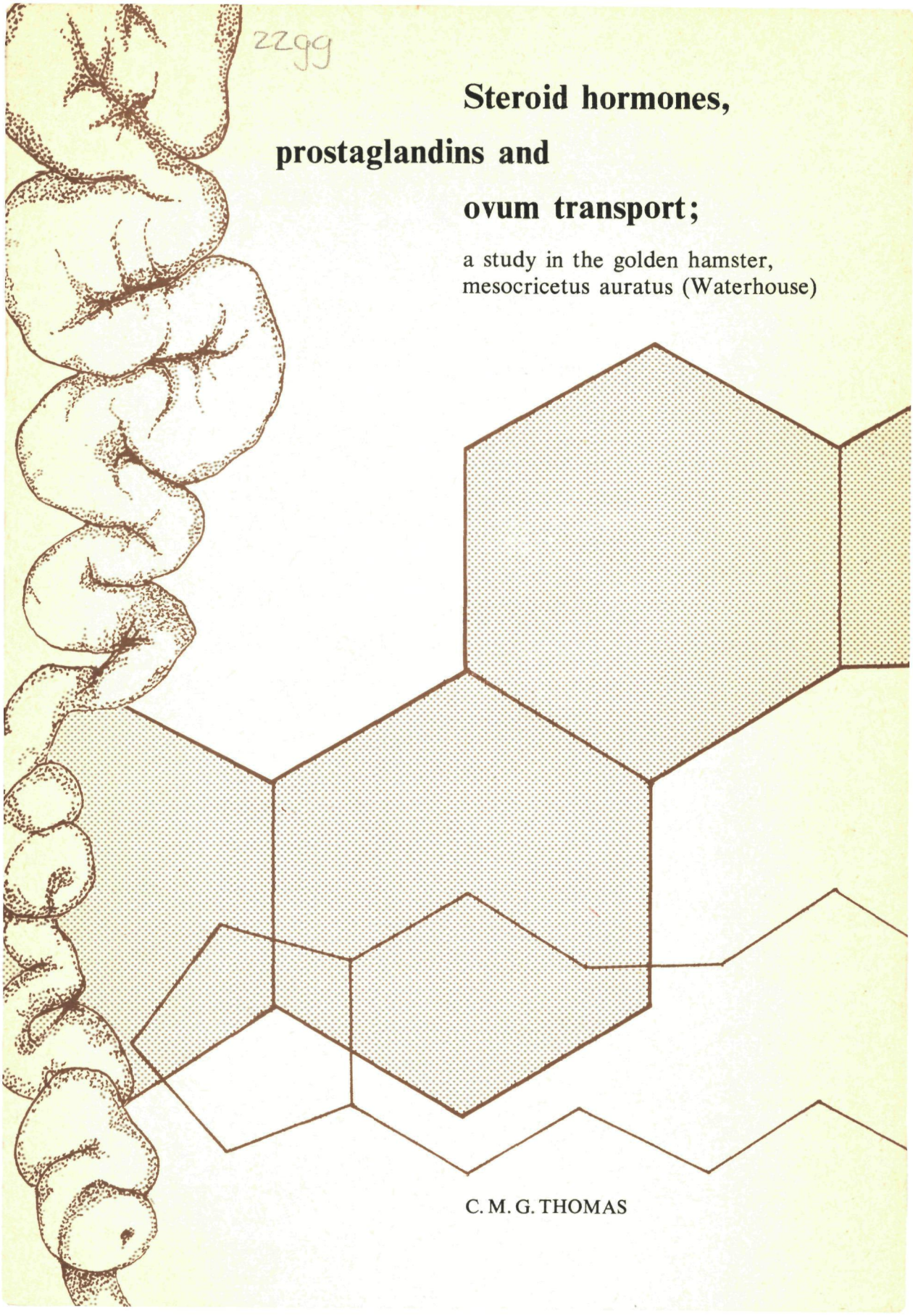
Steroid hormones, prostaglandins and ovum transport;

a study in the golden hamster, mesocricetus auratus (Waterhouse)



C. M. G. THOMAS

STEROID HORMONES, PROSTAGLANDINS AND OVUM TRANSPORT; A STUDY IN THE GOLDEN HAMSTER, MESOCRICETUS AURATUS (WATERHOUSE)

PROMOTOR

PROF DR T K A B ESKES

CO-REFERENTEN

DR L A BASTIAANS

DR R ROLLAND

STEROID HORMONES, PROSTAGLANDINS AND OVUM TRANSPORT; A STUDY IN THE GOLDEN HAMSTER, MESOCRICETUS AURATUS (WATERHOUSE)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN
DOCTOR IN DE GENEESKUNDE
AAN DE KATHOLIEKE UNIVERSITEIT TE NIJMEGEN
OP GEZAG VAN DE RECTOR MAGNIFICUS
PROF DR A J H VENDRIK
VOLGENS BESLUIT VAN HET COLLEGE VAN DECANEN
IN HET OPENBAAR TE VERDEDIGEN OP VRIJDAG 1 DECEMBER 1978
DES NAMIDDAGS TE 4.00 UUR

DOOR

CHRISTIANUS MARIA GERARDUS THOMAS

GEBOREN TE HEERLEN

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Het onderzoek, dat in dit proefschrift is beschreven, werd verricht in het Laboratorium (hoofd: Drs. P. C. W. Houx) van de Kliniek voor Gynaecologie en Obstetrie (hoofden: Prof. Dr. J. L. Mastboom en Prof. Dr. T. K. A. B. Eskes) van het St. Radboudziekenhuis, Katholieke Universiteit te Nijmegen. De experimenten met proefdieren werden verricht door Dr. L. A. Bastiaans, verbonden aan bovengenoemd laboratorium. De voorbereiding van de experimenten en de bewerking van de verkregen resultaten vond plaats in de Afdeling Gynaecologische Endocrinologie en Infertiliteit (hoofd: Dr. R. Rolland) en werd kritisch begeleid door Dr. L. A. Bastiaans en Dr. R. Rolland.

Door veel personen en instanties werd een bijdrage geleverd in het tot stand komen van dit proefschrift. Voor al deze hulp ben ik iedereen zeer erkentelijk. Met name wil ik noemen:
De heer J. Jansen, die een groot gedeelte van de dierexperimenten zeer vakkundig heeft verzorgd.
Mej. H. W. M. Knaps en de heer M. G. Heijman, die de steroidbepalingen mee hebben ontwikkeld en zorgvuldig de talrijke radioimmunoassays hebben uitgevoerd.
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De benodigde faciliteiten voor het dierexperimentele werk werden door het Centraal Dierenlaboratorium (hoofd: Dr. W. J. I. v.d. Gulden) ter beschikking gesteld.
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De heer P. J. B. A. Eisenburger heeft de omslag ontworpen.
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Aan José, Marcel en Eveline

CONTENTS

ABBREVIATIONS AND SYSTEMATIC NAMES

DPM	desintegrations per minute
ECP	oestradiol cyclopentylpropionate
EDTA	ethylene diaminetetraacetate
FSH	follicle stimulating hormone
g	gram
g	relative centrifugal force
hCG	human chorionic gonadotrophin
IM	indomethacin
indomethacin	(1-p-chlorobenzoyl-5-methoxy-2-methylindol-3-yl) acetic acid
i.p.	intraperitoneal
LH	luteinizing hormone
min	minute
μg	microgram (10^{-6} gram)
ng	nanogram (10^{-9} gram)
OE_2	oestradiol (-17β)
oestradiol (-17β)	1, 3, 5 (10)-oestratriene-3,17β-diol
p	probability
pg	picogram (10^{-12} gram)
PG	prostaglandin
PGE_1	11α,15L-dihydroxy-9-ketoprost-13trans-enoic acid
PGE_2	11α,15L-dihydroxy-9-ketoprosta-5cis, 13trans-dienoic acid
$PGF_{1\alpha}$	9α,11α,15L-trihydroxyprost-13trans-enoic acid
$PGF_{2\alpha}$	9α,11α,15L-trihydroxyprosta-5cis, 13trans-dienoic acid
progesterone	4-pregnene-3,20-dione
RIA	radioimmunoassay
s.c.	subcutaneous
SD	standard deviation
SEM	standard error of the mean
$\bar{X}$	mean value

INTRODUCTION

This study investigates the role of steroid hormones and prostaglandins in oviductal ovum transport of hamsters in the first stage of pregnancy.

For some decades now it has been known that both natural and synthetic oestrogens influence the development of ova and their rate of transport through the oviduct in the case of various species (Burdick and Pincus, 1935; Whitney and Burdick, 1936; Burdick et al., 1937; Burdick and Whitney, 1937, 1938; Burdick and Vedder, 1941; Harrington, 1964; Yanagimachi and Sato, 1968).

Furthermore, the administration of oestrogens to laboratory animals can lead to a complete interruption of pregnancy (Smith, 1926; Dreisbach, 1959; Edgren and Shipley, 1961; Greenwald, 1961b, 1967; Banik and Pincus, 1964; Yanagimachi and Sato, 1968).

Progestational agents are also considered to be involved in the regulation of oviductal ovum transport (Black and Asdell, 1959; Greenwald, 1961a; Chang, 1966, 1967; Chang and Hunt, 1970).

The data reported so far are rather confusing and contradictory. It has been proposed that oestrogens stimulate the contractile frequency of oviductal musculature, while progestins would decrease this frequency (Greenwald, 1961a; Harper, 1964, 1965a, 1965b, 1966; Chang and Harper, 1966; de Mattos and Coutinho, 1971; Chang, 1976).

Other investigators (Boling, 1969, 1971; Boling and Blandau, 1971a, 1971b), however, support the idea that the oviductal musculature is minimally active when high levels of physiological oestrogen concentrations are maintained, but becomes contractile with decreasing oestrogen levels or with increasing progesterone concentrations.

More recent observations indicate that also prostaglandins are involved in a number of reproductive processes in mammals. These substances may cause luteolysis, delayed implantation, both tubal retention of ova and acceleration of tubal transport, enhance sperm transport and the rate of fertilization and also influence mechanisms involved in ovulation (Labhsetwar, 1974). Administration of oestrogens to certain species such as monkeys (Auletta et al., 1972) and guinea-pigs (Blatchley et al., 1971, 1972) is associated with increased blood levels of prostaglandins.

In the present study the well-known effects of oestrogens and the possible role of prostaglandins in ovum transport of the golden hamster are combined in the hypothesis that oestrogens influence this process through alterations in oviductal prostaglandin synthesis.

In order to test this hypothesis, the four-day oestrous cycle and the first four days of pregnancy were studied.

The moment of ovulation during the cycle was defined and the pattern of normal ovum transport was studied in great detail. These processes were related to the plasma levels of oestradiol and progesterone that were measured transversally in groups of hamsters during the two periods of time studied. The prostaglandins in oviductal tissue were measured and the patterns compared with the plasma levels of ovarian steroid hormones that are known to fluctuate, that is to say during the periovulatory period. Furthermore, the effects of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) upon ovum transport and pregnancy were determined following its subcutaneous or intraperitoneal administration. The final tests of the abovementioned hypothesis included the induction of prostaglandin synthesis in oviductal tissue through oestradiol administration, the effect of this treatment on ovum transport itself, and the effects on oviductal transport of inhibited endogenous prostaglandin synthesis following the exogenous administration of prostaglandin synthetase inhibitors.

LITERATURE

1. The anatomy of the genital tract of hamsters

The length of the oviduct of the hamster is approximately 15 mm (Oeri, 1960). This strongly twisted organ can be divided into an infundibulum, ampulla, isthmus and junctura (Figure 1). As is the case with other rodents, the ovaries of the hamster are enclosed in a membranous periovarial sac, the ovarian bursa, which is filled with liquid.

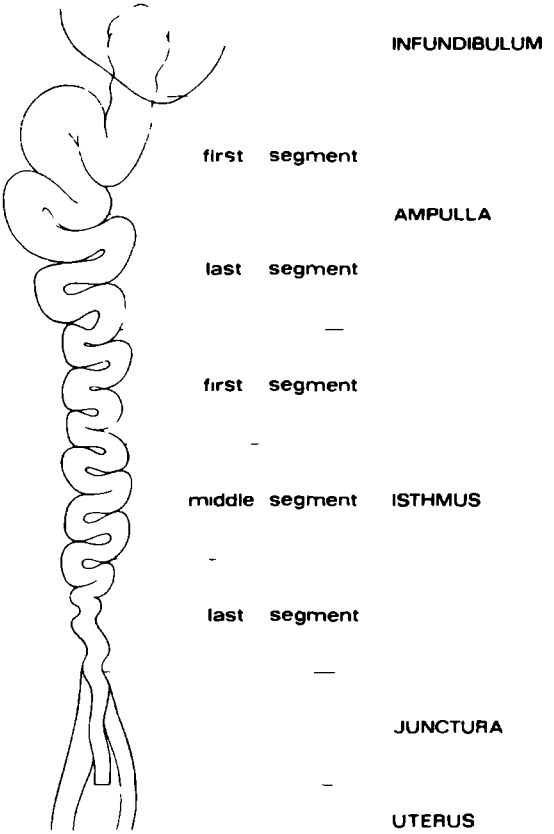

FIGURE 1 Schematic representation of the oviduct of the hamster

The infundibulum or proximal portion of the oviduct terminates in the fimbriated end and projects into this bursa, but it occupies only a small area of the periovarial space. The measure of contact between the fimbriated tip and the surface of the ovary is very limited (Alden, 1942; Wimsatt and Waldo, 1945). The infundibulum, including the fimbriae, does not contain muscular tissue (Oeri, 1960) but is lined with ciliated cells which beat in the direction of the tubal ostium (Blandau and Verdugo, 1976).

The ampulla is connected to the infundibulum and consists of a tunica muscularis with deep longitudinal mucosal folds which are covered by a continuous layer of ciliated epithelial cells (Bastiaans, 1973). The muscular layer of the isthmus is much more developed than that of the ampulla, as is also the case with the circular muscular layer of the junctura.

Like other rodents the hamster has a uterus duplex, consisting of two uterine horns and two cervical channels. These channels remain subdivided for approximately two-thirds of the cervical length before they fuse (Deanesly, 1938).

2. Reproductive cycles and ovulation

The reproductive pattern of mammals may be classified according to the type of ovulation (reflex or spontaneous), and according to the type of cycle (oestrous or menstrual). Both cycles are very similar in nature but major differences are the very short period of sexual receptivity in the oestrous (heat) cycle of rodents, and the menstrual bleeding that occurs in the menstrual cycle of primates. This bleeding is mainly brought about by a sudden change in hormonal levels. Although such hormonal changes also take place in the oestrous cycle, the stimulation of the epithelium is followed by a resorptive process without haemorrhage.

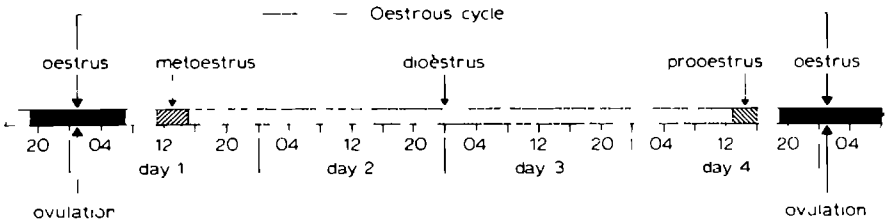

FIGURE 2 The reproductive cycle of female golden hamsters

The golden hamster has an extremely rhythmic oestrous cycle. The period of approximately 96 hours between the onset of one oestrus and the next is similar to that of the other laboratory rodents that are commonly used.

The oestrous cycle is represented in Figure 2 and consists of four main

stages; oestrus (9-15 hours), metoestrus (about 4 hours), dioestrus (75 hours or more) and prooestrus (about 3 hours).

The day of ovulation is often defined as Day 1, the day of the oestrous and metoestrous period. Days 2 and 3 are designated dioestrus 1 and dioestrus 2, respectively, and the day preceding ovulation is Day 4, prooestrus.

The various phases of the oestrous cycle are defined by a number of behavioural patterns, e.g. sexual receptivity which is shown by lordosis and the allowance of intromission, but also by other characteristics, such as the cytology of vaginal smears and the appearance of a vaginal plug (Fox and Laird, 1970). During the oestrous cycle several ovarian follicles pass through a number of successive developmental stages in order to reach maturity. Although many primordial follicles start to grow, only a few will go through all stages and ultimately become Graafian follicles. The number of Graafian follicles that reach their maximum size just prior to ovulation depends on hereditary and environmental factors (Hafez, 1970).

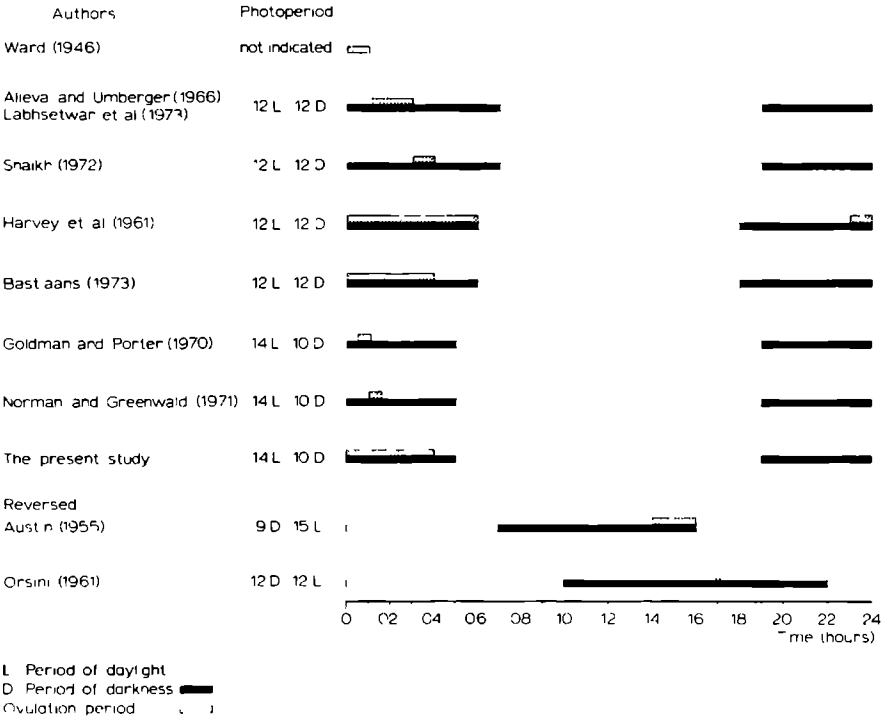

FIGURE 3 Relationship between photoperiod and time of ovulation in various hamster colonies

The time of ovulation in various hamster colonies and its relationship with the period of light as reported in the literature is illustrated in Figure 3. In most of the cases ovulation starts 4 to 6 hours prior to the break of day and ends with the beginning of room illumination, or up to 4 hours earlier. The same events are seen during reversed periods of light. Elongated periods of light of 14 hours per day between 05.00 and 19.00 (Norman and Greenwald, 1971; Seegal and Goldman, 1975) show consistent four-day ovulatory cycles, with an estimated ovulation time between 00.30 and 01.10 as examined by the presence of ova in the oviducts (Goldman and Porter, 1970).

3.The function of the oviduct

The oviduct has the unique function of conveying sperm and oocytes in opposite directions, almost simultaneously, around the time of ovulation (Hafez, 1973).

Processes essential to reproduction that have been shown to occur in the oviducts of various species are the picking up of oocytes and the transport of these cells and of the sperm to the site of fertilization. It also provides a temporary environment for the conditioning of sperm by means of capacitation, and of oocytes prior to fertilization. Following fertilization the oviduct supports the further development of the ovum during the period between ovulation and implantation when ovum transport takes place (Mastroianni and Komins, 1975).

4. Oviductal ovum transport

Recently, in a number of reviews edited by Pauerstein (1975a) and by Harper et al. (1976), a compilation was made of the present knowledge of gamete transport through the oviduct from the ovulation site to the site of implantation. The experimental studies discussed in these reviews deal with an impressive variety of factors that play a part in the above processes and they render a wealth of information. Even so, it is still impossible to give a complete explanation of the mechanism of gamete transport for any single mammal (Blandau and Verdugo, 1976).

Oviductal ovum transport till implantation takes place about 3 to 4 days in the case of most species and is fairly constant for each of them (Bennett, 1976). However, the opossum with 24 hours and the dog for which this process takes as long as 8 to 10 days (Andersen, 1927) are exceptions to this rule.

