FERTILITY CONTROL THROUGH ACTIVE IMMUNIZATION USING PLACENTA PROTEINS

By

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ABSTRACT

Numerous studies have demonstrated that antibodies to placental proteins in a variety of species are capable of preventing or disrupting gestation. Early work in this area was primarily directed towards the passive immunization of rodents with heterologous antisera to whole placental extracts. Toxicity and renal damage often accompanied fertility inhibition. More recent studies reported less toxicity and a higher specificity of antibodies to reproductive function when anti-placental antibodies were absorbed with serum and extracts of non-reproductive organs. Few studies have been reported in which active immunization with placental proteins was employed. The most detailed studies of active immunization have employed highly purified placental hormones. Immunizations of rats and rabbits with human placental lactogen have resulted in marked reduction in reproductive function. Immunization of human females with chemically altered (hapten-coupled) HCG resulted in the production of antibodies reacting with unaltered HCG and pituitary LH. These antibodies were capable of reducing the level of endogenous serum LH in pre- and postmenopausal women. They also altered the events of the menstrual cycle in premenopausal women. More specific inhibition of chorionic gonadotrophin has been obtained by immunization of baboons with the beta subunit of HCG. Antifertility effects without alterations in the menstrual cycle of female baboons immunized with the beta subunit of HCG have been reported. The antibodies produced in these animals reacted significantly with human LH in vitro. The possibility of using hormonal and non-hormonal placental proteins as antigens for the specific immunological inhibition of fertility remains.
Antibodies raised to placental proteins have been shown to disrupt pregnancy in laboratory animals in several studies. Few reports have demonstrated that the action of such antibodies were specific to the products of conception and did not react to antigens of non-reproductive organs. Further, no application of passive immunization procedures has been suggested for application to human fertility control because of problems associated with sensitivity reactions to repeated injections of foreign serum proteins. In recent years, progress has been made toward the isolation of highly purified proteins from placental tissue and preliminary experiments have been conducted using active immunization techniques to prevent or disrupt gestation. These latter experiments have provided data which now indicate that a method of human fertility control may be developed by immunization with placental proteins.

Although at our current state of knowledge there is no opportunity to apply passive immunization methods to human fertility control, many data suggesting feasibility of methods and mechanisms of action have been obtained in passive immunization studies. In order to provide background information, some of the more significant studies in this area will be reviewed in this report.

**Passive immunization studies**

Disruption of pregnancy by antisera to placental antigens was demonstrated early in this century (Dobrowski 1903); however, few further studies were reported until much later. Antisera to whole rat placental extracts were administered to normal pregnant rats on days 11 and 12 of gestation (Seegal & Loeb 1940). These workers found that the treatment produced resorption of 66 per cent of foetuses; however, equal or greater damage to pregnancy could be produced by injections of antisera to rat blood. Therefore, no conclusions could be reached on the basis of this study regarding specific action of the antibodies. Later, the same authors (Loeb et al. 1949) demonstrated that abortion was induced in 100 per cent of pregnant rats injected with antiserum to rat placenta together with deoxycorticosterone acetate. Although the antiserum used in these latter experiments was more effective in producing abortion, all treated rats showed renal hypertrophy and nephritis. Another report (Cohen & Nedzel 1940) described the abortion-inducing action of antisera to guinea pig placenta when administered to guinea pigs. Although antibodies to several organs were present in the antisera, in vitro absorption removed non-placenta specific antibodies and five of six pregnant animals aborted after the administration of absorbed antiserum.

Similar studies as above were conducted in mice (Koren et al. 1968). An antiserum raised to whole mouse placenta in rabbits was injected into pregnant mice on days 10–14 of gestation. Although nearly all mice aborted, kidney
and liver damage was observed in treated animals and approximately 10 per cent died following immunization. A control group of animals receiving normal rabbit serum did not suffer total loss of pregnancies and renal lesions were not correlated to the disruption of pregnancy in the treated animals. These studies suggested that at least some of the abortions were produced by specific antibodies to placentae.

