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ACTION OF PROSTAGLANDIN $F_{2\alpha}$ DURING EARLY PREGNANCY IN THE MOUSE

by

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To two very special people-

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ABSTRACT

The prostaglandins are a unique group of biologically active compounds that appear to be present in, or at least can be elicited from, all body tissues. Moreover, they have been implicated in numerous physiological processes. In particular, reproductive functions in the female have received considerable attention. Prostaglandin $F_{2\alpha}$ is capable of inducing a decidual reaction when instilled into uteri of prepubertal rats primed with progesterone while elevated levels of E and I prostaglandins are present at implantation sites.

To investigate the role of $PGF_{2\alpha}$ in implantation and early embryonic development, pregnant Swiss-Webster mice were injected subcutaneously with a 100 µg dose of $PGF_{2\alpha}$ on days 4.5 and 5.5 or 5.5 and 6.5 p.c. and killed 24 hours later by intracardiac perfusion with Karnovsky's fixative. A central slide of each implantation site was removed, processed and embedded in Araldite. Thick sections were cut on an LKB ultramicrotome and stained with toluidine blue. Seventy-five percent and 43% of implantation sites appeared abnormal when examined on days 6.5 and 7.5 p.c. respectively. Changes which were observed included degeneration of the conceptus and decidua, and leucocytic infiltration. Subsequently, groups of pregnant mice were treated with a 50 µg, 100 µg or 200 µg dose of prostaglandin $F_{2\alpha}$ on day 3, 4, 5 or

6 p.c. Animals were killed 24 hours before parturition or 12 hours after treatment. In the latter case, implantation sites were removed and prepared for electron microscopic examination.

$PGF_{2\alpha}$ treatment on day 5 p.c. caused a significant reduction in mean fetal weight in all groups at term. After a dose of 200 µg/animal on day 3 or 6 p.c., mean fetal weights were also significantly reduced. Placental weights showed no consistent alterations following $PGF_{2\alpha}$ treatment. Litter size was significantly reduced after 50 µg or 100 µg $PGF_{2\alpha}$ on day 6 p.c., while the number of resorptions was increased after treatment on day 6 p.c. at a dose of 50 µg/animal. No changes were detected in the number of dead fetuses. Light microscopic examination of the placentae showed dilated labyrinthine channels associated with $PGF_{2\alpha}$ treatment. Implantation sites from animals that received 50 µg $PGF_{2\alpha}$ on day 4 p.c. showed numerous intraepithelial spaces beneath the blastocyst and deficient decidual reaction around the implantation chamber. Animals treated with 100 µg $PGF_{2\alpha}$ on day 4 p.c. exhibited incomplete formation of the implantation chamber and alterations in the association between the blastocyst and uterine epithelium. At the cellular level, the trophectoderm and endoderm displayed dense lysosome-like bodies, rarefied cytoplasmic and nuclear areas and decreased numbers of microvilli. Occasional electron dense cells were also encountered. In animals treated on day 5 p.c. with 50 µg or 100 µg $PGF_{2\alpha}$,

some egg cylinders were enlarged and the yolk sac cavity was obliterated. Few changes were observed after prostaglandin treatment on day 6 p.c.

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1. INTRODUCTION

Discovery of the ability of human seminal fluid to stimulate smooth muscle and the identification of prostaglandins as the active agents have provided the initial impetus for investigations of the roles and actions of these compounds in virtually every system in the body.

Clinically, the prostaglandins have proven effective as abortifacients due to their ability to stimulate the myometrium during the first and second trimesters of pregnancy, obviating surgical intervention. Modification of the chemical structure of the parent prostaglandins protects against enzymatic degradation and alleviates some of the undesirable side effects. Recent investigations have also shown that these compounds induce menstruation; ultimately analogues may prove effective as a "morning-after" pill.

Prostaglandins occur ubiquitously in the body and have many physiological functions at the cellular level. However, no consistent mode of action has been elucidated. Different prostaglandins may have either a stimulative or suppressive effect on the action of hormones and it is this opposing influence of these compounds that has proven most inexplicable. Different prostaglandins may have reverse actions in the same tissue; likewise a prostaglandin may have reverse actions in different tissues. Comparing

studies of the role of prostaglandins, consideration must be given not only to the effect of these compounds in producing a response but also to variations in the latter due to changes in endogenous prostaglandin production. Thus estrogen-induced luteolysis during the progestational phase in the Rhesus monkey increases prostaglandin production by the ovary, whereas inhibition of prostaglandin production prevents luteolysis (Auletta et al., 1978). Furthermore, administration of prostaglandin or inhibition of PG-production with indomethacin does not necessarily produce opposite effects at the tissue level. When dealing with prostaglandin treatment in vivo, reverse actions may occur with different concentrations. In fact, administration of varying dose levels provides the most meaningful results for both in vivo and in vitro investigations.

