

Possibility and potential of a vaccine against human chorionic gonadotropin for family planning

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Human chorionic gonadotropin (hCG) is a unique hormone produced soon after fertilization in women. It is an ideal target for contraception. Antibodies against hCG prevent pregnancy without blocking ovulation and derangement of menstrual regularity. Previous work on development of a vaccine against hCG is reviewed including the first ever safety and efficacy trials in women with a birth control vaccine. The possibility of a recombinant vaccine with enhanced immunogenicity is indicated.

Keywords: Efficacy, human chorionic gonadotropin, recombinant vaccine, safety.

THE population of the world is continuing to grow and is projected to reach 7 billion by 2012, according to the 2008 revision of the official United Nations population estimates and projections (UN Population Division/DESA). India has a population of 1.16 billion and it is growing at the rate of 1.548% (15.5 millions per year according to 2009 estimate) every year, which is roughly equal to the entire population of Australia. This many extra numbers pose demands for food, water, education and jobs. India has 2.4% of geographical area of the world, whereas its population is 15% of the world's population. We are already about six times over populated. Leave aside land, water essential for life and agriculture is becoming scarce day by day. Although efforts are being made to harness water resources and enhance productivity of the land, there is need to limit the size of the family voluntarily by providing safe and acceptable methods for family planning.

Currently, the methods available for contraception are many. However these are not used by those in need for various reasons. The most dependable single time interventions are tubal ligation for women and vasal ligation for men. In spite of incentives, these are resorted to fairly late in the reproductive life of the couple after engendering many children. Intrauterine devices (IUDs) entail extra bleeding; Indian women who are already anaemic cannot support it for long. Spermicides increase the effectiveness of certain barrier methods of contraception such as diaphragm. However, they do not provide reliable contraception when used alone¹. Steroidal contraceptives in

form of pills, injectibles and implants are highly effective but they have side effects varying from weight gain², decrease in bone mineral density (BMD)³ spotting and derangement of menstrual regularity and bleeding profiles. Condoms bring in besides contraception extra safety against transmission of human immunodeficiency virus (HIV) and sexually transmitted diseases (STD), but in spite of wide publicity for 'safe sex', their use is still not widespread nor consistent. With this background, additional methods are required as options for family planning, specially those that do not require day-to-day motivation, are reversible and do not derange menstrual profiles nor entail extra bleeding. It is in this context that work was initiated many years ago to develop a potential birth control vaccine based on eliciting antibodies inactivating human chorionic gonadotropin (hCG). A safe, effective and reversible birth control vaccine would be a valuable addition to the currently available fertility-regulation methods.

Role of hCG

The choice of hCG as target was based on the rationale that it comes into play only when the embryo reaches the endometrium. No hCG is secreted by the pituitary. Follicle stimulating hormone (FSH), lutenizing hormone (LH) are the gonadotropins made and secreted by pituitary. They travel to the ovaries to develop follicles, secrete estradiol and progesterone and induce ovulation. Antibodies against hCG would not intervene in this axis and women would keep on ovulating and normally producing their sex hormones. It may be recalled that the steroidal contraceptives employed in the pills, injectibles and implants inhibit the hypothalamic-pituitary gonad axis.

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They block the secretion of FSH and LH thereby blocking ovulation and normal estradiol and progesterone production.

