MATERNAL ACCEPTANCE OF THE FOETAL ALLOGRAFT:

ROLE OF PLACENTAL GONADOTROPHINS

BY

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DECLARATION

I, Charanjit Singh Bambra, hereby declare that the work contained in this thesis is my original work and has never been submitted for a degree in any other University.

Signed .....Charanjit.....

This Thesis has been submitted for examination with my approval as University supervisor.

Signed .....Samson Gombe.....

Professor Samson Gombe

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CONTENTS

SUMMARY

Treatment of rats with anti-PMSG and anti-hCG for 5 days from days 6, 11 or 16 of pregnancy caused variable necrosis of foetal tissue, the extent of which increased from the chorionic cells to the inner trophoblastic layers with the increase in dose from 4 mg to 10 mg anti-PMSG. At 10 mg/1 day, foetal death and resorption was invariable in early to mid-pregnancy, whilst in late pregnancy some litters were carried to term. In antibody treated hyraxes placental damage, which started with the destruction of the columnar cell monolayer and then progressed towards the deeper-lying spongy trophoblast with the duration of the treatment, was observed, whilst goats treated with these antibodies showed variable necrosis of foetal tissue, the extent of which increased from columnar trophoblast cells apposed to maternal connective tissue, to the innermost foetal mesenchyma. The main histological change in corpora lutea of these animals was hyperaemia in anti-PMSG treated ones. In addition, the primary follicles showed degeneration concomitant with the appearance of sausage shaped eosinophilic bodies.

Immunofluorescence studies with conjugates of anti-hCG and anti-PMSG were carried out on the placentae of rat, cane-rat, spring-hare, hyrax, goat and dikdik. Conjugates of anti-hCG and anti-PMSG showed sharp localization at the region of the foeto-maternal junction in the placenta of rat, spring-hare and hyrax, and in the sub-placenta of the cane-rat. In ruminants only anti-PMSG conjugate was sharply localized on the outermost chorionic cells apposed to the maternal connective tissue.

Treatment of rats with anti-hCG and anti-PMSG for 5 days from day 6 of pregnancy caused an increased clearance and *in vitro* hepatic metabolism of progesterone. Oestrogen clearance increased in anti-PMSG treated rats and decreased in anti-hCG treated ones. Biochemical and histological changes showed an increase in hepatic GOT and GPT concomitant with hyperaemia and fatty infiltration. No alteration in kidney function was evident.

It was concluded that PMSG/hCG - like gonadotrophin (s) is produced by the chorionic cells of the rat, cane-rat, sping-hare and the hyrax, whilst those of the goat and dik-dik produced a PMSG - like gonadotrophin. That in all of these animals, except the cane-rat, gonadotrophin - like materials are interposed between the foetus and the mother constituting a barrier. Neutralization of the 'gonadotrophin' leads to degenerative changes which start with the destruction of the feto-maternal junction. These 'gonadotrophins' may be involved in regulating the synthesis and metabolism of steroid hormones, both at the ovarian and hepatic levels.

INTRODUCTION

The foetus, carrying as it does, the paternal antigens, should be susceptible to maternal immune attack. In practice this does not happen. Several hypotheses have been advanced to explain the acceptance of the fetal allograft. Of these the presence of an immunological barrier between the foetus and the mother is the most plausible. Amoroso and Perry (1975) have proposed that this barrier is comprised of a gonadotrophin/oestrogen glycocalyx coat.

The requirement of this theory is that there be a continuous production of placental gonadotrophins/oestrogens throughout gestation in sufficient quantities to provide this coat. The theory also pre-supposes the presence of gonadotrophins (in situ, at least) in all placentalia.

This study was undertaken (a) to demonstrate the presence of gonadotrophin-like material at the foeto-maternal junction in some species which have hitherto not been shown to have them and (b) to see if the removal of this material would induce maternal immune attack of the foetus as would be expected in the Amoroso and Perry hypothesis.

Since antibodies to oestrogens have been shown not to terminate pregnancy after implantation (Murphy and Mead, 1976) efforts were concentrated on the role of hCG and PMSG-like material on immune suppression during pregnancy.

ANIMALS

Three mammalian orders were chosen for this study: Rodentia, Hyracoidea and Artiodactyla. The rodents were primarily used because of lack of documented evidence as to the presence or

absence of hCG - and PMSG-like substances, and secondly because of their discoidal haemochorial placentation, a situation present in the primates. The rodents used were Wistar rats (Myomorph, Rattus rattus), African spring hare (Sciuromorph, Pedetes capensis) and African cane rat (Hystricomorph, Thryonomys swinderianus). The cane-rat is particularly unique among the mammals studied in that it possesses a placenta inside a placenta - the sub-placenta (Oduor-Okello, personal communication). The rock hyrax (Procavia capensis) was chosen because of the exceptionally long gestation for its size (7 months) and the belief in traditional medicine that its urine cures certain infertility conditions. The Artiodactyls were chosen because of their epitheliochorial placentation, which differs from both the rodents and the hyrax. In these animals there is no invasion of maternal tissue and the placenta forms a loose connection with maternal tissue. These animals would thus serve as good comparisons for studies on both the removal of the residual coat of glycoproteins, and the location, if any, of the gonadotrophins on the placenta (see Appendix 1). The Artiodactyls were the goat (Capra hircus) and Dikdik (Madaqua curtz).

LITERATURE REVIEW

Evolution of viviparity involved a reduction in the yolk content of the egg, as a consequence of which, animals acquired a new set of inovations: reduction in the number of offspring; retention of young within the female genital tract; formation of the placenta; and parental care of the young. This was achieved by the animals undergoing considerable changes in their reproductive physiology, with the hormones (especially placental ones) assuming a major role.

Before the retention of the young inside the mother could be achieved, the animals had to overcome the problem of immunological rejection. As Anderson (1971) points out, 'Viviparous species depend entirely upon this apparently precarious immunological situation for their survival'. How viviparous animals have achieved this, has been a puzzling problem.

Immunological aspects of the maternal-foetal relationship

By virtue of two gametes, one from each parent, fusing together to form a gamete; the foetus will inherit genetic material from both parents. Among other things, this will be reflected in the transplant or histocompatible antigens the foetus will display on its surface tissue. Recognition of the paternally derived transplant antigens by the maternal immunological surveillance system should then initiate an immune-attack culminating in foetal death and termination of pregnancy. However, pregnancy does occur and succeed despite profound histocompatibility antigen differences and even in inter-species matings (Gray, 1954). Indeed no convincing evidence concerning death of the foetus from maternal immunological death has been recorded (Kerr, 1968).

Many reviews have been written in an attempt to explain this enigma (Medawar, 1953; Boyd, 1959; Billingham; 1963; 1964; Lanman, 1965; Park, 1965; Papiernik-Behaver, 1966; Scott, 1966; Simmons and Russel, 1967a; Currie, 1968; Kirby, 1968a; Billington, 1969; Wolstenholme and O'Connors, 1969; Beer and Billingham, 1971; and Amoroso and Perry, 1975), and various theories proposed: antigenic immaturity of the conceptus (Little, 1924); diminished immunological reactivity of the mother (Medawar, 1953); uterus as an immunological privileged site (Billingham and Silvers, 1963); local placental steroid production (Zipper, Ferando, Saez and Tchernitchin, 1966); invasiveness of the trophoblast (see Currie, 1968); and anatomical separation of foetus and mother (Woodruff, 1958).

Antigen immaturity of the foetus

Since recognition of transplant antigens is the first requirement of an immune response, it occurred to Little (1924) that the fetus could not be rejected if these antigens were absent. Very few reports appear showing lack of histocompatible antigens on the foetal tissues (Medawar, 1953; Haskova, 1962; and Nelson, 1965).

The observation that murine placenta contained antigenic determinants capable of inducing homograft immunity in mice isogeneic with the mother (Uhr and Anderson, 1962); the demonstration that 13 day-old mouse embryo X-irradiated cells could induce antibody production (Muller, 1963); and the very early appearance of transplant antigens in the embryonic life of mice (Simmons and Russel, 1962; Billingham and Silvers, 1963; and Edidin, 1964) and chicken (Billingham and Silvers, 1963) provided

the earliest evidence for the presence of histocompatibility antigens on foetal tissues.

The fact that mouse tubal cells failed to develop if transferred beneath the renal capsule of specifically prehyperimmunised hosts (Kirby, Billington and James, 1966; Simons and Russel, 1965) hinted that histocompatible antigens were present in the ova. Presence of transplantation antigens on the surface of mouse egg cells at morula stage was established by Olds (1968) and James (1969). Antigenicity of the mouse egg has been demonstrated also by in vitro studies (see Billington, 1971). Most convincing evidence for presence of both major and minor histocompatible antigens comes from direct visualization of antigenic sites on cells by fluorescent antibody techniques (Davies, 1968; Billingham and Silvers, 1963; Palm, Heyner and Brinster, 1971; Palthey and Ediden, 1973; Searle, Johnson and Billington, 1974). Direct evidence for presence of histocompatible antigenic determinants on human trophoblast (Loke, Joysey and Borland, 1971), and its counterpart choriocarcinoma (Rudolph and Thomas, 1971) is also available.

There is no doubt, in view of this overwhelming evidence then, that transplant antigens are present on foetal tissues. It has recently been proposed that embryonic cells shed their transplant antigens in a soluble form, and these compete with the tissue for effector processes of the immune response (Alexander, 1974). It was suggested that these antigens formed adducts with antibody as well as cytotoxic cells which could then no longer attack embryonic cells. No experimental evidence in support of this concept is available, and it is unlikely to be the mechanism responsible for foetal protection.

DIMINISHED IMMUNOLOGICAL REACTIVITY OF THE MOTHER

Pregnancy depresses maternal immunological mechanisms:

Earliest evidence in support of the concept of diminished maternal immunological reactivity during pregnancy was provided by Sturgis and Bethel (1943) who observed a decrease in numbers of circulating lymphocytes during pregnancy in women. Later, Heslop, Krohn and Sparrow (1954) demonstrated that the survival of skin allografts was longer in pregnant rabbits. At about the same time Mitchison (1953) and Woodruff (1953) showed that pregnancy was unaffected in mice artificially sensitized against paternal antigens. Similar results were obtained by Medawar and Sparrow (1956) and Simmons and Russel (1962) in mice and Andersen and Munroe (1962) in women.

Indirect supporting evidence for this concept is provided by the observation that in mice (Knox, 1957) and women (Baker and Baker, 1947) there was a decreased resistance to diseases such as poliomyelitus, and the reported increase in mortality in women in second half of pregnancy (Kottmeier, 1962; Niemenen and Remes, 1970). Increased infections caused by intracellular micro-organisms including hepatitis, variola, influenza, varicella and coccidiodomycosis have also been documented (See Purtilo, Hallgren and Unis, 1972) during pregnancy. This evidence does indicate that there is a depression in maternal immunity in pregnancy.

Depression of maternal cell-mediated immunity:

Conflicting evidence exists as to whether there is any alteration of maternal lymphocyte function during pregnancy (Lewis, Whang, Nagel, Oppenheim and Perry, 1966; Harrison, 1972; Purtilo,

Hallgren and Yunis, 1972; Watkins, 1977; Finn, St. Hill, Goven, Ralfs, Gurrey and Denye, 1972).

Evidence indicating that cell-mediated immune responses are depressed during pregnancy includes impaired cutaneous (Lichtenstein, 1942) and in vitro lymphocyte (Smith, Caspary and Field, 1972) responses to tuberculin preparation (P.P.D.); the prolonged survival of skin allografts in pregnant women (Anderson and Munroe, 1962); and the more recent demonstration of depressed cellular immunity in pregnant mouse (Fabris, 1973; Carter, 1976).

Hormonal milieu of pregnancy results in thymic atrophy during pregnancy (Pritchard and Micklem, 1972) and could alter immune responses (Fabris, 1973; Carter, 1976). The hormones shown to have immunosuppressive properties include corticosteroids, oestrogens, progestogens and placental gonadotrophins (Billingham, Krohn and Medawar, 1951a; b; Medawar and Sparrow, 1956; Kaye and Jones, 1971; Munroe, 1971; Han, 1975; Stimson and Hunter, 1976).

Injection of corticosteroids is followed by thymic involution (Nelson, Hall and Limson, 1967), caused by rapid efflux of cortical thymus cells, the residual cells being greatly enriched in immunocompetent medullary cells (Jacobson and Blomgren, 1972). Systemically these animals are non-specifically defficient in both hormonal and cellular immunity (Cohen and Claman, 1971; Carter, 1976). A similar mechanism is known to operate in pregnant mice (Baines, Fross, Millar and Facogg, 1977). Cortisone has been shown to be the effective corticosteroid in rabbit (Billingham, Krohn and Medawar, 1951 a, b; Morgan, 1951) and in guinea-pig (Sparrow, 1953). The observed decrease in numbers of circulating lymphocytes

(Germuth, 1956) and the increased period of skin graft survival in rabbits (Krohn, 1956) following administration of adreno-corticotrophic hormone is probably mediated via the release of adrenocortical hormones from adrenal cortex. However, maternal adrenocorticosteroids are not known to increase significantly during pregnancy and there is no evidence for their binding on foetal membranes. Moreover, these immunosuppressive effects of the hormones can only be produced at pharmacological levels and not at physiological levels.

Oestrogens can depress cell-mediated immune responses, including homograft rejection (Lajos, Gorcz, Szehely, Casaba and Domany, 1964; Hulka, Mohr and Liberman, 1965; Zipper, Ferrando, Saez, Tchernitchin, 1966; Beer and Billingham, 1971; Beer, Billingham and Hoer, 1971a; Waltman, Burde and Fikrig, 1971; and Stimson and Hunter, 1976). Oestrogen acts by causing thymic involution, which occurred progressively during pregnancy (Millar, Mills and Baines, 1973). In the rat it is believed that oestrogen acts on the thymus causing it to release a factor, which depresses lymphocyte responses (Stimson and Hunter, 1976). Such a factor has been identified in the late pregnancy serum present in the humans (Stimson and Blackstock, 1975). Very few reports appear on immunosuppressive property of progestogens (Nelson, Hall, Manvel-Limian, Friedherg and O'Brien, 1967; Munroe, 1971; and Carter, 1976). A major shortcoming of all experiments done to demonstrate steroidal immunosuppression is the large quantities of the steroids required to elicit these responses, which are in excess of the normal physiological values.

Evidence that glycoproteins can reduce maternal cell-mediated immune responses includes reports that placental glycoproteins (Riggio, Pairrilo, Bull, Schwartz, Stenzel and Rubin, 1971) and alpha-globulin (Niosi et al., 1972) impair lymphocyte responses to phytohaemmagulutinin. Conflicting evidence exists for immunosuppressive properties of human chorionic gonadotrophin (Nelson, 1966; Nelson, Hall, Manuel-Limson, Friedberg and O'Brien, 1967; Simmons, Price and Ozerkis, 1968; Pearse and Curtis, 1969; Burke and Roulet, 1970; Kaye and Jones, 1971; Belling and Weksler, 1974). A major point of disagreement is the findings by Jenkins and coworkers (Jenkins, Acres, Peters and Riley, 1972) that the quantity of hCG required to inhibit lymphocyte reactions is far in excess of the actual physiological levels. However, Adcock, Teasdale, August, Cox, Meschia, Battaglia and Naughton (1972) have shown that hCG can cause immunosuppression at very low levels. Recently, Caldwell, Stites and Fudenherg (1975) have warned that the evidence of immunosuppressive role of hCG should be viewed with caution in view of the finding that purified hCG cannot suppress lymphocyte responses. Human placental lactogen (or human chorionic somatomammotrophin) has also been demonstrated to have immunosuppressive properties (Kaye and Jones, 1971; Christienssen, Anderson, Osther, Peterson, Bach-Martensen and Lebech, 1976).

Serum from pregnant animals (Harrison, 1972) and pregnant humans (Kasakura, 1971; 1973; Gratt, Yunis and Good, 1973) was found to suppress lymphocyte transformation in vitro. This property is either due to presence of hormones in the serum, or due to the presence of lymphocyte depressing factor which was found to increase during pregnancy (Smith, Caspary, Field, 1972). Presence

of foetal proteins such as alpha feto-protein (Purves and Geddes, 1972; Purtilo and Yunis, 1971) and carcinogenic antigen (Caron, 1967) in pregnancy serum of women have also been shown to depress cell-mediated immunity by coating maternal lymphocyte antigen - receptor sites, and recently even bovine uterine proteins have shown to inhibit lymphocyte responses in vitro (Roberts, 1977). Unfortunately, in vitro lymphocyte responses cannot be regarded as conclusive tests for immunosuppression.

Recently it has been suggested that from evolutionary point of view, it is of more benefit for the mother to have specific immune tolerance towards fetal antigens rather than generalised immunological depression (see Watkins, 1972). Specific immune enhancement mediated by antigen-antibody complexes (Sjogren, Hellstrom and Bansal, 1971) has been hypothesized to aid allogenic foetal survival.(Hellstrom, Hellstrom and Brown, 1969). Such mechanisms have been reported to operate in man (Lewis, Whang, Naggel, Oppenheim and Perry, 1966) and rat (Billingham and Barker, 1969; Wynn, 1971). Anderson (1971) postulated that during pregnancy foetal antigens cross the placenta in small amounts, but at gradually increasing rates with the progressing pregnancy. He suggested that these rising antigen titres caused immune mechanisms to dysfunction or inactivate adaptations of immunity, resulting in a state of 'immunoloigcal inertia of viviparity'.

It would thus appear that many factors can depress maternal cell-mediated immunity. What remains to be determined is whether this is the sole mechanism responsible for non-rejection of the fetal allograft and whether these factors can interfere with immunological responses at normal physiological levels.

Immune responses are not affected by pregnancy

In mice, (Medawar and Sparrow, 1956; Hulka and Mohr, 1968) rat and rabbit, (Woodruff, 1958) and man (Watkins, 1972; and Commings, 1968) pregnancy does not alter immune responses. Evidence for active immunological rejection of the conceptus includes reports of binding of maternal immunoglobulin to placental chorionic vill, (Burstein, Blumenthal, 1969) lymphocyte infiltration in the decidua (Burstein, Berns, Franhel and Blumenthal, 1965) and accellerated rejection of homografts of skin in women with history of habitual abortion (Bardawil, Mitchell, McKeogh and Marchant, 1962), and the haemolytic disease of the newborn (see Beer and Billingham, 1971). Recent findings indicated that the female was capable of expressing humoral and cullular immunity to foetal transplantation antigens of paternal origin (Maroni and Souza, 1973). Indeed antibodies against the paternal component have been detected in the blood of multiparous women (Amos, 1969; and Teresaki, Michey, Yamazaki and Vredovoe, 1970) and mice (Herzenberg and Gonzales, 1962; Goodlin and Herzenberg, 1964; Kaliss and Dagg, 1964).

In view of these findings it would appear that depression of cell-mediated immunity could play a minor role in protection of the foetal allograft, but cannot account fully for the success of mammalian pregnancy.

Uterus as an immunological privileged site

It has been suggested that the uterus (Billingham and Silvers, 1962; Kirby, Billington and James, 1966), like the anterior chamber of the eye, the cheek pouch of Syrian hamster (Medawar, 1948; Billingham and Silvers, 1962), subcutaneous fat pads and the testes

(Russel, 1961) is a privileged site on the basis of a deficient lymph supply. However, it has been shown that the only privileged sites in which grafts can neither elicit sensitivity nor be affected by a state of sensitivity are the anterior chamber of the eye and substantia propria of the cornea (Medawar, 1948; Billingham and Boswell, 1953).

Other evidence refuting the concept of the privileged status of the uterus included the survival of ectopic pregnancies (Eales, 1932; Nicholas, 1934; Theirsch, 1941; Williams, 1941; Thomas, 1943; Wiener, 1944; and Jarcho, 1949); the demonstration that blastocysts implanted and developed more or less normally to relatively advanced stage when deliberately placed in ectopic sites such as the brain, the spleen, the cryptorchid testes, the mesentry, the spleen and the abdominal cavity (Fawcett, 1950; Kirby, 1960, 1963a and b; 1968; McLaren and Tarkowoski, 1963; Billington, 1965b; and McLaren, 1965a) and the fact that blastocysts readily implant of their own accord and develop to an advanced stage in dubious extra uterine sites as the ovary, rectum or pouch of Douglas in man (Beer and Billingham, 1971). More direct evidence was provided by Schlesinger (1962) who studied the affect of implanting small test grafts into the uterine horn, and more recently by Poppa, Simmons, David and Russel, (1964). They demonstrated that homografts of a normal non-invasive tissue - the parathyroid, transplanted to the uterus of pseudopregnant and pseudopregnant parathyroidectomized rats were consistently rejected within 20 days. These studies indicated that where non-embryonic tissue was concerned, transplantation immunity was both incitable and expressible in the uterine milieu. Similar results were obtained by Beer, Billingham and Hoer (1971a).

Since the transplantation immunity can be evoked and expressed in the uterus as elsewhere and since choriocarcinoma (the counterpart of the conceptus), continues to survive inspite of extensive medical therapy (Benirschke and Driscoll, 1967; Bagshawe, 1969; Beer and Billingham, 1971) the survival of the foetal allograft must be related to the peculiarity of the conceptus rather than the uterus.

Local placental steroid production

Zipper, Ferrando, Saez and Tchernitchin (1966) suggested that local production of immunosuppressant steroids by the placenta and their release in high concentrations may interfere with local immunological reactions, a view also held by Nelson, Hall, Manuel-Limson, Friedlberg and O'Brien (1967) and Hulka and Mohr (1969), and has recently been extended to encompass placental polypeptide hormones (Adcock et al., 1973). Medawar and Sparrow (1956), however, have demonstrated that such steroids will not suppress graft rejection in pre-immunized animals whereas normal pregnancy can occur in animals immune to paternal antigens (Michison, 1953).

It would thus appear that local hormonal production can alter maternal immune reactions. Since the hormones would only influence the efferent limb of the immune mechanism, this localized production cannot fully account for the acceptance of the foetal allograft.

Invasiveness of the trophoblast

The invasive trophoblast has cytolytic activities and it was felt that this could interfere with cullular components of the immune response by destroying the lymphoctyes, thereby inhibiting the detection of foetal antigens (See Currie, 1968). However, this

theory can be easily dismissed. It has been shown that late pregnancy trophoblast is non-invasive and yet still has the same immunological properties as the earlier invasive trophoblast (Simmons and Weintraob, 1969).

Anatomical separation of the mother and the foetus

Basis for the presence of the anatomical barrier:

Many attempts have been made to prejudice either the incidence or the outcome of heterospecific pregnancies in mice (Medawar and Sparrow, 1956; Mitchison, 1953; and Haskova, 1961), rats (Woodruff, 1958) and rabbits (Heslop, Krohn and Sparrow, 1954; Woodruff, 1958; Lanman, Dinnerstein and Fikrig, 1962) by sensitizing the mothers before mating or during pregnancy with paternal homografts without success. These findings can only be explained on the basis of an anatomical barrier. Most definitive studies done to indicate the presence of a barrier between the conceptus and the mother are those by Lanman; Dinnerstein and Fikrig (1962); Lanman, Herod and Fikrig (1964); and Lanman and Herod (1965). These investigators examined the fate of conceptuses conceived in one animal and subsequently transferred to a recipient of the same species. Survival of the conceptuses were found to be uninfluenced by the hostile immune environment and could only be explained on the basis of an anatomical barrier.

Further support for the barrier concept is derived from the findings that the barrier could be produced experimentally by interposing a sheet of porous membrane - permeable to the largest of molecules but impermeable to cells - between a free homograft and the host to which it is located (Woodroff, 1957). Observations with

such membranes using homografts of tumours and Harderian glands in mice (Algure, Weaver and Prehn, 1954, 1957; Weaver and Prehn, 1955) and skin homografts in rats (Woodruff, 1956, 1957) suggest that graft survival is only limited by the extent to which nutrition can be maintained by diffusion through the membrane. Homografts insulated in this way do not immunize the host and moreover survive as readily in hosts immunized by a previous orthodox graft from the same donor as in the non-immunized hosts.

In view of the fact that foetal red cells (Gregor and Steeple, 1957; Macris, Hellman and Watson, 1958; Zippursky, Hull, White and Israels, 1959; Lee and Vazques, 1962; Zipirsky, Pollock, Neelands, Chown and Israels, 1962; Woodruff and Fin, 1966); syncytiotrophoblast (Douglas, Thomas, Carri, Cullen and Morris, 1959; Salvaggio, Nigogosyan and Mack, 1960); maternal erythrocytes (Lee, Vazques, 1962; Duhring, Smith, Greene, Rochlin and Blakemore, 1959); maternal lymphocytes (Desai and Greger, 1963; Woodraw and Finn, 1966), and mouse cells (Tuffrey, Bischum and Barnes, 1969) can move across the anatomical barrier, the over simplified view of the membrane permeability (since cells do move across) cannot explain the immunological acceptance of the foetus.

Nature of the immunological barrier

Simmons and Russel (1962, 1963) analysed the immunological response to trophoblastic cells and other embryonic cells implanted beneath the kidney capsule in previous sensitized and non-sensitized allogeneic recipients, at various stages of its development, and they showed that trophoblastic cells survived and were uninfluenced by the immune status of the immunized and non-immunized mouse hosts.

Further evidence in support of the trophoblast as the immunological barrier was provided by Wynn (1968, 1969, 1971) who showed that in all the placentae examined under electron microscope at least one layer of the trophoblast persisted throughout gestation.

Since the trophoblast has got histocompatible antigens (see page 10) it stands to reason that these antigens must be masked if the trophoblast is to serve as the barrier. Very good evidence (Kirby, Billington, Bradbury and Goldstein, 1964; Bradbury, Billington and Kirby, 1965; Currie, Dooninck and Bagshawe, 1968) indicated that some form of immunologically inert material closely associated with maternal surface of the barrier masks the histocompatible antigens.

On the basis of electron microscope and histochemical studies of murine placentae (Kirby et al., 1964; Bradbury et al., 1965; Kirby, Billington and James, 1966) suggested that the insulating pericellular coating was a layer of 'fibrinoid' mucopolysaccharide material rich in hyaluronic and sialic acid, and that it constituted the foeto-maternal junction, enveloping each trophoblastic cell in the placenta. What appeared to be unequivocal evidence of the presence of transplantation antigens and of their normal masking by pericellular sialomucin was subsequently presented by Currie and his co-workers (Currie, Van Doorninck and Bagshawe, 1968). They found that treatment of ectoplacental cone cells from $7\frac{1}{2}$ day mouse embryos with neuroaminidase in vitro enabled them to sensitize unrelated adult hosts against subsequent test skin homografts of their own genetic makeup.

Fibrinoid or fibrinoid like material lying between maternal and foetal components have been described in a number of species including the rat (Schiebler and Knoop, 1959; Bulmer and Dickson, 1961), guinea-pig (Davies, Dampsey and Amoroso, 1961); the seal (Harrison and Young, 1966) and man (Bardawil and Toy, 1959). In mice it has been established that the trophoblast is non-antigenic (Simmons and Russel, 1966) and that neuraminidase removes terminal moieties (Guttschalk, 1957) so that underlying transplantation antigens are detected (Currie et al., 1968). Indirect evidence in support of the fibrinoid concept is provided by the observed conspicious thickness of fibrinoid material at the maternal foetal junction of the placenta of hybrid foetuses as opposed to isogeneic foetuses (Kirby et al., 1964). An interesting hypothesis by Rambourg and Lebland (1967) suggested that all animal cell types possessed surface coatings of glycoproteins which contained varying amounts of sialic acid, and that this increased during pregnancy, thereby causing non-antigenicity of the foetal trophoblast. No corroborative evidence for this theory is available.

Histochemical studies on the human term placenta, ectopic trophoblast and hydatidiform mole have demonstrated an extreme degree of sulphatian of the mucoprotein coating of both normal and abnormal human trophoblast (Bradbury, Billington, Kirby and Williams, 1969) which confers a high negative charge on the tissues and this would be responsible for the electrostatic repulsion of negatively charged lymphocytes (Currie and Bagshawe, 1967) thus preventing them from crossing to the foetal antigens. An alternative theory suggests that this is accomplished by the material rendering the trophoblast 'non adhesive' to the lymphoctye (Jones and Kemp, 1969).

The fibrinoid theory is definitely more attractive and plausible than the over-simplified concept of the barrier porosity as advocated by Woodruff (1957).

Limitations of the 'fibrinoid' concept

A major limitation of the fibrinoid concept is that a prominent 'fibrinoid' barrier cannot be demonstrated around trophoblastic cells of all mammals (Wynn, 1969; 1971), nor can it be found around each of the cells of pure trophoblastic transplants beneath the kidney capsule (Simmons, Cruse and McKay, 1967). In epithiliochorial placentata no 'fibrinoid' is present (Amoroso, 1952; Lawn, Chiquvine and Amoroso, 1969), and histologically fibrinoid cannot be demonstrated around trophoblast bathed by maternal blood in the intervillous spaces of human placenta (Wynn, 1971) nor on the trophoblastic microvillie of the rabbit (Tai and Halasz, 1967).

In view of these shortcomings Amoroso and Perry (1975) suggested that if the acellular (Sialomucin) trophoblast of murine and human placenta described by Kirby et al., (1964), Currie and Bagshawe (1967), Bradbury et al., (1965) and Bradbury, Billington, Kirby and Williams (1969) could be identified with sialoglycoprotein hormones, a suitable explanation for the immunological privilege enjoyed by the placental trophoblast could then be provided. They proposed that placental gonadotrophins, in conjunction with oestrogens could form an immunosuppressive glycocalyx coat on foetal membranes, and thereby render the foetuses safe from maternal immunological attack. This theory presupposes that all mammals have placental gonadotrophins, and that these are produced in sufficiently large quantities to provide the immunosuppressive coat.

No direct experimental evidence in support of the Amoroso and Perry hypothesis is available to-date although indirect evidence is available. Borland and co-workers (Borland, Like, and Wilson, 1974; Loke, Joysey and Borland, 1971) analysed the killing effect of rabbit lymphocytes sensitized against trophoblast target cells and showed that human trophoblast cells were not susceptible to this killing action. If, however, the human chorionic gonadotrophin layer around the trophoblast cells was first removed by enzymatic action, then they were killed by sensitized lymphoctyes. When such trophoblast cells were treated with hCG after exposure to the enzyme, their resistance to specific lymphoctye damage was restored. These experiments do suggest that human chorionic gondadotrophin masks the antigens displayed on the human trophoblast, and its removal results in an immune attack.

Placental glycoproteins in mammalian pregnancy

Five polypeptide hormones have been identified from placentae of various mammals: human chorionic gonadotrophin (hCG - Ascheim and Zondek, 1927); pregnant mare serum gonadotrophin (PMSG - Cole and Hart, 1930; and Zondek, 1930), human chorionic somatomammotrophin or human placental lactogen (hCS - Josimovich and MacLaren, 1962); placental uterotrophic hormone (Beas and Flores, 1969); and human chorionic thyrotrophin (Myers, 1961). Of these hormones only three are sialoglycoproteins - hCG, PMSG and hCS.

Presence of glycoproteins in mammals

Human chorionic gonadotrophin

Human chorionic gonadotrophin has been shown to be present in urine and blood of pregnant women (Ascheim and Zondek, 1927;

Gey, Seegar-Jones and Helmann, 1938; Waltz, Tulner, Evans, Hartz and Earle, 1954). Substances closely allied to hCG are known to be present in most sub-human primates including gorilla, orangutan, chimpanzee and monkey (Zuckerman, 1935; Hamlet, 1937; Kneil, Johannson and Knobil, 1969; Hobson, 1971, 1975; Hobson and Wide, 1972; Hodgen, Vaitukaitis, Ross and Tulner, 1974).

A positive Ascheim-Zondek test has been reported for the pig (Phillip, 1929) and the cow (Menzani and Gentile, 1934). Substances similar to hCG are thought to be present in mouse and mice (see Amoroso and Perry, 1975), in giraffe (Wilkinson and de Fremary, 1946; Amoroso, 1955), in rabbit (Fujimoto, Woody and Duhelow, 1973; Haour and Saxena, 1974) and Beagle dog (Etreby, 1975).

Pregnant Mare Serum Gonadotrophin

A potent gonadotrophin has also been demonstrated in the blood of pregnant mares (Cole and Hart, 1930; Day and Rowlands, 1940, 1947; Rowlands, 1949, 1964; Brady, 1948) and donkeys (Samodehin, 1940; Zondek and Sulman, 1945; Calisti and Oliva, 1955; Oliva and Chiechini, 1961; Shukla and Mammon, 1972). It is thought to be present in the zebra (Zondek, 1915) and fallow deer (Unterberger, 1932).

Human chorionic somatomammotrophin

Placental chorionic somatomammotrophin has been shown to be present in man and monkey (Fukuschima, 1961; Ito and Higashi, 1961; Josimovich and MacLaren, 1962; Friesen, 1965; Shome and Friesen, 1965; and Grant, Kaplan and Grumbach, 1970). To-date hCS-like materials have been identified in rats (Astwood and Greep, 1938; Lyons, 1944; Matthies, 1967; Shiv, Kelly and Friesen, 1973;

Talamantes, 1973; Kelly, Roberts and Shiv, 1975; and Gusdon, Caudle and Glenn, 1976); mouse (Caruti and Lyons, 1960; Kohomoto and Bern, 1970; Talamantes, 1975), hamster (Kelly, Shiv, Friesen and Robertson, 1973; Talamantes, 1973; Matthies, 1974), chinchilla (Talamantes, 1973); guinea-pig (Kelly et al., 1973); field and bank moles (Forsyth and Blake, 1975), goat (Buttle, Forsyth and Knaggs, 1972; Forsyth, 1974); sheep (Forsyth, 1974; Kelly, Robertson and Friesen, 1974; Chan, Robertson and Friesen, 1976); fallow deer (Forsyth, 1974) and beagle dog (Etreby, 1975). It is also thought to be present in pig, cat, dog, horse and rabbit (Gusdon, Leake, Van Dyke and Atkins, 1970).

It is quite apparent that although the presence of hCS-like materials is well established throughout the mammalian group, the same cannot be said of the two gonadotrophins. For example their presence has yet to be established in insectivores, carnivores, bats and herbivores.

Localization of gonadotrophin activity

Many of the early studies, particularly on the in vitro culture of human trophoblast (Jones, Gey and Gey, 1943) and histochemical examination of the placenta (see Wislocki and Padykula, 1961) indicated that the cytotrophoblast was involved in chorionic gonadotrophin secretion. However, numerous reports appear showing the inability of the cytotrophoblast to synthesize ultrastructurally (Pierce and Midgley, 1963; Yoshida, 1964 a, b, and Enders, 1965).

Use of histochemical and immunofluorescent techniques coupled with electromicroscopy have confirmed that both hCG and hCS are a product of the syncytiotrophoblast (Midgley and Pierce, 1962;

Sciarra, Kaplan and Grumbach, 1963; Thiede and Choate, 1963; Currie, Beck, Ellis and Read, 1966; Beck and Currie, 1967; Beck, Gordon, Donald and Melvin, 1969; Dreskin, Spicer and Greene, 1970; Hamanaka, Tanizawa, Hashimoto, Yoshinare, and Okudaira, 1971; Genehacev, Robyn and Pantic, 1972; Ikonicoff and Cedard, 1973; and Osada, 1976).

Pregnant mare serum gonadotrophin is produced by endometrial cups (Cole and Goss, 1943; Rowlands, 1947; Amoroso, 1952; Clegg, Boda and Cole, 1954) which were thought to be of maternal origin, but have now been shown to be of trophoblastic origin (Allen and Moor, 1972; Allen, Hamilton and Moor, 1973; Cole, 1975).

All the three sialoglycoprotein hormones (hCG, hCS and PMSG) are produced by the foetal syncytio-trophoblast which is apposed to the maternal tissues. Recently, hCG has been reported to be produced by the extraplacental sites: colon and liver (Yoshimoto, Wolfsen and Odell, 1977); the testes (Braunstein, Rasor and Wade, 1975); and the sperm (Asch, Ferandes and Paverstein, 1977). The significance of these production sites and the hormone they produce is not clear.

Possible Functions of Placental Hormones

Human Chorionic Gonadotrophin

Removal of the placenta terminates the functional corpus luteum in rabbit (Hammond, 1917); rat, hamster and guinea-pig (Klein, 1939a, b); and cat (Amoroso and Porter, 1970). In seeking the role of the placenta in corpus luteum maintainence, a pre-requisite for success of the pregnancy, attention was directed to the placental gonadotrophins. It is generally accepted that in

women the regression of the corpus luteum is prevented by the luteotrophic effect of hCG (Brown and Broadbury, 1947; Bradbury, Brown and Gray, 1950; Segaloff, Sternberg and Cashill, 1951; Henzl and Segre, 1970; Wiele, Bogumil, Dyenfirth, Fern, Junelwicz, Warren, Rizkavah and Mikhail, 1970; Hanson, Powell and Silvers, 1971). A similar situation exists in the monkey (Hisaw, 1944; Neil, Johannson and Knobil, 1969) and rats (King, 1938; Rasenfield, Lapan and Baron, 1941; Yochim and Defeo, 1962; Mantalenalis, 1968). Major arguments in favour of the luteotrophic function are based on the measurement of gonadotrophin levels and correlating these with functional status of the corpus luteum.

For instance, hCG levels were at their highest when the progesterone levels were at their highest also (Diczfaluasy and Troen, 1961). Since hCG levels were detectable in blood by 15th day post coitus (Goldstein, Aono, Taymor, Jachelson, Todd and Hines, 1968; Wide, 1969), some 4 - 5 days before the expected luteal regression, it was felt that hCG was responsible for maintaining the declining corpus luteum (Wiele et al., 1970). This view, however, is questionable. It has been shown that hCG is first detectable in blood, using radioimmunoassay techniques, at a time which coincides with increasing progesterone concentrations (Goldstein et al., 1968; Wide, 1969; Braunstein, Grodin, Vaitukaitis, Ross, 1973) rather than before this increase as would be expected (Johannson, 1969; Yoshimi, Strott, Marshall, Lipsett, 1969). Moreover in monkeys and man chorionic gonadotrophin continues to rise while progesterone levels are decreasing (Heap and Perry, 1974; Friesen, 1973; Strott et al., 1969), and in women in last trimester no correlation was found between serum progesterone and hCG levels (Boroditsky, Francisco, Jeremy and

Studies on the 'resuce' of the corpus luteum too have been re-evaluated (Short, 1969) and the evidence is considered weak in favour of the hypothesis. Short (1961) postulates that in these studies menstruation was prolonged because the large doses of hCG used caused formation of corpus luteum. Even if hCG does influence the maintainence of the declining corpus luteum, the continued secretion of large amounts of hCG after this function has been fulfilled is puzzling.

