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EXPERT OPINION

Advances in development of a contraceptive vaccine against human chorionic gonadotropin

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Introduction: There is continuing need for contraceptives. According to World Health Organization, 210 million pregnancies occur each year, out of which some 80 million are unintended. A vaccine offering privacy and periodic intake would be an attractive proposition.

Areas covered: The article is a brief review of three vaccines developed against human chorionic gonadotropin (hCG) with progressively better attributes. Clinical trials have proven in more than one country the complete safety and reversibility of the anti-hCG vaccine(s) in women. Vaccination does not entail any disturbance in levels of reproductive tract hormones of the woman or any disturbance in menstrual regularity and bleeding profiles. Phase II clinical trials show the effective prevention of pregnancy in sexually active women of proven fertility. A recombinant vaccine amenable to industrial production has been developed; it induces substantially higher antibody titers in mice of four different genetic strains than those required to prevent pregnancy in women. Rigorous toxicology studies have been completed on this vaccine in rodents and marmosets.

Expert opinion: This unique vaccine, requiring periodic intake and demonstrating no impairment of ovulation, hormonal profiles and menstrual regularity, is on the verge of final clinical trials under the aegis of the Indian Council of Medical Research and should be a valuable addition to the available contraceptives.

Keywords: contraception, fertility control, human chorionic gonadotropin

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1. Introduction

The idea of a vaccine for contraception arose from the experience of Family Planning Clinics in India. Although government offered a basket of methods free of charge, their acceptance was low. Tubal or vasal ligation was considered as practically permanent procedures, and men or women opted for these fairly late in their reproductive life after having produced many children. Males did not like to use condoms for apparent compromise of their pleasure of sexual intercourse. Pill demanded daily motivation for intake by women. Intrauterine devices (IUDs) caused extra bleeding, which women, already anemic (hemoglobin usually < 9 gm/100 ml) could hardly sustain. A vaccine, if possible, would require only periodic intake, provide privacy of use and will be free of the disadvantages described above. As women bear the brunt of pregnancy, we decided to develop a vaccine for use by women.

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Article highlights.

- This contraceptive vaccine is needed to prevent unwanted pregnancies with no discernible side effects.
- The human chorionic gonadotropin (hCG) is a unique target for reversible control of fertility in women without impairment of ovulation, sex steroid levels and regularity of menstrual cycles.
- This article updates the research and clinical trials data on progressively better anti-hCG vaccines developed over years.

This box summarizes key points contained in the article.

2. What could be the ideal target?

Antibodies would have ease of binding and inactivating an antigen in circulation rather than the one present on gametes or other reproductive organs. A number of hormones act as a cascade to enable the production of the egg in the female. The symphony starts with luteinizing hormone releasing hormone, a decapeptide originating from hypothalamus, common to both males and females. It causes the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) by the pituitary, which act on gonads. In females these cause ovulation, as well as the production of estrogen and progesterone. Neutralization of any of these hormones by antibodies would also entail side effects. Hence these would not be advisable for a vaccine intended for contraception purposes. However, a vaccine against luteinizing hormone releasing hormone could be useful for control of fertility of stray animals. It could also be useful for some cancers such as prostate cancers for cutting off the androgens.

Of interest is the hormone, which surfaces from the early embryos following the fertilization of the egg. Human chorionic gonadotropin (hCG) is present in the culture fluid of *in vitro* fertilized eggs, an observation first reported by Bob (Robert) Edward [1] in course of his work along with Streptoe to generate the first 'Test Tube' baby. The hCG is not made by any organ of normal non-pregnant female – the reason why it serves as a reliable test of pregnancy. The hCG plays a crucial role in implantation and onset of pregnancy. Hearn *et al.* [2] observed that marmoset embryos exposed to anti-hCG antibodies fail to implant, whereas those exposed to irrelevant immunoglobulins implant normally. We, therefore, chose hCG as the target for a potential birth control vaccine.

