

## Effects on pregnancy in mice of passive immunization against ovine LH and human chorionic gonadotrophin\*

A. Tandon†, C. Das, B. L. Jaikhani, Amitabh Gaur, S. Sehgal‡, G. Gopinath§ and G. P. Talwar

*Departments of Biochemistry and §Anatomy, All India Institute of Medical Sciences & National Institute of Immunology, New Delhi-110029, and ‡Post-Graduate Institute of Medical Education and Research, Chandigarh-160012, India*

**Summary.** Mice given daily i.p. injections of immunoglobulins against ovine LH on Days 3–7 of pregnancy were devoid of implantation sites on Day 8 whereas mice treated with antibodies to hCG had embryos of normal number and appearance on Day 8. These antibody treatments reduced the mean  $\pm$  s.d. serum progesterone concentrations from  $65.4 \pm 15.3$  ng/ml (control globulins) to  $8.6 \pm 4.9$  ng/ml (anti-LH) and  $9.2 \pm 3.1$  ng/ml (anti-hCG) on Day 8 and had no differential effect on serum oestrogen levels on Day 4. However, the mice treated with anti-hCG did not litter; resorption of the embryos took place between Days 10 and 14 of pregnancy.

Indirect immunofluorescence and quantitative immunoenzymic assays showed the presence of anti-ovine LH and anti-hCG reacting antigens in the mouse fetoplacental unit. On Day 6, the values of reacting antigens (mean  $\pm$  s.d. absorbance units/10  $\mu$ m section of embryo) were  $0.050 \pm 0.002$  with control globulins,  $0.059 \pm 0.002$  with anti-hCG-Ig and  $0.196 \pm 0.018$  with anti-LH-Ig; the corresponding values on Day 12 were  $0.075 \pm 0.009$ ,  $0.402 \pm 0.02$  and  $0.416 \pm 0.015$ . The quantitative disposition of the reacting antigens to the two types of anti-gonadotrophins seems to bear a temporal relationship to their respective antifertility action.

The pregnancy terminating action of immunoglobulins to ovine LH (Days 6, 7 & 8) and hCG (Days 8, 9 & 10) was counteracted by administration of 2 mg medroxyprogesterone acetate on Days 6, 9 and 12, indicating the importance of progesterone in the maintenance of pregnancy in the mouse.

### Introduction

Immunization against LH or chorionic gonadotrophin (CG) is able to terminate early pregnancy in a variety of animals (mouse: Munshi, Purandare & Rao, 1972; rat: Loewit & Laurence, 1969; Madhwa Raj & Moudgal, 1970; Bambra & Gombe, 1978; hamster: Rao, Madhwa Raj & Moudgal, 1972; rabbit: Spies & Quadri, 1967; bonnet monkey: Prahalada, Mukku, Rao & Moudgal, 1975; Moudgal, Mukku, Prahalada, Murty & Li, 1978; marmoset: Hearn, Short & Lunn, 1979; baboon: Stevens, 1976; Tandon, Das, Jaikhani & Talwar, 1981). The mechanism by which such immunization causes abortion is not fully understood. The antibodies could abrogate the gonadotrophin-induced rescue of the corpus luteum. The rise of serum progesterone during the

\* Reprint requests to Professor G. P. Talwar, National Institute of Immunology, New Delhi-110029, India.

† Present address: Department of Obstetrics & Gynaecology, Hyogo Medical College, Nishinomiya, Hyogo 663, Japan.

second part of the luteal cycle is temporally related to the appearance of CG in man (Yoshimi, Strott, Marshall & Lipsett, 1969) and other primates (Knobil, 1973) and stimulation of progesterone production by the corpus luteum by CG is demonstrable *in vivo* (Garner & Armstrong, 1977) and *in vitro* (Rice, Hammerstein & Savard, 1964). The antigonadotrophin may also have an action locally at the implantation site. Immunoreactive hCG-like material is detectable along the trophoblast lining in villi (Midgley & Pierce, 1962; Paul, Jaikhani, Gupta & Talwar, 1979) and it has been suggested that lining by a 'self' immunologically tolerated protein provides protection to the fetoplacental unit against immunological rejection (Borland, Loke & Wilson, 1975). The experiments reported in this paper evaluate the contributory role of these factors in mice immunized against gonadotrophins. Antibodies against a widely cross-reacting gonadotrophin, ovine LH, as well as hCG which has a narrower specificity were employed. We used mice as the experimental animals because of the reported presence of a CG-like material in implantation sites and placentae from Day 5 until Day 19 of gestation (Wide & Wide, 1979).