The importance of prostaglandins in reproduction appears obvious since these compounds were first identified in semen. Subsequently, their role in female reproductive processes has been extensively examined. In particular, the prostaglandins play a role in ovulation and luteal regression in rats, guinea pigs, mice and rabbits. In addition, endometrial tissue is capable of producing prostaglandins and these compounds may be the inductive factor for stimulating the decidual cell reaction. Elevated levels of prostaglandins are present in the serum and at implantation sites during early pregnancy in rodents. Moreover, the blastocyst itself

may be capable of producing prostaglandins. Yet the role of these compounds in implantation and development is obscure.

The present study was undertaken to examine the:

i) abortifacient action of prostaglandin $F_{2\alpha}$ during early pregnancy in mice.

ii) cellular aspects of $PGF_{2\alpha}$ action in implantation and blastocyst-uterine interaction.

iii) placental and ovarian changes near term following prostaglandin treatment.

2. REVIEW OF LITERATURE

2.1 EMBRYOLOGY OF THE MOUSE

Robinson (cited by Jenkinson, 1900) and other early investigators (Born, 1892; Duval, 1895; Jenkinson, 1900) examined blastocyst development to approximately the time of mesoderm formation; while Huber (1915) examined a similar stage of development in the rat embryo. The accuracy of these early investigations is underscored by the fact that most subsequent studies of mouse or rat embryology have focused on a particular aspect of development rather than a re-examination of previous observations.

Fertilization of the mouse ovum occurs in the ampulla of the oviduct and morulae or early blastocysts enter the uterus approximately 72 hours later (Biggers et al., 1971). At ninety-six hours post coitum the blastocyst lies in a uterine crypt (Snell and Stevens, 1966) at the antimesometrial side of the uterine lumen (Mossman, 1937). The zona pellucida has been lost and the uterine lumen has become occluded (Potts, 1968). The manner in which the blastocyst is tightly clasped by the uterus forms the implantation chamber for the rat conceptus (Enders, 1975). Similar associations develop in the mouse (Nilsson, 1974).

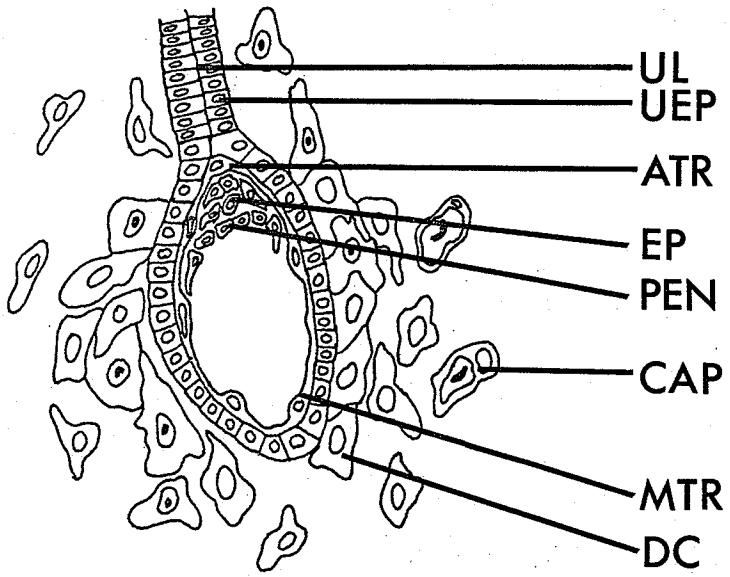
Development of the mouse blastocyst has been divided into four substages (Nadijcka and Hillman, 1974). Initial development of the blastocoele (substage 1) and elongation of trophectoderm cells (substage 2) overlying the inner

cell mass occurs before hatching from the zona pellucida thus before the initial phases of implantation. Cytological differentiation is first evident at substage 3 with development of the endoderm along the blastocoelic surface of the inner cell mass. These endoderm cells exhibit microvilli and increased numbers of cisternae of endoplasmic reticulum (Enders et al., 1978). Substage 3 is associated with hatching of blastocysts. Finally substage 4 is marked by further flattening of trophectoderm cells over the epiblast and extension of the endoderm peripherally as the parietal endoderm beneath the mural trophoblast. A basal lamina lines the juxtacoelomic surface of the trophoblast. At this stage of development, the blastocyst consists of three basic tissues: trophoblast, epiblast and endoderm (Fig.1). Recent investigations have indicated the relationship between these. High cell proliferation rates occur in the mouse trophectoderm overlying the inner cell mass, and it has been suggested that the latter induces this increased mitotic rate (Copp, 1978). Labelling experiments of trophectoderm cells at the mesometrial point of the blastocyst have indicated that these cells move away from the inner cell mass to become mural trophoblast cells (Copp, 1979). Consequently, trophoblast at the abembryonic pole represents the oldest population of these cells and it is from this region that the primary giant cells which have high levels of Δ^5-3β-hydroxysteroid dehydrogenase (Chew and Sherman, 1975) first form (Dickson, 1963).