Another study using rabbit anti-mouse placenta serum in mice showed abortion and toxicity despite prior absorption of the antisera (Nemirovsky 1970). However, in 1971 Behrman and co-workers reported studies of passive immunization of mice with rabbit anti-mouse placenta serum in which much less toxicity was observed (Kometani & Behrman 1971). Antisera were absorbed repeatedly with normal mouse serum and toxicity levels were established prior to the antifertility studies. Abortion could be produced in all animals if sera were administered on day 11 or later in gestation. Fewer abortions were observed when immunizations were performed during early pregnancy. Evidence was presented to suggest that cytotoxic effects produced by antisera were complement dependent. Immunodiffusion tests of effective antisera indicated antibodies to at least four different placental proteins.

Perhaps the most carefully conducted study of the effects of passive immunization in rodents using antisera to placental proteins was reported by Beer et al. (1972). Rat placenta at 12–14 days of gestation were homogenized and partially fractionated by centrifugation. The resulting trophoblast cells were used to immunize rabbits and the antisera obtained were used to passively immunize pregnant rats at 7–18 days of gestation. The unabsorbed sera produced abortion in 100 per cent of the animals but 2 of 12 rats died from this treatment. In the same study, rabbit anti-rat lymphocyte serum caused abortion in 25 per cent of the treated rats but 67 per cent of the animals died. Subsequently, rabbit anti-rat placenta serum was absorbed with rat lymphocytes; the toxicity of the antisera disappeared but its abortifacient activity was retained. Conversely, when the anti-rat placenta serum was absorbed with rat trophoblast tissue, the abortifacient activity was no longer present. Histological examination of uteri, decidua, ovaries, kidneys, lungs and livers of female rats aborted with absorbed anti placenta serum revealed no detectable damage. The abortion-inducing action of anti-rat placental antisera was not effective in disrupting pregnancy in hamsters or mice. No effects upon pregnancy in female rats were observed when antisera to rat trophoblast produced in male rats were used.

Studies have also been conducted using antibodies to purified placental proteins. El Tomi et al. (1971a) demonstrated that abortion could be produced in rats by administering antisera to highly purified human placental lactogen (HPL) raised in rabbits. Antisera were shown to cross-react with rat LH and rat prolactin and the number of implantations were reduced in animals in-
jected with antisera early in gestation. Animals treated later in gestation had normal implantation numbers but significant foetal resorption occurred. This study suggested that sera to HPL reacted with rat placental antigen(s) necessary for normal gestation. Whether such substance(s) had any functional similarity to placental lactogen was not apparent from this study.

A similar study to the above was reported the following year (Gusdon 1972). In this study, total foetal resorption was observed in pregnant rats after the injection of rabbit anti-HPL sera. No effects were found in non-treated controls or rats injected with normal rabbit serum. The striking observation from this report was that those females receiving anti-HPL serum returned to normal oestrous cycles after abortion but failed to conceive upon repeated matings for 11 months after the initial immunization. No explanation for this unusual observation was provided. Similar abortifacient activity of anti-HPL sera was also observed in mice (Yamini et al. 1972). These authors demonstrated a complement dependent cytotoxic action of anti-HPL and suggested that pregnancy disruption may be caused by the anti-lymphocyte antibodies contained in the anti-HPL sera. Stevens et al. (1971) reported that abortions were produced in pregnant baboons using baboon anti-HPL sera. No detailed characterization of the antisera used was made although cross-reaction with baboon placental extract was demonstrated. The studies reported using antibodies to HPL leave many questions unanswered and the study of the effects of antibodies specific for placental lactogen in a primate species would provide more meaningful data.

Antisera to human chorionic gonadotrophin (HCG) have shown the capability of interfering with reproduction in non-human species. Highly cross-linked HCG injected into rabbits resulted in the production of anti-HCG sera which cross-reacted with endogenous mouse gonadotrophin (Schlumberger & Anderer 1969). In these studies, injections of anti-HCG sera into female mice induced prolonged oestrous periods for 18–20 days and disrupted fertility for periods of 37–100 days. No antisera were administered to pregnant mice and the relationship of anti-HCG sera and mouse placental proteins was uncertain. Although the effects of HCG antisera upon fertility were not determined, Mougadal et al. (1971) demonstrated that such sera were capable of reducing the secretion of progesterone from the corpus luteum in non-pregnant rhesus monkeys. These sera apparently cross-reacted with endogenous monkey LH, but reactivity with chorionic gonadotrophin from pregnant monkeys was not studied.