The hCG is composed of two subunits, the α subunit is common to FSH, thyroid-stimulating hormone and LH, and it is the β subunit which accords identity to these hormones. The β subunit of hCG however shares a large homology with β subunit of human luteinizing hormone (hLHβ), but it has an extra C-terminal of 35 amino acids which is non-existent in hLHβ. This region can serve as a hCG-specific antigen. Unfortunately the C-terminal was found to be a poor antigen and antibodies were of low affinity for binding with hCG, $K_m = 10^8\ M^{-1}$ as compared to binding of hCG to its receptors which has a $K_m = 10^9\ M^{-1}$. Elongation of the C-terminal to 45 amino acids improved immunogenicity as compared to terminal 30 or 37 amino acids long peptides linked to tetanus toxoid (TT) [3]. We elongated further the terminal peptide to 53 amino acids, which enhanced hCG neutralization capacity of the antibodies as compared to those generated by 30, 35 and 45 terminal amino acids linked to TT as carrier [4]. However, while specificity to hCG and non-reactivity to hLH was retained by this vaccine, the neutralization capacity of antibodies of hCG bioactivity remained substantially poorer than the sera against the entire hCGβ. Extensive survey of the C-terminal peptides discouraged their eventual use for a vaccine which has to generate antibodies capable of neutralizing fairly high amounts of hCG encountered in early stages of pregnancy. We thus opted for the entire hCGβ as antigen. It may be mentioned that Steven *et al.* with support from World Health Organization worked on the C-terminal hCGβ-specific peptide linked to carrier [5]. It had to be given along with strong oily adjuvants to generate antibodies. The vaccine after nearly 20 years of persistent work in animals including baboons had to be abandoned after unacceptable reactions in women immunized with this vaccine in Sweden.

2.1 Rendering hCGβ immunogenic in humans

Being given that fetuses bathe in large amounts of hCG during pregnancy, both men and women are immunologically tolerant to hCG (and its subunits). To render hCGβ immunogenic in women, we linked it to a carrier competent to mobilize T-cell help to enable B cells to produce antibodies against it. We chose TT for use as carrier. It was cheap and available in plenty from industrial sources. We expected that hCGβ conjugated to TT would generate antibodies against both tetanus and hopefully hCG. Antibodies against tetanus will be useful to prevent a fairly large number of women dying in India in 1970s of tetanus following delivery in aseptic conditions. After a variety of toxicology and safety studies, reported in an entire issue of *Contraception* [6], we conducted a probing study in four tubal ligated women to see whether our hypothesis of conjugation to a carrier rendered hCGβ immunogenic to generate antibodies inactivating the whole (αβ) hCG. This indeed was the case. Women produced antibodies against both hCG and tetanus [7], administration of 5000 IU of hCG to the immunized woman diminished anti-hCG titers indicating their recognition and binding to hCG. The titers returned to their original titers in course of time. The hCG challenge had no effect on anti-TT titers, indicating that the antibodies against TT were independent of those against hCG. The safety, reversibility and ability of the first anti-hCG vaccine inducing bioeffective anti-hCG antibodies in women were established in Phase I trials conducted not only in India but also in Finland, Sweden, Chile and Brazil under the auspices of the International Committee on Contraception Research of the Population Council [8].

2.2 Enhancement of immunogenicity

Having determined that by linking hCGβ to a carrier TT rendered it immunogenic, the next task was to make it more immunogenic to obtain higher titers of antibodies, without employing oily adjuvants. We explored the advantage of employing a heterospecies dimer (HSD) of hCGβ rather than the β subunit. The ability of the β subunits to associate noncovalently with α subunit is retained across species in mammals. We linked β subunit of hCG with α subunit of ovine LH. The heterodimer thus created was conjugated to either TT or diphtheria toxoid (DT). Although TT had proved to be a good carrier, repeated immunization with TT led to carrier-induced immunosuppression of immune response to hCGβ, which was overcome by presenting hCGβ on DT. HSD-DT induced distinctly higher titers of anti-hCG antibodies [9], which were devoid of cross-reaction with hTSH and hFSH indicating that α subunit of ovine luteinizing hormone did not generate antibodies reactive with α subunits of two other hormones secreted by the human pituitary.

