Contraceptive vaccines

D. K. Giri and G. P. Talwar

National Institute of Immunology, JNU Complex, Aruna Asaf Ali Marg, New Delhi 110 067, India

Fertility control by immunological approaches is no longer a fantasy. It can be achieved in both male and female with no significant side-effects. Besides the feasibility of using these vaccines in animal fertility control, birth control vaccines for human use, have reached the stage of clinical trials. A vaccine directed against hCG is at the most advanced stage and is the first to provide evidence on the ability of the vaccine to prevent pregnancy in women at and above 50 ng/ml hCG antibody titre. The vaccine is safe and reversible.

On the grounds that the hormonal subunits made ectopically by such tumours act as autocrine growth factors for the cancer cells, the recombinant hCG vaccine is under trial in Mexico in lung cancer patients of the type that secrete hCG. Another vaccine directed against LHRH is under trial in hormone-dependent prostate carcinoma patients. Two CMI vaccines employing purified extract of neem seed hold promise for regulating female and male fertility without impairment of sex steroid production, vigour and libido.

VACCINES were traditionally developed against pathogens ‘foreign’ to the body. It has, however, been possible to devise vaccines that can induce either antibody or cell-mediated immune (CMI) response selectively directed against a hormone or a gamete antigen involved in reproduction. What began as an idea in the mid-seventies has become a feasible reality today. Vaccines against three hormones, viz. hCG, LHRH and FSH, have reached the stage of clinical evaluation. A vaccine against the human chorionic gonadotropin (hCG) has not only passed through Phase I clinical trials in India and four countries abroad, but has also completed successfully Phase II clinical trials in three major centres in the country, demonstrating the safety, reversibility and efficacy of the vaccine to prevent pregnancy in women. Novel CMI vaccines for males and females are on the anvil employing immunomodulators from neem (Azadirachta indica). Finally, some vaccines originally made for fertility control are likely to find applications in hormone-dependent cancers. The hCG vaccine is in clinical trial in Mexico in lung cancer patients carrying a type of tumour that secretes hCG and its subunits. Similarly, LHRH vaccine is approved for trials in prostate carcinoma patients. What follows is a brief review of these developments. In the context of this special issue, discussion will be largely confined to the vaccines developed by us.
HCG vaccines

HCG as a target for immunointerception was the choice made by both WHO and our group due to several reasons. HCG is an early signal of conception and is essential for establishment and maintenance of pregnancy during the first seven weeks. It is normally responsible for the missing of the menstrual period, the first biological sign of pregnancy to a woman, by its action on corpus luteum to continue the production of progesterone. The neutralization of the hormonal signal of HCG by circulating antibodies negates the HCG support to corpus luteum and the immunized women menstruate at the expected time even if the month is fertile (Figure 1). There are more than one indications that the action of hCG vaccine is exercised at the preimplantation stage; thus, pregnancy, which is deemed to start after the attachment of the embryo to the uterus, does not take place. This contention is also backed by the logic that continued synthesis of progesterone from corpus luteum of ovaries is essential for preparation of the endometrium to receive the embryo. The denial of corpus luteum support by the anti-hCG antibodies cuts off the progesterone production and endometrial receptivity. Thus, in choosing hCG, one intervenes at a point which is post-ovulatory but prior to the onset of pregnancy. The immunointervention does not disturb the normal physiological functions of ovulation and secretion of normal sex steroid hormones takes place in the same manner as in the non-pregnant state. In this respect the vaccine approach will be preferable to the contraceptives, which act by blocking ovulation and stopping the secretion of normal sex steroids, which are replaced by the synthetic steroids given.

Other reasons for the choice of hCG was the fact that this hormone, in contrast to gamete antigens, was available from natural sources and that the subunit composition and the primary structure of subunits were known.

