The preparation and application of N-terminal 57 amino acid protein of the follicle-stimulating hormone receptor as a candidate male contraceptive vaccine

Cheng Xu¹,², Ying-Chun Li¹,³, Hua Yang⁴, Yan Long⁵, Min-Jian Chen¹,², Yu-Feng Qin¹,², Yan-Kai Xia¹,², Ling Song¹,², Ai-Hua Gu¹,², Xin-Ru Wang¹,²

Follicle-stimulating hormone receptor (FSHR), which is expressed only on Sertoli cells and plays a key role in spermatogenesis, has been paid attention for its potential in male contraception vaccine research and development. This study introduces a method for the preparation and purification of human FSH 57-amino acid protein (FSHR-57aa) as well as determination of its immunogenicity and antifertility effect. A recombinant PET-28a(+)-FSHR-57aa plasmid was constructed and expressed in Escherichia coli strain BL21 Star™ (DE3) and the FSHR-57aa protein was separated and collected by cutting the gel and recovering activity by efficient refolding dialysis. The protein was identified by Western blot and high-performance liquid chromatography analysis with a band of nearly 7 kDa and a purity of 97.4%. Male monkeys were immunized with rhFSHR-57aa protein and a gradual rising of specific serum IgG antibody was found which reached a plateau on day 112 (16 weeks) after the first immunization. After mating of one male with three female monkeys, the pregnancy rate of those mated with males immunized against FSHR-57aa was significantly decreased while the serum hormone levels of testosterone and estradiol were not disturbed in the control or the FSHR-57aa groups. By evaluating pathological changes in testicular histology, we found that the blood-testis barrier remained intact, in spite of some small damage to Sertoli cells. In conclusion, our study demonstrates that the rhFSHR-57aa protein might be a feasible male contraceptive which could affect sperm production without disturbing hormone levels.

Asian Journal of Andrology (2014) 16, 623–630; doi: 10.4103/1008-682X.125910; published online: 28 March 2014

Keywords: follicle-stimulating hormone receptor; prokaryotic recombinant expression; male contraception; vaccine

INTRODUCTION
The world’s population is growing at a tremendous rate despite the availability of various contraceptive modalities. Females are target for most current contraceptives, which might bring burdens to their health, such as hormonal disorders and even the risk of endometrial cancer,¹–³ and males aspire to contribute to contraception.¹ However, for men the available choices are still limited to condoms and vasectomy. There is an urgent requirement for a better male contraception method, which is safe, highly effective, reversible, easily accessible and suitable for all stages of reproductive life.

Data from animal experiments and some clinical trials provide several good male contraception targets.³–⁴ Among these, follicle-stimulating hormone (FSH) has been paid more attention for its importance in quantitatively and qualitatively normal spermatogenesis and spermatogonial maturation.⁵–⁶ Data from nonhuman primates also support the crucial role of FSH in the regulation of spermatogenesis in the presence of well-maintained, intratesticular testosterone content.¹¹,¹² However, after years of testing, it seems that the contraceptive approach based on selective FSH withdrawal is not making good progress.¹³ So studies have switched to the alternative strategy of optimizing spermatogenic suppression by saturating the FSH receptor (FSHR).¹⁴,¹⁵ Although previous research has shown that FSH-deficient male mice are fertile with small testes,¹⁶ and that FSHR mutations are associated with variable degrees of spermatogenic failure, they regrettably do not show azoospermia or infertility.¹⁷ Other conflicting results have revealed that in male bonnet monkeys¹⁸ and mice¹⁵ there is the possibility of using the FSHR protein as a male contraceptive vaccine. Nevertheless, these studies have some deficiencies: (i) purity: the protein product inevitably carries some impurities of the expression system to different degrees, owing to the limit of the preparation method and purification; (ii) cross-talk reactions: because of the high homologies of FSHR and luteinizing hormone receptor (LHR), the full-length or the N-terminal-134aa of FSHR proteins, although giving good contraception, are not suitable for developing safe vaccines.¹⁸