### Materials and Methods

**Antibodies.** Purified hCG (biological activity: 12 000 i.u./mg, provided by the Population Council, New York, U.S.A.) or ovine LH (NIH-LH-S17) was dissolved (100 µg of each) in sterile saline (8.5 g NaCl/l) and emulsified with an equal volume of Freund's complete adjuvant (Difco Laboratories, Detroit, MI, U.S.A.). The emulsion was injected intradermally at multiple sites on the shaven backs of rhesus monkeys: 6 monkeys (3 for each hormone) were immunized. Booster injections of 50 µg hCG (or ovine LH) emulsified with incomplete Freund's adjuvant (Difco) were given at 4 intramuscular sites every 4 weeks. Two monkeys were injected intradermally with Freund's complete adjuvant alone (without antigen) and were given Freund's incomplete adjuvant intramuscularly to serve as donors of control sera. Control and immunized monkeys were bled every 14 days by saphenous venepuncture. Sera were stored at 4°C with 0.1% sodium azide as preservative. Sera having a binding capacity for <sup>125</sup>I-labelled hCG of more than 40–50% at 1:1000 dilution in a radioimmunoassay system (Shastri, Dubey, Vijaya Raghavan, Salahuddin & Talwar, 1978) using ethanol: ammonium acetate (final concentrations, 6.6% ammonium acetate and 66% alcohol) instead of second antibody were pooled. Immunoglobulins (Ig) were precipitated from pooled immune or control serum at 50% ammonium sulphate saturation, dissolved in 10 mM-phosphate-buffered saline (PBS), pH 7.4, and dialysed against PBS for 72 h with 6 changes of PBS. The dissolved dialysed globulins were centrifuged at 12 000 g for 45 min to remove suspended undissolved particles and the solution was passed through a Millipore disc (0.22 µm pore size) under sterile conditions.

The hCG-neutralizing ability of the antibodies was determined by the mouse Leydig cell assay (having a sensitivity of 15 µi.u. hCG), supplemented by the weight gain assays for the mouse uterus and rat prostate gland (Das, Salahuddin & Talwar, 1976; Das *et al.*, 1978).

**Mouse pituitary gonadotrophin.** Pituitaries from adult mice of the Swiss strain were suspended (10 mg wet weight/ml) in phosphate-buffered saline (containing 6.5 mM-Na<sub>2</sub>HPO<sub>4</sub> · 2H<sub>2</sub>O, 1.4 mM-KH<sub>2</sub>PO<sub>4</sub>, 135 mM-NaCl and 0.1% BSA) pH 7.4 and sonicated at 20 000 Hz for 15 min. The sonicate was centrifuged at 20 000 g for 45 min at 4°C. The supernatant was tested in the mouse Leydig cell assay (Das *et al.*, 1978) for gonadotrophic activity. The ability of immunoglobulins to ovine LH and hCG to neutralize mouse pituitary gonadotrophic activity was checked in the same bioassay system. Pituitary extract and antibodies were incubated at 37°C for 2 h and then at 4°C overnight, after which the mouse Leydig cells were added to this mixture and the testosterone produced was measured by radioimmunoassay. The antiserum (S-741 No. 7) used in this assay was obtained from Dr G. A. Abraham, California. Labelled testosterone (tritium at four places) was purchased from New England Nuclear (Boston, MA, U.S.A.).

