

[Indian J Med Res.](#) 2014 Nov; 140(Suppl 1): S73–S77.

PMCID: PMC4345757

PMID: [25673547](#)

Development of antifertility vaccine using sperm specific proteins

[A.H. Bandivdekar](#)

National Institute for Research in Reproductive Health (ICMR), Mumbai, India

Reprint requests: Dr A.H. Bandivdekar, National Institute for Research in Reproductive Health (ICMR), J.M. Street, Parel, Mumbai 400 012, Maharashtra, India e-mail: batmaram@gmail.com

Received 2013 Apr 12

[Copyright](#) : © Indian Journal of Medical Research

This is an open-access article distributed under the terms of the Creative Commons Attribution-Noncommercial-Share Alike 3.0 Unported, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

Sperm proteins are known to be associated with normal fertilization as auto- or iso-antibodies to these proteins may cause infertility. Therefore, sperm proteins have been considered to be the potential candidate for the development of antifertility vaccine. Some of the sperm proteins proved to be promising antigens for contraceptive vaccine includes lactate dehydrogenase (LDH-C4), protein hyaluronidase (PH-20), and Eppin. Immunization with LDH-C4 reduced fertility in female baboons but not in female cynomolgus macaques. Active immunization with PH-20 resulted in 100 per cent inhibition of fertility in male guinea pigs but it induced autoimmune orchitis. Immunization with Eppin elicited high antibody titres in 78 per cent of immunized monkeys and induced infertility but the immunopathological effect of immunization was not examined. Human sperm antigen (80kDa HSA) is a sperm specific, highly immunogenic and conserved sperm protein. Active immunization with 80kDa HSA induced immunological infertility in male and female rats. Partial N-terminal amino acid sequence of 80kDa HSA (Peptide NT) and its peptides (Peptides 1, 2, 3 and 4) obtained by enzymatic digestion did not show homology with any of the known proteins in gene bank. Peptides NT, 1, 2 and 4 were found to mimic immunobiological activity of native protein. Passive administration of antibodies to peptides NT, 1, 2 and 4 induced infertility in male and female rats and peptide 1 was found to be most effective in suppressing fertility. Active immunization with keyhole limpet haemocynin (KLH) conjugated synthetic peptide 1 impaired fertility in all the male rabbits and six of the seven male marmosets. The fertility was restored following decline in antibody titre. All these findings on 80kDa HAS suggest that the synthetic Peptide-1 of 80kDa HSA is the promising candidate for development of male contraceptive vaccine.

Keywords: Fertilization, immunocontraception, infertility, peptide, sperm

Introduction

With continuously increasing world population efforts are being made to develop the newer methods for male as well as female fertility control which will be safe, reversible and acceptable. Though several hormonal and non-hormonal methods for contraception are available for the women, only the condoms for spacing and terminal method of vasectomy are available for male fertility regulation. The acceptability of these methods is not satisfactory, suggesting the need for additional methods for fertility regulation which should be safe, reversible and acceptable.

Contraceptive vaccine using sperm specific, conserved, immunogenic candidate antigens has been considered to be one of the promising approaches for fertility regulation¹. The feasibility of the approach is also supported by the relatively large numbers of healthy men and women whose infertility is associated with the presence of anti-sperm antibodies². Sperm proteins are known to be essential for normal fertilization as auto-or iso-antibodies to these proteins are associated with infertility. Therefore, sperm proteins have been considered to be the potential candidate for the development of antifertility vaccine.

Many sperm specific, conserved, immunogenic proteins have been identified and evaluated for the development of safe, efficacious contraceptive vaccine^{3,4,5,6}. A few of these have been proved to be promising antigens which include lactate dehydrogenase C4 (LDH C4), protein hyaluronidase-20 (PH-20), and Eppin. Immunization with LDH-C4 reduced fertility in female baboons but not in female cynomolgus macaques⁷. Active immunization with PH-20 resulted in 100 per cent inhibition of fertility in male guinea pigs but it induced autoimmune orchitis⁸. Immunization with Eppin elicited high antibody titres in 78 per cent of immunized monkeys and induced infertility but the immunopathological effect of immunization was not examined⁹.