Thus, in this study, a peptide produced and expressed by the Escherichia coli strain targeted the FSHR N-terminal 57aa sequence and overlapping 18–74 residues was designed and prepared, and its immune effects in the rhesus monkey were observed.
MATERIALS AND METHODS

Antigenic epitope prediction and plasmid construction
Antigenic epitopes of the human FSHR (GenBank No. AAI25271) were predicted by DNASTAR software (DNASTAR Inc., Madison, USA) from the characteristics of its antigenic index analysis. Sequence alignment and homology comparisons were performed on the homology between the FSHR and LHR proteins (LHR, GenBank No. NP_000224) to avoid the design of a protein that may cross-talk with LHR.

Total RNA was extracted from a human testis (three adult testes; 32, 33 and 35-years-old, stored at -70°C for a month) by using TRIzol reagent and reverse transcribed to cDNA according to the manufacturer's protocol. The FSHR-57aa fragment (GeneBank No. NM_181446) (nucleotides 162–322) was amplified by polymerase chain reaction with the following primers: 5’-GTTC-CCATGG(NcoI)-GCTGTCATCATGGATCTGTCA (162–181)-3’ (forward) and 5’-GTTC-GGATCC(BamHI)-TTATTTCTCCAGGTCCCCAAAT (314–332)-3’ (reverse). The PCR products were digested by NcoI and BamHI enzymes (TaKaRa, Otsu, Japan) and after agarose gel purification, were inserted into the pET-28a (+) clone plasmid (Novagen, Darmstadt, Germany).

Expression and purification of recombinant protein

Transformation
The construct plasmid pET28a-FSHR was then transformed in E. coli strain BL21 Star™ (DE3). After confirmation by DNA sequencing, the positive clones were cultured in Luria broth medium (0.5% w/v yeast extract, 1% (w/v) tryptone, 1% (w/v) NaCl) with 50 mg l⁻¹ kanamycin at 37°C and shaken at 200 rpm for 4 h until the absorbance at 600 nm (A600 nm) reached 1.2.

Intermediate culture and induced expression
The culture mixture (5 µl) was further inoculated into fresh 500 ml Luria broth medium containing kanamycin and grown at 37°C, with shaking at 200 r.p.m overnight until A600 nm was near 2.1. Then, 0.5 mol l⁻¹ isopropyl-D-thiogalactopyranoside 0.5 ml was added and the incubation continued at 37°C and shaken at 200 r.p.m for 4 h.

Collection, solubilization and washing of inclusion bodies
The bacterial pellets were collected by centrifugation at 4°C, 4500g for 10 min and resuspended in 10 ml sonication lysis buffer (100 mmol l⁻¹ NaCl, 50 mmol l⁻¹ Tris-Cl, 1 mmol l⁻¹ ethylenediaminetetraacetic acid and pH 8.0). After sonication (400 W for 2 s at intervals of 1 s three times) on ice, the recombinant protein was extracted from the pellet of bacterial lysate (inclusion bodies) and collected by centrifugation at 4°C, 13 000g for 25 min and resuspended in 15 ml washing buffer (100 mmol l⁻¹ NaCl, 20 mmol l⁻¹ Tris-Cl, 5 mmol l⁻¹ ethylenediaminetetraacetic acid and pH 8.0). The resuspended inclusion bodies were then collected, followed by a solubilization procedure including sonication, centrifugation (4°C, 13 000g, 25 min) and washing (15 ml washing buffer), repeated five times and the final washing buffer contained 4 mol l⁻¹ urea.

Purification of recombinant protein by gel cutting
After centrifugation at 4°C, 18 000g for 30 min, 3 ml 5 × sodium dodecyl sulfate (SDS) gel-loading buffer was added to the collected supernatant, which was separated by 15% w/v SDS-polyacrylamide gel electrophoresis (SDS-PAGE; 4°C, 100 V and 15 h).