Three hCG vaccines devised

HCG is composed of two subunits, α and β. The α-subunit is common to three other pituitary hormones. The choice was thus to use β-subunit of HCG or a sub part of it. Accordingly, two types of vaccines were made. Stevens, with support from WHO Task Force, employed the 37 amino acids carboxy terminal peptide (CTP) of β-hCG, whereas Talwar et al.3 opted to employ the entire β-hCG. The rationale for choosing CTP was the non-occurrence of this peptide in β-hLH, thus the expectation that the antibodies would be non-cross-reactive with hLH. Subsequent findings have, however, unexpectedly shown that CTP-induced antibodies react with somatostatin-producing cells of the pancreas. The entire β-subunit of HCG is much more immunogenic than CTP and the antibodies have better capacity for inactivation of the bioactivity of hCG5,6. Furthermore, the antibodies have no autoantibody reactivities7, and do not react with pancreas or other tissues8. These are no doubt partly cross-reactive with hLH but the degree of cross-reaction does not impair ovulation in primates or in humans. Long-term (5–7 years) toxicity studies in monkeys with β-oLH, which induces antibodies cross-reactive with monkey LH and monkey CG did not produce any pituitary or kidney pathology9,10. These findings are understandable on the grounds that LH receptors do not exist on membranes of pituitary gonadotrophs and that the antibodies do not enter viable cells to react with intracellular material. The cross-reaction of antibodies takes place with LH in circulation and it does not deplete the monthly LH surge below the amount necessary for ovulation.

Carrier linkage to overcome immunological tolerance

β-HCG or CTP being ‘self’ molecules do not by themselves elicit antibody response. These were either hapten-modified11 or linked to carrier12 to elicit immune response. The first prototype vaccine linked β-hCG with tetanus toxoid (TT). This vaccine evoked antibodies concomitantly against hCG and TT. The vaccine thus brought in the additional benefit of immunoprophylaxis
against tetanus, an infection responsible for a very large number of maternal and fetal deaths in the country where the deliveries take place at home or in rural surroundings. The antibodies recognized hCG and bound to it; however, hCG by itself did not act as a booster. The antibody titres declined to near-zero level in the course of time in the absence of booster injection. Immunization did not derange menstrual regularity nor had any other side effects on endocrine, metabolic or other body functions. The vaccine conceived and developed in India was approved for Phase I clinical trial not only in India but also in Chile, Finland, Sweden and Brazil under the auspices of the International Committee on Contraception Research (ICCR) of the Population Council. The results from all centres were confirmatory of the safety of the vaccine and its reversibility. A drawback of this vaccine was the large variability of titres amongst the recipients.

**Heterospecies dimer (HSD) – the improved hCG vaccine**

Three steps were taken to improve the immunogeni
city of β-hCG-TT vaccine:

1. Sodium phthalyl derivative of lipopolysaccharide (SPLPS) from *Salmonella enteritidis* was included as an adjuvant in the first injection. SPLPS is a non-pyrogenic but powerful B-cell mitogen. It stimulates also the local production of cytokines potentiating antibody response.

2. Diphtheria toxoid (DT) was selected as a carrier in addition to TT to make two different conjugates. These were administered in alternating sequence to prevent hyperimmunization against a given carrier. This strategy also overcomes the carrier-induced immunosuppression noticed in a few women on repeated immunization with the same carrier. Presenting the antigen on an alternate carrier overcame the suppression and evoked antibody response (Figure 2).

3: The intrinsic immunogenicity of β-hCG was augmented by associating it with a heterospecies α-subunit of ovine origin. The property of association between these subunits is conserved across species. Thus, the ovine α combines with the human β to generate a heterospecies dimer (HSD). HSD, a man-made hormone, is superior to hCG for its steroidogenic property (Figure 3). The antibodies generated by it have better bioneutralization capacity. More importantly, it is a better immunogen, presumably because of the ‘foreignness’ brought in by the heterospecies. No cross-reaction of antibodies made by it is seen with hTSH and hFSH.