**Animals.** Cyclic female mice of the Swiss and NMRI strains, randomly bred at AIIMS and

weighing 25–30 g, were used. A total of 80 mice was taken for the study and females were caged in groups of 3 with a male of proven fertility. The day of finding a vaginal plug was taken as Day 1 of pregnancy. Mice were killed by cervical dislocation at different stages of pregnancy. Uterine horns with embryonic implantation sites were frozen at  $-70^{\circ}\text{C}$  and stored until required.

*Medroxyprogesterone acetate.* An aqueous suspension of medroxyprogesterone acetate (6 $\alpha$ -methyl-17 $\alpha$ -hydroxy-pregn-4-ene-3,20-dione acetate), supplied by Upjohn Company, Kalamazoo, MI, U.S.A. as Depo-Provera, was emulsified with ground-nut oil. In mice, the dose of MPA required for pregnancy maintenance after removal of ovaries on Day 6 of pregnancy was determined. The MPA emulsion was injected intramuscularly on Days 6, 9 and 12 of pregnancy.

*Steroid assays.* Serum progesterone/oestradiol concentrations were measured by a competitive protein-binding method as described by Aso, Guerreo, Cekan & Diczfalusy (1975). The antisera were supplied by the WHO Programme for the Provision of Matched Assay Reagents for the Radioimmunoassay of Hormones in Reproductive Physiology. The specificity of the progesterone antiserum was expressed by its cross-reactions at 50% binding as follows: 5 $\alpha$ -dihydroprogesterone (7%), 20 $\alpha$ -dihydroprogesterone (<3%), 17 $\alpha$ -hydroxyprogesterone (<3%), testosterone (<0.2%), and cortisol (<0.01%). The specificity of the oestradiol antiserum was expressed by its cross-reactions at 50% binding as follows: oestrone (1.7%), testosterone (<0.0002%) and cortisol (<0.0001%).

Due to the high specificity of the antisera used, it was possible to perform the radioimmunoassay directly on the ether extracts of serum without employing chromatographic purification of the steroids. Samples for the progesterone assay (0.1 ml) were extracted with a 20-fold volume of anhydrous diethyl ether by vortexing for 1 min. The aqueous layer was frozen in an ethanol:solid  $\text{CO}_2$  mixture and the ether solution was decanted into a test tube. The residue, obtained after the evaporation of ether at room temperature overnight, was dissolved in 0.2 ml gelatin-phosphate-buffered saline, 0.01 M, pH 7.4 (containing 0.1% sodium azide) by heating the mixture in a  $60^{\circ}\text{C}$  water bath for 10 min and vortexing for 15 sec.

The aqueous extract (0.2 ml) was combined with a 0.1 ml mixture of equal volumes of appropriately diluted antiserum ( $\text{Bo} = 40\%$ ) and radioactive progesterone/oestradiol solution. The resulting solution (0.3 ml) was incubated for 10 min at  $60^{\circ}\text{C}$ , followed by 18 h at  $4^{\circ}\text{C}$  for separating the bound and unbound fractions. Then 0.5 ml chilled 1% charcoal prepared in gelatin-phosphate-buffered saline was added to the tubes. After keeping in an ice bath for 15 min, the tubes were centrifuged at 1000 g for 5 min. The charcoal supernatant was decanted in a scintillation vial containing 1 ml double-distilled ethyl alcohol. After putting 10 ml scintillation fluid (0.4% PPO and 0.05% POPOP in toluene) in each vial, the radioactivity of the bound fraction was counted in Packard Scintillation Spectrometer. The non-specific binding was 2–4%. For the progesterone assay, the limit of sensitivity was 12.5 pg with the standard ranging from 12.5 to 400 pg/assay tube. For the oestradiol assay the limit of sensitivity was 6.25 pg with the standard ranging from 6.25 pg to 400 pg/assay tube. The inter- and intra-assay coefficients of variation were in the range of 10–12% and 4–6% respectively for these steroids.