The gel slices containing FSHR-57aa proteins (7 kDa) were cut out and put into a dialysis sac with 3.5 ml electrophoresis buffer. The sac underwent horizontal electrophoresis at 150 V for 2 h at 4°C and then reversed-pole electrophoresis for 5 min. The buffer in the dialysis sac with target FSHR-57aa proteins was collected. The dialysis-electrophoresis procedure was repeated and about 7 ml protein sample was harvested.

Refolding dialysis
The protein was put into a dialysis sac and the urea gradually dialedyzed out. The gradual removal of the urea gave time for the polypeptide to refold. The flow sheet is shown in Figure 1.
Identification of the FSHR-57aa protein

The recombinant FSHR-57aa protein was analyzed in Western blots. The purity of FSHR-57aa protein was assessed by Agilent HPLC 1100 (Agilent Technologies, Santa Clara, CA, USA). The other essential characteristics of FSHR-57aa protein (including the N-terminal sequencing, the molecular weight assessment and amino acid components analysis) were analyzed by Shanghai Applied Protein Technology Co. Ltd. Briefly, the N-terminal sequencing of the rhFSHR-57aa was performed by Edman degradation in a ABI 491A protein sequencer (Applied Biosystems, Foster City, CA, USA), the molecular weight was assessed by mass spectrometry (Bruker Autoflex II, Ettlingen, Germany) and the amino acid components of FSHR-57aa were analyzed by an amino acid analyzer (Hitachi Co. Ltd, Tokyo, Japan).

Immunization procedure and antibody titration

Healthy adult monkeys (Macaca radiata) of proven fertility were included in this study (Asia Primate Research Center, Kunming, China). Twelve fertile males were randomly divided into two groups (control and FSHR group) and continuously caged with females at a ratio of 1:3. Among the males in the control and FSRH groups, the mean age ranged from 7 to 10 years and mean ± standard deviation was 8.7 ± 1.0 and 8.3 ± 0.8 years, respectively; likewise the body weight ranged from 6.82 to 17.56 kg and mean ± standard deviation was 11.1 ± 3.9 and 8.0 ± 0.9 kg, respectively. All animal experiments were approved by the Ethics Committees of Nanjing Medical University.

The male adult monkeys were inoculated once every 3 weeks with a dosage of 200 µg rhFSHR in 0.5 ml phosphate buffered saline (PBS) with an equal volume of aluminium adjuvant in the FSRH group. Injections were intramuscular at two sites (gluteus maximus and deltoid muscle, 0.5 ml per site). The control monkeys were injected with adjuvant and boosters in PBS were given every 3 weeks. Blood was collected from the great saphenous vein every 5 weeks (from 0 week) and the blood was centrifuged and the serum was collected, stored at -70°C. Semen analyses were performed on fresh ejaculates obtained by electrostimulation along with the last five blood collections.

The antibody titration was determined by ELISA in 96-well microtitre plates. The plates were coated with optimized concentrations of rhFSHR protein (100 ng rhFSHR in 100 µl PBS each well) at 4°C overnight. Then, the antigen was removed, followed by blocking with 3% (w/v) bovine serum albumin at 37°C for 1 h and then washing with PBS containing 0.05% (v/v) Tween-20. The serum of immunized monkeys was diluted (1:1000) with PBS and added to the 96-wells. After incubation at 37°C for 1 h, the plates were washed thoroughly and reacted with horseradish peroxidase (HRP)-conjugated goat anti-monkey IgG antibodies (Sigma, 100 µl per well, 1:5000 dilution), and detected at A450 nm. Seroconversion criterion: mean absorbance for immunized value compared to the preimmunized value to reach 2 (A450 nm, with serum dilution of 1:1000).

Fertility evaluation

After 12 immunizations (168 days), the reproductive ability was investigated by mating each immunized male with three females during the breeding season (from November to the next February). Abdominal B-ultrasound was used to determine gestation and the pregnancy rate was recorded (Medison Digital Color MT; Medison Co. Ltd, Seoul, Korea).