A cross-reaction of antisera to the subunits of HCG with non-human primate chorionic gonadotrophins has, however, been shown by other workers (Hodgen et al. 1973). A relatively low cross-reaction of antisera to the beta subunit of HCG with human or baboon LH has also been observed (Stevens, unpublished). In this latter study, a sheep antisera prepared against the beta subunit of
HCG which cross-reacts with baboon CG was administered to a pregnant baboon on day 20 of gestation. This resulted in an immediate fall in endogenous baboon chorionic gonadotrophin and ovarian steroid levels. Heavy vaginal bleeding was observed approximately 36 hours after the intravenous injection of the antisera (Fig. 1). This work clearly confirmed the classical concept that chorionic gonadotrophin is necessary for maintaining the steroid secretion from the primate corpus luteum of pregnancy.

In recent years, extensive efforts have been made to evaluate the effects upon reproduction of antisera to non-hormonal primate placental proteins. In an extension of earlier work using antisera to whole placental extracts, Behrman and co-workers recently reported antifertility effects of antisera directed to specific placental proteins of rhesus and squirrel monkeys (Behrman et al. 1974). These workers isolated a fraction from monkey placental extracts that reacted with antisera raised against whole placentae even following absorption
with normal serum. Although no antisera were produced to this fraction, the antisera reacting to it produced abortions in 63–80 per cent of the rhesus and squirrel monkeys studied. While this report suggests that antibodies producing abortions may be specifically directed to a placental protein, the criteria of purity of the reacting fraction were not documented and an exhaustive characterization of the abortifacient antisera was not presented.

Two "placenta-specific" proteins have been isolated from human term placentae by *Bohn* (1972). One of these proteins is present in large amounts in pregnancy sera (SP₁) while the other is not (PP₃). Antisera raised in rabbits to human SP₁ have been shown to react with extracts of placentae and pregnancy sera from rhesus monkeys, Cynomolgus monkeys and baboons (*Bohn*, unpublished), but not with pregnancy sera of rats and guinea pigs (*Bohn* & *Ronneberger* 1973). Antibodies to human SP₁ were effective in producing abortions in 8 of 10 Cynomolgus monkeys at 19–55 days of gestation following a single injection (*Bohn*, unpublished). Thus it appears that antibodies to SP₁ will cross-react readily with antigens from primate placentae but not with placental proteins from animals of lower phylogenetic levels. Further studies are necessary to determine the specificity and mechanism of abortifacient action of antibodies to SP₁.

**Active immunization studies**

Relatively few studies have been conducted employing active immunization techniques with homologous or heterologous antigens. Even fewer reports have appeared in which highly purified proteins were used to elicit antibody responses. In 1966 *Okunda* & *Groilman* reported studies in which female rats were immunized with whole rat placental extracts. Although 8 of 12 immunized rats conceived following mating, only one rat had any living foetuses by day 20 of gestation. The antisera produced in these animals reacted strongly with kidney extracts, and upon examination all animals showed nephritis. Whether disruption of fertility in these animals was related to antibodies to placental proteins could not be ascertained. Similar procedures were used and similar results were obtained in another study, in which rabbits were immunized with total rabbit conceptus from the ninth day of gestation (*Menge* 1968).

Another study employed the guinea pig as the species immunized with whole placental extracts (*Paine* & *Kennedy* 1968). Five animals were immunized using Freund's complete adjuvant. The number of oestrus periods required for conception was greater than normal in the treated animals. Reduced fertility was observed in two animals, one died and the other two had normal pregnancies. A somewhat more detailed study was conducted by *Boss* & *Neken* (1970) in which females were immunized with fractions of rat placental homogenates. No significant difference was noted in antibody responses to different placental
fractions obtained by centrifugation, and injections of most fractions caused antibody responses in 50 per cent or more of the rats in each group. Only about 30 per cent of the immunized rats became pregnant, but in those that did conceive there was no detectable disruption of gestation. A similar type of experiment was performed by Menge (1968) in cattle. Ten heifers were injected with homogenates of whole bovine conceptus (32–38 days gestation) in Freund’s complete adjuvant and were then bred by artificial insemination. Immunized heifers required a mean of 3.8 inseminations per conception while control animals needed only an average of 1.2. In pregnant immunized animals, no significant difference in the number of viable foetuses at day 16 of gestation was observed when compared to control animals.