The other main role of hCG has been postulated to be on steroidogenesis. It has been demonstrated that hCG can stimulate steroidogenesis *in vitro* (Rice, Hammerstein and Savard, 1964; Savard, Marsh and Rice, 1965; Toru and Troen, 1975). It can increase production of oestrones by ovaries of dogs (Hollander and Hollander, 1958) and rabbits (Gospodarwicz, 1964) stimulate conversion of cholesterol to products such as pregnenolone and progesterone (Villee, Van Leuschen and Zeleswki, 1968); and enhance production of progesterone and 17α-hydroxyprogesterone in women (Strott *et al.*, 1969). Perfusion of the human placenta with hCG increases the rate of conversion of dehydroepiandrosterone or testosterone to oestradiol (Varangot and Coyle, 1966; Varangot, Cedard and Yannotti, 1965). It is thought that hCG stimulates placental steroid biogenesis by accelerating aromatization of neutral steroids (Liggins, 1972).

However, a lot of uncertainty exists as to whether hCG plays any part in regulation of placental steroidogenesis under normal conditions. Thus it does not influence *in vitro* oestrogen conversion in cows (Paton, 1969) or the pig (Preumont, Cooke and Ryan, 1969). Neither is hCG found to have any influence on oestrogen

secretions in sheep (Domanski, Skrzeczkowski, Stupnicks, Fitko and Dobrowolski, 1967; Short, McDonald and Rowson, 1963) nor does it influence 3β hydroxysteroiddehydrogenase acitivity in the mouse (Salomon and Sherman, 1976). In humans hCG does not influence side chain cleavage or aromatization in placental preparations (Macome, Bischoff, Uma Bai and Diczfalusy, 1972) and no correlation was found to suggest that it regulated placental steroidogenesis (Savchenko and Strel'tsova, 1975).

Even though uncertainty exists as to the exact influence of hCG on steroidogeneis, it would seem to have some action on the ovary and thus steroid secretion or metabolism as shown by the binding of this hormone on ovarian receptor sites (Kazeto and Hreschyshn, 1970; Vaitukaitis, Hammond, Ross, Hichman and Ashwell, 1971; Kammerman and Canfield, 1972; Kuhn, 1972; Takahashi, Koyomo, Isojima and Adacki, 1972; Ashitaka, Tsong and Koide, 1973; Rajaniemi, Hirschfield, and Midgley, 1974; Zeleznik, Midgley and Reichert, 1974; Mizeyewiski, 1975; Mizejewiski and Gerald, 1976).

Less certain functions attributed to hCG include the development of foetal interstitial tissue (Brody and Carlstrom, 1965); stimulation of growth of foetal adrenal glands (Paverstein and Salomon, 1966; Lavritzen, 1967; Laurizen and Lehman, 1967; Shackleton and Mitchell, 1969; Villee, 1969), possible lactogenic function in the rat (Kuhn, 1972) and sex determination (Brody and Carlstrom, 1965; Effer, Sidney, Gupta and Edwards, 1973; Honsob and Wide, 1974, 1975).

It is apparent then that hCG cannot be said to play any one function with certainty. Most of the experiments were conducted in

animals which have not been shown to have hCG, and the hCG values used exceeded physiological values.

Pregnant mare serum gonadotrophin

The appearance of PMSG was observed in the blood and lymph at the time of implantation of the blastocyst and was concomitant with the appearance of the accessor corporalutea in the mare (Amoroso, 1952). Since the ovarian activity has been shown to precede the appearance of PMSG in the serum (Bain, 1967) its possible role in the formation of accessor corporalutea, if any, and its role during gestation has not been very clear.

PMSG possesses a high follicular stimulating activity and some luteinizing activity (Cole and Hart, 1930). Thus it stimulates ovarian interstitial cells, induces ovulation and luteinizes granulosa cells (Cole, 1970; Carithers and Green, 1972; Kaufmann and Whittington, 1972; Shuji and Kennan, 1972; Vilenbroek and Van Der Werf, 1972; Ying, 1973). It can inhibit oestrus cycle and ovulation (Allen, 1970). Recently, it has been suggested that PMSG can influence progesterone synthesis in rat ovaries (Armstrong and Hixon, 1969), but in sheep it has been shown to have no influence on oestrogen secretions (Domanski et al., 1967; Short et al., 1963).

The role of PMSG in the mare is far from clear. All the suggested functions were found in animals which have not yet been shown to have PMSG.

Human Chorionic Somatomammotrophin

No definite role has been assigned to hCS. The hormone is thought to have somatotrophic (Kaplan and Grumback, 1964; 1965;

Friesen, 1965, 1973; Grumbach, Kaplan, Sciarro and Burr, 1968; Florini, Tonelli, Brever, Cappala Ringler and Bell, 1970) and lactogenic effects (Josimovich and MacLaren, 1962; Friesen, 1965; Forsyth, 1967; Ito and Kyoho, 1974).

It has growth promoting effects in rats (Diczfalusy, 1953; Genazzani, Benuzzi, Badieni and Felber, 1969; Florini et al., 1970) and man (Grumbach et al., 1968; Friesen, 1973). These growth promoting effects are reviewed by Friesen, (1973).

Some of the changes attributed to hCS include the rise in concentration of plasma free fatty acids and increased mobilization of fat stores; the lack of sensitivity to endogenous and exogenous insulin levels in response to a glucose load; and the islet of Langerhan cell hyperplasia observed in pregnancy (Grumbach, Kaplan, Abrams, Bells, and Conte, 1966; Beck and Daughaday, 1966; and Turtle, Beck and Daughaday, 1966). More recently it has been shown to increase level of ketone bodies in fasted rats; monkeys and rabbits (Friesen, 1973). It also antagonizes insulin and thereby increases amount of glucose and amino acids available to the foetus (Friesen, Suwa and Pare, 1969). However, in the sheep, glucose, amino-acid nitrogen concentration and insulin levels are lowered (Handwerger, Fellows, Crenshaw, Hurley, Barett and Mawer, 1976).

The second major biological action of hCS appears to be on the mammary gland (Josimovich and MacLaren, 1962; Friesen, 1969, 1966; Forsyth, 1967). It has no effect on areolae or nipples (Grumbach et al., 1968). Turkington and Topper (1966) showed that hCS in the presence of insulin and hydrocortisone caused

histological development of alveoli and stimulated casein synthesis in organ cultures of mouse mammary gland. The mechanism of action of hCS on mammary gland explants were studied extensively by Turkington (1969, 1970), and the action was thought to be exerted by a membrane effect.

Apart from these effects placental lactogen (hCS) stimulates erythropoiesis (Jepson and Friesen, 1968) and aldosterone excretion in urine (Melby, Dale, Wilson and Nicholes, 1966). It is also thought to be involved in stimulation of progesterone synthesis (Robertson and Friesen, 1971; Kelly, Robertson and Friesen, 1974) although there is disagreement on this point (Conly, Spellacy and Cleveland, 1972). In rats in can inhibit secretion of prolactin (Nagasawa, Yanai, Yamanouch, 1976). The significance of this activity is not know.

Although many sites of action of hCS have been suggested, the mechanisms involved in these complex actions, and exactly where hCS acts in these are not clearly understood.

Of the three sialoglycoprotein hormones, hCS and hCG have been shown to have immunosuppressive properties (already discussed, see page 14), while such a role has not been demonstrated for PMSG. The role of these hormones, especially for PMSG and hCG, during pregnancy are not known. The suggested role of these hormones in immunological tolerance of the foetus (Amoroso and Perry, 1975) have been all in the conjectoral stage.

Neutralization of endogenous gonadotrophins using specific antibodies

In recent years one of the major methods of *in vivo* demonstration of investigating the gonadotrophin role has been the neutralization

of the endogenous hormone by using specific antibodies against that hormone. The experiments done to-date, and the results found have been tabulated below. Most work has been done on the monkey and rat.

In the rat for example antibodies to LH caused complete foetal resportion in rat treated during early pregnancy but had no effect in those treated from mid-pregnancy onwards (Loewit and Laurence, 1969; Madhwa Raj, Sairam and Moudgal, 1968; Madhwa Raj and Moudgal, 1970). Subsequently, Loewit, Badawy and Laurence (1969) attributed this to luteal regression as evidenced by the reappearance of 20αhydroxysteroid dehyrdrogenase. Luteal regression was also found to precede foetal resporption by Ray and Moudgal (1970) and Sairam, Rao and Li (1974). Since the α subunit of hCG and LH are similar antigenically, it is generally accepted that hCG will cause similar effects (see table).

However, these experiments can be criticised on several grounds. Firstly, in none of these experiments was the level of progesterone (or oestrogen) measured. Secondly, a strong challenge has come against the concept that LH is necessary for progesterone secretions during pregnancy. It has been demonstrated in hypophysectomised and hysterectomised pregnant rats that secretion of progesterone continued for three days but at a lower rate (Rothchild, Billiar, Kline and Pepe, 1973). They concluded that placental luteotrophin was responsible for increasing rate of progesterone secretion in absence of LH, thus LH was not necessary for progesterone secretion during pregnancy. Thirdly, the results obtained with antibodies to hCG are based on the belief that hCG can

maintain corpus luteum of pregnancy, a point which is on no account agreed upon. Histology of the ovaries or the conceptuses, and uterus was never done. Finally, it has never been established whether ovarian dysfunction precedes foetal resorption or vice-versa.

Neutralization of Endogenous Gonadotrophins during Pregnancy

Antiserum against	Animal	Stage of Gestation	Results	Reference
Luteinizing hormone	Rabbit	Early	**	Spies & Quadri (1967)
Luteinizing hormone	Rat	Early	*	Madhwa Raj, Sairam & Moudgal (1968)
				Madhwa Raj & Moudgal (1970)
				Loewit & Laurence (1969)
Luteinizing hormone	Rat	Early	**	Loewit, Badawy & Laurence (1969)
				Ray & Moudgal (1970)
Luteinizing hormone	Hamster	Early	**	Rao, Madhwa Raj & Moudgal (1970)
Luteinizing hormone	Monkey	Early	**	Prahalada, Venkataramiah, Rao & Moudgal (1975)
B-Interstitial Cell Stimulating hormone	Rat	Early	**	Sairam, Rao & Li (1974)
B-Interstitial Cell Stimulating hormone	Monkey	Early	*	Moudgal, Prahlada, Rao & Venkataramia (1974)
B-Human Chorionic Gonadotrophin	Monkey	Not Pregnant	***	Moudgal, MacDonald & Greep (1971)

* Termination of pregnancy

** Termination of pregnancy preceded by luteal regression

*** Progesterone decrease interferred with menstruation

MATERIALS AND METHODS

ANTIBODY

Production

Highly purified hCG, Lot 90679 of potency 3,200 i.u. per mg, and PMSG, Lot 10876 of potency 3,000 i.u. per mg (Organon, Surrey, U.K.) were dissolved in 0.01M-Sodium phosphate-buffered saline (pH 7.2) at a concentration of 10 mg/ml. Aliquots of the hormone solutions (0.3 ml) were combined with equal volumes of complete Freund's adjuvant (Difco, Detroit, Michigan) and emulsified by constant mixing. Two male Nubian goats (one for each hormone) were injected intramuscularly with 3 mg emulsion in multiple sites on the hind limbs. Booster doses were given after 5 weeks. Two further boosters were given at 10-day intervals with the hormone preparations in incomplete Freund's adjuvant before bleeding. A maximum of 50 ml blood was drawn by jugular venepuncture at one bleeding.

Purification

Ice-cold saturated $(NH4)_2SO_4$ solution (3 molar concentration) was added drop by drop to an equal volume of the goat plasma in an ice-bath, mixed and stirred gently for 30 minutes. After centrifugation at 1500 g for 15 minutes, the precipitate was dissolved in minimal quantities of 0.01 M-sodium phosphate-buffered saline (pH 7.2) and extensively dialysed against 0.01 M-sodium phosphate buffer (pH 7.5) containing 0.1 M-NaCl. After 2 days the globulin mixture was run in an ion-exchange column containing diethylaminoethyl cellulose (DE-52) pre-equilibrated with 0.01 M-sodium phosphate buffer (pH 7.5) containing 0.1 M-Nacl.

The immunoglobulin fraction eluted with the void volume and was later confirmed to be IgG by immunoelectrophoresis as described by Graber and Burtin (1964). The IgG solution (containing IgG-anti-PMSG or IgG-anti-hCG) was concentrated by negative-pressure dialysis and centrifuged at 10,000 g for 10 minutes. Sterilization was effected by passage through 0.2 µm Sartorius Membrane Filter, type SM 16519 (Sartorius Filter Co., West Germany). The immunoglobulin solutions were then stored at -20°C in 1 ml aliquots containing 10 mg IgG. Protein concentrations were determined spectrometrically at 280 nm (Bechman DB-GT, Grating Spectromphotometer), using 14.3 as the absorption coefficient in calculations.

The antibody titre was determined by the microhaemagglutination test of Boyden (1951) and found to be 1:4096 for PMSG and 1:6144 for hCG. The antibodies were analysed for specificity and cross-reaction by the gel-precipitation method (Ouchterlony, 1962), double counter-current immunoelectrophoresis and in radioimmunoassary system as described by Nixon, Tullner, Rayford and Ross (1971). The cross-reactivities of the antisera were checked against hCG, hFSH, hLH and human prolactin for the PMSG antibody and against PMSG, hFSH, hLH and human prolactin for the hCG antibody. The purified antisera were highly specific, with anti-PMSG reacting only against PMSG and anti-hCG reacting only against hCG (see Appendix II).

Experiments

One hundred and one rats, thirty-five hyraxes, three goats, four cane-rats, four dikdiks and four spring hares were used in this study.

Rats

One hundred and one female Wistar rats (250-300 g) were used. Each female was caged separately with a male until mating occurred (Day 1), as determined from vaginal smears every morning and afternoon. The male was then removed. Pregnant rats were assigned to one of 4 experimental groups.

Group 1 comprised 15 animals which received 4 mg IgG-anti-PMSG interperitoneally for 5 days each morning between 08:00 and 08.30h. Five of the animals (A) were injected from Day 6, five from Day 11 (B) and five from Day 16 (C).

At 5 days after the end of treatment, the animals in Groups 1A and 1B were bled and killed. One ovary, the uterus, mammary tissue and pituitary of each animal were fixed in 5% formol saline for histology. The other ovary was frozen for subsequent steroid extraction and assay. Animals in Group IC were allowed to go to term and litter. The number and weight of the foetuses was recorded.

Group II: 4 rats were injected with 10 mg IgG-anti-PMSG on Day 6 (A), Day 11 (B) or Day 16 (C) of gestation and the subgroups were treated in the same way as those in Group 1. In addition 14 rats were treated as in Group IIA. Of these 4 animals were used for progesterone clearance test (D), 4 for oestrogen clearance test (E) and the remaining 6 (F) used for *in vitro* progesterone metabolism and enzymatic analysis.

Group III: The six subgroups (A-F) were treated as in Group II but each rat was injected with 10 mg IgG-anti-hCG.

Group IV was the control group: 5 rats were killed on Day 16 (A), 5 on Day 21 (B) and the remaining 5 were allowed to litter (C). Five rats were treated with 10 mg non-immune IgG for 5 days from Day 6 to serve as additional controls. Control rats for subgroups D, E and F were all treated with 10 mg non-immune IgG. Portions of the placentae were frozen with solid CO_2 and stored at $-20^{\circ}C$ for subsequent immunofluorescence studies.

Hyraxes

Thirty-five pregnant female rock hyraxes (800 - 900 g) in early pregnancy (2-3 months) were used. The stage of gestation was confirmed by autopsy by recording foetal weight and length parameters. They were captured at Lukenya, some 20 km from Nairobi, and brought to the laboratory. To reduce stress of capture and transportation, the animals were given glucose (20 ml orally) and acetyl-promazine (5 mg intramuscularly). An antibiotic umbrella (0.5 ml "combiotic") was also given routinely. Hyraxes invariable harbour tapeworm infestation. Because of this, a taeniacide (250 mg "Yomesam") was given on the day of arrival. After a week, the animals were assigned to one of experimental groups.

Group I comprised of 10 animals and received 10 mg IgG-anti-hCG intraperitoneally for 5 days each morning between 08.00 and 08.30 h. The animals were then killed in duplicate on Day 1, 2, 3, 4 and 5 after the end of treatment. The ovaries and uteri were removed and fixed in 5% formol saline for routine histology.

Group II: 10 animals were treated as in Group 1, but each animal received 10 mg IgG-anti-PMSG.

Group III was the control group. Fifteen animals were treated with 10 mg non-immune IgG. They were killed at the same time interval as in Group 1. The placental portions and the pituitaries of all of these animals were frozen with solid CO_2 and stored at -20°C for subsequent immunofluorescence, and pituitary extract analysis.

Goats

Three female pregnant Nubian goats in late pregnancy were used. The animals were brought to the laboratory and given an antibiotic umbrella (2 ml "combiotic") routinely. Since these goats invariably harbour round worms, 10 ml "Nilverm" was given, to remove them. After two weeks, one goat received 100 mg IgG-anti-hCG, one 100 mg IgG-anti-PMSG and the third acted as a non-treated control. Prior to this, the animals had been bled for five days through jugular vene-puncture, bleeding being continued during treatment and for 5 days thereafter. On the seventh day post treatment, a biopsy was performed under general anaesthesia and a placentome obtained. The ovary of antibody-treated animals was also obtained at the same time. Immediately on removal, a portion of the placentome from the control animal was frozen on solid CO_2 and stored at -20°C for subsequent immunofluorescence study.

Cane-rats, Springhares and Dikdiks

Placentae from these animals were removed, frozen on solid CO_2 immediately and stored at -20°C for subsequent immunofluorescence studies.

Autopsy

Histology - The formol saline-fixed ovaries, uteri, pituitaries

and mammary-tissue were processed routinely for histology. Sections were cut at 7μm and stained with Ehrlich's haemotoxylin and eosin. Corpora lutea, implantation sites, placentae, foetuses, mammary tissue and pituitaries were studied by light microscopy.

Steroids

All reagents used for extraction and assay of steroids were Analar grade and were purchased from a variety of sources. Ovarian homogenates and plasma samples were ether extracted as described by Gombe and Oduor-Okelo (1977) for testosterone. Mean ± s.e.m. recoveries for ovarian and plasma samples were 85 ± 2 and 96 ± 2% respecitvely for progesterone and 94 ± 3 and 89 ± 3% respectively for oestrogens. Similarly, recoveries for the goat plasma was 91 ± 2% for the former and 89 ± 3% for the latter. Progesterone and total unconjugated oestrogens were measured by radioimmunoassays. The antiserum to progesterone (B456 No.6) was kindly donated by Dr. R.B. Heap, Cambridge. The mean recovery of authentic progesterone (25 - 1000 pg/1 ml stripped plasma) was 89.6 ± 2.7% for the rat and 88.2 ± 2% for goat, and the within-assay coefficient of variation was 5.2% and 6.0% (n = 12) respectively. The working range of the assay was 20 - 1000 pg. All plasma samples were assayed together and all tissue samples were assayed together but separately from the plasma samples. The antiserum to oestradiol - 17β (S-52-S) was pruchased from Dr. G.E. Abrahams, California. This antiserum cross-reacts with oestradiol - 17α(40%), oestrone (35%) and oestriol (8%). Therefore, the values given represent total unconjugated oestrogens. The recovery was 88.3 ± 2.0% for the rat and 81.7 ± 2.4% for the goat from 10 - 500 pg authentic

oestradiol-17β added to 1 ml stripped plasma. The within-assay coefficient of variation was 5.4% and 6% (n = 12) respectively and the working range of the assay was 5-500 pg.

Pituitary and Placental Extracts

The hyrax pituitaries and placentae were homogenized with sodium phosphate-buffered saline (0.01M, ph 7.4). The supernatant collected after centrifugation at 3000 g, was concentrated by negative-pressure dialysis. The cross-reactivity with antibodies against PMSG and hCG was checked using the gel precipitation method (Ouchterlony, 1962).

Steroid Clearance and Metabolism

Progesterone and Oestrogen Clearance

Animals were anaesthetised with 15 mg sodium pentobarbitone ('Sagatal', May and Baker, England) interperitoneally. Right external iliac artery and left femoral vein were exposed and cannulated using small calibre Portex intravenous set (19G, Ref. 200/500/010, Kent, England). Duplicate (0.1 ml) blood samples were drawn through the artery using 1 ml syringe and ether extracted.

Heparinised physiological saline (0.1 ml) containing 72,000 cpm ^{3}H-progesterone (1,2 (n) H^{3} progesterone, 42 Ci/m Mol) or 98,000 cpm H^{3} - oestradiol (6,7-^{3}H oestradiol 44 Ci/m Mol) was infused through the vein. Aliquots (0.6 ml) of blood samples were subsequently collected from the artery at 5, 15, 30, 60, 90, 120, 150 and 180 min. post infusion.

Blood samples were ether extracted and purified by TLC as described by Heap, Gombe and Sale (1975) for progesterone and

Challis, Harrison and Heap (1973 b) for oestradiol. The purified samples were counted to determine the progesterone and oestrogen clearance.

Progesterone Binding and Metabolism

Tritiated progesterone (8000 cpm) was added in triplicate to 1 ml plasma or water. After incubation at 37°C for 1 hour the unbound steroid was separated by adding 0.5 ml dextran-coated charcoal (625 mg Norit Siema Charcoal and 62.5 mg Dextran T70 in 100 ml 0.01M phosphate buffer pH 7.5). After brief mixing (1 minute at 2°C) and centrifugation the supernatant was counted.

Immediately on killing an animal, the liver was removed and cooled in ice-cold (2°C), 0.01 M sodium phosphate buffer (pH 7.4). Portions of the liver (250 mg) were carefully weighed and cut into thin slices, which were transferred to an incubation flask containing 30 ml 0.01M sodium phosphate buffer (pH 7.4), 50 mg D-glucose and 106,000 cpm ^{3}H progesterone. The flask was flushed with a mixture of 95% O_2 and 5% CO_2, tightly stoppered and incubated at 37°C for 3 hours with constant agitation. After incubation 1 mg cold progesterone was added for correction of procedural losses and to aid identification of the progesterone spot on the TLC.

Control incubations were carried out in quadruplicate under identical conditions except for the absence of liver.

The steroid metabolites were extracted and separated by TLC as described by Heap et al (1975).

Liver Function

Plasma and liver GPT and GOT were measured using BDH kits

(BDH Chemicals Ltd., Poole, England). Recovery of this method was found to be 99.8 ± 0.1% and 99.7 ± 0.1% (n = 10) for GPT and GOT respectively, using the Moni - Trol - 1 standard (Lot LTD 133, Dade Abbot Lab., California, U.S.A.).

Portions of the liver were fixed in 5% formol saline and processed histologically, cut at 7 µm and stained with Ehrlich's haemotoxyline with eosin. Livers were studied under light microscopy.

Kidney Function

Plasma urea and creatinine levels were determined using Roche Diagnostic Kits (Hoffman - La Roche and Co., Basle, Switzerland). Recoveries were found to be 99.7 ± 0.2% (n = 10) for urea and 98.9 ± 0.1% (n = 10).

Calculations and Statistics

Calculation of $t\frac{1}{2}$ values

The $t\frac{1}{2}$ values of progesterone and oestrogen were calculated by regarding the zero time value as unity, and determining the time taken (graphically) to halve this value.

Statistical Tests

The stastical significance of the difference between the means was tested by student's t test and Duncan's new multiple range test.

Localization of antigonadotrophic activity

The site of activity of the IgG-anti-PMSG and IgG-anti-hCG was examined by immunofluorescence studies. Thin sections (4 mm) of

fresh rat placentae from all the 20 animals in control Group IV were frozen on microscope slides, previously cooled with solid CO_2, with a freezing medium (OCT Compound, Lab-Tek. Products, Naperville, Illinois) and then stored at -20°C until required. These were subsequently sectioned at 7 µm on a cryostat set at -26°C. The sections were mounted on acid-cleaned slides, dried in a cold stream of air in dry slide boxes at 4°C, and wrapped in airtight polythene bags. Silica gel was used as a dessicant.

Fluorescien Conjugation

The IgG-anti-PMSG, IgG-anti-hCG and non-immune IgC (100 mg) were each conjugated with fluorescein isothiocyanate (Isomer 1, Lot No. 9101581), Cockeyville, Maryland, U.S.A.) according to the method of Clark & Sheppard (1963). Each conjugate was dialysed for 24 hours against 0.01 M-sodium phosphate-buffer, pH 7.5; then applied to an ion-exchange column containing diethyl aminoethyl cellulose (DE-52) pre-equilibrated with the above buffer to which 0.05 M-NaCl had been added. The conjugate was eluted from the column with 0.01 M-sodium phosphate buffer (pH 7.5) to which increasing concentrations of NaCl solution (0.05, 0.1, 0.3, 0.5 and 1.0 M-NaCl) were added. Three peaks were obtained at salt concentrations of 0.05, 0.3 and 1.0 M-NaCl. The fraction eluting with 0.3 M-NaCl was retained whilst the early and late fractions were discarded. The usable fraction was then concentrated by negative-pressure dialysis. The same procedure was followed for conjugating tetramethyl rhodamine isothiocyanate (lot no. 1031464, Cockeyville, Maryland, U.S.A.) except that rhodamine was passed through a sephadex G25 column pre-equilibrated with 0.01M-sodium

phosphate buffer containing 0.05 M NaCl, to remove the unbound rhodamine, before the ion-exchange step.

The protein concentration and the fluroescein/protein (F/P) ratio were determined spectrometrically, according to the method of Brandtzaeg (1973), on a spectrometer (Bechman BD-GT Grating Spetrophotometer) used at 280 nm (for protein measurement) and 495 nm and 525 nm (for fluorescein and rhodamine measurement) respectively. The protein concentration was 20m7 mg/1 ml for the antiserum to PMSG and 26.4 mg/1 ml for the antiserum to hCG for fluorescein and 20.1 mg/1 ml and 22.7 mg/1 ml respectively for rhodamine. The corresponding F/P ratios were 5.2 and 6.4 and the R/P ratios were 3.9 and 4.5. The conjugates were centrifuged at 10 000 g for 1 hour, sterilized by millipore dialysis (see above) and stored.

Demonstration of Fluorescence

The conjugates were tested at various dilutions to determine the optimal working dilution. The methods of Johnson and Holborow (1971) were used. The placental sections were fixed for 1 minute in ice-cold (4°C) 40% ethyl alcohol, then transferred to sodium phosphate buffer (0.01 M, pH 7.2) changed every 10 minutes, and left for 30 minutes. The slides were gently wiped with absorbent tissue paper and put into a humid chamber. Three drops of the respective fluorescent antibody were added over the tissue. After 30 minutes, unbound fluorescent antibodies were washed off with sodium phosphate-buffered saline (PBS), pH 7.2, and the slides passed through three changes of the buffer in 45 minutes. The slides were dried, mounted in 5% glycerol in PBS, and the edges of the mount sealed with nail polish. The slides were viewed with a Leitz-

Ortholux microscope with a 200-W HBO mercury lamp, a 3-mm BG 12 transmission filter and a K510 barrier filter for fluoroscein and S546 transmission filter and K610 barrier filter for rhodamine. Contiguous sections were processed for routine histology. Immunofluorescent controls were provided by mixing conjugated antibodies with excess antigens (HCG and PMSG), leaving overnight, and staining the placental sections as described above. A further control was provided by the use of non-immune, fluorescein-conjugated goat IgG.

RESULTS

Neutralization Experiments

Rat

All the rats treated with anti-PMSG and anti-hCG remained in good health for the duration of the experiment. At autopsy an abundance of mesentric fat was particularly noticeable. There were no signs of peritonitis.

Litter size and weight

The rats in Group IC all littered, the number of fetuses (58) and the litter weight (6.8 ± 0.5 (s.e.m.) g) being comparable to those of the controls in Group IVC (n = 47, 6.9 ± 0.4 g). Only 1 rat in Group IIC gave birth to an apparently normal litter (n = 11, 6.6 ± 0.4 g). Two rats in Group IIIC gave birth: the number of fetuses (25) was normal but the mean ± s.e.m. weight of the litters (5.9 ± 0.3 g) was significantly ($P<0.05$) less than that of controls (Group IVC).

Dead degenerating fetuses were removed on Day 26 *post coitum* from the 3 rats in Group IIC and the 2 in Group IIIC which did not litter.

Histology

Foetal

The conceptuses in Groups IA (4 mg IgG anti-PMSG) and IB appeared macroscopically normal but definite degenerative changes were detectable in the placentae when compared with Group IV (Pl.1, Fig. 1 and 2). These changes consisted of scattered necrotic foci of the giant cell layer in Group IB (Pl.2, Fig. 3) and scattered

necroses of both the giant cell layer and the adjoinging spongy trophoblast in Group IA (Pl.2, Fig. 4). Congestion of the trophoblast was evident (Pl.3, Fig. 5). The necrotic foci were invariably invaded by lymphocytes and neutophis (Pl.3, Fig. 6). In Groups IIA (10 mg IgG anti-PMSG) and IIIA (10 mg IgG anti-hCG) foetal resorption was complete in 3 of the animals and nearly complete in the other (Pl.4, Fig. 7 and 8, Pl.5, Fig. 9). The implantation sites in the former were recognized histologically by small eosinophilic hyaline nodules surrounded by haemosiderin deposits (Pl.6, Fig. 10 and 11). There was partial foetal resorption in 2 of the Group IIB and IIIB rats (Pl.7, Fig. 12) and the other two contained dead but unresorbed foetuses. In all the rats of Group IIB and IIIB the placentae were entirely necrotic and massively infiltrated by leucocytes.

The conceptuses in Group IV treated with non-immune IgG were macroscopically and microscopically normal.

Ovarian

The CL of the animals in Groups IA (4 mg IgG anti-PMSG), IB and IIA (10 mg IgG anti-PMSG) were histologically hyperaemic but otherwise were not different from those of rats in Groups IV A (non-treated) and IVB (Pl.8, Fig. 13 and Pl.9, Fig. 14 and 15). Group IIB rats also had apparently normal luteal cells although the CL were very congested. In Groups IIIA (10 mg IgG - anti-hCG) and IIIB the CL were macroscopically normal but central necrosis (Pl.10, Fig. 16), larger in the CL of IIIB animals, was apparent. The CL of rats in Group IIC and IIIC that did not litter and had dead foetuses *in utero* were macroscopically and microscopically normal, as were those of the animals treated with non-immune IgG.

Pituitary and Mammary Tissues

No changes, both macroscopically and microscopically were noticed when the pituitaries and mammary tissues of rats in Groups I, II and III were compared with those of Group IV (Pl.11, Fig. 17 and 18, Pl.12, Fig. 19, Pl.12, Fig. 20 and 21, Pl.14, Fig. 22 and 23).

Steroid Concentrations

These are shown in Table 1. Compared with controls, ovarian progesterone concentrations were significantly higher in Groups IA, IB and IIIA, while plasma progesterone levels were significantly lower in Groups IIA, IIB, IIIA and IIIB. Plasma oestrogen concentrations were significantly higher in Group IIB animals than in those of Group IVB. The CL of the Group IIC and IIIC rats with dead fetuses appeared to contain normal amounts of progesterone.

Hyrax

Histology

Group 1 (10 mg IgG anti-hCG)

The hyraxes dissected on Day 1 (post-treatment) had live fetuses. Histological examination of the placentae revealed cellular necrosis of columnar cell monolayer (the maternal-foetal junction) with some necrosis spreading to the adjoining spongy trophoblast. No white blood cells were evident in these regions. The maternal myometrium and endometrium and the deeper lying palisade zone were comparable to the control animals (Pl.15, Fig. 24 and 25, Pl.16, Fig. 26 and 27, Pl.17, Fig. 28, Pl.18, Fig. 29). The ovaries showed no differences to the ovaries of control animals (with

respect both to the corpora lutea and the follicles).

In Day 2 hyraxes, the fetuses were also alive. The placental necrosis had spread to encompass the whole of the spongy trophoblast (Pl.18, Fig. 30). The palisade zone showed massive congestion (Pl.19, Fig. 31). The corpus luteum showed no changes (Pl.20, Fig. 32 and 33). The primary follicles showed a definite increase in the number of damaged primary follicles. Careful histological examination established that this damage was systematic and followed a pattern. In the initial stages the primary follicles underwent disruption of the nucleus, and this was accompanied by the formation of pink staining granules. As the graafian follicles increased in size, the oocyte nuclei disappeared, the cytoplasm became vacuolate and the granules coalecsed into ovoid, intensely eosinophilic bodies (Pl.21, Fig. 34 and 35, and Pl.22, Fig. 36).

Dead generating fetuses were removed from Day 3 hyraxes. The columnar cell layer, now represented as a hyaline structure, had separated from the myometrium (Pl.23, Fig. 37). The spongy trophoblast was extensively damaged and disorganised. Lymphocytes and neutrophils were present in these areas (Pl.23, Fig. 38). The palisade zone was massively congested with blood. The corpora lutea of these animals appeared normal, and primary follicles were destroyed, as was the case for Day 2 animals.

Day 4 and 5, too, had dead degenerating fetuses. The placentae were extensively necrotic and congested; some damage to the palisade zone was observed. The corpora lutea appeared normal, and the follicular changes resembled those described for Day 2 animals.

Group 2 (10 mg IgG anti-PMSG)

Both hyraxes killed on Day 1 had live foetuses. The placentae and ovaries appeared normal. Day 2 animals, too, had live fetuses. The placentae showed congestion in the spongy trophoblast zone, while the maternal uterine and the palisade zone were normal. The corpora lutea and the follicles appeared as was the case in the control animals.

Day 3 animals also had live fetuses, but examination of the placentae revealed foci of necrosis at which columnar monolayer were extensively disorganised, the damage tending to spread to the deeper spongy trophoblast (Pl.24, Fig. 39). The palisade zone was massively congested (Pl.24, Fig. 40). The corpora lutea appeared hyperaemic (Pl.25, Fig. 41). The graafian follicles showed the degenerative changes described above.

Day 4 animals revealed live fetuses on dissection. Foci at maternal-foetal junction and the spongy trophoblast were extensively necrotic. Some separation of the columnar cell layer from the maternal myometrium was evident. Lymphocytes and neutrophils were visible in this area. The palisade zone was massively congested. The corpora lutea were hyperaemic and follicles appeared as in Day 3 animals.

Day 5 animals had dead degenerating fetuses. Placentae were extensively necrotic and congested. The appearance resembled the placentae of Day 5 anti-hCG treated animals. Corpora lutea and follicles appeared as in Day 4 animals.

Group 3 (10 mg non-immune serum)

All the 15 hyraxes had alive fetuses. No obvious damage to the placenta was observed both macroscopically and microscopically. The corpora lutea appeared normal in the control animals.

Pituitary and Placental extract analysis

No precipitin lines were observed on Ouchterlony plates, indicating lack of cross-reactivity of the hyrax pituitary with antibodies to hCG and PMSG. The placental extract on the other hand showed weak precipitin lines with both anti-hCG and anti-PMSG. Cross-reactivity data for the placental extract using these antibodies in a radioimmunoassay system is presented in Table 2.

Goat

All the goats remained in good health for the duration of the experiment.

Anti-hCG treated goat

The gross examination of the placenta on biopsy revealed massive necrosis of the foetal membranes and pus was evident on the cotyledonary tissue (Pl.26, Fig. 43 and 44). The foetus was alive but under-developed. The ovary appeared normal. Microscopic examination of placentome revealed massive disorganisation of foetal tissue. The columnar trophoblastic cells lying adjacent to the maternal connective tissue were degenerate and their nuclei were pyknotic. There were large expanses of empty spaces in the cotyledons, where the trophoblastic tissue was absent (Pl.27, Fig. 45 and 46). In places, the damage had spread into the foetal connective mesenchyme. The corpora lutea were congested although luteal cells did not show any damage (Pl.29, Fig. 48 and 49).

The levels of plasma progesterone and oestrogen did not reveal any significant changes during or after treatment with anti-hCG (see Table 3).

Anti-PMSG treated goat

Gross histological appearance of the anti-PMSG treated placenta and ovary resembled that of the anti-hCG treated animal. Microscopic examination of the cotyledon showed massive disorganisation of the foetal tissue. The damage started with the destruction of the columnar trophoblastic cell layer lying adjacent to maternal connective tissue and in places had spread to the foetal mesenchyma (Pl.28, Fig. 47). No damage to the maternal connective tissue was visible and only a few leucocytes were seen.

As in the anti-hCG treated goat, there were no significant changes in the plasma steroidal levels (see Table 4). This animal subsequently gave birth to an under-developed kid which was grossly underweight and died twenty-four hours after birth.

Immunofluorescence Studies

The optimal working dilutions were 1:64 for IgG-anti-hCG and 1:96 for IgG-anti-PMSG conjugates. At these dilutions, no immunofluorescence was visible in the placental sections stained with antigen-adsorbed fluorescein conjugates.

Rat

The anti-hCG-fluorescein conjugate stained the rat placentae positively (see Pl.30). The greatest immuno-fluorescence was present at the feto-maternal junction on the giant cells and outermost chorionic cells (Pl.30, Fig.53). Under oil-immersion

the fluorescence broke down to very small discrete stained globules. Some positive staining was evident in the spongy trophoblast, but none was seen in the labyrinthine zone. The maternal myometrium showed no staining, whilst the positive staining in the endometrium was restricted to the area adjacent to the feto-maternal junction, and was of very low intensity. The anti-PMSG-fluorescein conjugate stained the same areas as did the anti-hCG conjugate except that the intensity of immunofluorescence was much weaker (Pl.30, Fig. 51). Staining with non-immune IgG-Fluorescein conjugate was not observed anywhere in the placenta (Pl.30, Fig. 52).

Hyrax

Anti-hCG-immunofluorescence was present in very high intensity at the feto-maternal junction, and appeared as a thin band running the length of the placenta, being located at the site of the columnar cell mono-layer (Pl.31, Figs. 54 and 55). Under oil immersion this band appeared composed of masses of small positively staining globules. Some positive staining was evident at the zone of spongy trophoblast, but none at the palisade zone. The intensity of this staining decreased towards the deeper-lying palisade zone. Maternal myometrium showed no staining, whilst the positive staining in the endometrium was restricted to the area adjoining the feto-maternal junction and was of the lowest intensity. Anti-PMSG-fluorescein conjugate stained the same areas as did anti-hCG except that the intensity of immunofluorescence was much weaker (Pl.32, Fig. 56). Non-immune IgG-fluorescein conjugate did not stain any region of the hyrax placenta.

Goat

Anti-PMSG-fluorescein conjugate stained the goat placenta positively. The highest immunofluorescent material was present as a bright staining band at the columnar tophoblastic cell layer lying adjacent to the maternal connective tissue. Maternal and fetal connective tissue showed no staining (Pl.33, Figs. 57 and 58, Pl.34, Fig. 59). Both anti-hCG-fluorescein conjugate and non-immune IgG-fluorescein conjugate did not stain the goat placenta.