3. Efficacy of HSD-DT to prevent pregnancy

After toxicology studies and obtaining Ethics and Regulatory approvals, Phase I clinical trials were conducted on HSD-DT vaccine in women. The vaccine was free of any untoward side effects and did not block ovulation nor did it alter the normal production of reproductive hormones of the immunized women [10,11]. Their menstrual periods remained regular and bleeding profiles remained normal in contrast to frequent effects on these traits noted for women employing IUDs and other contraceptives.

The next obvious question was whether this vaccine against hCG is effective in preventing pregnancy. The crucial Phase II efficacy trials were conducted in 148 women of proven fertility, with at least one or two live children, who were sexually active. Many had come to the hospitals of the reputed institutions: the All India Institute of Medical Sciences, New Delhi, and the Postgraduate Institute of Medical Education and Research, Chandigarh, for medical termination of pregnancy (MTP). They were asked to wear IUD, till such time as anti-hCG bioefficacy titers exceeded 50 ng/ml, which was tentatively fixed as the protective threshold. Boosters were given periodically to keep the titers > 50 ng/ml.

Although all women immunized with the vaccine formed anti-hCG antibodies, only 119 women (80%) had titers > 50 ng/ml. Their IUDs were removed in order to expose them to pregnancy. They were asked to keep a diary of the sexual intercourses and menstrual periods. Luteal phase bleeds were taken to determine the progesterone levels.

These trials indicated a very high efficacy of the vaccine to prevent pregnancy [12]. Only one pregnancy occurred in 1224 cycles. Eight women completed > 30 cycles without becoming pregnant, 9 between 24 and 29 cycles, 12 completed 18 – 23 cycles, 15 completed 12 – 17 cycles and 21 women had completed 6 – 11 cycles of continuous exposure to the risk of pregnancy. Women kept on ovulating normally and had regular menstrual cycles (**Figure 1**). The vaccine was fully reversible. Pregnancies occurred at titers < 35 ng/ml titers. **Figure 2** is a representation of a woman, who was protected for 12 cycles, while she was receiving boosters as and when titers were tending to go < 50 ng/ml. After 12 months, she decided to have another child. She became pregnant in the very next cycle when her titers were < 30 ng/ml. A study was carried out to determine whether low anti-hCG antibodies in circulation had any adverse effect on pregnancy and on developmental landmarks and cognition ability of the newborns. Four women opting for another child were enrolled by informed consent. They were examined clinically at monthly interval till delivery. The babies born were followed up to 2 – 3.5 years. The anthropometric indices of babies born were comparable with elder siblings, as also their cognitive abilities [13].

It may be stated that when decision was taken to conclude the trial to analyze the findings, many women offered to pay for the vaccine to continue to be immunized. It reflects in a way that they were happy and satisfied with this mode of contraception.

4. The big gap and revival

Soon after the conclusion of Phase II Trials, GPT retired from the Directorship of the National Institute of Immunology in 1994. After a gap of nearly 12 years, GPT was asked by an Indo-US Committee on Contraception Research to revive the vaccine. It was decided to make a recombinant vaccine, which could be amenable to industrial production. By adopting this route, we would also assure the consistency of carrier linkage to the β subunit of hCG. We chose β subunit of enterotoxin of *Escherichia coli* as carrier instead of TT or DT, as besides humoral antibody response, it will also extend the immune response to mucosal surfaces. The construct hCGβ-B subunit of heat-labile enterotoxin of *Escherichia coli* (LTB) (**Figure 3**) was expressed in yeast *Pichia pastoris*, a production cell in which recombinant hepatitis B was expressed. This vaccine received the approval of the Drugs Controller General of India (DCGI), Regulatory Agency of France and other countries for manufacture for human use. The purified hCGβ-LTB vaccine was adsorbed on alum. Heat killed, *Mycobacterium indicus pranii* (MiP) was employed as the adjuvant. MiP is a non-pathogenic mycobacterium, developed as an immunotherapeutic vaccine for leprosy [14]. MiP is approved by DCGI and USFDA and is being used not only as adjunct to multidrug regimen in leprosy but also in 'difficult to treat' category II patients of tuberculosis. The immunogenicity of hCGβ-LTB was initially investigated in BALB/C mice. All mice generated bioeffective antibodies against