*Immunofluorescence test.* Uterine horns with embryos kept at  $-70^{\circ}\text{C}$  were brought to  $-20^{\circ}\text{C}$  and embedded in OCT compound (Lab. Tek. Products, Naperville, IL, U.S.A.). They were sectioned longitudinally at 10  $\mu\text{m}$  in a cryostat set at  $-20^{\circ}\text{C}$ . The sections were mounted on a microscope slide and stored at  $-20^{\circ}\text{C}$  overnight. Storage prevented the loss of sections during subsequent washing operations. The slides were brought to room temperature and the sections of implantations were allowed to react with immunoglobulins to hCG or to ovine LH for 30 min at  $37^{\circ}\text{C}$  in a humid chamber. Embryos at the same stage of development were also independently treated with control monkey globulins. After 30 min, unbound globulins were washed off with 50 mM-phosphate-buffered saline, pH 7.4: the slides were passed through 3 changes of the buffer in 45 min. Implantation sites were subsequently treated with a 1:16 dilution of fluorescein conjugated with antihuman immunoglobulin (Burroughs Wellcome Laboratories, Beckenham, Kent, U.K.) for 30 min. Excess of conjugate was washed off, as described above, in PBS for 45 min. The slides

were mounted in 5% glycerol in PBS and viewed under a Reichert u.v. microscope using exciter filter E<sub>1</sub> and barrier filter SP<sub>3</sub>. Stained sections could be stored at 4°C for 2–3 weeks without any loss of intensity.

**Immunoenzymic test.** Longitudinal sections (10 µm) of mouse embryos at two developmental stages (Days 6 and 12) were exposed to control monkey globulins or to anti hCG/ovine LH immunoglobulins at 1:50 dilution in PBS for 60 min at 37°C. The sections were then washed by immersing in PBS for 45 min with 3 changes of the buffer at 37°C. Endogenous peroxidase activity was blocked by dipping the slides in methanol containing 0.3% hydrogen peroxide for 30 min at room temperature. Methanol was removed by washing in PBS with 3 changes of buffer over a period of 45 min. The sections were then treated with Protein A tagged with horseradish peroxidase (Sigma Type VI) at 1:200 dilution in PBS for 60 min at 37°C, and washed in PBS for 45 min with 3 changes of the buffer. The embryo sections were then dissolved in 1 ml citrate-phosphate buffer, 200 mM, pH 5.5, containing 0.1% Triton X-100 and collected in a tube. Citrate-phosphate buffer (1 ml) containing ortho-phenylenediamine (2 mg/ml) and H<sub>2</sub>O<sub>2</sub> (0.12%) was added to all tubes and allowed to react in the dark for 15 min. The enzyme action was stopped by adding 0.2 ml 5 N-H<sub>2</sub>SO<sub>4</sub>. The colour density was read at 492 nm in a Pye Unicam SP8-100 spectrophotometer. The absorbance obtained was then divided by the number of embryos present in the section.

## Results

### *Effects on pregnancy of immunization against LH and hCG*

Pregnant mice were injected intraperitoneally with the immunoglobulin fraction of the antisera to ovine LH or hCG or to control globulins daily between Day 3 and 7 of pregnancy (Table 1). Laparotomy on Day 8 showed that most of the mice treated with antibodies to LH had no implantation sites. Two of the mice in this group had resorbing embryos. To check whether the lack of implantation sites was due to failure to ovulate, the experiment was repeated but with a unilateral laparotomy, under ether anaesthesia, on Day 3 of pregnancy. The ovaries were examined for ovulation points and then the mice were treated with antibodies to LH as before. The 5 mice treated with 50 mg control globulins/0.5 ml PBS had  $3.9 \pm 2.8$  (s.d.) ovulations on the right ovary on Day 3 and  $6.9 \pm 1.8$  embryos in the uterine horns on Day 8. In the 7 anti-LH-treated mice (47 mg Ig/0.5 ml) there were  $4.1 \pm 2.1$  ovulations on Day 3 but no implantations in 6 of the mice and 7 resorbed embryos in the other mouse on Day 8.