Cane-rat

High intensity of immunofluorescence was observed with anti-hCG-fluorescein conjugate in the sub-placental region of the cane-rat placenta (Pl.35, Figs. 60 and 61, Pl.36, Fig. 62). A lower intensity of immuno-fluorescence was present in the region of placental labyrinthine whilst the maternal myometrium and endometrium showed no immunofluorescence. The anti-PMSG-fluorescein conjugate stained the same areas as did anti-hCG-fluorescein conjugate, except that the intensity of immunofluorescence was much weaker. Under oil-immersion the sub-placental region consisted of very small discrete globules. Non-immune IgG-fluorescein conjugate did not stain the cane-rat placenta. Some of the maternal uterine vessels which had hypertrophied endothelium showed immunofluorescence with both anti-hCG- and anti-PMSG-fluorescein conjugate (Pl.36, Fig. 63).

Spring-hare

Immunofluorescence with both anti-hCG and anti-PMSG conjugates was sharply localized at the region of outer-most chorionic cells lying adjacent to the maternal endometrium. No immunofluorescence

was observed in the other areas of the conceptus (Pl.37, Fig. 64, and Fig. 65). Non-immune IgG-fluorescein conjugate did not stain any region of the spring-hare conceptus. As in the cane-rat maternal blood vessels which had hypertrophied endothelium showed strong immunofluorescence with both conjugates (Pl.38, Figs. 66 and 67).

Dik-dik

Only anti-PMSG-fluorescein conjugate stained the dik-dik placenta. The region and intensity of staining were similar to goat.

Metabolic Clearance Studies

Progesterone and Oestrogen Clearance

The half lives of progestins and oestrogens of unextracted blood are represented in Text Fig. 68 and 69 (Plates 39 and 40 respectively). The fast $t_{\frac{1}{2}}$ for progestins was 13.2 ± 0.3, 11.0 ± 0.4, and 9.4 ± 0.7 minutes for control, anti-hCG-treated and anti-PMSG-treared rats respectively, from which it is apparent that antigonadotrophin treatment increased the rate of progestin clearance ($P<0.001$). The corresponding figures for the slow pools were 72.8 ± 1.1, 66.8 ± 3.1 and 58.3 ± 4.0 for the control, anti-hCG-treated and anti-PMSG-treated rats respectively. Although the difference between the control and anti-hCG-treated rats was not significant for the slow pool, that between the control and the anti-PMSG-treated rats was significant ($P<0.01$).

In contrast to the above, the oestrogen clearance for anti-hCG-treated rats was slower than the controls. Thus, for $t_{\frac{1}{2}}$ for the fast pool was 47.0 ± 0.4, 105.0 ± 1.4 min. for control and anti-

hCG-treated rats respectively. Similarly, the slow pool $t_{\frac{1}{2}}$ values were 145.0 ± 1.3 and 339.0 ± 3.8 min. for control and anti-hCG-treated group.

Surprisingly the oestrogen clearance of anti-PMSG-treated rats was faster than the controls treated with non-immune globulin. Thus the fast and slow $t_{\frac{1}{2}}$ for anti-PMSG-treated and control animals were 37.8 ± 0.5 vs 47.0 ± 0.4 min. and 132.5 ± 6.1 vs 145.0 ± 1.3 min. respectively ($P<0.05$).

Progesterone binding and metabolism

Whereas percentage binding of plasma progesterone in the controls (55.7 ± 0.6%) and anti-PMSG-treated rats (57.5 ± 1.3%) were similar, there was a considerable decrease in the anti-hCG-treated rats (42.7 ± 0.7%, $P<0.001$).

Text Fig. 70 (Plate 41) gives the percentage recovery of progesterone and its metabolites after *in vitro* incubation with slices of liver. There was a significant decrease of H^3-progesterone in antogonadotrophin-treated rats ($P<0.001$). Authentic progesterone was present only in fraction 12; thus activities in fractions 2, 3, 9, 13, 14 and 15 represent metabolites. It will be seen that the amount of metabolites was greater in anti-PMSG and anti-hCG-treated rats livers. In addition, the appearance of a metabolite in fraction 3 was confined to the antibody treated rat livers. Although the nature of the metabolites was not determined, the pre-progesterone fractions (2, 3 and 9) possibly represent poly-hydroxy-derivatives, whilst the post-progesterone fractions (13, 14 and 15) represent the reduced keto-derivatives as found by Heap *et al.*, (1975).

Liver Function

Gross examination of the livers of both anti-hCG- and anti-PMSG-treated rats showed no obvious changes. Microscopically, however, the antibody-treated animals showed antralobular hyperaemia which was especially marked in anti-hCG-treated rats [illegible]. Additionally fatty infiltration of the liver was noticeable in the experimental groups. Anti-hCG rats appeared to have foamy fat infiltration which made the cells to have a centrally located nuclei with radiating strands of protein. The anti-PMSG-treated rats had eccentrically placed nuclei with much of the cytoplasm taken up by large globules of fat.

Both the serum and hepatic GPT and GOT levels were highly elevated in the antigonadotrophin-treated rats (see Table 5).

Kidney Function

The kidneys of both anti-hCG and anti-PMSG-treated rats were normal as could be judged from gross appearance and the values of plasma urea and creatinine as depicted in Table 6.

Table 1. The effect of treatment (mean ± s.e.m.) of pregnant rats for 5 days with antibodies to PMSG and hCG on concentrations of progesterone and total oestogens.

Treatment	Group	No. of rats	Progesterone		Oestrogen	
			Ovarian (ng/g)	Plasma ng/ml	Ovarian (ng/g)	Plasma (pg/ml)
Anti-PMSG, 4 mg/day						
-from Day 6	IA	5	444.7±52.5*	6.1±1.3	-	-
-from Day 11	IB	5	309.8±81.5*	7.1±0.9	-	-
Anti-PMSG, 10 mg/day						
-from Day 6	IIA	4	199.8±52.1	4.9±0.6*	15.2±11.0	82.3±24.7
-from Day 11	IIB	4	117.4±34.5	5.7±0.6	2.0±1.4	118.3±28.3*
-from Day 16	IIC	3**	94.4±24.3	5.6±1.3	0.8±0.2	106.7±63.3
Anti-hCG, 10 mg/day						
-from Day 6	IIIA	4	286.4±125.5	4.9±0.4	5.0±0.1	175.0±86.7
-from Day 11	IIIB	4	125.8±37.4	3.0±1.6	3.0±1.2	88.8±24.1
-from Day 16	IIIC	2**	106.7	3.5	0.4	91.5
None						
Killed Day 11	IVA	5	96.7±22.0	7.2±1.4	8.6±1.5	81.6±24.7
Killed Day 16	IVB	5	100.3±23.5	8.4±0.9	3.0±1.6	55.8±12.8

All rats in Group IVC were allowed to litter, therefore no steroid levels are available

* These values are significantly different from those of corresponding control groups, $P<0.05$

** Animals with dead fetuses at autopsy (see Text)

Table 2. Radioimmunoassay system analysis of the hyrax placental extract with closely related hormones.

The antiserum to hCG gave the following cross-reaction in specificity studies.

Hormone	% Binding
hCGα	<0.01
hCGβ	100
hCG	20
FSHα	<0.01
FSHβ	<0.01
FSH	<0.01
HGH	<0.01
h prolactin	<0.02
hpl	0.05
LHα	0.25
LHβ	10
LH	15
hyrax placental extract	40
hyrax pituitary extract	11

Table 3. Influence of anti-hCG treatment on plasma progesterone and oestrogen levels

	Pre-treatment	Treatment	Post-treatment
Progesterone (ng/ml)	4.8±1.4	3.5±0.4	3.6±0.6
Oestrogen (pg/ml)	161.4±18.2	196.0±20.0	196.3±22.0

Errors are quoted as ± s.e.m.

Table 4. Influence of anti-PMSG treatment on plasma progesterone and oestrogen levels

	Pre-treatment	Treatment	Post-treatment
Progesterone (ng/ml)	4.0±0.6	3.2±0.6	3.7±0.8
Oestrogen (pg/ml)	170.0±7.1	196.4±19.9	199.5±23.0

Errors are quoted as ± s.e.m.

N.B. All statistics done using 'students t test' and Duncan's new multiple range test.
Note lack of statistical significance between all sample.

Table 5. Influence of antibody treatment on serum and liver transaminases

	No. of animals	Plasma GPT lu/l	Plasma GOT lu/l	Liver GPT lu/g/l	Liver GPT lu/g/l
Control rats	6	50±1	94±2	3422±141	3038±64
Anti-hCG-treated rats	6	95±4***	122±4**	4196±41***	3766±260***
Anti-PMSG-treated rats	6	98±2^{aaa}	118±4^{aa}	3922±88^{aa}	3881±107^{aaa}

Errors are quoted as ± s.e.m.

All statistics done using "students t test" and Duncan's new multiple range test

* Denotes statistical significance of anti-hCG-treated rats with respect to control animals.

a Denotes statistical significance of anti-PMSG-treated rats with respect to control animals.

** aa $P<0.01$

***aaa $P<0.001$

Table 6. Influence of antibody treatment on kidney clearance

	No. of animals	Urea mg/100 ml	Urea N mg/100 ml	Creatinine mg/100 ml
Control rats	6	39.6±0.9	19.6±0.4	1.1±0.1
Anti HCG-treated rats	6	40.8±0.7	18.5±0.3	1.1±0.1
Anti PMSG-treated rats	6	40.8±0.8	18.7±0.6	1.1±0.1

Errors are quoted as ± s.e.m.

N.B.

Note the lack of statistical significance between the control and antibody treated rats.

All statistical analysis carried out using "students t test" and Duncan's new multiple range test.

PLATE 1

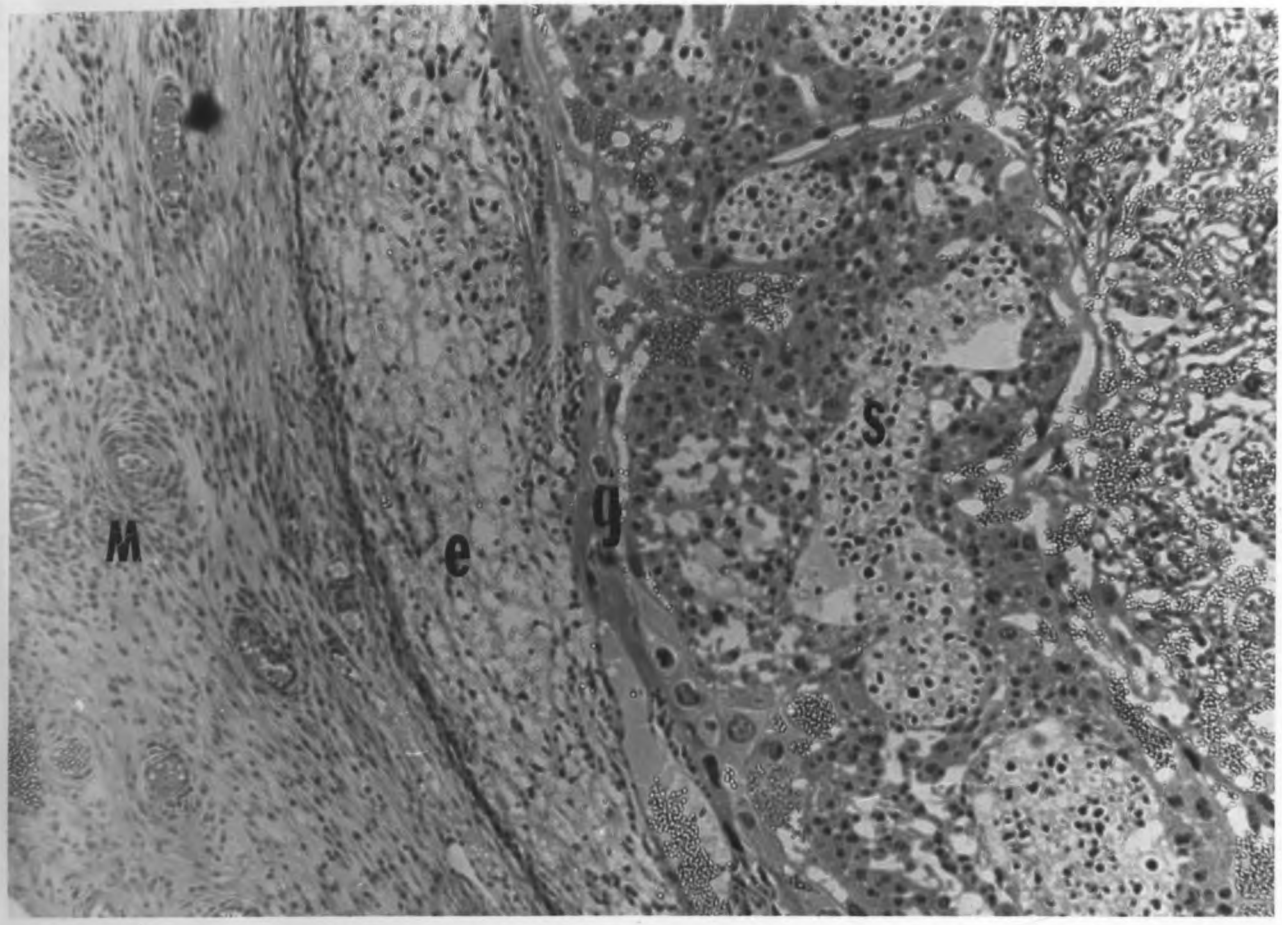
Sections of Group IV, non-immune lgG treated rat placenta X200.

Fig. 1

Light micrograph of Group IV non-immune lgG treated rat showing the labyrinthine zone (l), the spongy zone (s), giant cells (g), maternal endometrium (e), and myometrium (m).

Fig. 2

Light micrograph of the same section as Fig. 1 showing labryinthine zone (l) and the foetal membrane (f).


M
e
g
S

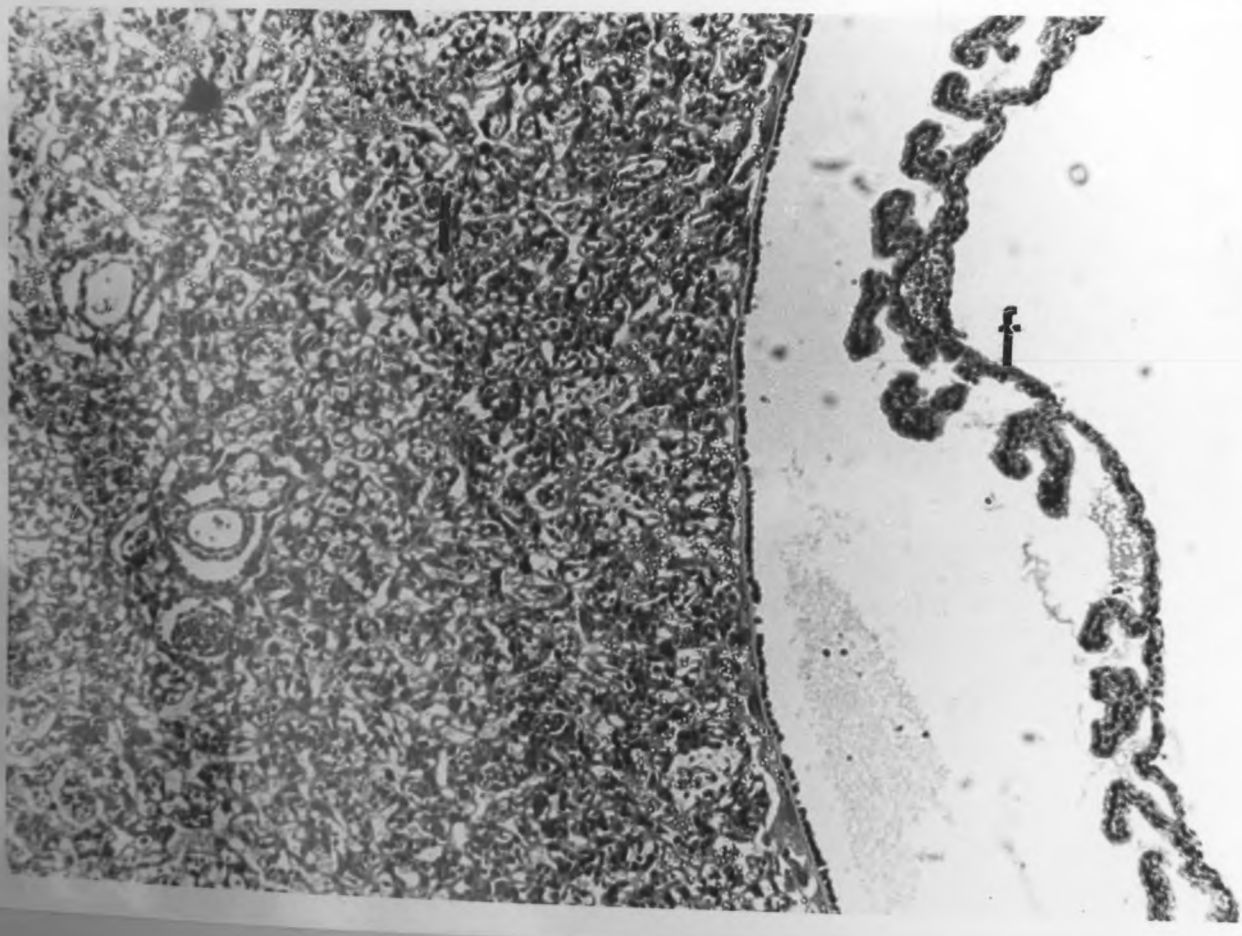

f

PLATE 2

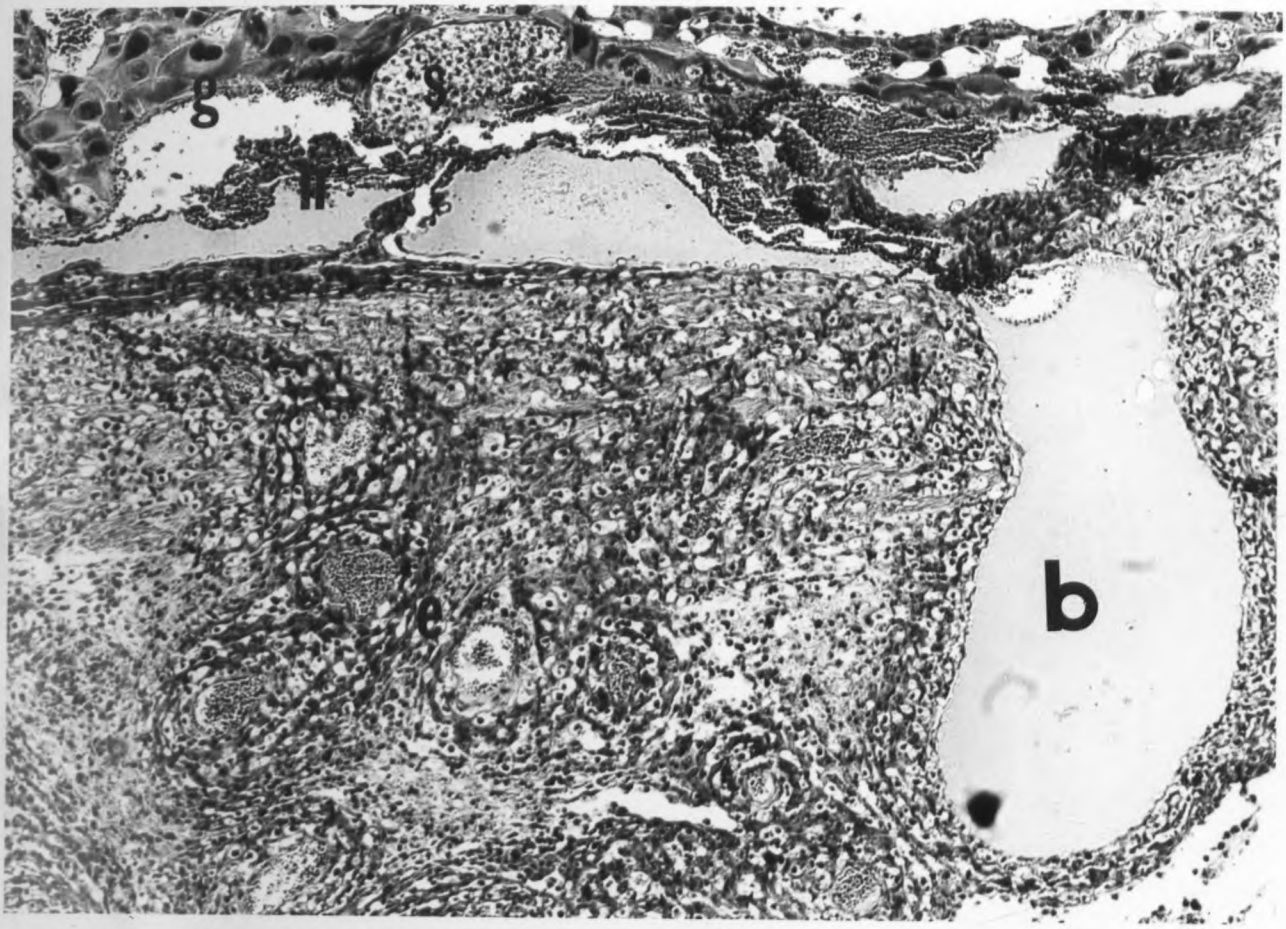
Placental changes in Group 1 rats (4 mg IgG-anti-PMSG) X200.

Fig. 3

Showing the congestion of blood vessles (b), scattered necrotic foci of the giant cells (g) and necrosis of the spongy zone in a Group IV rat.

Fig. 4

Showing the necrosis (n) and congestion of blood vessels (b) in spongy zone (s), in a Group IA rat.


g
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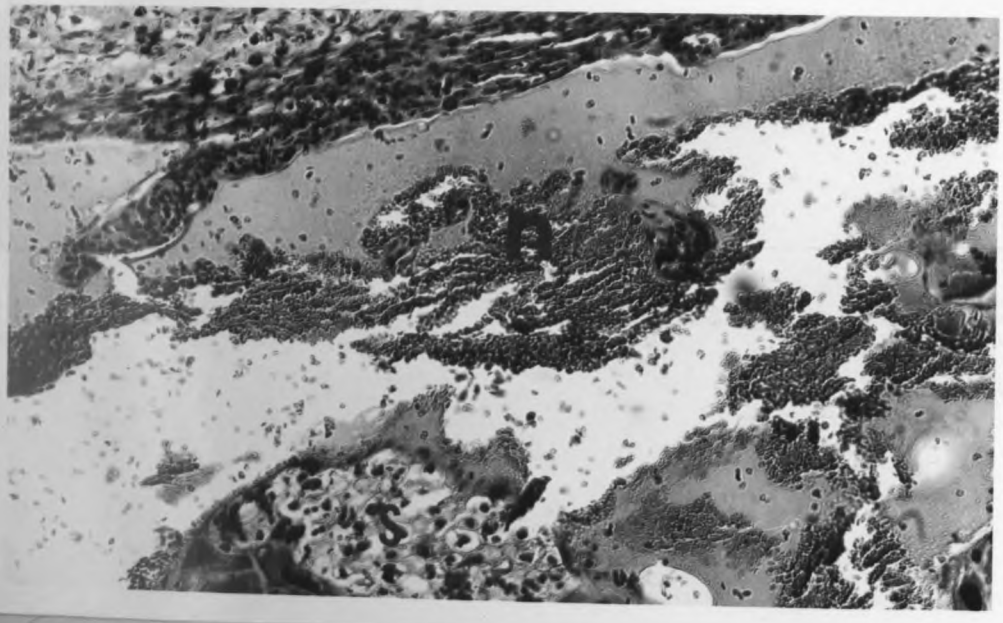

n
s

PLATE 3

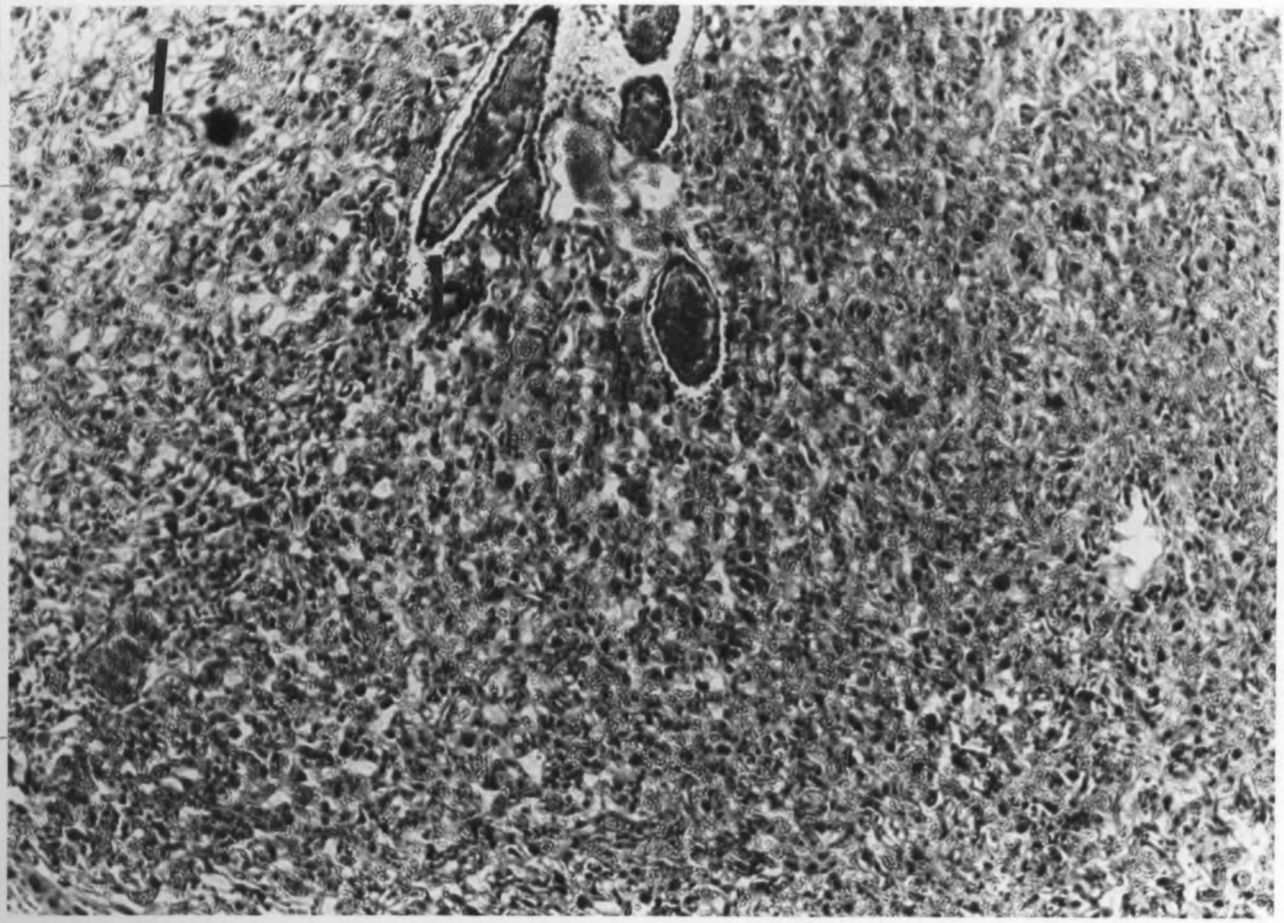
Congestion in Group 1 rat (4 mg IgG-anti-PMSG)

Fig. 5

Showing congestion of blood (b) in the labryinthine trophoblast (l) in a Group 1b rat X200

Fig. 6

Section of the spongy trophoblast ($\frac{S}{l}$) of the placenta showing necrosis and lymphocytic invasion (l) X300.


l
b

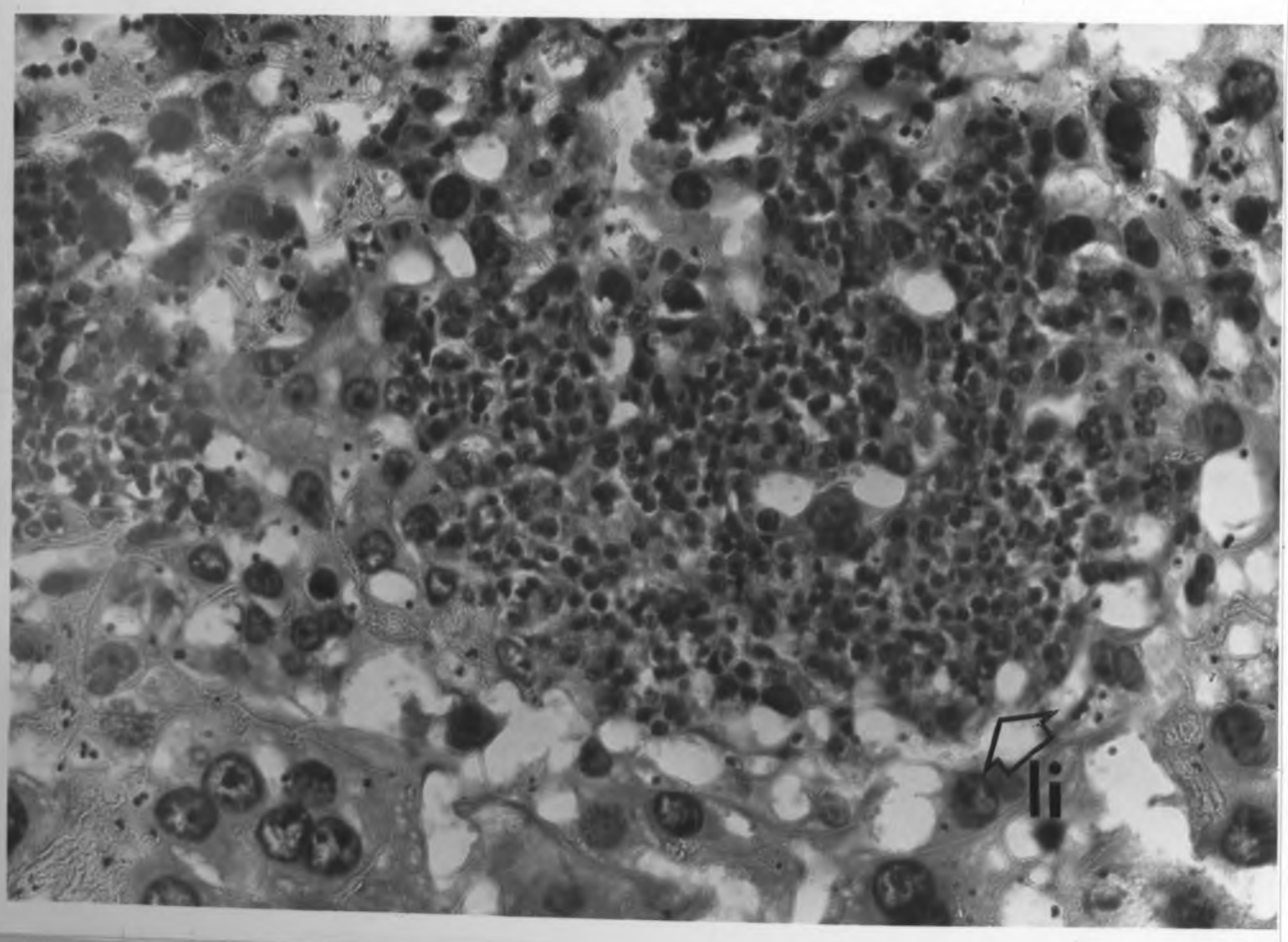

li

PLATE 4

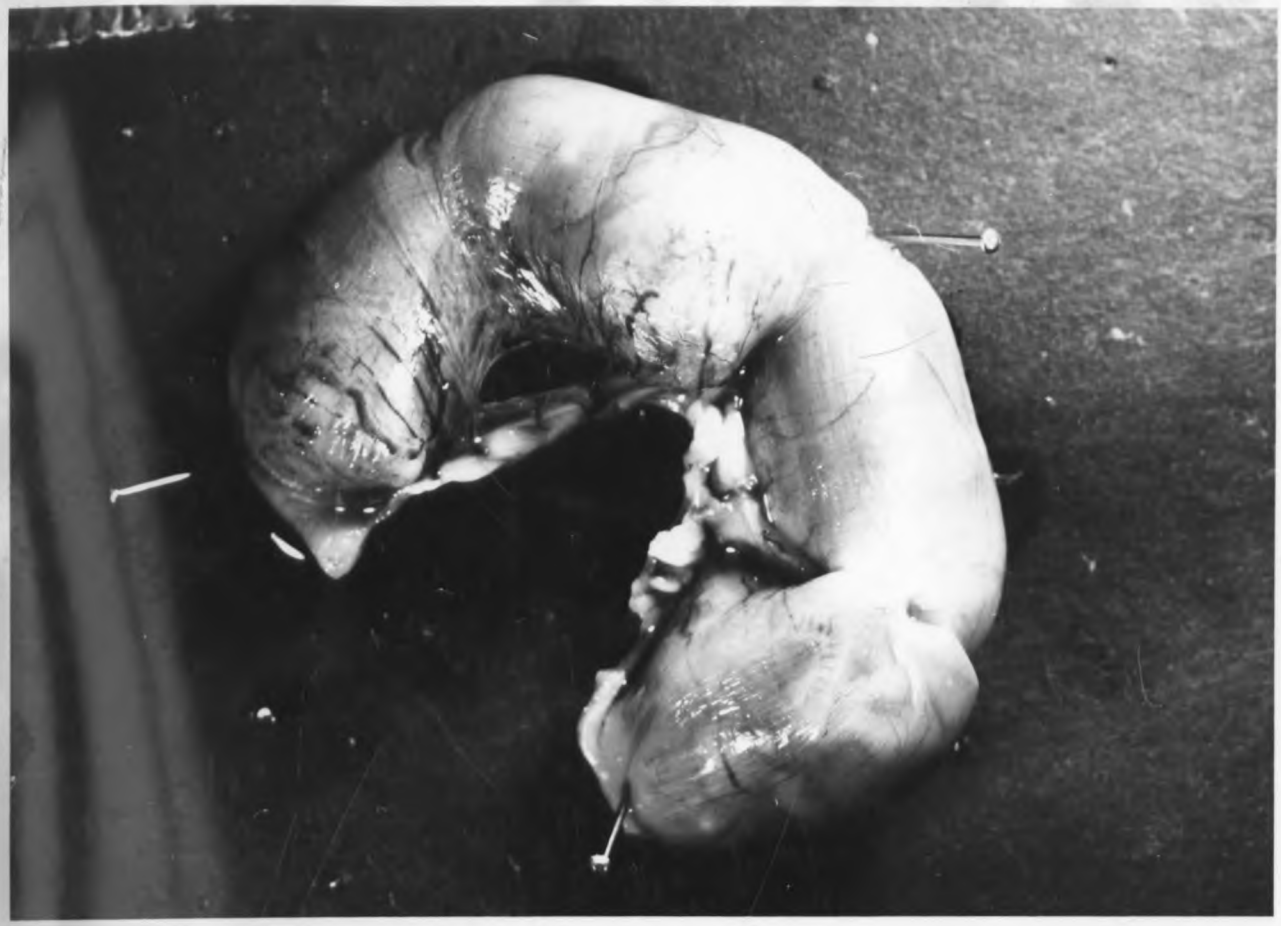
Gross photograph of Group IIIA (10 mg IgG-anti-hCG) rat conceptuses X3.

Fig. 7

Control rat conceptus (Group IV non-immune IgG treated) showing normal growth of the placenta at Day 16.

Fig. 8

Group IIIA rat conceptuses showing partial resorption.



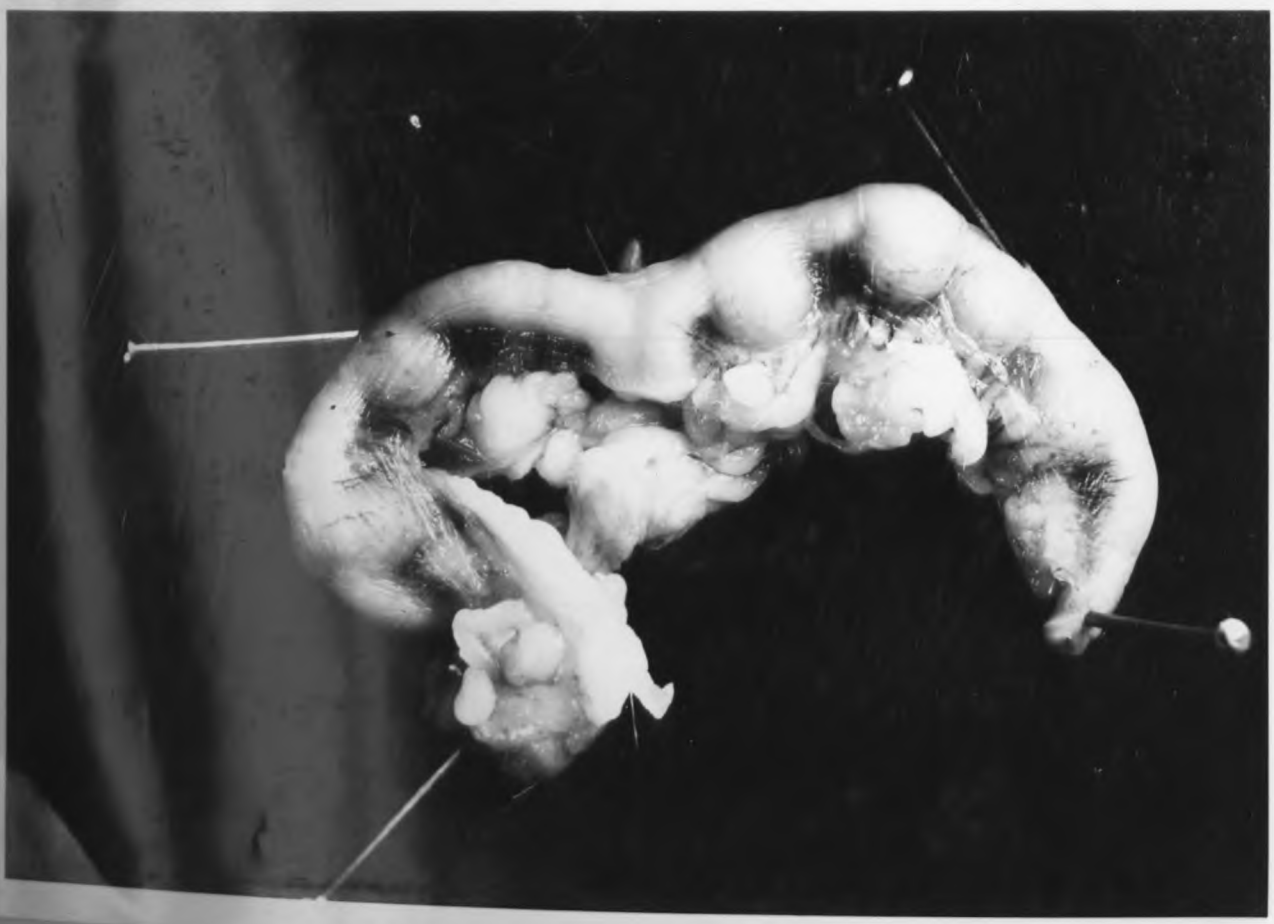


PLATE 5

Gross photographs of Group IIIA (10 mg IgG-anti-hCG) rat uteri X3.