The pattern of ovum transport has been studied in a discontinuous way, but this pattern has also been studied by continued direct observation of ova in situ. The former approach uses a reasonably small number of points localizing the ova at selected intervals of time. It has been employed for a number

of species (Humphrey, 1968; Bastiaans, 1973; Pauerstein et al., 1974).
The second, continuous manner of observation has been applied to the study of ovum transport in the case of the rabbit. Several motion pictures (Harper, 1961a, 1961b; Blandau and Boling, 1973) show that net forward movement of ova in the oviduct of the rabbit is not a smooth continuous or intermittent progression but the result of a back and forth motion. After release of the oocytes from the ovarian surface, they are passively transported to the tubal ostium. The ovaries are in constant motion within the ovarian bursa due to contractions of the mesovarium. The liquid in the bursa is also in constant motion, causing the passive transport of the oocytes to the infundibulum (Blandau, 1961).
After arrival at the ostium the oocytes are quickly transported to the distal part of the ampulla. This transport is brought about by the ciliated epithelium and the abovarial contractions of the ampulla. Here, the oocytes are fertilized and stay in the ampulla for a relatively long period. The duration of this period is 15 hours for the hamster (Bastiaans, 1973), 24 hours for the mouse (Burdick et al., 1942; Humphrey, 1968), 30 hours for the guinea-pig (Squier, 1932), and as long as 48 hours for the rabbit (Greenwald, 1961a). Then, the ova start to traverse the isthmus fairly rapidly. Transport is due to contractions of the circular muscle. The stimulus for these contractions is the distention of the isthmus by accumulated fluid and by the presence of the ova themselves. They are moved rather vigorously in the isthmic lumen and retained in the final segment, until the utero-tubal junction sufficiently relaxes and a peristaltic rush carries them through the directional flow of luminal fluid into the uterine cavity (Hafez, 1973).
The average duration of ovum transport across the oviduct in the hamster is about 55 hours (Bastiaans, 1973). This period is shorter than that of other laboratory animals such as the rabbit (Greenwald, 1961a), mouse (Humphrey, 1968), guinea-pig and rat in which this process takes about 60, 72, 82 and 96 hours, respectively (Bennett, 1969).
Implantation in the hamster has generally finished 87 hours after ovulation (Bastiaans, 1973).

5. Factors involved in ovum transport

Several anatomical and physiological mechanisms play a part in the control of ovum transport (Hafez, 1976). Factors that can be mentioned here are a possible functional integrity of the ampullary-isthmic and utero-tubal junction, the rheology and hydrodynamics of oviductal secretions, ciliary movement, and the contractile activity of oviductal musculature and ligaments (Croxatto and Ortiz, 1975; Pauerstein, 1975b, Hafez, 1976). Pharmacological

and neural mechanisms are also involved, but the relative importance of each is unknown (Hafez, 1976). Mechanisms suggested in attempts to explain the rate of ovum transport are focused on oviductal secretions (Koester, 1969, 1970), muscular activity (Boling, 1969) and isthmic resistance to ovum passage (Pauerstein, 1974).

Koester (1969, 1970) postulated that the speed of oviductal ciliary activity and the rate of flow for oviductal secretions work together in controlling the process of ovum transport. This theory includes increased ciliary beating due

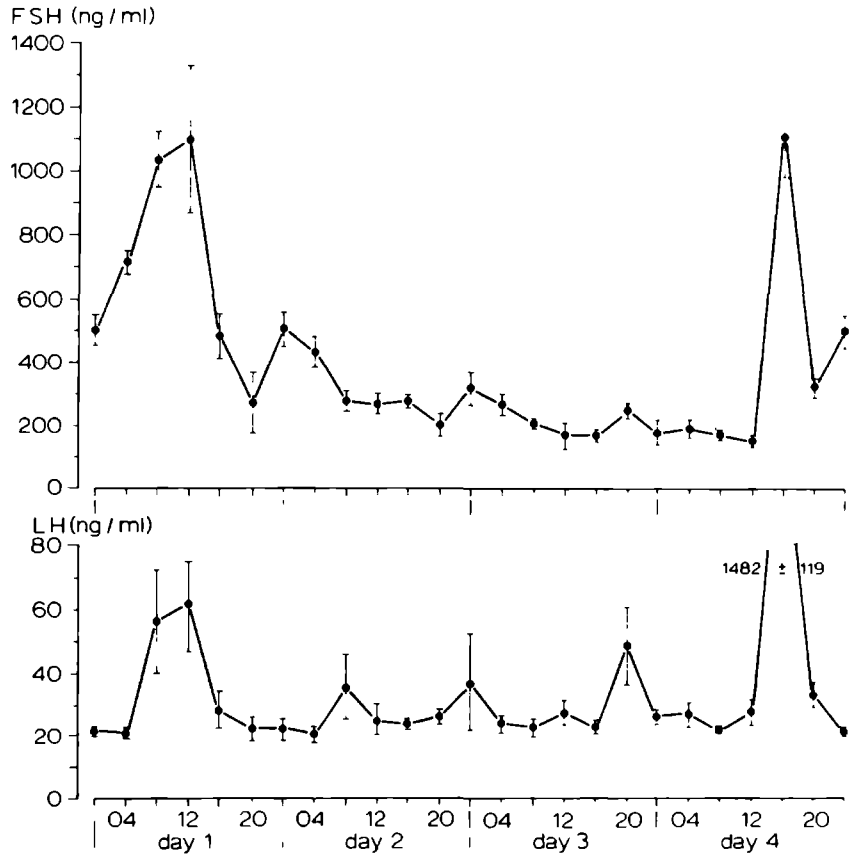

FIGURE 4 Serum FSH en LH concentrations* at 4h intervals during the oestrous cycle of the hamster (Data from Bex and Goldman, 1975)

* Mean ± SEM

to rising progesterone levels on the third postovulatory day, but the hypothesis discounts oviductal muscular activity as a regulator of ovum transport.
Pauerstein (1974) was the first to suggest an explanation for the mechanism of oviductal ovum transport that emphasizes the isthmic delay during this process. The dense adrenergic innervation of the oviductal isthmus indeed supports the idea that this part may act as an adrenergic sphincter. Experimental results of Howe and Black (1973), Hodgson and Eddy (1975), and Moawad et al. (1976) support the hypothesis of Pauerstein (1974) that oestrogens enhance alpha adrenergic activity, with concomitant isthmic constriction. This leads to an arrest of ova in the oviductal isthmus.
Corpus luteum formation after ovulation results in production of progesterone. The increased progesterone levels on the third postovulatory day are high enough to enhance the beta adrenergic receptor activity to such an extent that the isthmic constriction is released, thus allowing the ova to move into the uterus. However, on the basis of additional studies, Pauerstein (1975b) also concluded that adrenergic mechanisms play a relatively minor role in the regulation of normal ovum transport. Furthermore, he suggested that these mechanisms are apparently involved in the pharmacologic effects of oestrogens and progesterone on ovum transport (Pauerstein, 1975b).
The mechanisms for the control of ovum transport as described above are regulated by many signals that are generated in various sources. Thus, the autonomic nervous system already mentioned, the hypothalamic pituitary system, the ovum itself (Van Niekerk, 1976; Betteridge et al., 1976), the seminal plasma (Croxatto and Ortiz, 1975), and also the possibility for the oviduct to produce prostaglandins (Saksena and Harper, 1975) may all contribute to the control of normal ovum transport. However, the ovary with its capacity to release oestrogens and progestins into the circulation has been recognized as the main source of regulatory signals for all phases of the reproductive process in the female including ovum transport (Croxatto and Ortiz, 1975).
The conclusion that ovum transport is under endocrine control is proved by the numerous experiments in which steroids as well as prostaglandins and many other compounds (Bennett, 1976) are administered to laboratory animals. This is done to establish their individual effect on the rate of ovum transport by means of acceleration or arrest ('tube-locking').
Furthermore, the plasma levels of oestradiol and progesterone during the oestrous cycle of the hamster as reported in the literature will be discussed. Special emphasis will also be given to the first period of pregnancy when ovum transport takes place. In the same way the literature on prostaglandins during the oestrous cycle and the first stage of pregnancy will be reviewed as

well as the effects of exogenously administered steroid hormones and prostaglandins on ovum transport.

6. *Hormonal patterns in the oestrous cycle and in the first stage of pregnancy*

The endocrine mechanisms regulating the ovarian functions in mammals with either an oestrous or a menstrual cycle have much in common. The influence of the pituitary on the growth and ripening of ova in the ovary is mediated through FSH whereas the serum level of LH exerts its effect on ovarian steroid hormone production.

Figure 4 illustrates the LH and FSH serum levels as measured by Bex and Goldman (1975) in the oestrous cycle of the hamster. Both hormones demonstrate peak values between 15.00-16.00 on prooestrous Day 4 (Goldman and Porter, 1970; Bex and Goldman, 1975). Minimum FSH concentrations are measured on Day 3 and in the morning of Day 4 (Bast and Greenwald, 1974; Bex and Goldman, 1975). This is in agreement with the earlier hypothesis of Greenwald (1961c) that the decreasing FSH secretion in this period is responsible for the follicular atresia occurring between dioestrus 2 (Day 3) and prooestrus (Day 4) which eliminates approximately half the number of the large follicles.

The most detailed reports on peripheral plasma levels of oestradiol (Baranczuk and Greenwald, 1973) and progesterone (Ridley and Greenwald, 1975) as measured during the oestrous cycle of the hamster, are presented in Figure 5. For oestradiol, baseline levels are maintained on Days 1 and 2 of the cycle. A sustained level of oestradiol is detected between 14.00 and 22.00 of Day 3. On Day 4, a sharp transistory peak occurs at 15.00, followed by a rapid decline to basal levels. Comparable maximum oestradiol peak levels as measured on cycle Day 4 are reported by Saidapur and Greenwald (1978). However, due to the low frequency of sampling, this study does not provide more than a rough impression of peripheral steroid profiles for both oestradiol and progesterone during the oestrous cycle of the hamster. The progesterone measurements reported in the same study confirm the observations of others (Lukaszewska and Greenwald, 1970; Leavitt and Blaha, 1970; Norman and Greenwald, 1971) about the occurrence of a fairly constant level of progesterone during the afternoon and evening of cycle Day 4. Shaikh and Saksena (1973) made the same observation, but their data show a prolongation of this level until at least 04.00 of Day 1.

In all reports on peripheral progesterone in cyclic hamsters, the concentrations on Days 1 and 2 are comparably low, whereas minimum values are

recorded on Day 3 and in the morning of Day 4. In contrast to the high degree of uniformity for all data cited here, the most detailed progesterone pattern measured by Ridley and Greenwald (1975), given in Figure 5, shows a somewhat different shape. Especially the duration of the period with maximum progesterone levels as reported by others is extended with cycle Day 1.

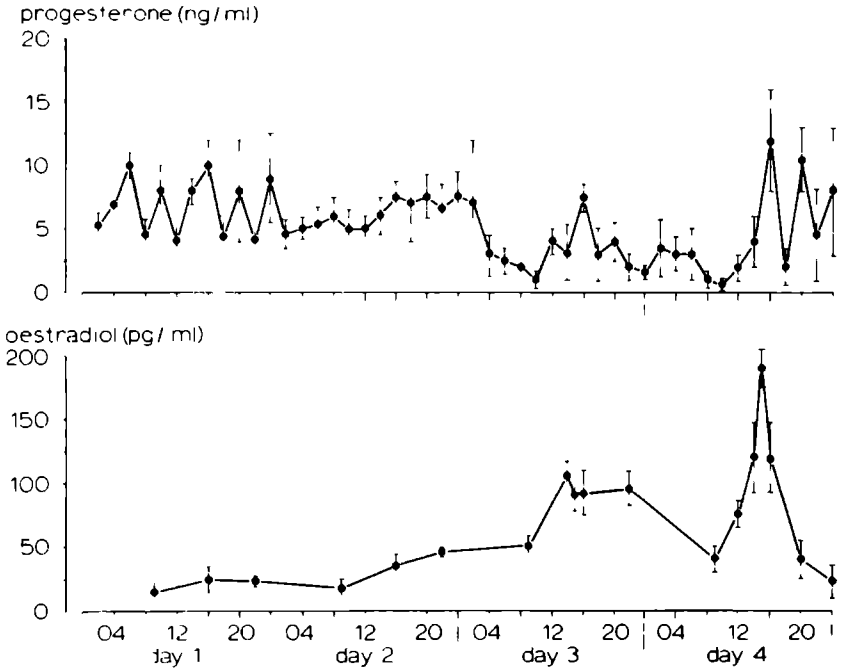

FIGURE 5 Peripheral plasma levels* of progesterone and oestradiol in cyclic hamsters [Data on progesterone from Ridley and Greenwald (1975) and data on oestradiol from Baranczuk and Greenwald (1973)

* Mean ± SEM

Data on steroid plasma levels during the first stage of pregnancy of the hamster are scarce and limited to measurement of progesterone. On Day 2 of pregnancy, Lukaszewska and Greenwald (1970) have observed a progesterone concentration in peripheral plasma of about one-half the maximum value of this hormone recorded during the oestrous cycle. A somewhat lower progesterone level is measured on Day 4 of pregnancy. These data accord with those of Leavitt et al. (1970), Leavitt and Blaha (1970), and Blaha and Leavitt (1974). Furthermore, they reported that progesterone levels increase between Day 1 and Day 2 of pregnancy.

7. The effect of steroid hormones on ovum transport and on pregnancy

The endogenous patterns of oestradiol and progesterone as released by the ovary into the circulation are important factors in the regulation of normal ovum transport (Hafez, 1973). These ovarian hormones affect both the quality and the quantity of the secretory activity and the secretions of the oviductal epithelium (Hafez, 1973, 1976). They also influence the contractility of the oviductal musculature and furthermore regulate the pattern and the rate of gamete transport.

At the end of oviductal ovum transport, the ovum reaches the uterus at a time when the endometrium has been stimulated sufficiently by ovarian oestrogens and progesterone to ensure the successful continuation of pregnancy (Chang and Pickworth, 1969). Although the normal developmental synchrony between the embryo and endometrium has been demonstrated in the case of only a few species (Chang and Pickworth, 1969), these observations are an important argument for the contribution of ovarian steroid hormones to the control of normal ovum transport.

However, a common and well-defined pattern of hormonal changes, responsible for the uniform regulation of normal ovum transport in different species of mammals is unlikely. This is supported by the difference in the patterns of their ovarian cycles; the most constant factor here was the presence of a short, but distinct increase in the blood levels of oestrogens just prior to ovulation, as demonstrated e.g. in the case of the human female (Thorneycroft et al., 1971), the rhesus monkey (Hotchkiss et al., 1971; Bosu et al., 1972) and the hamster (Baranczuk and Greenwald, 1972, 1973).

Ovarian progesterone secretions show many more different patterns. Maximum levels are detected prior to ovulation in the rabbit (Hilliard and Eaton, 1971) and the hamster (Lukaszewska and Greenwald, 1970; Leavitt et al., 1970; Leavitt and Blaha, 1970; Norman and Greenwald, 1971; Saidapur and Greenwald, 1978). After ovulation, they have been established in the human (Mishell et al., 1971) and the rhesus monkey (Kirton et al., 1970; Monroe et al., 1970).

The non-uniformity in the regulation of normal ovum transport by ovarian steroids in the case of various species of mammals is also supported by the numerous experiments from which is concluded that the effects of exogenous oestrogens upon ovum transport and pregnancy vary with the species, the time of administration and the dose used. Greenwald (1967) has shown that a single subcutaneous injection of oestradiol cyclopentylpropionate (ECP) given to various animal species shortly after mating resulted in termination of pregnancy in most of them, in acceleration of ovum transport, or in arrest of ova at the ampullary-isthmic junction.

The doses of ECP required to terminate pregnancy in the mouse, the rat, the guinea-pig, the rabbit, and the hamster are 1, 10, 10, 50, and 25 μg, respectively. Comparable amounts of ECP administered resulted in an acceleration of ovum transport in the mouse (1 μg or more), the rat (10 μg or more), and the rabbit (25 μg), whereas the guinea-pig (50-100 μg) and the hamster (100 μg) required greater doses in order to obtain the same effect.

Tube-locking of ova is induced by means of relatively high dosages of ECP in the rabbit (100 μg), the guinea-pig (250 μg) and the hamster (250 μg). As opposed to this, not more than 1 μg of ECP given at the same time shortly after mating, is sufficient to cause oviductal retention of ova in the mouse. Finally, tube-locking of ova in the rat could not be obtained with ECP. Moreover, such an effect has never been reported to occur in this species with any oestrogen.

In a detailed study, Bastiaans (1973) has reported the effects of some oestrogens and progestins on ovum transport and pregnancy in the hamster.

The effects on ovum transport after a single subcutaneous injection of 500 μg oestradiol or 250 μg oestrone can be summarized as follows:

Day/time of treatment (hours from ovulation)	4/10.00 −16	4/22.00 −4	1/10.00 8	2/10.00 32
Oestradiol (500 μg)	substantial delay	complete arrest	substantial delay	accelerate
Oestrone (250 μg)	substantial delay	complete arrest	delay + accelerate	accelerate

Ova were substantially delayed or even arrested when these steroids were injected prior to ovulation, whereas ovum transport was delayed or accelerated when oestrogens were administered after ovulation.

Comparably high doses (100-500 μg) of these oestrogens also terminated pregnancy. The strongest effect was observed with oestrone rather than with oestradiol, and the oestrogens were most effective when administered 4

hours prior to ovulation. The repeated subcutaneous injection of far lower quantities of oestradiol or oestrone also affected ovum transport:

Treatment relative to ovulation (hours)	Dose of oestrogen (μg)	Effect on ovum transport of	
		Oestradiol	Oestrone
-16, -4, 8	25	delay	substantial delay
20, 32, 44	25	none	accelerate
-16, -4, 8 20, 32, 44	10	complete arrest	complete arrest

The same dosages of oestradiol and oestrone also caused clear effects on the termination of pregnancy following the same repeated treatment schedule. A high percentage of abnormal ova was found after treatments with these oestrogens, whereas oestriol (25 μg) affected neither transport nor ovum development.

Massive doses (10 mg) of several progestins did not affect pregnancy or ovum transport when either given by themselves, or in combination with oestrogens. Then, only the oestrogen effects were observed.

8. The effect of steroid hormones on oviductal contractility

The effects of steroid hormones on the contractile activity of the oviduct during ovum transport have been the subject of many studies. Treatment of intact and ovariectomized rabbits with oestrogens and progestins (Chang and Harper, 1966; Harper, 1964, 1965a, 1965b, 1966) influences the pattern of normal ovum transport, due to changes in oviductal contractility. Harper (1966), and Spilman and Harper (1974) concluded that oestrogens can stimulate and progesterone can depress the activity of the ampullar musculature, which in turn results in an acceleration or retention of oviductal ovum transport. This theory explains the strong increase of the ampullar contractility around ovulation, as just prior to this process the levels for oestradiol are at their maximum.

In contrast to the theory of Harper, Boling and Blandau (1971a, 1971b) have formulated an opposite conclusion from their experiments dealing with the effects of the ovarian hormones on oviductal motility in the rabbit.

It is observed that the oviductal musculature is minimally active in the presence of high oestrogen concentrations within the physiological range whereas the muscular activity increases when oestrogen levels decrease.

The latter effect results in an acceleration of ovum transport. These authors strongly emphasize that it is withdrawal rather than dominance of oestrogens that increases the contractile activity of the oviduct and, at the same time, results in the acceleration of ova in the case of the rabbit.
It has also been demonstrated in experiments with oocytes of donor animals that progesterone administered to ovariectomized rabbits does not significantly change the rate of transport. However, when progesterone is given to intact or ovariectomized oestrogen-pretreated animals, progesterone increases the amplitude and decreases the frequency of contractions, which might facilitate ovum transport (de Mattos and Coutinho, 1971).