After four immunizations (56 days), the serum testosterone and estradiol levels of immunized and control males were measured by radioimmunoassay with commercial radioimmunoassay kits (Beijing North Institute of Biological Technology, China). Four internal and external quality control samples were included in each assay. Detection

![Figure 2: The bioinformatics analysis of FSHR-57aa protein. Homology comparison of FSHR and LHR protein (a) and Antigenic Index-Jameson Wolf analysis (b) showed the FSHR-57aa protein is an ideal candidate epitope for an FSHR vaccine. FSHR: follicle-stimulating hormone receptor; LHR: luteinizing hormone receptor.](image-url)
Peptide of FSHR as male contraceptive vaccine
C Xu et al

The recombinant human FSHR-57aa immunogen was immunogenic in the male monkey. The serum antibody titre remained fairly constant during the entire immunization period. Boosters significantly enhanced the antibody titre on day 56 (8<sup>th</sup> week) after first immunization and reached a plateau on day 112 (16<sup>th</sup> week) after first immunization (Figure 5a).

The antibody titre in seminal plasma, when the dilution was 1:100, showed a slight but nonsignificant increase on day 140 (20<sup>th</sup> week) after first immunization (Figure 5b).

Transmission electron microscope (TEM) analysis
Testicular tissue sections for electron microscopic analysis were fixed in a 5% (v/v) glutaraldehyde, buffered with 0.1 mol L<sup>-1</sup> sodium cacodylate, containing 3 mmol L<sup>-1</sup> calcium chloride and pH 7.4 for 24 h. Ultrathin sections approximately 60–90 nm thick were placed on copper grids and stained with a mixture of uranyl acetate and lead citrate, then viewed in a transmission electron microscope (JEM-1010, JEOL Ltd, Tokyo, Japan).

Statistical analysis
The software Statistical Package for Social Sciences 17.0 (SPSS Inc., Chicago, IL, USA) was used and differences were regarded as statistically significant if P < 0.05. Data were analyzed by independent-samples t-test and between three or more groups by one-way analysis of variance. The Games-Howell test for heterogeneous variances was used for multiple comparisons among the groups. The difference in pregnancy rates was assessed by the Pearson chi-squared test between control and case groups.

RESULTS
The construction of recombinant pET-28a (+)-FSHR-57aa plasmids
From the results of antigenic epitope prediction and sequence comparison alignment,<sup>16</sup> the N-terminal FSHR-57aa (overlapping 18–74 residues), with a high antigenic index and a low-homology compared with the LH receptor protein, was designed to be the potential peptide of vaccine (Figure 2). The recombinant pET-28a (+)-FSHR-57aa plasmid was successfully constructed (Figure 3a) and sequenced (Figure 3b).

Identification of the protein
The result of SDS-PAGE analysis showed that the recombinant FSHR-57aa monomer was nearly 7 kDa and the bandscan analysis suggested its purity was over 95% (Figure 4a). The purity of rhFSHR-57aa was 97.4% from high-performance liquid chromatography analysis (Figure 4b). The molecular weight of the protein was 7420.582 Da detected by time-of-flight mass spectrometry (Figure 4c), which is consistent with the result of the FSHR-57aa monomer in SDS-PAGE gels. The N-terminal 15 residues identified by chromatography were NH2-G-C-H-H-R-I-C-H-C-S-N-R-V-P-L, which exactly matches the FSHR-57aa N-terminal sequence. The results of the amino acid components analysis suggest that the recombinant protein was the exact protein of FSHR-57aa (Figure 4d). The amino acid sequence of protein of FSHR-57aa (Table 1) indicated near homology with that of Homo sapiens and Macaca radiata.

Antibody titration

Table 1: The amino acid sequence of protein of FSHR-57aa

| Genera | Amino acid sequence |
|--------|---------------------|
| H      | CHHRICHCSNRVFLCQESKYTEPSDLPRTAELRFVLTKLRVIQKGA |
| M      | CHHRICHCSNRVFLCQESKYTEPSDLPRTAELRFVLTKLRVIQKGA |

FSHR: follicle-stimulating hormone receptor; H: Homo sapiens; M: Macaca radiata. The greyed letters represent those with distinction amino acid between Homo sapiens and Macaca radiata.