Promising candidate antigens for inducing immunological infertility

LDH-C4, a testis specific isoenzyme is one of the best studied sperm antigens. Its primary and secondary structure has been elucidated¹⁰. A synthetic peptide representing immunodominant B-cell epitope of human LDH-C4 conjugated to diphtheria toxoid has been shown to decrease fertility by 75 per cent in female baboons upon active immunization¹¹. Naz and his group^{12,13} demonstrated that the antibodies to 23kDa glycoprotein (FA-1) isolated from human and murine testes caused complete impairment of fertility in *in vitro* fertilization (IVF) assay. However, active immunization studies showed significant reduction in fertility in majority but not in all the animals immunized.

Another intra-acrosomal sperm antigen identified using monoclonal antibodies and characterized extensively is SP-10¹⁴. Both monoclonal and polyclonal antibodies to SP-10 were found to impair fertilization *in vitro*, its efficacy *in vivo*, however, has still not been established. Liu *et al*¹⁵ identified and sequenced cDNA for human sperm membrane protein (hSMP-1) of 55kDa. It did not show sequence homology with any other protein in the database. The *hSMP-1* gene is expressed in testis but not in liver or kidney.

Primakoff and his group^{16,17} reported a guinea pig sperm antigen, pH-20, which caused 100 per cent infertility upon active immunization in male and female guinea pigs. The fertility has been demonstrated to be restored following decline in antibody titre. However, in the males it induced autoimmune orchitis. Later the protein was found to have hyaluronidase activity. In addition, a number of sperm antigens are being investigated and most of these have been proved to be effective in reducing sperm-egg interaction *in vitro*, but immunization trials have been largely unsuccessful in terms of contraceptive efficacy.

Human seminal plasma inhibin (hSPI), a follicle stimulating hormone (FSH) regulating peptide of molecular size of 10.5kDa has been reported to be another candidate antigen for fertility regulation. It was found to be of prostatic origin¹⁸. This 94 amino acid peptide has been sequenced and its small

fragments which immunobiologically mimic native protein have been identified and synthesized. Active/passive immunization with hSPI or its synthetic peptides results in about 75 per cent inhibition of fertility in male and female rats^{19,20,21}. Active immunization of male bonnet monkeys with N-terminal 17 amino acid peptide (R-17) elicited antibody titre with loss of motility of the ejaculated spermatozoa. This ongoing study demonstrated that the effect was reversible following decline in antibody titres.

80kDa Human sperm antigen (80kDa HSA)

An 80kDa HSA was initially identified from human sperm extract as an antigen responsible for inducing immunological infertility and later demonstrated to be the promising candidate for development of antifertility vaccine^{22,23,24}. Protein band of 80kDa from human sperm extract reacted specifically with the serum of an immunoinfertile woman, 80kDa HSA was purified from human sperm extract. Active immunization with 10µg of purified 80kDa HSA elicited gradual increase in antibody titre and induced infertility in male and female rats. Immunized animals regained fertility with decline in antibody titre²².

The partial N-terminal amino acid sequence of 80kDa HSA (peptide NT) and its peptides obtained by enzymatic digestion with endoproteinase Lys-C (peptides 1, 2, 3 and 4) and Glu-C (peptides 5 and 6) did not show sequence homology with any of the proteins in Gene database²².