9. Prostaglandin patterns in the oestrous cycle and in the first stage of pregnancy

A number of reports have indicated that prostaglandins are present in the plasma (Blatchley et al., 1971, 1972, 1975a, 1975b; Cox et al., 1973; Earthy et al., 1975; Saksena and Harper, 1972b; Saksena et al., 1974; Shaikh et al., 1973; Shaikh and Saksena, 1973), the uterus (Ham et al., 1975; Lamothe et al, 1977; Poyser, 1972, 1973; Saksena and Harper, 1972a, 1972b), and the oviduct (Ogra et al., 1974; Saksena and Harper, 1975; Vastik-Fernandez et al., 1975) of several species. It has been established that the plasma or tissue concentrations of prostaglandins vary during the oestrous cycle of the rat (Ham et al., 1975; Saksena and Harper, 1972b), the guinea-pig (Blatchley et al., 1971, 1975a, 1975b; Earthy et al., 1975; Poyser, 1972, 1973), and the hamster (Saksena and Harper, 1972a; Shaikh and Saksena, 1973). Prostaglandin levels in peripheral plasma of pseudopregnant and pregnant rats (Saksena et al., 1974) or hamsters (Shaikh et al., 1973) have also been reported to show fluctuations, as is observed for the prostaglandin concentrations in oviductal tissue of rabbits in the period of ovum transport (Saksena and Harper, 1975).
Information on prostaglandin levels in cyclic hamsters is scarce and limited to measurement of PGF in uterine tissue samples which were taken at seven occasions during the cycle. Maximum PGF concentrations were detected on Day 2, and between 24.00 and 00.30 of Day 1, while lowest concentrations were discovered in the morning of Day 1 (Saksena and Harper, 1972a).
The uterine venous, and peripheral plasma values for PGF were determined at nine different periods during the oestrous cycle of the hamster (Shaikh and Saksena, 1973). The lowest value for PGF in the uterine venous plasma was recorded between 10.00 and 12.00 of Day 4 and the peak concentration occurred between 19.00-21.00 of the same day. The uterine venous and peripheral plasma values for PGF correlated well, but it was concluded that

there were discrepancies as compared to the pattern of uterine tissue concentrations (Saksena and Harper, 1972a). In peripheral blood of pregnant hamsters, the PGF concentration measured on Day 3 was similar to the results of Day 4, except for a sharp decline between 19.00-21.00 on that day. The measurements of peripheral PGF concentrations were continued once a day up to Day 9 of pregnancy. The levels determined over this period remained rather constant (Shaikh et al., 1973).

10. The effect of prostaglandins on ovum transport

It is probable that prostaglandins have several physiological regulatory functions in reproductive processes including ovum transport (Labhsetwar, 1974).

The exogenous subcutaneous administration of prostaglandins in several dosages during the period of ovum transport may either be ineffective, or it may cause an acceleration or arrest of ova. Under these circumstances, hamsters appear extremely resistant to prostaglandins: PGE_1, PGE_2, and $PGF_{2\alpha}$ did not affect ovum transport in the case of this species (Labhsetwar, 1972a, 1972b, 1973).

PGE_1 also proved to be ineffective with regard to mice (Horton and Marley, 1969), but accelerated ovum transport in rabbits (Chang et al., 1973; Ellinger and Kirton, 1974), whereas it led to oviductal arrest of ova in rats (Nutting and Cammarata, 1969; Labhsetwar, 1973).

For PGE_2, no effects were observed with regard to rabbits (Nutting, 1969; Chang et al., 1973), but ova were arrested in the oviducts of mice (Liu et al., 1974) and rats (Nutting and Cammarata, 1969; Labhsetwar, 1972a).

The effectiveness of $PGF_{2\alpha}$ on ovum transport has been established in rabbits (Chang and Hunt, 1972; Chang et al., 1973; Ellinger and Kirton, 1974) in the case of which it caused an acceleration of ova, while in the case of rats the result was an oviductal arrest (Labhsetwar, 1972b). No effects for $PGF_{2\alpha}$ have been reported to occur with regard to mice (Liu et al., 1974). The results are summarized in the diagram:

Species	PGE_1	PGE_2	$PGF_{2\alpha}$
mouse	none	arrest	none
rabbit	accelerate	none	accelerate
rat	arrest	arrest	arrest
hamster	none	none	none

11. The effect of prostaglandins on pregnancy

Close to the effects of prostaglandins on ovum transport are their influences

on pregnancy and implantation The termination of pregnancy and the reduction in the number and size of implantation sites observed following prostaglandin treatment may be the result of disturbances in the rate of ovum transport, although other factors, mainly luteolysis appear more important. Termination of pregnancy in rats and hamsters did not occur when several dosages of PGE_1 (Labhsetwar, 1973), PGE_2 (Labhsetwar, 1972a, or $PGF_{2\alpha}$ (Gutknecht et al , 1969; Labhsetwar, 1972a) were administered subcutaneously during the days of ovum transport Outside this period in a later stage of pregnancy, treatment of hamsters with PGE_1 (Labhsetwar, 1973), PGE_2 (Labhsetwar, 1972a) or $PGF_{2\alpha}$ (Gutknecht et al , 1971, Labhsetwar, 1971, 1972a, 1972b) invariably resulted in termination of pregnancy, whereas in the case of rats this effect was only achieved with PGE_2 (Labhsetwar, 1972a) and $PGF_{2\alpha}$ (Gutknecht et al , 1969; Labhsetwar, 1972a, 1972b) Termination of pregnancy following the subcutaneous administration of $PGF_{2\alpha}$ after the period of ovum transport was also reported for the rabbit (Gutknecht et al., 1969) and the mouse (Bartke et al., 1972, Labhsetwar, 1972c) A reduction in the number and size of implantation sites was mainly observed in rats and occurred when PGE_1 was administered subcutaneously during the days of ovum transport, until Day 7 of pregnancy (Labhsetwar, 1973), or following PGE_2-treatment during Day 1 up to and including Day 4 of pregnancy (Labhsetwar, 1972a), whereas $PGF_{2\alpha}$ was ineffective (Gutknecht et al , 1969; Labhsetwar, 1972a) Neither were any effects observed on implantation when hamsters were treated with several dosages of PGE_1 (Labhsetwar, 1973), PGE_2 of $PGF_{2\alpha}$ (Labhsetwar, 1972a) during the days of ovum transport. Finally, Horton and Marley (1969) reported that no effects on pregnancy or implantation occurred in mice following the subcutaneous administration of PGE_1 during the first nine days of pregnancy The diagram below summarizes these results·

Species	Treatment (days)	PGE_1	PGE_2	$PGF_{2\alpha}$
mouse	1-9	none	–	–
	4-7	–	–	termination
rabbit	3-7	–	–	termination
rat	1-4	reduction	reduction	none
	4-7	reduction	termination	termination
hamster	1-3	none	none	none
	4-9	termination	termination	termination

12. The effect of prostaglandins on oviductal contractility

Considering the various effects of exogenously administered prostaglandins on ovum transport, this group of compounds is apparently associated with oviductal function and may be responsible for the spontaneous contractility of the oviduct (Spilman and Harper, 1975). In vivo studies on oviductal muscle activity (Spilman and Harper, 1972, 1973, 1975; Spilman, 1976) have demonstrated that the effects of E- and F-series prostaglandins are mutually antagonistic. Increased muscular activity of the oviduct caused by $PGF_{1\alpha}$ or $PGF_{2\alpha}$ with concomitant occlusion of the isthmus could be suppressed by a subsequent injection of either PGE_1 of PGE_2, which also resulted in a relaxation of oviductal occlusion. Furthermore, spontaneous spasmodic contractions could be interrupted by E prostaglandins at any time after their onset (Spilman and Harper, 1973).

In ovariectomized rabbits, progesterone treatment, in contrast to treatment with oestradiol, increased the duration of oviduct response to PGE_1, whereas progesterone was more effective than oestradiol in decreasing the response to $PGF_{2\alpha}$ (Spilman, 1974). This author also reported that the increased oviductal activity caused by $PGF_{2\alpha}$ lasted longer in untreated ovariectomized animals than in those treated with either oestrogens or progesterone (Spilman, 1974). The same result was reported upon comparison of the former group with the effects of $PGF_{2\alpha}$ on intact oestrous rabbits (Heilman and Strainer, 1976). The above data suggest that steroid hormones affect the responsiveness of the oviduct to prostaglandins.

Tissue binding of PGE_1 and $PGF_{2\alpha}$ to the oviduct has been demonstrated with regard to oestrous rabbits and for rabbits 72 hours after mating (Wakeling and Spilman, 1973). A decreasing gradient of specific binding was detected in the ampulla, distal isthmus, and proximal isthmus for both groups of animals, although no striking differences between oestrous and pregnant rabbits were observed.

13. Oviductal tissue concentrations of prostaglandins

The observations with regard to changing tissue concentrations of F prostaglandins in oviducts of rabbits at several points of time after an injection of human chorionic gonadotrophin (hCG) also support the role of prostaglandins in the regulation of normal ovum transport (Saksena and Harper, 1975). Maximum PGF levels in the ampulla as well as in the distal isthmus were recorded 10 hours after hCG injection and thus they coincided with the presence of all the ova in the ampulla. About 60% of the ova were located in the distal portion of the isthmus 30 hours following hCG treatment. Then, the PGF concentrations in this oviductal segment as well as in the ampulla were

at their lowest level with a gradual increase afterwards. The concentrations of PGF in the proximal isthmus were maximal by that time. A fall to very low levels of PGF after a period of 50 hours and more allowed the ova to traverse the proximal portion of the isthmus in order to subsequently move into the uterus where more than 80 % of all the ova were recovered 70 hours after hCG treatment.

14. Proposed role of prostaglandins in ovum transport

On the basis of the above data, Spilman and Harper (1975) suggested a mechanism through which prostaglandins contribute to the physiological control of ovum transport. According to their theory, the brief increase of oestradiol to maximum levels just prior to ovulation, and the dramatic fall of ovarian steroids as observed around ovulation and maintained in the period of ovum transport (Hilliard and Eaton, 1971) causes a delayed increase in oviductal tissue PGF concentrations, with peak values occurring at a time when the oviductal isthmus is most sensitive to stimulation by $PGF_{2\alpha}$.

Additional evidence for this theory is provided by Spilman (1974) who observed that the proximal isthmus of the rabbit oviduct is most sensitive to stimulation by $PGF_{2\alpha}$ during the first few days after ovariectomy, i.e. during surgically -induced reduction of circulating steroid levels. Thus, the steroid-induced changes in the oviductal tissue concentrations of PGF and changes in the sensitivity to $PGF_{2\alpha}$ could increase the occlusion of the isthmus and prevent premature passage of ova into the uterus. Spilman and Harper (1975) further hypothesize that the gradual increase of progesterone secretion after ovulation in the case of the rabbit may be responsible for a decrease in oviductal tissue concentrations of PGF as has been reported after progesterone treatment (Saksena and Harper, 1975). A concomitant decrease in the response of the proximal isthmus to $PGF_{2\alpha}$ stimulation and an increase in the responsiveness to PGE_1 (Spilman, 1974) would also contribute to a progressive movement of ova through the isthmus into the uterine cavity (Spilman and Harper, 1975).

However, the proposed role of progesterone within this mechanism appears questionable, because an important assumption in the hypothesis is the existence of a postovulatory rise of progesterone levels whereas Hilliard and Eaton (1971) reported maximum progesterone output previous to ovulation in the rabbit, with low steroid levels afterwards.

Further evaluation of the proposed mechanism includes an investigation into the question whether oviductal tissue concentrations of PGE in rabbits can be detected and whether they fluctuate during the period of ovum transport (Spilman and Harper, 1975; Spilman, 1976). Recently, it has been established

that the human oviduct is capable of producing both PGE and PGF (Elder et al., 1977).

It is not known if oviductal PGE is affected by steroid treatment as has been reported to be the case with PGF (Saksena and Harper, 1975).

Recently, however, utilizing an in vitro incubation technique, the presence of a prostaglandin synthetase system in the rabbit oviduct has been identified (Valenzuela and Harper, 1976). In connection with this, it was found that indomethacin, a prostaglandin synthetase inhibitor, failed to alter ovum transport in the case of the rabbit (Hodgson, 1976; El-Banna et al., 1976). Neither did the drug have any effect on the in vitro spontaneous motility of the human oviduct during either the follicular or luteal part of the cycle (Elder et al., 1977). This suggests that prostaglandins may not play an important role in human ovum transport. This opinion is also shared by Maia et al. (1977) who concluded that the accelerating effect of oestradiol on ovum transport in the case of the guinea-pig is apparently not mediated by prostaglandins, since indomethacin was ineffective in antagonizing it, whereas neither PGE_2 nor $PGF_{2\alpha}$ accelerated ovum transport in the case of this species. However, in these experiments no report was given of the tissue levels of prostaglandins and the magnitude of prostaglandin synthesis inhibition. Furthermore, several drugs which are known to interfere at different levels with prostaglandin synthesis, metabolism, or release from tissue have been tested in order to evaluate the role of prostaglandins in ovum transport (Valenzuela et al., 1977). Indomethacin administered locally to rabbits in a dose of 2 mg/kg body weight significantly accelerated ovum transport, whereas a dose that was six times as big proved to be ineffective. It was suggested that the higher dose affected other sites, e.g. inhibition of prostaglandin action, in addition to interference of this drug with the transformation of arachidonic acid into the endoperoxides, the intermediaries between arachidonic acid and primary E and F prostaglandins, or thromboxanes (Valenzuela et al., 1977). Both indomethacin and meclofenamate have the latter quality, which is also known as inhibition of prostaglandin synthetase. Again, no data concerning oviductal tissue concentrations of prostaglandins or drugs tested by Valenzuela et al., (1977) are reported, but the results suggest that prostaglandins play a physiological role in the control of the oviductal function in the case of the rabbit.

In conclusion: Many details of the proposed mechanism for prostaglandins in oviductal function (Spilman and Harper, 1975) need further investigation. It is in particular data concerning tissue concentrations of prostaglandins in oviductal tissue that may show whether prostaglandins are of any importance or not. Up till now these data were lacking.

MATERIALS AND METHODS

1. The golden hamster, Mesocricetus auratus (Waterhouse)

The golden hamster was selected as laboratory animal for this study. It belongs to the order RODENTIA of the family CRICETIDAE.

The female animal reaches sexual maturity at four to six weeks, whereas the male becomes fertile between the sixth and seventh week of its life.

Female hamsters show outstandingly regular oestrous cycles during reproductive life, with ovulations occurring every four days (Orsini, 1961). Following ovulation, a white opaque, viscous smear fills the vaginal space completely. Synchronization of animals for the stage of their cycle is accomplished by checking the characteristic postovulatory discharge for at least three consecutive regular oestrous cycles. Female hamsters selected in this manner make it possible to study the period of ovulation in more detail. Therefore, these animals are eminently suited for the administration of hormones or drugs at pre-arranged intervals relative to ovulation. If rats or mice had been selected for these experiments, this would have caused difficulties due to the irregularity of their ovarian cycles.

Another advantage of the regular oestrous cycle and the appearance of a postovulatory discharge in the case of the hamster is the fact that it is possible to predict the time during which the animal may be bred.

The duration of pregnancy in hamsters is sixteen days (Soderwall et al., 1960). Day 1 of pregnancy is defined as the day when sperm are found in the vaginal smear after mating.

Some investigators report a diminution in the number of fertile matings and a reduction in litter size during the winter months (Magalhaes, 1970). However, seasonal changes in reproductive behaviour can be anticipated by keeping the animals under standardized laboratory conditions (Magalhaes, 1970).

2. Biological techniques

In all the experiments young healthy female golden hamsters with body weights between 80 and 120 g were used.

The animals were housed at a temperature between 22 and 24 °C, with daylight periods between 05.00 and 19.00.
According to the vaginal discharge method, the hamsters were checked for at least three consecutive cycles. Only animals with a regular oestrous cycle of four days were used.
For several experiments it was necessary to have pregnant hamsters. This was realized by caging a male together with the selected female hamster during the night of ovulation. Next morning, mating was considered successful when sperm were present in the vaginal smear.
Anaesthesia of the hamsters was performed with sodium pentobarbital (Nembutal, Abbott S.A., Saint-Rémy-sur-Avre, France) in a concentration of 60 mg/ml. It was injected intraperitoneally in quantities of 90 mg/kg body weight. Blood samples for the measurement of oestradiol and progesterone plasma concentrations were obtained by right ventricular heart puncture. From each animal approximately 4 ml of blood was obtained and collected in a polystyrene tube containing 5.5 mg EDTA as anticoagulant. After centrifugation for 10 min at 1200 x g and at room temperature, the plasma samples were kept frozen at -20 °C until assaying.
Autopsy on cyclic or pregnant hamsters was performed by opening the abdominal cavity and removing of the ovaries, oviducts and uterus. The organs were immediately transferred into an isotonic saline solution of 37 °C. The bursa was removed from each ovary and the ruptured follicles were counted in order to detect the number of ovulation sites. The oviducts were dislocated from the uterus just behind the junctura. If a pair of oviducts was destined for prostaglandin determination, the organs were immediately frozen in liquid nitrogen and stored at -70 °C until assaying.
In other hamsters, normal ovum transport was studied. The oviducts were stretched to some extent by disrupting the mesosalpinx at various intervals. After transferring the oviducts on glass slides and adding saline solution to prevent the tissue from drying out, the slides were placed under a light microscope. Then, the position of ova within the oviducts was determined. Implantation was investigated by flushing the uterus with isotonic saline solution and checking this fluid for the presence of ova.
The contractility of the oviduct in vitro was studied according to the technique described by Burdick et al. (1942). The ovary including its intact bursa, the oviduct, and a small portion of the uterus were transferred to a dry glass slide. The mesosalpinx was carefully disrupted in order to make some stretching of the strongly twisted oviduct possible. A small quantity of isotonic saline solution was added and a cover glass slide was pressed against the wall

of the ovarian bursa. In this manner the in vitro contractility of the oviduct could be observed for a short period.

Oestradiol and indomethacin were administered to cyclic and to pregnant hamsters in order to evaluate the effects on oviductal tissue levels of prostaglandins.
Oestradiol (Sigma Chemical Company, St. Louis, Mo., USA) was dissolved in a mixture of 10% benzyl alcohol in arachis oil. The animals received a single subcutaneous injection of 0.2 ml solution, corresponding to 250 μg oestradiol. Indomethacin (Sigma Chemical Company, St. Louis, Mo., USA) was dissolved in 0.1 M phosphate buffer (pH 7.4) and administered orally in doses of either 0.1 or 0.5 mg indomethacin/0.2 ml.
Oestradiol and indomethacin were administered to pregnant hamsters in order to study their effect on the rate of ovum transport. This was investigated by flushing the oviducts with isotonic saline solution and checking this fluid for the presence of ova. However, a far more comprehensive study was made of $PGF_{2\alpha}$ with respect to its ability to modify ovum transport and to affect pregnancy.
The prostaglandin $PGF_{2\alpha}$ (Prostin $F_{2\alpha}$, Tromethamine (THAM) salt, The Upjohn Company, Kalamazoo, Mich., USA) was dissolved in 0.1 M phosphate buffer (pH 7.4) and several concentrations were injected either subcutaneously (0.4 ml) or intraperitoneally (0.8 ml) at both 10.00 and 16.00 of the same day. Controls were treated equally with vehicle only.
For the prostaglandin tested, the total daily doses administered were between 1-4 mg $PGF_{2\alpha}$/kg body weight. In some experiments the injected quantities were fixed amounts of prostaglandin per animal, and were in fact the same quantities as above on the basis of animals all weighing 100 g.
The significance of differences in the results between the groups of treated animals and the control groups was calculated by making use of a two-tailed Student's t-test (Snedecor and Cochran, 1967).

3. Analytical procedures
The period of ovulation and the time course of ovum transport and implantation were studied in groups of cyclic and pregnant hamsters. After the total bleeding of these animals according to the procedure described above, oestradiol and progesterone concentrations were determined in the plasma samples obtained, using highly specific radioimmunoassay (RIA) methods.
Analogous to the transversally measured patterns for steroid hormones in groups of cyclic and pregnant hamsters, the tissue concentrations of prostaglandins $F_{2\alpha}$ and E_2 were determined in the oviducts of another series of

animals which were bled and autopsied at the same intervals as those of the first series. The RIA methods for the measurement of $PGF_{2\alpha}$ and PGE_2 were applied to the oviducts and plasma samples of these groups of hamsters. However, the plasma patterns of prostaglandins obtained are not reported in this study, but will be published elsewhere.