The synthesis of hCG starts soon after fertilization of the egg. Eggs fertilized *in vitro* by assisted reproductive technology secrete hCG in the culture medium⁴ before embryo is transferred to the uterus. The hCG secreted at this stage is critical to implantation, which leads to the onset of pregnancy. Antibodies neutralizing the bioactivity of hCG prevent the establishment of pregnancy. The role of hCG in implantation is supported by observations in primates as well as in humans. Marmoset embryos exposed to antibodies against hCG fail to implant, whereas those exposed to normal immunoglobulins implant normally⁵. The prevention of implantation by antibodies against hCG is also evident from the observations in women immunized with a vaccine against hCG. Whereas circulating antibodies against hCG above the protective threshold prevented pregnancy in sexually active women, no lengthening of the luteal phase was observed in them⁶. They continued to ovulate normally and had regular menstrual cycles. Had the antibodies been acting as abortifacients post-implantation, the length of the luteal phase would have been longer and menstruation more copious. This was not the case in trials conducted in women. Thus the vaccine by generating antibodies neutralizing the bioactivity of hCG prevents the nidation or implantation of the embryo and thereby the establishment of pregnancy. No effect is exercised on the hypothalamic-pituitary gonad axis. Hence, neither ovulation is impaired nor the synthesis by the women of natural sex steroids.

Rendering hCG immunogenic

The mother and as the foetus are exposed to high levels of hCG during pregnancy; hence both are immunologically tolerant to this hormone. To make it immunogenic, it was conjugated to a carrier mobilizing the T-cell help. The carrier selected initially was tetanus toxoid (TT). In the 1970s, a large number of women used to die of tetanus by infection contracted during delivery taking place at home or in the field⁷. The use of tetanus as carrier was aimed to accomplish two purposes; to override the immunological tolerance against hCG as well as to induce antibody response against tetanus thereby preventing post-delivery deaths due to this infection.

The hCG is a heterodimer of two subunits α and β ; α is common to three other pituitary hormones, viz. h-thyroid stimulating hormone (hTSH), hFSH, hLH. The β subunit in each case confers the hormonal identity. Hence, hCG β instead of the whole hCG was chosen as antigen with the hope that its linkage with TT would induce antibodies reacting with hCG and not with other pituitary hormones containing the common α subunits. This was indeed found to be the case^{8,9}. Furthermore, sera from three

women immunized with hCG β -TT showed no reaction with human thyroid, pituitary, parathyroid, adrenal, testes and ovaries as examined by immunohistochemical techniques¹⁰. hCG β -TT vaccine not only generated hCG neutralizing antibodies in rodents and monkeys¹¹ but also in four women immunized with this vaccine in a probing clinical trial conducted after thorough reversibility and safety studies¹². Anti-hCG response was obtained in all women along with the antibodies reacting against TT. Figure 1 *a* recalls the pertinent findings. The duration of antibody response in subject ND was 500 days.

Whether the antibodies thus generated recognize *in vivo* hCG or not, was tested by challenge with two doubling doses of hCG. Purified authentic hCG administered to immunized women bound to the circulating antibodies,