Figure 1.

Blastocyst lying in the implantation chamber. Gestational age is four and a half days p.c. UL, closed uterine lumen, UEP, uterine epithelium, ATR, apical trophectoderm cell, EP, epiblast, PEN, primitive endoderm, CAP, endometrial capillary, MTR, mural trophoblast, DC, primary decidual cell, M, mesometrial direction (after Enders and Schlafke, 1967, with additions).

M

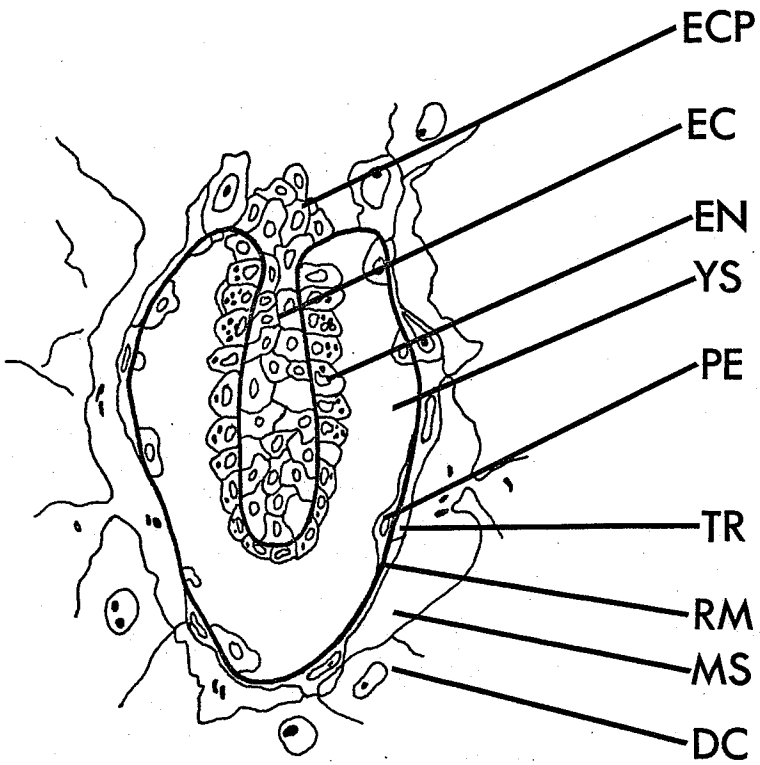


Functional differences between cells of the trophoblast and inner cell mass have been demonstrated before the blastocyst implants. Isolated trophoblastic vesicles are capable of stimulating a decidual cell reaction, and implanting when transferred to uteri of pseudopregnant mice, whereas isolated inner cell masses are incapable of producing such a response (Gardner, 1972; Surani and Barton, 1977). In addition, cells of the inner cell mass have been shown incapable of producing trophectoderm cells even when exposed to a similar environment as outside cells of the blastocyst (Rossant, 1975a; Rossant and Lis, 1979); this mass of cells is capable of endoderm formation when isolated and placed in the mouse oviduct in vivo (Rossant, 1975b).

Following initial apposition and adhesion of the blastocyst in the implantation chamber (Schlafke and Enders, 1975), transformation of the blastocyst into the egg cylinder by germinal inversion occurs (Fig. 2). Until this point in development there is no increase in total volume of the conceptus (Snell and Steven, 1966). Prior to implantation, as noted previously, trophectoderm cells over the inner cell mass move toward the mural trophoblast regions. However, immediately after implantation, mitotic activity in embryos is very intense (Snow, 1976). Copp (1978) suggested that size constraints imposed on the blastocyst at implantation and the continued proliferation in trophectoderm over the inner cell mass accounts for the antimesometrial growth and development of the egg

Figure 2.

Typical egg cylinder structure at five and a half days of gestation. ECP, ectoplacental cone, EC, ectoderm, EN, endoderm, YS, yolk sac cavity, PE, parietal endoderm, TR, trophoblast, RM, Reichert's membrane, MS, maternal sinus, DC, decidual cells (after Enders and Schlafke, 1967, with additions).