Radioimmunoassays for oestradiol and progesterone

The determination of steroid hormone concentrations in hamster plasma was based on a technique originally developed and described for human serum samples (Thomas et al., 1977). An evaluation was made as to whether the same technique could be applied to the measurement of oestradiol and progesterone concentrations in hamster plasma. These experiments led to the conclusion that chromatographic purification of plasma sample extracts on Sephadex LH-20 was necessary in order to obtain reliability characteristics which were comparable to those described previously for human serum samples (Thomas et al., 1977). The procedure used consisted of the following steps:

After addition of tritiated oestradiol and progesterone in equal quantities (10^3 DPM, ± 1 pg) to 2 ml plasma, the samples were extracted twice with 5 quantities of diethyl ether. The combined organic layers were dried and the residues were taken up in 0.2 ml toluene/methanol (9 : 1, v/v ; mixture A). Chromatographic purification of these preparations was performed on columns containing Sephadex LH-20 (Pharmacia Fine Chemicals AB, Uppsala, Sweden). Previous to use, glass columns of 0.5 x 30 cm were packed with a suspension of 0.5 g Sephadex LH-20 in 5 ml mixture A, and subsequently treated with 9 ml solvent A, 7 ml toluene/methanol (1 : 1, v/v) and finally with 9 ml of mixture A. Then, the 0.2 ml sample preparations were applied to these columns which were developed with a total volume of 5 ml mixture A. The first fraction (1.6 ml) contained progesterone. The next 0.5 ml of effluent contained less than 5 % of the total radioactivity added to plasma samples previous to extraction and was, therefore, rejected. Oestradiol was collected in the third fraction of about 3 ml.

In general, the two steroids were recovered for more than 80 %. The distribution patterns of the chromatographic procedure revealed the complete separation of progesterone from oestradiol, and these steroids were collected in the first and third fraction, respectively. The hormone concentrations in these column effluents were then determined by RIA, taking two different volume aliquots for measurement. The latter procedures were, however, identical to those already described, whereas the reliability characteristics reported for human serum (Thomas et al., 1977) resembled those determined

now for hamster plasma samples. This is demonstrated by the estimate of assay precision that was quantified after the repeated measurement of one hamster plasma pool in 10 consecutive experiments. The between-assay coefficients of variation (CV_B) for means of duplicate measurements were 11.7 % for oestradiol and 12.4 % in the progesterone assay. These figures are in agreement with those reported for human serum (Thomas et al., 1977).

Radioimmunoassays for prostaglandins $F_{2\alpha}$ and E_2

In two previous reports (Thomas et al., 1978a, 1978b) a simple and reliable RIA technique was introduced for the measurement of four different prostaglandins in human plasma. Comparable reliability characteristics for all the RIA systems were also established with other biological materials including hamster plasma (Thomas et al., 1978b).

In addition to these results, the same technique was applied and validated for the measurements of the prostaglandins in oviductal tissue of hamsters. Only one addition was made to the procedure delineated previously (Thomas et al., 1978a, 1978b), i.e. the preparation of tissue homogenates using a small Potter-Elvehjem tube. No further modifications were necessary. Hence, the complete procedure can be summarized as follows:

Each pair of oviducts, ranging in weight between 25 and 40 mg was assayed within 10 days after autopsy. The frozen tissue was weighed and immediately transferred to a small Potter-Elvehjem glass homogenizer. Then, 1 ml of assay buffer, composed of 0.05 M Tris(hydroxymethyl)aminomethane-HCl (pH 7.4) with 0.15 M NaCl and 0.1 % gelatin (w/v) (Thomas et al., 1978a) and also containing 125 µg calcium acetyl salicylate for the prevention of prostaglandin synthesis (Vane, 1971) was added to the oviducts. The mixture was homogenized for 1.5 min and after decanting the homogenate, the tube was washed once with 1 ml of assay buffer.

To the combined tissue homogenate, 10^3 DPM tritiated $PGF_{2\alpha}$ was added, acidified with 0.1 ml 1 N HCl (final pH 3.5) and extracted twice with 5 quantities of ethyl acetate. After drying the combined organic layers, the residues were taken up in 1 ml distilled water and applied to columns filled with Sephadex G-25 (1.5 x 25 cm). Subsequent elution was performed with distilled water. The first 15 ml of effluent which contained the material of high molecular mass was collected and discarded.

The next 15 ml of effluent containing the prostaglandins was collected and lyophilized. The residue was taken up in distilled water and the solution obtained was subsequently used for process recovery determinations and for the measurement of the prostaglandins by means of RIA. The recovery for ^{3}H-$PGF_{2\alpha}$ added to the oviduct samples before extraction ranged from 50 - 70 %.

The RIA method for the measurements of $PGF_{2\alpha}$ and PGE_2 concentrations in the chromatography samples was highly identical to the technique described elsewhere (Thomas et al., 1978a, 1978b).
The precision of the method used was evaluated according to the procedure reported previously (Thomas et al., 1977).
Since it is impossible to use an oviduct pool in the same way as a plasma pool (i.e. to repeat in each assay the complete assay procedure including tissue homogenization in the case of an oviduct pool) the precision of RIA determinations for $PGF_{2\alpha}$ and PGE_2 was monitored with a hamster plasma pool which was freshly collected and kept frozen at -70 °C. Although no absolute guarantee can be given as to whether the repeated measurement of prostaglandins in one plasma pool will mirror the between-assay precision for oviductal tissue concentrations of these compounds, a number of arguments justify this approach: snap-freezing of plasma or tissue samples in liquid nitrogen and subsequent storage at -70 °C have been shown to stabilize and to significantly decrease the between-assay variability as a measure of assay precision, when compared to more generally accepted procedures of sample handling such as slow freezing and storage at -20 °C. Hence, the between-assay coefficients of variation for means of duplicate measurements of a hamster plasma pool which was kept frozen at -70 °C for more than six months were 20.4 % for $PGF_{2\alpha}$ (after 15 assays) and 22.1 % for PGE_2 (after 9 assays). The within-assay variation for a single measurement of the same plasma pool was 10.1 % in the two systems after 15 and 9 consecutive experiments, respectively. These data were also obtained with other biological fluids, such as human plasma and amniotic fluid.

OVULATION, OVUM TRANSPORT AND PLASMA CONCENTRATIONS OF OESTRADIOL AND PROGESTERONE IN CYCLIC AND PREGNANT HAMSTERS

INTRODUCTION

Previous to the examination of the hypothesis that oestrogens influence ovum transport through alterations in oviductal prostaglandin content, it was necessary to make an inventory of some physiological events taking place during the oestrous cycle and the first four days of pregnancy of golden hamsters. This chapter deals with data that make it possible to define the moment of ovulation in cyclic hamsters. Next, the time course of normal ovum transport during the first stage of pregnancy is determined. Finally, these processes are related to the patterns of steroid hormones as measured in the plasma of the same groups of hamsters during the oestrous cycle and the first period of pregnancy.

RESULTS

1. The oestrous cycle

Ovulation and ovum transport to the oviduct

Table 1 gives the numbers of follicles ruptured and of ova recovered from the oviduct between 23.00 and 24.00 of Day 4 and between 01.00 and 08.00 of Day 1. No ovulation at all occurred until at least 24.00 on Day 4, but this process

TABLE 1 Ovulation and ovum transport to the oviduct on Day 4 and Day 1 of the oestrous cycle of hamsters

Autopsy day / period	Number of animals	Number of follicles	Percentage of follicles ruptured*	Percentage of ova in oviduct**
4 23 00-24 00	6	69	0	0
1 01 00-02 00	6	77	61 0	60 3
1 03 00-04 00	5	71	98 6	97.1
1 05 00-06 00	5	77	98.7	100 0
1 07.00-08 00	5	68	100 0	100 0

* Percentage based on number of follicles

** Percentage based on number of follicles ruptured

started between 01.00 and 02.00 of Day 1. By that time, 61% of all the follicles had been ruptured and approximately 60% of the expelled ova had reached the oviduct. Between 03.00 and 04.00, ovulation had almost been completed and more than 97% of the ova was recovered from the oviduct. These data lead to the conclusion that ovulation starts later than 24.00 of Day 4 and has almost been accomplished at 04.00 of Day 1. Half the number of all the

TABLE 2 Plasma levels of oestradiol and progesterone in cyclic hamsters

Autopsy day / period	Number of animals	Oestradiol* pg / ml	Progesterone* ng / ml
1 09.00-11.00	5	14 ± 1	7.5 ± 0.9
15.00-17.00	10	42 ± 4	6.7 ± 0.8
21.00-23.00	6	37 ± 7	8.4 ± 1.0
2 03.00-05.00	5	23 ± 6	6.3 ± 0.5
09.00-11.00	5	26 ± 5	6.1 ± 0.8
15.00-17.00	5	28 ± 7	9.7 ± 1.1
21.00-23.00	5	39 ± 14	8.9 ± 1.2
3 03.00-05.00	5	42 ± 14	3.8 ± 0.4
09.00-11.00	8**	89 ± 11	1.2 ± 0.3
15.00-17.00	5	122 ± 16	1.9 ± 0.3
21.00-23.00	4	188 ± 7	1.9 ± 0.3
4 03.00-05.00	5	164 ± 11	0.1 ± 0.1
09.00-10.00	5	120 ± 5	0.6 ± 0.1
11.00-12.00	5	107 ± 7	1.6 ± 0.2
13.00-14.00	5	148 ± 22	2.3 ± 0.3
15.00-16.00	7	274 ± 23	17.0 ± 1.9
17.00-18.00	11	142 ± 16	21.9 ± 6.6
19.00-20.00	6	64 ± 7	25.0 ± 1.9
21.00-22.00	5	18 ± 2	25.0 ± 1.7
23.00-24.00	6	15 ± 1	18.9 ± 1.2
1 01.00-02.00	6	10 ± 1	17.5 ± 1.7
03.00-04.00	5	10•	10.4 ± 2.0
05.00-06.00	5	10•	8.0 ± 1.7
07.00-08.00	5	10•	3.3 ± 0.7

* Mean ± SEM

** 8 animals for oestradiol determinations and 7 animals for progesterone determinations

• Minimum detectable dose

Graafian follicles had been ruptured between 01.00 and 02.00 of Day 1. It seems justified to use 02.00 as the time of ovulation in cyclic hamsters.

Plasma levels of steroid hormones in cyclic hamsters

Steroid measurements were performed in plasma samples obtained after total bleeding of cyclic hamsters including those in which the time of ovulation and the initiation of ovum transport was monitored. Table 2 shows the plasma concentrations of oestradiol and progesterone as measured during the entire oestrous cycle. The patterns for these steroid hormones are also displayed in Figure 6. Oestradiol was below 15 pg/ml plasma in the period of ovulation and in the morning of Day 1. Then, the levels became somewhat higher and fluctuated until 09.00 - 11.00 on Day 3. Afterwards, oestradiol concentrations rose significantly to reach a maximum between 21.00 - 23.00. A continuous decline until noon of Day 4 preceded the sharp rise to a distinct peak level observed between 15.00 - 16.00 on Day 4. This maximum oestradiol concen-

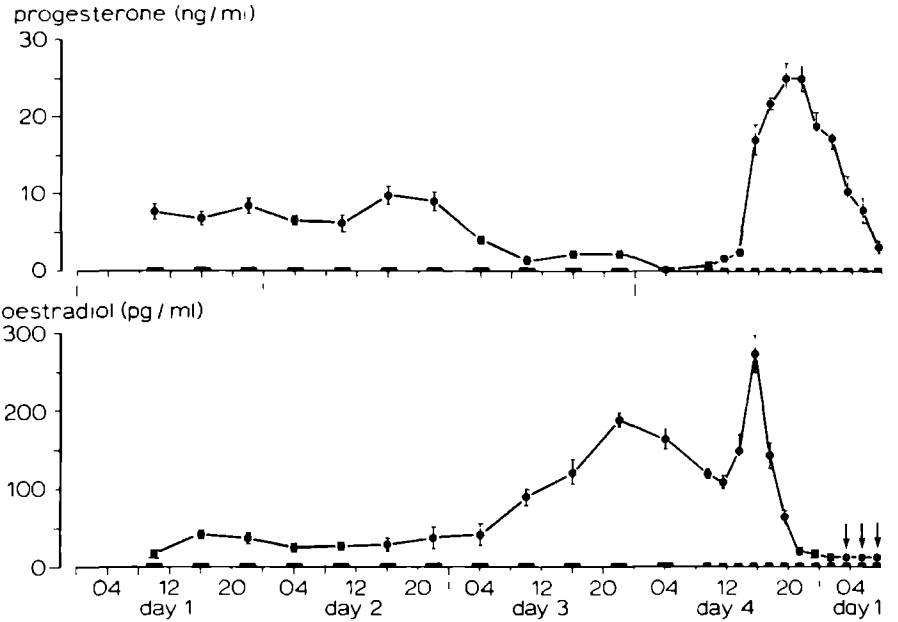

FIGURE 6 Plasma levels* of progesterone and oestradiol in hamsters throughout the oestrous cycle

* Mean ± SEM

➔ Minimum detectable dose

tration was followed by a very rapid decline to the lowest levels of the entire cycle.
Fluctuating progesterone concentrations were measured on Days 1 and 2. Much lower levels were observed on Day 3 and in the morning of Day 4, with almost undetectably minimal concentrations between 03.00 - 05.00. In the afternoon of Day 4, a sudden sharp increase was measured between 15.00 - 16.00, with maximum preovulatory progesterone concentrations between 19.00 - 22.00. This was followed by a sharp decline to one-third of the peak levels between 05.00 - 06.00 on Day 1.

2. The first stage of pregnancy

Ovum transport through the oviduct
Normal ovum transport was determined in the various oviductal segments of untreated hamsters during the first four days of pregnancy. Table 3 shows the results of these experiments. During the first period of Day 1, all the ova were recovered from the ampulla. With the time of ovulation fixed at 02.00 during the preceding night, all the ova remained at least 7 - 9 hours in the ampullary segment of the oviduct. The majority of ova were found in the foremost portion of the isthmus (segment 1) in the afternoon of Day 1. On Day 2, all the ova remained in the isthmus with the majority localized in the last segment near the junctura. In the morning of Day 3, however, less than 10% were found in this segment and approximately 25% had entered the uterus by then. The rest remained in the junctura. In the afternoon of Day 3, more than 75% had completed their journey. Hence, the maximum duration of ovum transport was approximately 64 hours (18.00 on Day 3). The data of Day 4 show that nearly half the number of ova (43.1%) could be flushed out of the uterus 79 - 81 hours after ovulation. This indicates that implantation had occured for more than 50% of all the ova. In the afternoon of Day 4 all the ova were implanted. During the entire period investigated, the total number of unfertilized ova ranged between 0 and 9%.

Contractility of the oviduct in vitro
The contractility of the oviduct in vitro was observed for groups of five animals at different times in the pre- and postovulatory period. The oviduct did not show peristaltic contractions 11 to 10 hours before the assumed time of ovulation, and the ampullary lumen was narrow. This changed completely in the periovulatory period. Between 03.00 - 04.00 and 09.00 - 10.00 on Day 1, the ampulla was strongly expanded, due to the accumulation of fluid. There were strong rhythmic abovarian contractions with relaxations that ended at

TABLE 3 Ovum transport through the various segments of the oviduct of untreated hamsters

Autopsy day / period	Number of animals	Number of corpora lutea	Percentage of ova recovered* from						Percentage of unfertilized ova
			ampulla	isthmus+ 1	isthmus+ 2	isthmus+ 3	junctura	uterus	
1 09 00-11.00	5	63	100.0	–	–	–	–	–	–
15.00-17 00	9	114	22 8	77.2	–	–	–	–	–
2 09 00-11.00	7	100	–	–	21 0	78 0	–	–	9 0
15.00-17 00	7	94	–	–	10.5	90 4	–	–	6 4
3 09 00-11.00	6	81	–	–	–	8.6	63 0	24.7	4 9
15 00-17 00	5	58	–	–	–	–	19 0	79 3	5 2
4 09.00-11.00	5	58	–	–	–	–	–	43 1	3.4
15 00-17.00	5	65	–	–	–	–	–	0 0	0.0

* Percentage based on total number of corpora lutea counted

+ Isthmus is divided into a first (1), a middle (2) and a last (3) segment

the ampullary-isthmic junction. Between 15.00 - 16.00 of Day 1 contractions were seen in the ampulla and in the foremost part of the isthmus. During these intervals the lumen of the isthmic entrance was clearly enlarged. Between 09.00 - 10.00 of Days 2 and 3, contractions were seen at the ampullary-isthmic junction and in those segments of the oviduct where ova were present. The ova were shaken rather vigorously through the entire lumen. During these intervals, the ampullary lumen was again as narrow as 11 to 10 hours preovulatory. These data lead to the conclusion that the oviductal contractions mainly occur in those segments where the ova are localized.

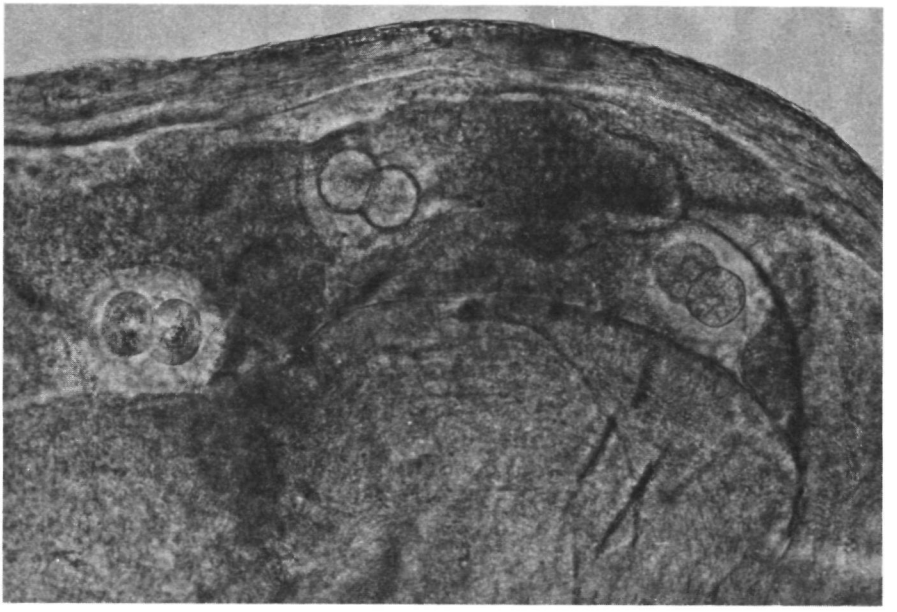

PHOTO 1 Ova in the isthmus 31-32 hours after ovulation (x 128)

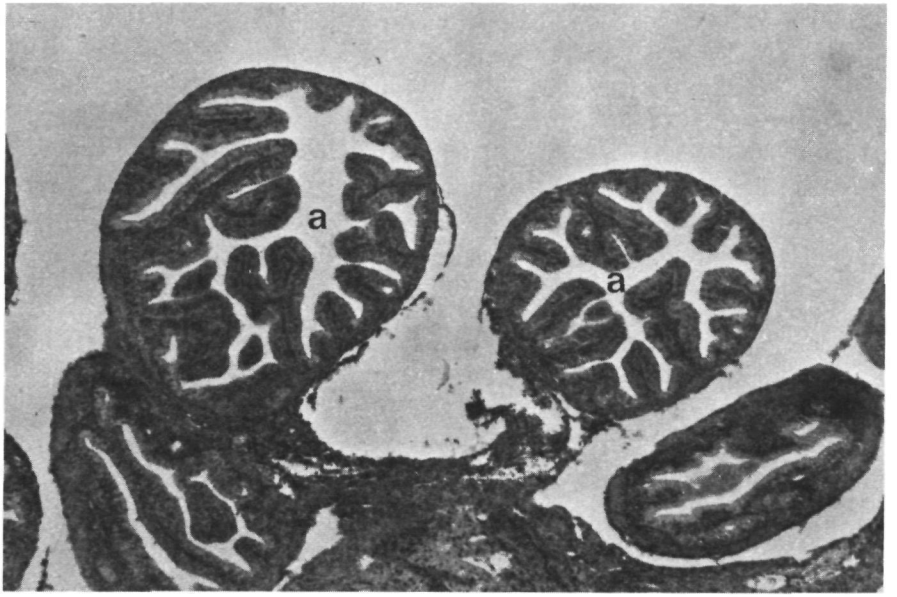

PHOTO 2 Cross section through the ampulla (a) 11-10 hours prior to ovulation (x 40)

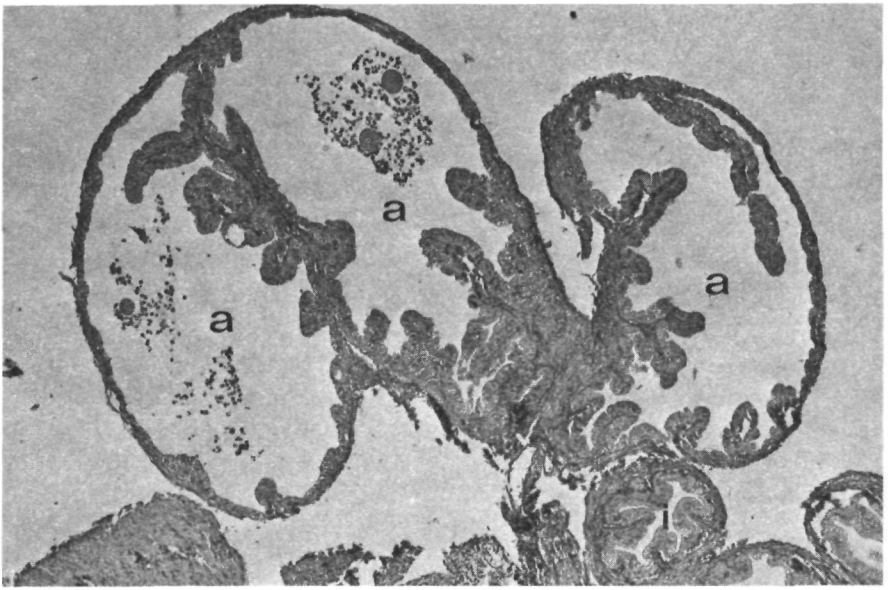

PHOTO 3 Cross section through ampulla (a) and isthmus (i) 7-8 hours after ovulation (x 25)

Plasma levels of steroid hormones in pregnant hamsters

Oestradiol and progesterone concentrations were also measured in the plasma of the hamsters that were used for the study of normal ovum transport (cf. Table 3). The values obtained for the two steroid hormones are summarized in Table 4 and further illustrated in Figure 7.