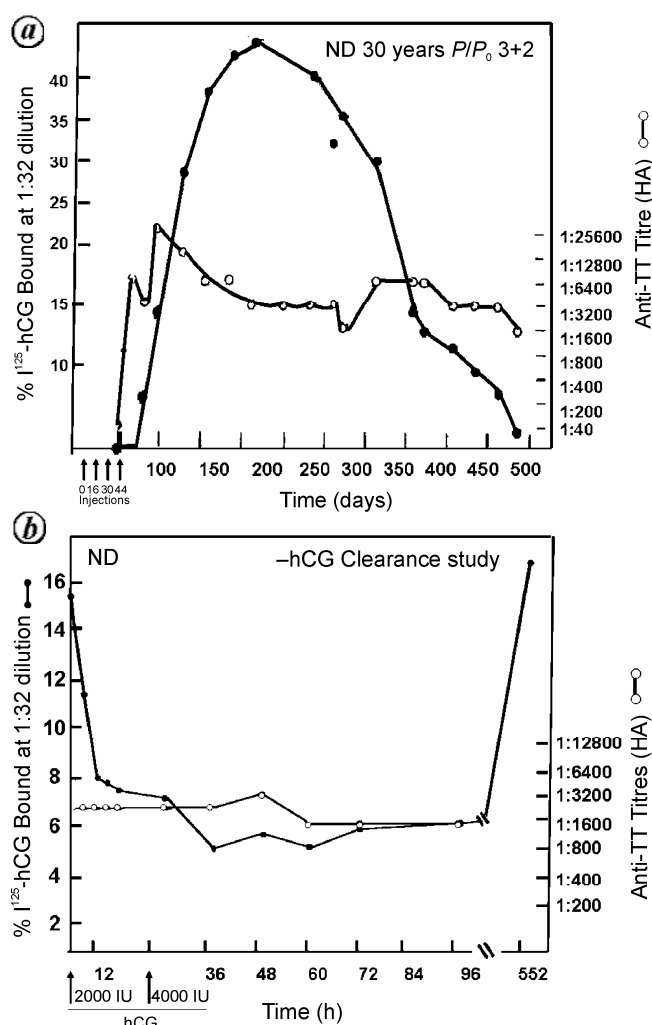


Figure 1. *a*, Time kinetics of anti-hCG and anti-tetanus antibody response in subject ND immunized with four injections of hCG β -TT at fortnightly intervals (adapted from Talwar *et al.*¹²). *b*, Anti-hCG and anti-tetanus titres in the subject ND after injection of 2000 and 4000 IU of hCG. Circulating antibodies bound with the administered hCG causing decline of titers, which in course of time returned to the same level as at the time of challenge. No effect of hCG was seen on anti-tetanus titres (adapted from Ramakrishnan *et al.*⁵⁷).

causing a decline of the antibody levels (Figure 1 b). hCG alone did not act as booster, the titres came back to the normal range in course of time. Thus, the immunization strategy employed could not be expected to cause an internal auto-boosting every time hCG is secreted by women in fertile cycles. This deduction is supported by Phases I and II clinical trials conducted by us in 253 women^{6,13,14}. Interestingly, the administered hCG did not cause any change in the anti-TT titres confirming that the vaccine was generating antibodies independently against both hCG β and TT.

hCG β versus carboxy terminal peptide

hCG β shares 85% homology with hLH β . The hCG β however has an additional 35 amino acid carboxy terminal peptide (CTP), which is not present in hLH β . On basis of its presence in hCG β and not in hLH β , the logic was to employ the CTP instead of the entire hCG β as an immunogen. Vernon Stevens and the World Health Organization (WHO) Task Force in fact chose the CTP¹⁵ for developing an anti-hCG vaccine. We however decided to use the entire hCG β on the following grounds.

- hCG β was much more immunogenic than CTP, which obligatorily demanded the use of strong oily adjuvants before any antibody is detectable¹⁵. In contrast, hCG β -TT was immunogenic with alum as adjuvant which is permitted for human use by the drugs regulatory authorities in India and elsewhere.
- The avidity of the antibodies induced by CTP vaccine was two logs lower than that induced by the hCG β -TT (Ka 10⁸ versus Ka 10¹⁰ M⁻¹). In view of the reported high affinity of hCG for its receptor (Ka 10⁹ M⁻¹), it was our belief that the antibodies elicited by the CTP vaccine would have low bioneutralization capacity. In fact, data is not available on the ratio of hCG bioneutralization capacity and binding capacity index (B/I) of the CTP-TT antibodies, whereas more than one labora-

tory has confirmed the bioneutralization ability of the antibodies generated by the vaccines employing the entire hCG β -TT¹⁶. Table 1 summarizes the properties of these vaccines.

The expected advantage of using CTP as immunogen was only partially realized. The anti-CTP antibodies were no doubt nonreactive with hLH. However, much to the consternation of all, these antibodies reacted with somatostatin producing cells of pancreas and pituitary, whereas the sera generated by hCG β -TT and HSD-TT/DT vaccines did not have such cross-reaction¹⁷. This was presumably due to the fact that the CTP vaccine generates predominantly sequence reading antibodies, whereas the hCG β vaccine elicits predominantly conformation reading antibodies.