Table 2: Fertility evaluation

| Groups (n, %) | Enabled pregnancy | No pregnancy |
|--------------|-------------------|--------------|
| Control      | 17 (94.4)<sup>1</sup> | 1 (5.6) |
| FSHR-57      | 11 (61.1)<sup>1</sup> | 7 (38.9) |

FSHR: follicle-stimulating hormone receptor. *Statistical analysis indicates chi-square test=5.8, Fisher’s exact P = 0.041.
Peptide of FSHR as male contraceptive vaccine
C Xu et al

Fertility evaluation

The pregnancy result is shown in Table 2. The pregnancy rates of females in the control and FSHR-57 groups were 94.4% and 61.1%, respectively, with the chi-squared test = 5.8, and Fisher's exact P value was 0.041. The sperm parameter values are shown in Figure 6 as total concentration (10^6 ml^-1), motion (%) and linearity (%) of the FSHR-57 and control groups. The total sperm concentration of the control and FSHR-57 groups was 578.8 ± 281.8 (10^6 ml^-1) and 419.5 ± 204.3 (10^6 ml^-1), respectively (Figure 6a). Sperm motion of the control and FSHR-57 groups was 70.2% ± 10.9% and 60.9% ± 7.9%, respectively (Figure 6b), and the linearity of the control and FSHR-57 group was 45.4 ± 14.8 and 27.8 ± 8.3, respectively (Figure 6c). All of results represent the mean ± standard error of the mean. The P value of sperm parameter values were all >0.05. Although serum levels of testosterone and estradiol ranged widely within and between monkeys at different times, no significant statistical differences were observed in them between the FSHR-57aa and control group (Figure 7). In the FSHR-57aa protein immunized group, we did not observe obvious degeneration of testicular tissue. However, some slight changes were found in the FSHR-57aa immunized group, such as apparent slight damage to Sertoli cells and a possible decreased number of round spermatids and spermatozoa in the seminiferous tubules (Figure 8). Morphological quantification indicated that the diameters of the seminiferous tubules was significantly different between the two groups, while there were no significant changes in the thickness of seminiferous epithelium (Table 3).

At the electron microscopic level, two arresting changes were found in the immunized group compared with the control group (Figure 9a and 9b): (i) apparently larger interstitial spaces (Figure 9c) and (ii) an apparent large area of vacant matrix in the lumen (Figure 9d).
DISCUSSION

Globally there is a clear desire and need for more contraceptive options.22,23 Couples desire more choices for fertility control and unintended pregnancies continue to occur.24 Development of contraceptive vaccines for males is of great importance for global population control. Up to the present, many efforts have been put into developing male contraceptive vaccines.4,7,25 Although the hormonal approach to male contraception has made significant progress in the last decade, it has encountered bottlenecks because of the non‑ignorable side effects.26‑28 Scientists have switched to hormone‑based targets or sperm‑specific molecules,25,29,30 but none of these studies have yet produced a successful contraceptive agent.

Because of its role of FSH in spermatogenesis, the FSH receptor is a promising target in developing safe and effective contraceptive vaccines.31,32 For it can be used to generate a specific antibody that can block fertility. In 1997, Kumar et al.16 and Tapanainen et al.17 found that the deletion of FSH in mice and mutations in the FSH receptor in humans did not show infertility or azoospermia. In the same year, Moudgal et al.18 drew the conclusion that the FSHR protein can substitute for FSH in the development of a contraceptive vaccine in male bonnet monkeys. Recently, Yang et al.15 successfully inhibited fertility of mice with a new immunization strategy, with priming with rhFSHR protein (1–140) and boosting with a peptide containing amino acids 32–44. The full‑length FSHR protein is not suitable for developing a vaccine because of safety,33,34 and FSHR protein produced by current methods has not achieved satisfactory yields and purity. In this study, the N‑terminal 57aa of the FSHR protein, which contains the major determinant epitopes in eliciting antibodies in adult monkeys, has been developed and tested for its ability to impede male fertility.