In yet another study, no effects on conception or gestation were observed when extracts of homologous placentae were injected into rabbits; however, the number of pregnancies was reduced by one-half if the injections of extracts were followed by chronic administration of HCG (Goldzieher et al. 1970). No effects upon conception or on the course of pregnancy were noted from injections of HCG alone in this study. Similarly, Glass & Mroueh (1967) found that rabbits immunized with HCG readily became pregnant and exhibited normal gestation.

Significant disruptions of the oestrous cycle and gestation were observed in female rats following active immunization with purified human placental lactogen (El Tomi et al. 1970). The mean cycle length was nearly doubled in HPL injected rats over the control animals which were treated with Freund’s adjuvant. The conception rates were also slightly reduced in the immunized animals; however, there was no significant reduction in implantation rates in those animals conceiving. However, the number of foetuses surviving until term was markedly reduced in HPL immunized rats, and in the animals immunized at 90 days of age, no young were born from any of 28 females of this group. In another group, immunized at 21 days of age and mated 3 months later, 24 of 37 animals conceived and about 60 per cent of the foetuses survived. These authors speculated that a rat placental protein cross-reacted with antibodies generated to HPL which resulted in a diminished fertility.

Another report from the same laboratory suggested that HPL immunizations exert an effect on reproduction also in the rabbit (El Tomi et al. 1971b). Female animals immunized with purified HPL and Freund’s adjuvant readily produced antibodies to HPT. However, no disruption of gestation was apparent. No difference was found in the number of corpora lutea or implantation sites between control and immunized rabbits. On the other hand, all animals showed some foetal resorption and the number of young born in surviving litters was only about 15 per cent of control litters. Both the rat and rabbit studies discussed above suggested immunological similarity of a human placental protein and one or more components of placentae of lower species.
Since none of the studies discussed above described effects of active immunization with a highly purified protein to a homologous species, few data were available to suggest feasibility of using such methods as a means of human fertility control. The design of studies for potential human application of active immunization was plagued by two major problems. The first of these is that isoantigens are rarely antigenic and the second is the unacceptability of Freund's complete adjuvant for human use. In an attempt to circumvent these problems, Stevens (1973) conducted studies in which homologous antigens (pituitary gonadotrophins) were hapten-coupled with a diazonium salt and injected into female baboons in an oil vehicle. The findings from this study indicated that such antigen alteration could result in the production of antibodies cross-reacting and neutralizing biological effects of endogenous unaltered antigens without the aid of conventional adjuvants. The data obtained in this study stimulated another study in which postmenopausal women were immunized with hapten-coupled HCG in saline (Stevens, unpublished). Low levels of antibodies to HCG and human LH were detected 5–7 weeks after the first of four injections. The concentration of endogenous LH was reduced in

![Graph](image_url)

**Fig. 2.**

Effects of immunization with hapten-coupled HCG upon FSH and LH levels in a postmenopausal woman (Stevens, unpublished).
8 of 10 women during the period of significantly elevated antibody levels. Antibodies were abundant for only 1–2 months (Fig. 2). In the same study, 4 previously sterilized premenopausal women were similarly immunized and a higher level of antibodies was observed. Likewise, endogenous LH levels were reduced for a longer period of time (Fig. 3). Subsequent detailed studies showed that these premenopausal women were anovulatory, but unfortunately, no control studies were performed to determine their ovulatory status prior to the immunization.

In order to demonstrate the effectiveness of immunological procedures for disrupting hormonal levels in endocrinologically normal women, another study was conducted in which control data showed normal hormonal patterns prior to the start of immunization (Stevens & Crystle 1973). In this latter study, nine premenopausal women, previously sterilized by bilateral salpingectomy, were studied through a control menstrual cycle by assessing serum levels of pituitary and ovarian hormones. Only six of these exhibited normal patterns. Subsequently, these six were immunized with 10 mg of HCG coupled with 40
haptenic groups per HCG molecule. Antibodies reacting with HCG and LH were detected in all subjects at 3–5 weeks of immunization. During a second menstrual cycle, the effect of the immunization upon hormonal patterns was assessed. Midcycle LH peaks were reduced or abolished in all six subjects and baseline LH values were reduced in four women. Patterns of serum progesterone indicated that 4 of the 6 did not ovulate but the other two showed elevated luteal phase progesterone levels despite reduced LH peaks at midcycle. The data obtained from one of these subjects are shown in Fig. 4.