The CTP vaccine of Stevens given with strong oil-based adjuvants was withdrawn during clinical trials in Sweden, when it produced unacceptable reactions in the first seven women immunized with it. The WHO task force ascribed the reactions to the instability of the adjuvant. No further trials have however taken place with the CTP vaccine till now, nor any paper published on the subject.

Reversibility and safety clinical trials

The International Committee on Contraception Research (ICCR) of the Population Council, New York carried out a Phase I safety trial on our hCG β -TT vaccine with the approval of the US Food and Drug Administration (FDA) and other agencies in Finland, Brazil, Chile and Sweden. The findings confirmed not only the safety but also the reversibility of the hCG β -TT vaccine¹⁸. Women continued to have regular menstrual cycles and had normal levels of gonadotropins and sex steroids after vaccination. The antibodies neutralized hCG bioactivity in *in vitro* assays.

Table 1. Comparative properties of antibodies generated by the three vaccines formulated against human chorionic gonadotropin

	Immunogen (adjuvant and vehicle)		
	CTP-DT ^a (Squalene, MPD, Arlcel A)	β hCG-TT ^b (Alum)	HSD-TT/DT ^c (alum + SPLPS)
Peak titre (ng/ml)	36–127	120–1800	222–6000
Avidity (M ⁻¹) ^d	10 ⁸	10 ¹⁰	10 ¹⁰
B/I index ^e	?	52	61
Cross-reactivity with hTSH and hFSH	–	–	–
hLH	–	+	+
Pancreatic cells	+ ^f	–	–

^aFrom Jones *et al.*⁵². ^bFrom Thau *et al.*⁵⁵. ^cFrom Om Singh *et al.*¹⁶. ^dAvidity data from collaborative studies. ^eB/I (bioneutralization capacity/antigen binding capacity) index data from Talwar and Singh⁵⁶. ^fData from Rose *et al.*¹⁷.

How hazardous would the cross reaction with hLH be?

The tonic levels of hLH are low throughout the menstrual cycle, but a surge occurs as a peak in the midcycle causing ovulation. The fact that women immunized with hCG β -TT vaccine in India and in other countries continued to ovulate indicates that the partial cross-reaction with hLH of the antibodies induced by the vaccine did not reduce the levels of hLH below the threshold required for inducing ovulation. Nonetheless, sustained cross-reaction with LH over prolonged periods could be detrimental. Also, the antigen-antibody complexes formed as a result of this cross-reaction may get deposited in the kidney, pituitary or the ovaries causing pathological problems. To seek answers to these natural queries, Sheldon Segal planned a long-term study (5–7 years) at the Population Council which was conducted in 63 rhesus monkeys. They were immunized with ovine LH β (oLH β), inducing antibodies frankly cross-reacting with monkey LH (RhLH). Although the cross-reaction of the oLH β antibodies with RhLH was significant, the females continued to ovulate and had regular menstrual cycles. Histopathological examinations were carried out on the ovary and pituitary of 48 of the 63 immunized monkeys. There was no difference between the pituitaries of immunized and non-immunized animals¹⁹. Systematic toxicology²⁰ evaluation in comparison with age-matched controls was carried out in 10 of these animals. Neither the gross pathology, nor the organ weights revealed evidence of adverse side-effects of immunization. Studies on the effects of immunization on atherosclerotic lesions, and coronary and carotid arteries and in the aorta showed no differences between immunized and control monkeys with respect to the arterial characteristics (plaque area, maximal plaque thickness, per cent lesions and per cent stenosis) that were used as indicators of the extent and/or severity of atherosclerosis²¹. Thus, the antibodies with variable degree of cross-reaction with LH in either monkeys or in women did not reduce LH below the critical amount required to induce ovulation (being given that the surge has surplus levels of LH). Long-term (five years) toxicology in monkeys did not put in evidence damage of any tissue due to possible immune complexes.