Table 3: Histomorphometry data in the testis (mean±s.d.)

| Groups   | Diameters (µm) | Thickness (µm) |
|----------|----------------|---------------|
| Control  | 192.8±7.7      | 54.0±14.9     |
| FSHR‑57  | 202.4±12.0*    | 54.2±15.4     |

Diameters: diameters of seminiferous tubules; FSHR: follicle‑stimulating hormone receptor; s.d.: standard deviation; Thickness: thickness of seminiferous epithelium. Data were analyzed by independent‑samples t test. *P<0.05 compared with control.
effects. We determined first if the recombinant hFSHR-57aa residues elicited a satisfactory immune response in the male bonnet monkey, and second if the new purification method for recombinant FSHR-57aa vaccine was feasible. Third, we observed if the antibody was able to suppress fertility in vivo by virtue of its ability to bind to FSH receptors.

How to increase the yield of target protein from inclusion bodies and keep its biologic activity is always the bottleneck of protein expression. Our study provides detailed information about recombinant FSHR-57aa protein preparation, which is expressed in inclusion bodies. This preparation procedure removed most of the non-target proteins and achieved high purity in gel slices. Meanwhile, it contained denaturation and renaturation procedures which ensure the recovery of active soluble proteins from the inclusion body. By this method, the purity of FSHR-57aa protein obtained nearly reached 98% and all the immunized monkeys produced a satisfactory equivalent antibody response with the aluminum adjuvant.

Further fertility evaluation showed a significant difference in fertility between the FSHR-57aa and control groups, although the pregnancy rate in the treated group (61.1%) was far from our expectation for a highly efficient contraceptive vaccine. The contraceptive efficiency of FSHR-57aa in our study was slight, which is consistent with previous conclusions in humans and rodents. The road to male contraception appears to be long and winding, especially by active immunization methods. Our work nevertheless contributes to the development of male contraception.

The semen analysis revealed no significant differences in concentration, motion or linearity of spermatozoa between the two groups, which conflicts with our anticipation that the protein would cause a complete block in sperm production. However, the results of serum hormone demonstrated that the recombinant protein may not do harm to the endocrine system, including the hypothalamic-pituitary-gonadal axis, which corresponds to previous studies on the mechanism of action of FSH.

It is worth noting that in the histology and transmission electron microscope analysis of the testes, the diameters of the seminiferous tubules was slightly increased in FSHR-57aa group and the Sertoli cells were mildly disturbed, but the blood-testis barrier was not destroyed during the immunization procedure. As a previous study has reported that in rodents there was an apparent decrease in tubule size in the testis in the treatment group and another study presented no pathological change in the seminiferous tubules, perhaps FSHR-57aa induces a relatively less pathological effect than the FSHR-134aa reported previously or tissues could 'self-repair' promptly. Nevertheless, the destruction and repairing mechanism needs to be confirmed in our further studies.

In conclusion, our detailed study has shown that rhFSHR-57aa is a potential male contraception vaccine. Although, the antifertility effect needs to be improved, the present availability of recombinant FSH receptor preparations and that anti-FSHR sera act as FSH antagonists open this area to further detailed study.

AUTHOR CONTRIBUTIONS
AHG, YKX and XRW conceived and designed the experiments. YL, MJ_DC and YFQ performed the experiments. CX analyzed the data. LS contributed reagents, materials and analysis tools. CX, YCL and HY wrote the paper.

COMPETING INTERESTS
The authors declare that they have no competing interests.

ACKNOWLEDGMENTS
This study was supported by the National Key Technologies R and D Program (No. 2012BAI31B07 and No. 2006BAI03B12), the National Science Foundation of China (No. 81112694). This project was also funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions.