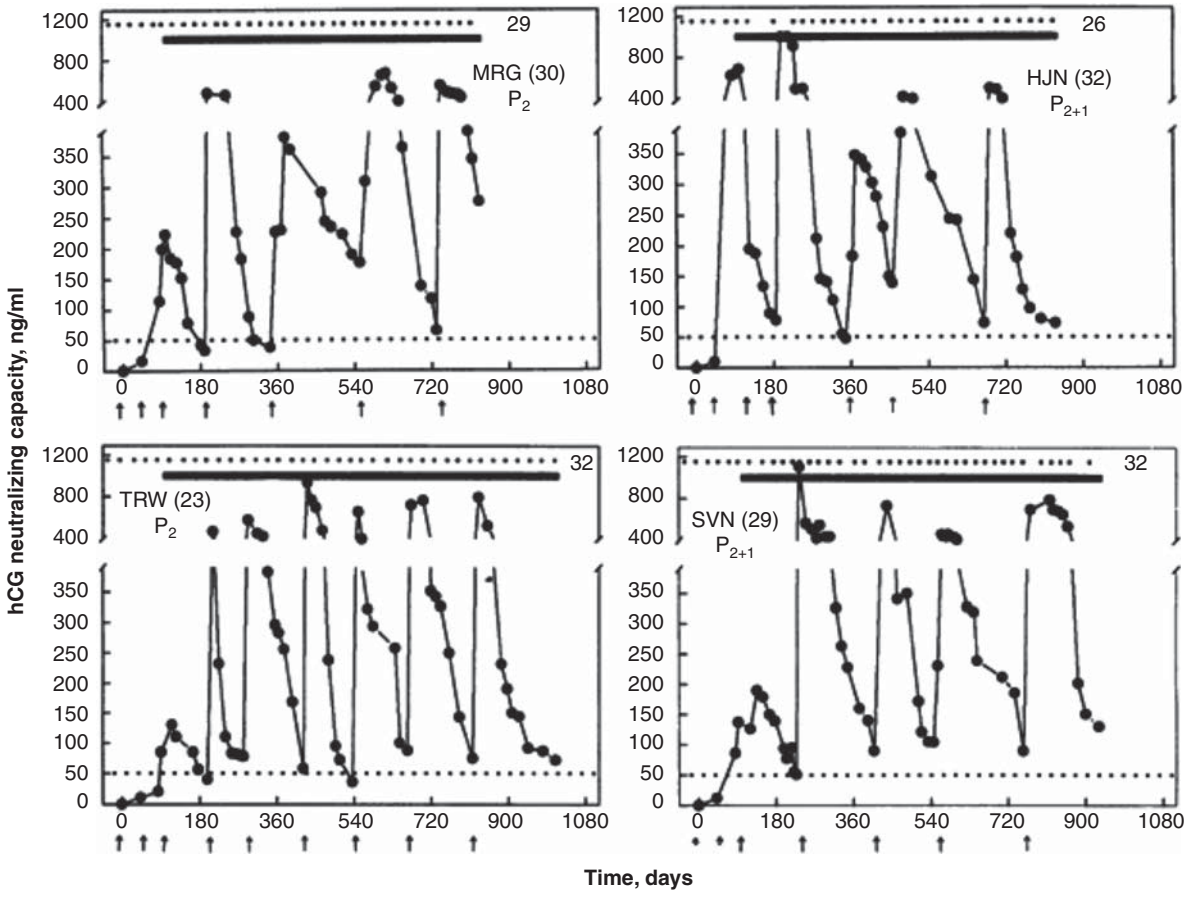

Figure 1. Anti-hCG response to the HSD vaccine in four sexually active women of proven fertility is shown. MRG 30-year-old and TRW 23-year-old had two children each; HJN 32-year-old and SVN 29-year-old had two children each and one elective termination of pregnancy. All of them remained protected from becoming pregnant over 26 – 32 cycles. The dotted lines at the top edge represent the menstrual events which remained regular, solid lines denote the period over which they were exposed to pregnancy. Booster injections were given to keep antibody titers > 50 ng/ml.

Adapted from [12] © (1994) National Academy of Sciences, USA.

hCG: Human chorionic gonadotropin.

hCG [15]; the antibody titers in each mouse were high, ranging from 3600 to 17,830 ng/ml (**Figure** 4). The vaccine was also immunogenic in four other genetic strains of mice [16], although the quantum of response varied as would be expected.

4.1 Synergy of DNA and protein versions of recombinant hCGβ-LTB vaccine

DNA vaccines are not only cheaper to make but also thermostable without requiring cold chain. Therefore, we made the recombinant hCGβ-LTB vaccine as DNA, in addition to proteinic form of the vaccine expressed in yeast *Pichia pastoris*. The hCGβ-LTB DNA vaccine was prepared by cloning the gene encoding