![Figure 2. Alternate use of carriers to prevent immunosuppression. A volunteer in Phase I clinical trial receiving repeat immunization with the vaccine (two LH-β-hCG-TT)-generated antibodies against gonadotropin and TT. Booster immunization with β-hCG-TT in a few other women (not all) failed to induce anti-hCG response. Such immunosuppressed women produced anti-hCG antibodies on immunization with gonadotropin linked to alternate carrier, diphtheria toxoid (DT) or cholera toxin chain B. (From Reference 18)](image1)

![Figure 3. Superior steroidogenic property of HSD composed of β-hCG associated with α-subunit of LH of ovine origin. The production of testosterone by Leydig cells in response to hCG (9000 IU/mg) (●–●) and hSD (O–O) are given as a function of the dose of the hormone. Response computed on the basis of hormone equivalent to purified hCG of 12,000 IU/mg potency (O–O). The isolated subunits by themselves do not recognize the receptor and have little steroidogenic property. Their association results in a conformation recognizing their receptor. (From Reference 20)](image2)

**Phase I clinical trials**

After due toxicology, drug regulatory and ethical approvals, various formulations of the hCG vaccine went through Phase I clinical trials in five centres of the country. These trials demonstrated the lack of any significant contraindications. No immediate or delayed
reactions related to immunization were discernible. Blood chemistry and haematology parameters remained largely unchanged. The women kept ovulating. Their menstrual cycles remained regular. No increase in the number of bleeding days or amount was seen. The degree of cross-reaction with hLH had no relationship with the menstrual cycle length\textsuperscript{22}. No difference in autoimmune reactivities could be seen in bleeds before and after immunization\textsuperscript{8}. The antibody titres declined with time, pointing to the reversibility of the phenomenon.

**Phase II efficacy trials with the HSD vaccine**

After ascertaining fully the safety and reversibility of the vaccine, it was crucial to determine whether the vaccine is effective in humans. Previous data on efficacy were in baboons\textsuperscript{23,24} and bonnet monkeys\textsuperscript{25}. Clinical trials were conducted at the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, Safdarjung Hospital and All India Institute of Medical Sciences, New Delhi, in women of reproductive age who were of proven fertility with two live children and who had active sex life. One hundred and sixty-one women attending the family planning clinics volunteered for this study. Of these, 148 completed the primary immunization of three injections. All of them generated anti-hCG antibodies. But only 119 of them (80\%) who had titres above 50 ng/ml were further followed up. The protocol of this study had fixed a putative threshold of 50 ng/ml of hCG bioneutralization capacity for evaluation as to whether this titre (and above) is adequate for prevention of pregnancy. It has been subsequently seen that this threshold is on the higher side and no pregnancy, in fact, took place above 35 ng/ml. The women were asked to use IUD or an alternate contraceptive for the first three months during the primary immunization schedule of three injections given at six-weekly intervals. On attainment of the titres above 50 ng, the IUD was removed and women exposed to pregnancy without the use of any alternate contraceptive. Figure 4 illustrates typical antibody kinetics in a woman. The solid bar is the period during which she was exposed but did not become pregnant. Boosters were provided to those desirous of continuing the study. They had the option of moving out of the study and not take further injections. Women were protected from becoming pregnant for periods ranging from a minimum of 3 months to two-and-a-half years. Only one pregnancy was recorded in over 1200 cycles at or above 50 ng/ml titre. The antibodies generated by the vaccine were thus highly effective in preventing pregnancy at and above this titre. HSD is the first birth control vaccine, for which evidence of efficacy has been established\textsuperscript{26}.

**Further development for large-scale use of the vaccine**

Two important milestones have been crossed. The vaccine is safe and reversible in humans. It is effective in preventing pregnancy. The following lacunae have,
however, to be filled in order to make the vaccine logistically usable on a large scale:

1. The lag period of about three months for antibody titre build-up during primary immunization requires to be covered by a dependable, compatible, companion approach.

2. The multiple injections presently necessary for primary immunization require to be given in a single dose to avoid the risk of incomplete immunization on account of the failure of the recipient to turn up for subsequent injections.

3. A simple assay which could be performed by the individual would be required for the woman to check each month whether she has adequate antibody titres.

4. The vaccine has to be made at a reasonable cost and in large quantities for which recourse to DNA recombinant techniques may be necessary.