REFERENCES
1. Mueck AO, Seeger H, Rabe T. Hormonal contraception and risk of endometrial cancer: a systematic review. Endocr Relat Cancer 2010; 17: R263–71.
2. Verhaeghe J. Clinical practice: contraception in adolescents. Eur J Pediatr 2012; 171: 895–9.
3. Veljkovic M, Veljkovic S. The risk of breast cervical, endometrial and ovarian cancer in oral contraceptive users. Med Prg 2010; 63: 657–61.
4. Moudgal NR, Jeyakumar M, Krishnamurthy HN, Sridhar S, Krishnamurthy H et al. Development of male contraceptive vaccine—a perspective. Hum Reprod Update 1997; 3: 335–46.
5. Page ST, Amory JK, Brenner WJ. Advances in male contraception. Endocr Rev 2008; 29: 465–93.
6. Diekman AB, Norton JE, Klotz KL, Westbrook VA, Herr JC. Evidence for a unique N-linked glycan associated with human infertility on sperm CD52: a candidate contraceptive vaccinogen. Immunol Rev 1999; 171: 203–11.
7. Luo J, Yang J, Cheng Y, Li W, Yin TL, et al. Immunogenicity study of plasmid DNA encoding mouse cysteine-rich secretory protein-1 (mCRISP1) as a contraceptive vaccine. Am J Reprod Immunol 2012; 68: 47–55.
8. Powers JG, Baker DL, Ackerman MG, Spraker TR, et al. Passive transfer of maternal GnRH antibodies does not affect reproductive development in elk (Cervus elaphus nelsoni) calves. Theriogenology 2012; 78: 830–41.
9. Fouikles NS, Schlottter F, Pevet P, Sassone-Corsi P. Pituitary hormone FSH directs the CREM functional switch during spermatogenesis. Nature 1993; 362: 264–7.
10. Hedger MP, Winnall WR. Regulation of activation and inhibin in the adult testis and the evidence for functional roles in spermatogenesis and immunoregulation. Mol Cell Endocrinol 2012; 359: 30–42.
11. Rao AJ, Ramachandra SG, Ramesh V, Couture L, Abdenenre L, et al. Induction of infertility in adult male bonnet monkeys by immunization with phage-expressed peptides of the extracellular domain of FSH receptor. Reprod Biomed Online 2004; 8: 385–91.
12. Srinaman V, Rao AJ, FSH, the neglected sibling: evidence for its role in regulation of spermatogenesis and Leydig cell function. Indian J Exp Biol 2005; 43: 993–1000.
13. Mathiesssen Kl, McLachlan RI, O’Donnell L, Frydenberg M, Robertson DM, et al. The relative roles of follicle-stimulating hormone and luteinizing hormone in maintaining spermatogenic maturation and spermatogenesis in normal men. J Clin Endocrinol Metab 2006; 91: 3962–9.
14. Yan P, He W, Liang Z, Chen Z, Shang X, et al. A novel dominant B-cell epitope of FSHR identified by molecular docking induced specific immune response and suppressed fertility. Gynecol Endocrinol 2009; 25: 828–38.
15. Yang LH, Li JT, Yan P, Liu HL, Zeng SY, et al. Follicle-stimulating hormone receptor (FSHR)-derived peptide vaccine induced infertility in mice without reproductive organs. Reprod Dev Biol 2011; 21: 54–50.
16. Kumar TR, Wang Y, Lu N, Matzuk MM. Follicle stimulating hormone is required for ovarian follicle maturation but not male fertility. Nat Genet 1997; 15: 201–4.
17. Tapanainen JS, Attoomaki K, Min J, Vaskivuo T, Huhtaniemi IT. Men homogous for an inactivating mutation of the follicle-stimulating hormone (FSH) receptor gene present variable suppression of spermatogenesis and fertility. Nat Genet 1997; 15: 205–6.
18. Moudgal NR, Sairam MR, Krishnamurthy HN, Sridhar S, Krishnamurthy H, et al. Immunization of male bonnet monkeys (M. radiata) with a recombinant FSH receptor preparation affects testicular function and fertility. Endocrinology 1997; 138: 3065–8.
19. Brindley GS. Electroejaculation: its technique, neurological implications and uses. J Neurol Neurosurg Psychiatry 1981; 44: 9–18.
20. World Health Organization. 2010 WHO laboratory manual for the examination and processing of human semen. Geneva: World Health Organization; 2010.
21. Soding J, Remmer M. Protein sequence comparison and fold recognition: progress and good-practice benchmarking. Curr Opin Struct Biol 2011; 21: 404–11.
22. Ogihara JP, Unnara-Darapah IC, Odesensam YU, Etuk IP, Okoli IC. The potentiality of medicinal plants as the source of new contraceptive principles in males. N Am J Med Sci 2011; 3: 255–63.
23. Ferro VA, Garside DA. Reproductive component vaccine developments for contraceptive and non-contraceptive uses. Expert Opin Ther Pat 2011; 21: 1473–82.
24. Singh S, Deirdre W, Rubina H, Akinnrinola B, Gilda S. Abortion Worldwide: A Decade of Unyielding Progress. New York: Guttmacher Institute; 2009.
25. Li H, Ding X, Guo C, Guan H, Xiong C. Immunization of male mice with B-cell epitopes in transmembrane domains of CatSper1 inhibits fertility. Fertil Steril 2012; 97: 445–52.
26. Grimes DA, Lopez LM, Gallo MF, Halpern V, Nanda K, et al. Steroid hormones for contraception in men. Cochrane Database Syst Rev 2012; 3: CD004316.
27. Ilani N, Swerdloff RS, Wang C. Male hormonal contraception: potential risks and benefits. Rev Endocr Metab Disord 2011; 12: 107–17.
28. Halpern V, Lopez LM, Grimes DA, Gallo MF. Strategies to improve adherence and acceptability of hormonal methods of contraception. Cochrane Database Syst Rev 2011: CD004317.
Peptide of FSHR as male contraceptive vaccine
C Xu et al