the vaccine antigen hCGβ-LTB in eukaryotic plasmid VR1020(DJ) approved by USFDA. *E. coli* was transformed by the plasmid construct VR1020 (DJ)/hCGβ-LTB, and endotoxin-free plasmid DNA was isolated for use as DNA vaccine. Notable enhancement of immunogenicity of the vaccine was observed in mice with prime-boost approach, when the first two doses of primary immunization were given by the DNA form of the vaccine followed by the proteinic form of hCGβ-LTB vaccine. Being a genetically engineered product, the vaccine was considered and approved by the Review Committee on Genetic Manipulation, government of India.

5. Preclinical safety and efficacy studies of hCGβ-LTB vaccine in marmosets

Preclinical toxicology, safety and efficacy studies were carried out in a subhuman primate species, the marmosets, at the National Institute for Research in Reproductive Health, Mumbai. Notable enhancement of immunogenicity of the vaccine was observed, when the first two doses of primary

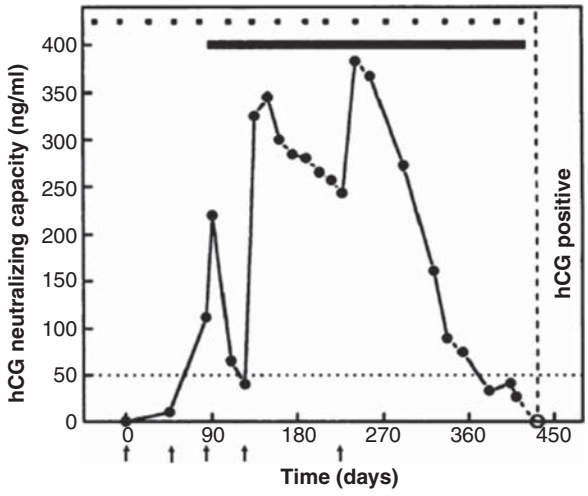

Figure 2. Regain of fertility on decline of antibodies is shown. Woman STS 30-year-old with two children and one termination remained protected from becoming pregnant over 12 cycles. She conceived in the cycle when titers were < 20 ng/ml.
Adapted from [12] © (1994) National Academy of Sciences, USA.