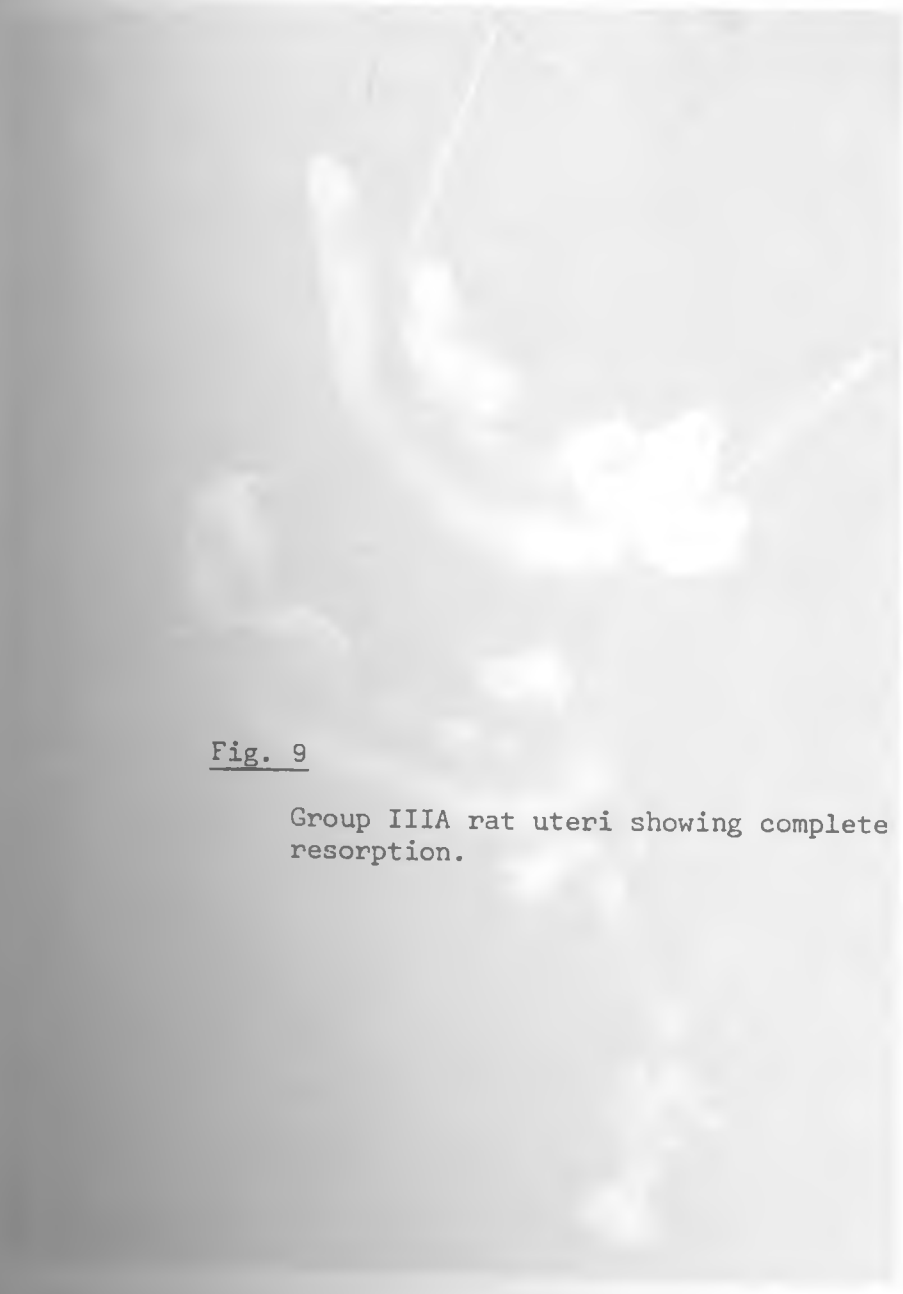


Fig. 9

Group IIIA rat uteri showing complete resorption.

PLATE 6

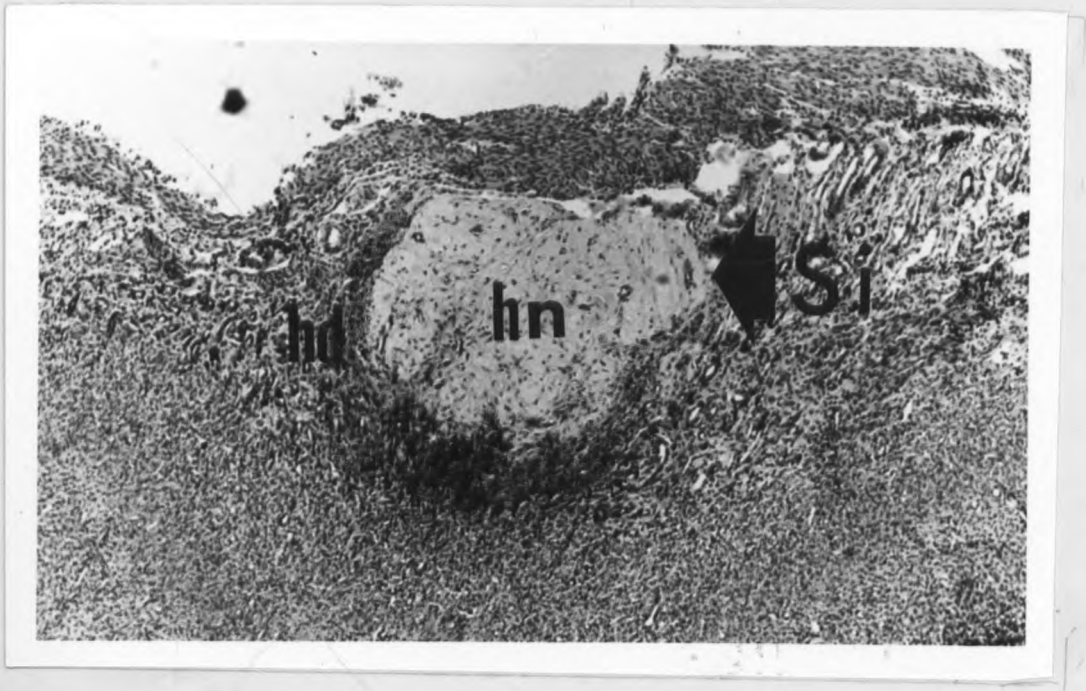
Photomicrograph of complete resorption in Group IIA (10 mg anti-PMSG-treated) rat.

Fig. 10

Section of the implantation site (si) of a rat from Group IIA showing the lack of fetal tissues, hyaline nodules (hn) and haemosiderin deposits (hd) X125.

Fig. 11

Same section as above at a higher magnification X200.


hd
hn
Si

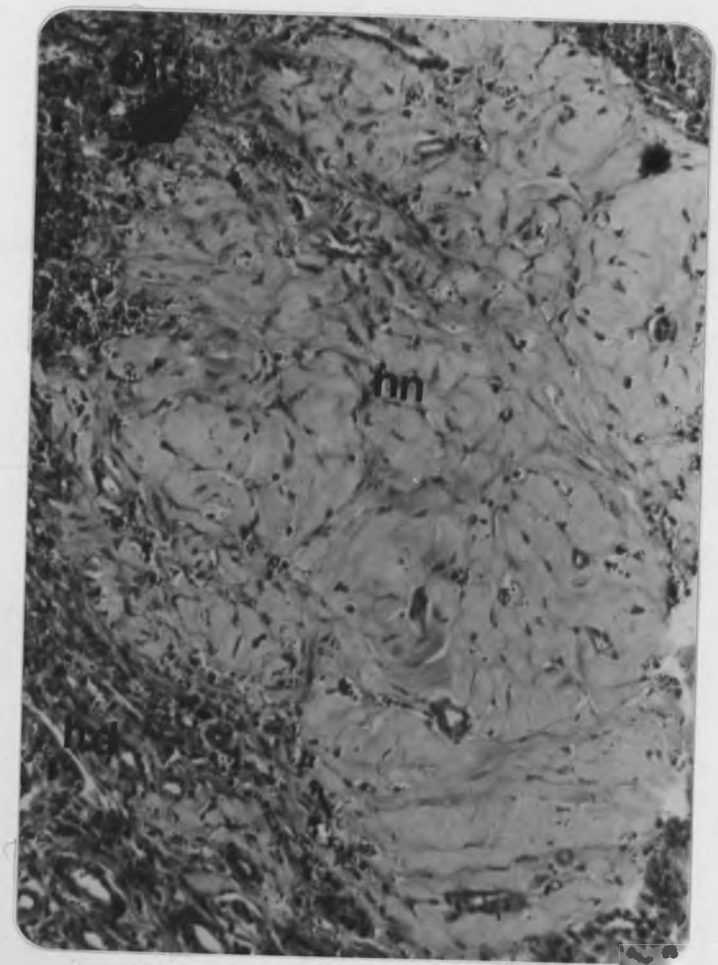

hn
hd

PLATE 7

Photomicrograph of partial resorption in Group IIIB (10 mg anti-nCG treated) rat X125.

Fig. 12

Section showing remains of the conceptus. Note the necrosis of the conceptus (c) but lack of cellular damage to the maternal tissue (e).

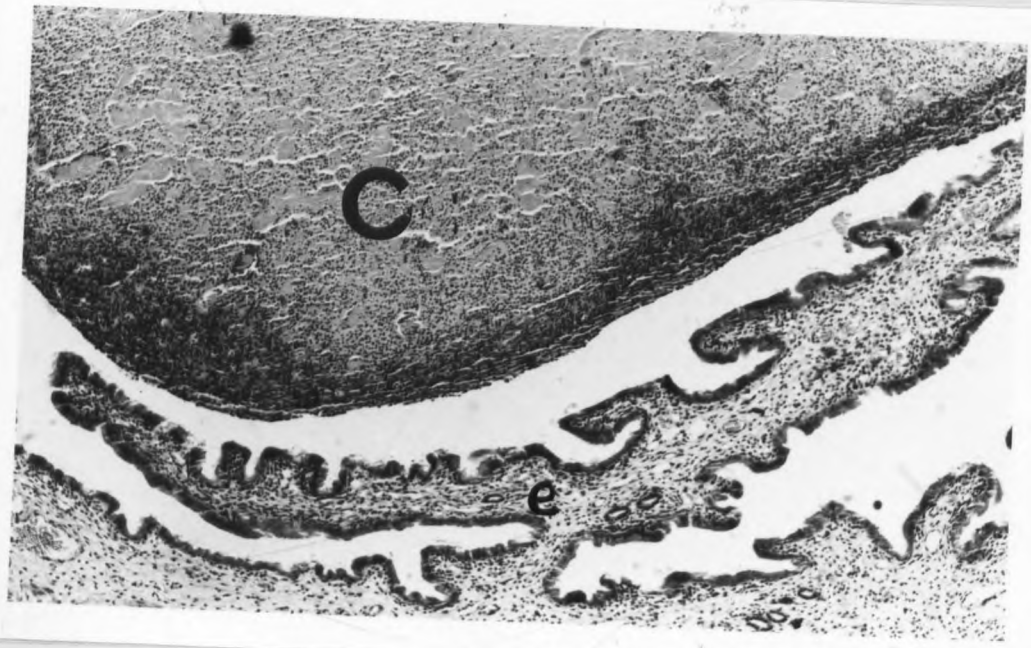


PLATE 8

Corpus luteum of Group IVA (non treated) rat.

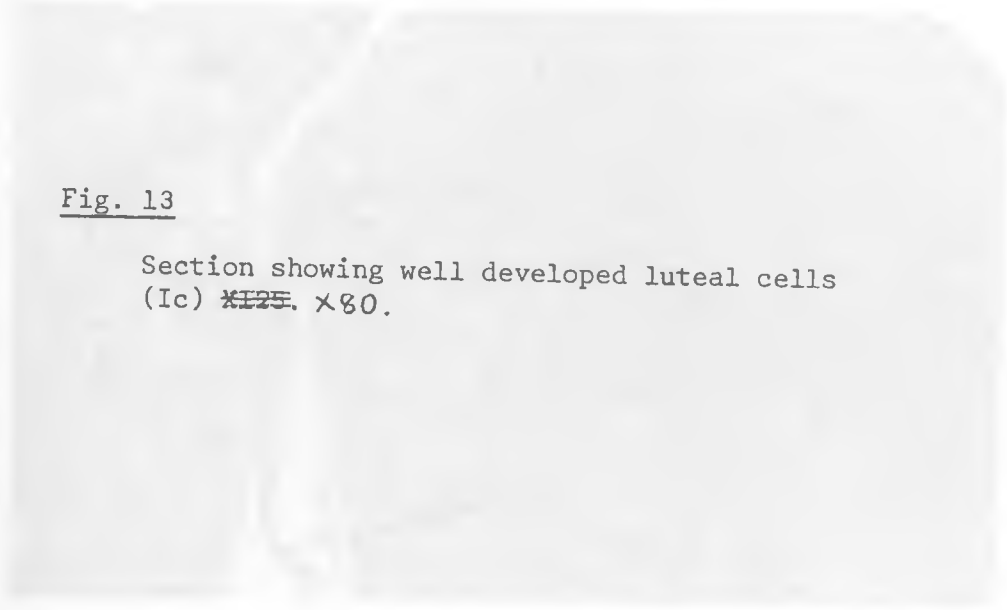


Fig. 13

Section showing well developed luteal cells (Ic) ~~XI25~~. ×80.

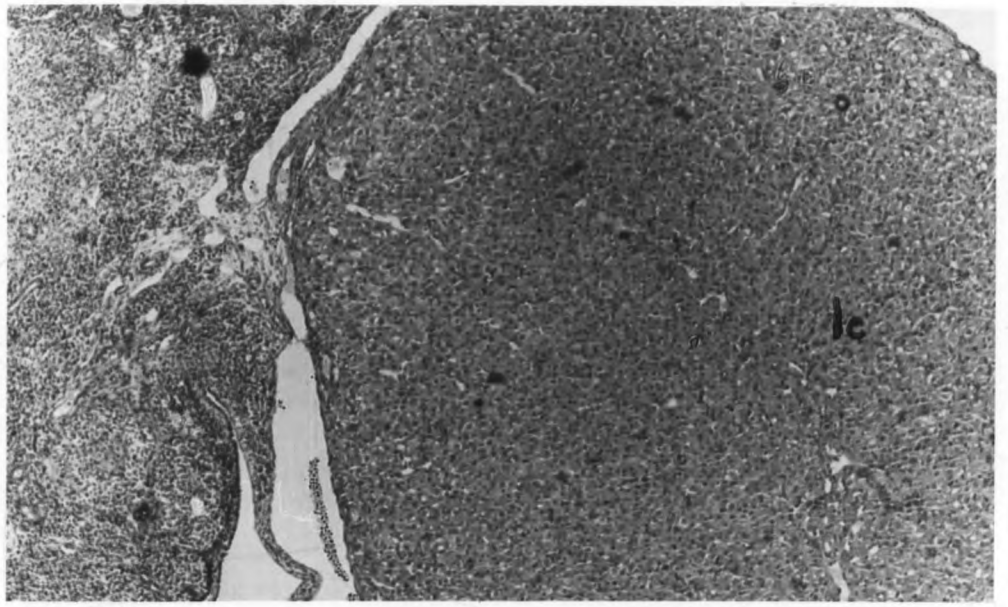

lc

PLATE 9

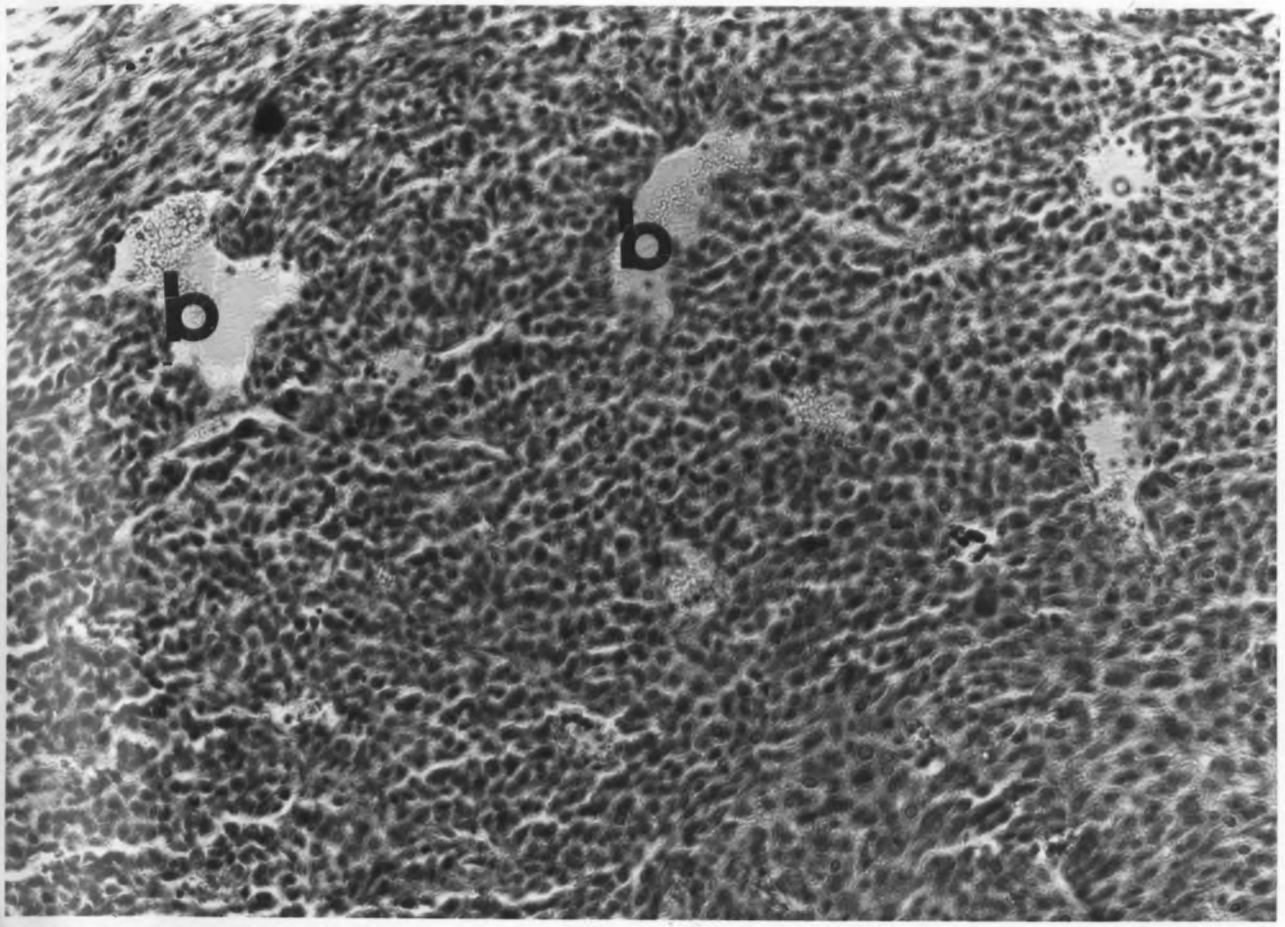
Photomicrograph of corpus luteum showing congestion.

Fig. 14

Section from Group 1A (4 mg IgG-anti PMSG treated) rat showing congestion (b) X125.

Fig. 15

Section from Group IIA (10 mg IgG-anti-PMSG treated) rat showing massive congestion (b) and luteal cells (Ic). X200


b
b

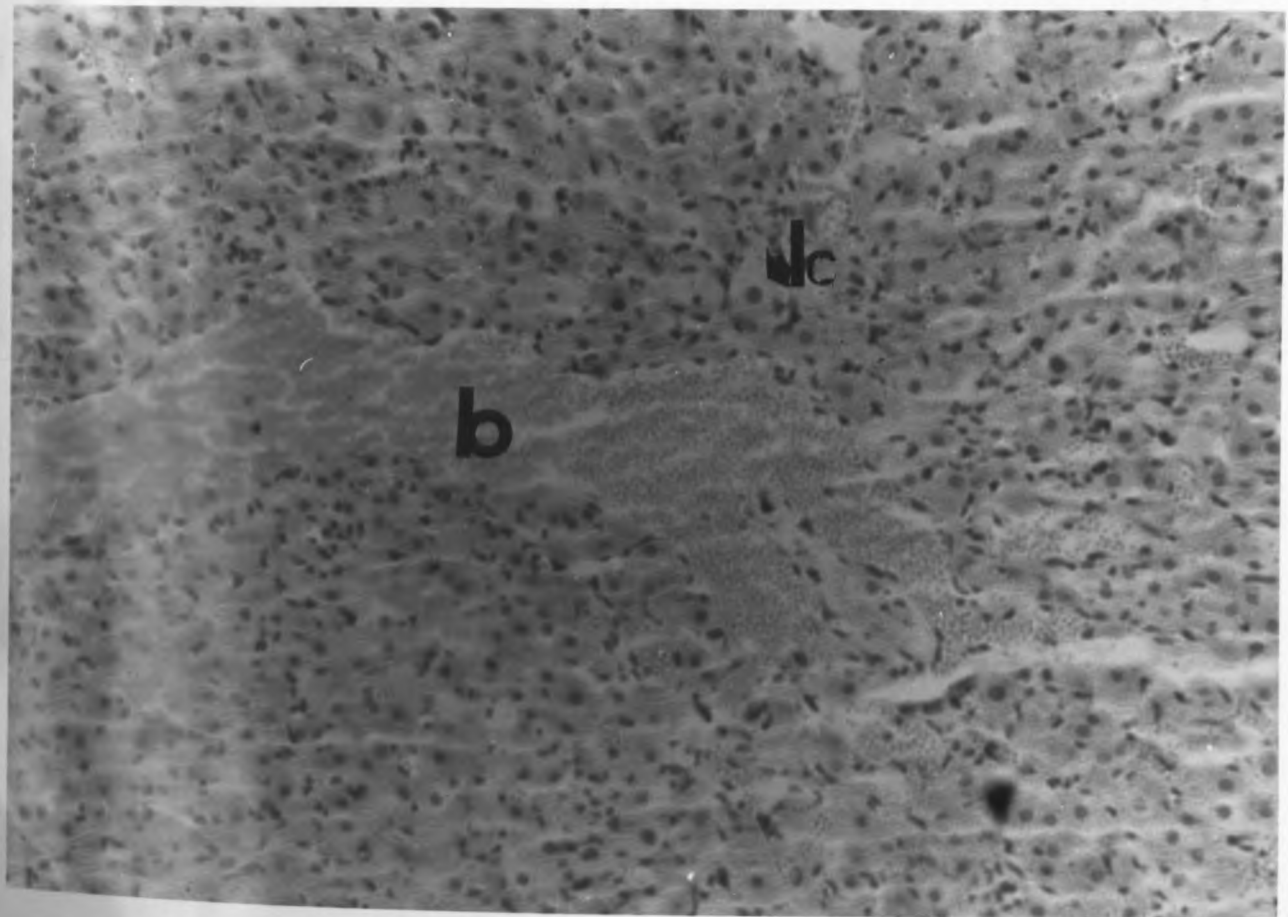

c
b

PLATE 10

Central necrosis of the corpus luteum.

Fig. 16

Photomicrograph of Group IIIA (10 mg IgG-anti-hCG treated) rat corpus luteum, showing the central necrosis (Cn) and luteal cells (Ic) X200.

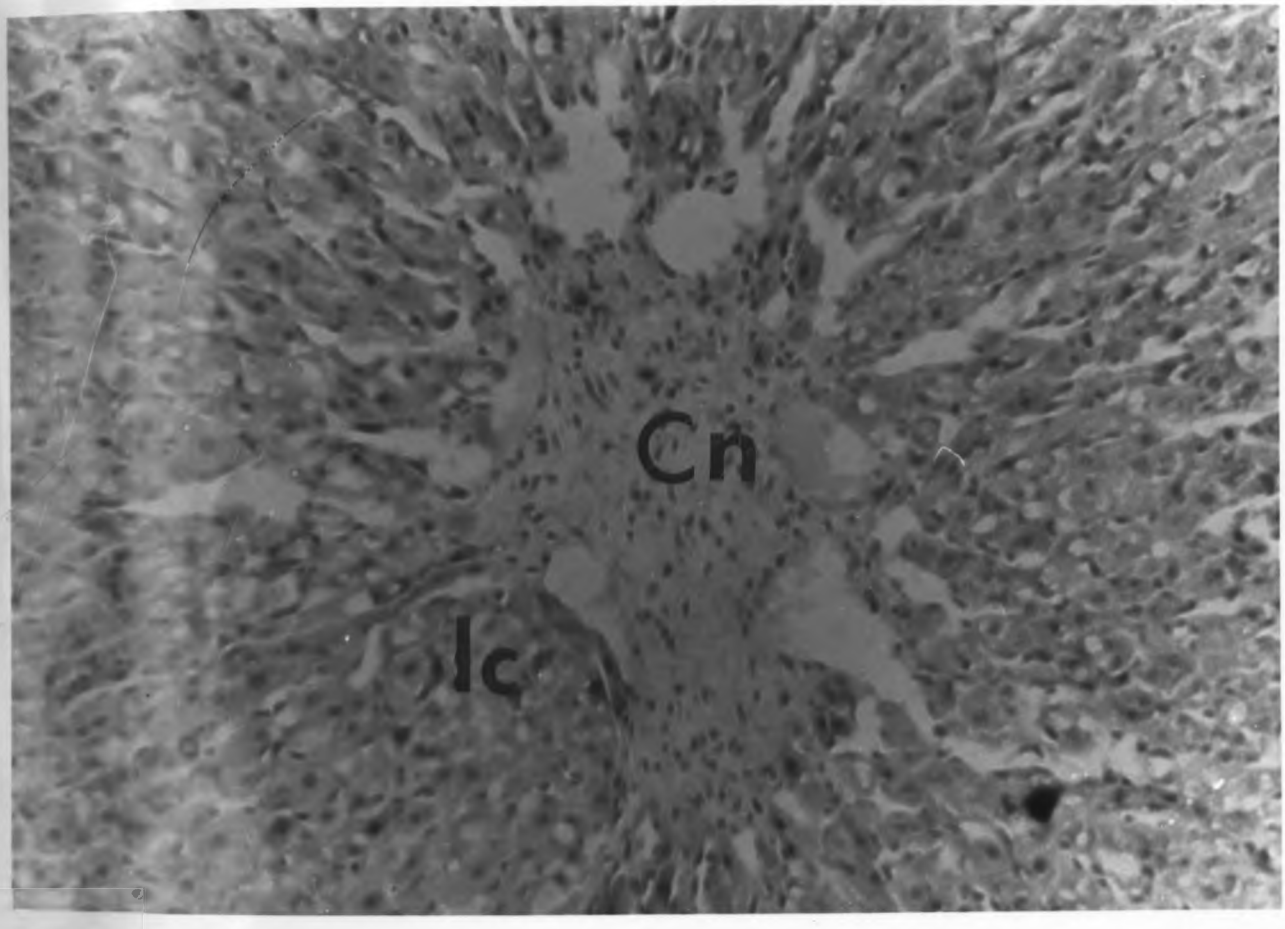

Cn
Ic

PLATE 11

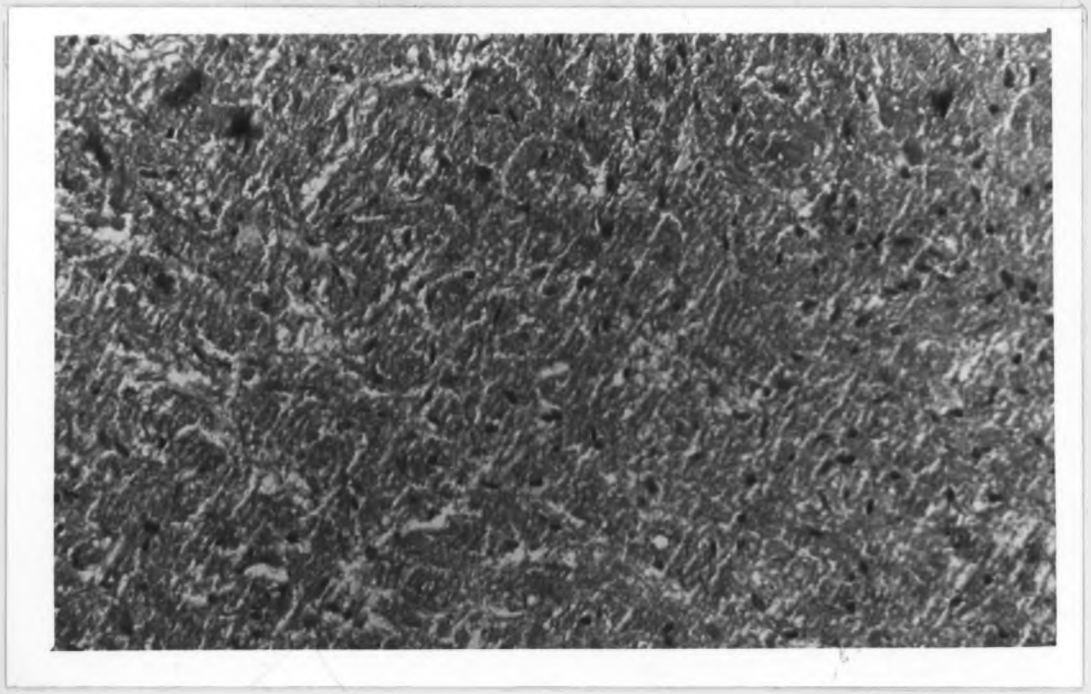
Photomicrographs of the pituitary gland of Group IVA (non-treated) rat. X500.

Fig. 17

Section showing the neurohypophysis.

Fig. 18

Section showing the gonadotroph containing region of the adenohypophysis.



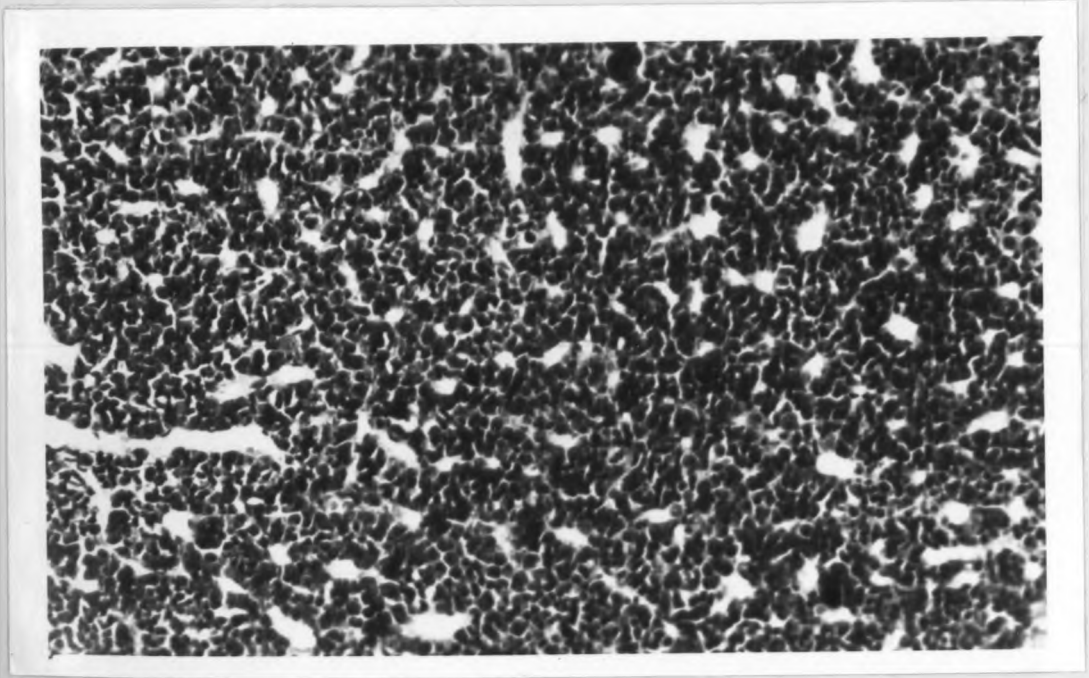


PLATE 12

Photomicrograph of the pituitary gland of Group IIIA (10 mg anti-hCG treated) rat. X200

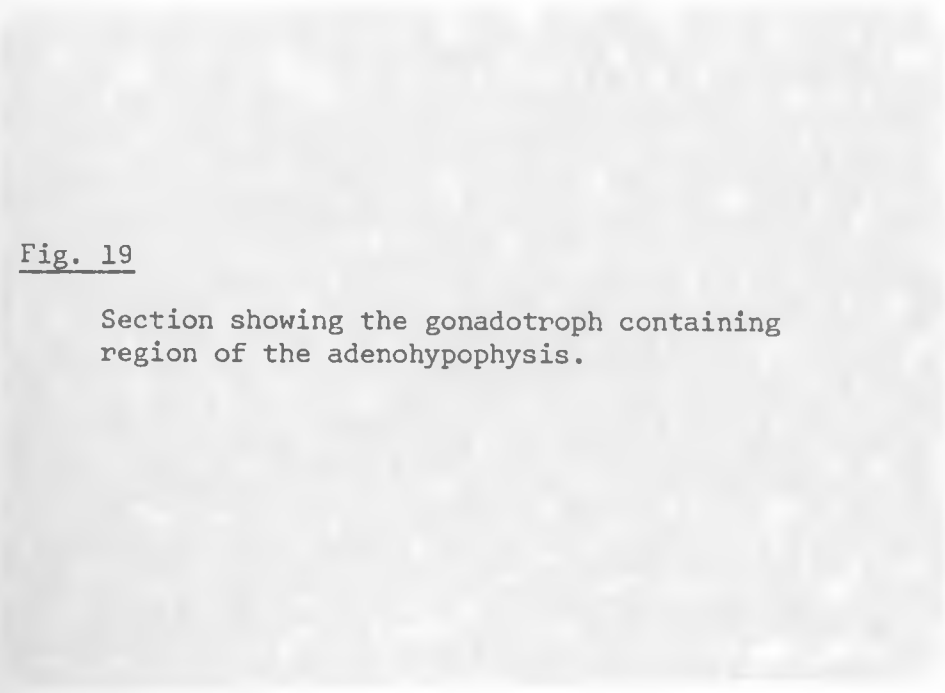


Fig. 19

Section showing the gonadotroph containing region of the adenohypophysis.

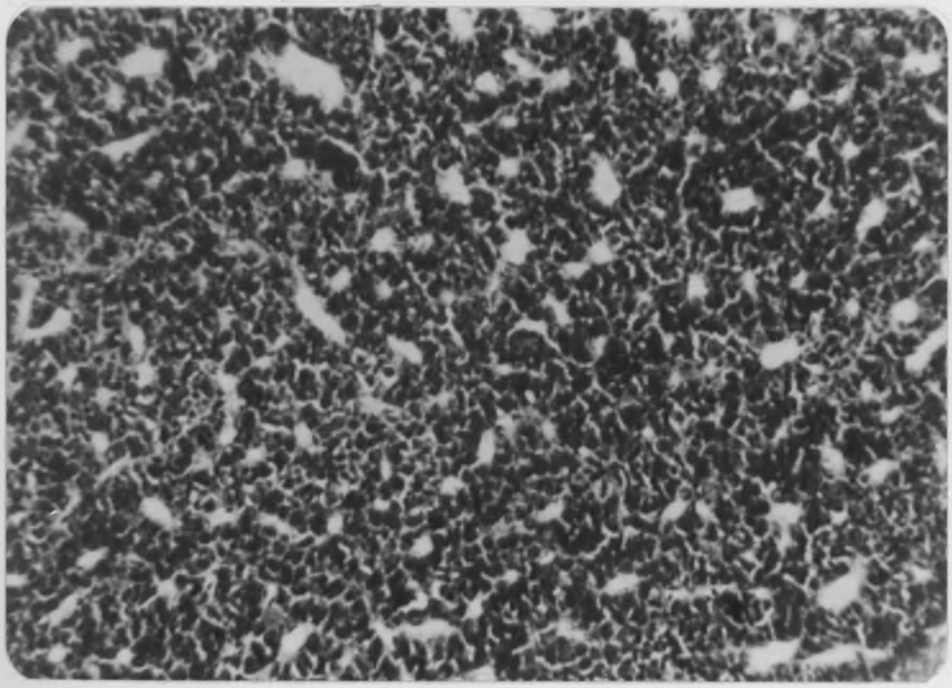


PLATE 13

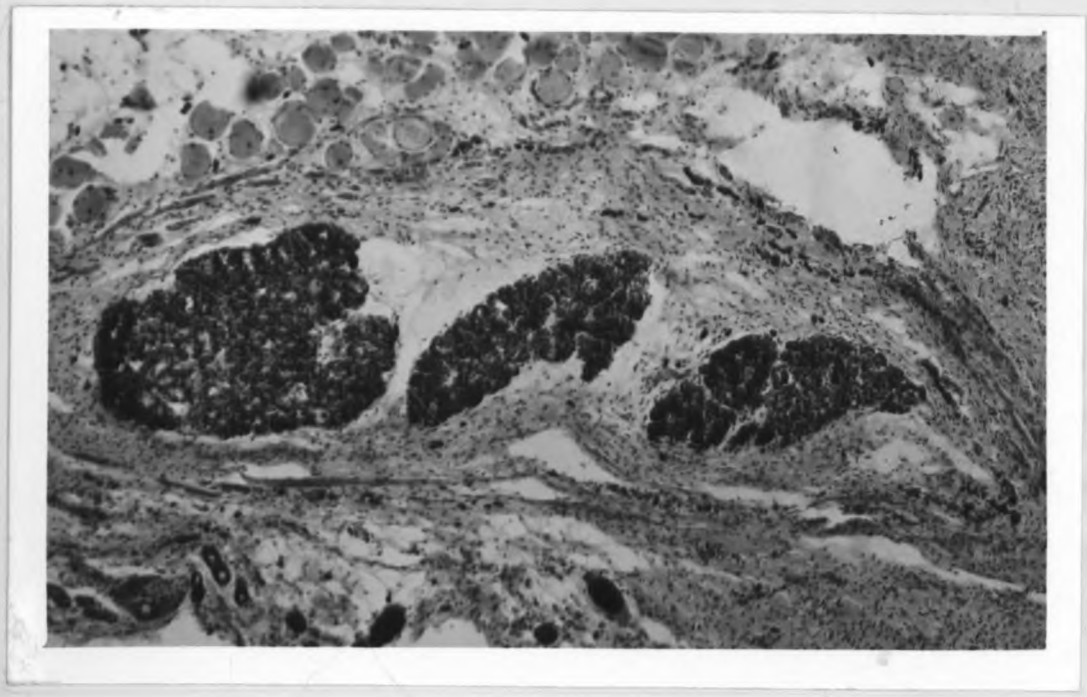
Photomicrograph of the mammary tissue of Group IVA (non-treated) rat.