Four of these six subjects were studied during another cycle six months after the first immunization. Significantly elevated antibody levels were found in 3 of the 4 cycles. Midcycle LH peaks were absent in 3 of the 4 cycles but the progesterone patterns suggested that two of the cycles were ovulatory. Levels of serum oestrogens were generally reduced in all four cycles and one of those showed only slight elevation at midcycle (Fig. 5). Throughout the entire study of HCG immunization of premenopausal women, menses and cycle length remained normal in all subjects. Lymphocyte transformation tests for delayed hypersensitivity revealed normal findings.

![Graph](image-url)

**Fig. 4.**

Normal hormonal patterns and effects of immunization with hapten-coupled HCG upon hormonal pattern in a premenopausal woman (Stevens & Crystle 1973).
Hormonal patterns in a premenopausal woman six months after immunization with hapten-coupled HCG (Stevens & Crystle 1973).

Although the human studies described above illustrated the feasibility of reducing the circulating levels of a human hormone by isoimmunization, it should be borne in mind that HCG antibodies normally do not discriminate between HCG and LH neither biologically nor immunologically. Therefore, the presence of persistent antibodies to HCG in young women may not only render them anovulatory but is likely to result (upon prolonged treatment) in amenorrhoea and clinical symptoms of ovarian deficiency. Thus, while these studies were significant as a model for immunological investigations in humans, no direct application to human fertility control was provided.

Recent studies have indicated that antibodies produced to the beta subunit of HCG have a relatively low cross-reactivity to human LH (Ross et al. 1972).
Since the subunit preparations used were not completely pure, it was hoped that a “pure” beta subunit of HCG might elicit antibody response to HCG, but not to LH. Consequently, a highly purified preparation of the beta subunit of HCG was used to immunize sheep, rabbits and baboons (Stevens, unpublished). The antibodies produced reacted with HCG, baboon CG and human LH, but not with baboon LH. These findings discouraged the use of beta HCG in humans as a means of fertility control but permitted antifertility evaluation of HCG immunization in baboons. During this study, 10 fertile baboons were immunized and were subsequently mated with males of proven fertility. Preliminary evaluation of the antifertility effect of the immunization revealed that no pregnancy lasted past expected time of menses following 30 matings (Table 1). The previous conception rate in non-immunized baboons in the colony was about 90 per cent. These studies indicate that in case an antigen could be prepared that elicited antibodies specific for HCG and not reacting with human LH, an immunological method for human fertility control may become feasible.

Table 1.

Effects of immunization of female baboons with beta HCG on fertility.

<table>
<thead>
<tr>
<th>Baboon No.</th>
<th>No. of matings</th>
<th>Ovulations</th>
<th>Pregnancy*</th>
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* Pregnancy defined as any animal not having menses at or before expected time for normal cycle.
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DISCUSSION

Cinader: The strategy of immunization with chemically modified autologous antigens (Cinader 1963), now being applied to hormones and to placental antigens, is based on model experiments in which animals with neonatally induced tolerance were immunized with para-azo-sulphonic acid derivatives of the tolerogen (Cinader & Dubert 1955, 1956). The ensuing response results in antibody which can combine with tolerogen. This reactivity is attributable to peptide determinants which are conformationally altered, as a consequence of conjugation with the hapten (Cinader et al. 1967; St. Rose
& Cinader 1967). The proportions of hapten/tolerogen must be determined as a function of the number of responding individuals and of the highest antibody quantity directed against peptide determinants. This hapten/tolerogen proportion is lower than that which induces maximal antibody response, directed against hapten. In this context, it should be noted that the capacity to respond with tolerance breakdown is under genetic control (St. Rose & Cinader 1973). Individuals which are resistant to tolerance circumvention can be made responsive by adjuvant administration. Some thought might be given to the possible use of adjuvants which do not have harmful side effects.

Talwar: About two years ago, a WHO Research and Training Centre (RTC) was established in New Delhi, and one of the major projects of that RTC is to try to develop antiplacental approaches to fertility control.