hCG auto-antibodies

The occurrence of auto-antibodies to hCG is a rare phenomenon. In the literature only two female cases have been reported. One of these was a patient with nine years history of secondary infertility²². She had a normal pregnancy at the age of 16 years with normal vaginal delivery. After three years of contraception, the patient tried for several years to become pregnant. She had regular menstrual cycles and was shown to be ovulating but

experienced recurrent pregnancy loss. On thorough investigations, it was found that she had auto-antibodies to hCG which had low affinity (K_a 1.4×10^6 l/mol), but high capacity (418 nmol/l) for hCG. The circulating antibodies had low cross-reaction with recombinant hFSH, recombinant human LH, hCG α and hCG β (<0.019%, 0.021%, 0.039% and 0.006%) respectively, but prevented the whole dimer hormone hCG action on establishment and sustenance of pregnancy²².

The second case reported in the literature is of a 32-year-old woman, whose infertility was zeroed into circulating antibodies against hCG²³. No clear understanding is available on how these women developed auto-antibodies to hCG. These 'experiments of Nature' in a way confirm the anti-fertility action of antibodies against hCG. They further showed that though such antibodies prevent pregnancy, they do not interfere with ovulation and normal menstrual regularity.

Johnson *et al.*²⁴ examined a large group of infertile women, none of them carried antibodies against hCG or other trophoblast products. Besides these two women, four male patients have been reported²⁵. They were hypogonadotropic, hypogonadic and they developed low affinity anti-hCG antibodies on long-term treatment with exogenous urinary derived hCG.

hCG β based vaccine

hCG β has to link with α subunit to trigger the hormonal response. It is the dimer and not the α or β subunits individually that exercise biological activity. While linking with the homologous human α subunit was contraindicated in view of its identity with α subunit of hTSH, hFSH, hLH, we thought of creating a heterospecies dimer (HSD) by joining non-covalently hCG β with α ovine subunit. The ability of subunits to link is conserved across species in mammals. Ovine LH α (oLH α) can intrinsically link with hCG β to generate HSD. The dimer, HSD, fully recognized the hCG receptor and even evoked a better steroidogenic response than hCG²⁶. Furthermore, conjugates of HSD with TT had better immunogenicity in rats and bonnet monkeys, and the antibodies had a better capacity for neutralization of the bioactivity of hCG⁹. None of the immunized sera was reactive with hFSH and hTSH as determined by binding with radio-iodinated hormones⁹.

Phase I safety trials

After due preclinical toxicology studies and permissions from the Drugs Controller General of India and Institutional Ethics Committees, comparative Phase I clinical trials with the hCG β -TT and HSD-TT vaccines were conducted in five centres in India under the auspices of the Indian Council of Medical Research (ICMR). All women

immunized with these vaccines generated anti-hCG and anti-TT antibodies. Clinical examinations were carried out at intervals of 4–6 weeks. The cycles were ovulatory and menstrual regularity was unaffected¹³. Immunization with these formulations had no significant effect on haematological, clinical chemistry and other metabolic parameters¹⁴. The bionutralization capacities of antibodies generated by the heterospecies dimer-TT vaccine were 25% higher on an average than those generated by hCG β -TT²⁷. On repeated immunization with TT as carrier, few subjects demonstrated suppression of antibody response against hCG due to carrier-induced epitope specific-suppression²⁸, which could be overcome by immunization with an alternate carrier such as diphtheria toxoid (DT) or cholera toxin chain B (CTB)²⁹. Subjects immunized with formulation HSD-TT gave a more consistent antibody response (with not as much variation amongst individuals) and the mean duration of the antibody titres was better in subjects immunized with HSD-TT compared to hCG β -TT.