The oestradiol concentrations in the first stage of pregnancy were always very much higher in samples collected at night as compared to those obtained at moments during the day.

For progesterone, the average values ranged between 6.7 and 12.9 ng/ml plasma, with only moderate fluctuations from one period to the next over all four days.

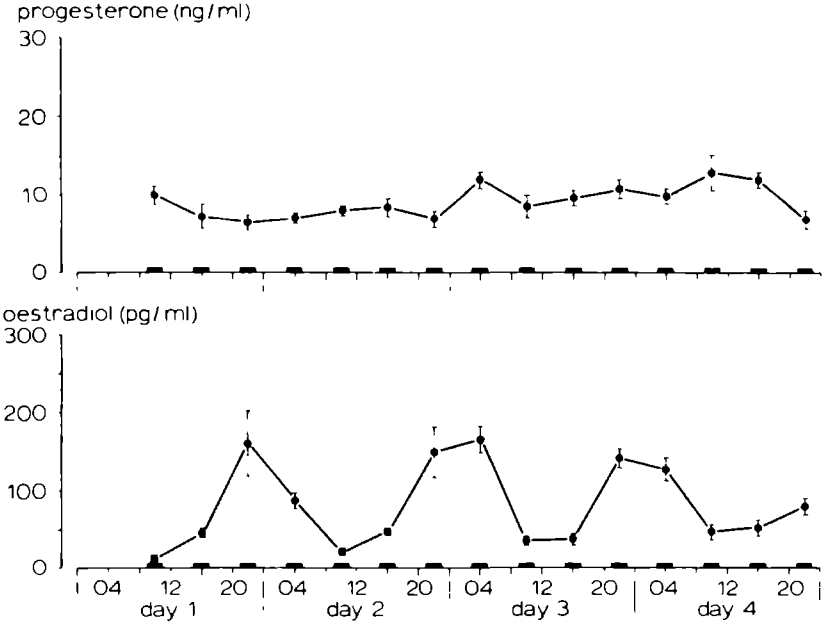

FIGURE 7 Plasma levels* of progesterone and oestradiol in hamsters during the first stage of pregnancy

* Mean ± SEM

TABLE 4 Plasma levels of oestradiol and progesterone for hamsters during the first four days of pregnancy

Autopsy day / period	Number of animals	Oestradiol* pg / ml	Progesterone* ng / ml
1 09.00-11.00	5	10 ± 1	9.9 ± 1.2
15.00-17.00	9	46 ± 6	7.3 ± 1.5
21.00-23.00	5	161 ± 42	6.7 ± 1.0
2 03.00-05.00	4	87 ± 10	7.0 ± 0.7
09.00-11.00	7**	20 ± 5	8.1 ± 0.6
15.00-17.00	7	44 ± 5	8.3 ± 1.2
21.00-23.00	5	149 ± 32	7.0 ± 1.0
3 03.00-05.00	5	167 ± 17	12.0 ± 1.1
09.00-11.00	6	34 ± 5	8.5 ± 1.3
15.00-17.00	7	37 ± 5	9.8 ± 1.0
21.00-23.00	5	139 ± 12	10.8 ± 1.3
4 03.00-05.00	5	126 ± 13	10.2 ± 1.0
09.00-11.00	5	46 ± 10	12.9 ± 2.2
15.00-17.00	5	53 ± 9	12.1 ± 0.9
21.00-23.00	4	81 ± 7	6.7 ± 1.0

* Mean ± SEM
** 7 animals for oestradiol determinations and 8 animals for progesterone determinations

DISCUSSION

The present chapter dealt with the determination of the ovulation period and with the measurement of oestradiol and progesterone levels in the peripheral plasma of hamsters during the oestrous cycle. After ovulation and the establishment of successful mating, it was registered how long oviductal ovum transport during the first stage of pregnancy takes. Furthermore, the peripheral plasma levels of oestradiol and progesterone were determined. Finally, the contractility of the oviduct in vitro was studied previous to ovulation and during the first three days of pregnancy when ovum transport takes place.

Ovulation occurred between 00.00 and 04.00 of Day 1 (Table 1). This was also reported by the majority of authors cited in Figure 3. The moment of ovula-

tion, when 50% of all the Graafian follicles were ruptured was at 02.00 on Day 1. This was determined on the basis of the data presented here (Table 1) as well as on the basis of those of Bastiaans (1973), and indicates the high rate of synchronization for ovulation in cyclic hamsters. Oestradiol and progesterone concentrations were measured in hamster plasma during the entire four-day oestrous cycle (Table 2). The demonstrated oestradiol pattern (Figure 6) fits in quite well with the one determined by Baranczuk and Greenwald (1973) (Figure 5). However, they observed rather constant high levels for oestradiol between 14 00 and 22.00 on Day 3. Labhsetwar et al. (1973) also established a significant rise in oestradiol values after 14.00 on Day 3. Our data indicate maximum oestradiol levels between 21.00 on Day 3 until 05.00 of Day 4. The differences observed are probably due to the fact that blood samples were taken more frequently at moments that were equally spaced out over the observation period. Peak levels for oestradiol on Day 4 were observed in all cases between 15.00 and 16 00. This is consistent with previous reports for the rat (Ferin et al., 1969; Hori et al., 1969).

It is assumed that the functional significance of the rise in ovarian oestradiol secretion on Day 3 lies in the fact that it triggers off the release of LH on Day 4 (Labhsetwar et al., 1973) and that it subsequently leads to ovulation (Baranczuk and Greenwald, 1973).

The results of progesterone plasma concentrations measured during the oestrous cycle of the hamster (Table 2, Figure 6) are comparable with those reported by Lukaszewska and Greenwald (1970), Leavitt and Blaha (1970), Leavitt et al. (1970), Norman and Greenwald (1971), Shaikh (1972), Labhsetwar et al. (1973) and Saidapur and Greenwald (1978).

However, all these reports, including the present one, differ from the detailed study of Ridley and Greenwald (1975) (Figure 5). The most striking differences are the strong fluctuations between maximum and minimum plasma levels of progesterone in the afternoon of cycle Day 4, and the repeated occurrence of maximum progesterone concentrations which are reached over the entire Day 1. The authors attribute the differences between this and previous studies (Leavitt and Blaha, 1970; Norman and Greenwald, 1971) to the use of a different assay technique (RIA) or to random animal variation (Ridley and Greenwald, 1975), although these reasons cannot fully explain the discrepancies in the patterns reported.

The maximum progesterone values in most reports appear to be the consequence of the ovulatory LH release (Greenwald, 1971; Norman and Greenwald, 1971; Labhsetwar et al., 1973). Fluctuations in the timing of the critical period for LH release between 15.00 - 18.00 on Day 4 (Goldman and Porter, 1970; Labhsetwar et al., 1973; Bast and Greenwald, 1974; Bex and Goldman,

1975) may explain the slight differences in the periods of maximum progesterone secretions: Labhsetwar et al. (1973) recorded this on Day 1, whereas Lukaszewska and Greenwald (1970), Shaikh (1972) and our study indicate maximum progesterone levels already on Day 4. The low progesterone concentrations measured on Day 3 and on Day 4, previous to the progesterone peak coincided with the first oestradiol elevation. Hence, it seems likely that luteolysis occurs on cycle Day 3, as indicated by the low progesterone values measured during this time (Figure 6), and that the functional significance of the elevated preovulatory oestradiol levels observed on Day 3 (Figure 6) may be related to the initiation of follicle maturation necessary for the next ovulation.

The pattern of oviductal ovum transport in pregnant hamsters as shown in Table 3 is identical to a high degree with the observations of Bastiaans (1973). His results and the data reported here were compared by calculating the time necessary for 50% of all the ova to pass through the various segments of the oviduct during ovum transport. The average duration of the entire process lasted 56½ and 58½ hours, respectively. This was determined by calculations of the time between ovulation and the moment when half the number of ova could still be flushed out of the uterus.

The ampullary-isthmic junction had been passed by 50% of the ova 12 hours after ovulation. The isthmus was traversed about 47 and 48 hours after ovulation, respectively. Half the total number of fertilized ova was implanted 77 hours (Bastiaans, 1973), and 79 hours postovulatory (this study). Implantation was completed 87 hours after ovulation.

The high degree of correlation between the two sets of data indicate the reliability of the techniques used and the regularity of the pattern of ovum transport in the case of hamsters.

The steroid milieu during the first two days of the cycle (Table 2, Figure 6) was rather similar to the hormonal levels during the same period of pregnancy (Table 4, Figure 7) when ovum transport takes place. The only differences observed concerned the oestradiol levels at night. The end of ovum transport was determined to be 58½ hours postovulatory (i.e. at 12.30 on Day 3). At this moment both the oestradiol and progesterone values in the case of cyclic hamsters were significantly different from the corresponding steroid levels measured for pregnant animals. This indicates that after at least 48 hours (i.e. on Day 3), there must be a signal to the corpus luteum causing it to persist, as is evidenced by the continuation of the progesterone and oestradiol production in pregnant hamsters on Day 3 and Day 4.

There might be two speculations as to the sources of this signal: Firstly, the fertilized ovum produces a chorionic gonadotrophin as early as three days

postovulatory, thus preventing the initiation of new follicle maturation previous to the forthcoming ovulation. Recently, chorionic gonadotrophin activity has been demonstrated in placental extracts of rats, mice and hamsters (Wide and Hobson, 1977), whereas Haour and Saxena (1974) have detected a gonadotrophin similar to hCG-LH in rabbit blastocysts prior to implantation. Secondly, copulation itself causes the persistence of a functional corpus luteum. This is evidenced by the duration of pseudopregnancy, which lasts 9 days (Orsini, 1961), although it occasionally varies in length from 7 to 13 days following ovulation (Magalhaes, 1970). Cessation of oestrous cycles and lack of ovarian follicle maturation are its main characteristics. Conclusive evidence for the role of copulation in the persistence of a functional corpus luteum can be derived from an experiment with pseudopregnant hamsters only when it can be shown that the individual steroid profiles for oestradiol and progesterone are equal in both pregnant and pseudopregnant animals on the third postovulatory day.

Steroid hormones influence the regulation of oviductal contractility.
This is evidenced by numerous experiments in which exogenously administered oestrogenic and progestational steroids interfere with normal ovum transport (Chang, 1976).
The theories of Harper (1966) and of Boling and Blandau (1971a, 1971b) concerning the role of steroid hormones on the contractile activity of the oviduct during ovum transport, as described in Chapter II (ad 8) can be applied to our data. It was observed that a slight tonic (i.e. a continuous but moderately) ampullary activity with a narrow ampullar lumen as seen 11 - 10 hours prior to ovulation coincided with the oestradiol peak on Day 4.
Between 1 - 2 and 7 - 8 hours postovulatory, the ampulla contained fluid and showed strong abovarian contractions followed by relaxations. During these intervals, the oestradiol levels were low which is in accordance with the theory of Boling and Blandau (1971a, 1971b). Thus, the conclusion appears to be justified that the hamster oviduct becomes contracted due to oestradiol at the time prior to ovulation when oestradiol secretions are at maximum, and that contractions of the oviduct start as soon as the high oestradiol values decline. Furthermore, the maintenance of oviductal contractility over the entire period of ovum transport may be related to the decreasing oestradiol levels which are observed in the morning of each Day (Figure 7), as a consequence of lower concentrations during the days than in the preceding nights.
The muscular layer of the isthmus is stronger developed than the ampullar segment of the oviduct. The isthmus reacts later upon the declining oestradiol

levels than the ampullar segment of the oviduct, for which the anatomical differences may at least in part be responsible. This then causes the ova to stay in the distal part of the ampulla for a relatively long time.
It is not clear which role progesterone plays in oviductal contractility. The maximum progesterone concentrations prior to ovulation occurred later, and lasted longer than those of oestradiol. Furthermore the nadir in progesterone values between 07.00 - 08.00 on cycle Day 1 was followed by a significant rise during the next hour in both mated (Table 4, Figure 7) and unmated (Table 2, Figure 6) animals. Afterwards, the plasma progesterone concentrations remained constant until ovum transport was completed. These sequences of change in the progesterone levels may all facilitate a relaxation of ampullar activity, with a further increase of the amplitude and a decrease in the frequency of contractions.
The conclusion seems justified that there must be a delicately balanced relationship between the physiological patterns of oestrogens and progestins on the one hand and the initial stages of ovum transport and thus the contractile activity on the other.

TISSUE CONCENTRATIONS OF PROSTAGLANDINS $F_{2\alpha}$ AND E_2 IN OVIDUCTS OF HAMSTERS DURING THE CYCLE, IN PREGNANCY, AND FOLLOWING TREATMENT WITH INDOMETHACIN AND OESTRADIOL

INTRODUCTION

The hypothesis outlined in the introduction of this study entails tests in order to establish whether there exists a relationship between oestrogens and oviductal tissue levels of prostaglandins. The following experiments were designed in order to investigate this theory.
First of all, tests were conducted in order to determine the tissue concentrations of prostaglandins $F_{2\alpha}$ and E_2 in the oviducts of untreated golden hamsters throughout the oestrous cycle and during the first stage of pregnancy. Next, the influence was tested of exogenously administered oestradiol on oviductal tissue concentrations of the two prostaglandins for hamsters on Days 1 and 2 of the cycle and on the same days of pregnancy. The effect of indomethacin on oestradiol-induced oviductal tissue concentrations of prostaglandins was studied, as well as the influence of indomethacin on the endogenous tissue levels of prostaglandins in the case of non-pretreated pregnant hamsters.

RESULTS

1. Prostaglandin levels in the oviducts of untreated hamsters

The oestrous cycle
Table 5 shows tissue concentrations of $PGF_{2\alpha}$ and PGE_2 measured in the oviducts of cyclic hamsters. On the basis of the concentrations in each animal, the $PGF_{2\alpha}/PGE_2$ ratios were calculated. The average for every group of cyclic animals is given in this Table. The $PGF_{2\alpha}$ and PGE_2 patterns and their $PGF_{2\alpha}/PGE_2$ ratios in the oviducts of hamsters throughout the oestrous cycle are illustrated in Figure 8.

TABLE 5 Tissue concentrations of prostaglandins $F_{2\alpha}$, E_2, and their ratios in oviducts of untreated hamsters throughout the oestrous cycle

Autopsy day / period	Number of animals	$PGF_{2\alpha}$* ng/g oviduct	PGE_2* ng/g oviduct	Ratio* $PGF_{2\alpha}/PGE_2$
1 09 00-11 00	5	2256 ± 169	27 5 ± 2 8	85 ± 9
15 00-17 00	5	767 ± 91	11 5 ± 1 5	71 ± 12
21 00-23 00	5	1147 ± 79	25 0 ± 1 7	46 ± 3
2 03 00-05 00	5	884 ± 112	15 2 ± 1 6	58 ± 4
09 00-11 00	5	459 ± 41	7 2 ± 0 5	65 ± 6
15 00-17 00	5	388 ± 64	5 8 ± 0 7	66 ± 7
21 00-23 00	5	891 ± 78	19 4 ± 1 2	46 ± 2
3 03 00-05 00	5	716 ± 103	17 2 ± 1 4	41 ± 4
09 00-11 00	5	861 ± 137	9 7 ± 0 7	88 ± 13
15 00-17 00	5	862 ± 104	9 1 ± 1 0	94 ± 5
21 00-23 00	5	1797 ± 119	31 5 ± 3 2	59 ± 7
4 03 00-05 00	5	2135 ± 232	37 9 ± 1 9	56 ± 5
09 00-10 00	5	1849 ± 114	9 6 ± 1 0	198 ± 15
11 00 12 00	5	1915 ± 98	10 7 ± 0 4	178 ± 5
13 00-14 00	4**	2003 ± 170	12 0 ± 1 9	168 ± 21
15 00-16 00	5	1477 ± 84	12 0 ± 0 5	124 ± 9
17 00-18 00	4	1557 ± 151	12 7 ± 0 9	123 ± 4
19 00-20 00	5	3269 ± 116	43 9 ± 1 6	75 ± 4
21 00-22 00	5	4486 ± 311	54 0 ± 4 4	85 ± 7
23 00-24 00	4	4236 ± 755	47 1 ± 3 2	89 ± 14
1 01 00-02 00	5	3632 ± 378	67 0 ± 11 8	58 ± 7
03 00-04 00	5	4274 ± 341	65 6 ± 5 4	67 ± 7
05 00-06 00	3	2984 ± 367	79 4 ± 11 5	38 ± 2
07 00-08 00	4	2534 ± 302	63 0 ± 5 4	40 ± 2

* Mean ± SEM

** 5 animals for $PGF_{2\alpha}$ measurements

Minimum $PGF_{2\alpha}$ and PGE_2 levels in oviducts were detected on cycle Day 2 (Figure 8). $PGF_{2\alpha}$ concentrations were doubled to 891 ng/g oviduct at 21 00 – 23 00 of Day 2 These values were maintained until they doubled at 21 00 – 23 00 of Day 3 The concentrations of about 2000 ng/g tissue remained constant until 14 00 of Day 4 Then, significantly lower levels ($p < 0\ 05$) were observed during the next four hours, followed by an extremely sharp increase to maximum levels lasting throughout the night until the early morning of Day 1 (Table 5)

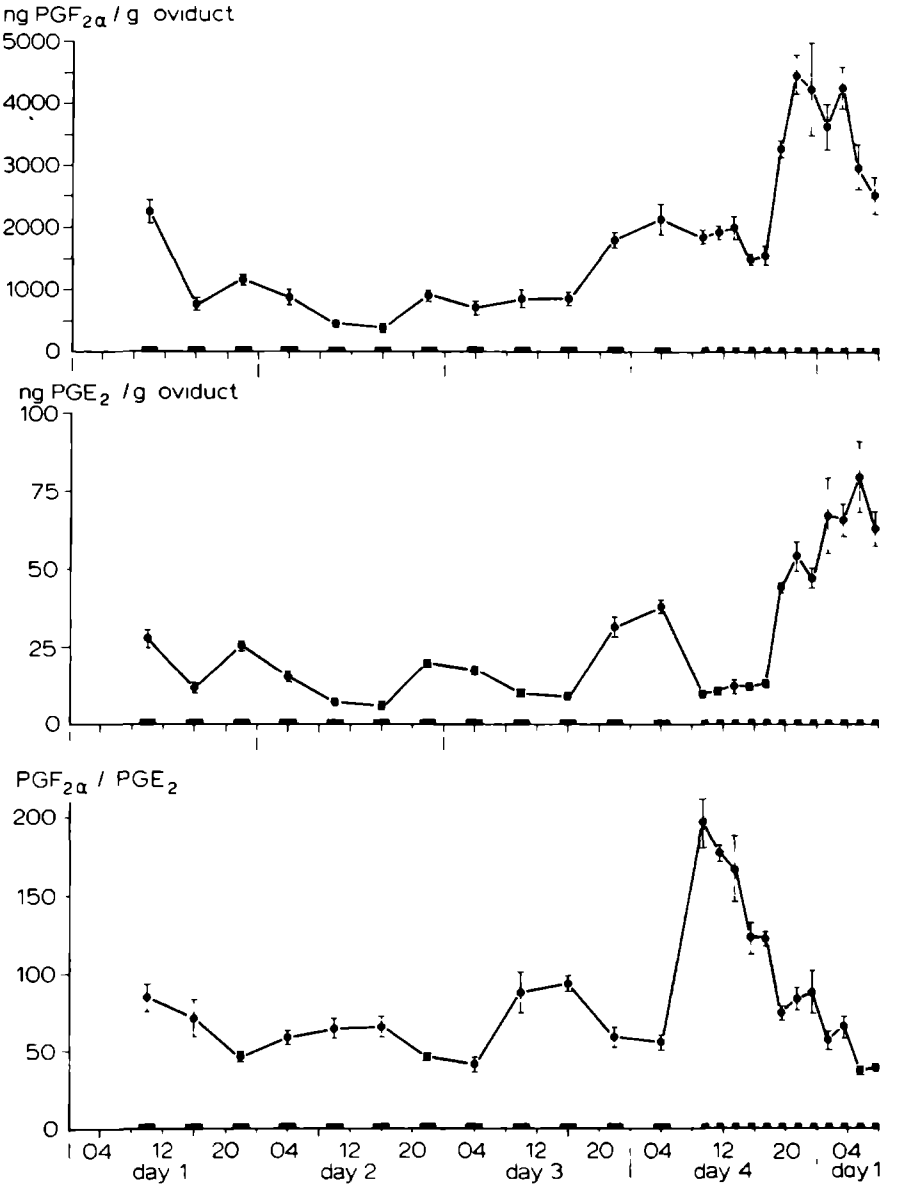

FIGURE 8 Tissue concentrations* of prostaglandins $F_{2\alpha}$, E_2, and their ratios in oviducts of hamsters throughout the oestrous cycle

* Mean ± SEM

PGE_2 concentrations showed a rather similar rhythmic pattern: The lower levels (6-7 ng/g oviduct) were detected on Day 2 with somewhat higher levels on Day 3 and Day 4 until 18.00. Maximum oviductal PGE_2 concentrations for cyclic hamsters also remained at a constantly high level during the evening and night of Day 4 following a similar pattern as observed for $PGF_{2\alpha}$.
The two prostaglandins measured also showed an equal maximum increase during the cycle since the lower values detected on Day 2 increased to maximum levels that were 10 – 12 times higher. At the same time, differences for the individual prostaglandin patterns were confirmed by the rate of change observed for prostaglandin ratios during the cycle (Figure 8).