Our approach is slightly different from the one that Dr. Stevens had adopted. Let me emphasize that in none of our experiments have we used whole HCG. We have only worked with the beta subunit. For the non-endocrinologists in this audience I may mention that the alpha subunit of HCG is identical with that of three other hormones, TSH, FSH and LH, and consequently there is a danger of crossreaction if one uses whole HCG. The beta subunit, even in the purest form available, when analysed by electrophoresis on polyacrylamide gels shows microheterogeneity. It resolves in about 8 bands. We have studied the antigenic properties of each of these 8 bands, and the reactivity of each of these bands along the polyacrylamide gel has been analyzed with the anti-HCG serum and anti-HLH serum. These are competitive type experiments where the binding of labelled HCG to anti-beta HCG, or of labelled LH to anti-LH is determined. When the antigenic determinants for beta HCG are analyzed, the HLH reactivity partly lies outside the spectrum, partly within the spectrum. The outside part can be removed chemically, but to remove the one overlapping the reactivity against HCG we used an immunosorbent against a heterologous LH preparation. With gradient absorption one can remove the high affinity absorbing sites to arrive at a preparation which gives almost negligible crossreaction, practically no crossreaction with HLH. On the other hand, it preserves the determinants against HCG. At this stage, it is essentially one band, though at high concentrations one may see two bands. Another major difference is that this highly purified beta HCG is conjugated to a proteinic carrier rather than modified by haptenic groups. The complex of beta HCG with tetanus toxoid in appropriate proportions is immunogenic in a large variety of species, ranging from mouse, rabbit, goat, monkey to human. The immunity is of a long-lasting type; there is a rise in the antibody titer gradually reaching a plateau around the 30th week in the goat and slightly earlier in the monkey. Later there is a tendency to decay, but with another injection a powerful booster response is obtained. The antiserum does not react with any of the hormones we usually come across, i.e., HGH, HPL, FSH, TSH, even HLH at physiological concentrations. On the other hand, the antiserum reacts immunologically with the total HCG molecule. Immunological reactivity does not necessarily mean that the antiserum would also neutralize the biological effects. The biological effectiveness has been tested in at least two or three systems, such as the ventral prostate assay and the production of progesterone by isolated corpus luteum slices. The antiserum is able to neutralize the HCG-induced effects in these systems.

In short, the main differences between Dr. Stevens’ approach and ours is that we are dealing with a chemically and immunochemically purified preparation of beta subunit of HCG with almost negligible or no reaction with LH, and then the complex
that we are studying is the subunit of the hormone conjugated in discrete proportions with a proteinic carrier, a type of vaccine approved for human use, i.e., the tetanus toxoid, which I think is highly immunogenic and has desirable immunization properties.

Stevens: I think this is very nice work that Dr. Talwar has done and I would agree with him that antisera to beta subunit of HCG react very little with LH. Even with our preparations, which were not chemically characterized so extensively, we have produced antisera in animals with as low as less than one per cent of the reaction with HLI as they do with HCG. However, the Review Group of WHO decided that this was still too much crossreaction to attempt studies in humans, and, therefore, did not recommend to follow this line of experimentation in human females.

Simons: You said that lymphocyte transformation tests revealed negative or normal findings in your active immunization studies. Could you clarify what you mean please? Secondly, you referred to the desirability of avoiding adjuvants, such as Freund's, in immunizing procedures which have application to humans. It may be possible to achieve an adjuvant effect in humans by combining antigen immunization with a BCG immunization programme. Such an approach would have the additional advantage of association with a widely practised public health procedure. Do you know of a reason which might preclude such an approach?

Stevens: First, lymphocyte transformation tests were not performed directly under my supervision. As I understand it, the procedure was the blast formation type of test in which the modified HCG was introduced with the peripheral lymphocytes of the patients and observation of the number of cells that had been transformed. There was no difference between the control transformation and the samples taken at various periods during and following immunization.

As far as the use of adjuvants is concerned, what I meant to say was that one could not preclude the use of all adjuvants but should not use adjuvants of the Freund's complete type. Certainly, there are many types of adjuvants acceptable for human use, such as the ones you have suggested, and we are currently performing an extensive evaluation of many adjuvants that could be used with various kinds of altered or chemically modified proteins and peptides.

Fudenberg: With regard to the use of BCG, if BCG is to be given repeatedly, this can result in humoral immunity. Some cancer patients so treated clearly have developed immune complex disease. There have also been a wide variety of other very undesirable side effects, so I think repeated use of BCG is precluded. In this context, have you looked at your immunized baboons to see if they had any urinary abnormality findings or renal function that are suggestive of latent antigen-antibody complex renal disease?

