A vaccine that prevents pregnancy in women

(human chorionic gonadotropin/birth control vaccine)

G. P. Talwar*†, Om Singh*, Rahul Pal*, N. Chatterjee*, P. Sahai*, Kamala Dhall‡, Jasvinder Kaur‡, S. K. Das§, Sushma Suri§, Kamal Buckshee¶, L. Saraya¶, and Badri N. Saxena∥

*National Institute of Immunology, New Delhi 110067, India; ‡Departments of Obstetrics and Gynecology, Postgraduate Institute of Medical Education and Research, Chandigarh, India; §Safdarjung Hospital, New Delhi, India; ¶All India Institute of Medical Sciences, New Delhi, India; and ∥Indian Council of Medical Research, New Delhi, India

ABSTRACT   We report here results of clinical trials on a birth control vaccine, consisting of a heterospecies dimer of the β subunit of human chorionic gonadotropin (hCG) associated noncovalently with the α subunit of ovine luteinizing hormone and conjugated to tetanus and diphtheria toxoids as carriers, that induces antibodies of high avidity ($K_a \approx 10^{10}$ M^{-1}) against hCG. Fertile women exposed to conception over 1224 cycles recorded only one pregnancy at antibody titers of >50 ng/ml (hCG bioneutralization capacity). The antibody response declines with time; fertility was regained when titers fell to <35 ng/ml. This study presents evidence of the feasibility of a vaccine for control of human fertility.

A number of contraceptive methods are available; they do not, however, suit all potential users. There is need to develop additional methods, in particular those that are reversible, require only periodic intake, and do not disturb menstrual regularity or bleeding. Vaccines regulating fertility offer promising prospects to meet these specifications. The rationale for these vaccines is to induce the formation of antibodies and/or cell-mediated immunity to intercept selectively a process critical to the success of reproduction. A number of potential antigens are being investigated (1–7). Among these are hormones, which play an important role in the regulation of fertility. Human chorionic gonadotropin (hCG) is an early signal of conception and is considered essential for establishment and maintenance of early pregnancy. An advantage in choosing hCG as a target for immunocontraception is that its inactivation would not interfere with other physiological processes in the female, such as ovulation and production of sex steroid hormones.

Carrier conjugation with tetanus toxoid (TT) was proposed as a strategy to overcome the immunological tolerance of a woman against hCG (8). This initial prototype vaccine (βhCG-TT adsorbed on alum) had limitations. It induced high antibody titers in only a small percentage of women, and those with low titers were not protected from pregnancy (9). Three changes were made to enhance immunogenicity.

(*i*) An adjuvant, the sodium phthalyl derivative of lipopolysaccharide, was included in the first injection. This nonpyrogenic adjuvant is usable in aqueous phase (10). Its use doubled, on average, anti-hCG titers and increased the frequency of high responders.

(*ii*) The intrinsic immunogenicity of βhCG was enhanced by associating it noncovalently with the α subunit of ovine luteinizing hormone (LH) to form a heterospecies dimer (HSD), a laboratory-made hormone that attains a conformation which recognizes receptors on target tissues (whereas isolated subunits do not) and generates a steroidogenic response even superior to that by hCG (11). HSD linked to carriers was indeed found to be more immunogenic than βhCG in rats and monkeys (12). Moreover, the antibodies had better capacity to neutralize the bioactivity of hCG (11, 13).

(*iii*) HSD was conjugated to one of two different carriers, TT or diphtheria toxoid (DT); these conjugates were used in an alternating sequence. This schedule was adopted since carrier-induced immunosuppression, observed in a small percentage of women on repeated injection of conjugates with the same carrier, could be overcome by presenting the hormonal subunit on an alternate carrier (14).