TABLE 6 Tissue concentrations of prostaglandins $F_{2\alpha}$, E_2, and their ratios in oviducts of hamsters during the first four days of pregnancy

Autopsy day / period	Number of animals	$PGF_{2\alpha}$* ng/g oviduct	PGE_2* ng/g oviduct	Ratio* $PGF_{2\alpha}/PGE_2$
1 09.00-11.00	5	1966 ± 221	19.4 ± 1.7	102 ± 11
15.00-17.00	5	1070 ± 19	11.0 ± 0.2	98 ± 3
21.00-23.00	4	1533 ± 93	31.8 ± 1.6	48 ± 2
2 03.00-05.00	3	1715 ± 159	43.4 ± 1.1	40 ± 4
09.00-11.00	5	812 ± 48	6.5 ± 1.2	136 ± 19
15.00-17.00	5	690 ± 42	5.3 ± 0.4	132 ± 3
21.00-23.00	5	2167 ± 251	44.9 ± 5.1	49 ± 5
3 03.00-05.00	4**	1379 ± 55	29.1 ± 2.7	49 ± 6
09.00-11.00	5	1249 ± 117	11.2 ± 0.6	114 ± 13
15.00-17.00	5	900 ± 77	7.4 ± 0.9	130 ± 21
21.00-23.00	4	1176 ± 91	25.7 ± 1.2	37 ± 8
4 03.00-05.00	4+	1225 ± 86	34.0 ± 3.1	39 ± 17
09.00-11.00	5	1336 ± 108	11.9 ± 1.2	117 ± 13
15.00-17.00	5	667 ± 66	8.2 ± 0.8	81 ± 3
21.00-23.00	5	1524 ± 139	38.4 ± 3.7	40 ± 3

* Mean ± SEM
** 5 animals for $PGF_{2\alpha}$ determinations
\+ 5 animals for PGE_2 determinations

The first stage of pregnancy

Tests were also conducted in order to determine the tissue levels of $PGF_{2\alpha}$ and PGE_2 in oviducts of hamsters during Day 1 up to and including Day 4 of pregnancy. The mean concentrations of the prostaglandins and their ratios are given in Table 6 and are illustrated in Figure 9. The prostaglandin concentrations in the oviducts of mated hamsters decreased from ovulation onwards to minimum levels at 15.00 – 17.00 of Day 1. Then, the values increased to reach maxima during the night, and they were followed by a rapid fall to minimum levels throughout the day for both prostaglandins on Days 3 and 4 of pregnancy. Values fluctuated rather equally with much higher levels occurring during the nights than in the mornings and afternoons of

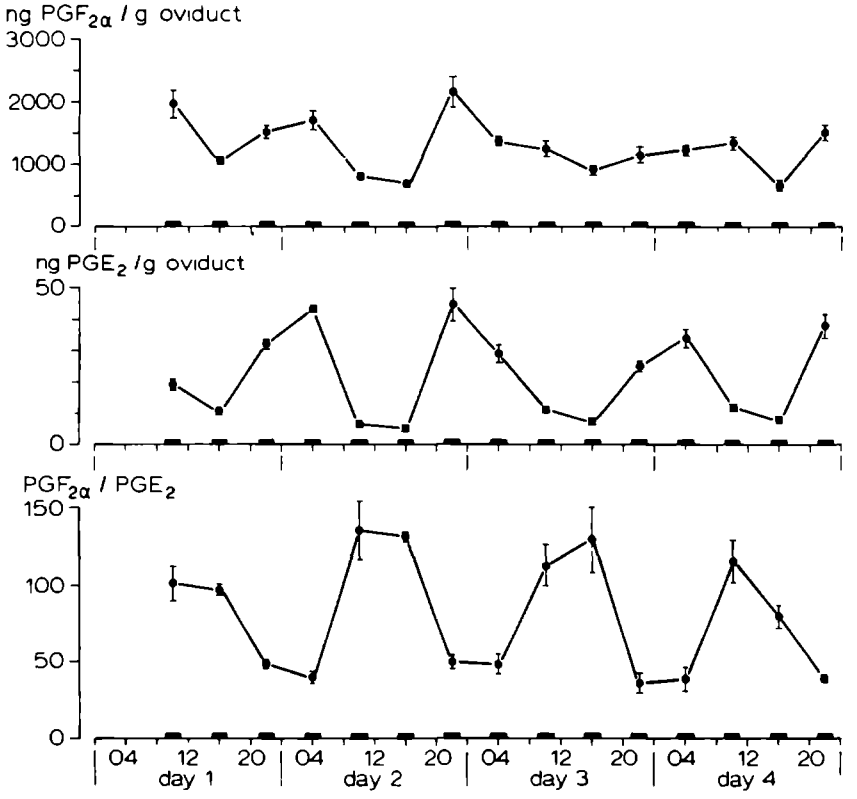

FIGURE 9 Tissue concentrations* of prostaglandins $F_{2\alpha}$, E_2, and their ratios in oviducts of hamsters during the first stage of pregnancy

* Mean ± SEM

these Days (Figure 9). The levels of PGE_2 were subject to greater fluctuations than those of $PGF_{2\alpha}$, as can be derived from their individual rates of change (Table 6) as well as from the patterns of their ratios over the entire period (Figure 9).

2. Prostaglandin levels in the oviducts of hamsters after various treatments

The effect of oestradiol given to cyclic and to pregnant hamsters

The exogenous administration of oestradiol to both cyclic hamsters and animals in the first stage of pregnancy (Table 7) always resulted in significant

TABLE 7 The effect of s.c. injected oestradiol on tissue concentrations of prostaglandins $F_{2\alpha}$, E_2, and their ratios in oviducts of cyclic and pregnant hamsters

Autopsy day / period	Number of animals	Treatment dose+ route day	$PGF_{2\alpha}$* ng/g oviduct	PGE_2* ng/g oviduct	Ratio* $PGF_{2\alpha}/PGE_2$
1 15.00-17.00	5	cycle control	767 ± 204	11.5 ± 3.4	71 ± 26
1 15.00-17.00	5	250µg OE_2 s.c. 1	1695 ± 289 [b]	27.7 ± 7.9 [a]	65 ± 17
2 15.00-17.00	5	cycle control	388 ± 144	5.8 ± 1.6	66 ± 15
2 15.00-17.00	5	250µg OE_2 s.c. 2	1147 ± 250 [b]	17.5 ± 3.4 [b]	73 ± 21
1 15.00-17.00	5	pregnancy control	1070 ± 42	11.0 ± 0.4	98 ± 6
1 15.00-17.00	5	250µg OE_2 s.c. 1	4300 ± 1165 [b]	70.1 ± 7.0 [b]	61 ± 14 [b]
2 15.00-17.00	5	pregnancy control	690 ± 94	5.3 ± 0.9	132 ± 6
2 15.00-17.00	5	250µg OE_2 s.c. 2	2443 ± 399 [b]	44.8 ± 9.4 [b]	55 ± 9 [b]

* Mean ± SD

\+ 250 µg oestradiol (OE_2) injected subcutaneously (s.c.) at 10.00 of Days 1 or 2 into cyclic or pregnant hamsters

a $p < 0.01$ as compared to control group

b $p < 0.001$ as compared to control group

increases ($p < 0.01$) of the oviductal tissue concentrations of $PGF_{2\alpha}$ (Figure 10) and PGE_2 (Figure 11) as compared to their controls. In the case of hamsters treated with oestradiol, the maximum increments of oviductal prostaglandin concentrations were observed on both days of pregnancy, with stronger increases for PGE_2 than for $PGF_{2\alpha}$ levels.

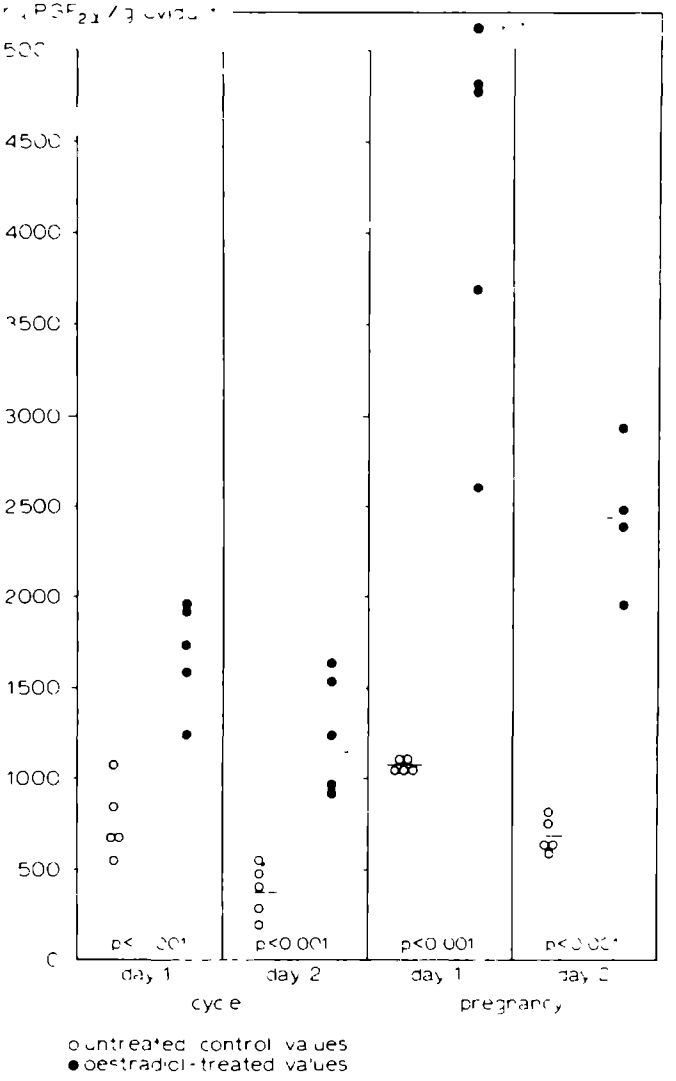

FIGURE 10 The effect of s.c. injected oestradiol on tissue concentrations of prostaglandin $F_{2\alpha}$ in oviducts of cyclic and pregnant hamsters

The effect of indomethacin and oestradiol administered during pregnancy
The stimulation of prostaglandin tissue concentrations in oviducts of hamsters in the first stage of pregnancy after oestradiol administration (Table 7) could be reversed by treating animals with indomethacin previous to oestra-

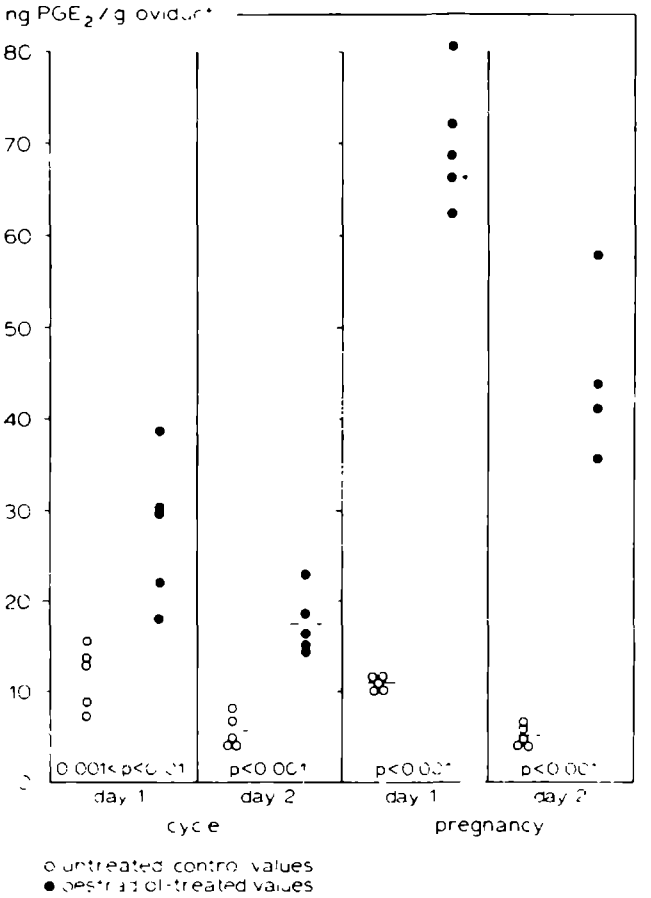

FIGURE 11 The effect of s.c. injected oestradiol on tissue concentrations of prostaglandin E_2 in oviducts of cyclic and pregnant hamsters

diol injection. The results of these experiments are shown in Table 8. The combined treatment of pregnant hamsters with both indomethacin and oestradiol always resulted in prostaglandin tissue concentrations and ratios that were significantly lower ($p < 0.01$) than those obtained after a single subcutaneous oestradiol injection. On Day 2 of pregnancy, the decrease of prostaglandin tissue levels, as opposed to their ratios, depended on the dose of indomethacin administered: The higher quantity of the prostaglandin synthetase inhibitor was more effective in decreasing $PGF_{2\alpha}$ ($p < 0.05$) and PGE_2 ($p < 0.001$) levels than the lower amount of indomethacin.

TABLE 8 The effect of exogenously administered oestradiol and the influence of combined treatment with indomethacin and oestradiol on tissue concentrations of prostaglandins $F_{2\alpha}$, E_2, and their ratios in oviducts of pregnant hamsters

Autopsy day / period	Number of animals	Treatment dose+	route	day	$PGF_{2\alpha}$* ng/g oviduct	PGE_2* ng/g oviduct	Ratio* $PGF_{2\alpha}/PGE_2$
1 15.00-17 00	5	250µg OE_2	s.c	1	4300 ± 1165	70.1 ± 7.0	61 ± 14
1 15.00-17.00	4	100µg IM 250µg OE_2	orally s.c.	1	571 ± 161 b	29 2 ± 7 8 b	21 ± 7 b
1 15.00-17.00	5	500µg IM 250µg OE_2	orally s c	1	619 ± 151 b	22 9 ± 2.5 b	27 ± 5 b
2 15 00-17 00	4	250µg OE_2	s c	2	2443 ± 399	44 8 ± 9 4	55 ± 9
2 15.00-17 00	5	100µg IM 250µg OE_2	orally s c.	2	466 ± 129 b } c	19 0 ± 2 8 a } d	24 ± 5 b
2 15 00-17.00	5	500µg IM 250µg OE_2	orally s c	2	292 ± 86 b	11.3 ± 1 8 b	27 ± 10 a

* Mean ± SD

\+ Oestradiol (OE_2) injected subcutaneously (s.c) at 10 00 of Days 1 or 2 into pregnant hamsters and
100 or 500 µg indomethacin (IM)/animal administered orally to hamsters at 08 00, previous to oestradiol treatment

• 5 animals used

a $p < 0$ 01 as compared to control (= oestradiol-treated) group

b $p < 0$ 001 as compared to control (= oestradiol-treated) group

c $p < 0.05$ between groups treated with different doses of indomethacin

d $p < 0$ 001 between groups treated with different doses of indomethacin

The effect of indomethacin treatment on pregnant hamsters

Table 9 illustrates the influence of indomethacin on endogenous tissue levels of $PGF_{2\alpha}$ and PGE_2 in oviducts of hamsters in the first stage of pregnancy as compared to controls

In the majority of all tests performed, the oral administration of indomethacin 7 hours prior to the moment of autopsy caused a significant decline of prostaglandin tissue concentrations The $PGF_{2\alpha}$ tissue levels decreased more markedly than those of PGE_2 (Table 9)

TABLE 9 The effect of indomethacin on tissue concentrations of prostaglandins $F_{2\alpha}$, E_2, and their ratios in oviducts of pregnant hamsters

Autopsy	Number of	Treatment			$PGF_{2\alpha}$*	PGE_2*	Ratio*
day / period	animals	dose+	route	day	ng/g oviduct	ng/g oviduct	$PGF_{2\alpha}/PGE_2$
1 03 00-05 00	4	pregnancy control			3889 ± 449	129 ± 17	31 ± 7
1 03 00-05 00	5	100µg	IM orally	4•	2451 ± 599 b	83 ± 12 b	30 ± 7
1 03 00-05 00	5	500µg	IM orally	4•	2700 ± 568 b	90 ± 11 b	30 ± 5
1 21 00-23 00	4	pregnancy control			1533 ± 186	32 ± 3	48 ± 4
1 21 00-23 00	5	100µg	IM orally	1	723 ± 186 c	32 ± 6	23 ± 7 c
2 03 00-05 00	3	pregnancy control			1715 ± 276	43 ± 2	40 ± 8
2 03 00-05 00	3**	100µg	IM orally	1	393 ± 89 c	17 ± 5 c	27 ± 9
2 03 00-05 00	4	500µg	IM orally	1	390 ± 105 c	12 ± 2 c	33 ± 6
3 03 00-05 00	5	pregnancy control			1379 ± 122	29 ± 5	49 ± 12
3 03 00 05 00	5	100µg	IM orally	2	761 ± 105 c	23 ± 5	34 ± 4 a
3 03 00-05 00	4	500µg	IM orally	2	494 ± 97 c	17 ± 3 b	25 ± 6 a

* Mean ± SD

\+ 100 or 500 µg indomethacin (IM)/animal administered orally to pregnant hamsters 7 hours before the autopsy period

• Treatment at 20 00 of cycle Day 4 (preovulatory)

** 4 animals for $PGF_{2\alpha}$ determinations

a $p < 0\ 05$ as compared to pregnancy control

b $p < 0\ 01$ as compared to pregnancy control

c $p < 0\ 001$ as compared to pregnancy control

If prostaglandins play a role in ovum transport they must be present in oviductal tissue and their concentration should vary in relation to the changes that take place during this process Little information on prostaglandin levels in oviductal tissue is available Prostaglandins have been identified by immunohistologic techniques in the human oviduct (Ogra et al , 1974, Vastik-Fernandez et al , 1975) Saksena and Harper (1975) have measured PGF concentrations in oviducts of rabbits treated with hCG The data presented here establish the occurrence of $PGF_{2\alpha}$ and PGE_2 in the oviduct of hamsters Up till now this fact had not been reported by other groups