Stevens: The only studies we have done were to look for the excretion of excess albumin in urine. The results were negative.

Talwar: With the preparation that we use we have not observed any untoward effect at any level. Last September, our National Agency gave us permission for some controlled human trials. With the help of our Obstetrics & Gynecology Department, a few cases have been studied and very carefully monitored for a variety of systems, such as kidney, liver, thyroid, adrenal and pituitary functions, and up till now there is nothing untoward to report.
Amino acid Sequence of β-hCG & β-hLH

Fig. A.
The amino acid sequence of beta subunits of human LH and HCG as reported by Morgan et al. (1973).
* indicates location of carbohydrate attachment on native hormone.

Amino acid Sequence of a C-Terminal Peptide of β-hCG

Fig. B.
The amino acid sequence of a C-terminal peptide of the beta subunit of HCG that is not represented in the beta subunit of LH. The two structures represent sequences reported by two different groups of workers.
* indicates location of carbohydrate attachment on native hormone.
Mitchison: Dr. Stevens, could you tell us about the C-terminal peptide, which seems to be unique to HCG?

Stevens: This is a point that I hoped to discuss. That long tail that you saw earlier on the beta subunit of HCG when comparing the structures of beta subunits of HCG and LH (Fig. A) is represented by its amino acid structure on this slide (Fig. B). Slightly different sequences have been reported by two different groups of workers. There was a deletion of a serine at position 121 by one worker and there is a difference in three amino acids at the C-terminal end; however, these structures are essentially the same. We have made preparations of this, and a slightly larger peptide from natural HCG by chymotrypsin digestion. We have also prepared a synthetic peptide using this structure with 4 more amino acids at the N-terminal end. Antibodies have been produced to these peptides in rabbits. We have not had sufficient quantities to immunize baboons yet, but hopefully, we can start very soon. The antibodies produced to these peptides react with the native HCG, the beta subunit of HCG, but not with human LH. We have found that these peptides are not immunogenic under all circumstances. They need to be conjugated with a larger molecule or haptenic group to render them antigenic. We plan to prepare a variety of these synthetic peptides of different lengths and with different conjugations and to test with many types of adjuvants to try to find the most desirable immunization reagents and schedule that will produce long term antibody levels with a single injection.

Fox: Have you made any histological studies on target organs for the gonadotrophins of immunized animals?

Stevens: Yes, in the animals that were immunized with the HCG beta subunit we have done some studies. Fluorescent-labelled antibodies were reacted with pituitary tissue and with tissues of a host of other organs. In the animals that had been immunized for a long period of time, we have done histopathological examinations on some organs, including the pituitary. We have not found any evidence of tissue damage. However, one must remember that we did not show any evidence of crossreacting antibodies to baboon pituitary hormones, so our negative findings are not terribly surprising. We hope that we can obtain some guidelines for the kinds of safety studies that should be done in this area in order to conduct a critical evaluation of this method.

Rowe: Dr. Stevens, you assess immune response by antibody measurement and then you observe an effect on hormones and on fertility. Do you have direct evidence that these effects are due to antibody? Can you, for instance, transfer antibody passively and see the same effect?

Stevens: I could show you another series of slides showing data obtained when normal pregnant baboons were immunized with hormones raised in the baboons as well as antibodies raised in other animals. We have shown that we can abort baboons with anti-HCG sera. Using placental lactogen, we have immunized pregnant baboons with baboon antiplacental lactogen sera and observed similar effects. I am not willing to conclude that this action is directly due to the neutralization of the circulating or intracellular hormone. The antigen-antibody reaction with the hormone might have caused some secondary damage, resulting in abortion. The answer to these questions must await more detailed studies.

Lunenfeld: Dr. Stevens, I wonder whether you could elucidate a technical point that
you did not mention either in your written or in your oral presentation. How did you measure LH in the presence of LH crossreacting antisera, and vice versa, how did you measure antiserum levels in the presence of endogenous crossreacting hormone?

Stevens: To make a very short answer, I cannot explain it. However, you must realize that the antibodies were produced to altered antigen that did not have identical determinants as the native molecule. The only explanation I can give is that perhaps some of the antibodies were not reactive with native hormone, but were with the $^{125}$I-labelled antigen used to detect them. The antibodies to native antigen used in the radioimmunoassay would react with the native endogenous hormone, thus reflecting a measurable level of hormone in the same serum sample in which antibodies were found.