Partial N-terminal amino acid sequence of 80kDa HSA (peptide NT) and its peptides obtained by digestion with endoproteinase Lys-C and endoproteinase Glu-C

N-terminal sequence of 80kDa HSA: X Asn Thr Arg Val Ala Gly Gln Thr Val Ala Phe Leu Peptide NT

Partial sequence of peptides obtained by endoproteinase Lys-C digestion of 80kDa HSA Leu Phe Pro Gln Tyr Val Ala Tyr Ile Thr Asn Leu Lys Ala Peptide 1

Val The Phe Leu Asp Asn The Asp Trp Gly Asn Trp Asp Thr Tyr Thr Peptide 2

Ala Gly Phe Asn Asp Gly Pro Gly Phe Ser Tyr Arg Val Ala Peptide 3

Arg Cys His Asn Asp Asn Asp Ile Asp Val Leu Thr Leu Peptide 4

Partial sequence of peptides obtained by endoproteinase Glu-C digestion of 80kDa HSA Tyr Glu Leu Trp X Trp Asp Asn Peptide 5

Leu Gln Gly Leu Lys Ser Ala Cys

Ala Pro Asn Peptide 6

Peptides of 80kDa HSA were synthesized, conjugated with keyhole limpet haemocyanin (KLH) and the antibodies were raised in rabbits. Peptides NT, 1, 2 and 4 were found immunogenic and mimicked the immunobiological properties of the native protein as the anti-peptide antibodies recognized the native protein in ELISA and also by Western blot analysis showed the specific reactivity with native 80kDa HSA from human sperm extract²². Anti-peptide antibodies also agglutinated human, rat and monkey spermatozoa, *in vitro*¹⁹. Passive administration of anti-peptide antibodies induced infertility in male and female rats (Table I)²⁵.

Table I

Antifertility effect of passive administration of antibodies to 80kDa HSA and its synthetic peptides

	Number of males fertile (% infertile animals)	Number of females fertile (% infertile animals)
Control	7 (12.5)	8 (0)
Ab 80kDa HSA	2 (75)	2 (75)
Ab peptide NT	3 (62.5)	2 (75)
Ab peptide 1	2 (75)	2 (75)
Ab peptide 2	4 (50)	4 (50)
Ab peptide 4	6 (25)	4 (50)

Peptides NT and 1 were found to be more effective than other peptides in inhibiting fertility following passive administration of antipeptide antibodies in rats ([Table I](#)). Six of the eight animals passively immunized with antibodies to 80kDa HSA and peptide 1 became infertile and the remaining two animals were found fertile. Peptides NT and 1 were further investigated for fertility regulation by active immunization of male rabbits and marmosets²⁴.

Active immunization with KLH conjugated synthetic peptides NT and 1 elicited gradual increase in antibody titre in male rabbits. All male rabbits immunized with peptide 1, and four of the six rabbits immunized with peptide NT failed to impregnate normal females. The two animals which could impregnate the females showed the reduction in litter size. All the animals regained fertility following cessation of antibody titre ([Table II](#)).

Table II

Antifertility effect of active immunization with peptides 1 and NT in male rabbits

	Peptide 1		Peptide NT	
	Control	Treated	Control	Treated
No of animals immunized	6	6	6	6
No. of animals fertile	6	0	6	2
% Inhibition of fertility	0	100	0	66
Minimum antibody titre Require to cause infertility	1:800		1:800	
<i>Source: Ref. 24</i>				

Active immunization with most effective KLH conjugated synthetic peptide 1 elicited gradual increase in antibody titre and induced infertility in six of the seven immunized male marmosets having antibody titre > 1:400 (Table III). Histological examination of testes, epididymis, seminal vesicle and prostate did not show any changes following active immunization with synthetic peptide 1²⁴. The data suggest the potential of synthetic peptide1 of 80kDa HSA for development of antifertility vaccine.

Table III

Antifertility effect of active immunization with KLH conjugated peptide1 in male marmosets

Groups	Number of animal immunized	Number of animal infertile	Number of animals regained fertility
Treated	7	6	5/5
Control	6	1	-
Minimum antibody titre required for infertility : 1:400			
<i>Source: Ref. 24</i>			