For this reason the significance of these findings can only be illustrated by comparing the data reported here for the oviduct of the hamster with the PGF levels measured by Saksena and Harper (1975) in the oviduct of the rabbit Although one should bear in mind that certain differences in analytical results are probably due to differences in assay procedures and to varying degrees of sensitivity and specificity of the antibodies used, the $PGF_{2\alpha}$ concentrations in the oviduct of the hamster (between 400 and 4500 ng $PGF_{2\alpha}$/g oviduct) are about 75 times higher than those reported by Saksena and Harper (1975) for the oviduct of the rabbit This fits in very well with the great differences in oestrogenicity for the two species if one assumes a relationship between this feature and the prostaglandins in the oviduct The hamster is rather insensitive to oestrogens (Greenwald, 1976), as can be derived from its relatively high endogenous oestradiol plasma levels during the oestrous cycle (cf Chapter IV) compared to those in the rabbit (Wu et al , 1977)

The significance of the high prostaglandin levels in the oviducts of hamsters also becomes clear when one compares these levels with the much lower concentrations (between 7 and 20 ng PGF/g tissue) in the uterus of the hamster (Saksena and Harper, 1972a) The same tendency, i e much higher PGF levels in the oviduct (between 6 and 62 ng PGF/g tissue) than in the uterus (between 2 and 15 ng PGF/g tissue) has also been reported for rabbits by Saksena and Harper (1975)

Ogra et al (1974) suggested the existence of an endocrine control mechanism for the $PGF_{2\alpha}$ production in the human oviduct With regard to the rabbit, Saksena and Harper (1975) speculated that the sudden increase of PGF 10 hours after the induction of ovulation with hCG was caused by an augmented ovarian oestradiol secretion Hence, although one assumes that there is an endocrine control mechanism for oviductal prostaglandin synthesis in various species, reports of experiments specifically designed to confirm the presence of such mechanisms were lacking

The data given here show that, in the case of the oviduct of the hamster, not only $PGF_{2\alpha}$ levels but also PGE_2 levels vary during the oestrous cycle and during the period of ovum transport. In contrast to these results no significant changes were found in the prostaglandin concentrations in hamster plasma during the cycle and in the first stage of pregnancy (manuscript in preparation). This does not correspond with the results of Shaikh and Saksena (1973) who reported that PGF values in peripheral plasma of hamsters show a cyclic pattern similar to that of PGF in uterine venous plasma during the cycle. However, it can be argued that the peripheral plasma levels measured by these authors are at least questionable: Their PGF concentrations were in between 1 and 10 ng/ml plasma while our data are invariably substantially lower with ranges in between 100 and 200 pg $PGF_{2\alpha}$/ml plasma. A satisfactory explanation for these differences can be found in the rigorous conditions that have to be maintained in the collection of blood samples (Thomas et al., 1978a) and the complete unreliability of prostaglandin measurements in plasma samples that have not been snapfrozen at a low temperature in liquid nitrogen (unpublished data). As the latter criterion was not fulfilled by the authors cited, one can conclude that for this reason alone the cyclic pattern for F prostaglandins in peripheral plasma of hamsters during the oestrous cycle is questionable.
Additional evidence to justify this conclusion can also be found in the absence of cyclicity in the peripheral plasma levels of PGE_2 and the 13, 14-dihydro-15-keto metabolites of the F and E series in the same groups of hamsters (manuscript in preparation).

The $PGF_{2\alpha}$ and PGE_2 concentrations in oviducts of cyclic hamsters show a rhythmic pattern that correlates well with the ovarian oestradiol secretion reported in Chapter IV. Comparable cyclic variations of PGF levels during the oestrous cycle are also known to occur in uterine tissue of hamsters (Saksena and Harper, 1972a), rats (Saksena and Harper, 1972b) and guinea-pigs (Poyser, 1972). Maximum uterine PGF concentrations have been observed when endogenous levels of oestrogens were high (Saksena and Harper, 1972a; Poyser, 1972) or following the exogenous administration of oestradiol (Saksena and Harper, 1972a). It has also been reported that the latter relationship also occurs in the uterus of the rabbit as well as in its ovary and oviduct: Administration of oestradiol caused significant increases of PGF levels in these organs while by contrast progesterone depressed them to basal levels (Saksena and Harper, 1975). Furthermore, in vitro data on prostaglandin production after incubation of 'microsomal' fractions of uteri of oestrogen-treated, castrated rats indicated that oestrogens increase the PGF/PGE

ratio in these preparations (Ham et al., 1975). When one relates these data to the results presented here, the plasma peak level for oestradiol detected in the evening of cycle Day 3 indicates an adequate stimulation of both $PGF_{2\alpha}$ and PGE_2 synthesis in the oviduct. However, the duration of this oestradiol-induced effect lasted longer for $PGF_{2\alpha}$ than for PGE_2, leading to a sharp increase of the $PGF_{2\alpha}/PGE_2$ ratio in the morning of cycle Day 4. The observation that the oviductal PGF concentrations in rabbits were depressed to basal levels by progesterone (Saksena and Harper, 1975) might also be in accordance with our data concerning the oviducts of hamsters. The sudden increase of plasma progesterone concentrations at 15.00 – 16.00 of cycle Day 4 (cf. Table 2) immediately led to a significant fall of oviductal $PGF_{2\alpha}$ levels ($p < 0.05$), although no basal levels were reached, whereas PGE_2 concentrations remained unchanged. However, both $PGF_{2\alpha}$ and PGE_2 values increased substantially to maximum levels in the evening of cycle Day 4, when the main oestradiol peak occurring between 15.00 – 16.00 on the same day fully stimulated oviductal prostaglandin synthesis.

Hence, this process appeared to be facilitated by the constantly high progesterone levels that were measured during the same period (cf. Table 2). Similar observations confirming these results were made in animals in the first stage of pregnancy. The stimulating effect of endogenous oestradiol plasma levels on oviductal prostaglandin tissue concentrations of pregnant hamsters was demonstrated (Figure 12) by the correlation between the elevated oestradiol plasma levels observed throughout the nights during the first period of pregnancy (cf. Figure 7) and the concomitant substantial increases of the prostaglandin tissue concentrations (cf. Figure 9). PGE_2 concentrations were more effectively stimulated than the tissue levels of $PGF_{2\alpha}$; this was also observed in the oviducts of cyclic hamsters during the night of Day 3 and the early morning of Day 4 (Figure 8). During the latter period of the cycle and also throughout the first stage of pregnancy, the facilitating effect of progesterone on prostaglandin synthesis in oviducts of hamsters is lacking. At this stage of the cycle, progesterone is at nadir, whereas the moderate progesterone plasma levels during the first days of pregnancy are fairly constant, with ranges in between 7 and 12 ng/ml. Hence, oviductal tissue levels of $PGF_{2\alpha}$ and PGE_2 are adequately stimulated by oestradiol, but only for a shorter period of time due to the relatively low levels of progesterone. The steroid milieu involved affected the tissue levels of PGE_2 much more than those of $PGF_{2\alpha}$, as the former prostaglandin concentrations fluctuated more strongly than $PGF_{2\alpha}$ according to the rhythm of the oestradiol plasma levels during the first stage of pregnancy (Figure 12). These conclusions are most strikingly demonstrated by the patterns of change for

oestradiol plasma levels (Figure 7) and of the $PGF_{2\alpha}/PGE_2$ profile (Figure 9) that had been shifted about 12 hours over the entire first period of pregnancy (Figure 12). The changing ratios for $PGF_{2\alpha}/PGE_2$ suggest a differential control of biosynthesis and/or secretion of these compounds in the oviduct. Its prostaglandin production could also be induced by exogenous administration of oestradiol to cyclic or pregnant animals (Table 7). In addition, this stimulation could be reversed by combined treatment of pregnant hamsters with

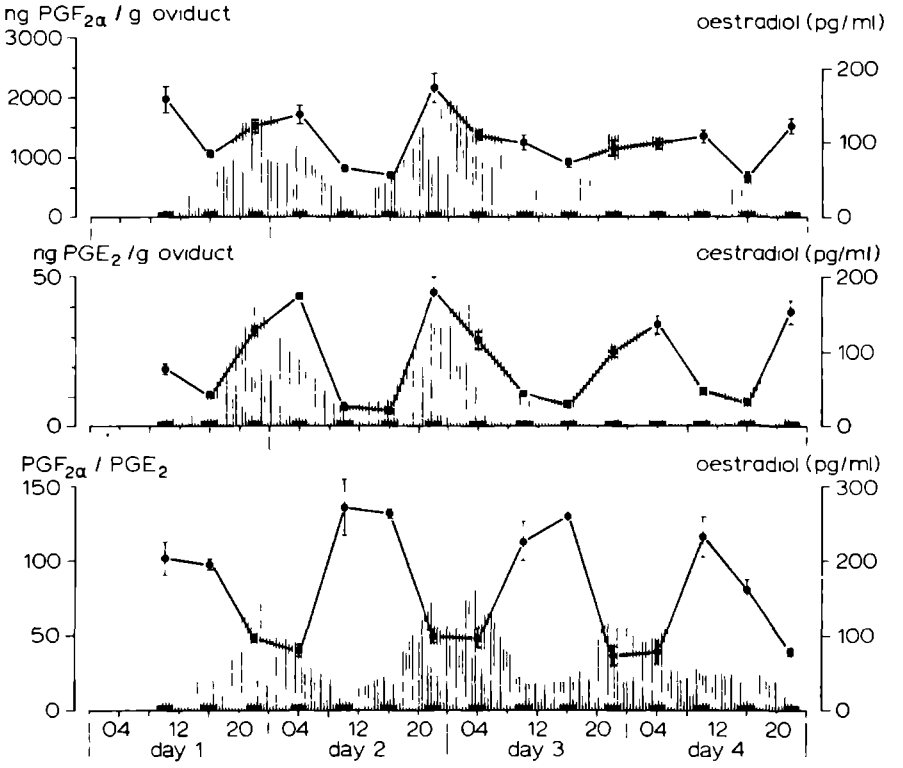

FIGURE 12 The relationship between the plasma levels* of oestradiol and the tissue concentrations* of prostaglandins $F_{2\alpha}$, E_2, and their ratios in oviducts of hamsters during the first stage of pregnancy

* Mean ± SEM

indomethacin previous to oestradiol administration. Finally, the tissue concentrations of prostaglandins in oviducts of hamsters during the first period of pregnancy could be quickly suppressed by the single oral administration of

indomethacin at a time when oestradiol started to increase or was still elevated and thus stimulated oviductal prostaglandin synthesis (Table 9). On the basis of these data it was concluded that ovarian steroid hormones are at least in part responsible for the regulation of oviductal prostaglandin production.

THE EFFECTS OF PROSTAGLANDIN $F_{2\alpha}$, INDOMETHACIN AND OESTRADIOL ON OVUM TRANSPORT AND ON PREGNANCY

INTRODUCTION

First of all it was shown that plasma levels of ovarian steroid hormones vary throughout the oestrous cycle and in the first days of pregnancy of golden hamsters (Chapter IV). Next, it was demonstrated that prostaglandins are present in the hamster oviduct. A rhythmic pattern of changing tissue concentrations for prostaglandins $F_{2\alpha}$ and E_2 during the two reproductive states was established and showed a good correlation with the changing plasma levels of oestradiol (Chapter V). Following the exogenous administration of oestradiol to cyclic hamsters and to hamsters in the first stage of pregnancy, the oviductal tissue concentrations of these prostaglandins increased whereas the combined treatment with indomethacin blocked the oestradiol-induced synthesis of prostaglandins in this organ. Finally, the administration of indomethacin to otherwise untreated pregnant hamsters decreased the normal prostaglandin production in oviductal tissue significantly. In this chapter the effects of $PGF_{2\alpha}$, indomethacin and oestradiol on ovum transport and pregnancy will be reported in order to clarify the role of prostaglandins in the process of ovum transport, especially with respect to the hypothesis that the familiar effects of exogenously administered oestrogens on ovum transport are mediated through alterations in oviductal prostaglandin synthesis.

RESULTS

1. Prostaglandin $F_{2\alpha}$

The effects of prostaglandin $F_{2\alpha}$ on ovum transport

The results of these experiments are presented in Table 10 for the subcutaneously treated groups. Table 11 gives the results following administration of $PGF_{2\alpha}$ via the intraperitoneal route.

When a total dose of 0.4 mg $PGF_{2\alpha}$ was injected subcutaneously into hamsters on Days 1 or 2 post coitum, the percentages of ova recovered from oviducts and uteri at autopsy on Days 2, 3, or 4 were not different from the percentages of ova recovered from oviducts and uteri of control groups.

TABLE 10 Effect of s.c. injected prostaglandin $F_{2\alpha}$ on ovum transport

Autopsy	Number of	Treatment			Corpora lutea	% of ova recovered from	
						oviduct	uterus
day / period	animals	dose+	route	day	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$
2 15.00-17.00	5	control	groups•	1	13.4 ± 1.9	100	0
3 09.00-11.00	5				13.8 ± 0.8	78.0 ± 15.0	17.7 ± 11.0
3 15.00-17.00	5				11.8 ± 2.5	29.7 ± 27.7	67.0 ± 32.1
4 09.00-11.00	5				11.6 ± 1.3	0	45.1 ± 36.0
2 15.00-17.00	5	0.4	s.c.	1	15.2 ± 0.8	98.8 ± 2.8	0
3 09.00-11.00	11				12.9 ± 2.3	80.7 ± 16.8	17.0 ± 13.7
3 15.00-17.00	10				13.2 ± 1.8	14.1 ± 14.6	84.5 ± 13.7
4 09.00-11.00	9				14.2 ± 1.6	0	27.1 ± 14.6
3 09.00-11.00	5	control	groups•	2	11.4 ± 1.2	67.1 ± 24.5	31.4 ± 23.0
3 15.00-17.00	5				10.6 ± 1.0	19.1 ± 14.0	80.9 ± 14.0
4 09.00-11.00	5				11.2 ± 1.6	0	32.0 ± 22.6
3 09.00-11.00	5	0.4	s.c.	2	12.8 ± 1.3	75.6 ± 15.3	23.0 ± 13.4
3 15.00-17.00	5				11.6 ± 0.9	16.6 ± 16.3	78.5 ± 18.2
4 09.00-11.00	5				12.4 ± 0.9	0	39.1 ± 10.5

+ Total dose (mg) $PGF_{2\alpha}$/animal

• Control groups treated equally with 0.4 ml of vehicle

The treatment with $PGF_{2\alpha}$ via the intraperitoneal route (Table 11) showed statistically significant differences for all doses given on Days 1 and 2, as compared to controls. When $PGF_{2\alpha}$ was given on Day 1, the percentages of oviductal ova were in all cases significantly lower on the first occasion of autopsy after treatment than those of their controls. The most striking effect on ovum transport was obtained with the higher dose: 4 mg $PGF_{2\alpha}$ /kg body weight resulted in significant differences at two subsequent moments selected for autopsy, the latter falling between 09.00 - 11.00 on Day 3. The rate of ovum transport acceleration nearly doubled at that time, as can be concluded from the mean percentages of ova recovered from the treated groups and their controls (33.6 and 60.8 %, respectively). Furthermore, significant differences were observed on Day 3 between the percentages of uterine ova from groups treated with dosages of 0.4 mg $PGF_{2\alpha}$ /animal and with dosages of 2 or 4 mg $PGF_{2\alpha}$ /kg body weight, as compared to the controls.

The intraperitoneal administration of $PGF_{2\alpha}$ on Day 2 appeared to be even more effective in disturbing the rate of ovum transport by means of acceleration.

When 0.4 mg $PGF_{2\alpha}$ /animal was given on Day 2, the average number of oviductal ova recovered between 09.00 - 11.00 on Day 3 was only 14.8 % and differed significantly from the control value of 75.9 %. The percentage of oviductal ova between 15.00 - 17.00 on Day 3 was also substantially lower than that of the controls.

During the same period in the afternoon of Day 3, the percentage of ova that could be flushed out of the uterus was 28.2 % for the treated group, and also differed significantly from the 75.1 % of uterine ova in the control group.

The administration of $PGF_{2\alpha}$ on the basis of body weight indeed confirmed the above results; an intraperitoneal dose of 4 mg $PGF_{2\alpha}$ /kg body weight given on Day 2 resulted in even lower percentages for oviductal ova and for ova in uterine flushings on Day 3. However, the degree of significance for each of the groups tested was the same as described above. The lower dosages of $PGF_{2\alpha}$ injected on Day 2 also revealed significant differences in the rate of acceleration for tubal ova between 09.00 - 11.00 and between 15.00 - 17.00 on Day 3. In these cases, the mean percentages of oviductal ova of treated groups were always much lower than those of controls. The uterine flushings contained significantly less ova than those of controls as determined for the period between 15.00 - 17.00 on Day 3, with the exception of the group treated on Day 2 with 0.2 mg $PGF_{2\alpha}$ /animal.

The effects of prostaglandin $F_{2\alpha}$ on pregnancy

The results of the experiments after subcutaneous administration of $PGF_{2\alpha}$ are presented in Tabel 12 and those after intraperitoneal treatment are given in Table 13.

When hamsters were injected subcutaneously (Table 12) with dosages between 0.05 and 0.4 mg $PGF_{2\alpha}$ /animal on Days 1 and 2, the mean numbers of corpora lutea were equal to those of control groups.

Pregnancy was terminated in 6 out of 36 treated hamsters. These animals (16.7 %) did not show new ovulations. In the hamsters still pregnant on Day 6, no significant decrease in the percentages of embryos was observed.