ECP
EC
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cylinder. Consequently cells accumulate over the inner cell mass forming the extra-embryonic regions. This is substantiated by the demonstration of melanin-labelled apical trophectoderm cells of the blastocyst occuring in the extra-embryonic ectoderm of the egg cylinder (Copp, 1979). Consequently the egg cylinder develops as a core of ectoderm lined peripherally by the visceral endoderm. Embryonic and extra-embryonic regions can be distinguished. Early egg cylinders show small ectoplacental cones but well developed regions antimesometrially (Copp, 1979) which indicates growth in this direction while the conceptus is tightly clasped by the uterus. Subsequent extension of the ectoplacental cone toward the uterine lumen is associated with loosening and phagocytosis of the uterine epithelium by trophoblast of the cone mesometrially from the egg cylinder (Enders and Schlafke, 1967).

Endoderm lines the outer aspect of the ectoderm in both embryonic and extra-embryonic regions. Regional variations in structure have been described (Solter et al., 1970). Recent injection experiments using isolated endoderm and ectoderm cells from the blastocyst have indicated that primitive endoderm gives rise only to extra-embryonic structures; endodermal derivatives in the fetus arise from ectoderm (Gardner, 1976; Gardner and Rossant, 1979).

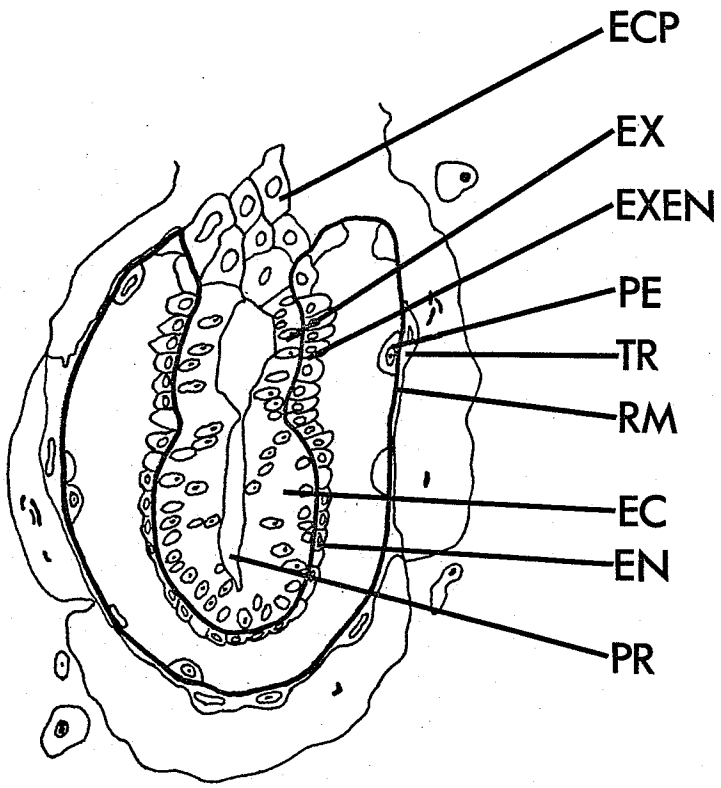
A basal lamina separates visceral embryonic and extra-embryonic endoderm from the ectoderm and is reflected as Reichert's membrane to enclose the yolk sac cavity around the egg cylinder (Reinius, 1965b). Occasional parietal endoderm

cells lie along the embryonic side of Reichert's membrane and extend processes along this surface (Enders et al., 1978). The peripheral surface of this membrane is lined by a single layer of trophoblast (Snell and Stevens, 1966). The maternal blood sinus or periembryonic sinus is formed around the conceptus at this point. Thus, the egg cylinder is separated from the maternal circulation by a parietal yolk sac composed of these three layers, parietal endoderm, Reichert's membrane and trophoblast. Various substances including peroxidase, invertase and trypan blue have been shown to cross this barrier readily (Schultz, 1966; Seibel, 1974; Batten and Haar, 1979a;Beck, 1979).

Following the egg cylinder stage, the proamniotic cavity forms in the centre of the ectodermal core (Fig. 3) and amniotic folds subsequently divide amniotic, exocoelomic and ectoplacental cavities. At this stage of gestation (approximately 160 hours p.c.) distinct morphological differences are evident in visceral extra-embryonic and visceral embryonic endoderm (Batten and Haar, 1979b). The conceptus is still surrounded by the parietal yolk sac. However parietal endoderm cells are less frequently encountered than at previous stages resulting in many regions of a barrier formed only by trophoblast and Reichert's membrane separating maternal blood sinus from the yolk sac cavity. The thickness of the trophoblast lining the membrane has been variously reported as one or two cells in width (Reinius,1965a) to non-existent (Reinius, 1965b) in the mouse. While the close association of these cells and

Figure 3.