Fig. 20

Photomicrograph of mammary tissue of a Group IVA rat. X200

Fig. 21

Same section as above, but at a higher magnification. X500



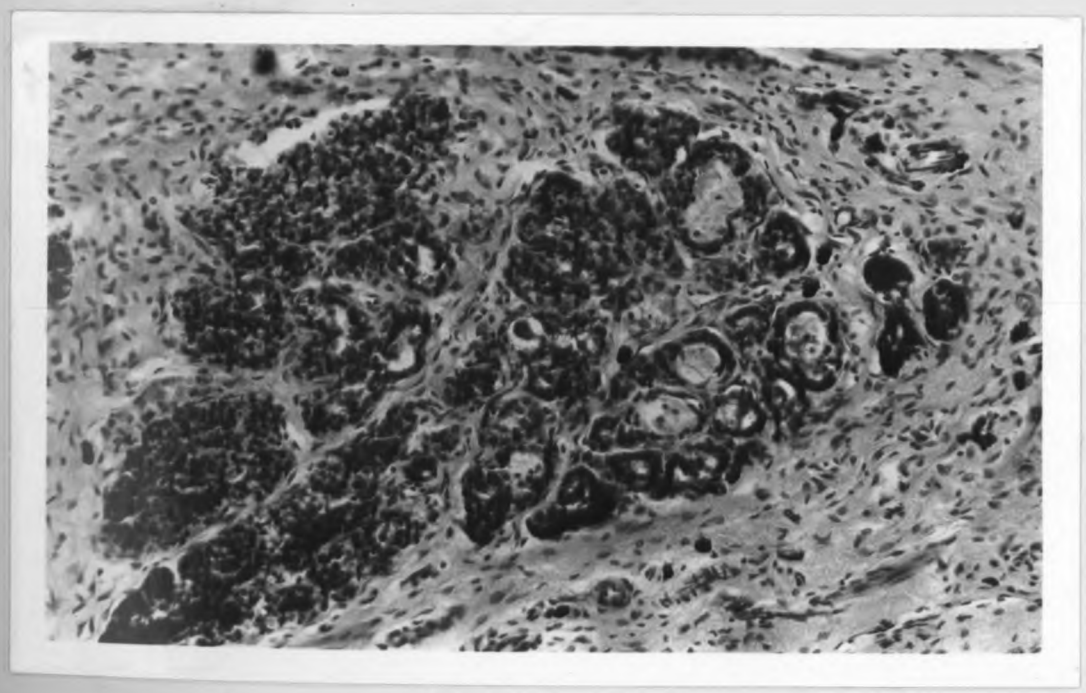


PLATE 14

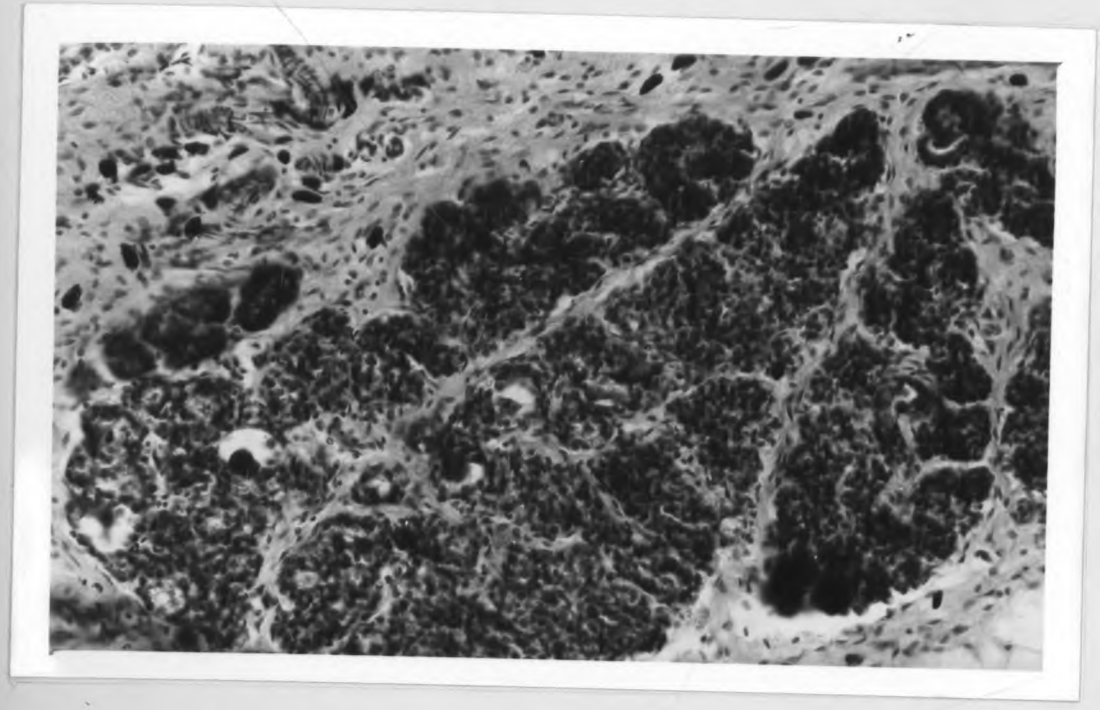
Photomicrographs of the antibody treated rats mammary tissue X500.

Fig. 22

Section of a mammary gland of Group IIA (10 mg IgG-anti-PMSG treated) rat.

Fig. 23

Section of a mammary gland of Group IIIA (10 mg IgG-anti-hCG treated) rat.



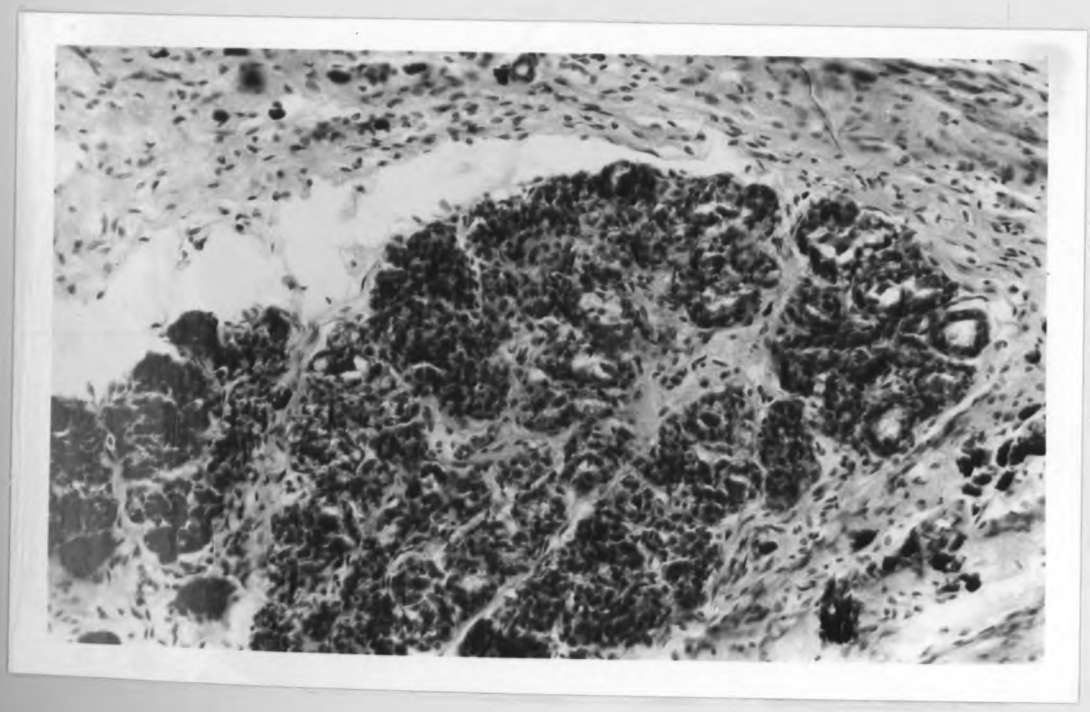


PLATE 15

Photomicrographs of Group III (10 mg non-immune IgG-treated) hyrax placenta. X200.

Fig. 24

Section of the hyrax placenta showing spongy zone (s), the junctional zone (j), maternal endometrium (e) and the maternal myometrium (m).

Fig. 25

Section of the hyrax placenta showing the spongy (s) and palisade (p) zones.

m
e
J
S

P
S

PLATE 16

Photomicrographs of Group III (10 mg non-immune IgG treated) hyrax placenta. X200.

Fig. 26

Section of the hyrax placenta showing the spongy and palisade zones

Fig. 27

Section of the hyrax placenta showing the palisade zone (p) and the foetal tissues (f).

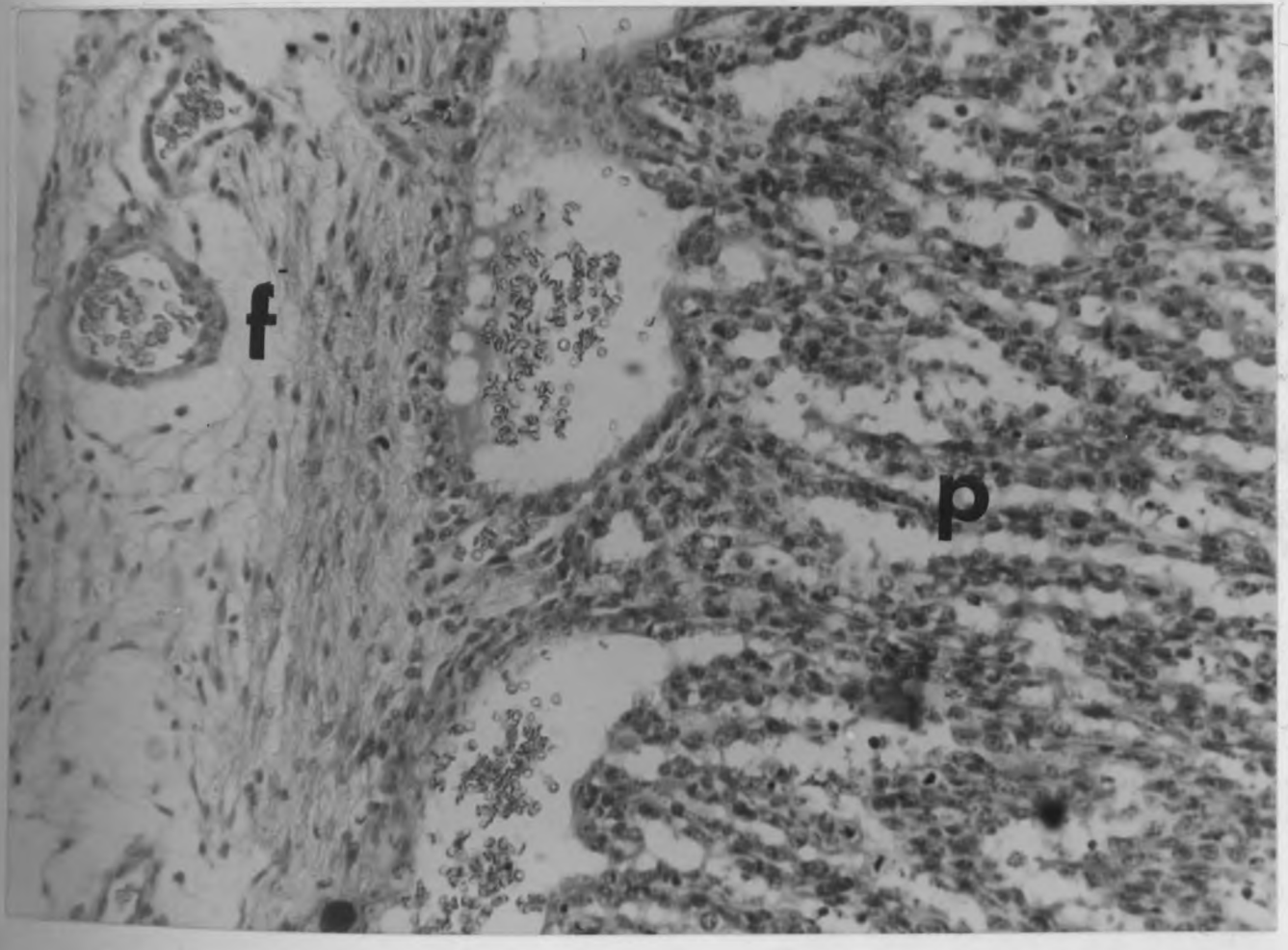

f
p

PLATE 17

Photomicrograph of Group III (10 mg non-immune IgG-treated) hyrax placenta. X500.

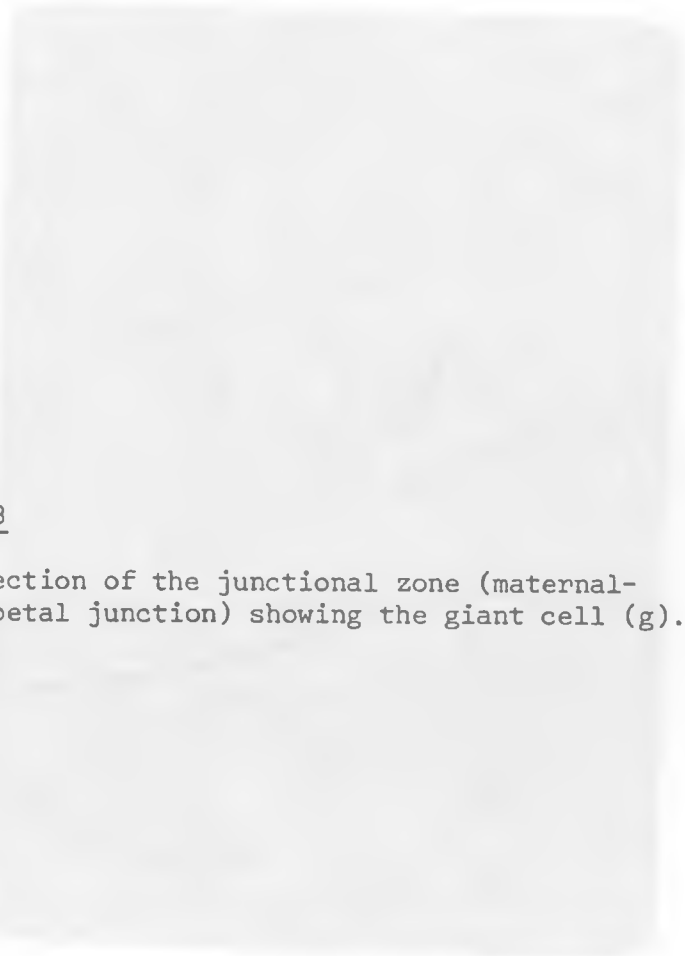


Fig. 28

Section of the junctional zone (maternal-foetal junction) showing the giant cell (g).

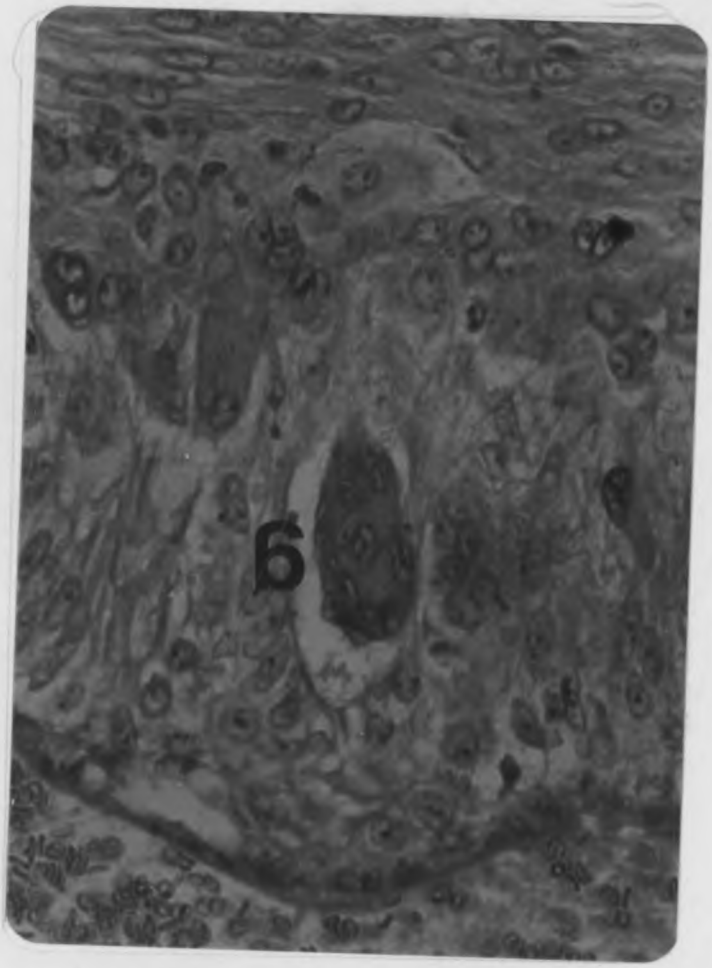

б

PLATE 18

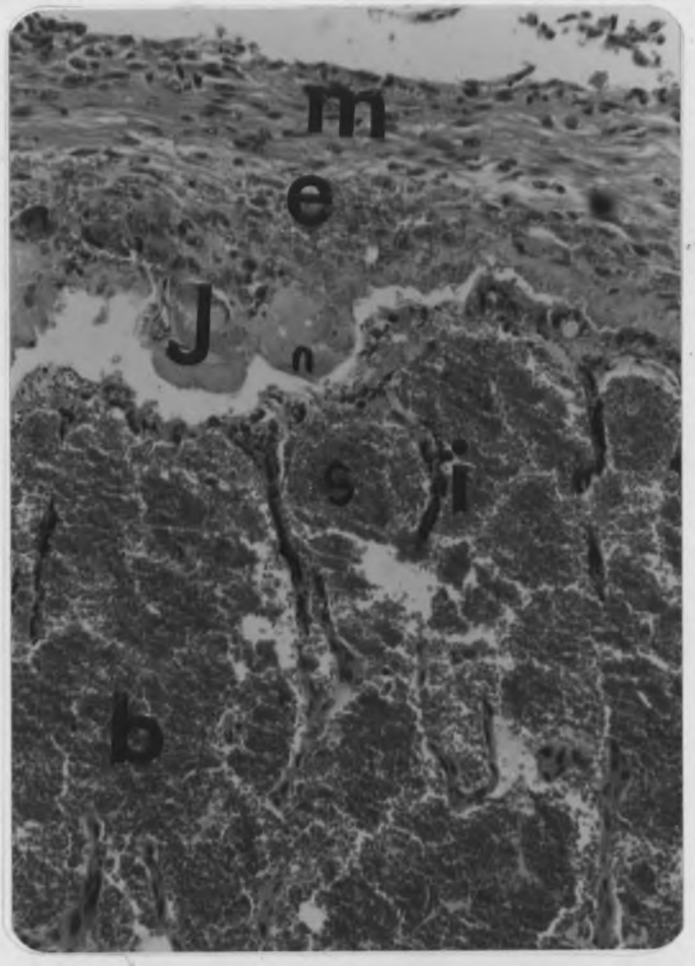
Photomicrographs of Group I (10 mg IgG anti-hCG treated) hyrax placenta. X200

Fig. 29

Section of Day 1 hyrax placenta showing necrosis (n) and conjestion (b) of the spongy (s) and junctional (j) zones. Note that the junctional zone is represented by a hyaline mass, the deposition of iron (i), and the lack of damage to maternal endometrium (e) and myometrium (m).

Fig. 30

Section of Day 2 hyrax placenta showing the necrosis (n) of the junctional and spongy (s) zones.


m
e
J
n
s
i
b

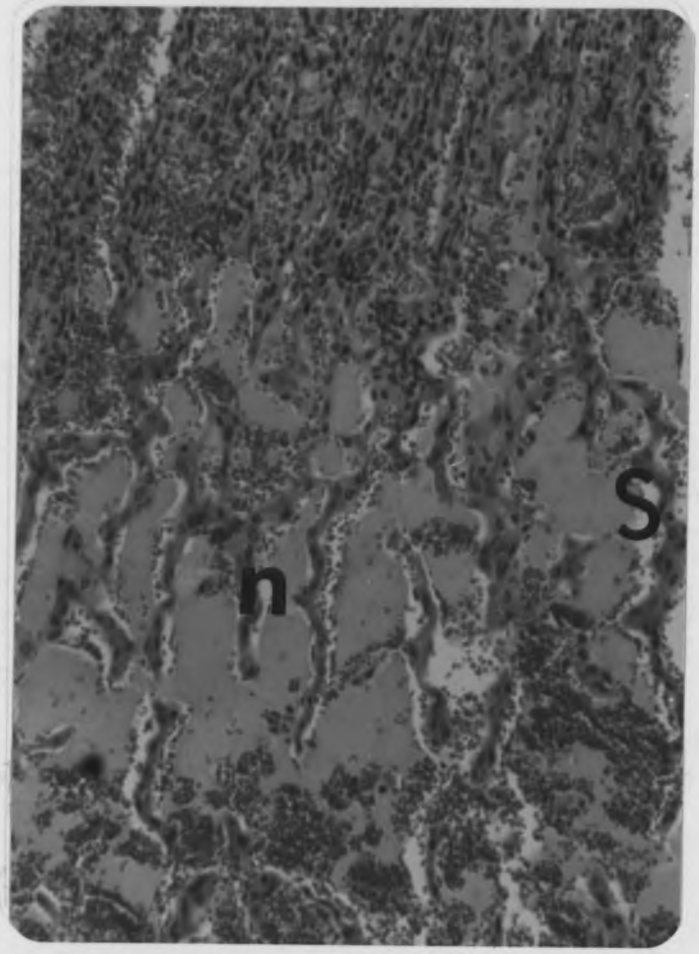

S
n

PLATE 19

Photomicrograph of Group 1 (10 mg IgG-anti-hCG treated) hyrax placenta.

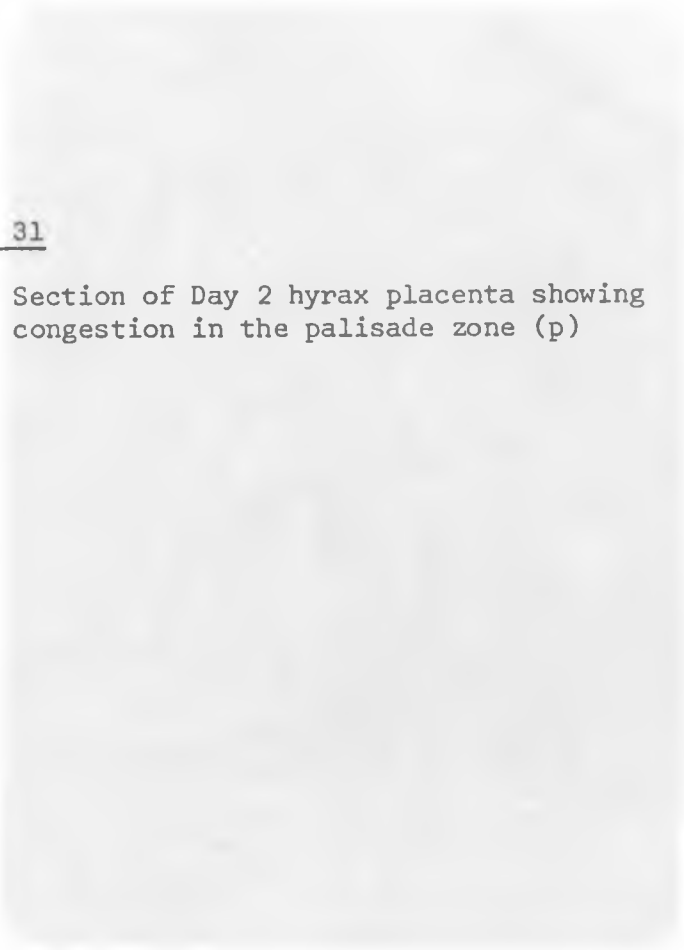


Fig. 31

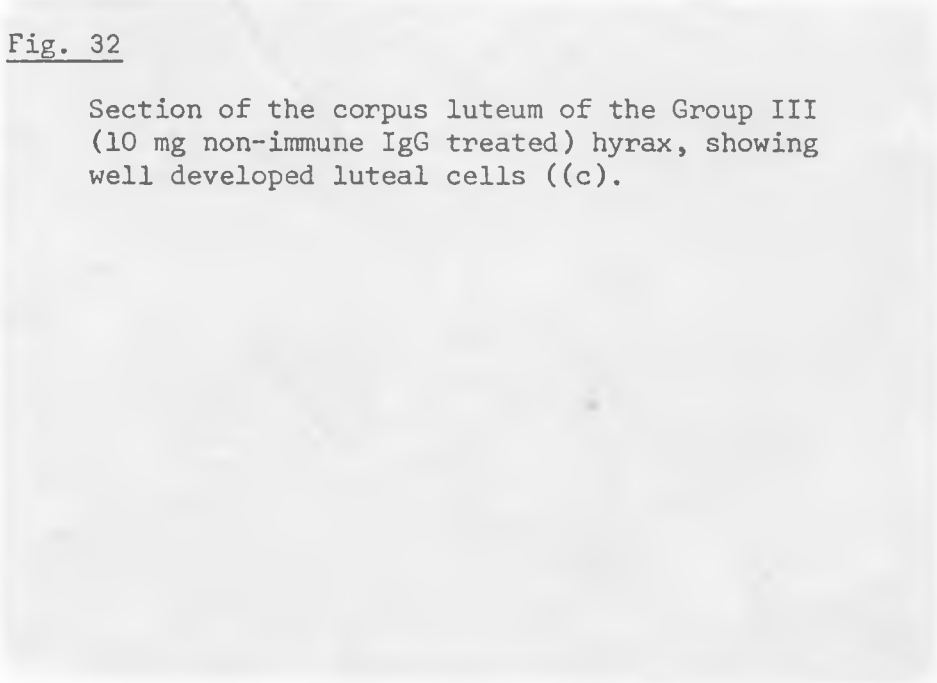
Section of Day 2 hyrax placenta showing congestion in the palisade zone (p)


P

PLATE 20

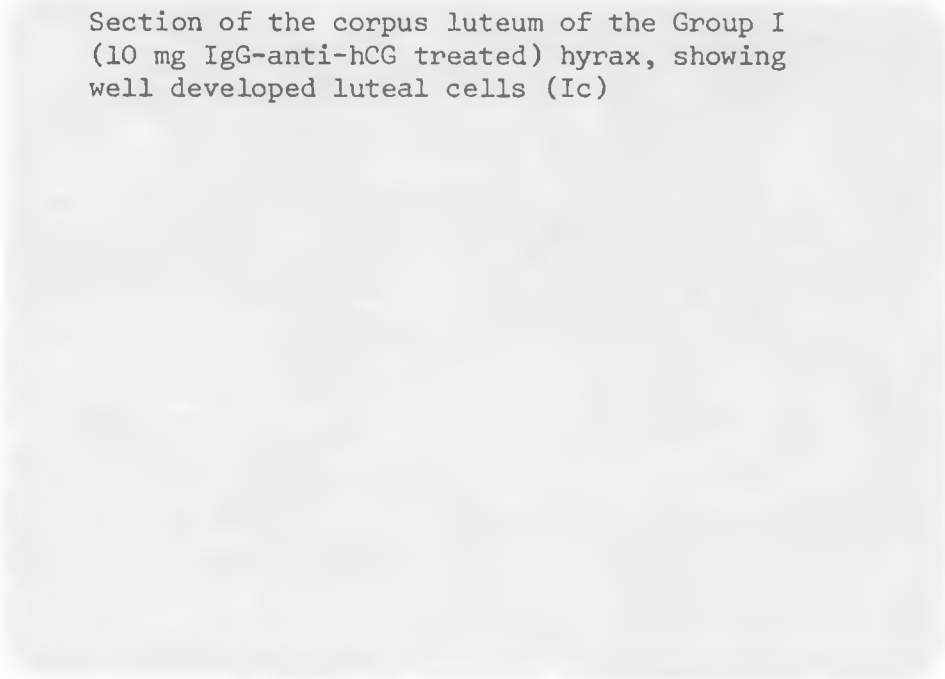
Corpora lutea of Day 3 hyraxes. X1250

Fig. 32

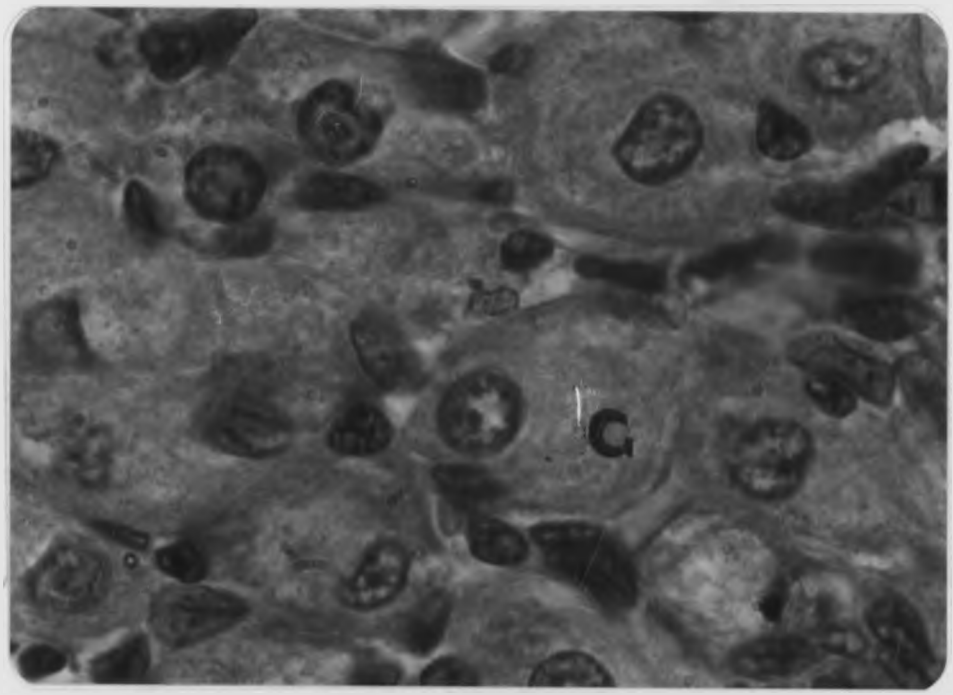


Section of the corpus luteum of the Group III (10 mg non-immune IgG treated) hyrax, showing well developed luteal cells ((c).

Fig. 33



Section of the corpus luteum of the Group I (10 mg IgG-anti-hCG treated) hyrax, showing well developed luteal cells (Ic)


G

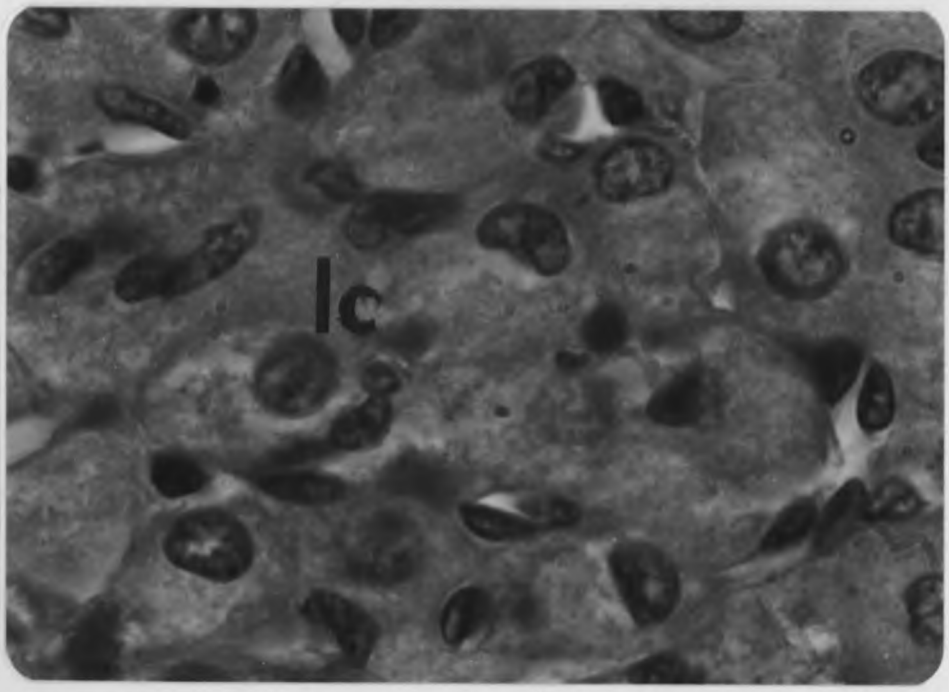

lc

PLATE 21

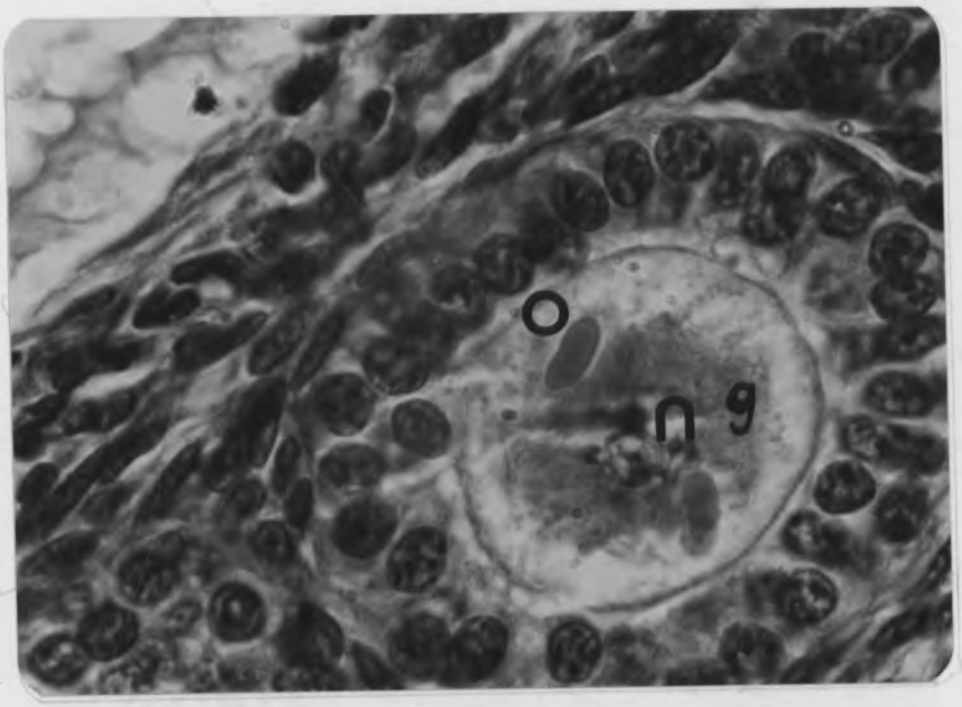
Destruction of the ovarian follicles in Day 2 (10 mg IgG-anti-hCG-treated) hyrax. X1250.

Fig. 34

Destruction of primary follicles. Note the appearance of pink granules (g) with disruption of the nucleus (n), and the final form of vacuole containing pink staining strongly eosinophilic ovoid particle (o).

Fig. 35

Same ovary as above, but a different area.


o
n
g

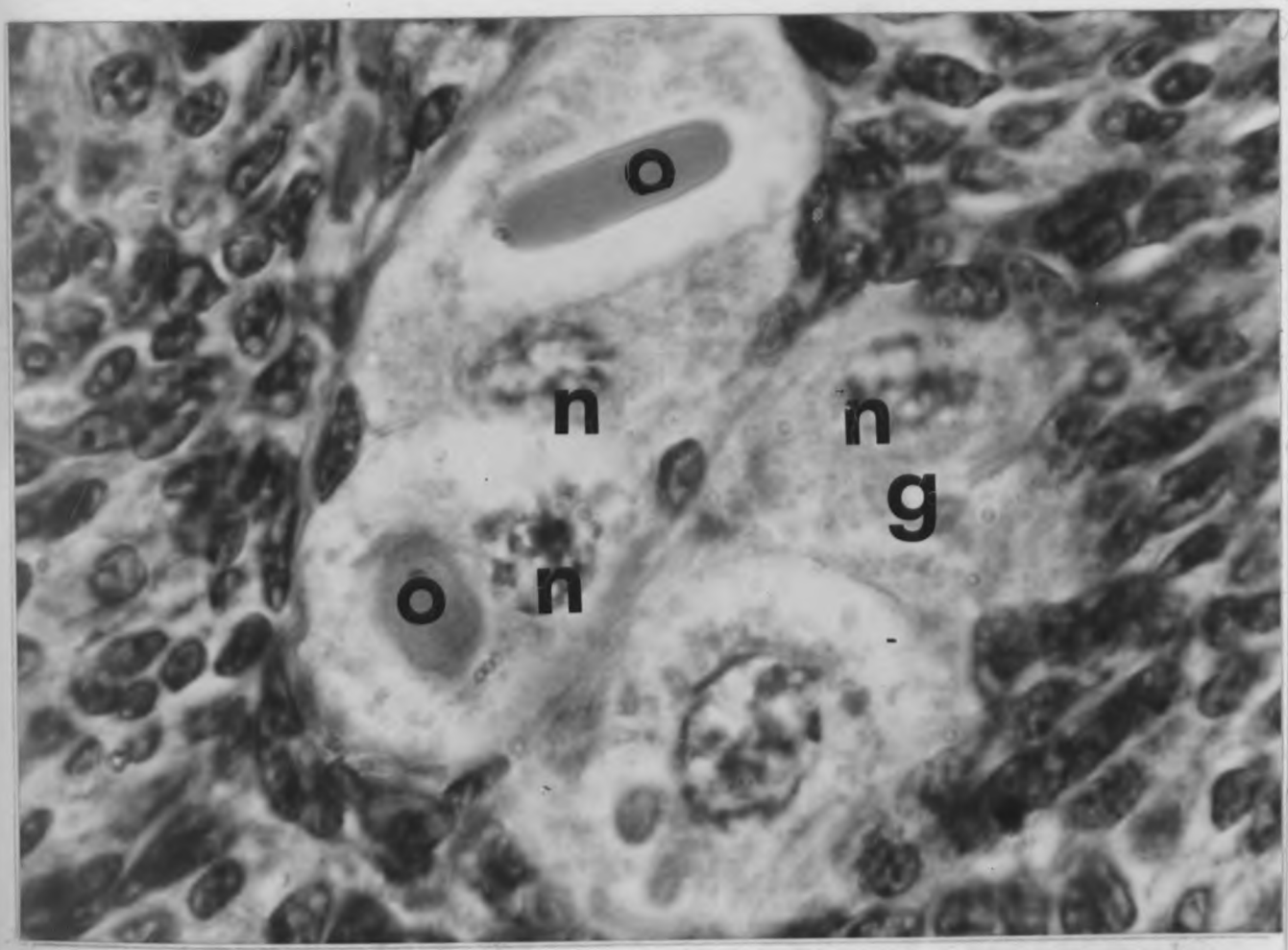

o
n
n
g
o
n

PLATE 22

Tertiary follicle in the ovary of the Group I hyrax (10 mg IgG-anti-hCG treated). X1250.