**Table 1.** Effect of immunoglobulins (Ig) against ovine LH and hCG on implantation sites on Day 8 of pregnancy in mice

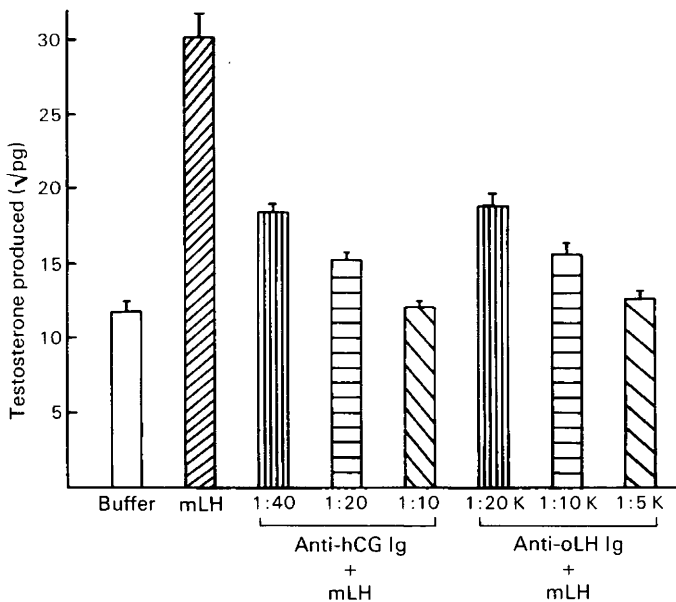
Group	Treatment*	No. of mice	Implantation sites on Day 8 (mean $\pm$ s.d.)
I	Control monkey globulins (injected with Freund's complete adjuvant alone) (50 mg Ig/0.5 ml)	7	$8.8 \pm 1.8$
II (a)	Monkey anti-oLH Ig (47 mg Ig/0.5 ml)	12	0 in 10 mice $7.9 \pm 1.0$ resorbing in 2 mice
II (b)	Monkey anti-oLH Ig diluted 500 times (94 µg/0.5 ml)	8	0 in 7 mice 8 resorbing in 1 mouse
III	Monkey anti-hCG Ig (45 mg/0.5 ml)	9	$8.4 \pm 2.1$

\* Globulins equal to the amount indicated were injected i.p. daily on Days 3–7.

Treatment with antibodies to hCG had no effect on the number and gross appearance of embryos on Day 8 of pregnancy (Table 1). The globulin preparation used was able to bind hCG, as determined by radioimmunoassay, with a titre of 13  $\mu\text{g}$  hCG/ml and was also effective in neutralizing the activity of hCG in the mouse Leydig cell, mouse uterine weight and rat ventral prostate weight gain assays.

*Reactivity of antibodies to ovine LH and hCG with mouse pituitary gonadotrophin*

The two types of immunoglobulins were examined for reaction with mouse pituitary extract in the mouse Leydig cell assay. The results in Text-fig. 1 show neutralization of the effect of the pituitary gonadotrophin by the anti-LH and anti-hCG immunoglobulins. However, the antibodies against ovine LH were about 500 times more potent than those against hCG. Another set of experiments was therefore performed using immunoglobulins against ovine LH diluted 500 times. This preparation had the same effect as was obtained with greater amounts of antibodies.



**Text-fig. 1.** Action of immunoglobulins (Ig) against ovine LH or hCG on the mouse pituitary extract-induced rise in testosterone production by mouse Leydig cells. See 'Materials and Methods' for experimental details. The antibodies were tested at three dilutions. K is equal to 1000. Values are mean  $\pm$  s.d. for triplicate determinations.

*Effect of antibody treatment on sex steroid levels*

Serum progesterone levels were measured on Day 8 of pregnancy in mice treated with immunoglobulins against LH and hCG. The progesterone concentrations in the antibody-treated mice were of the same order and substantially lower than those in mice receiving control globulins (Table 2).

However, the serum oestradiol concentrations on Day 4 of pregnancy, i.e. around the day of implantation, were not affected (Table 2).