These issues have been the subject of our ongoing research. It has been established that a single instillation of purified neem seed extract (Praneem) into the uterus, by a technique easier than insertion of IUD, can prevent pregnancy in rodents and monkeys without impairment of ovulation. Figure 5 is an evocative example of how this treatment works. Praneem vaccine induced local cell-mediated immunity (VILCI) and was given in the right horn, the left horn received an equal volume of 0.1 ml of peanut oil. The treatment did not change libido and the animals mated. The peanut-oil-treated horn had normally developing embryos whereas the VILCI-treated horn had no implantation sites in spite of the fact that the right ovary showed ovulation points. The receptivity of the uterus to estrogens and progesterone remained unchanged by treatment with VILCI. The duration of the effect varied from 50 to 180 days in the rodents depending upon the dose. The animals regained fertility in the course of time. After due toxicology, drug regulatory and ethical approvals, Phase I clinical trial has been completed on Praneem VILCI at PGIMER, Chandigarh. These studies have shown that treatment with VILCI is safe for humans. Extended Phase II trials with the hCG vaccine in combination with VILCI are planned with the idea to determine whether the combined use of the two can provide protection right from the day of enrollment of the subject. A parallel group with VILCI alone would furnish data on the efficacy, if any, of a novel CMI vaccine to be a contraceptive in its own right.

The mechanism by which VILCI prevents implantation is not fully known. The genital tract is normally immunosuppressed to react against the sperm, which is foreign to the woman's immune system. After treatment with Praneem VILCI, the immunological response is activated against the sperm, which is translated into local production of cytokines, such as γ-interferon, IL2 and TNF, that reject implantation.

**Figure 5.** Prevention of implantation in the uterus by local treatment with purified extract of neem seed (VILCI) The right horn received 0.1 ml of neem seed extract and the left horn an equal volume of peanut oil. The animals mated and normally developing embryos could be seen in the horn treated with peanut oil. No implantation sites were visible in the neem-treated horn. The effect varied from 50 to 180 days and was reversible.

**Single contact point immunization**

Biodegradable microspheres of polylactide-polyglycolides (Figure 6) have been developed, in which multiple doses of the vaccine are encapsulated. These erode with time, releasing the vaccine either as a continuous antigenic stimulus or in pulsatile fashion (depending upon the formulation). We are also working on microspheres encapsulating TT for eventual use as a single delivery vaccine against neonatal tetanus. Microspheres loaded with the hCG vaccine have generated sustained antibody response in monkeys for over 6 months without the need of a booster. Further improvements are being carried out to obtain a sustained antibody response for 12 months by a single contact immunization.

**A simple assay for antibody titres**

The sera from women immunized during Phase II efficacy trials with the H1S vaccine were valuable to investigate the determinants to which antibodies are raised in humans. Analysis has been done by a competitive enzyme immunoassay employing a panel of monoclonal antibodies. These studies have put in evidence a dominant response to an epitope recognized by two monoclonal antibodies (206 and P3W-80). A basis is
Figure 6. Biodegradable microspheres of polylactide–polyglycolide encapsulating multiple doses of the vaccine that can be given at a single contact point (a). These erode with time releasing the encapsulated vaccine (b).
therefore laid for developing a single-step competitive capture assay using these monoclonals. A more detailed discussion on the antibody profile is available elsewhere\(^\text{30}\).

**Recombinant products**

The genes for \(\beta\)-hCG and \(\alpha\)-oLH have been cloned and expressed in the following systems: vaccinia\(^\text{31,32}\) and baculovirus\(^\text{33-36}\). The vaccinia-expressed products are fully glycosylated and identical to the native hormonal subunits. The baculovirus expression system has better yield but the products are partially glycosylated. In both cases, the \(\beta\)-hCG is immunoreactive with conformation reading monoclonals specific to \(\beta\)-hCG. The \(\beta\)-subunit can also bind with the \(\alpha\)-subunit to generate bioactive hormone. More recently, Batra and Mukhopadhyay et al. have described the \(\beta\)-hCG in *E. coli* with a higher yield (unpublished data). The peptide made has the same amino acid composition as the human \(\beta\)-hCG. It is, however, not glycosylated at all. The feasibility of using these subunits made by the recombinant techniques for vaccination purposes is being investigated.