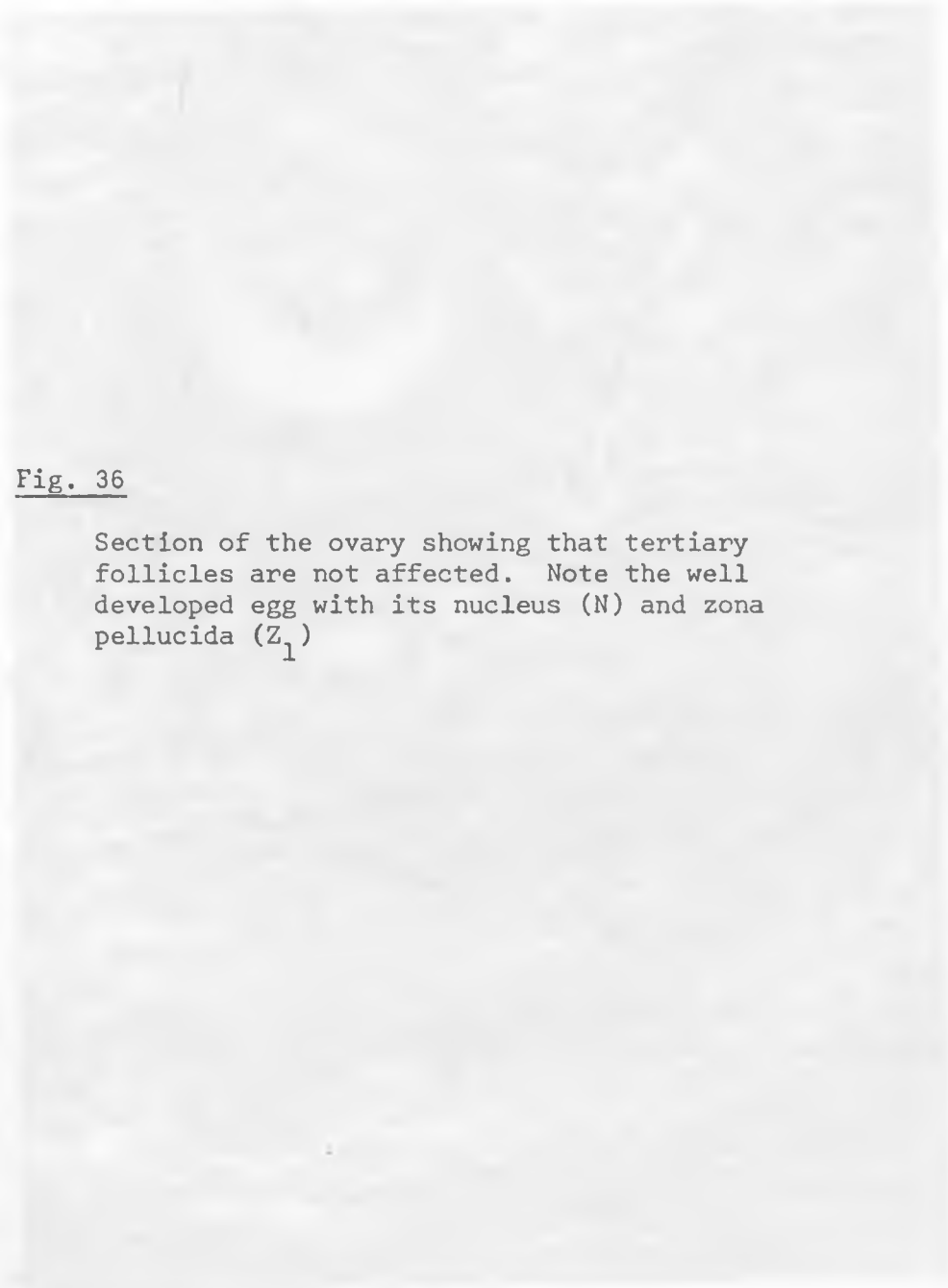


Fig. 36

Section of the ovary showing that tertiary follicles are not affected. Note the well developed egg with its nucleus (N) and zona pellucida (Z_1)

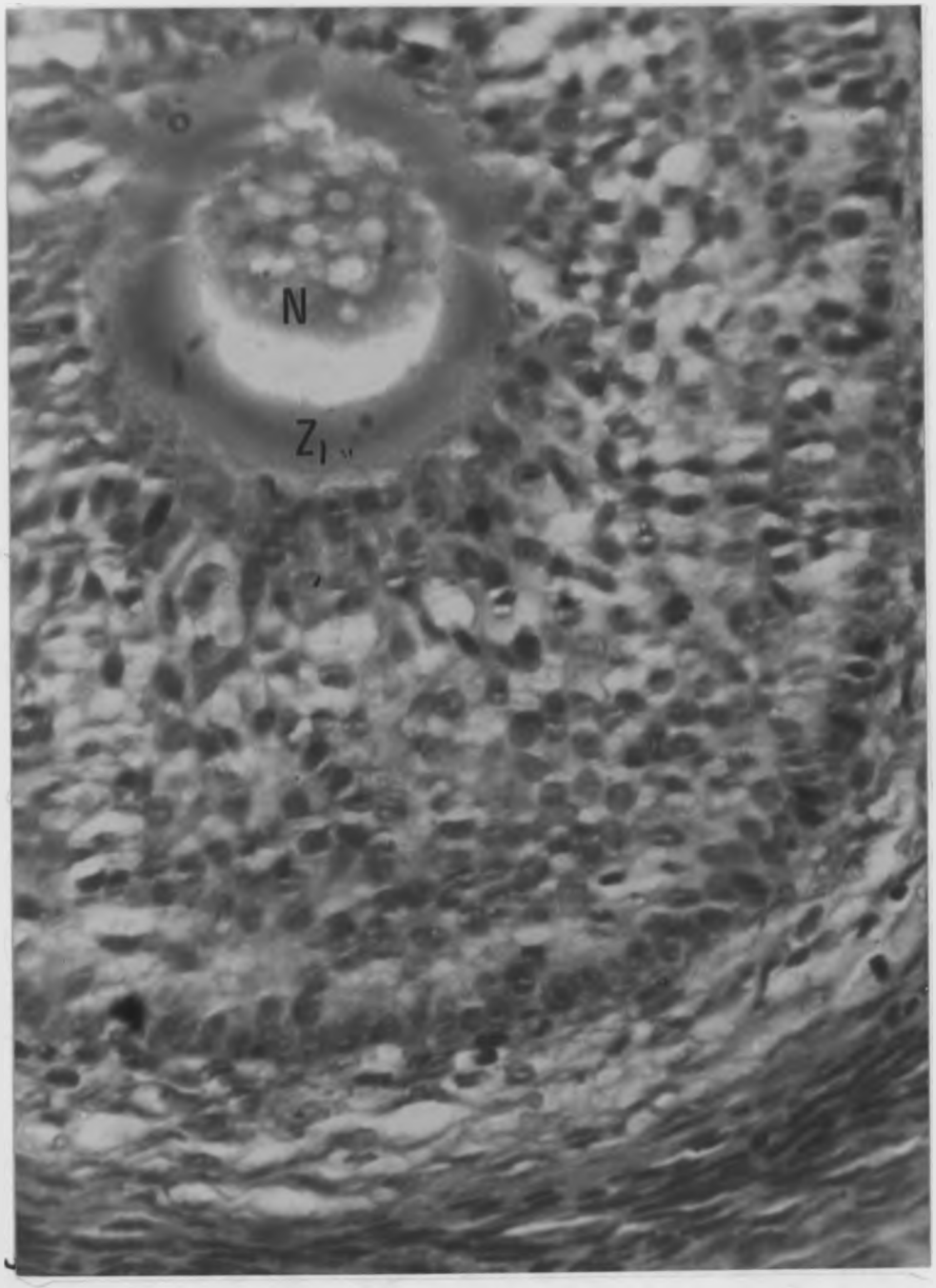

N
Z_1

PLATE 23

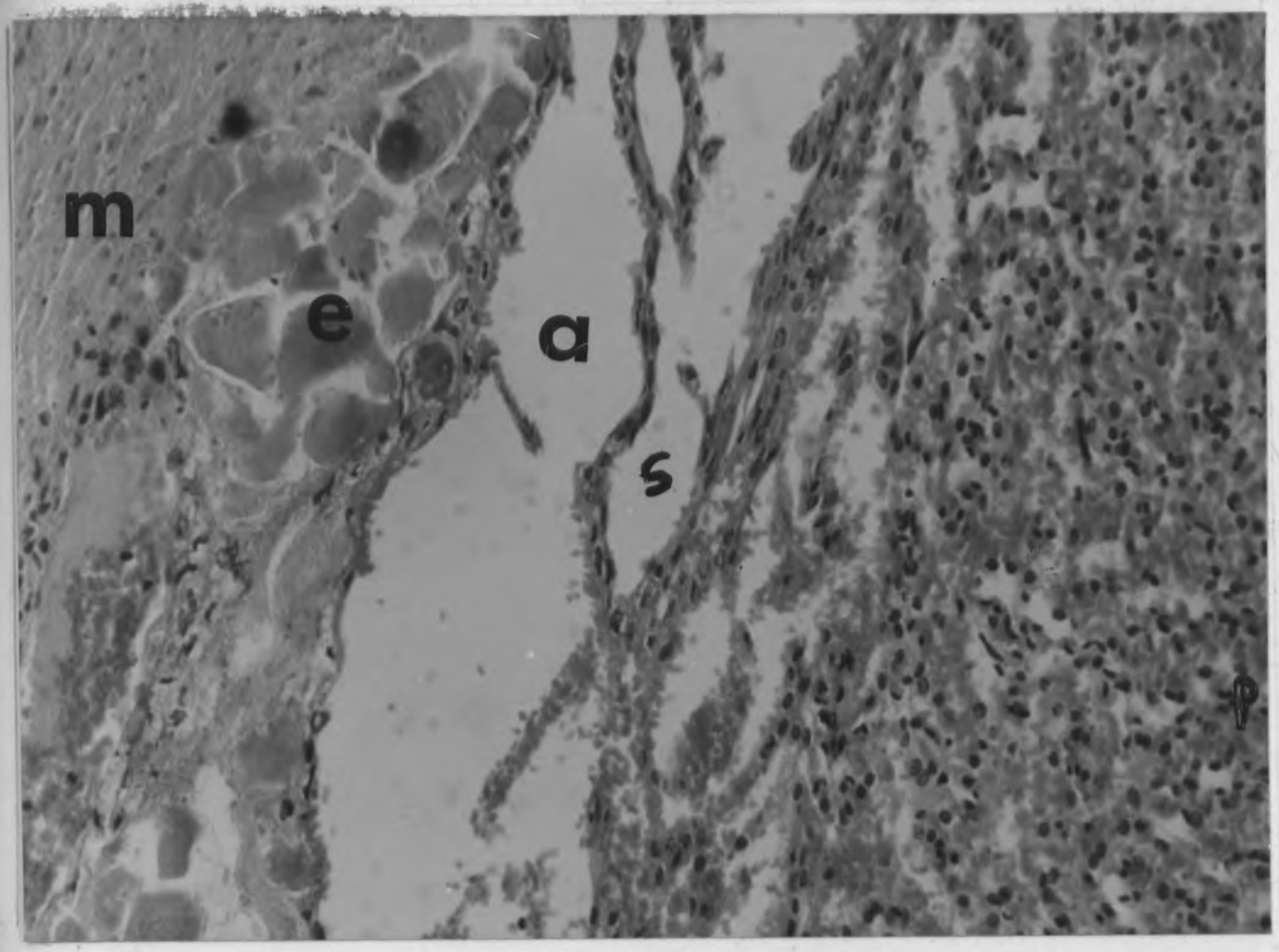
Photomicrograph of Day 3, Group 1 (10 mg IgG-anti-hCG-treated) hyrax placenta.

Fig. 37

Section showing the columnar cell monolayer (junctional zone) represented by a hyalene structure, with the separation (a) of the spongy zone from the maternal endometrium (e). Note the massive destruction of the spongy zone (s) and palisade zone (p) and the intact maternal myometrium (m). X200.

Fig. 38

Section showing palisade zone under oil immersion. Note presence of lymphocyte (L) and neutrophil (N) in foetal blood vessel and the hyalinization of the cells of this zone. X1250.


m
e
a
s
p

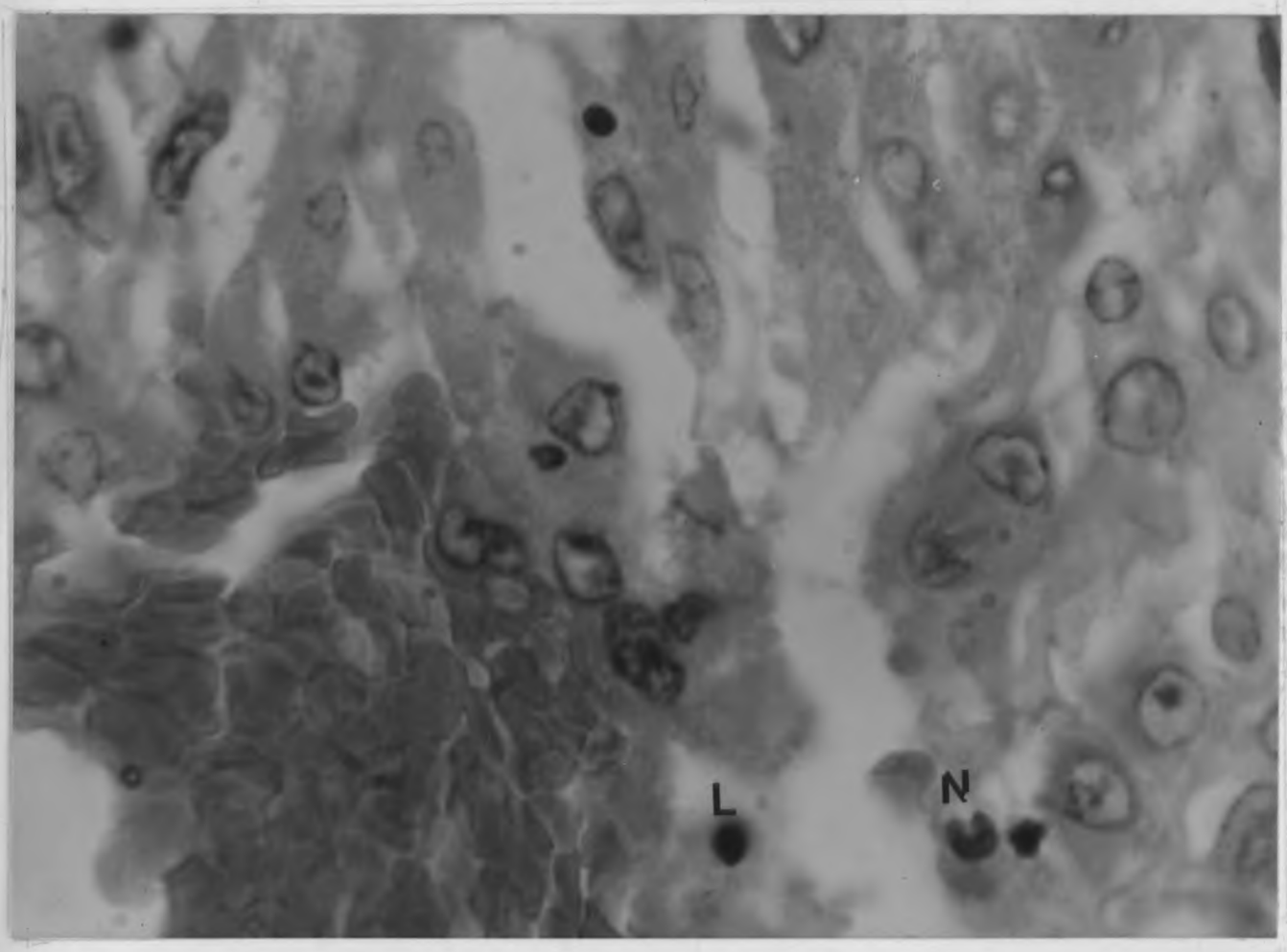

L
N

PLATE 24

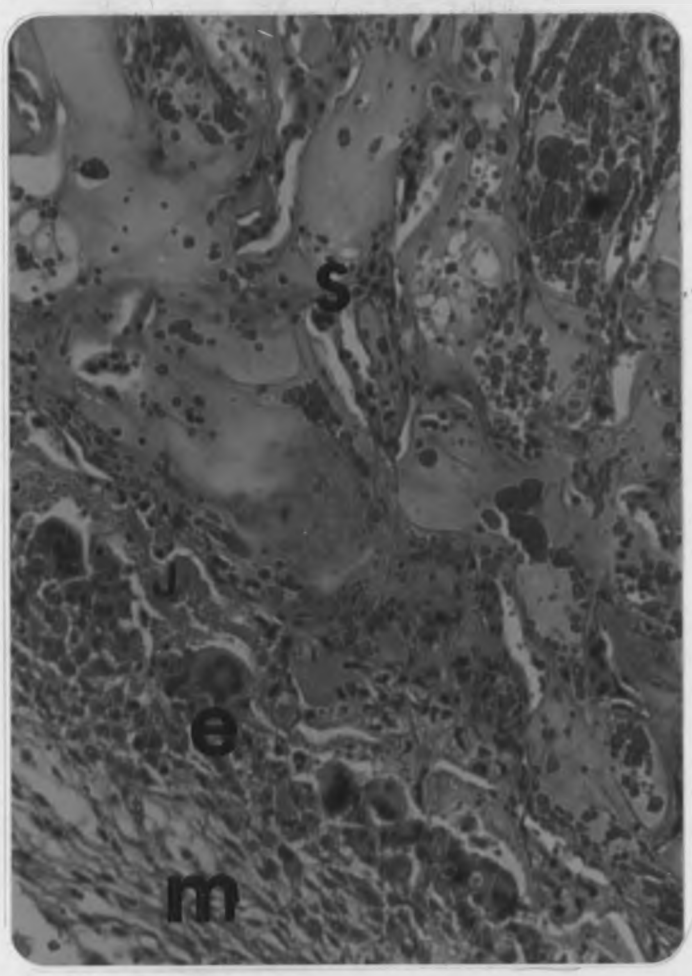
Photomicrograph of Day 3, Group II (10 mg IgG-anti-PMSG treated) hyrax placenta. X200

Fig. 39

Section showing necrosis of the junctional (j) zone and the spongy trophoblast (s). Note the lack of damage to the maternal endometrium (e) and myometrium (m).

Fig. 40

Section showing congestion in the palisade zone. p - palisade zone ; f - fetal mesenchyma


s
J
e
m

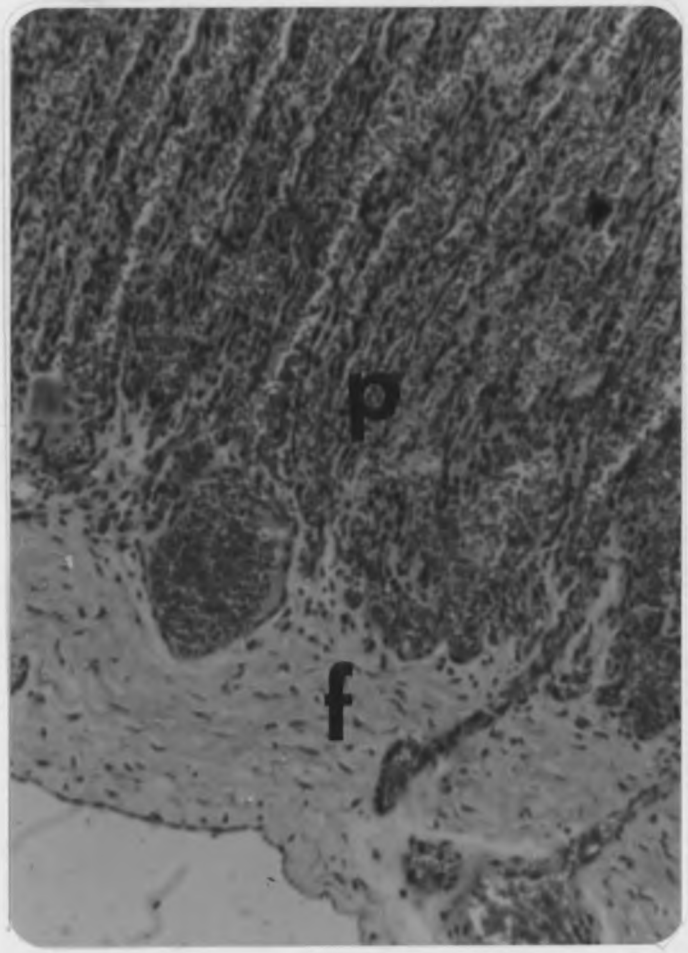

p
f

PLATE 25

Corpora lutea of Day 3, Group II (10 mg IgG-anti-PMSG treated) hyrax.

Fig. 41

Section showing congestion (b) in the corpus luteum X200. (c1)

d
b

PLATE 26

Necrosis of the IgG-anti-hCG treated goat.

Fig. 43

Gross photograph of the placenta showing the necrosis (n) of the cotyledonary tissue.

Fig. 44

Gross photograph of the underdeveloped foetus.

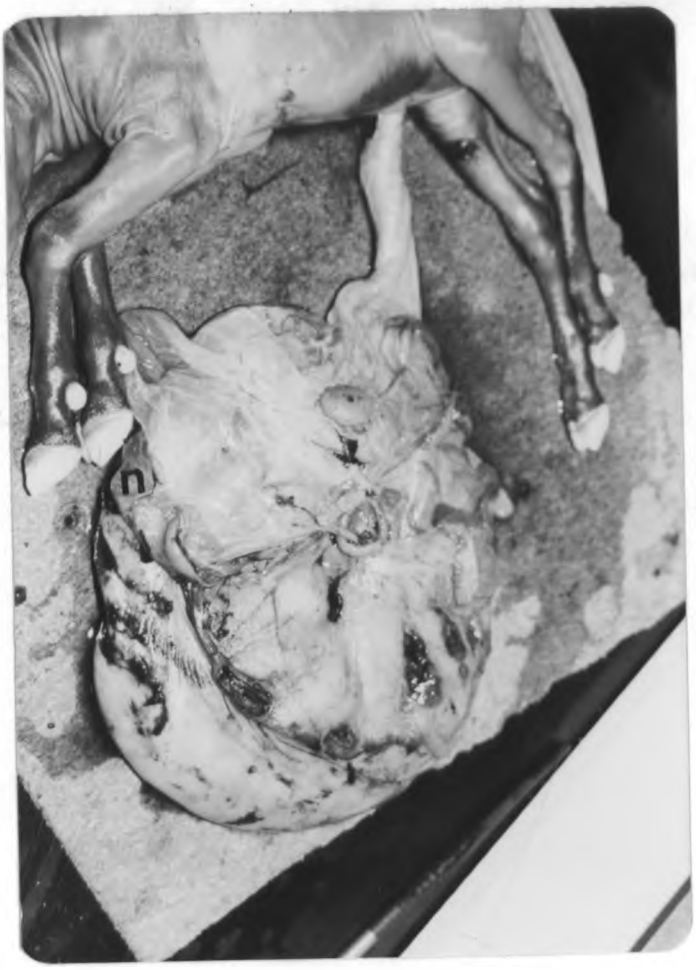


PLATE 27

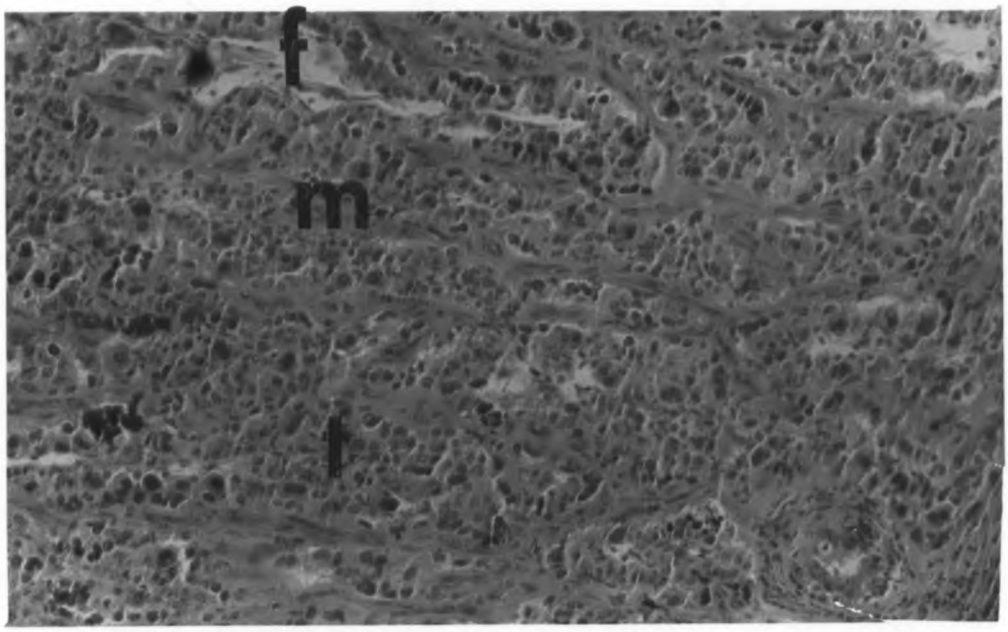
Placentae of the non-treated and antibody treated control goats. X200

Fig. 45

Section showing the maternal connective tissue (m), the foetal mesenchyma (f) and the columnar trophoblastic cells (t).

Fig. 46

Section showing massive disorganisation of the foetal tissue in the IgG-anti-hCG treated goat. Note the absence (a) of the foetal mesenchyma and columnar trophoblastic cells. The maternal tissue is intact.


f
m
l

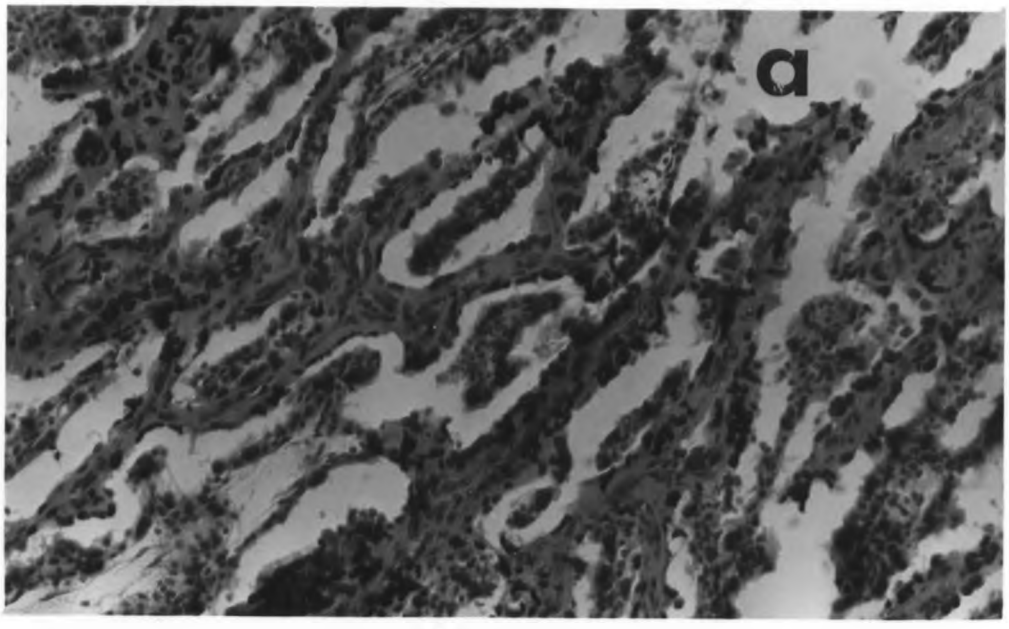

a

PLATE 28

Placental damage in antibody treated goats X200

Fig. 47

Section showing massive disorganisation of the foetal tissue in the IgG-anti-PMSG treated goat. Changes as in Fig 46 .

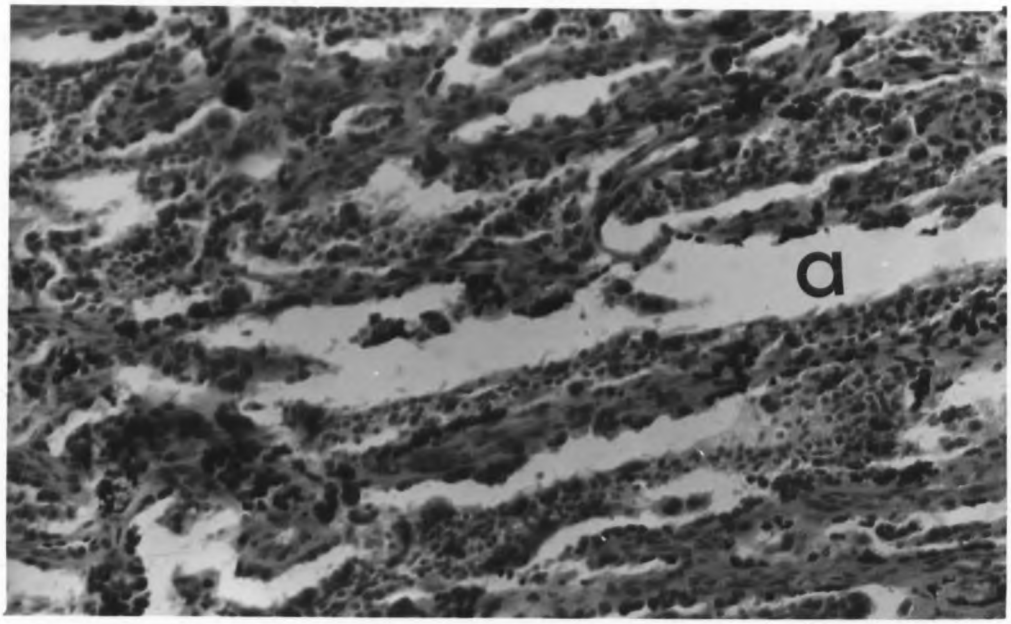

a

PLATE 29

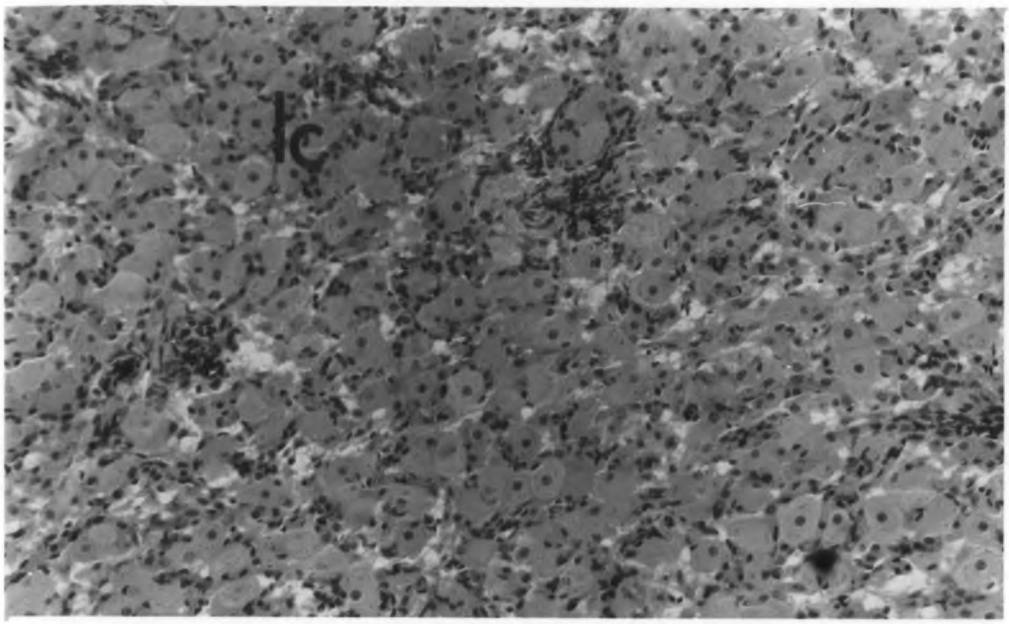
Corpora lutea of the goats. X200

Fig. 48

Section of the non-treated goat corpus luteum showing well developed luteal cells.

Fig. 49

Two Sections from the same corpus luteum showing typical conjestion (b) and the well developed luteal cells (Lc)


lc

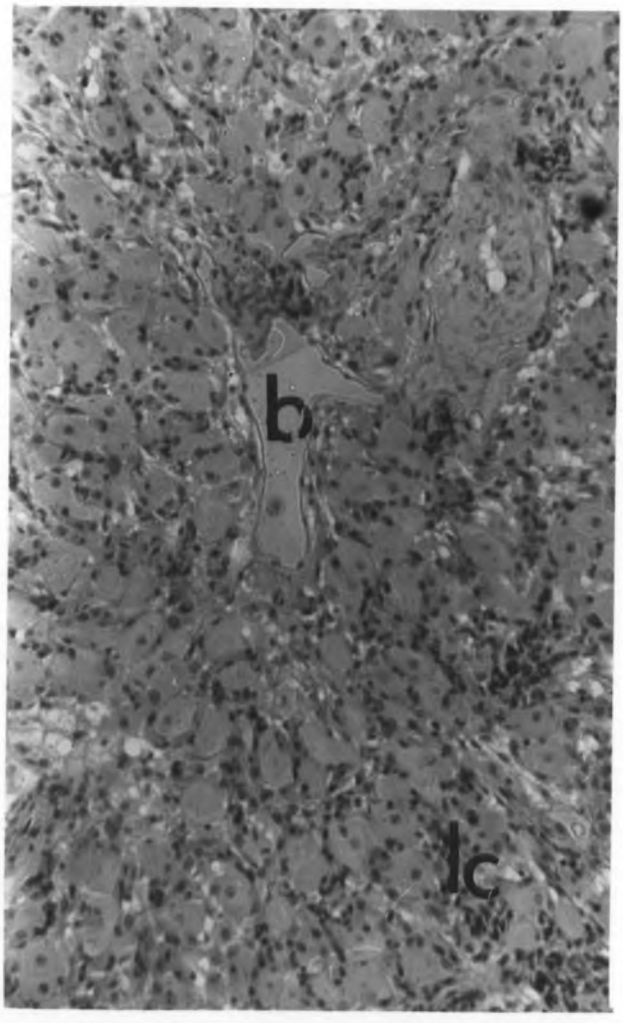

b
lc

PLATE 30

Sections of the placenta of a control rat (Group IVA) at Day 16 of gestation. X125.

Fig. 50

Light micrograph showing the spongy zone (s), giant-cells (g), maternal myometrium (m), and maternal endometrium.

Fig. 51

Section treated with anti-PMSG-fluorescein conjugate showing the intensity of immunofluorescence in the region of giant cells and the outermost chorionic cells.

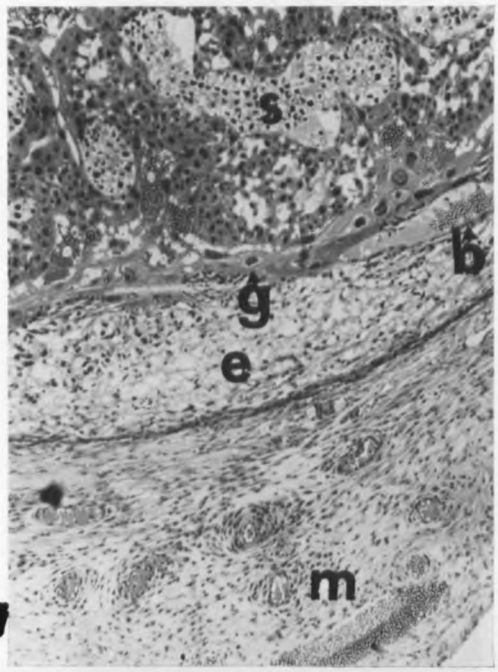
Fig. 52

Section treated with non-immune-IgG-fluorescein conjugate. Note the absence of giant and chorionic cell staining and only light background staining in the bottom right hand corner.

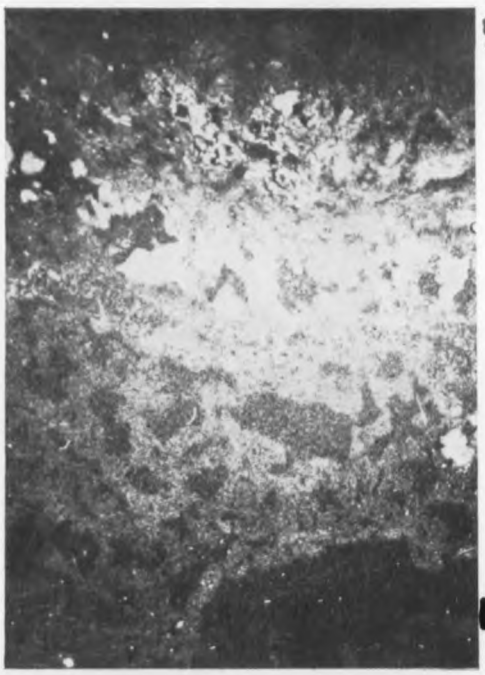
Fig. 53

Section treated with anti-hCG-fluorescein conjugate showing a distribtion of fluorescein similar to but brighter than, that in Fig. 72.