After preclinical toxicology studies, ethical and drug regulatory agency approvals, phase I clinical trials were undertaken with the HSD vaccine in five institutions in India. Volunteers were women who had previously undergone elective tubal ligation. The vaccine was able to induce anti-hCG antibodies in all women (15). The response was reversible. Ovulation and menstrual regularity were unaffected and the duration and amount of bleeding were normal (16). A careful study of 36 hematological, biochemical, metabolic, and endocrinological parameters in the immunized women revealed no significant side effects (17). Similar results had earlier been obtained in trials carried out with the βhCG-TT vaccine in 16 women at Helsinki, Uppsala, Bahia, and Santiago under the auspices of the International Committee for Contraception Research of the Population Council, New York (18).

It was important to determine whether the antibodies induced by the HSD- and βhCG-based vaccines in monkeys and in women (phase I clinical trials) had any reactivity with somatic tissues. Immunopathological studies demonstrated the lack of nuclear, DNA, parietal cell, smooth muscle, islet cell, adrenal cortex, thyroid mitochondrial, thyroglobulin, C-reactive protein, and rheumatoid factor reactivities (19).

Chronic toxicology studies have also been carried out in monkeys that were hyperimmunized for 7 years with an analogous vaccine inducing antibodies against both monkey CG and LH. No significant immunopathology was observed in any organ, including the pituitary and the kidney (20, 21). Moreover, women immunized with the vaccine in 1977–1978 during phase I clinical trials did not manifest any symptoms of autoimmune disease nor did their sera have reactivity with human and primate somatic tissues. Nevertheless, the potential for tissue cross-reactivity needs to be considered as vaccine development proceeds.

The objective of the present trial was to determine whether a vaccine based on the heterospecies dimer of the α subunit of ovine LH and βhCG can prevent pregnancy in women and, if so, to determine the level of antibody titers necessary for efficacy.

MATERIALS AND METHODS

Vaccine. The improved vaccine is HSD coupled to either TT or DT. HSD was generated by incubating equimolar concentrations of βhCG and the α subunit of ovine LH in 10 mM phosphate buffer (pH 7.2) at 37°C for 24 hr as described (11). Conjugation of HSD with carriers was carried out as described (14, 15). The first injection contained 150 μg each of HSD-TT and HSD-DT adsorbed on alum, along with 1.0 mg of the sodium phthalyl derivative of lipopolysaccharide. Subsequent injections were with 300 μg of HSD-TT or HSD-DT adsorbed on alum in an alternating sequence.

Study Protocol and Procedures. The trials were conducted at the All India Institute of Medical Sciences, and Safdarjung Hospital, New Delhi; and Postgraduate Institute of Medical Education and Research, Chandigarh. Approvals of the Drugs Controller of India and institutional ethics committees were obtained. Women attending the family planning clinics in these institutions were enrolled for the study after providing informed, written consent. They were of reproductive age (20–35 years), had an active sexual life, and reported having regular menstrual cycles. All volunteers had at least two living children. Blood samples on days 18 and 24 of two preimmunization cycles were analyzed for progesterone levels to confirm ovulation; these ranged from 16 to 51 nM. Women in whom pregnancy was suspected or who were breast-feeding were not included in the trial. In addition, women who had histories of recurrent abortions; secondary infertility; or allergic, autoimmune, or endocrine disorders were also excluded, as were women with known or suspected malignancies.

Immunization consisted of three primary injections of the vaccine at 6-week intervals. Booster injections were given subsequently as and when necessary in order to maintain antibody titers above 50 ng/ml (hCG bioneutralization capacity) to those desiring to continue in the study. Subjects were required to report to the clinic for follow-up in the 1st and 3rd week of each cycle. Blood was withdrawn on each visit and sent to the National Institute of Immunology for determination of antibody titers.

