INTRODUCTION
**Introduction**

As the population of this planet keeps rising unabated, one realizes that there is an unmistakable need for improved and reliable methods for birth-control. Safe, effective and reliable birth-control vaccines would be a great addition to currently available methods and in fact may turn out to be superior in some respects (Fathalla and Brazelatto, 1988). Thus while birth-control vaccines which are safe, reversible, devoid of interference in the normal physiological functions of the female (except for preventing pregnancy) and which require a periodic intake would find much use in developing countries, such methods would also be equally acceptable and useful in economically developed countries.

Vaccines were traditionally made to deal with external pathogens bacteria, toxins, viruses, and parasites. The idea of using selected "self" target antigens as immunogens, is on the one hand still relatively novel, on the other is an idea which has been actively pursued by a number of investigators over the last two decades. That birth-control can be brought about by immunological means is evidenced by naturally occurring immunologic infertility and by experimental data. A large number of authenticated cases of infertility caused by immunological means have been reported over the years in subjects in whom all other genetic, anatomic and
endocrinological causative factors have been clearly ruled out (Talwar, 1980).

Fertility control vaccines are aimed at intercepting events leading to successful reproduction. The National Institute of Immunology is engaged in developing an antifertility vaccine based on human chorionic gonadotropin (hCG), immunization with which leads to the development of anti-hCG antibodies and a block in pregnancy (Talwar 1980, Talwar et al, 1991). hCG is a self protein in humans and is therefore usually non-immunogenic in humans; it can be made immunogenic, however, by conjugating it to a foreign carrier such as tetanus toxoid (TT), diphtheria toxoid (DT) or cholera toxin chain B. Presumably, these carriers induce a helper T cell response which provides help to hCG-specific B cells for the production of hCG-specific antibodies.

The prototype hCG-based vaccine consisted of βhCG linked covalently to tetanus toxoid (TT) (βhCG-TT); the ability of this conjugate to induce anti-hCG antibodies was confirmed in probing clinical trials in India, Finland, Sweden, Brazil and Chile (Talwar et al, 1976).

Another formulation takes advantage of the fact that βhCG can specifically form dimers not just with αhCG but also with heterospecies αLH (Strickland et al, 1980). This
vaccine formulation which consists of a heterospecies dimer (HSD) of BhCG and aoLH linked to TT, is currently under phase II efficacy trials. This HSD has a higher steroidogenic potency than the native hCG molecule, is more immunogenic in animals and it elicits antibodies with superior bioneutralizing capacity (Talwar et al, 1988; Pal et al, 1990).

The success of these vaccines is dependent on a humoral response, which in turn depends on T cell help. This T cell help is crucial in the case of BhCG as it is a "self" molecule and thus presumably may not have T cells reactive to it, since the self-reactive T cells may have been deleted during thymic education. An appropriate carrier linked to hCG thus elicits T cell help. To obtain a good antibody response it is necessary for the conjugate to have an optimal ligand:carrier stoichiometry. While conjugate preparations made earlier using periodate, glutaraldehyde or heterobifunctional reagents did evoke antibody responses against BhCG, they had sub-optimal ligand:carrier ratios of (3.5:1). In the first part of this thesis is presented a new method for conjugation of BhCG to carriers using two heterobifunctional reagents and provides evidence for a relationship between ligand:carrier ratios and anti-hCG antibody responses. Formulations with higher BhCG:carrier ratios (upto 10:1) and higher immunogenicity were obtained by this new method. The method is also superior in a
predictable and reproducible manner the conjugation of
the ligand to the carrier.

During Phase I clinical trials on the hCG vaccine, it was
observed that some of the subjects failed to manifest a
clear booster anti-hCG response upon secondary
immunization with the vaccine even though they had
initially developed substantial anti-hCG titres after
primary immunization (Gaur et al, 1990). Antibody
responses in these women was restored by immunization
with the same αoLH-βhCG dimer linked to an alternate
carrier (Gaur et al, 1990). This form of hypores-
ponsiveness may be partially attributable to the
phenomenon of 'epitope specific suppression' initially
described by Tada et al (1972, 1979) and Eardley and
Sercarz (1976), then studied extensively by Herzenberg
and colleagues (1982, 1983) and by others (Schutze et al
John et al 1989). These studies have confirmed that
preimmunization with a carrier (such as TT) often results
in an inhibitory effect on the production of antibodies
to new epitopes or ligands linked to the same protein.

Previous experiments relating to this phenomenon have
been done with small molecules or haptens such as
dinitrophenyl (Herzenberg et al 1982) and synthetic
peptides (Lise et al 1987, Schutze et al 1987). We were
interested in ascertaining whether antibody responses to
large molecules such as βhCG and αoLH (molecular weights 24,000 and 14,000 daltons respectively) conjugated to TT, can be affected similarly by preimmunization with TT. The principal objective of this study was to investigate the effects of carrier preimmunization on levels of anti-hCG antibodies in the βhCG-TT system and on levels of anti-βhCG and anti-αoLH antibodies in αoLH-βhCG-TT system, after subsequent immunization with the appropriate conjugates. An experimental animal system was developed to stimulate the phenomenon of carrier induced immunosuppression. This thesis reports data which show that presensitization with TT does inhibit anti-βhCG responses in BALB/c mice subsequently immunized with βhCG-TT. Similarly anti-βhCG responses are inhibited in two out of four strains subsequently immunized with αoLH-βhCG-TT. Interestingly enough, this hypo-responsiveness is directed only at βhCG and not against αoLH even though both these ligands are presented in a composite manner, conjugated to the same carrier, TT. This effect appears to correlate with the subclass of IgG antibody response; the anti-αoLH response is predominantly of the IgG\textsubscript{1} type, while the anti-βhCG response is IgG\textsubscript{1}, IgG\textsubscript{2a} and IgG\textsubscript{2b}. The final part of this thesis describes two ways to bypass carrier-induced suppression, one involving the use of an alternate carrier, DT, and the other involving the use of a synthetic T helper epitope from TT as a carrier.
Thus, this thesis focuses on basic immunochemical and immunoregulatory aspects of the βhCG vaccine. It contributes to the optimization of the vaccine formulation and to understanding the nature of antibody responses to ligand-protein conjugates in general.