Talwar: May I ask Dr. Stevens whether in the six cases where whole HCG was given, you have seen the effect on thyroid or TSH?

Stevens: No, we have not studied the effects on thyroid or TSH.

Voisin: What we are aiming at in this meeting is the induction of immune reactions directed against antigens involved in the reproduction mechanisms with the idea that these reactions must counteract these processes. We must, however, keep in mind that a significant part of the immune reaction acts in the opposite direction and that this seems to be of great use in physiological situations. In the experiments I shall refer to, which were done in our laboratory with Gerard Chaouat, we studied non-hormonal placental antigens, but antigens that are linked to the surface of trophoblastic cells, as Dr. Talwar alluded to. We made use of two strains of mice, C57 BL/KS and A/J, differing both at the H-2 and non-H-2 loci. KS females were made pregnant by either KS or A/J males for 1-5 pregnancies. Their placentae were examined for the presence and specificity of maternal immunoglobulins bound to the placenta. What was found, in brief, was as follows: First, maternal immunoglobulins were strongly fixed on and not washable off the cell membrane of trophoblast. This was much more prominent in allogeneic than in syngeneic pregnancies and even more so in placentae from multi-

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**Table A.**

Facilitation (enhancement) action of placental eluates on Sarcoma 1 (A/Jax) tumours\(^*\) grafted on C57B1Ks mice\(^**\).

<table>
<thead>
<tr>
<th>Origin of placental eluates</th>
<th>Number of grafted mice</th>
<th>Lethal takes</th>
<th>Rejections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Father</td>
<td>Mother</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/Jax</td>
<td>C57Ks</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>C57Ks</td>
<td>C57Ks</td>
<td>15</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^*\) $2 \times 10^6$ Sa1 cells grafted on either male or female C57Ks.

\(^**\) Male or female.
parous females. Immunofluorescence studies showed that maternal immunoglobulins are strongly fixed at the surface of placental, trophoblastic cells, even after a long perfusion followed by several washings of the slides. These antibodies were mainly of the IgG₁ type (mouse IgG₁ like guinea pig IgG₁ are the anaphylactic, non-complement fixing antibodies). These antibodies can be eluted. They are specific of the paternal transplantation antigens, and they have the properties of facilitating antibodies, that is, when injected into normal KS mice they render them able to accept the graft of paternal strain A/J sarcoma cells. As shown by the data of Table A, out of 10 mice there were 9 lethal takes of the sarcoma graft, while, when the eluates – prepared in the same way – came from isogenic placentae, there was no one take out of 15 grafted mice. The simple message brought by these experiments is that immunization may go two ways, facilitation reaction as well as rejection reaction, the former counteracting the latter, and this is as true in reproduction immunology as it is true in cancer immunology and in transplantation immunology.

Stevens: I think it is important to point out that there are several possible mechanisms which may be operative for the antifertility effects of HCG antibodies. One possibility is that the antibodies might be cytotoxic to the cells of the trophoblast. This has been carefully described by Morisada et al. (1972). There is also the possibility that when the zona pellucida comes off, there may be a coating of the blastocyst with antibodies, thereby preventing implantation in much the same way that antibodies to the zona pellucida may act. Furthermore, you must remember that chorionic gonadotrophin is necessary endocrinologically for extending the life of the corpus luteum of pregnancy so that the uterine environment will be maintained during the early stages of gestation. Antibody neutralization could prevent this maintenance. Lymphocyte suppressing activity of HCG has also been suggested. Should antibodies remove HCG from trophoblast cell surfaces, normal immunological surveillance may terminate the pregnancy.

We plan now to prepare several different synthetic peptides, all representing part or parts of the C-terminal portion of the HCG beta subunit that is exclusive to the chemical structure of HCG. We further plan to conjugate these peptides with varying types of acceptable conjugates that could be used in humans, and then evaluate each of these types with varying vehicles (adjuvants) and time schedules to obtain the ideal method of immunization. When these experiments are completed, we will immunize baboons and perform critical studies of safety and efficacy. Then, hopefully, we will get permission from our Ethics Committee to perform human trials.

References: