7.16±0.95 |
| Sperm Count (million/ml) | Control             | 62.00±3.18     | 68.60±2.10 | 75.50±3.76 | 78.00±2.11 |
|                     | GnRH-BSA            | 49.10±2.65     | 35.22±3.21 | 21.00±2.20 | 16.42±1.78 |
|                     | GnRH-BSA + KA       | 44.18±2.82     | 26.12±2.00 | 18.30±1.90 | 12.34±1.10 |

Mean ± SEM of five animals (Accuracy of calculation up to two decimal digits)

*p* = Significant difference with the controls in the same column ($p<0.01$)

* = Significant difference with the GnRH-BSA groups in the same column ($p<0.01$)

* = Significant differences ($p<0.05$)

Figure 1. Normal morphological sperm forms in the controls (A) and morphologically abnormal sperms after GnRH-BSA and Kamdhenu ark along with GnRH-BSA administration (B-I) in male *Mus musculus.*
Kamdhenu ark and GnRH-BSA immunization

Recently, Ganaie and Shrivastava reported the modulatory effects of Kamdhenu ark on GnRH-BSA immunization in female mice (29). In corroboration to above studies, our study also revealed that GnRH-BSA immunization significantly decreased the values of GSI, sperm motility, count and morphology in male Mus musculus. The aforesaid parameters diminished more significantly in the group supplemented with Kamdhenu ark along with GnRH-BSA immunization. All these changes in GSI and sperm parameters suggested that GnRH-BSA immunization could have directly suppressed the activities of gonadotropins and testosterone through hypothalamic-hypophysial-gonadal axis or might have indirectly affected the testicular tissue. However, more significant decreases in the parameters after Kamdhenu ark supplementation may be because of its modulatory and bioenhancing properties.

Acknowledgement

The authors gratefully acknowledge the help and financial support of Professor Meenakshi Benerjee, the head of Department of Biosciences, Barkatullah University, Bhopal (M.P), India.

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(2):992-100.

2. Hoskinson RM, Rigby RD, Mattner PE, Huynh VL, D'Occhio M, Neish A, et al. Vaxstrate: an anti-reproductive vaccine for cattle. Aust J Biotechnol. 1990;4(3):166-70, 176.

3. Prendiville DJ, Enright WJ, Crowe MA, Vaughan L, Roche JF. Immunization of prepubertal beef heifers against gonadotropin-releasing hormone: immune, estrus, ovarian, and growth responses. J Anim Sci. 1995;73(10):3030-7.

4. Bonneau M, Enright WJ. Immunocastration in cattle and pigs. Livest Prod Sci. 1995;42(2-3):193-200.

5. Robbins SC, Jelinski MD, Stotish RL. Assessment of the immunological and biological efficacy of two different doses of a recombinant GnRH vaccine in domestic male and female cats (Felix catus). J Reprod Immunol. 2004;64(1-2):107-19.

6. Miller LA, Johns BE, Killian GJ. Immunococontraception of white-tailed deer with GnRH vaccine. Am J Reprod Immunol. 2000;44(5):266-74.

Table 3. Percentage of normal and abnormal sperm morphology in the experimental and control groups of male mice, Mus musculus, after different intervals

| Days | Groups           | Normal (%) | Abnormal (%) |
|------|-----------------|------------|--------------|
|      |                 | Pin head | Large head | Oval head | Double head | Head less | Bent neck | Looping mid piece | Coiled tail | Double tailed | Tail-less |
| 30   | Control         | 58.44±2.12 | 5.0±0.35 | 4.0±0.79 | 12.0±1.65 | 4.0±1.77 | 3.0±0.86 | 4.4±1.60 | 1.6±0.30 | 0.0±1.0 | 2.5±1.00 |
|      | GnRH-BSA        | 27.77±0.78 | 8.4±1.03 | 5.0±0.79 | 5.8±0.10 | 6.4±1.03 | 6.2±0.65 | 2.4±0.75 | 2.2±0.96 | 5.4±1.15 |
|      | GnRH-BSA+KA     | 24.00±1.22 | 7.6±0.57 | 4.2±0.65 | 6.4±1.03 | 2.4±0.75 | 8.6±0.90 | 5.6±1.3 | 6.5±2.19 | 1.80±0.24 | 1.66±0.51 | 2.5±0.75 |
| 60   | Control         | 64.00±1.41 | 5.4±0.57 | 4.2±0.65 | 3.4±0.57 | 0.60±0.00 | 5.0±1.36 | 3.4±0.57 | 4.2±0.65 | 2.5±0.55 | 1.33±0.51 | 0.80±0.14 |
|      | GnRH-BSA        | 23.20±1.16 | 11.2±0.65 | 10.20±1.74 | 7.20±0.50 | 2.60±0.90 | 7.80±0.65 | 9.4±1.15 | 10.80±1.38 | 4.0±0.70 | 2.00±0.00 | 3.5±0.87 |
|      | GnRH-BSA+KA     | 20.65±0.94 | 10.80±0.45 | 6.4±0.59 | 7.4±0.90 | 0.00±0.00 | 11.80±1.43 | 10.00±1.76 | 9.60±0.51 | 2.50±0.48 | 3.0±1.11 | 5.80±2.38 |
| 90   | Control         | 67.10±1.74 | 4.5±0.65 | 2.60±1.03 | 2.2±0.41 | 0.00±0.00 | 3.40±1.71 | 2.60±0.90 | 1.60±0.83 | 0.00±0.00 | 2.60±1.03 | 1.0±0.21 |
|      | GnRH-BSA        | 14.24±0.48 | 13.60±1.15 | 5.80±0.93 | 6.0±1.00 | 1.20±0.65 | 10.40±0.75 | 12.0±0.79 | 17.80±1.94 | 2.50±1.15 | 1.0±0.25 | 8.0±0.65 |
|      | GnRH-BSA+KA     | 12.72±0.23 | 13.20±1.19 | 8.80±0.96 | 5.80±1.55 | 2.2±0.96 | 13.40±1.03 | 9.5±2.07 | 10.80±1.29 | 3.0±0.79 | 2.5±0.83 | 11.0±1.76 |
| 120  | Control         | 72.00±1.66 | 2.60±0.41 | 2.20±0.39 | 1.0±0.50 | 0.00±0.00 | 2.80±1.29 | 1.20±0.54 | 2.0±0.44 | 1.00±0.00 | 0.00±0.00 | 2.5±1.15 |
|      | GnRH-BSA        | 10.33±0.25 | 12.80±1.55 | 7.00±0.79 | 6.00±0.79 | 2.80±0.96 | 16.00±1.36 | 17.20±0.96 | 20.60±1.95 | 5.00±1.11 | 2.80±0.41 | 9.5±0.68 |
|      | GnRH-BSA+KA     | 7.95±0.40 | 14.60±1.03 | 11.00±0.79 | 7.20±0.82 | 3.80±0.96 | 17.60±1.07 | 15.00±1.83 | 14.25±2.21 | 4.60±1.15 | 4.0±1.17 | 12.50±0.65 |

Mean ± SEM of five animals (Accuracy of calculation up to two decimal digits)

a = Significant difference with the controls in the same column (p<0.01)
b = Significant difference with GnRH-BSA groups in the same column (p<0.01)
* = Significant differences (p<0.05)
7. Simms MS, Scholfield DP, Jacobs E, Michaeli D, Broome P, Humphreys JE, et al. Anti-GnRH antibodies can induce castrate levels of testosterone in patients with advanced prostate cancer. Br J Cancer. 2000;83(4):443-6.

8. Kumar N, Savage T, DeJesus W, Tsong YY, Didolkar A, Sundaram K. Chronic toxicity and reversibility of anti-fertility effect of immunization against gonadotropin-releasing hormone in male rats and rabbits. Toxicol Sci. 2000;53(1):92-9.

9. Bhadauria H. Gomutra-Ek Chamatkari Aushadhi (Cow urine- A Magical therapy). Vish Ayur Patrika. 2002;5:71-4.

10. Chauhan RS, Singh BP, Singhal NK. Immunomodulation with kamdhenu ark in mice. J Immunol Immunopathol. 2001;3(1):74-7.

11. Garg N, Chauhan RS, Kumar A. Assessing the effect of cow urine on immunity of white leghorn layers. International Society for Animal Hugges (ISAH). 2005;2:81-3.

12. Mathivanan R, Kalaarasi K. Panchagavya and Andrographis paniculata as alternatives to antibotonic growth promoters on haematological, serum biochemical parameters and immune status of broilers. J Poult Sci. 2007;44(2):198-204.