**Table 2.** Effect of administration of immunoglobulins (Ig) against ovine LH and hCG on Days 3–7 on serum progesterone and oestradiol levels in pregnant mice

Group	Treatment	Progesterone conc. on Day 8 (ng/ml)	Oestradiol conc. on Day 4 (pg/ml)
I	Control monkey globulins	65.4 ± 15.3 (6)	31.6 ± 2.3 (6)
II	Monkey anti-oLH Ig	8.6 ± 4.9 (12)	29.8 ± 1.4 (6)
III	Monkey anti-hCG Ig	9.2 ± 3.1 (7)	32.5 ± 1.7 (6)

Values are mean ± s.d. for the no. of mice in parentheses.

#### *Delayed action of anti-hCG immunoglobulins*

Some of the animals treated with antibodies to hCG were allowed to go to term so that the normality of the progeny could be assessed. However, no young were born. Laparotomy on Day 22 of pregnancy revealed the presence of embryonic sites. Similarly treated mice were then laparotomized on Days 10 and 14 of pregnancy. On Day 10 the uterus appeared similar to that on Day 8, except that the embryos had grown. However, by Day 14 the embryos had begun to resorb.

#### *Antigonadotrophin-reacting antigens on the feto-placental unit*

These experiments were carried out to see whether antigens reacting to the antibodies against LH and hCG are present on the mouse feto-placental unit and, if so, whether these bear any relation to the antifertility effect exercised by the two types of antibodies.

Two techniques were employed to evaluate the reaction of the anti-gonadotrophin antibodies with the frozen sections of uterine horns containing implantation sites: the immunofluorescence technique gave essentially qualitative information, but the method utilizing horseradish peroxidase was amenable to quantitation by measurement of the enzyme activity in conditions in which the reaction products formed over unit time were proportional to the amount of the enzyme fixed with the antibodies on the tissue. With the immunofluorescence method, on Day 6 of gestation there was a slight reaction with anti-LH immunoglobulins, but on Day 12 both immunoglobulin types exhibited intense reactions (Pl. 1, Figs 1–5).

In the horseradish peroxidase test, the antibodies bound to tissue were determined in the second stage of incubation with Protein A conjugated to horseradish peroxidase. On Day 6, the mouse embryos had very few anti-hCG-reacting antigens but greater amounts of anti-LH-reacting material (Text-fig. 2). By Day 12 the amount of reacting antigen was increased to similar levels for both immunoglobulin types.

#### PLATE 1

Photographs of tissue of the mouse feto-placental unit stained by the direct immunofluorescence test to detect the presence of antigonadotrophin-reacting antigens.

