

REVIEW ARTICLE

RESEARCH IN REPRODUCTION: THE INDIAN SCENARIO
IN THE LAST DECADE [II]*

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Abstract : Developing new, improved and totally safe, effective and acceptable contraceptives based on the recent advances in cellular and molecular biology of reproduction is a new challenge to biomedical scientists involved in research in reproductive biology. The present article reviews some of the major contributions made during the last decade by scientists working in India in developing new strategies and technologies for better human reproductive health and fertility regulation.

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INTRODUCTION

In a UNICEF report it has been stated, "Family planning could bring more benefits to more people than any other single technology now available to the human race" (1). The necessity of developing a strategy for woman centred agenda had been highlighted at the Cairo International Conference on Population and Development (2) and it emphasized on three specific needs which were previously unmet by the currently available methods: women-controlled methods that provide additional protection against sexual transmitted infections; methods which a woman can use as a back-up when exposed

to unprotected sexual intercourse and which will decrease the resort to abortion; expanded male contraceptive choices, participation and responsibility (3). Now the challenge for scientists in the related areas is to translate this concept into developing new, improved and totally safe, effective and acceptable contraceptives based on the recent advances in cellular and molecular biology of reproduction. In this review I have attempted to tell the story of the contributions made during the last decade by scientists working in India in developing new strategies and technologies for better human reproductive health and fertility regulation.

*For part I of the article, see *Indian Journal of Physiology and Pharmacology* 2000; 44(3): 237-254

Physico-chemical methods for fertility regulation in the male

At the Centre for Biomedical Engineering of the All Indian Institute of Medical Sciences, Guha and colleagues developed an injectable method for inducing vas deferens occlusion using maleic anhydride with dimethyl sulphoxide (DMSO) as its solvent (4). A special feature of this treatment was that of spontaneous re-establishment of sperm passage with time, and the restoration period could be regulated from three months to five years. Phase I clinical trials on male volunteers showed that the treatment was well tolerated with only minimal side effects and with no long-term adverse effects (5). These results paved the way for the phase II clinical trials and the drug, styrene maleic anhydride (SMA) in DMSO [now named as *Risug*] was successfully administered to sexually active healthy male volunteers whose wives were without any form of contraceptive support. The results obtained from this study were encouraging as one year treatment led to azoospermia and provided pregnancy protection (6). At the University of Rajasthan, Jaipur, Lohiya and colleagues examined the feasibility of a spacing method for contraception using SMA as a vas occlusive agent in male langur monkeys (7). The results suggest that the SMA-based spacing technique for male contraception could be extrapolated to the human by use of no-scalpel injection and non-invasive reversal. Ultrastructural studies revealed that after non-invasive reversal, the vas epithelium regained a state of normalcy as evidenced by prominent plasma membrane, nucleus, cytoplasmic organelles, and stereocilia. The results

suggest that exfoliation of epithelium due to vas occlusion by SMA is reversed after 150 days of noninvasive reversal (8), while necrospermic status of the spermatozoa during initial ejaculations have been suggested to offer instant sterility after vas occlusion with SMA (9).

Use of plant products for the development of novel contraceptive strategies

As an alternate approach to vasectomy, long-term contraception in the male was tested at the National Institute of Immunology, New Delhi, by injecting a single dose of oil extracted from a traditional Indian plant, Neem (*Azadirachta indica*) into the vas deferens of the rat (10). The authors reported an antifertility response throughout the 8 month observation period without any inflammatory or obstructive changes in epididymis and vas deferens, and with no change in testosterone levels, however spermatogenic block was observed which was presumably mediated by local immune mechanism, though anti-sperm antibody could not be detected. Subsequently, polyherbal neem as a cream preparation was developed by this group and was tested in rabbits and monkeys; it showed contraceptive efficacy after intravaginal application and was found to be safe in subacute toxicity studies performed in monkeys (11). The spermicidal activity of neem oil was first reported by Riar and colleagues of the Defense Institute of Physiology and Allied Sciences, Delhi (12). Subsequent studies revealed that the spermicidal action with loss of motility was due to the formation of pores and vesicles on sperm head indicating damage to cell membrane; supplementation of

pentoxifylline which is known to enhance motility could not reverse the spermicidal action of neem oil (13). In addition, the use of neem as a spermicidal agent would provide protection to women against a variety of microorganisms and sexually transmitted diseases (14).