Phase II efficacy trials with HSD-hCG vaccine

These were the first ever efficacy trials conducted with any birth control vaccine in women⁶. About 148 women

of proven fertility with two living children were enrolled in the trial. All enrolled women were sexually active and hyperfertile. Many had come to the investigating institutions, the All India Institute of Medical Sciences (AIIMS, New Delhi), Post Graduate Institute of Medical Education and Research (PGIMER, Chandigarh) and Safdarjung Hospital, New Delhi, for medical termination of pregnancy. The available methods for family planning either did not suit these women or were not used consistently.

All women generated antibodies against hCG. No pregnancy took place in the women at and above 50 ng/ml titres bionutralization capacity. About 119 (80%) had titres above 50 ng/ml bionutralization capacity. However, only 60% women maintained antibody titres above 50 ng/ml threshold for three months or longer. All women continued to have regular menstrual cycles and had luteal progesterone indicative of normal ovulation. The antibody titres declined with time but booster injections raised the levels (Figure 2). Eight women completed more than 30 cycles by voluntary intake of boosters without becoming pregnant, nine completed 24–29 cycles, 12 completed 18–23 cycles, 15 completed 12–17 cycles and 21 subjects completed 6–11 cycles. Women became readily pregnant in absence of boosters on antibodies declin-

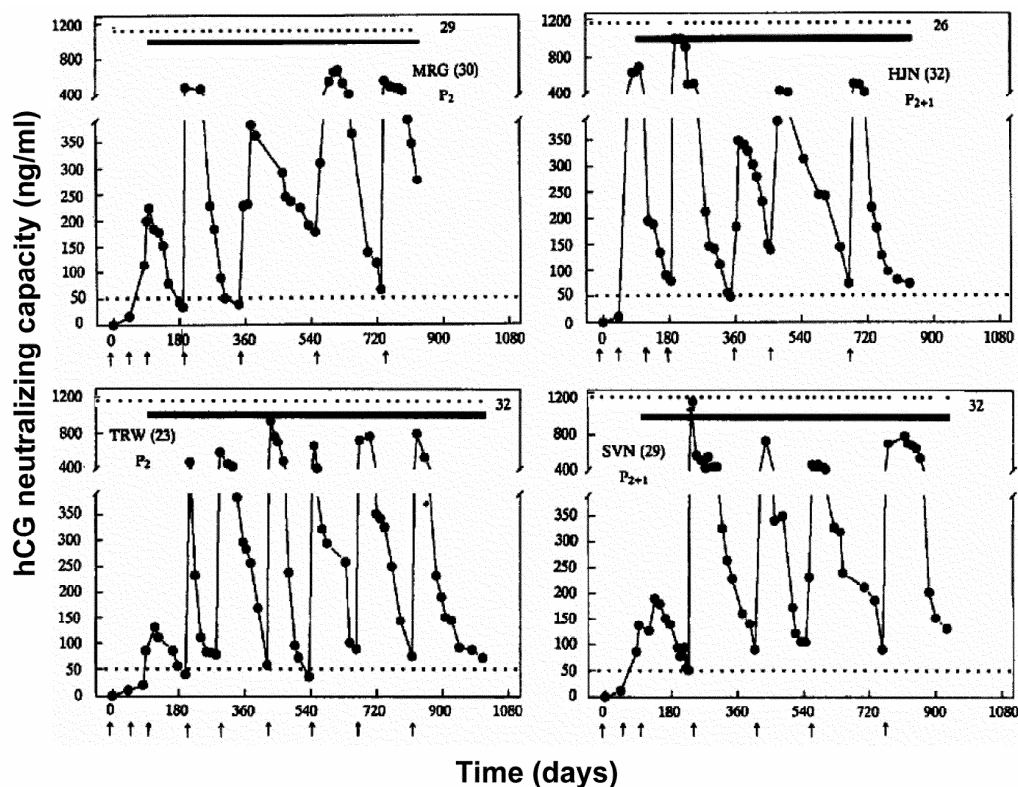


Figure 2. Kinetics of anti-hCG response in four subjects (MRG, HJN, TRW, SVN) after immunization with the HSD vaccine. All subjects were of proven fertility with two live children (P_2); HJN and SVN had also undergone elective termination of an unwanted pregnancy (P_{2+1}). Square at the top edge of the figure denote menstrual events, which were by and large regular and of normal duration. Solid horizontal lines denote the period during which these women were exposed to possible pregnancy with no alternate contraceptive used. Arrows denote injections of the vaccine at a gonadotropin dose of 300 μ g (adapted from Talwar *et al.*⁶).