50



53



51

52

PLATE 31

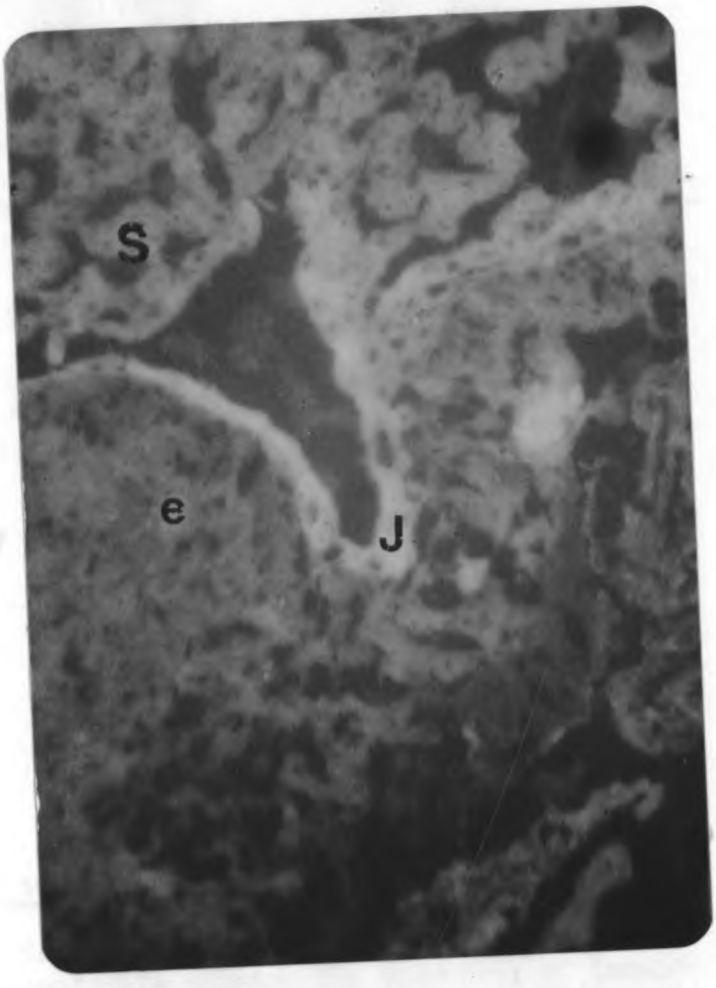
Immunofluorescence studies on the placenta of a Day 4 control hyrax.

Fig. 54

Section stained with anti-hCG-fluorescein conjugate showing the intensity of immuno-fluorescence at the foeto-maternal junction (j), and the decreasing intensity of staining on the spongy zone (s) and maternal endometrium (e). X200.

Fig. 55

Section of the foeto-maternal junction (j) stained with anti-hCG-fluorescein conjugate showing the intensity of immunofluorescence. X500.


S
e
J

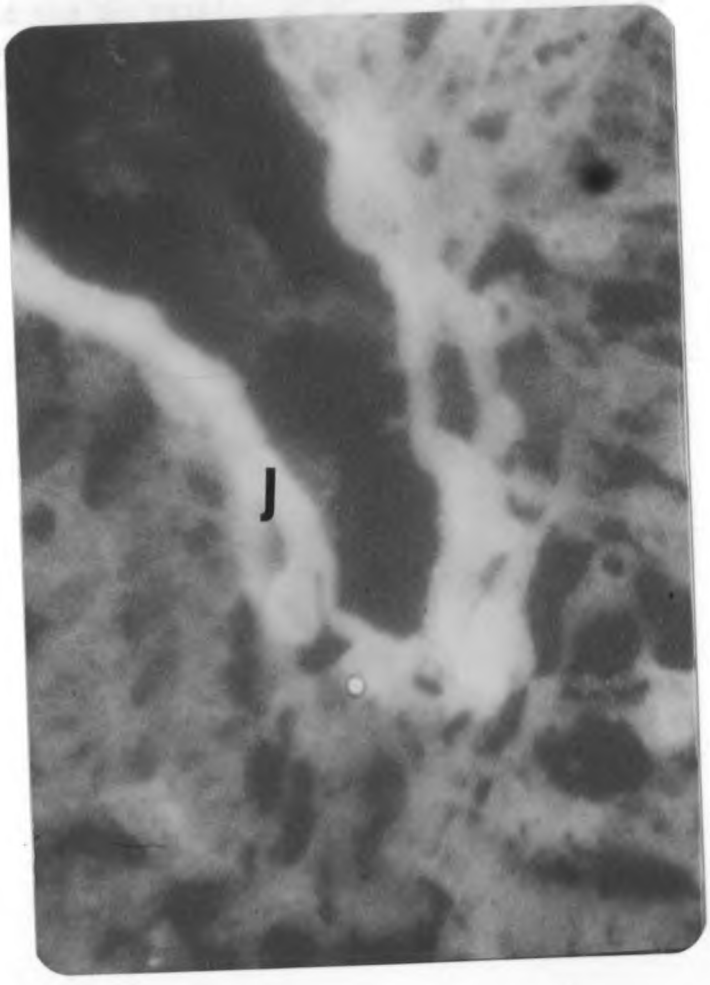

J

PLATE 32

Immunofluorescence studies on the placenta of a Day 4 control hyrax.

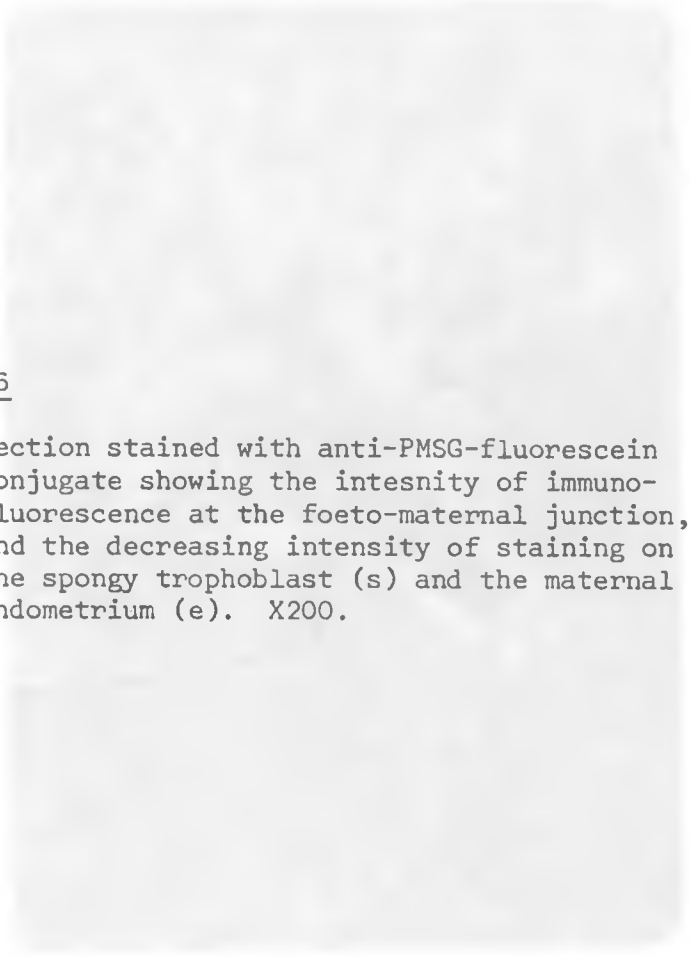


Fig. 56

Section stained with anti-PMSG-fluorescein conjugate showing the intesnity of immuno-fluorescence at the foeto-maternal junction, and the decreasing intensity of staining on the spongy trophoblast (s) and the maternal endometrium (e). X200.

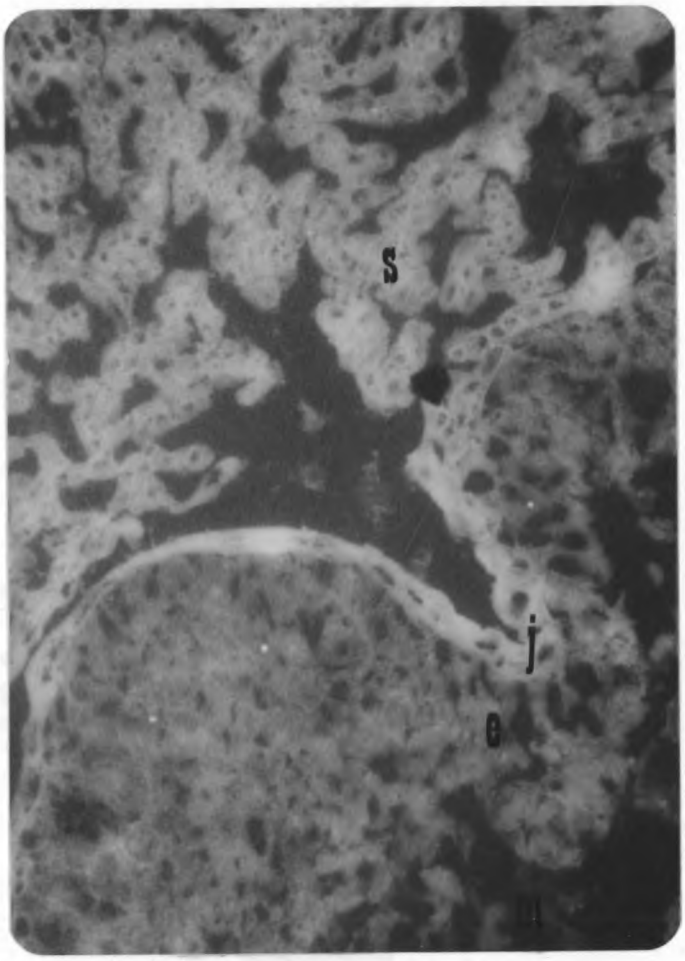

S
j
G

PLATE 33

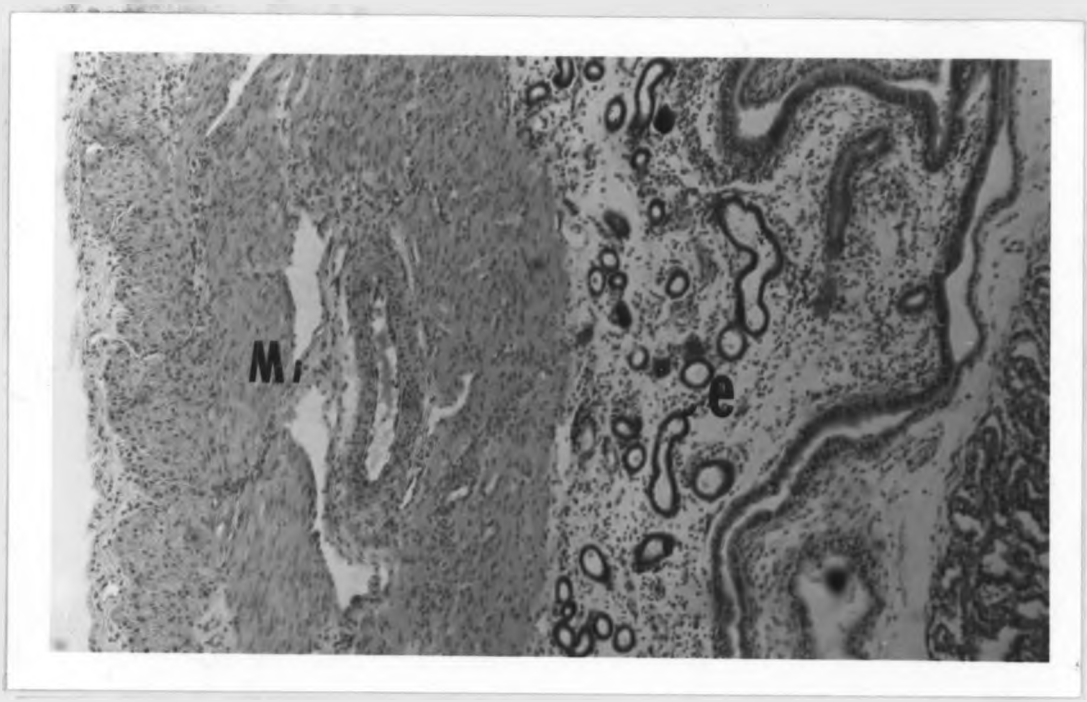
Placenta of the goat. X200

Fig. 57

Section of the maternal tissues showing the myometrium (m) and the endometrium (e).

Fig. 58

Section of the placentome showing the epitheliochorial placentation (2) and the maternal uterine tissue (1).


M
e

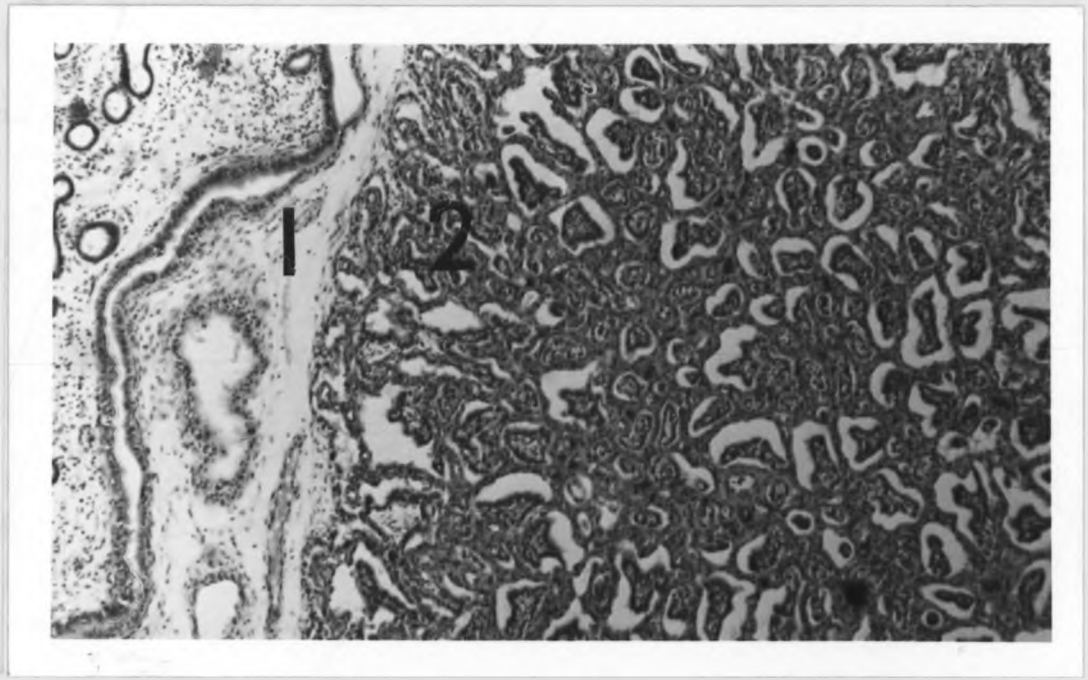

1
2

PLATE 34

Immunofluorescence studies on the goat placenta X500.

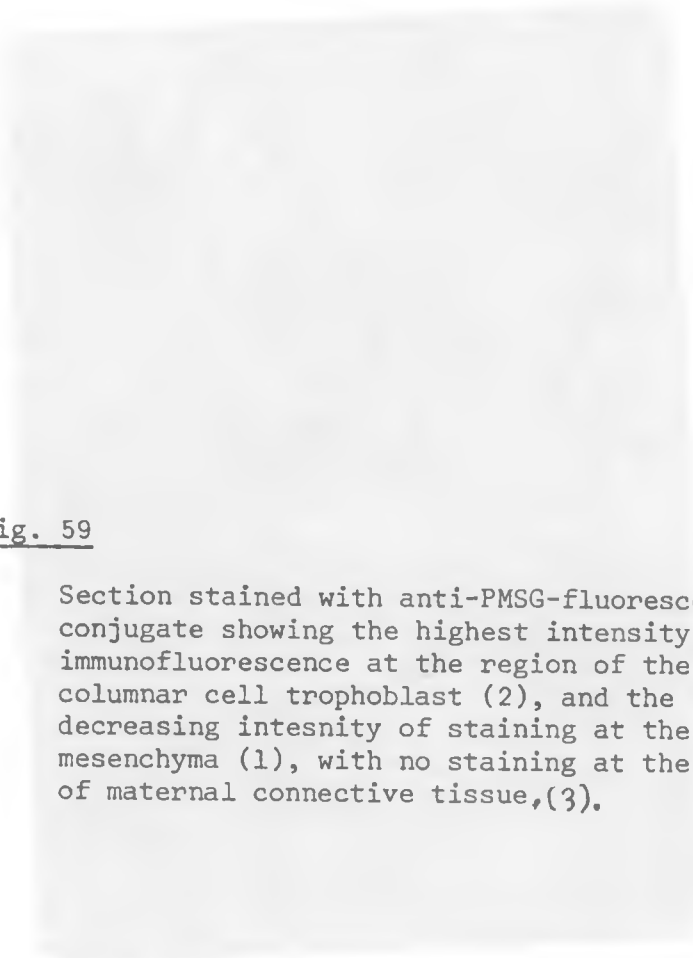


Fig. 59

Section stained with anti-PMSG-fluorescein conjugate showing the highest intensity of immunofluorescence at the region of the columnar cell trophoblast (2), and the decreasing intesnity of staining at the foetal mesenchyma (1), with no staining at the region of maternal connective tissue,(3).

1
2
3

PLATE 35

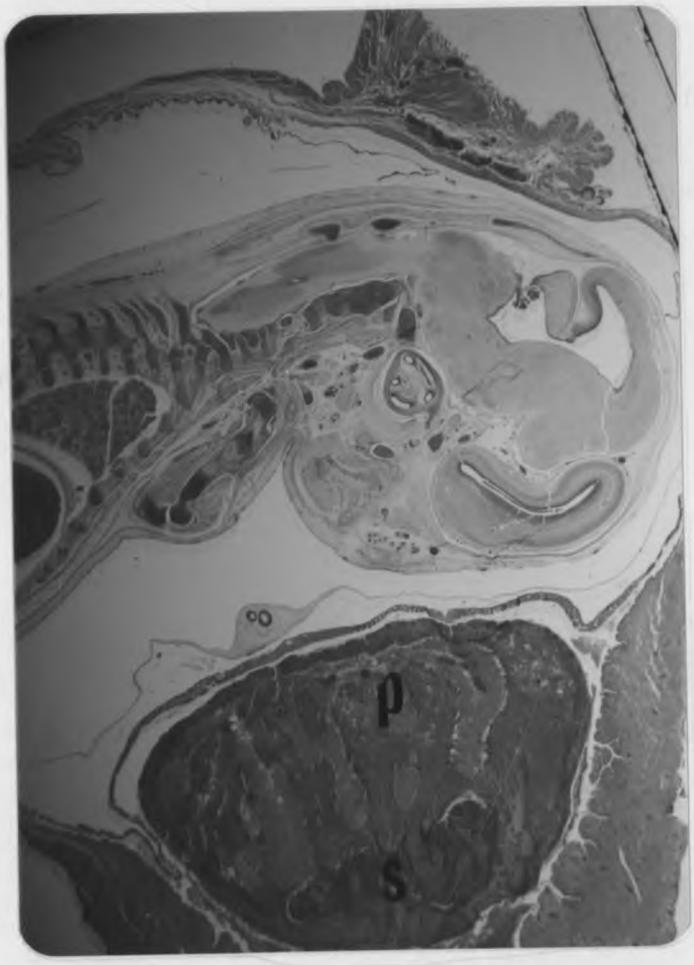
Photomicrographs of the sub-placenta of the cane-rat.

Fig. 60

Gross photograph of the conceptus of the cane-rat to show location of the placenta (p) and the sub-placenta (s). X5

Fig. 61

Light micrograph of the placenta (p) of a cane-rat showing the location of the sub-placenta (s). X125.


p
s

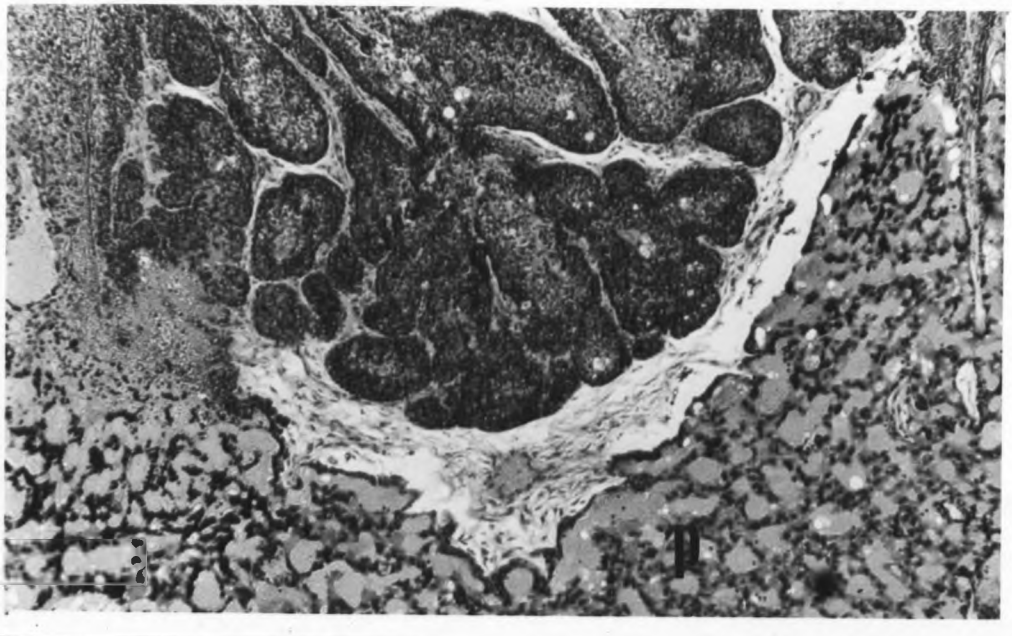


PLATE 36

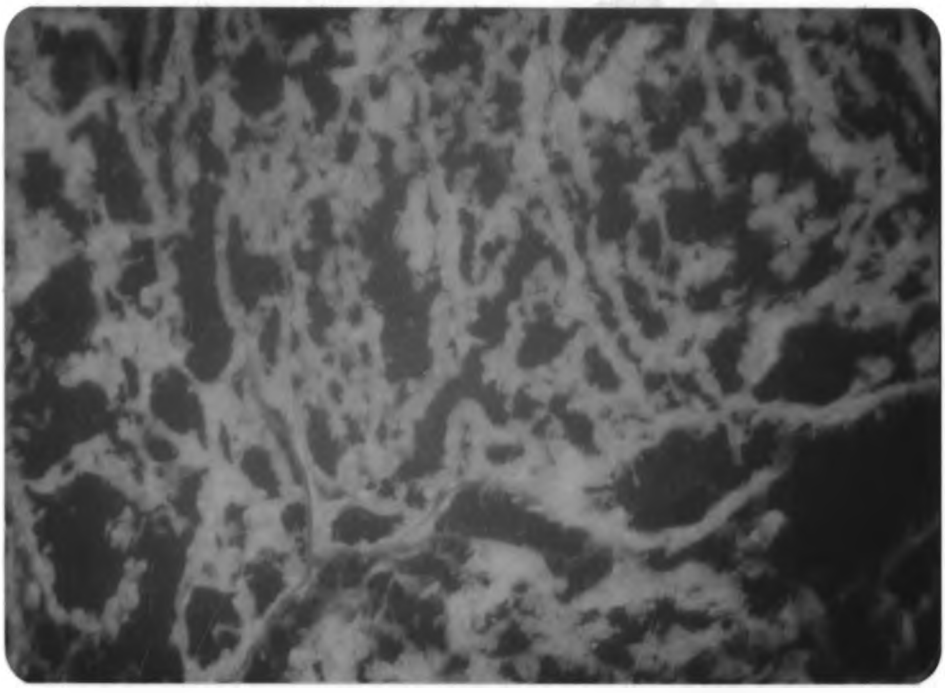
Immunofluorescence studies on the placenta of the cane-rat.

Fig. 62

Section stained with anti-hCG-fluorescein conjugate showing the immunofluorescence in the sub-placenta. X200

Fig. 63

Section stained with anti-hCG-fluorescein conjugate showing the intensity of staining on maternal blood vessel. X500.



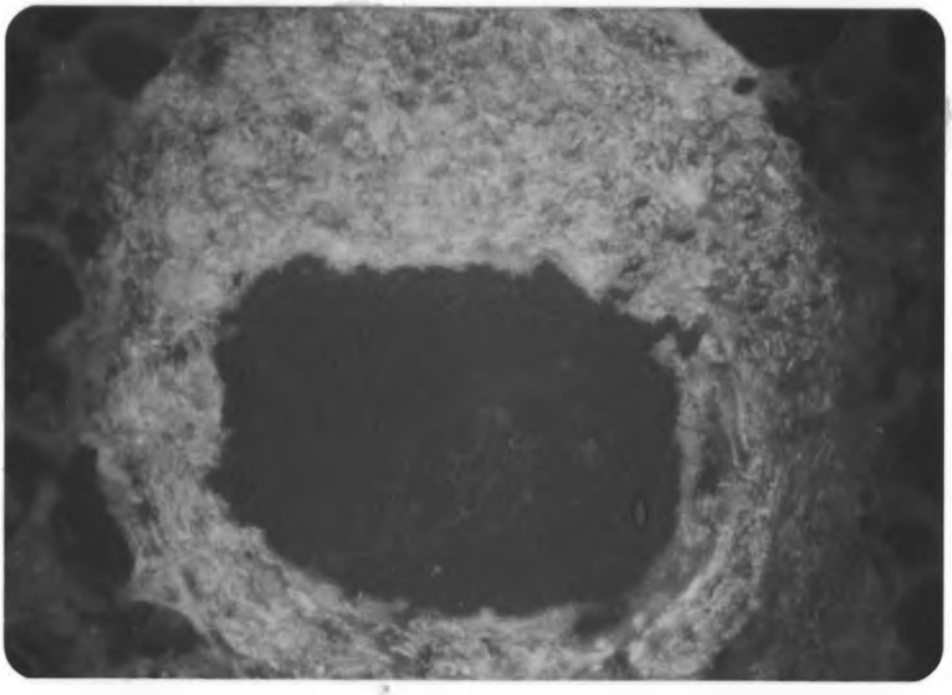


PLATE 37

Photomicrograph of the placenta of a spring-hare. X200

Fig. 64

Light micrograph of the placenta of a spring-hare at 2 weeks of gestation showing the placenta (3), allantois (2) and maternal endometrium (1).

Fig. 65

Section stained with anti-hCG-rhodamine conjugate showing the intensity of staining at the region of the foeto-maternal junction. (Indicated by 3 in the above figure).


3
2
1

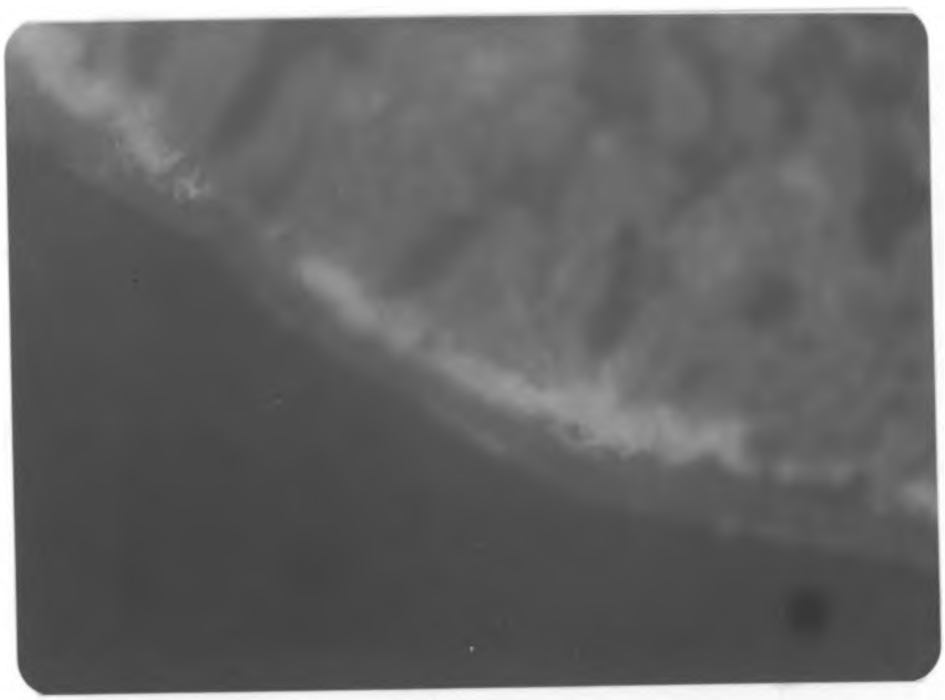


PLATE 38

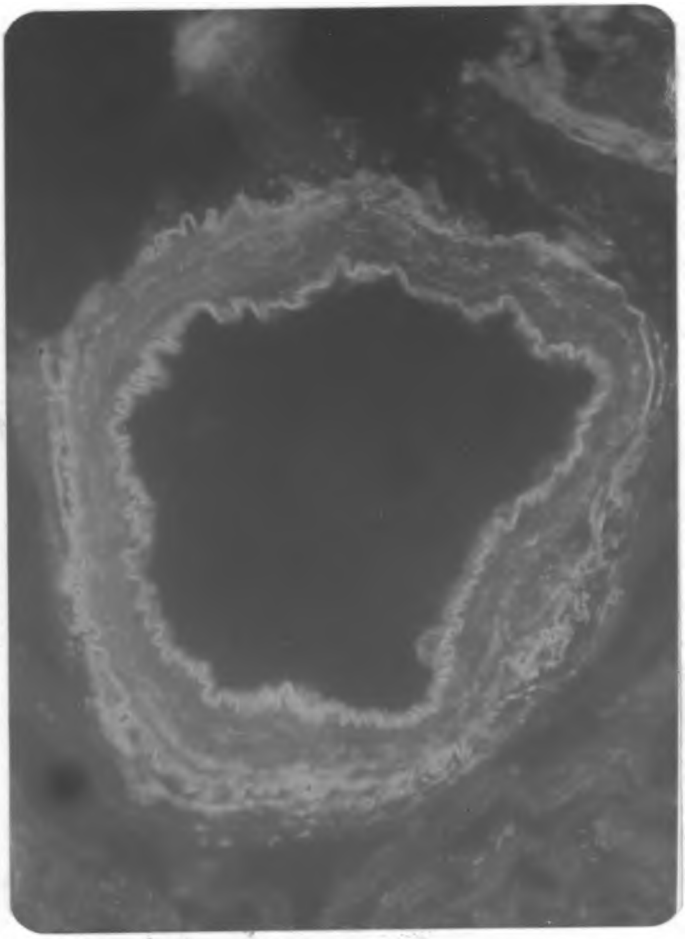
Photomicrographs of maternal uterine blood vessels in the placenta of a spring-hare.

Fig. 66

Light micrograph of a maternal uterine blood vessel (b), showing hypertrophy of the endothelial cells (t). X200

Fig. 67

Section stained with anti-hCG rhodamine conjugate showing the intesnity of staining on maternal blood vessel. X500.



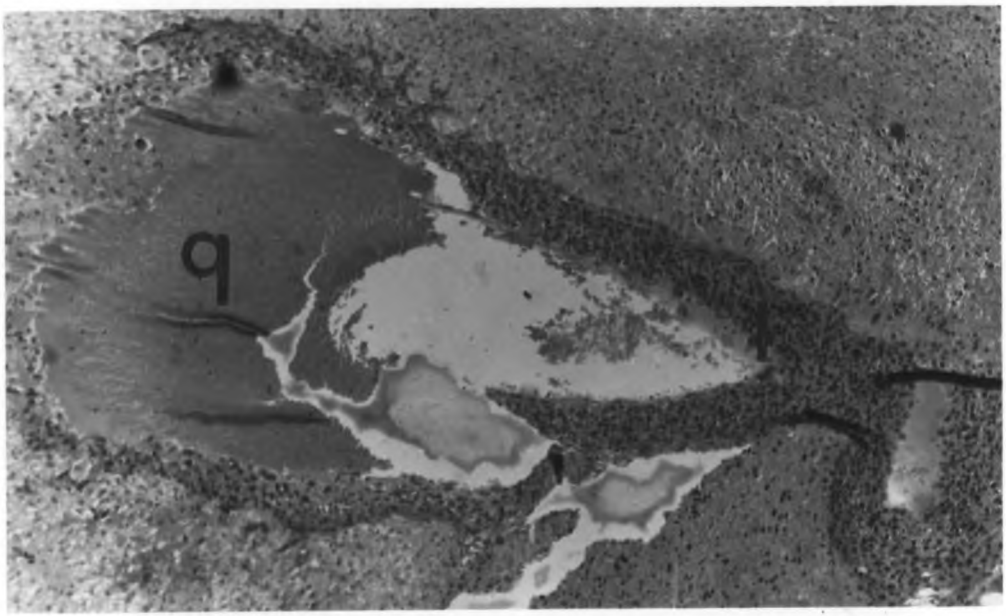

9

PLATE 39

Metabolic Clearance Graph

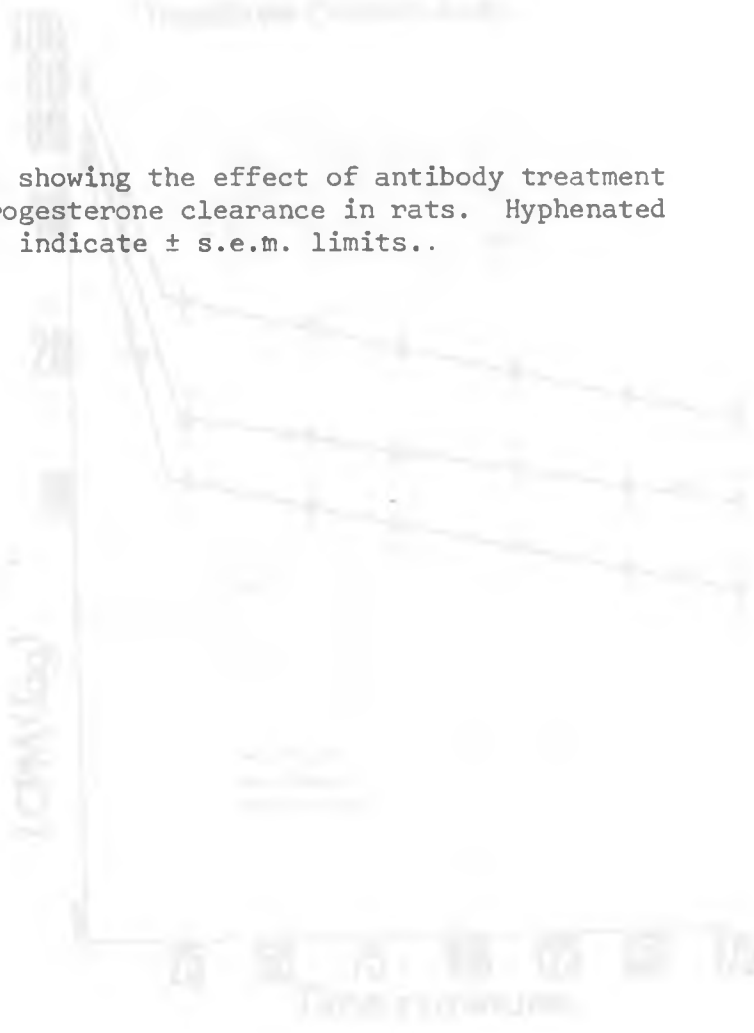


Fig. 68

Graph showing the effect of antibody treatment on progesterone clearance in rats. Hyphenated lines indicate ± s.e.m. limits..

: Progesterone clearance in rats.

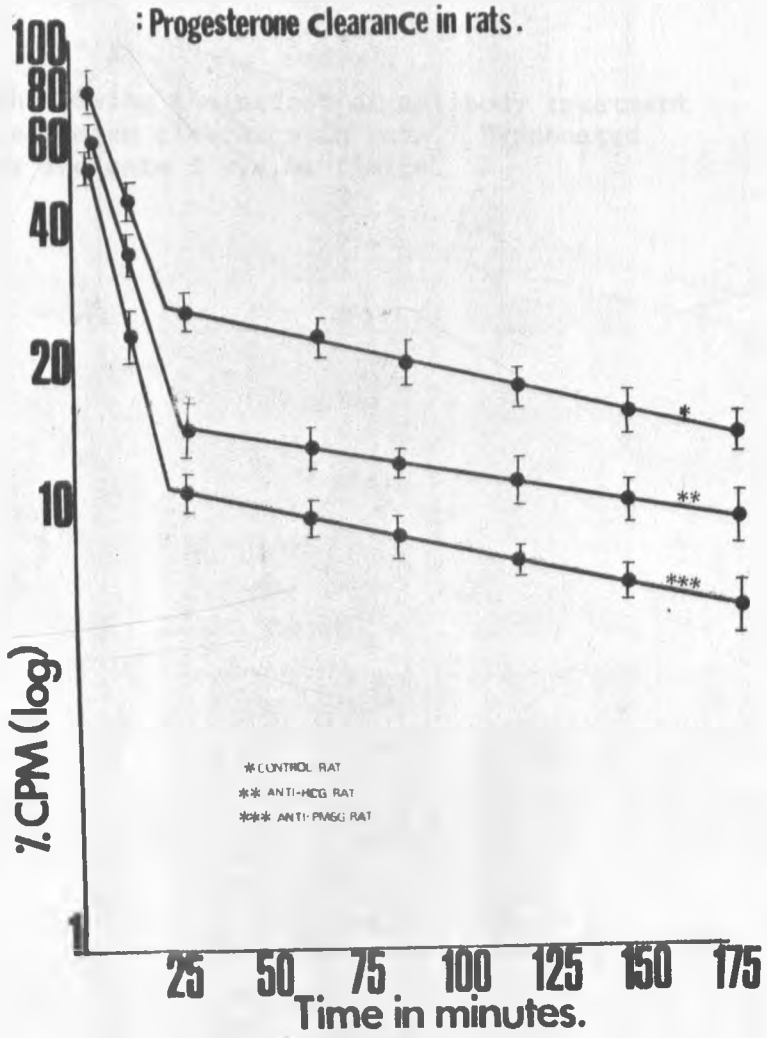


PLATE 40

Metabolic clearance graph.

Fig. 69

Graph showing the effect of antibody treatment on oestrogen clearance in rats. Hyphenated lines indicate ± s.e.m. limits.