The neutralization capacity of antibodies was estimated by a modification of a receptor binding inhibition assay described earlier (13). Briefly, testes of 2- to 3-month-old Wistar outbred rats were decapsulated and homogenized in a Polytron homogenizer in 50 mM Tris·HCl buffer (pH 7.4) containing 5 mM MgCl$_2$, 0.1% bovine serum albumin, and 0.1% sodium azide. The homogenate was filtered through a nylon mesh and centrifuged at 500 × g for 10 min at 4°C. The resulting pellet was resuspended in 8 ml of Tris buffer per pair of testes; 50 μl of testicular homogenate was incubated with 100 μl of various dilutions of human sera and 1 ng of ^{125}I-labeled hCG (^{125}I-hCG) (8–12 μCi/μg; 1 Ci = 37 GBq) for 2 hr at 37°C. The assay was terminated by the addition of 1 ml of cold Tris buffer. Tubes were centrifuged at 2000 × g; the resulting pellet was washed with Tris buffer and assayed for radioactivity. Dilutions of sera causing a 50% inhibition in the binding of ^{125}I-hCG were computed by regression analysis. Bioneutralization capacity was expressed in ng per ml of serum. The avidity of antibodies for hCG was determined by the cold displacement method essentially as described (15). Briefly, diluted antisera were incubated with labeled hCG in the presence of increasing amounts of unlabeled hCG at 4°C for 24 hr. Bound fractions precipitated by 12.5% PEG 8000 were separated by centrifugation at 1500 × g for 20 min and assayed in an LKB multi-γ-counter. Association constants were computed by nonlinear regression analysis. Cross-reactivity of antisera with human thyroid-stimulating hormone, human follicle-stimulating hormone, and human LH was determined by direct binding assays (15). Progesterone levels in luteal

phase blood samples were estimated by RIA, using reagents supplied by the World Health Organization Matched Reagents Program. Menstrual records and follow-up forms were sent every month to the National Institute of Immunology and Indian Council of Medical Research.

An antibody level of 50 ng/ml (hCG bioneutralization capacity) was fixed as the putative threshold for testing efficacy. For 95% confidence, observations were to be made for at least 750 cycles. Alternative contraception (intrauterine devices or condoms) was prescribed until antibody titers above the threshold were attained, after which all alternative methods were terminated. Results were conveyed to the respective centers within 1 week. In the event of antibody titers declining below 50 ng/ml, subjects had the option of taking a booster injection or withdrawing from the trial. In the latter case, they were followed up until antibody titers declined to levels near 0. In case the cycle was delayed, a pregnancy test was conducted and if pregnancy was confirmed, medical termination was offered by the clinics free of cost, but the option of continuing or terminating the pregnancy was left to the woman.

RESULTS

Of 162 women interviewed, 148 completed the schedule of three primary injections. While all women made antibodies to hCG, 119 (80%) generated titers that were clearly >50 ng/ml. On March 31, 1992, observations were completed on 750 cycles when enrollment was stopped and booster injections were withheld unless specifically requested by the subjects, in accordance with trial conditions approved by institutional ethics committees. Follow-up was continued on women with circulating antibodies. As of August 1, 1993, observations had been recorded on 1224 cycles. One pregnancy had occurred in a woman having an antibody titer of >50 ng/ml (the pregnancy was terminated by vacuum aspiration upon the subject's request). Fig. 1 gives the kinetics of antibody response and period of protection in four representative women. Booster injections to those willing to continue in the study were given at an average of 3 months. Eight women had completed >30 cycles without becoming pregnant, 9 had completed 24–29 cycles, 12 completed 18–23 cycles, 15 completed 12–17 cycles, and 21 subjects had completed 6–11 cycles of continuous exposure to the risk of pregnancy. Twenty-six pregnancies occurred among participants who had titers below the level of 50 ng/ml and who had not used alternative contraceptives effectively (see below).