**Fig. 1.** Day 6 pregnant mouse treated with anti-hCG Ig.

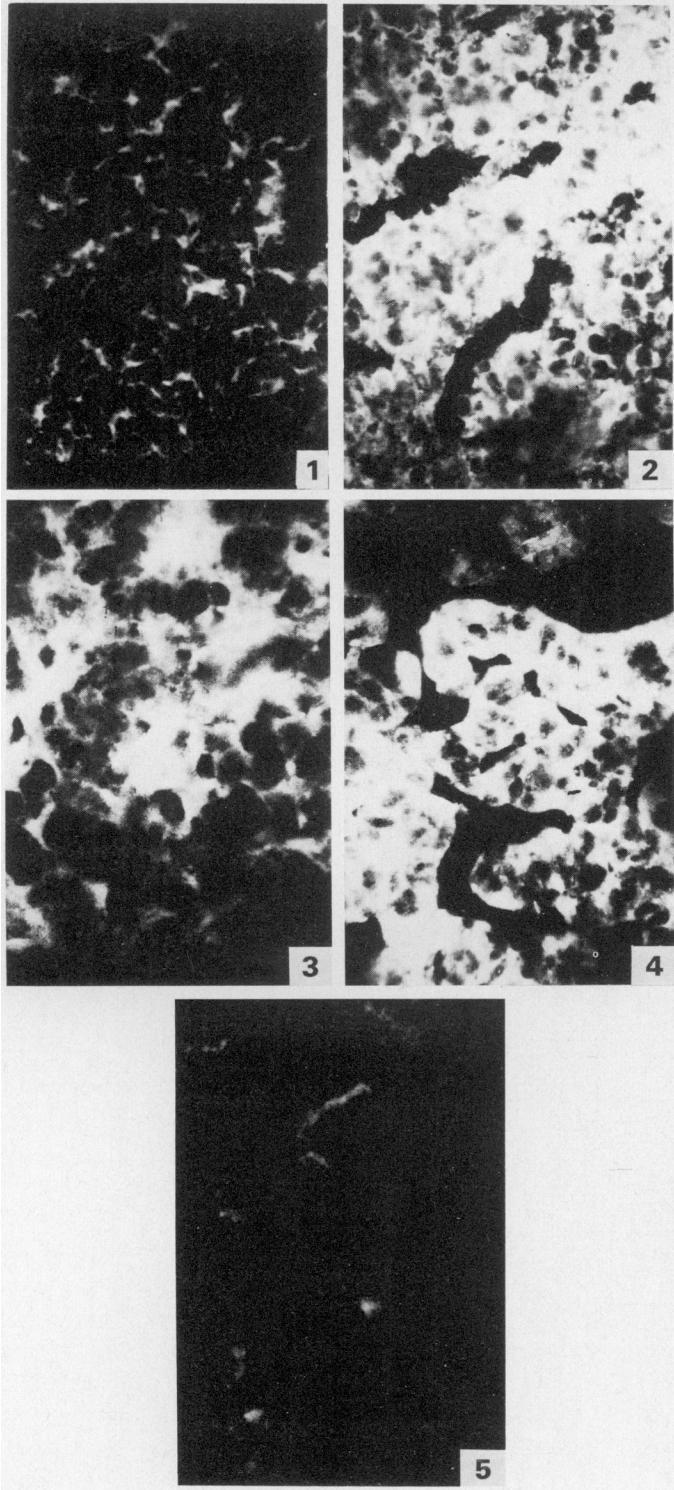
**Fig. 2.** Day 6 pregnant mouse treated with anti-oLH Ig.

**Fig. 3.** Day 12 pregnant mouse treated with anti-hCG Ig.

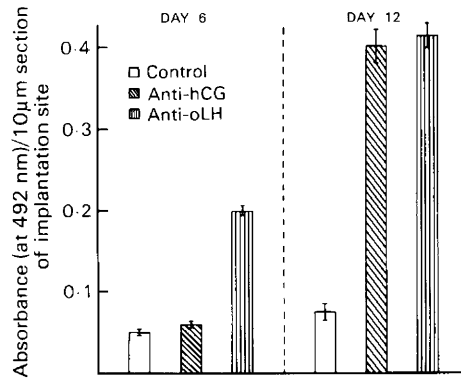
**Fig. 4.** Day 12 pregnant mouse treated with anti-oLH Ig.

**Fig. 5.** Day 12 pregnant mouse treated with control globulins.

PLATE 1



(Facing p. 374)



**Text-fig. 2.** Immunoenzymic quantitation, using horseradish peroxidase conjugated to Protein A, of antigens reacting to anti-ovine LH and anti-hCG immunoglobulins in mouse embryos at Days 6 and 12 of pregnancy. Ten typical sections from a representative mouse were processed as described in the 'Methods'. Values are mean  $\pm$  s.d.

*Pregnancy rescue by progestational steroids*

It was determined that 3 injections of antibodies to ovine LH on Days 6, 7 and 8 or to hCG on Days 8, 9 and 10 were as effective in terminating pregnancy as 5 injections given on Days 3-7 of pregnancy (Table 3). To evaluate the ability of progestational steroids to overcome the pregnancy terminating action of antigonadotrophins, experiments were performed as indicated in Table 4. Ovariectomy of pregnant mice on Day 6 accompanied by administration of 2 mg MPA on Days 6, 9 and 12 sustained the embryonic development and well developed embryos could be seen on Day 16. Administration of immunoglobulins against LH or hCG did not terminate pregnancy under these conditions (Table 4).