13. Prasad MR, Chinoy NJ, Kadam KM. Changes in succinic dehydrogenase levels in the rat epididymis under normal and altered physiologic conditions. Fertil Steril. 1972;23(3):186-90.

14. Highland HN, Rao MV, Chinoy NJ, Shah VC. Analysis of the functional and nuclear integrity of human spermatozoa. Int J Fertil. 1991;36(1):43-7.

15. Esbenshade KL, Britt JH. Active immunization of gilts against gonadotropin-releasing hormone: effects on secretion of gonadotropins, reproductive function, and responses to agonists of gonadotropin-releasing hormone. Biol Reprod. 1985;33(3):569-77.

16. Johnson HE, DeAvila DM, Chang CF, Reeves JJ. Active immunization of heifers against luteinizing hormone-releasing hormone, human chorionic gonadotropin and bovine luteinizing hormone. J Anim Sci. 1988;66(3):719-26.

17. Meloen RH, Turkstra JA, Lankhof H, Puijk WC, Schaaper WM, Dijkstra G, et al. Efficient immunocastration of male piglets by immunoneutralization of GnRH using a new GnRH-like peptide. Vaccine. 1994;12(8):741-6.

18. Grizzle TB, Esbenshade KL, Johnson BH. Active immunization of boars against gonadotropin in releasing hormone. I. Effects on reproductive parameters. Theriogenology. 1987;27(4):571-80.

19. Dowsett KA, Pattie WA, Knott LM, Jackson AE, Hoskinson RM, Rigby RP, et al. A preliminary study of immunological castration in colts. J Reprod Fertil Suppl. 1991;44:183-90.

20. McLachlan RJ, Wreford NG, Tsonis C, De Kretser DM, Robertson DM. Testosterone effects on spermatogenesis in the gonadotropin-releasing hormone-immunized rat. Biol Reprod. 1994;50(2):271-80.

21. Cook RB, Popp JD, Kastelic JP, Robbins S, Harland R. The effects of active immunization against GnRH on testicular development, feedlot performance, and carcass characteristics of beef bulls. J Anim Sci. 2000;78(11):2778-83.

22. Janett F, Stump R, Burger D, Thun R. Suppression of testicular function and sexual behavior by vaccination against GnRH (Equity) in the adult stallion. Anim Reprod Sci. 2009;115(1-4):88-102.

23. Levy JK, Miller LA, Cynda Crawford P, Ritchey JW, Ross MK, Fagerstone KA. GnRH immunoncontraception of male cats. Theriogenology. 2004;62(6):1116-30.

24. Ross MK, Miller LA, Crawford PC, Ritchey JW, Fagerstone KA. GnRH immunoncontraception in cats. In: Baker H, Boyle S, Griffin B, editors. Proceedings of the 2004 ACCD International Symposium on Non-surgical Methods for Pet Population Control; 2004 June 24-27; Denver, Colorado, Belle Court (Portland): ACCD Press; 2005. p.113-5.

25. Prabhakar K, Singh GK, Chauhan RS, Singh DD. Effect of cow urine on lymphocyte proliferation in developing stages of chicks. Indian Cow. 2004;1(2):3-5.

26. Kumar R, Chauhan RS, Singhal LK, Singh AK, Singh DD. A comparative study on immunostimulatory effects of Kamdhenu Ark and Vasant Kusumakar in mice. J Immunol Immunopathol. 2002;4(1-2):104-6.

27. Garg N, Chauhan RS. Kamdhenu arc changes humoral immunity in rat. In: National Symposium on Molecular Biology in India- A Post Graduate Update; 2003 Jan 18, Gwalior, India, Gwalior (Madhya Pradesh): Cancer Hospital and Research Institute; 2003. p. 98.

28. Chauhan RS, Singhal LK, Singhal RK, Kumar R. Effect of cow urine on interleukin-1 and 2. J Immunol Immunopathol. 2004;6(1):38-9.

29. Ganaie JA, Shrivastava VK. Effects of gonadotropin releasing hormone conjugate immunization and bioenhancing role of Kamdhenu ark on estrous cycle serum estradiol and progesterone levels in female Mus musculus. Iran J Reprod Med. 2010;8(2):70-5.