29 Khan SA, JadHAV SV, Suryawanshi AR, Bhonde GS, Gajbhiye RK, et al. Evaluation of contraceptive potential of a novel epididymal sperm protein SFP2 in a mouse model. *Am J Reprod Immunol* 2011; 66: 185–98.

30 Gupta SK, Kothan PT. Relevance of immuno-contraceptive vaccines for population control. I. Hormonal immunocontraception. *Arch Immunol Ther Exp (Warsz)* 1990; 38: 47–60.

31 Tapanainen JS, Aittomaki K, Huhtaniemi IT. New insights into the role of follicle-stimulating hormone in reproduction. *Ann Med* 1997; 29: 265–6.

32 Narula A, Gu YQ, O’Donnell L, Stanton PG, Robertson DM, et al. Variability in sperm suppression during testosterone administration to adult monkeys is related to follicle stimulating hormone suppression and not to intratesticular androgens. *J Clin Endocrinol Metab* 2002; 87: 3399–406.

33 Curtis PD, Richmond ME, Miller LA, Quimby FW. Pathophysiology of white-tailed deer vaccinated with porcine zona pellucida immunocontraceptive. *Vaccine* 2007; 25: 4623–30.

34 Govind CK, Srivastava N, Gupta SK. Evaluation of the immunocontraceptive potential of *Escherichia coli* expressed recombinant non-human primate zona pellucida glycoproteins in homologous animal model. *Vaccine* 2002; 21: 78–88.

35 Burgess RR. Refolding solubilized inclusion body proteins. *Methods Enzymol* 2009; 463: 259–82.

36 Nieschlag E, Henke A. Hopes for male contraception. *Lancet* 2005; 365: 554–6.

37 Dias JA, Van Roey P. Structural biology of human follitropin and its receptor. *Arch Med Res* 2001; 32: 510–9.

38 Plant TM, Marshall GR. The functional significance of FSH in spermatogenesis and the control of its secretion in male primates. *Endocr Rev* 2001; 22: 764–86.

How to cite this article: Xu C, Li YC, Yang H, Long Y, Chen MJ, Qin YF, Xia YK, Song L, Gu AH, Wang XR. The preparation and application of N-Terminal 57 amino acid protein of the follicle-stimulating hormone receptor as a candidate male contraceptive vaccine. *Asian J Androl* 28 March 2014. doi:10.4103/1008-682X.129910. [Epub ahead of print]