**Live recombinant hCG vaccine**

A live recombinant vaccine has been made where \(\beta\)-hCG has been inserted in vaccinia along with a transmembrane 48 amino acid fragment\(^\text{17}\). This vaccine is highly immunogenic in rodents. In monkeys sustained antibody response of long duration has been obtained after a single immunization with the recombinant vaccine, followed after several months by a plain booster in alum. Monkeys were rendered infertile during the phase of high antibody titre.

This genre of vaccine is highly interesting. It is cost-effective, does not require an adjuvant or carrier and can be made industrially by established technology. Vaccinia as a vector has been approved for a vaccine against AIDS. However, it may not be acceptable universally for contraception, to be practised by young healthy persons, as vaccinia can lead to undesirable virulence in a very small percentage of recipients, specially those who are immunocompromised. We are currently engaged in transferring the \(\beta\)-hCG-transmembrane cassette in an alternate vector, e.g. fowl pox virus. Fowl pox virus is species-restricted and does not replicate in humans, even though it expresses early proteins.

**Applications of hCG vaccine in cancers making hCG**

Ectopic synthesis of hCG has been demonstrated in a variety of human malignancies, including lung cancers\(^\text{18}\). High circulating levels of hCG and its subunits are often used as biochemical markers of malignancy, and decreased levels often used as indicators of successful treatment. The function of the aberrant synthesis of hCG or its subunits has been studied in a human cancer cell line (ChaGo), established from a bronchogenic squamous cell carcinoma. ChaGo cells secrete predominantly the \(\alpha\)-subunit of hCG. These cells were observed to lose their characteristic transformation phenotypes, including the tumourigenic potential in athymic mice, following the block of intracellular synthesis of \(\alpha\)-hCG by antisense RNA\(^\text{39}\). In contrast, the stimulation of \(\alpha\)-hCG synthesis by c-AMP stimulated cell proliferation and cell progression into the S-phase. These observations provided indications on the autocrine growth-promoting role of \(\alpha\)-hCG of maintaining the cells in a continuously proliferating state. When anti-hCG antibodies were passively administered to athymic mice bearing ChaGo cell-induced tumour, necrotic degeneration of the tumour was seen\(^\text{40}\). The antibodies were ineffective against non-\(\alpha\)-hCG-producing tumours. Histological picture of anti-\(\alpha\)-hCG antibody-treated tumour revealed progressive necrosis. In case the ChaGo cells were exposed to antibodies prior to implantation in addition to passive administration, a concentration-dependent inhibition of tumour growth was seen (Figure 7). In the highest dose group treated with 500 ng of anti-\(\alpha\)-hCG antibodies, no tumour could be seen at any stage up to 10 weeks of observation. Two more lung cancer cell lines of different histological type (H460 and H520), both known to synthesize and secrete \(\alpha\)-hCG, have been tested *in vitro* and *in vivo* using anti-\(\alpha\)-hCG antibodies. A concentration-dependent inhibition of growth was observed of both cell lines *in vitro*, and on the H460 tumour *in vivo* in nude mice. The effect of the antibodies is thus evident on several human lung cancer cell lines.

The non-small cell lung cancers making \(\alpha\) or \(\beta\)-hCG are currently not treatable by any of the available drugs. Based on the observation that the hormonal subunits act as autocrine growth promoters and antibodies inhibit the tumour growth, a trial with our recombinant vaccinia-\(\beta\)-hCG vaccine has been approved in Mexico. Three patients diagnosed on the basis of radiography, histopathology and \(\beta\)-hCG in circulation have been immunized. After 12–16 months, all were alive. None of the patients had evidence of metastasis.

**LHRH vaccine**

Luteinizing hormone releasing hormone (LHRH) or gonadotropin releasing hormone (GnRH) is a decapeptide made by the hypothalami. It controls the secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the pituitary. These in
turn act on the gonads, male or female, to generate sperms or egg and also sex steroid hormones. Immunoactivation of LHRH leads to impairment of fertility and failure to make sex steroid hormones, be it male or female. (LHRH is the same in both male and female.) Since the decapeptide has a conserved sequence in mammals, the anti-LHRH vaccine has applications in animal fertility control, in addition to humans. It can, for example, be used for suppression of estrus in dogs and cats. The effect can be caused not only by active immunization but also by passive administration of bioeffective monoclonal antibodies. Immunization against LHRH can also be useful in animals raised for meat purposes, where suppression of androgens will have an impact on the quality of meat.