The antibodies had high affinity for hCG ($K_a \approx 10^{10}$ M^{-1}) and could inactivate hCG bioactivity *in vitro* and *in vivo*. Cross-reactivity of the antibodies to human LH ranged from 10% to 75%, but antibodies were totally devoid of cross-reactivity with human follicle-stimulating hormone and human thyroid-stimulating hormone. Immunization did not change the menstrual regularity; 85% of the cycles were within the normal range (22–35 days). Longer and shorter cycles occurred with similar frequencies in women with antibody titers of >50 ng/ml and in those with titers of <35 ng/ml (Fig. 2), a level at which women could conceive in the absence of alternative contraception. This is consistent with a previous study in which no relationship was seen between the degree of cross-reactivity with human LH and menstrual length (16). Luteal progesterone, estimated in single 3rd-week serum samples, ranged between 14 and 44 nM in ≈80% of the cycles. These levels are indicative of ovulation.

Reversibility. The response to the vaccine was reversible; antibody titers declined in all cases in the absence of booster injections. Although alternative contraceptives were provided, some women became pregnant when antibody titers
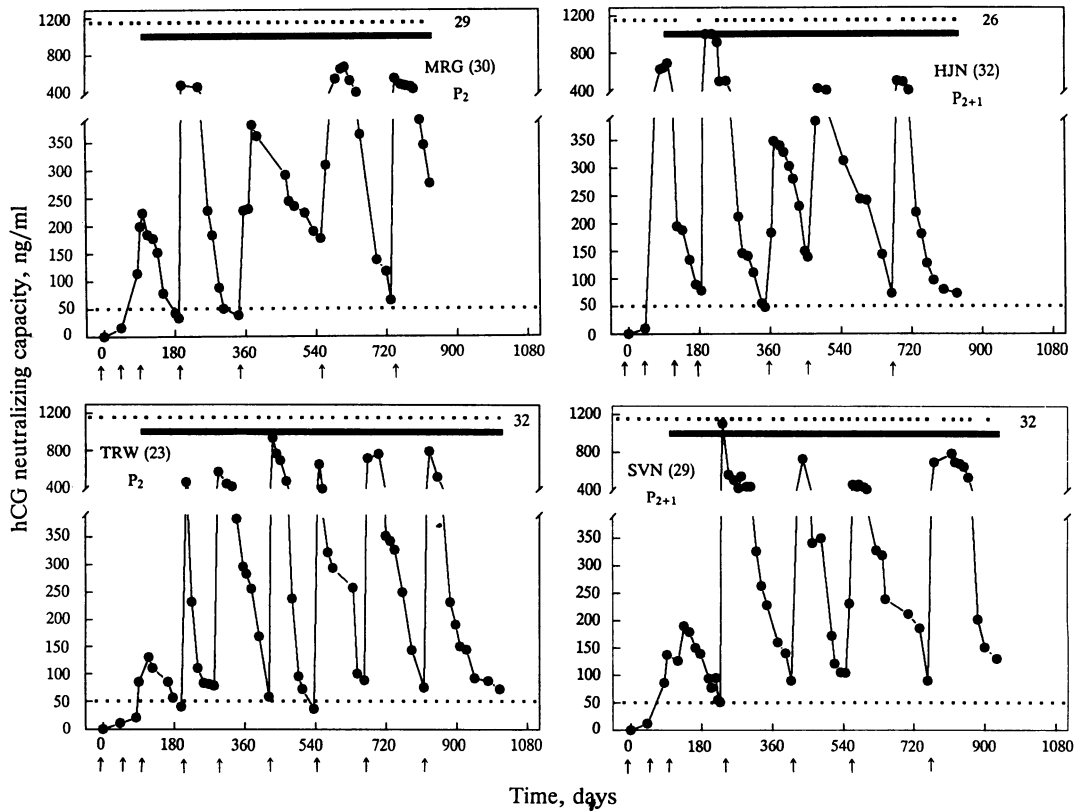
FIG. 1.   Kinetics of anti-hCG response in four subjects after immunization with the HSD vaccine. All subjects (MRG, HJN, TRW, SVN; 30, 32, 23, and 29 years old) were of proven fertility with two live children (P_2); HJN and SVN had also undergone elective termination of an unwanted pregnancy (P_{2+1}). Arrows denote injections with the vaccine at a gonadotropin dose of 300 μg. Rectangles near the top abscissa indicate menstrual events. Periods denoted by solid bars represent cycles in which the woman was exposed to the risk of pregnancy.

were <35 ng/ml. Fig. 3 depicts the case of a subject who, after being protected for 12 cycles, conceived when her antibody titers were <5 ng/ml. Four other women, who conceived at antibody titers of <5, <5, 10, and 20 ng/ml, respectively, carried their pregnancies to term and delivered normal offspring. Of the remaining pregnancies that occurred during periods of low titers, or due to nonadministration of the booster injection, 12 took place at titers at or <20 ng/ml

and 9 were between 21 and 35 ng/ml (Table 1). No late abortions were observed.

DISCUSSION

The present study provides evidence for prevention of pregnancy in women by circulating anti-hCG antibodies. The antibodies have to be directed against determinants that ensure bioneutralization of the hormone. Levels of >50 ng/ml are
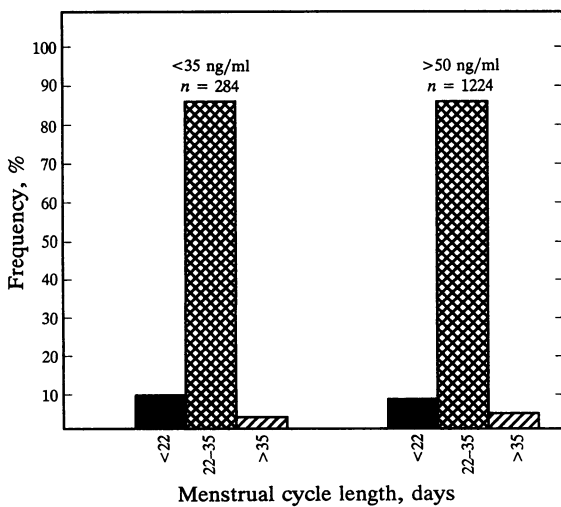

FIG. 2.   Regularity of menstrual cycles in women carrying anti-hCG antibodies of >50 ng/ml hCG bioneutralization capacity; 85% of the 1224 cycles were between 22 and 35 days. Percentage of longer and shorter cycles did not differ significantly from those recorded in women with antibody titers of <35 ng/ml, a level at which women could conceive in the absence of alternative contraception.
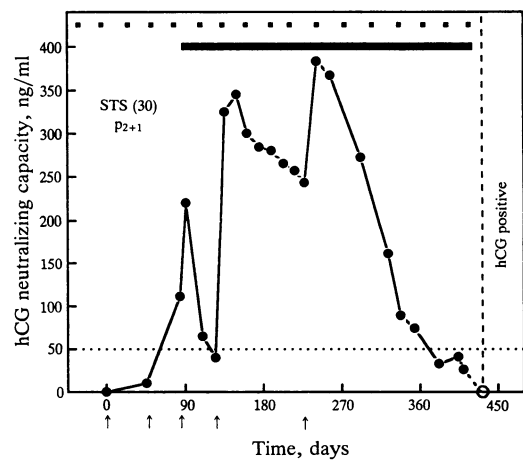
FIG. 3.   Regain of fertility on decline of antibodies. A 30-year-old subject (STS), with two gravidae and one elective abortion (P_{2+1}), on immunization with the vaccine, remained protected from pregnancy for 12 cycles. In the absence of a booster injection, antibody titers declined and she became pregnant in the cycle starting on day 417. The extrapolated antibody titers at midcycle in the fertile month, shown by the dotted line, were <5 ng/ml.

Immunology: Talwar *et al.*

Proc. Natl. Acad. Sci. USA 91 (1994) 8535

Table 1. Antibody titers in women who became pregnant

Subject	Titer at conception*	Cycles after titers fell to <50 ng/ml	Subject	Titer at conception*	Cycles after titers fell to <50 ng/ml
STS	<5	2	NMT	20	1
LLA	<5	2	PVN	20	1
PTB	<5	2	STP	20	1
GTD	<5	4	BLW	20	1
URM	<5	1	LTA	24	1
ANT	10	1	BML	25	1
SUN	10	1	RTS	26	1
DLY	10	1	BND	30	1
SVT	10	2	SRN	30	1
RNU	10	1	SRS	30	1
RMW	10	2	PVT	30	1
BNA	20	2	SNT	35	1
MRA	20	1	BNA	35	1

Subjects becoming pregnant had the choice of continuing pregnancy or seeking its termination. Twenty-two subjects had the pregnancy terminated, four subjects (GTD, URM, ANT, and NMT) carried the pregnancy to term and delivered normal babies (three boys, one girl).
*hCG bioneutralization capacity, ng/ml.

effective but those <35 ng/ml are inadequate to prevent pregnancy. The effective antibodies have high avidity ($K_a \approx 10^{10}$ M^{-1}) for hCG. They are partially cross-reactive with human LH, but this reactivity does not impair ovulation or cause luteal insufficiency. Nonneutralized LH appears to be sufficient to induce ovulation even after partial neutralization by antibodies. This has also been observed in rhesus monkeys immunized with the β subunit of ovine LH (22). The LH cross-reaction does not cause sufficiently deficient corpus luteum function since menstrual cycle regularity is maintained.

These observations provide the basis for a possible birth control vaccine. However, further work is necessary to make this vaccine workable as a general method for family planning. The period of $\approx$3 months before antibody titers build up to protective levels in a course of primary immunization must be covered by a compatible companion approach.

The immune response depends on genetic and nutritional factors. Although the immunogen used in the present vaccine has several determinants, all recipients of the vaccine do not generate a high enough antibody response to hCG. In the present study, 80% of the women produced antibody titers of >50 ng/ml. Further optimization may be required to enhance the immune response, such as the use of a better adjuvant. Encapsulation of the vaccine in microspheres to reduce the number of injections is also desirable, since the present schedule involving multiple injections would not be practical for general use. Such microspheres have been seen to generate a long-lasting antibody response in experimental animals after a single administration (23, 24). A live recombinant vaccine, using vaccinia as the vector, expressing βhCG in alignment with a transmembrane fragment, has also been made that generates a high antibody response of long duration in monkeys (25). This cassette is being transferred to a fowl pox vector, which expresses the inserted genes but does not replicate in humans. It remains to be seen whether this modality of immunization would improve further the antibody response.

The present study demonstrates the feasibility of a birth control vaccine that women could choose on a voluntary basis. Titers for protection have also been defined. Since antibody titers decline spontaneously unless booster injections are given, the duration of effective immunization can be controlled by the woman herself.

1. Primakoff, P., Lathrop, W., Woolman, L., Cowan, A. & Myles, D. (1988) *Nature (London)* **335**, 543–546.
2. Herr, J. C., Flickinger, C. J., Homyk, M., Klotz, K. & John, E. (1990) *Biol. Reprod.* **42**, 181–193.
3. Sacco, A. G. (1987) *Am. J. Reprod. Immunol. Microbiol.* **15**, 122–130.
4. Naz, R. K. (1993) in *Immunology of Reproduction*, ed. Naz, R. K. (CRC, Boca Raton, FL), pp. 279–291.
5. Ladd, A., Tsong, Y. Y., Prabhu, G. & Thau, R. (1989) *J. Reprod. Immunol.* **15**, 85–115.
6. Moudgal, N. R., Ravindranath, N., Murthy, G. S., Dighe, R. R., Aravindan, G. R. & Martin, F. (1992) *J. Reprod. Fertil.* **96**, 91–102.
7. Talwar, G. P., Pal, R., Arunan, K., Om Singh, Sad, S., Suri, A., Shaha, C., Gupta, S. K. & Upadhyay, S. N. (1992) in *Contraception: Newer Pharmacological Agents, Devices, and Delivery Systems*, eds. Sitrukware, R. & Bardin, C. W. (Dekker, New York), pp. 161–192.
8. Talwar, G. P., Sharma, N. C., Dubey, S. K., Salahuddin, M., Das, C., Ramakrishnan, S., Kumar, S. & Hingorani, V. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 218–222.
9. Shahani, S. M., Kulkarni, P. P., Patel, K. L., Salahuddin, M., Das, C. & Talwar, G. P. (1982) *Contraception* **25**, 421–434.
10. Om Singh, Shastri, N., Narang, B. S. & Talwar, G. P. (1982) in *Cellular and Humoral Mechanism in Immune Response* (Dept. of Atomic Energy, New Delhi, India), pp. 114–118.
11. Talwar, G. P., Om Singh & Rao, L. V. (1988) *J. Reprod. Immunol.* **14**, 203–212.
12. Talwar, G. P. & Om Singh (1988) in *Contraception Research for Today and the Nineties*, ed. Talwar, G. P. (Springer, New York), pp. 183–199.
13. Pal, R., Om Singh, Rao, L. V. & Talwar, G. P. (1990) *Am. J. Reprod. Immunol.* **22**, 124–126.
14. Gaur, A., Arunan, K., Om Singh & Talwar, G. P. (1990) *Int. Immunol.* **2**, 151–155.
15. Om Singh, Rao, L. V., Gaur, A., Sharma, N. C., Alam, A. & Talwar, G. P. (1989) *Fertil. Steril.* **52**, 739–744.
16. Kharat, I., Nair, N. S., Dhall, K., Sawhney, H., Krishna, U., Shahani, S. M., Banerjee, A., Roy, S., Kumar, S., Hingorani, V., Om Singh & Talwar, G. P. (1990) *Contraception* **41**, 293–299.
17. Talwar, G. P., Hingorani, V., Kumar, S., Roy, S., Banerjee, A., Shahani, S. M., Krishna, U., Dhall, K., Sawhney, H., Sharma, N. C., Om Singh, Gaur, A., Rao, L. V. & Arunan, K. (1990) *Contraception* **41**, 301–316.
18. Nash, H., Talwar, G. P., Segal, S., Luukkainen, T., Johannsson, E. D. B., Vasquez, J., Coutinho, E. & Sundaram, K. (1980) *Fertil. Steril.* **34**, 328–335.

19. Sehgal, S. (1992) in *Frontiers in Reproductive Physiology*, eds. Ghosh, D. & Sengupta, J. (Wiley, New Delhi, India), pp. 225–229.

20. Thau, R. B., Wilson, C. B., Sundaram, K., Phillips, D., Donelly, T., Halmi, N. S. & Bardin, C. W. (1987) *Am. J. Reprod. Immunol. Microbiol.* **15**, 92–98.

21. Thau, R. B. (1988) in *Contraception Research for Today and the Nineties*, ed. Talwar, G. P. (Springer, New York), pp. 217–230.

22. Thau, R. B., Sundaram, K. & Thornton, Y. S. (1979) *Fertil. Steril.* **31**, 200–204.

23. Stevens, V. C., Powell, J. E., Rickey, M., Lee, A. C. & Lewis, D. H. (1990) in *Gamete Interaction: Prospects for Immunocontraception*, eds. Alexander, N. J., Griffin, D., Spieler, J. M. & Waites, G. M. H. (Wiley–Liss, New York), pp. 549–563.

24. Singh, M., Om Singh, Singh, A. & Talwar, G. P. (1992) *Int. J. Pharm.* **85**, R5–R8.

25. Srinivasan, J., Om Singh, Pal, R., Lall, L., Chakrabarti, S. & Talwar, G. P. (1993) in *Local Immunity in Reproductive Tract Tissues*, eds. Griffin, P. D. & Johnson, P. M. (Oxford Univ. Press, New Delhi, India), pp. 477–481.