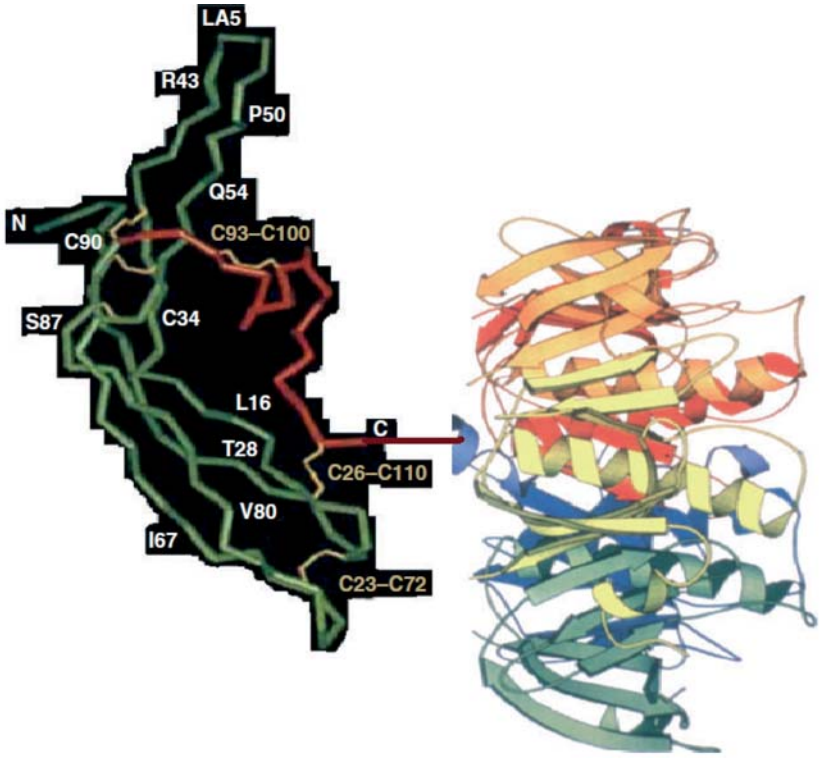

Figure 3. Conceptualized hCGβ-LTB vaccine is shown. The carrier β subunit of heat labile enterotoxin of *Escherichia coli* (LTB) is fused at C-terminal glutamine of hCGβ.

immunization were given with the hCGβ-LTB DNA vaccine followed by the third injection given with the proteinic form of the vaccine. Normal cycling adult female marmosets were immunized intramuscularly twice with DNA version of the vaccine along with autoclaved MiP as adjuvant at 2 weeks interval. DNA-injected animals were distributed in groups of three, and immunized with either 20, 40 or 80 μg of the recombinant hCGβ-LTB protein along with MiP as an