**Table 3.** Effect of immunoglobulins (Ig) against ovine LH and hCG on implantation sites on Day 16 of pregnancy in mice

Group	Treatment	No. of mice	Implantation sites on Day 16 (mean $\pm$ s.d.)
I	Control monkey globulins* (injected with Freund's complete adjuvant alone) (50 mg/0.5 ml)	6	6.7 $\pm$ 1.2
II	Monkey anti-oLH Ig* (47 mg Ig/0.5 ml)	8	6.8 $\pm$ 1.7 (resorbed)
III	Control monkey globulins† (injected with Freund's complete adjuvant alone) (50 mg/0.5 ml)	6	6.3 $\pm$ 1.0
IV	Monkey anti-hCG Ig† (45 mg/0.5 ml)	8	6.6 $\pm$ 1.7 (resorbed)

Globulins equal to the amount indicated were injected i.p. daily on \* Days 6-8; † Days 8-10.



**Table 4.** Effect of medroxyprogesterone acetate (MPA) in ovariectomized pregnant mice injected with control globulins or immunoglobulins (Ig) against ovine LH or hCG

Treatment*	No. of mice	Implantation sites on Day 16 (mean $\pm$ s.d.)
None	4	8.2 $\pm$ 1.6
Ovariectomy + MPA	4	8.0 $\pm$ 0.8
Ovariectomy + MPA + anti-oLH Ig on Days 6, 7 & 8	6	7.8 $\pm$ 1.6
Ovariectomy + MPA + control globulins on Days 6, 7 & 8	6	8.5 $\pm$ 0.7
Ovariectomy + MPA + anti-hCG Ig on Days 8, 9 & 10	6	8.1 $\pm$ 1.6
Ovariectomy + MPA + control globulins on Days 8, 9 & 10	6	8.1 $\pm$ 1.6

\* Ovariectomy on Day 6 of pregnancy; 3 i.m. injections of 2 mg MPA each on Days 6, 9 & 12 of pregnancy.

### Discussion

These experiments demonstrate the sensitivity of the mouse to the abortifacient action of immunoglobulins raised against ovine LH and human CG. Anti-LH immunoglobulins exerted their effect early in pregnancy, at the implantation or peri-implantation stage, whereas the antifertility effect of anti-hCG immunoglobulins was manifested only between Days 10 and 14 of pregnancy. This bears a temporal relation with the period of peak production of an hCG-like material by the mouse placenta. Wide & Wide (1979) have described the presence in mouse implantation sites of a substance akin to human chorionic gonadotrophin in terms of its physico-chemical properties and immunological reactivity. The content of this substance was reported to rise progressively in the post-implantation period, reaching a maximum on Day 11 of pregnancy.

Treatment with anti-ovine LH or anti-hCG immunoglobulins brought about an equivalent reduction of circulating progesterone concentration. Both antibodies were thus equally effective in preventing the pregnancy-induced rise in progesterone, leading to consideration of whether (1) progesterone is vital for the maintenance of early pregnancy and (2) if antibodies exercise an additional effect on embryonic antigens the target antigens to the two types of antibodies develop at two different times in pregnancy.

Immunofluorescence and immunoenzymic studies demonstrated the presence of antigens reacting to anti-LH and anti-hCG immunoglobulins in the mouse fetoplacental unit. The amount of anti ovine LH-reacting material was, however, substantially higher than that reacting to anti-hCG on Day 6, although the values were similar by Day 12. These observations suggest that antibodies to gonadotrophins may also act at the fetoplacental site.

The reversal of the pregnancy-terminating action of antibodies to ovine LH and hCG by medroxyprogesterone acetate emphasizes the importance of progestational steroids in the maintenance of pregnancy in mice (Spies & Quadri, 1967; Madhwa Raj & Moudgal, 1970; Rao *et al.*, 1972; Munshi *et al.*, 1972; Thau & Sundaram, 1980; Stevens, 1980; Tandon *et al.*, 1981), probably through its effect on myometrial contractions (Reynolds & Allen, 1932; Csapo, 1956).

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