The long-term contraceptive potential of neem for women was proposed by Upadhyay et al. (15), using an intrauterine mode of delivery. The numbers of MHC II antigen positive cells were found to be high in uterine endometrium of bonnet monkeys following such neem treatment. Oral administration of purified neem extract, *Praneem*, to pregnant rats led to resorption of embryos with elevated levels of interferon gamma (IFN- γ) and tumour necrosis factor-alpha (TNF- α) (16). A post-implantation abortifacient response to orally administered neem was similarly reported for the rabbit and the baboon, and the effect was shown to be reversible (17). The partially fractionated active principle of neem has been suggested to function as an immunomodulator in causing pregnancy failure with decline in chorionic gonadotropin (CG) and progesterone levels in the baboon, and increase in CD4+ and CD8+ cells in spleen and mesenteric lymph nodes (18). The active principal in neem responsible for reversible anti-fertility response in rodent, lagomorph and primate species was shown to be a mixture of six components which include saturated mono- and di-unsaturated free fatty acids and their methyl esters (19). A post-coital action of neem oil has been suggested based on the observation that *in vitro* exposure of two cell mouse embryos to neem oil led to failure of blastocyst development, trophoblast

attachment and proliferation and loss of fertility (20). At the Indian Institute of Chemical Biology, Calcutta, Pakrashi and colleagues investigated the anti-ovulatory (21) and anti-spermatogenic (22) potential of *Malviscus conzatti* flower in the rat.

Lohiya and colleagues at the University of Rajasthan, Jaipur have performed experiments with several plant products using small laboratory animals and non-human primate species. Using purified gossypol acetic acid alone and in combination with potassium chloride, the antifertility action and possible hypokalemia were tested in adult male langurs for 120 days. The treatment resulted in severe oligospermia, with impairment of sperm motility. The functions of accessory glands and libido, however, remained unimpaired. Complete reversal of these changes were noted after 90 to 105 days of withdrawal of treatment leading the investigators to suggest that, while oligospermia achieved was reversible, the hypokalemic response of langurs is similar to human and not related to impurity of gossypol (23, 24). Monkeys receiving gossypol plus potassium salt showed normal serum potassium level. Scanning electron microscopy of spermatozoa revealed deleterious abnormalities in head and midpiece; testicular morphology following gossypol exposure resulted in a decrease in seminiferous tubule diameter and arrest of spermatogenesis (25). The contraceptive efficacy of a chloroform extract of papaya seeds was investigated, and it was shown to induce a defect which was reversible and was observed to be mainly post-testicular in nature without influencing toxicological profile and libido in rats and rabbits

(26–28). Reversible sterility could also be induced in male rats by using an aqueous extract of papaya seeds without any adverse effects on libido and on toxicological profile (29).

The fields of family planning and the prevention and cure of sexually transmitted disease (STDs), including HIV/AIDS have traditionally been operated independently with the underlying operating assumption that the STDs are not of concern to married couples creating families; for them the risk of sexually transmitted diseases are generally considered trivial. However, epidemiological and sociologic facts now indicate that an increasing size of global population is simultaneously vulnerable to unwanted conception and unwanted infection (30). Thus, targeted drug delivery via the vaginal route may pave the way for the development of new methods for protection against pregnancy and STDs, thereby allowing improved reproductive health care. The hypothesis that potent anti-microbial, anti-tumour agents like magainin peptides, and antibiotic and anti-angiogenic agents like fumagillin can inhibit blastocyst implantation has been tested in the rhesus monkey model at the All India Institute of Medical Sciences. These agents were intravaginally administered through tampons to proven-fertile, mated rhesus monkeys and were observed to cause one hundred per cent pregnancy protection without any marked change in menstrual cycle length of treatment cycles and of subsequent cycles. Thus, intravaginal delivery of anti-microbial agents like magainins and fumagillol may lead to the development of potential anti-implantation strategy for interception of pregnancy (31, 32).

Hormonal contraception for the male

There is a need for more participation by men in fertility regulation, however, a significant degree of gap exists between the need and the demand for novel male contraceptives and the state of understanding of the basic knowledge of the functions of the male reproductive system. Sustained research efforts are therefore needed to provide broader contraceptive choices and to make men share the responsibility and the burden for fertility regulation with their partners.

Taking a lead from the report of Ready and Rao (33) that daily application of testosterone (T) to men resulted in suppression of spermatogenesis and azoospermia, and normal spermatogenesis regained with the withdrawal of the treatment, Rajalakshmi and coworkers at the All India Institute of Medical Sciences, New Delhi, studied the pharmacokinetics of a long acting androgen, testosterone enanthate (TE) in monkeys (34). They observed a spurt of supra-physiological levels of testosterone within 3 days of treatment which was followed by a gradual decline; dihydrotestosterone (DHT) and estradiol levels were also found to be elevated (34). Since similar data were also available from human studies with TE, a concern was voiced whether exposing the male to intermittent spurts of supra-physiological levels of androgens and estrogens could induce patho-physiological changes in the target organs. To this effect, the actions of long-term use of TE on prostate structure and function using the rhesus monkey as a primate model was critically evaluated (35). Long-term

exposure to TE (once in two weeks for 33 months) resulted in an increase in weight of cranial and caudal lobes of prostate, cellular hypertrophy and increased secretory function of cells, cellular hyperplasia, increased levels of estradiol and prostate-specific acid phosphatase and marked increase in fibromuscular stroma in central and peripheral zones of both lobes of prostate in adult male monkeys. In an attempt to understand the direct actions of steroid hormones and growth factors on prostate physiology, prostate epithelial cells were collected from rhesus monkeys, isolated and cultured in flasks coated with either collagen IV or laminin; the influence of growth factors such as IGF I and IGF II in combination with androgens such as dihydrotestosterone and androstenedione on cell number, thymidine incorporation and secretory activity were assessed. Of the two IGFs, IGF I was more effective than IGF II. DHT with IGFs was more potent in inducing proliferation, differentiation and secretion than androstenedione. It is considered that using such a primary culture system the physiology of prostatic epithelial cells can be monitored *vis-a-vis* the actions of drugs that can inhibit cell proliferation (36).

Though there was suppression of the nocturnal rise in T level, TE injection to rhesus monkeys led to increased bioavailability of T which favoured a change in its metabolism by the liver and instead of androstenedione, androsterone became the major metabolite as spermatogenesis became arrested. Liver function tests revealed that long-term TE exposure led to increased liver transaminases (SGOT and SGPT) and these enzymes returned to baseline values during the recovery period

(37). In monkeys subjected to controlled dietary conditions, long-term exposure to TE failed to show any changes in glucose tolerance test, however, serum insulin levels decreased significantly (38). The effects of a long-acting androgen ester testosterone buciclate (TB) administered on days 1 and 91 of a 360 day study period to bonnet monkeys revealed suppression of testicular and epididymal functions suggesting that this long-acting androgen may have the potentiality to induce and maintain reversible sterility (39). The rise in T levels following TB injection was gradual and peak values were attained by day 14 after injection and the levels were found to be within the normal range. Similarly, the levels of dihydrotestosterone was within the normal range and though estradiol level was elevated, it was lower compared to the rise observed following TE injection (40). At the National Institute of Health and Family Welfare, New Delhi, Das and colleagues observed that STS-557 (17-alpha-cyanomethyl-17-beta-hydroxy-estra-4,9(19)-dione-3-one) exhibited contraceptive potential when tested in the bonnet monkey. Daily treatment for 12 weeks resulted in significant decline in count, motility, acrosin and hyaluronidase activities and in the fertilizing ability of spermatozoa by sixth week of treatment. The blood testosterone profile declined by the second week coinciding with rising level of STS-557 in circulation (41, 42).

Hormonal antagonists for pregnancy interception

It is established that embryo implantation occurs in a favourable uterine milieu which exists in estrogen-primed, progesterone-dominated state of

endometrium (44). It is therefore likely that methods to intercept the process of embryo implantation in a potential conception cycle without compromising normal menstrual cyclicity could provide potential new tools for developing user-acceptable, safe and need-driven contraceptive strategies for women.

Centchroman, a non-steroidal antifertility agent with weak estrogenic and potent anti-estrogenic activities was approved for its use as 'once-a-week' pill in India since 1991. The drug discovered by the Central Drug Research Institute of Lucknow is thought to exert its contraceptive action by disrupting the balance of estrogen and progesterone necessary to prepare the uterus for implantation. Centchroman is also reported to accelerate ovum transport in animal models. The resulting asynchrony between uterine preparation and ovum transport prevents implantation of the fertilized egg (45). The contraceptive efficacy and safety of 30 mg centchroman administered bi-weekly for the first three months followed thereafter at weekly intervals was studied in a multicentre trial. A total of 377 women volunteers were covered for 3932 months of use with pregnancy protection and Pearl index of 1.83. About 90% menstrual cycles were within the normal range and about 4% were prolonged. The contraceptive effect was readily reversible and subsequent pregnancies were normal (46). Roy has chronicled the unfolding saga of an anti-estrogenic compound, clomiphene citrate in its failure to be used as a fertility regulating agent, while it obtained approval, on the other hand, as a drug now widely used for the treatment of ovulatory failure (47), and

has outlined the earlier studies of these two fertility regulating drugs, clomiphene citrate and centchroman and their current uses (48).

The development of novel anti-progestins which bind to progesterone receptor at the cellular level to inhibit progesterone action in target cells has allowed for the elucidation of the hormonal basis of the cellular and molecular events leading to embryo implantation. Anti-progestins are now being widely used to develop retro-active contraception which include emergency contraception, luteal contraception and menses-inducers to be used at the expected time of menstruation. It is anticipated that the development of such contraceptive technology will help to minimise the increasing incidence of unsafe abortion.

Antiprogestins such as lilopristone (ZK98734), onapristone (ZK98299) and mifepristone (RU486) have potent anti-progestational as well as variable degree of anti-glucocorticoid activities as judged from *in vivo* and *in vitro* animal experiments (49). At the Institute for Research in Reproduction, Mumbai, Puri and colleagues have used onapristone (ZK98299) to study its effects on pregnancy and fetal outcome in bonnet monkeys (50). Limited data on 8 animals demonstrated that onapristone given for four consecutive days between days 20 to 30 post-ovulation terminated pregnancy in 62% animals and in cases where treatment failed the endocrine function of placenta was affected and fetal growth retarded. Use of another antiprogestin, lilopristone at different time-points after ovulation resulted in luteolysis

and pregnancy failure due to its actions on gonadotrophin release in the bonnet monkey (51). Administration of onapristone at weekly intervals to adult bonnet monkeys for 10 weeks resulted in inhibition of ovulation in all monkeys studied with the arrest of typical follicular phase rise of estradiol and of the midcycle surge in the levels of bioactive LH along with very low level of progesterone throughout the cycle causing atrophy of endometrium (52). Lilepristone administered on a weekly regime to bonnet monkeys inhibited folliculogenesis which was reversed by exogenous FSH and LH administration (52). In a study using cyclic bonnet monkeys, Ishwad et al. (53) observed that weekly, low dose administration of onapristone did not block ovulation but had an inhibitory effect on endometrial development (54). Low dose onapristone administered throughout the menstrual cycle prevented pregnancy without disturbing menstrual cycle and ovulation in majority of cycles studied in bonnet monkeys (55). The binding characteristics of onapristone to progesterone receptors in human myometrial cytosol was also examined (56). The results of this study revealed that although progesterone and onapristone are mutually competitive for binding to progesterone receptor, onapristone also has distinctive binding sites. Further studies to characterize such binding sites for onapristone to progesterone receptor in human myometrial cytosol revealed that the antiprogesterin effects of onapristone may result from suboptimal nuclear binding/retention of antiprogesterin-receptor complexes (57). The relative binding affinity of onapristone for nuclear progesterone receptor was 33% of that for progesterone. Kumar and colleagues at the Institute for

Research in Reproduction, Mumbai have also studied the effects of daily interanasal administration of norethisterone (NET) on folliculogenesis, cervical mucus, vaginal cytology and endometrial morphology in the human, which indicated its potential use as an antifertility agent (58).

Understanding the cellular and molecular basis of blastocyst implantation, and the development of novel anti-implantation strategies using the rhesus monkey as a primate model is the focus of attention of Sengupta and colleagues at the All India Institute of Medical Sciences. In the pre-ovulatory phase, progesterone has been suggested to play a critical role in the regulation of mid-cycle gonadotrophin surge leading to delayed ovulation and altered profiles of ovarian hormones. Thus it is possible that administration of mifepristone, an anti-progesterin during the peri-ovulatory stage may render hostility to pregnancy establishment by acting at the ovarian level and at the endometrial level. Using the rhesus monkey as a primate model, Ghosh et al. (59) aimed to test two hypotheses: (i) inhibition of prevulatory progesterone action can delay or inhibit ovulation, and (ii) inhibition of preovulatory progesterone action can inhibit post-ovulatory phase endometrial receptivity for implantation. The results of this study supported earlier reports that follicular phase mifepristone can inhibit or disrupt follicular maturation and delay ovulation, however, it failed to inhibit implantation, because gonadal hormones including progesterone, resume normal functions once ovulation takes place. It appears that peri-ovulatory stage mifepristone treatment shall not provide pregnancy protection to women.

The use of low dose, single administration of mifepristone, an anti-progestin during the early luteal phase, however, leads to one hundred per cent pregnancy protection in the rhesus monkey without affecting menstrual cyclicity (60). Similar results were observed in women by a Swedish group of investigators (61). Mifepristone action during the early luteal phase led to endometrial contraception with significant degree of desynchronization and loss of receptivity for blastocyst implantation (62). Furthermore, as has been discussed earlier, the absence of dialogue between the developing preimplantation embryo and receptive stage endometrium was associated with failure of preimplantation embryo growth and viability and lack of gestational stage endometrial glandular hyperplasia and vascularity (63, 64). Anti-nidatory action of luteal phase mifepristone was found to be associated with changes in endometrial prostaglandin concentration during the implantation window (65). The hypothesis that prostaglandins are involved in the anti-nidatory action of mifepristone was further tested and it was observed that the anti-gestation activity of mifepristone and associated endometrial changes could not be accentuated or attenuated with co-administration of prostaglandin E or cyclo-oxygenase inhibitor (diclofenac), nor could these be mimicked by these agents alone (66, 67). The authors concluded that the anti-nidatory action of mifepristone is most likely mediated through a multifactorial mechanism and the prostaglandin milieu is one of the modules in the complex mechanism. However, for mifepristone to be used as an early luteal phase, once-a-month contraceptive, a simple

method to detect ovulation needs to be developed. Few leads have emerged from the use of mifepristone as a tool to induce endometrial de-synchronization, loss of endometrial receptivity and failure of implantation in the primate. The studies revealing the distribution, and regulation of local factors such as vascular endothelial growth factor (VEGF) and placental protein 14 (PP14) in receptive and non-receptive stage endometria suggest that they may serve as specific targets to modulate endometrial differentiation towards receptivity for blastocyst implantation (68, 69). The close association of VEGF and another angiogenic factor, placental growth factor (PIGF) in influencing vascular and endometrial functions at the time of implantation have been examined based on their localization and expression in the primate uterus (68).

Immuno-contraception

Vaccines for the control of fertility are likely to have an important impact in improving the currently available reproductive health options. Basic research in the field of gonadotropins and their actions on target cells led to the testing of the long-term contraceptive efficacy of ovine FSH vaccine by Moudgal and coworkers in healthy, fertile bonnet monkeys using pure FSH from sheep pituitaries at the Indian Institute of Science, Bangalore (70). The vaccine elicited an immunogenic response in all ten monkeys studied. Immunization for 4.7–5.7 years did not affect the health and libido of the animals. Concentration of T in serum remained normal, but within 150 days of immunization there was a marked decrease (75–100%) in the number

of spermatozoa in seminal ejaculates. All ten animals proved infertile in repeated matings with females of proven fertility and after stopping booster injections, nine out of ten animals regained fertility which closely correlated with the rate of decline of antibody titres. Srivastava and Das (71) reported that male bonnet monkeys were rendered oligospermic, but not azoospermic following active immunization with ovine FSH. The percentage of sperms having good motility was reduced with concomitant increase in sperm ATPase activity. Eight out of ten monkeys failed to impregnate females of proven fertility after mating for three consecutive cycles while the other males impregnated after cohabitation with females at a time when the antibody titre was reduced. Thus active immunization with ovine FSH may not produce azoospermia but renders infertility to male monkeys provided sufficient antibody titre is maintained. Immunization of proven fertile male adult monkeys with recombinant FSH receptor protein preparation representing 1-134 amino acids of the extracellular domain of the receptor results in production of receptor blocking antibodies. Serum T and LH levels remained unchanged following immunization but there was 50% reduction in transformation of spermatogonia (2C) to primary spermatocytes (4C) and the 4C: 2C ratio showed a correlative change with reduction in fertility index (sperm counts \times motility score) and monkeys became infertile between 242-368 days. The observed actions were near identical to that seen following immunization with FSH (72).

Human male volunteers were examined to test their response to ovine FSH after

immunization on days 1, 20, 40 and 70 (73). The kinetics of antibody production for both the immunogen (oFSH) and the cross reactive antigen (hFSH) were essentially similar. The volunteers responded to the first two immunizations with production of antibody which was specific for FSH and had no significant change in the values of related glycoprotein hormones (LH, TSH). Seminal plasma protein, transferrin, a marker of Sertoli cell and of seminiferous tubular function showed reduction (30-90%) following immunization with oFSH with reduction in sperm counts (30-74%). Thus, long-term blockade of endogenous FSH action in men using oFSH as an immunogen can potentially be used in reducing the quantity and the quality of spermatozoa resulting in infertility. Moudgal et al. (74) have commented that male contraceptive vaccines are well tolerated by the primate including the human and do not give rise to any known toxic symptoms or immediate health hazards. Contraceptive vaccines for the male thus appear to be a feasible strategy. Close attention is however needed to evaluate carefully the bioefficacy of antibodies raised to recombinant ovine FSH beta or FSH receptor over the LHRH/LH based vaccine since the former do not require exogenous testosterone supplementation to maintain accessory gland function and libido. While the LHRH/LH vaccine results in azoospermia, the FSH vaccine causes the production of low numbers of poor quality sperms which fail to impregnate cyclic females.

At the National Institute of Immunology, Gupta and coworkers have identified the peptide epitopes of porcine zona glycoproteins (pZPC) as an initial step in

an immuno-contraceptive vaccine based on synthetic peptides corresponding to pZPC or its homologues in other species (75). Immunization of female bonnet monkeys with pig zona pellucida glycoprotein ZP3 using adjuvants permissible for human use led to infertility; fifty per cent of animals regained fertility with declining antibody titre (76). The zona pellucida glycoprotein 3 alpha (ZP3alpha) is the primary sperm receptor ligand in porcine gamete interaction, and its epitopes have been mapped by using monoclonal antibodies. The authors suggest that such studies will help in designing synthetic peptide-based immuno-contraceptive vaccine (77, 78).

The immuno-contraceptive potential of vitamin carrier proteins in influencing pregnancy has been the focus of attention of Adiga and colleagues at the Indian Institute of Science, Bangalore. Studies in the rat revealed that bionutralization of endogenous maternal riboflavin carrier protein (RCP) by antibody against denatured avian vitamin carrier resulted in higher rates of fetal resorption as against animals immunized with the native protein (79). Passive immunization of pregnant rats on days 10 to 12 of gestation with monoclonal antibody to chicken thiamin carrier protein resulted in resorption (80). Leydig cells of adult rat testis have been shown to synthesize and secrete estrogen-inducible RCP under positive regulation of LH (81). Sertoli cells were shown to synthesize and secrete *in vitro* an estrogen-inducible RCP in culture suggesting a functional role of this protein as a carrier of riboflavin to developing germ cells in rodents (82). Riboflavin carrier protein (RCP) has also been localized on germ cells, and also on

acrosomal surface of mature spermatozoa from different mammalian species (83). The authors suggest that testicular RCP may play a role in cell-cell communication. Similar to RCP, thiamin carrier protein (TCP) has also been shown to be synthesized and secreted by rat Leydig cells (84) and Sertoli cells *in vitro* (85). The immuno-contraceptive potential of RCP has been highlighted by Adiga et al. (86) and it has been suggested that RCP or its defined fragments could be a novel, first generation vaccine for regulating fertility in both sexes. Immunohistochemically RCP has been localized on ovulated oocytes and early embryos, and active immunization in rats and bonnet monkeys with avian RCP prevents pregnancy without causing adverse physiological effects on the mother in terms of her vitamin status, reproductive cycles and reproductive endocrine profiles. Active immunization of male rats and monkeys with denatured RCP which is more effective in eliciting neutralizing antibodies markedly reduces fertility by impairing the fertilizing potential of spermatozoa (86).

At the National Institute of Immunology and at the International Centre for Genetic Engineering and Biotechnology, New Delhi, Talwar and colleagues have been developing a vaccine to prevent pregnancy. Talwar et al. (87) reported the development of a vaccine for inducing antibodies against human chorionic gonadotrophin (hCG). The vaccine which had earlier been shown to be reversible, and without any notable side effects on endocrine, cardiovascular and other body functions was observed to require an antibody titre ≥ 50 ng of hCG bionutralization capacity per ml. Talwar

and colleagues (88) now report that 148 women of proven fertility who were volunteers for the phase II efficacy trial for the HSD-hCG vaccine showed high efficacy with 1 pregnancy in 1224 cycles, at or above 50 ng/ml led to conceptions indicating regain of fertility (89). The antibody response was found to be predominantly against an epitope in the core part of the beta hCG molecule. Besides its use in the reversible control of fertility, hCG vaccine also has the potential to inhibit tumour growth as various types of cancers including lung carcinoma cells produce hCG (90). To minimize multiple injections for antibody titre maintenance, Singh et al. (91) have reported the development of a biodegradable delivery system for a birth control vaccine as tested in the rat and monkey models. A single injection of the immunogen entrapped in microspheres generated a response comparable to that obtained by the same immunogen on alum injected at a monthly interval and antibodies generated had good bioneutralization capacity indicating immunogen integrity.

The potential pitfalls in any immuno-contraceptive strategy must be seriously considered; these include cross-reactivity of antibody with non-target tissues, failure to produce antibody titre, failure to sustain

adequate antibody titre for a sufficient period of time, high degree of individual variation and induction of incomplete abortion (92). Thus the future considerations in the development of safe and effective vaccine for fertility regulation will need to take into account the mechanisms that exclusively cause cell-mediated immune responses and the definition of antibody titres to assess the role that each plays in infertility and cell and tissue damage (93).

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