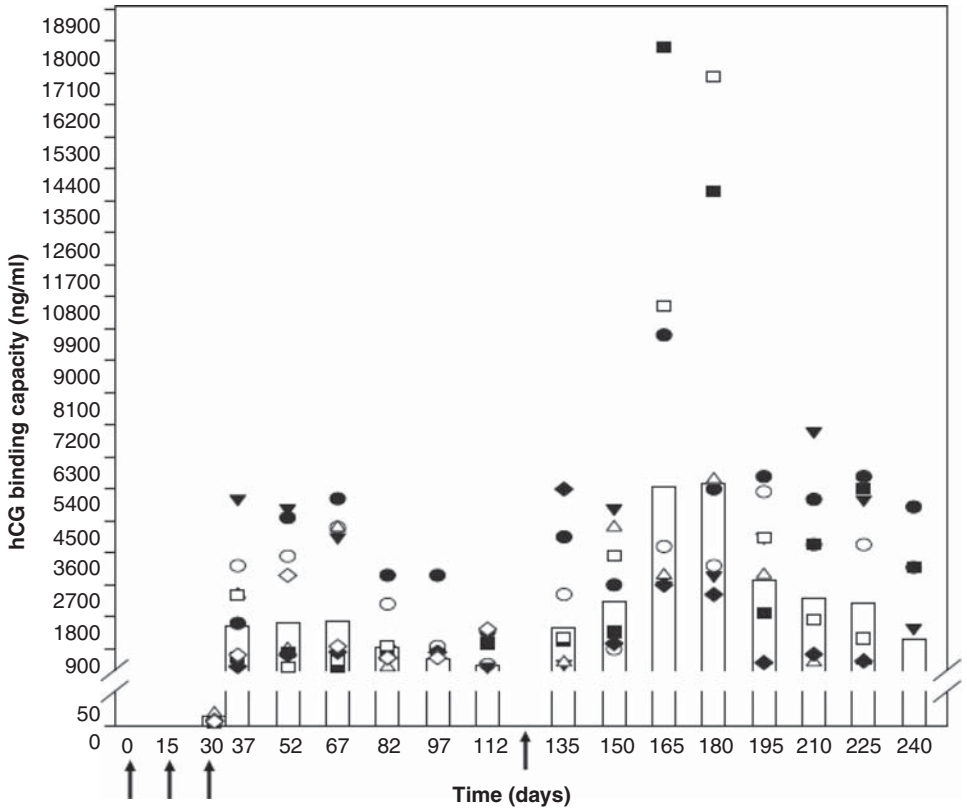

Figure 4. The hCG binding capacity (ng/ml) of BALB/c mice immunized with hCG-LTB adsorbed on alum along with MiP as an immune-modulator is shown. Immunization was done at fortnightly intervals (indicated by arrow) followed by booster on day 127. Geometric mean of hCG binding capacity is represented by the bars and symbols denote the individual animal's titers.

Adapted from [15].

hCG: Human chorionic gonadotropin; MiP: *Mycobacterium indicus pranii*.

adjuvant. Marmosets comprising the control group were immunized with only MiP adjuvant. Immunization with DNA and recombinant protein of hCGβ-LTB vaccine did not have any adverse effect on the body weight and on the general alertness of the immunized animals. Their hematological and biochemical parameters continued to remain normal.

All immunized and control animals were then cohabitated with adult fertile male marmosets for 2 weeks per cycle and their fertility was tested for 6 months. Except for one animal, in 80 µg dose group, none of the immunized animals became pregnant, whereas all animals in the control group conceived. After 6 months, with no booster injections given, all immunized animals regained fertility following the decline of antibody titers, indicating thereby not only the reversibility of the vaccine but also that the circulating anti-hCG antibodies were indeed responsible for preventing them from becoming pregnant.

6. Preclinical studies on hCGβ-LTB in rodents

Preclinical toxicology studies in rodents, were carried out by M/s Bioneeds at their GLP Facility in Bangalore, India, based on biosafety issues related to Genetically Modified Organisms, Schedule 'Y' guidelines on Drugs and Cosmetics and guidelines of Institutional Animal Ethics Committees.

It was observed that both DNA and protein vaccines were devoid of sensitizing the skin of guinea pigs. No clinical signs of toxicity and changes in body weight were noted. The two forms of hCGβ-LTB vaccine were non-mutagenic at the highest concentration tested both in Bacterial Reverse Mutation and Mammalian Chromosome Aberration Tests. Similar non-mutagenicity observations were made *in vivo* Mammalian Erythrocyte Micronucleus Test conducted in mice.

Single-dose acute toxicity study was conducted in Sprague-Dawley rats. Vaccinated rats were observed for mortality, clinical signs of toxicity, body weight and gross pathological examination. No mortality and clinical signs of toxicity were noted nor treatment-related changes in the body weight recorded. Changes in gross pathology (external and internal) were also not observed at even the highest dose tested. Both DNA and protein form of hCG-LTB were tested up to 10 times the human dose of the vaccines in rats with repeat

