Development of antifertility vaccine using sperm specific proteins

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Received April 12, 2013

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 haemocyanin (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 HSA suggest that the synthetic Peptide-1 of 80kDa HSA is the promising candidate for development of male contraceptive vaccine.

Key words 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 regulation. 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 antibodies. 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. 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 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.

**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 elucidated. 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 immunization. Naz and his group 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-10. 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. 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 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 origin. 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. 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. 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 titre.

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 database.
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 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 antipeptide antibodies recognized the native protein in ELISA and also by Western blot analysis showed the specific reactivity with native 80kDa HSA from human sperm extract\(^2\)\(^2\). Antipeptide antibodies also agglutinated human, rat and monkey spermatozoa, *in vitro*\(^1\)\(^9\). Passive administration of antipeptide antibodies induced infertility in male and female rats (Table I)\(^2\)\(^4\).

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 marmosets\(^2\)\(^4\).

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 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 required to cause infertility: 1:400 (Table II).

### 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 Ab 80kDa HSA    | 7 (12.5)                                      | 8 (0)                                         |
| Ab peptide NT          | 2 (75)                                        | 2 (75)                                        |
| Ab peptide 1           | 3 (62.5)                                      | 2 (75)                                        |
| Ab peptide 2           | 2 (75)                                        | 2 (75)                                        |
| Ab peptide 4           | 4 (50)                                        | 4 (50)                                        |

### 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 required to cause infertility | 1:800 | 1:800 |

*Source: Ref. 24*

### Table III. Antifertility effect of active immunization with KLH conjugated peptide 1 in male marmosets

|                         | Number of animal immunized | Number of animal fertile | Number of animals regained fertility |
|-------------------------|----------------------------|--------------------------|-------------------------------------|
| Treated                  | 7                          | 6                        | 5/5                                 |
| Control                  | 6                          | 1                        |                                     |

Minimum antibody titre required for infertility: 1:400

*Source: Ref. 24*
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 1\(^3\). The data suggest the potential of synthetic peptide 1 of 80kDa HSA for development of antifertility vaccine.

**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 infertility\(^2\). 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.

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