Design of the vaccine

Native LHRH has neither amino nor carboxy group available for linkage to carrier. Nonetheless, chemically synthesized molecule can be planned to have one or the other of the group open. Ladd et al. have found that linkage of TT at N-terminal amino position gives a vaccine with higher immunogenicity than the one where conjugation is done at the carboxyl end. The vaccine designed at NIH has adopted a different strategy, glycine at position 6 was substituted by D-lysine, which produced an analogue resistant to metabolic degradation in addition to creating an amino group for onward linkage to a spacer and diphtheria toxoid. The vaccine thus created has higher immunogenicity than achieved.
by linkages at amino or carboxy terminal positions. Knowledge-based computer graphic analysis of LHRH shows a bend in the middle of the molecule with the carboxy and amino terminal amino acids, proximating to each other (Figure 8). The folding of the molecule and the adjacent disposition of these are also supported by NMR. These two amino acids along with guanido group of arginine are believed to constitute the facet of LHRH interacting with the receptor on the target tissues.

Applications of the LHRH vaccine

The vaccine should find useful applications in animal fertility control, in particular, the domestic pets and the

Figure 8. Structure of GnRH modelled by a knowledge-based approach. Superimposed ribbon drawing highlights the type-Iβ-turn in the hormone. Computer graphics generated using Quanta version 3.1

Figure 9. Comparison of weights of prostates in rats immunized against LHRH-DT with those of adjuvant-injected controls. Student's t-test: b/a = P < 0.001 (From Reference 49)

Figure 10. An illustration of prostatic atrophy in a LHRH-DT vaccine-immunized rat compared to the normal prostate gland in an adjuvant-injected control rat (From Reference 49)
animals for meat purposes. Its application in humans as a contraceptive vaccine would be in conditions where LHRH is physiologically low, e.g. following parturition. The vaccine given to women soon after delivery can extend lactational amenorrhea. An agonist of LHRH was employed in women for keeping the ovulation suppressed for 11 months with no apparent ill effects. Scientists at the Population Council Laboratories, New York, are working on a possible male fertility control with the LHRH vaccine. Rodents are rendered infertile by immunization with the LHRH vaccine, and their libido is restored by employing an androgen in a way that aspermatogenesis persists.

We have directed this vaccine for evaluation in prostatic hypertrophy. Male rats immunized with the LHRH vaccine became infertile and their testosterone decreased to castration level. Accessory reproductive organs, in particular, the prostate atrophied markedly (Figures 9 and 10). On decline of antibodies and in the absence of booster immunization, the prostate regenerated, attaining near about the normal size. It is still not clear as to whether the regenerated prostate has the characteristics of a 'young' prostate. Were it to be so, this will be a marvellous way to do away with the 'old' prostate and let it be replaced with a prostate with 'young' characteristics. An anomalous situation is the unregulated growth of prostate with age in contrast to the regression and atrophy of several other tissues.

Immunizations with anti-LHRH vaccine of bonnet monkeys too resulted in atrophy of the prostate gland. Atrophic changes were also observed in other accessory sex organs and testes. The circulating level of testosterone declined as the anti-LHRH antibody titres increased.

Clinical trials in patients of prostatic adenocarcinoma

After due toxicology, drug regulatory and ethical approvals, the LHRH vaccine has gone for clinical trials in prostatic adenocarcinoma patients in two centres in India and at one in Austria. The LHRH vaccine of Population Council, New York, is on similar clinical trials in USA with the approval of FDA.