Embryo of six and a half days gestation. ECP, ectoplacental cone, EX, extra-embryonic ectoderm, EXEN, extra-embryonic endoderm, PE, parietal endoderm, TR, trophoblast, RM, Reichert's membrane, EC, embryonic ectoderm, EN, embryonic endoderm, PR, proamniotic cavity (after Snell and Stevens, 1966, with additions).


ECP
EX
EXEN
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TR
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Reichert's membrane has led investigators to suggest an ectodermal origin for the latter (Wislocki and Padykula, 1953), the demonstration of material similar to that of this membrane in parietal endoderm cells would indicate the endoderm as tissue of origin (Pierce et al., 1962; Jollie, 1968).

Mesoderm formation first occurs at this time as cells delaminate from the ectoderm at the posterior junction of embryonic and extra-embryonic regions (Snell and Stevens, 1966). Subsequently, with formation of the exocoelom, allantoic insertion occurs and development of the chorioallantoic placenta is initiated.

2.2 IMPLANTATION

2.2.1 Morphological Aspects

Numerous studies have examined the interaction of blastocyst and endometrial epithelium in both the rat and mouse. Schlafke and Enders (1975) identified stages of apposition and adhesion which occur as the first interaction between embryonic and maternal tissues at the implantation site. Apposition indicates the commencement of implantation and although luminal closure occurs around the blastocyst variable degrees of this stage are present during delayed implantation in the mouse (Nilsson, 1974). Following estrogen stimulation, adhesion involves the entire outer surface of the blastocyst in which the microvilli of the

uterine epithelium lose their typical shape and appear as bleb-like structures (Nilsson, 1966). Regions of primitive junctions take the form of densities of opposed membranes of trophoblast and epithelium in the mouse (Smith and Wilson, 1974) or simple regions of close membrane apposition in the rat (Enders and Schlafke, 1969). Penetration of the uterine epithelium by the trophoblast is the next step in the process of implantation. In the mouse and rat, trophoblast remains associated with the uterine epithelium for approximately one day (Schlafke and Enders, 1975). Subsequently, implantation in these rodents proceeds by a method termed displacement (Schlafke and Enders, 1975) in which the uterine epithelium is lost and phagocytosed by the trophoblast (Finn and Lawn, 1968). Attempting to determine a reason for sloughing of the epithelial cells, Smith and Wilson (1971) found a prominent border of lysosomes in the trophoblast cytoplasm at the light microscopic level. However several other investigators (Bergstrom, 1970; El-Shershaby and Hinchliffe, 1975) were unable to detect increased lysosomal activity of the trophoblast associated with epithelial penetration and displacement. Ultrastructural histochemical studies in the rabbit (Abraham et al., 1970) similarly found few alterations in acid phosphatase activity in the blastocyst during implantation but did detect increasing levels of activity in the uterine epithelium. Moreover, electron microscopic studies have failed to demonstrate a border of lysosome-like structures in either the mouse

(Reinius, 1967; Potts, 1968; Kirby, 1971; Nilsson, 1974; Nilsson and Lundkvist, 1979) or the rat (Nilsson, 1967; Enders and Schlafke, 1969; Tachi et al., 1970). Rather, evidence for an autolytic activity of the epithelium at least in the mouse and rat with accumulation of lipid and numerous dense bodies which are acid phosphatase-positive has been found (El-Shershaby and Hinchliffe, 1975). In support of this hypothesis, Finn and Bredl (1973) have shown that inhibiting transcription with actinomycin D during early implantation prevents the characteristic sloughing of the uterine epithelial cells.

In association with the implanting blastocyst, Wilson (1963) demonstrated the presence of "primary invasive cells" which were passed from the mouse blastocyst to the uterus and played a role in the implantation process. Subsequently, these structures have been shown probably to be epithelial cells which have degenerated singly and were phagocytosed by the trophoblast (Finn and Lawn, 1968; Smith and Wilson, 1974); portions of these structures appeared as dense bodies lying centrally in the trophoblast cell.

Following dislodging of the uterine epithelium, trophoblast penetrates to the basal lamina where in the mouse and rat it appears to pause (Schlafke and Enders, 1975). Subsequently the basal lamina disappears (Tachi et al., 1970) but the association between trophoblast which is still cellular in the rat (Enders and Schlafke, 1967) and the decidua is uncertain. An antigenic fibrinoid barrier