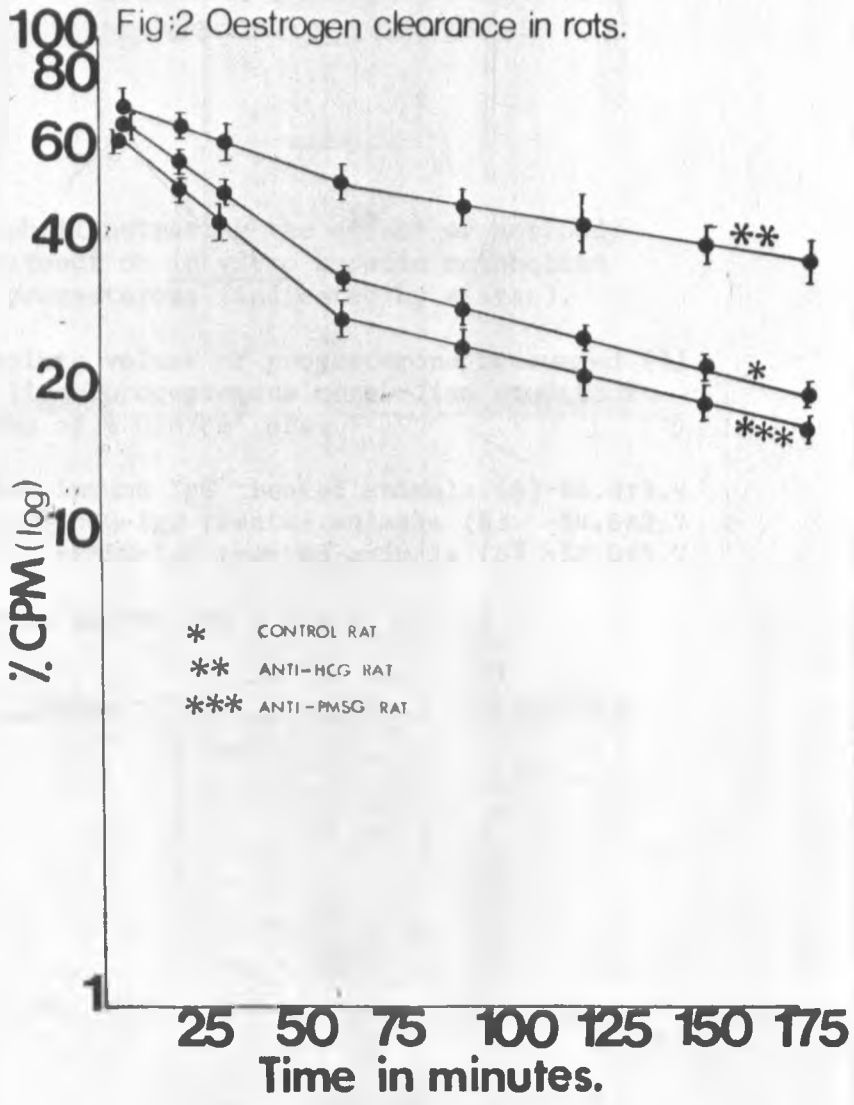


Fig:2 Oestrogen clearance in rats.
100
80
60
40
20
10
1
% CPM (log)
* CONTROL RAT
** ANTI-HCG RAT
*** ANTI-PMSG RAT
25 50 75 100 125 150 175
Time in minutes.

PLATE 41

Progesterone metabolism graph.

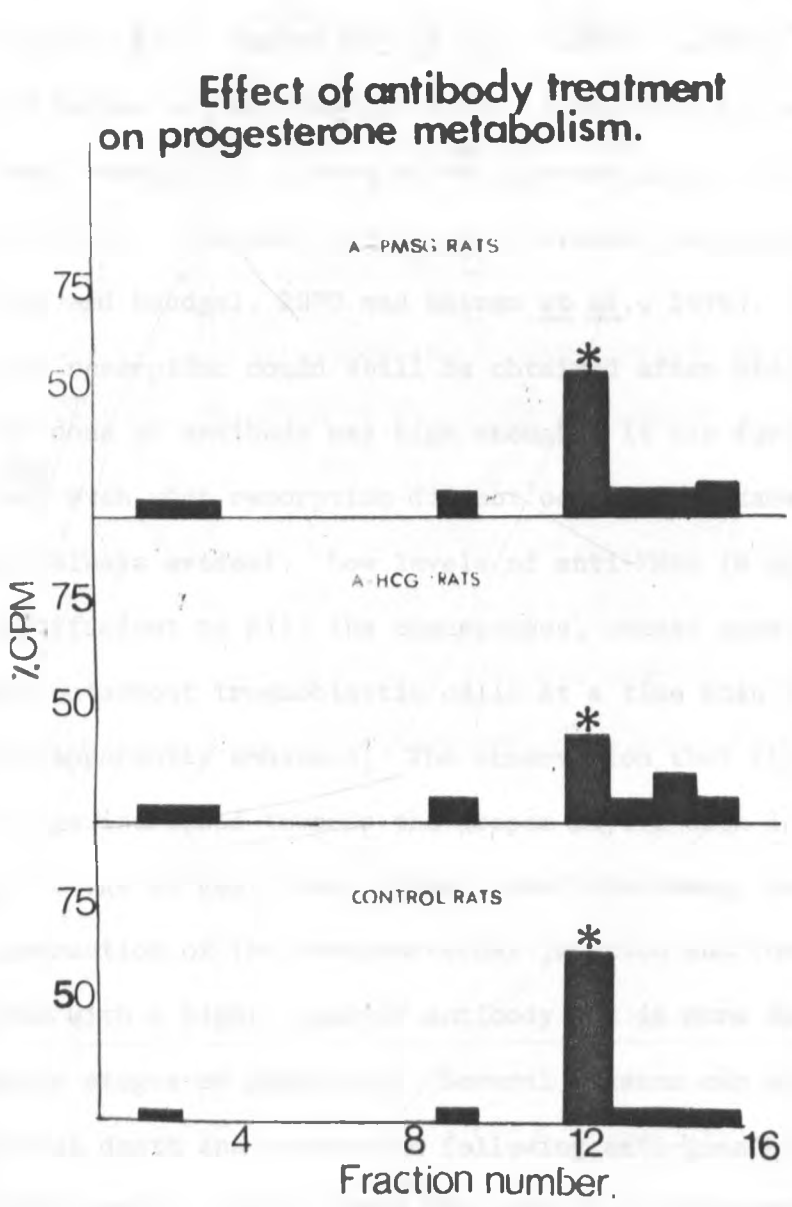
Fig. 70

Graph illustrating the effect of antibody treatment on in vitro hepatic metabolism of progesterone (indicated by a star).

Absolute values of progesterone recovered (*) in liver progesterone metabolism studies in terms of % CPM/cm^2 are:

Non-immune IgG treated animals (6)-65.3±3.4
Anti-hCG-IgG treated animals (6) -34.6±2.7
Anti-PMSG-IgG treated animals (6) -53.0±3.7

Errors quoted are ± s.e.m.


Effect of antibody treatment
on progesterone metabolism.
A-PMSG RATS
A-HCG RATS
CONTROL RATS
75
50
%CPM
4
8
12
16
Fraction number.

DISCUSSION

Neutralization Studies

In this study it has been shown that the response of pregnant rats to antigonadotrophic activity is dependant on dose and stage of gestation. Madhwa Raj et al., (1968); Loewit et al., (1969) and Madhwa Raj and Moudgal (1970) found that antiserum to LH caused fetal resorption in rats before mid-pregnancy and thereafter had no effect. Similar results were obtained using antiserum to hCG (Raj and Moudgal, 1970 and Sairam et al., 1974). It has been shown that resorption could still be obtained after mid-pregnancy provided the dose of antibody was high enough. It has further been shown that even when resorption did not occur, some damage to the placenta was always evident. Low levels of anti-PMSG (4 mg/day) although insufficient to kill the conceptuses, caused observable necrosis of the outermost trophoblastic cells at a time when luteal function was apparently enhanced. The observation that the trophoblastic damage increased towards the deeper layers with increase in dose and stage of gestation suggests that the damage begins with the destruction of the foeto-maternal junction and that it is accelerated with a higher dose of antibody and is more damaging during early stages of gestation. Several reasons can explain the lack of foetal death and resorption following anti-'gonadotrophin' treatment after Day 16: first, that the period of treatment was too short to allow the destruction of the foeto-maternal junction; secondly, that a decrease in placental gonadotrophin/gonadotrophin receptors occurs with advancing gestation; or thirdly, that both of these factors contributed.

In the hyrax, neutralization studies showed similar changes. The damage was first and foremost observed in the columnar trophoblast - constituting the foeto-maternal junction. It was initially in localised foci but increased in intensity and extent with the duration of neutralization. The observation that even where foetal death had occurred, the maternal and deeper foetal tissues were unaffected underscores the fact that only specific cells were damaged. Fetal death was probably caused by the separation of the foeto-maternal junctional membranes.

As in the rat and hyrax, 'gonadotrophin' neutralization in the goat was followed by placental damage and necrosis. This damage was always found to start at the chorionic cell layer adjoining the maternal connective tissue and spread towards the deeper lying foetal tissues. These results, in all the three animal types, are consistent with the contention that the placental degeneration always accompanied the destruction of the foeto-maternal junction.

The specificity of the antobodies used in these experiments; the observed lack of cross-reactivity of anti-hCG and anti-PMSG with the hyrax pituitary, and the reactivity of the hyrax placenta with these antibodies and the failure of these antibodies to elicit histological changes in the rat pituitaries and mammary tissues, are consistent with the belief that placental gonadotrophins were being neutralized with anti-hCG and anti-PMSG treatment, and that it was unlikely to be a placental lactogen.

Results on the influence of placental gonadotrophins on steroidogenesis have been well documented for both hCG (Rice *et al.*,

1964; Savard et al., 1965; Toru and Troen, 1975; Hollander and Hollander, 1958; Villee et al, 1968; Paton, 1969; Preumont et al., 1969; Domanski et al., 1967 and Short et al, 1963) and PMSG (Armstrong and Hixon, 1969; Domanski et al., 1967; Short et al, 1963. .). However controversy exists as to whether this influence is inhibitory or stimulatory. In the work done on rat (Loewit et al., 1969) it was contended that foetal resorption following anti-LH administration was a consequence of decreased ovarian function, the implication being that there was a fall in ovarian progesterone, and thereby plasma progesterone. Similar theories were advanced to explain foetal resorption following anti-hCG treatment in rats (Raj and Moudgal, 1970; Sairam et al, 1974). Present results indicate that in rats, from mid-pregnancy onwards, anti-PMSG and anti-hCG caused a slight but significant fall in plasma progesterone. This was, however, concomitant with a slightly elevated ovarian progesterone concentrations. Ovarian progesterone concentration was still quite high on Days 21 and 26 post coitum in groups IIC and IIIC, the latter being long after foetal resorption had occurred. In the goat, histological studies revealed well developed luteal cells, with no indication of luteal regression, whilst radioimmunoassays indicated absence of any significant differences between treated and control plasma steroidal levels. The contentions of Loewit et al., (1969), Raj and Moudgal (1970) and Sairam et al. (1974), that anti-LH or anti-hCG caused luteal regression which was responsible for fetal resorption in rats is thus not borne out by the results of the present investigation. This work suggests that the influence on steroidogenesis could be of stimulative nature.

It is not known, at the present time, why anti-hCG and anti-PMSG should cause necrosis of the primary oocytes in the hyrax. The observation that anti-PMSG caused greater ovarian changes would suggest that even during pregnancy the ovary still retains FSH-like receptors in its follicles and corpora lutea. The neutralization of the gonadotrophin receptors of the primary oocytes may have led to their subsequent dysfunction.

Immunofluorescence studies

It is generally accepted that foetal membranes have receptors which are capable of binding gonadotrophic substances. One line of evidence is based on studies done in an attempt to localise gonadotrophic activity on these receptors using histochemical and immunofluorescent techniques. Thus hCG and hCS activity has been localised on the human placenta (Midgley and Pierce, 1962; Beck and Currie, 1963; Sciarra et al, 1963; Thiede and Choate, 1963; Currie et al, 1966; Hamanaka et al, 1971; Genebaceu et al, 1972 and Osada, 1976) and PMSG on horse placenta (Cole and Grass, 1943; Amoroso, 1952 and Clegg et al, 1954). It was thought that this activity was located on receptors present in the syncytium. The results of the present investigation obtained using an immunofluorescent technique are in part agreement. In this study hCG/PMSG-like substances have been shown to be present in some none-primate, non-equid placentae. The specificity of the antibodies used, and the results of hyrax pituitary and rat pituitary and mammary tissue analysis suggests that the placental "gonadotrophin" localised in these animals was different from the pituitary gonadotrophins or placental lactogens.

In all these animals except the cane-rat, hCG/PMSG-like materials were shown to be present at the foeto-maternal junction. It was further demonstrated that these placental "gonadotrophins" are found in highest quantities on the outermost chorionic cells - the columnar cell layer, and that their concentration decreased towards the deeper foetal layers and on the adjacent maternal uterus. These observations suggest that the placental gonadotrophins are held on to cell membrane receptors which are present on the outermost trophoblastic cells in largest quantities.

In the rat it has been demonstrated that the giant cells of Duval give a positive PAS reaction (Bulmer and Dickson, 1960) and that they produce granules containing proteins (Dickson and Bulmer, 1960). These results taken together with the demonstration of "gonadotrophic activity" on rat giant cells in the present study, suggest that the rat giant cells produce a gonadotrophin substance allied to the glycoprotein hCG. As the origin of the giant cells is cytotrophoblastic, it would appear that the rat "gonadotrophin" has both a cytotrophoblastic and syncytiotrophoblastic origin unlike the human placenta.

Cane-rat sub-placenta was shown to have PMSG/hCG-like materials in large quantities in present studies. Electronmicroscopy studies of the sub-placenta reveal a structure consonant with a tissue involved with active protein synthesis. Furthermore the sub-placenta stained with PAS after amylane digestion (Oduar-Okelo, Ph.D. Thesis, University of Nairobi). These observations, strongly suggest that the secretion of the cane-rat subplacenta is a glyco-protein, and would support the belief that a placental glycoprotein

hormone is produced at this site. Since the origin of the sub-placenta is syncytial as well as cytotrophoblastic, it follows that as in the rat the cross-reacting material has a syncytial as well as a cytotrophoblastic origin, a finding contrary to that found in the human placenta.

In all these animals except the cane-rat the placental "gonadotrophin" was found to be present in form of a dense staining band located at the foeto-maternal junction - a region in direct contact with the maternal tissues. In the cane rat, the sub-placenta is located between the main placenta and the uterus in close proximity to the maternal tissues. Moreover the vascular supply is mainly from the mother and a reasonable quantity of blood flows towards the maternal side. Positive immunofluorescent staining, indicative of a placental gonadotrophin-like substance, in form of a thin band, was also observed at the luminal (maternal) margins of some placental blood sinuses. From these observations it is apparent that the gonadotrophin-like material is always observed in close proximity to the maternal tissues (uterus and blood). This suggests that these cross-reacting materials have an easy access to the maternal tissues and their presence has a strong influence on certain aspects of maternal physiology. Their possible function may be closely allied to their location or vice-versa.

Metabolic Clearance Studies

Loewit et al., (1969) attributed luteal regression, following treatment with antiserum to LH in rats, to the reappearance of 20α-hydroxysteroid dehyrdrogenase. They implied that there was

a reduction in ovarian progesterone concentrations which resulted in foetal resorption. These authors, however, did not monitor levels of progesterone. In this study, rats treated with antibodies to hCG and PMSG, between the 6th and 11th day of pregnancy, ovarian progesterone was actually increased. However, plasma progesterone was lower than normal. In these same animals, ovarian oestrogen concentrations were unchanged whilst plasma levels were unchanged in anti-PMSG treated animals, and slightly elevated in anti-hCG-treated rats. Ovarian progesterone concentrations were found to be still quite high on Days 21 and 26 of gestation, the latter long after the foetuses had been resorbed. Histological examination of the ovaries in these animals revealed no evidence of luteal regression in antibody treated animals, and the luteal cells in CL of these animals were apparently normal. Clearly the metabolism and biosynthesis of progesterone and oestrogen in the antibody treated animals is affected and it cannot be due to reappearance of 20α-hydroxysteroid dehyrdrogenase alone since there was no decrease in progesterone concentration, or any evidence of luteal regression.

The increase in the clearance time of progesterone in both anti-hCG and anti-PMSG treated rats is consonant with the observed low plasma progesterone levels in antibody treated rats inspite of the high ovarian concentrations. The increased clearance rate of progesterone in antigonadotrophin-treated animals could be partly explained in terms of binding of these steroids to plasma binding protein, as shown by the decreased binding of progesterone to plasma protein in anti-hCG treated animals. There was no change in binding of progesterone in anti-PMSG-treated animals. Similarly,

the observed increase in plasma oestrogen values in anti-hCG treated rats could be the result of increased time taken in clearing oestrogen

The hepatic metabolism of these steroids was altered as observed from the increased number and quantities of metabolites formed, and the decreased progesterone levels seen, in in vitro incubations of the livers of antibody treated rats with progesterone. That the liver was affected by gonadotrophin neutralisation was consonant with the increase in hepatic and plasma transaminane levels, and the microscopically observed fatty infiltration and hyperaemia of the livers, in treated rats. That the liver clearance is mainly affected is supported by the observation that the kidneys appeared normal, and their clearance was not altered, as indicated by unchanged plasma urea and creatinine levels.

Antibodies to hCG and PMSG have differential effects on the steroid metabolism. Thus anti-hCG treatment resulted in the fastest liver progesterone metabolism, slightly increased plasma progesterone clearance, and decreased plasma oestrogen clearance, whilst anti-PMSG-treatment resulted in slightly increased liver progesterone metabolism and increased clearance of both progesterone and oestrogen from the plasma.

The increased ovarian progesterone levels are indicative of increased ovarian synthesis of this hormone. The placental gonadotrophin could well have an inhibitory action on enzymes such as 20-21 desmolase and a decrease in NADP mediated conversion of pregnenolone to progesterone. Following antibody treatment, the gonadotrophin inhibition would be removed, and this would be followed by increased progesterone synthesis. However, this remains to be examined.

General Discussion

It could be argued that the antibodies injected into the animals were toxic, and that the effects of treatment observed were due to this toxicity. That this is not so is evident from the lack of damage to the maternal tissues, the progressive damage observed from superficial to deeper foetal tissues and the fact that non-immune antibody was without effect.

Passive administration of the antibody to hCG and PMSG seriously interfered with the process of pregnancy in rats, hyraxes and goats. Stevens (1976) observed similar results when baboons were immunised with β-hCG antibodies. He suggested that the effects observed were due to specific neutralization of the antigen. The results obtained in the present study could have been either due to specific neutralization of the antisera or non-specific effects of the antisera. Recent evaluations of antiserum to hCG in specific radioimmunoassay suggest that these antibodies can bind to labelled hormone to high levels (Stevens, 1976). In one of these studies hCG levels were measured with a specific radioimmunoassay before and after passive immunisation and the results showed that specific antibodies bind to their specific antigen. In the present study, antibodies used were specific. Moreover, non-immune IgG did not have any effect and only fetal tissues were affected. This would tend to suggest that the effects observed were a result of specific neutralization of the hCG/PMSG-like material. Injection of the antiserum can affect the antigen in three possible ways: (a) binding of antibody at or near the site on the hCG molecule which interacts with hormone receptor; (b) formation of hormone-

antibody complex alters tertiary structure of hCG; rendering it biologically inactive; (c) formation of a large complex of gamma globulin molecules with hCG-molecules resulting in a rapid clearance of the hormone by macrophage phagocytosis. No experimental evidence is available to support any of the mechanisms but the third model is generally accepted. This mechanism may apply in the present study and passive immunisation could have resulted in a rapid clearance of the 'hormone' and impairment of pregnancy due to the destruction of the foeto-maternal junction been the consequence.

It was shown in this study that the gonadotrophin-like material was present in all the animals examined and was located at precisely those areas of the placenta which were in contact with the maternal tissues - uterus (except in cane-rat) and maternal sides of blood sinuses - those areas from which the mother would be expected to initiate an immunological attack directed against the foetus. In the cane-rat the substance was localised in highest quantities on the sub-placenta, and this organ was in close proximity to the maternal uterus. In three groups of these animals (rats, hyraxes and goats) trophoblastic damage which started at the foeto-maternal junction and spread towards the deeper fetal layers with increase in dosage was observed. It was noted that although the maternal uterus adjacent to the feto-maternal junction indicated presence of "gonadotrophic" material, it was undamaged. It would thus appear that this destruction only affects the foetus and not the mother. One possible explanation which could be advanced to explain all these observations is that there was a progressive immune-attack from superficial to deeper tissues direct by the mother against its foetus.

However evidence in support of a maternal immune attack directed against the foetus requires a demonstration of either/or a cell-mediated response or a humoral response. In the rat, white blood cell infiltration was consistently observed. It could be argued that this was no more than may occur in a normal placenta. However the observation that large quantities of white blood cells were observed in antibody treated animals and that very few if any were observed in animals treated with non-immune IgG would suggest that the white blood cell infiltration was a consequence of antibody immunisation treatment. In the hyrax, invasion of foeto-maternal junction by white blood cells was only observed in later stages of treatment. This would suggest that the antibodies were simply lytic and did not act by neutralizing the gonadotrophic substance. However the specificity of the target cells (lack of damage to the maternal tissues), causation of foetal death in the later stages of treatment, and the lack of damage to non-immune IgG would tend to suggest the immunosuppressive role of the "gonadotrophin". In the goat only a few white blood cells were observed. This could be due to two reasons: either the time interval between end of treatment and operation was not long enough to allow a full immune attack or the low dose of antibody was used. This suggestion is supported by the rat experimental results which showed that fetal destruction was dependent on dose and time of treatment.

It is apparent that the destruction of the fetus is initiated at the feto-maternal junction in the rat, goat and hyrax. This would suggest that fetal death and damage to the deeper placental tissue was the result of detachment of the placenta. Present experimental work does not refute this suggestion. However the

initial detachment of the placenta was a direct result of passive immunisation with the antibodies directed against the "gonadotrophin".

The overall affects observed at the feto-maternal junction and the progressive degeneration from superficial to deeper tissues could have been due to: (a) maternal immune response to the injected goat immuno-globulin which had been bound to the trophoblast; (b) an immune-complex disease; or (c) a specific maternal immune response. Immunofluorescence studies demonstrated the binding of the goat-immunoglobulin to the maternal endometrium (hyrax, rat, goat) as well as to fetal tissues. Only fetal tissues showed signs of degeneration following passive immunisation with antibodies. This would tend to rule out the first possibility. Animals did not show any signs of sickness. Moreover both the goats were in good health and one had infact given birth to one kid (second pregnancy, no treatment being given) at the time of writing of this manuscript. Moreover passive immunisation with non-immune IgG was without apparent affect. This would tend to support the third possibility.

Since maternal lymphocytes do not normally gain access to the foetus in immunologicaly significant quantities, blocking factors against cell-mediated immunity need only operate at the placental level where feto-maternal interaction mainly occurs (feto-maternal junction, and maternal surface of blood sinuses) and would ensure a self-protective mechanism. It could be argued that if placental gonadotrophins were immunosuppressive the general immunity of pregnant animals would be affected. However for immunosuppression to take place, the organ being protected must have receptor sites for gonadotrophins. Further if the gonadotrophins were produced

and used *in situ*, there would be little interference with the general immune reactions, even if other tissues had appropriate receptors.

Five hypotheses have been proposed to explain the lack of fetal rejection by the mother: (a) Antigenic immaturity of the fetus; (b) Uterus as an immunologically privileged site; (c) Invasiveness of the trophoblast; (d) Depression of maternal immunological mechanisms during pregnancy and (e) Anatomical separation of the mother and the fetus.

Experimental evidence currently available effectively invalidates the second and third possibilities. This leaves the remaining three. If we accept the evidence that the fetus has transplantation antigens, is immunocompetent and is itself antigenic, and if we recognize the mother as being fully immunocompetent, the solution to the fetal allograft puzzle must lie in either the intrinsic properties of trophoblast and/or the unique manner in which it is handled by the mother, the end result being an "immunological barrier" between them (i.e. the feto-maternal junction, and to a lesser extend the blood sinuses). Evidence exists to indicate that some form of an immunologically inert material is closely associated with maternal surface of the barrier masking histocompatible antigens (Kirby *et al.*, 1964; Bradbury *et al.*, 1965 and Currie *et al*, 1968). This material-constituting the foeto-maternal junction has been shown to be rich in hyaluronic and sialic acid. Both PMSG and hCG are rich in these acid residues. Both PMSG and hCG have been shown to be present in primates and equids. Present studies establish their presence in rats, hyraxes, goats

dik-diks, cane-rats and spring-hares. Furthermore they have been shown to constitute the feto-maternal barrier in all animals except the cane-rat. In this animal most of the fluorescent activity was present in the sub-placenta which is in close contact with the uterus. The possibility exists therefore that a physiochemical activity or "barrier" composed of sialomucin may operate masking or obscurring trophoblast antigen. This suggestion was originally put forward by Kirby et al (1964) and recently by Amoroso and Perry (1975). Current study would tend to support this possibility.

That the trophoblast produces in relatively large but varying amounts, glycoprotein hCG or PMSG throughout pregnancy is well established and has been confirmed in the present study. Kaye and Jones (1971) demonstrated that hCG depressed maternal lymphocyte transformation induced by the mitogen, phytohaemmagglutinin (PHA) although Jenkins et al (1972) reported that concentrations comparable to those in the circulation were insufficient to affect this response in vitro. However Adcock et al (1973) established that hCG not only completely inhibited the response of lymphocytes to PHA at several dosage levels but that the effect was both reversible and non-cytotoxic. They concluded that the fetus was accepted by the mother because a glycoprotein-hCG represents trophoblastic surface antigens and blocks maternal lymphocyte reactivity. It has recently been suggested that the local concentrations of hCG far exceeds peripheral levels and is sufficient to obscure the trophoblast, preventing immune rejection (Braunstein et al, 1973). In the horse, PMSG has been shown to have immunosuppressive activities (Dr. T. Allen, ADRC, Cambridge, personal communication). These reports together with the results of passive immunisation

experiments obtained in the present study would tend to suggest that the fetal degeneration observed, may have been a result of unmasking this "barrier".

The principal purpose of the present study was to investigate the hypothesis put forward by Amoroso and Perry (1975) to account for the apparent failure of the mother to reject the foetus. They thought that the maternal-foetal barrier or the junction at the site of contact of trophoblast and maternal tissues was composed of a glycoprotein/oestrogen glycocalyx and that this was responsible for the protection of the foetus. This would suggest that the placental gonadotrophins occur in all viviparous animals, that they are located at the areas of contact between fetal and maternal tissues, and that their removal would be followed by a maternal immune response directed against the foetus.

Taking into account the results obtained in this study, the arguments suggesting that these degenerative changes observed may by construed as a maternal immunological attack directed against the fetus and the fact that the present studies did not involve any experiments designed to test the contribution of oestrogen to the glycocalyx as proposed by Amoroso and Perry (1975), one can argue that strong grounds for believing that the hypothesis of Amoroso and Perry holds exist, but the final proof awaits further investigations.

This work can be advanced further with design of experiments which can provide information on the maternal immune responses, specifically cell-mediated ones, during pregnancy. One of the experiments currently underway in the guinea-pig (Ph.D. project,

University of Nottingham - Bambra, Amoroso, Foxcroft, Robinson, Carter, Butt and Lynch) is that originally carried out by Borland and co-workers (Borland et al, 1974; Loke et al, 1971) who analysed the killing effect of rabbit lymphocytes sensitized against trophoblast target cells and showed that human trophoblast cells were not susceptible to this killing action. If, however, the hCG layer around trophoblastic cells was first removed by enzymatic action, then they were killed by sensitized lymphocytes. When such trophoblast cells were treated with hCG after exposure to the enzyme, their resistance was restored. Similar results are being observed in the guinea-pig studies. The presence of the β-hCG-like material in the guinea-pig has already been established and the syncytiotrophoblast has been shown to synthesise it (Bambra, Foxcroft, Amoroso, Robinson and Lynch, 1980a, b). This type of experiment has to be carried out on goat, hyrax and rat. Cane rat, because of its sub-placental localisation of gonadotrophins has got to be studied in more detail, as it did not show a specific feto-maternal localisation, It would be interesting to immunise this animal with antibodies and examine the effects of this passive immunisation. Experiments designed to test the immunosuppressive role of oestrogens (if any) have got to be carried out. The material identified as a gonadotrophin needs to be extracted and analysed to examine its structure in comparison to established placental gonadotrophins. Such studies are currently being done on the guinea-pig.

It can thus be concluded that PMSG/hCG-like gonadotrophin is produced by the chorionic cells of the rat, cane-rat, spring-hare and the hyrax, whilst those of the goat produce a PMSG-like substance. In all of these animals except the cane-rat, gonadotrophin-like

material is interposed between the fetus and the mother constituting a barrier. Neutralization of the "gonadotrophin" leads to degenerative changes which start with the destruction of the feto-maternal junction. These gonadotrophins may be involved in regulating the synthesis and metabolism of steroid hormones, both at the ovarian and hepatic levels.

APPENDIX 1

Animals used in this investigation

WISTAR RAT (Rattus rattus)

ROCK HYRAX (procavia capensis)

AFRICAN CANE-RAT .(Thryonomys swinderianus)

AFRICAN SPRING-HARE (Pedetes capensis)

GOAT (Capra hircus)

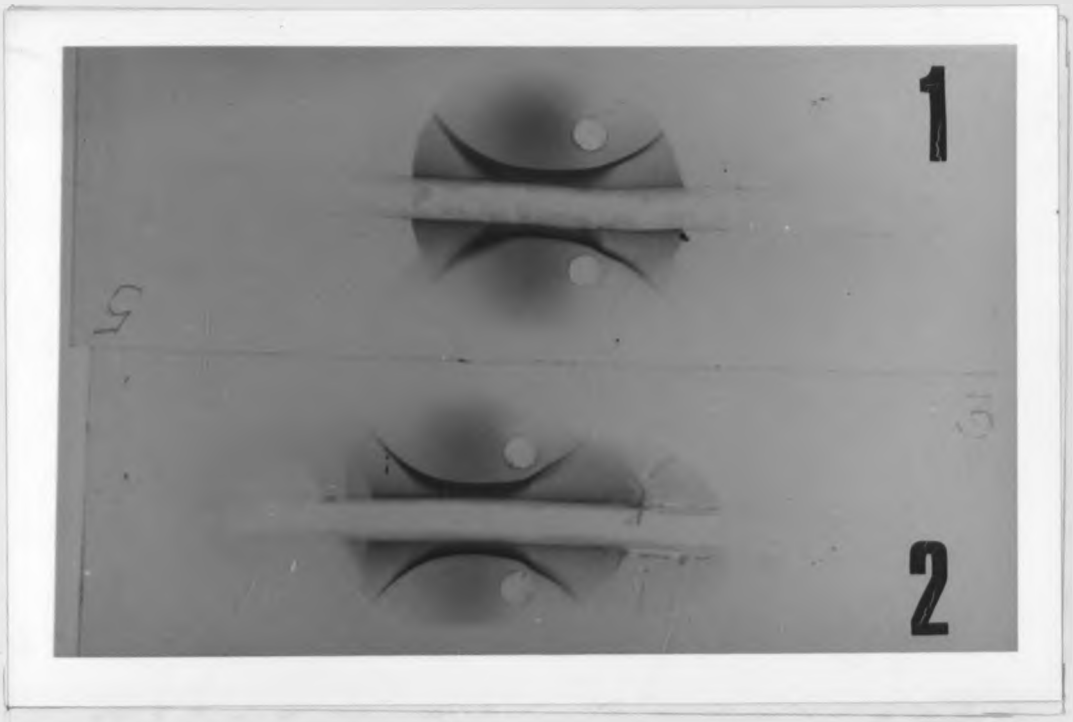
DIK-DIK (Madaqua curtz)

APPENDIX 2

Immunological testing of antibodies

Photograph of an immunoelectrophoresis plate confirming the presence of IgG, in anti-hCG (1) and anti-PMSG (2)

Photograph of an Ouchterlony plate showing presence of antibodies. Centre well contains either hCG or PMSG (1), the remaining wells contain serial dilutions of the respective pure IgG (2-6)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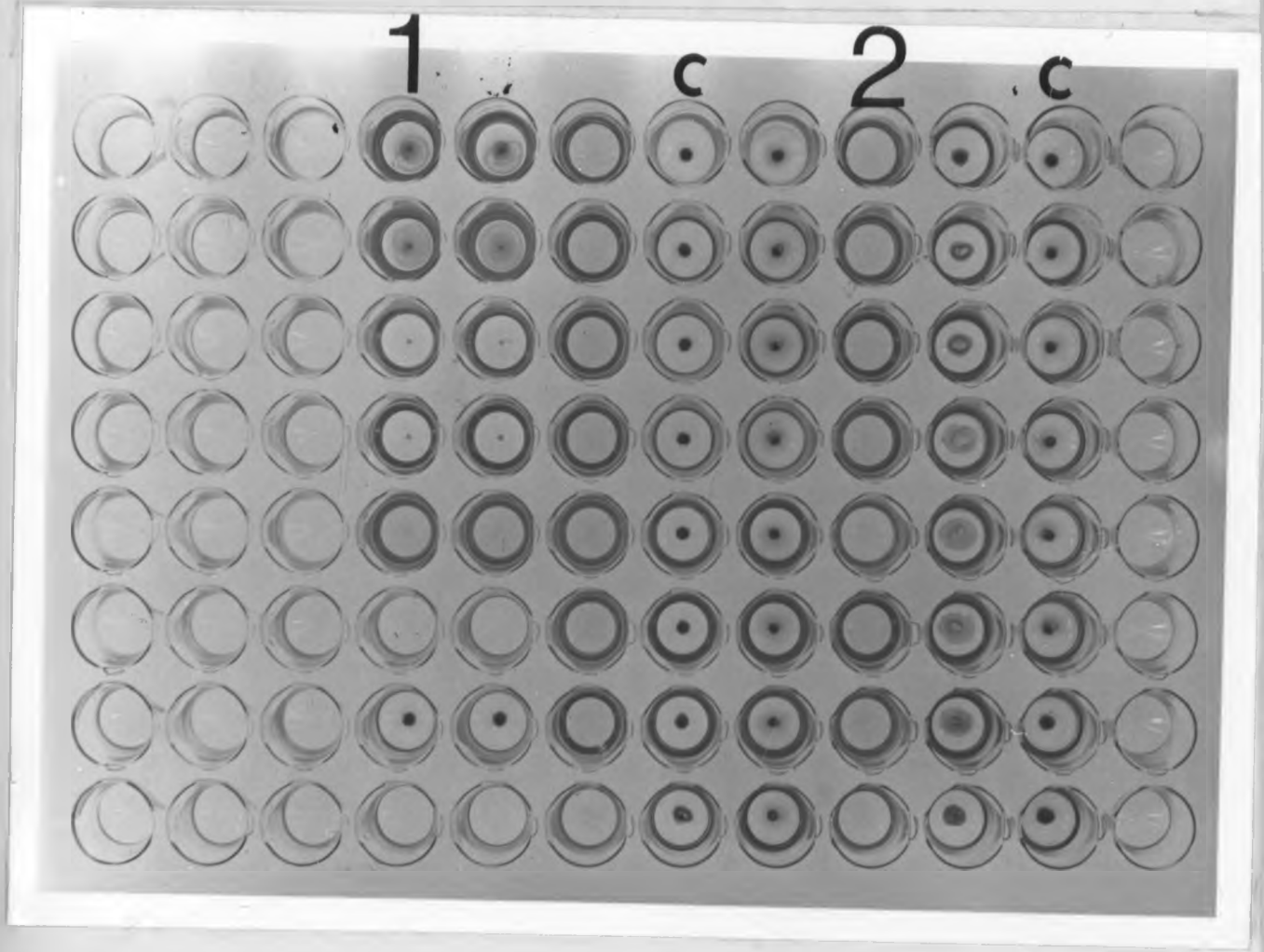
Photograph of passivehaemmaglutination plate for PMSG testing. Positive wells (1 and 2) and control wells (c) are shown

Photograph of the hCG test plate. Set of wells 1-4 contains test antisera, while the remaining wells contain heated control antisera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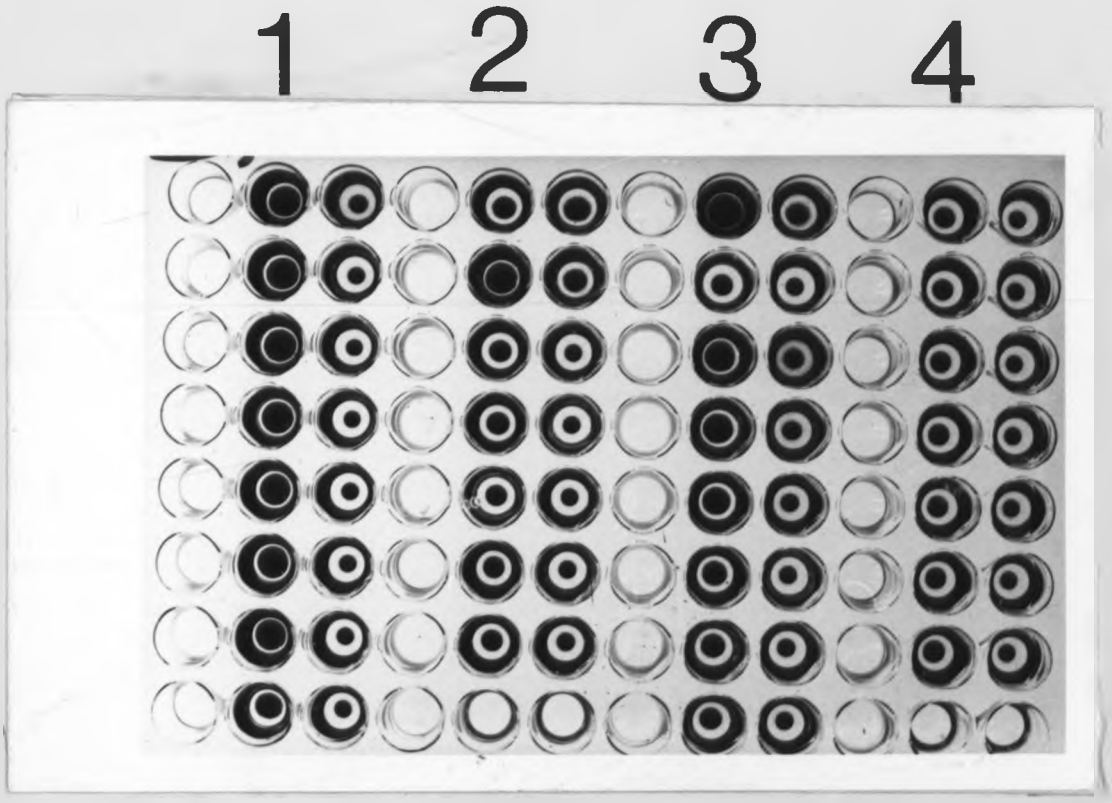
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APPENDIX 3

Apparatus designed to measure conductivity of the buffer

Photograph of the apparatus showing the milliamp-meter (1), the 6V power supply (2), the on-off switch (1), the testing beakers for simultaneous comparison of conductivities (4,5), the cross-over switch to monitor conductivity of either beaker 4 or beaker 5 (6), and the power adjustment rheostat (7).


MILLIAMPERES
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READY
READY
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4
5

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