ing to 35 ng/ml or lower levels. Figure 3 is a typical representation. The subject STS with two children and one medical termination of pregnancy was protected from becoming pregnant for 450 days. She became pregnant in the immediate cycle in which antibody titres fell below 35 ng/ml.

The efficacy of the vaccine to prevent pregnancy was high with only one pregnancy recorded in 1224 cycles above 50 ng/ml (refs 6 and 30). The vaccine was well tolerated. The response to the vaccine was reversible; antibody titres declined in all subjects in the absence of booster injections.

These historic trials provided hard evidence on the ability of circulating anti-hCG antibodies to prevent pregnancy without impairment of ovulation and without derangement of menstrual regularity and bleeding profiles. These also indicated 50 ng/ml bioneutralization capacity as protective threshold titres. Women regained fertility readily as and when titres declined below 35 ng/ml. No short- or long-term adverse effects of immunization were observed. Progeny born to previously immunized women had normal developmental landmarks and cognitive abilities compared to their siblings³¹.

The only shortcoming of this vaccine was the induction of above 50 ng/ml titres in only 60–80% of the women, which would be considered as adequate for a vaccine against infections but for fertility control, efficacy above 90% is required and desirable. Thus, immunogenicity of the vaccine had to be improved to make this candidate vaccine practicable for fertility control.

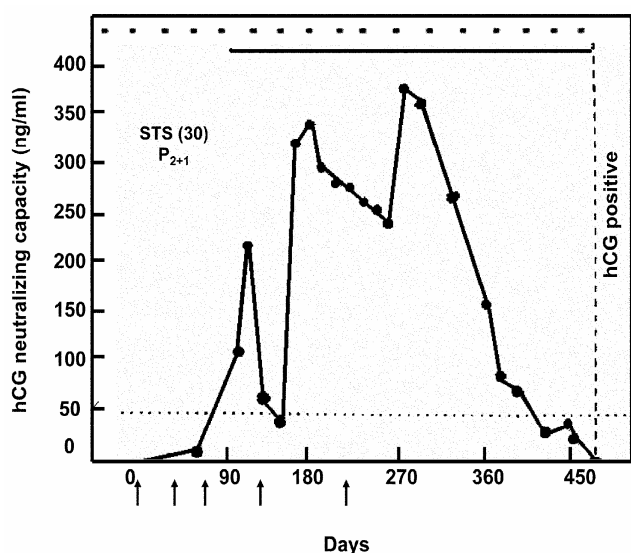


Figure 3. Regain of fertility on decline of antibodies. A 30-year-old subject with two gravidae and one elective abortion (P_{2+1}), on immunization with the vaccine, remained protected from pregnancy for 12 cycles. In the absence of booster injection, antibody titres declined and she became pregnant in the cycle starting on day 417 (adapted from Talwar *et al.*⁶).