The experience at this stage is that with the rise of antibody titres, the testosterone level declines. Ultrasound and CAT-scan have shown in many patients a regression of prostatic tissue mass accompanied by clinical improvement. The effect is, however, not observed in all patients. This could be either due to the deficiency of antibody titres achieved in these patients or, alternatively, due to the fact that all prostatic adenocarcinomas are not androgen-dependent. The vaccine, therefore, requires further developments, like improvement of immunogenicity with an appropriate adjuvant. Its incorporation in microspheres can help sustain antibody titres over a long period, necessary for immunotherapy. There will also be the requirement of a companion approach, which can address to androgen-independent prostatic growth. Paracrine/autocrine growth factors made by hyperplastic and hypertrophied prostatic cells deserve attention. The synthesis of several of these, including c-neu/HER-2, is increased in these tissues. Carcinoma of the prostate is the second biggest killer of males amongst the cancer-related deaths.

Male fertility control by CMI approach

Immunological approaches may be particularly suitable for male fertility control. Moudgal et al. are working on an FSH vaccine. An alternate approach would be to exploit the in-built developmental potential of selective immunosuppression of spermatogenesis by cell-mediated immune response. Several proteins whose ontogenesis takes place in pubertal years are 'foreign' to the body's immune system, whereas Leydig cells making testosterone are recognized as 'self', as these are present and functional at the fetal stage. Talwar et al. induced aspermatogenesis without decline of testosterone or libido by injection of a suspension of BCG.
(Bacillus Calmette Guerin) (Figure 11). The effect was caused in every mammal investigated, e.g. rodents, dogs, ram and monkeys. The effect was reversible and spermatogenesis was regained with time. Suri and his co-workers at this institute have kept on observation several monkeys who have been repeatedly made infertile by injection of BCG in the epididymis. In the phase when sperms reappear, the fertility is confirmed by mating and by the ability of the monkey to sire a progeny. In none of the monkeys are any antisperm antibodies detected in the circulation. Thus, the action is not mediated and exercised by antibodies. The action is highly localized and can even be obtained unilaterally in one testis without consequences on the other non-immunized testis.

Following these leads made 15 years back, a more recent and a more elegant achievement has been the possibility of inducing the same effect, e.g. aspermato genesis, without reduction of testosterone, by injecting purified neem seed extract into the vas deferens.

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New leads in contraception and innovations in existing contraceptive methods

Horacio B. Croxatto

Instituto Chileno de Medicina Reproductiva, José Ramón Gutiérrez 295, Depto 3, Correo 22, Casilla 96, Santiago, Chile

ONE quarter of the world population are women in the ages of 15 to 49 years and approximately 70% of these are coupled to men in an established relationship, most commonly marriage. It is estimated that 40–50% of these 800 million couples control their family size using modern contraceptive methods.

Current methods can be broadly classified into: behavioural, hormonal, intrauterine devices, barriers to sperm and sterilization. The percentage of contraceptive couples using each method is shown in Table 1.

Female sterilization which reflects the decision of the woman to have no more children for the rest of her life remains at the top of the list with 26% of all contraceptors choosing this method. Considering that the reproductive life span of the woman extends from the ages 15 to 49 years, that the age at marriage is close to 20 years and that the size of the family is decreasing and progressively approaching replacement level, it is understandable that for a significant proportion of women a definitive non-hormonal method is preferred after completing the desired family size.

Efforts to increase the variety of methods, their availability and the quality of their service delivery continue to be fundamental to expand contraceptive use to the level required for stabilizing the human population at the earliest possible time during the next century. As the rate of population growth is beginning to decline other motivations for contraceptive usage are getting increased recognition and contraceptive development begins to focus more and more on the needs of individuals and couples. Thus, a new generation of methods that can increase the choices and meet the diversity of needs that suit the gender, the different ages, the life styles and so on is much needed. Development of a larger variety of contraceptive methods is expected to lead to safer, more convenient and more affordable methods.

Regardless of population issues, the existing trend towards smaller family size is likely to be irreversible and implies that the use of contraceptive methods will be a common and widespread feature of human life from now on. Given this expectation, family planning should become an obligatory subject in general education and

| Table 1. Worldwide prevalence of use of various contraceptive methods |
|------------------------|---|
| Female sterilization   | 26 |
| Intrauterine devices   | 20 |
| Behavioural            | 15 |
| Oral contraceptives    | 14 |
| Male sterilization     | 10 |
| Condoms                | 10 |
| Others                 | 5  |