Conclusion

Several proteins have been shown to be present on the spermatozoa which are essential for the normal fertilization. Blocking these proteins due to the presence of antibodies may impair the fertilization process. The presence of antisperm antibodies has been shown to be associated with the individuals who are immunologically infertile but they are otherwise normal. Therefore, the use of sperm proteins has been considered as one of the promising approaches for the development of antifertility vaccine. Although there are number of antigens present on the sperm, but only the antigen which is specific to sperm may be the ideal candidate for the purpose of immunocontraception. 80kDa HSA was initially identified by Western blot analysis of human sperm extract using serum of a healthy woman having antisperm antibody as a cause of infertility²². This suggests that 80kDa HSA could be responsible for inducing immunological infertility and, therefore, would be the potential candidate antigen for the development of antifertility vaccine. Subsequently, 80kDa HSA was demonstrated to be sperm specific, highly immunogenic and conserved protein. Active/passive immunization with purified 80kDa HSA induced infertility in male and female rats. Active/passive immunization with its synthetic peptides also induced infertility in male rabbits and marmosets. These animals regained the fertility following decline in antibody titre with cessation of immunization. Availability of synthetic fragments which mimic native protein is the additional advantage for safety considerations. In conclusion, synthetic peptide 1 of 80kDa HSA has shown the potential for use in the development of contraceptive vaccine for males.

References

1. Naz RK. Vaccine for human contraception targeting sperm Izumo protein and YLP12dodecamer peptide. *Protein Sci.* 2014;23:857–68. [[PMC free article](#)] [[PubMed](#)] [[Google Scholar](#)]
2. Diekman AB, Norton EJ, Westbrook VA, Klotz KL, Nabby-Hansen S, Herr JC. Antisperm antibodies from infertile patients and their cognate sperm antigens: a review. Identify between SAGA-1, the H6-3C4 antigen and CD 52. *Am J Reprod Immunol.* 2000;43:134–43. [[PubMed](#)] [[Google Scholar](#)]
3. Jones R. Identification and functions of mammalian sperm egg recognition molecules during fertilization. *J Reprod Fertil.* 1990;42(Suppl):89–105. [[PubMed](#)] [[Google Scholar](#)]
4. Primakoff P. Sperm proteins being studied for use in a contraceptive vaccine. *Am J Reprod Immunol.* 1994;31:208–10. [[PubMed](#)] [[Google Scholar](#)]
5. Diekman AB, Herr JC. Sperm antigen and their use in the development of an immunocontraception. *Am J Reprod Immunol.* 1997;37:111–7. [[PubMed](#)] [[Google Scholar](#)]
6. Frayne J, Hall L. The potential use of sperm antigens as targets for immunocontraception, past, present and future. *J Reprod Immunol.* 1999;41:1–33. [[PubMed](#)] [[Google Scholar](#)]
7. Tollner TL, Overstreet JW, Branciforte D, Primakoff PD. Immunization of female cynomolgus macaques with a synthetic epitope of sperm-specific lactate dehydrogenase results in high antibody titers but does not reduce fertility. *Mol Reprod Dev.* 2002;62:257–64. [[PubMed](#)] [[Google Scholar](#)]
8. Tung KS, Primakoff P, Woolman-Gamer L, Myles DG. Mechanism of infertility in male guinea pigs immunized with sperm PH-20. *Biol Reprod.* 1997;56:1133–41. [[PubMed](#)] [[Google Scholar](#)]
9. O’Rand MG, Widgren EE, Sivashanmugam P, Richardson RT, Hall SH, French FS, et al. Reversible immunocontraception in male monkeys immunized with eppin. *Science.* 2004;306:1189–90. [[PubMed](#)] [[Google Scholar](#)]