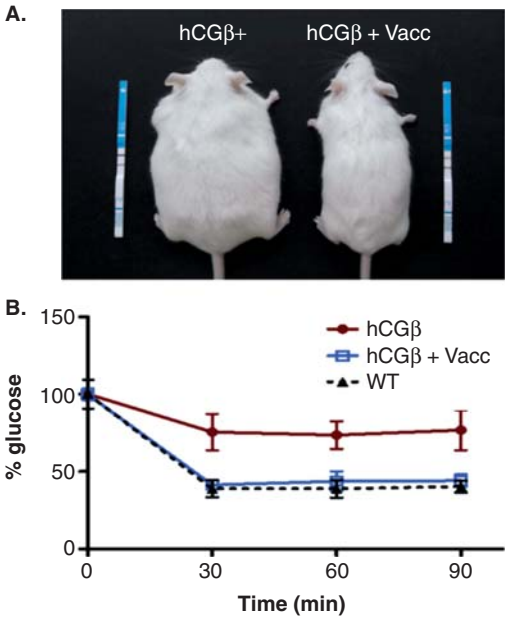

Figure 5. (**A**) Physical appearance of an hCGβ transgenic mouse and of another of the same age immunized with anti-hCG vaccine is shown. (**B**) Insulin response of transgenic hCGβ mice, and those immunized with anti-hCG vaccine at 9 months of age ($p < 0.05$) is shown. The response in corresponding non-transgenic mice is also given.

doses. Rats were followed up to 90 days post-immunization and showed no treatment-related changes in physical, physiological, clinical and hematological indices. No histopathological changes were observed in various organs. Segment II studies conducted in rats showed that vaccines did not affect the embryo-fetal development, body weight and food consumption. Gross pathology was normal in mothers and no abnormal effect was seen in fetal sex ratio, fetal weight, external and visceral skeletal examination of fetuses.

Thus, extensive toxicology studies on the hCGβ-LTB vaccine in two species of rodents and a subhuman primate species, the marmosets, have shown the total safety of the recombinant hCGβ-LTB vaccine. These are now ready to go to clinical trials. A clinical trial protocol has been developed and the Indian Council of Medical Research is awaiting approval from the regulatory authorities to initiate the clinical trials.

7. Additional benefits of the hCGβ-LTB vaccine: obesity and insulin resistance

Professor Hutaniemi at the Imperial College London and Susana Rulli of Instituto de Biologia y Medicina Experimental CONICET Buenos Aires, Argentina, have made mice transgenic for hCGβ [17]. The female transgenic mice become fat, as they mature. Quite interestingly, immunization with hCGβ-LTB vaccine prevents them from becoming obese (**Figure 5A**). The transgenic mice also become insulin resistant, which is also prevented by the hCGβ-LTB vaccine (**Figure 5B**). The lifespan of the transgenic mice also appears to be prolonged by immunization of these mice with the anti-hCG vaccine.

8. Expert opinion

A vaccine preventing pregnancy without disturbance of ovulation, menstrual regularity and bleeding profiles is an attractive option. The target chosen is a molecule not normally made by a healthy woman. It emerges only after fertilization of the egg and has a crucial role in implantation of the embryo. The immunological tolerance to hCG was overcome by linkage of the β subunit of hCG to a carrier, which confers additional advantage of immunoprophylaxis. In order to render the vaccine amenable to large-scale production, a recombinant vaccine has been made, which has passed through extensive toxicology and safety studies in two species of rodents and a subhuman primate species. Whether it would be able to induce high titers of antibodies in women and for how long and whether these would prevent pregnancy, remains to be seen. In the four strains of mice in which its immunogenicity has been investigated, the observations are: i) that all mice without exception respond immunologically to the vaccine; ii) they generate antibodies of titers varying from 3600 to 17,830 ng/ml, whereas the protective titers in women as per Phase II trials are 50 ng/ml. It is reasonable to expect that all women would respond to the vaccine in generating anti-hCG antibodies, and the titers would be considerably higher than the protective 50 ng/ml. How long will the response last will only be clear after trials are conducted with the recombinant vaccine in sexually active women of proven fertility.

Declaration of interest

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