Revival of the hCG vaccine

R&D on the hCG vaccine was revived under an Indo-US programme with a grant from the Department of Biotechnology in 2006. This enabled the making of a recombinant vaccine consisting of hCG β linked at C-terminal to B subunit of heat labile enterotoxin of *E. coli* (Figure 4). The engineered construct has been expressed in *Pichia pastoris* with good yield, rendering it amenable to industrial production at low cost. The vaccine adsorbed on just alum, a permitted adjuvant for human use, has generated antibody titres well above 50 ng/ml in every *BALB/c* mouse immunized with it. Studies in mice of five inbred strains of different genetic background, encompassing haplotypes H-2^d, H-2^k, H-2^b, H-2^s, H-2^q confirm the positivity of response with this vaccine in all animals. Inclusion of an adjuvant *Mycobacterium indicus pranii*³² (MIP, previously Mw), which is approved by the Drugs Controller General of India and USFDA as an immunotherapeutic vaccine against leprosy, has further enhanced the antibody titres to as high as 6500 ng/ml in *BALB/c* mice. Mw was incidentally developed by Talwar *et al.*^{33,34} as an immunotherapeutic vaccine for multi-bacillary leprosy. Employed as an adjunct to standard chemotherapy, it expedites the clearance of bacilli and clinical recovery of patients^{34,35}. MIP shares antigens with both *Mycobacterium leprae* and *Mycobacterium tuberculosis*. It is a potent stimulator of IL-12 and IFN- γ . This remarkable adjuvant brings in additional attributes to the vaccine. Ongoing work by Dipankar Nandi at the Indian Institute of Science, Bangalore indicates preventive and therapeutic

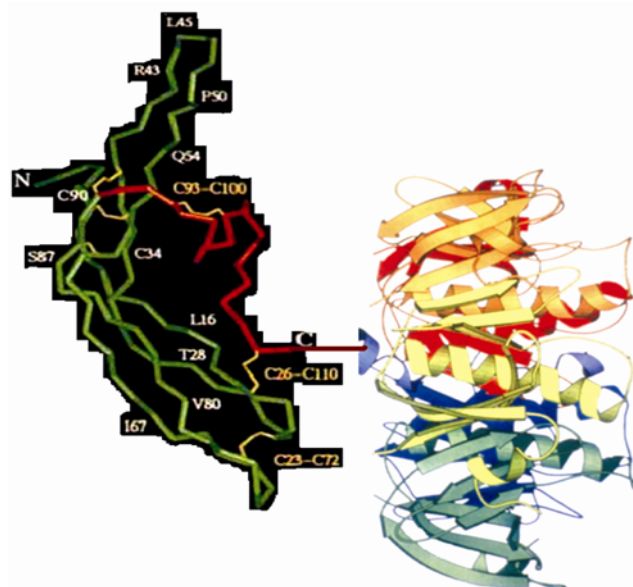


Figure 4. The new recombinant anti-hCG vaccine hCG β -LTB inducing antibodies in every mouse at several fold higher titres than 50 ng/ml. The carrier LTB (according to Salmond *et al.*⁵⁴) is linked at C-terminal amino acid glutamine of hCG β unit (according to Laphorn *et al.*⁵³).

tic action of Mw against two cancers tested in mice. Studies by Om Singh at the National Institute of Immunology, New Delhi show the therapeutic action of the hCG β -TT vaccine plus Mw in hCG synthesizing tumours. Of late, many reports have appeared on the ectopic or unexpected expression of hCG/hCG β by a variety of cancers like lung³⁶, bladder³⁷, colon³⁸, gastric^{39,40}, pancreatic⁴¹, breast⁴², cervical⁴³, oral^{44,45}, head and neck⁴⁶, vulva/vaginal^{47,48}, prostate⁴⁹ and renal cancers^{50,51}. Detection of hCG β expression in tumours is usually associated with poor prognosis and adverse survival^{33,47}. Thus a safe vaccine generating high titre bioeffective antibodies may find additional utility in treatment of advanced stage cancers expressing hCG/hCG β .

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ACKNOWLEDGEMENTS. This article is dedicated to the late Sheldon Segal, who with his sharp intellect and foresight rescued the hCG β -TT vaccine from closure by conducting valuable safety and reversibility trials in Finland, Sweden, Chile and Brazil under the International Committee on Contraception Research of the Population Council, New York. We thank the Department of Biotechnology for the grant under Indo-US programme for the hCG vaccine.

Received 30 June 2009; revised accepted 4 June 2010