10. Goldberg E, Shelton JA. Immunosuppression of fertility by LDH-C4. In: Talwar GP, editor. Immunological approaches to contraception and promotion of fertility. New York: Plenum Publishing Corporation; 1985. pp. 219–30. [[Google Scholar](#)]
11. O'Hern PA, Bambra CS, Isahakia M, Goldberg E. Reversible contraception in female baboons immunized with a synthetic epitopes of sperm specific lactate dehydrogenase. *Biol Reprod.* 1995;52:331–9. [[PubMed](#)] [[Google Scholar](#)]
12. Naz RK, Phillips TM, Rosenblum BB. The characterization of the fertilization antigen for the development of contraceptive vaccine. *Proc Natl Acad Sci USA.* 1986;83:5713–7. [[PMC free article](#)] [[PubMed](#)] [[Google Scholar](#)]
13. Naz RK, Menge A. Development of antisperm contraceptive vaccine for humans : Why and how? *Hum Reprod.* 1990;5:511–8. [[PubMed](#)] [[Google Scholar](#)]
14. Herr JC, Flickinger CJ, Homyk M, Klotz K, John E. Biochemical and morphological characterization of the intra-acrosomal antigen SP-10 from human sperm. *Biol Reprod.* 1990;42:181–93. [[PubMed](#)] [[Google Scholar](#)]
15. Liu Q, Wang LF, Miao SY, Catterall JF. Expression and characterization of a novel human sperm membrane protein. *Biol Reprod.* 1996;54:323–30. [[PubMed](#)] [[Google Scholar](#)]
16. Primakoff P, Woolman-Gamer L, Tung KSK, Myles DG. Reversible contraceptive effect of PH-20 immunization in male guinea pigs. *Biol Reprod.* 1997;56:1142–6. [[PubMed](#)] [[Google Scholar](#)]
17. Tung KSK, Primakoff P, Woolmen-Gamer L, Myles DG. Mechanism of infertility in male guinea pigs immunized with sperm PH-20. *Biol Reprod.* 1997;56:1133–41. [[PubMed](#)] [[Google Scholar](#)]
18. Bandivdekar AH, Gopalkrishnan K, Sheth AR. Antibodies to human seminal plasma inhibin cause sperm agglutination and impairment of cervical mucus penetration and sperm-egg attachment. *Adv Contracept.* 1987;3:1–12. [[PubMed](#)] [[Google Scholar](#)]
19. Vanage GR, Garde SV, Sheth AR. Passive immunization against prostatic inhibin peptide as a male contraceptive. *Int J Androl.* 1992;15:114–26. [[PubMed](#)] [[Google Scholar](#)]
20. Vanage GR, Mehta PB, Moodbidri SB, Iyer KSI. Effect of immunization with synthetic peptides corresponding to region 1-17 of human seminal plasma inhibin on fertility of male rats. *Arch Androl.* 2000;44:11–21. [[PubMed](#)] [[Google Scholar](#)]
21. Sheth AR, Garde SV, Joseph R, Patel D. Identification and evaluation of synthetic prostatic peptides as sperm vaccine for fertility regulation. In: Talwar GP, Rao KVS, Chauhan VS, editors. Recombinant and synthetic vaccines. New Delhi: Narosa Publishing House; 1994. pp. 275–82. [[Google Scholar](#)]
22. Bandivdekar AH, Gopalkrishnan K, Garde SV, Fernandez PX, Moodbidri SB, Sheth AR, et al. Antifertility effect in rats actively immunized with 80kDa human semen glycoprotein. *Indian J Exp Biol.* 1992;30:1017–23. [[PubMed](#)] [[Google Scholar](#)]
23. Vernekar VJ, Bandivdekar AH, Raghavan VP, Kamada M, Koide SS. Studies with synthetic peptides of 80kDa Human Sperm Antigen. *Am J Reprod Immunol.* 2004;51:106–111. [[PubMed](#)] [[Google Scholar](#)]
24. Khobarekar BJ, Vernekar VJ, Raghavan VP, Kamada M, Maegawa M, Bandivdekar AH. Evaluation of the potential of synthetic peptides of 80kDa human sperm antigen (80kDa HSA) for the development of contraceptive vaccine for male. *Vaccine.* 2008;26:3711–8. [[PubMed](#)] [[Google Scholar](#)]

25. Bandivdekar AH, Vernekar VJ, Kamada M, Raghavan VP. Antifertility effect of passive administration of antibodies to 80 kDa human sperm antigen and its synthetic peptides in male and female rats. *Am J Reprod Immunol.* 2005;54:332–341. [[PubMed](#)] [[Google Scholar](#)]

Articles from The Indian Journal of Medical Research are provided here courtesy of **Wolters Kluwer -- Medknow Publications**