Following the intraperitoneal administration of $PGF_{2\alpha}$ on Days 1 or 2 (Table 13), pregnancy was terminated in approximately 40 % of the 55 animals, although the mean number of corpora lutea for each of the groups was within the normal range for controls. In the pregnant animals, a significant impairment of the number of embryos was observed in 6 of the 10 groups, independent of the dose administered. In all the non-pregnant hamsters, multiple new

TABLE II Effect of i.p. injected prostaglandin $F_{2\alpha}$ on ovum transport

Autopsy	Number of animals	Treatment			Corpora lutea	% of ova recovered from	
						oviduct	uterus
day / period		dose+	route	day	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$
2 15.00-17.00	5	control	groups•	1	12.2 ± 1.3	98.5 ± 3.4	0
3 09.00-11.00	6				11.3 ± 1.5	60.8 ± 17.3	39.2 ± 17.3
3 15.00-17.00	5				12.0 ± 2.0	17.7 ± 10.5	82.3 ± 10.5
4 09.00-11.00	5				11.0 ± 1.9	0	25.3 ± 17.0
2 15.00-17.00	6	1*	i.p.	1	13.5 ± 1.4	77.3 ± 19.0 a	0
3 09.00-11.00	6				13.5 ± 0.8	54.8 ± 28.4	29.6 ± 26.1
3 15.00-17.00	5				15.2 ± 0.8	14.9 ± 23.4	65.0 ± 25.1
2 15.00-17.00	5	2*	i.p.	1	11.8 ± 0.4	71.4 ± 10.8 c	0
3 09.00-11.00	5				14.4 ± 1.5	56.3 ± 13.9	16.3 ± 11.3 a
3 15.00-17.00	5				14.8 ± 1.6	24.9 ± 23.7	55.5 ± 24.0
2 15.00-17.00	6	4*	i.p.	1	15.5 ± 1.0	56.8 ± 26.9 b	0
3 09.00-11.00	5				12.6 ± 1.3	33.6 ± 13.5 a	15.3 ± 25.3
3 15.00-17.00	5				14.2 ± 1.8	6.2 ± 13.8	43.5 ± 22.6 b
2 15.00-17.00	5	0.4	i.p.	1	13.8 ± 1.5	77.9 ± 18.2 a	0
3 09.00-11.00	5				14.2 ± 1.8	48.3 ± 25.4	25.1 ± 23.8
3 15.00-17.00	6				13.0 ± 0.6	9.8 ± 14.1	53.2 ± 22.9 a
4 09.00-11.00	5				14.8 ± 1.6	0	36.6 ± 18.3
3 09.00-11.00	6	control	groups•	2	11.5 ± 2.4	75.9 ± 13.0	24.1 ± 13.0
3 15.00-17.00	5				11.4 ± 1.3	24.9 ± 11.1	75.1 ± 11.1
4 09.00-11.00	5				11.6 ± 1.7	0	31.1 ± 15.0

3 09 00-11 00	6	1*	i p	2	12 7 ± 0 8	46 2 ± 8 2 c	12 0 ± 11 2
3 15 00-17 00	5				13 2 ± 1 3	5 7 ± 12 8 a	46 4 ± 21 6 a
3 09 00-11 00	6	2*	i p	2	15 5 ± 1 9	34 0 ± 13 0 c	23 6 ± 28 9
3 15 00-17 00	6				13 8 ± 1 2	5 8 ± 6 7 b	38 3 ± 34 5 a
3 09 00-11 00	5	0 2	i p	2	13 8 ± 1 5	25 2 ± 20 4 c	35 1 ± 22 2
3 15 00-17 00	5				13 6 ± 1 5	8 0 ± 11 1 a	71 7 ± 16 9
3 09 00-11 00	6	4*	i p	2	12 3 ± 2 0	1 5 ± 3 7 c	13 3 ± 10 2
3 15 00-17 00	5				14 4 ± 1 3	1 3 ± 2 8 b	19 9 ± 7 5 c
3 09 00-11 00	10	0 4	i p	2	14 1 ± 1 7	14 8 ± 14 4 c	39 1 ± 26 1
3 15 00-17 00	6				12 3 ± 1 0	4 0 ± 6 9 b	28 2 ± 11 2 c
4 09 00-11 00	6				14 8 ± 1 5	0	27 2 ± 9 0

+ Total dose (mg) $PGF_{2\alpha}$/animal
or
* total dose (mg) $PGF_{2\alpha}$/kg body weight (80-120 g)
• Control groups treated equally with 0 8 ml of vehicle
assuming a body weight of 100 g

Statistical significance against the control value of the same period of autopsy and the same day of treatment
a $p < 0\ 05$
b $p < 0\ 01$
c $p < 0\ 001$

TABLE 12 Effect of s.c. injected prostaglandin $F_{2\alpha}$ on pregnancy, implantation and luteolysis of hamsters

Autopsy	Number of animals	Treatment			Pregnant/ total	Pregnant animals		Number of non-pregnant animals	
day /period		dose+	route	day		number	% of embryos** $\bar{X} \pm SD$	without new ovulations	with new ovulations
6 15.00-17.00	5	control group•		1	5 / 5	5	89.5 ± 11.1	0	0
6 15.00-17.00	6	0.1	s.c.	1	6 / 6	6	95.8 ± 7.0	0	0
	5	0.2			4 / 5	4	83.1 ± 10.8	1	0
	5	0.4			4 / 5	4	90.8 ± 7.6	1	0
6 15.00-17.00	5	control group•		2	5 / 5	5	90.4 ± 8.9	0	0
6 15.00-17.00	5	0.05	s.c.	2	5 / 5	5	92.9 ± 16.0	0	0
	5	0.1			4 / 5	4	100.0 ± 0 a	1	0
	5	0.2			4 / 5	4	75.0 ± 30.4	1	0
	5	0.4			3 / 5	3	55.3 ± 32.2	2	0

+ Total dose (mg) $PGF_{2\alpha}$/animal
• Control groups treated equally with 0.4 ml of vehicle
** Percentage of embryos based on number of corpora lutea counted

Statistical significance against the control value of the same day of treatment
a $p < 0.05$

TABLE 13 Effect of i.p. injected prostaglandin $F_{2\alpha}$ on pregnancy, implantation and luteolysis of hamsters

Autopsy	Number of animals	Treatment			Pregnant/ total	Pregnant animals		Number of non-pregnant animals	
day / period		dose+	route	day		number	% of embryos** $\bar{X} \pm SD$	without new ovulations	with new ovulations
6 15.00-17.00	5	control	group•	1	5 / 5	5	95.7 ± 6.2	0	0
6 15.00-17.00	6	1*	i.p.	1	5 / 6	5	44.8 ± 17.5 c	1	0
	5	2*			3 / 5	3	42.6 ± 37.8	2	0
	6	0.2			4 / 6	4	47.6 ± 28.1 a	1	1
	5	4*			3 / 5	3	27.6 ± 14.8 c	2	0
	6	0.4			3 / 6	3	72.0 ± 36.1	0	3
6 15.00-17.00	5	control	group•	2	5 / 5	5	94.8 ± 8.2	0	0
6 15.00-17.00	5	1*	i.p.	2	3 / 5	3	66.3 ± 31.0	2	0
	5	2*			2 / 5	2	28.3 ± 24.3 a	3	0
	5	0.2			3 / 5	3	38.1 ± 26.3 a	0	2
	6	4*			4 / 6	4	24.6 ± 14.5 c	2	0
	6	0.4			2 / 6	2	70.0 ± 42.4	0	4

+ Total dose (mg) $PGF_{2\alpha}$/animal
or
* total dose (mg) $PGF_{2\alpha}$/kg body weight (80-120 g)
• Control groups treated equally with 0.8 ml of vehicle assuming a body weight of 100 g
** Percentage of embryos based on number of corpora lutea counted

Statistical significance against the control value of the same day of treatment
a $p < 0.05$
b $p < 0.01$
c $p < 0.001$

TABLE 14. The effect of indomethacin and oestradiol on ovum transport

Autopsy	Number of animals	Treatment			Corpora lutea	% of ova recovered from	
						oviduct	uterus
day / period		dose+	route	day	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$
3 15.00-17.00	5	control group•			12.6 ± 1.7	28.4 ± 19.9	68.6 ± 18.3
3 15.00-17.00	6	250µg OE_2	s.c.	4	11.8 ± 1.2	98.6 ± 3.4 a	0 a
3 15.00-17.00	5	500µg IM 250µg OE_2	orally s.c.	4	12.4 ± 1.4	95.3 ± 4.4 a	0 a

+ 500 µg indomethacin (IM) administered orally to hamsters at 20.00 of Day 4 (the day prior to ovulation) and/or 250 µg oestradiol (OE_2) injected subcutaneously (s.c.) at 22.00 of the same day.

• Control group treated with vehicles for indomethacin and for oestradiol.

Statistical significance against control group

a $p < 0.001$

TABLE 15 The effect of indomethacin on ovum transport

Autopsy day / period	Number of animals	Treatment dose+	route	day*	Corpora lutea $\bar{X} \pm SD$	% of ova recovered from oviduct $\bar{X} \pm SD$	% of ova recovered from uterus $\bar{X} \pm SD$
3 09.00-11.00	5	control group•			11.0 ± 1.2	78.1 ± 13.8	21.9 ± 13.8
3 09.00-11.00	5	500µg IM	orally	4 1 2	11.2 ± 1.1	78.0 ± 23.0	20.2 ± 23.8
3 15.00-17.00	5	control group•			11.8 ± 0.8	26.8 ± 24.9	71.4 ± 24.4
3 15.00-17.00	5	500µg IM	orally	4 1 2	13.0 ± 1.6	26.0 ± 11.4	71.2 ± 11.8

+ 500 µg indomethacin (IM) administered orally to hamsters at 20.00 for three consecutive days prior to the day of autopsy
* Cycle Day 4 and Days 1 and 2 of pregnancy
• Controls received 0.2 ml vehicle for three consecutive days of treatment

ovulations were seen after treatment with 0.4 mg $PGF_{2\alpha}$ /animal on Days 1 and 2. The corona radiata cells were absent from the new ova observed in hamsters treated on Day 1. These structures still surrounded the ova in animals injected on Day 2, thus indicating very recent ovulations.
The doses of 1, 2, or 4 mg $PGF_{2\alpha}$ /kg body weight did not permit new ovulations in the hamsters treated either on Day 1 or Day 2. When 0.2 mg $PGF_{2\alpha}$ / animal was injected on Day 1, pregnancy was terminated in the case of 2 out of 6 hamsters. Only one of the animals had ovulated again. The same dose of $PGF_{2\alpha}$ given on Day 2 showed new ovulations in the two non-pregnant hamsters, with ova surrounded by corona radiata cells.

2. Indomethacin and oestradiol

The effects of indomethacin and oestradiol on ovum transport
The results of these experiments are presented in Table 14. One subcutaneous injection of 250 μg oestradiol at 22.00 on the day prior to ovulation led to an almost complete arrest of oviductal ova, whereas the controls showed the normal pattern of ovum transport.
When treatment on the same day was extended with the oral administration of indomethacin 2 hours prior to the subcutaneous oestradiol injection, it was observed that once more nearly all the ova were retained in the oviduct in the afternoon of Day 3. No significant differences were present between the two treatment schedules. In one oviduct of each animal the number of ova were counted in the ampulla and the isthmus. The group of hamsters treated with oestradiol showed 40.6 ± 9.1 (SD) % and 12.3 ± 10.9 % of ova in these two oviductal segments, respectively. For the combined treatment group this was 36.0 ± 13.4 % and 17.0 ± 13.0%, respectively. Thus, the addition of indomethacin to the treatment schedule did not change the percentage of ova retained in the oviduct, nor did it change the distribution of the ova within the oviduct.

The effects of indomethacin on ovum transport
Hamsters were treated with 500 μg indomethacin which was given orally at 20.00 on three consecutive days as shown in Table 15.
The percentage of oviductal ova and of ova recovered from the uterine flushings was not different from their control groups at autopsy between 09.00 - 11.00 or between 15.00 - 17.00 on Day 3 post coitum.

The physiological pattern of ovum transport and the effects of prostaglandins on this process can be studied by establishing the changes brought about by administration of varying doses of prostaglandins at different times during the post coital period. The number of implanted blastocysts is a helpful parameter for the interpretation of changes in the rate of oviductal ovum transport: The number of embryos that can be counted on Day 6 post coitum depends mainly on the readiness of the uterus to conceive, and on the right moment for the ova to arrive in the uterus. A balanced relationship between these two processes is essential.

Prostaglandins have been reported to have several antifertility effects, such as the termination of pregnancy. $PGF_{2\alpha}$ administration during pregnancy causes its termination in several species, as discussed in Chapter II. This is primarily due to the effect of prostaglandins on the corpus luteum that becomes insufficient.

For this reason the latter process (luteolysis) may mask the influence of prostaglandins on implantation itself, and it may be difficult to establish whether the reduction of embryos is due to luteolysis, to inhibition of implantation, or is caused by a change in the rate of normal ovum transport, or a combination of these phenomena.

If prostaglandins indeed influence the passage of ova through the oviduct, they must be present in oviductal tissue, and their concentration should vary in relation to changes taking place during this process. In the previous chapter on prostaglandin levels in oviductal tissue of cyclic and mated hamsters, sufficient data have been given fulfilling these criteria. Finally, if prostaglandins indeed influence the process of ovum transport, their exogenous administration should disregulate this process. This should also be the case following the exogenous administration of endogenous prostaglandin synthesis inhibitors.

Prostaglandins are extremely sensitive to metabolic inactivation (Piper and Vane, 1971), and more than 80 % of the activity is destroyed by one single lung passage (Piper et al., 1970). It is probably for this reason that no clear effects on ovum transport were established after the subcutaneous administration of different $PGF_{2\alpha}$ dosages to hamsters (Table 10). This is in agreement with the findings of Labhsetwar (1972), although he applied a dose as low as 0.05 mg $PGF_{2\alpha}$, that was injected repeatedly for two or three days. However, when 0.05 to 0.4 mg $PGF_{2\alpha}$ were administered subcutaneously on Days 1 or 2 post coitum (Table 12), an antifertility effect resulting in a total loss of pregnancy was observed in 6 out of 36 hamsters. No significant

decrease in the number of embryos was observed at autopsy on Day 6 in the groups of the animals that remained pregnant. This illustrates that relatively high doses of $PGF_{2\alpha}$ if administered subcutaneously have little or no effect on the process of implantation.

The local administration of $PGF_{2\alpha}$ via the intraperitoneal route, however, shows quite different data: Although the uptake of $PGF_{2\alpha}$ by the peritoneum is fast, this mode of administration also allows immediate contact between $PGF_{2\alpha}$ and the oviducts.

As opposed to classic hormones, prostaglandins are thought to be produced and secreted within the tissue where they exert their target effects. Uptake into the systemic circulation leads to rapid destruction. Therefore it can be postulated that a direct contact between $PGF_{2\alpha}$ and the peritoneal surface of the oviducts will lead to an uptake of $PGF_{2\alpha}$ in the oviduct itself which results in higher tissue concentrations than would have been the case if the drug had been administered via different routes.

Indeed, intraperitoneal administration of $PGF_{2\alpha}$ between 1 and 4 mg/kg body weight (Table 11) always leads to an acceleration of oviductal ovum transport. This observation, however, is almost entirely limited to the first period selected for autopsy after $PGF_{2\alpha}$ treatment on Day 1. It is in particular treatment on Day 2 with the same dosages of $PGF_{2\alpha}$ that has shown significant acceleration of oviductal ovum transport. During the two periods selected for autopsy on Day 3, highly significant decreases were shown in the percentages of oviductal ova, whereas also the number of ova in uterine flushings were very much reduced in the afternoon of Day 3.

As might have been expected, the demonstrated acceleration of ovum transport in $PGF_{2\alpha}$ treated animals also disturbs normal implantation: The mean number of embryos, found at autopsy on Day 6 (Table 13) was significantly reduced ($p < 0.05$) in 6 out of 10 groups of intraperitoneally treated hamsters as compared to controls. In sharp contrast with these results, the subcutaneous administration of $PGF_{2\alpha}$ (Table 12) did not show any indication of impaired implantation in the 7 groups treated.

Hence, a decrease in the number of implantations originates primarily from a disturbance in oviductal ovum transport, and will depend to a much lesser degree on the presence of exogenously administered $PGF_{2\alpha}$ in the blood circulation.

However, the relationship between $PGF_{2\alpha}$ in peripheral blood and luteolysis as the main cause for loss of pregnancy is much clearer. In animals subcutaneously treated on Days 1 and 2 (Table 12) the injected quantities of $PGF_{2\alpha}$ incidentally cause a partial luteolysis in a minority of animals from virtually all the groups: Abortion occurs, but no subsequent ovulations are observed.

In contrast, the subcutaneous treatment of hamsters on Day 3 or Day 4 causes complete luteolysis in all the animals (data not shown), i.e. all hamsters aborted spontaneously and afterwards restored their ovarian cycle unanimously. The reason why older corpora lutea are much more sensitive to the luteolytic effects of $PGF_{2\alpha}$ than the much younger corpora lutea is unknown (Labhsetwar, 1972a).

As stated above, intraperitoneal injections allow a direct contact between $PGF_{2\alpha}$ and the genital tract which contains corpora lutea. Thus, in all the intraperitoneally treated groups on Days 1 and 2 (Table 13) partial luteolysis is observed in a substantially higher percentage of animals than is the case for the subcutaneously treated ones (Table 12). In a minority of cases the complete loss of pregnancy in a hamster is followed by spontaneous new ovulations indicating that within the same group both partial and complete luteolysis can occur (Table 13). These results confirm again that fresh corpora lutea are rather insensitive to the luteolytic action of $PGF_{2\alpha}$.

Furthermore, the relationship between $PGF_{2\alpha}$ and the disturbance of ovum transport appears to be dose-dependent resulting in an impairment of blastocyst implantation.

It has been reported previously (Bastiaans, 1973), that 500 µg of oestradiol administered subcutaneously to hamsters 4 hours prior to ovulation (i.e. at 22.00 on Day 4) completely arrested oviductal ova for the entire period during which transport takes place. It was shown (Table 14) that the same effect on ovum transport was obtained following treatment with half this quantity of oestradiol. This dose of 250 µg of oestradiol which was administered to hamsters at various times during the cycle or in the first stage of pregnancy invariably resulted in a highly significant increase of oviductal tissue concentrations of prostaglandins (Table 7). The stimulating effect of oestradiol on prostaglandin levels could easily be inhibited by additional treatment with indomethacin prior to oestradiol injection (Table 8).

On the basis of the accelerating effect of intraperitoneally injected $PGF_{2\alpha}$ on ovum transport, it seemed reasonable to postulate that the suppression of prostaglandin level following oral administration of indomethacin should bring about a change in the rate of normal oviductal ovum transport. Furthermore, indomethacin treatment should overcome the oestradiol-induced arrest of oviductal ova (Table 14) as the result of suppressing prostaglandin synthesis in the oviduct itself. This synthesis is brought about by exogenously administered oestradiol.

However, these assumptions were not confirmed by the experiments presented in Table 14 and 15. No definite conclusions should be drawn from these experiments because of the following arguments:

Other dosage-schedules of indomethacin, different prostaglandin synthetase inhibitors, or different routes of administration may suppress oviductal prostaglandin levels more markedly than the decreases in the tissue concentrations obtained in the experiments reported here.
As long as these possibilities have not been tested in full detail, final evidence for a definite role of oviductal prostaglandins in the regulation of ovum transport in the case of hamsters is lacking.

SUMMARY AND CONCLUSIONS

The hypothesis that oestrogens influence oviductal ovum transport through alterations in tissue concentrations of prostaglandins (Chapter I) is based on the familiar effects of oestrogens on ovum transport and the possible role of prostaglandins in this process (Chapter II).
In order to test this hypothesis, the golden hamster was selected as laboratory animal (Chapter III). First of all, an inventory was made of some physiological events taking place during the four-day oestrous cycle and during the first four days of pregnancy. The interval and the time of ovulation during the cycle was determined and the pattern of normal ovum transport was registered in great detail. These processes were related to the patterns of oestradiol and progesterone plasma levels which were measured transversally at frequent intervals throughout the entire cycle and during the first stage of pregnancy (Chapter IV). The plasma levels of the two steroid hormones were measured using highly specific radioimmunoassay techniques (Chapter III). Next, tests were conducted in order to determine the tissue concentrations of prostaglandins $F_{2\alpha}$ and E_2 in the oviducts of hamsters at frequent intervals throughout the same two stages.
The influence of exogenously administered oestradiol was tested on the oviductal tissue concentrations of the two prostaglandins for cyclic and for pregnant animals. Furthermore, the effect of indomethacin on oestradiol-induced oviductal prostaglandin levels was studied as well as the influence of indomethacin on the endogenous prostaglandin concentrations in the oviducts of pregnant hamsters (Chapter V).
Finally, the effects of prostaglandin $F_{2\alpha}$, indomethacin and oestradiol on ovum transport and on pregnancy were studied in order to clarify the role of prostaglandins in the process of ovum transport with respect to the hypothesis outlined above (Chapter VI).

Under specific laboratory-conditions (artificial light from 05.00 –19.00) the golden hamster ovulates between 00.00 and 04.00 on Day 1. Half the number of all the Graafian follicles had been ruptured at 02.00. This moment was taken as the time of ovulation.

The plasma levels of oestradiol during the cycle were low around ovulation and did not change much until 03.00 – 05.00 on Day 3. Substantially higher concentrations were measured between 21.00 – 23.00 on Day 3. A continuous decline until noon of Day 4 preceded the sharp rise to the peak levels measured preovulatory between 15.00 – 16.00.

Progesterone concentrations fluctuated on Day 1 and Day 2, and minimum levels were measured on Day 3 and in the morning of Day 4. A sudden, sharp increase was detected between 15.00 – 16.00 of Day 4, followed by maximum preovulatory progesterone concentrations lasting until midnight of the same day.

The oestradiol levels during the first four days of pregnancy were always much higher in samples collected during the night as compared to those obtained during the day. For progesterone, only moderate fluctuations between 6.7 and 12.9 ng/ml plasma were measured during the same days of pregnancy.

Ovum transport was studied by calculating the time necessary for 50 % of all the ova to pass through the various segments of the oviduct. Shortly after ovulation the ova had reached the distal part of the ampulla. The ampullary-isthmic junction had been passed by 50 % of the ova 12 hours after ovulation. The isthmus was traversed 36 hours later and this percentage of ova arrived in the uterus 58½ hours after ovulation. Implantation for half the number of ova was reached 77 hours postovulatory and the whole process was completed within 87 hours.

The observations on the contractility of the oviduct in vitro showed the absence of visible peristaltic contractions at different times prior to ovulation. In the periovulatory period, there were strong abovarian contractions of the expanded ampulla, with relaxations that ended at the ampullary-isthmic junction. At later moments, oviductal contractions mainly occurred in those segments where the ova were localized.

The prostaglandin levels in the oviducts of cyclic hamsters were low on Day 2. $PGF_{2\alpha}$ doubled in the evening of Day 2 and a twofold increase was observed between 21.00 – 23.00 of Day 3. The elevated level remained constant until it was followed between 19.00 – 20.00 on Day 4 by an extremely sharp increase to maximum levels lasting throughout the night until the morning of Day 1. The PGE_2 concentrations showed a rather similar cyclic pattern. As opposed to $PGF_{2\alpha}$, the pattern for PGE_2 moreover showed minimum levels in the morning and in the afternoon of Day 3 and Day 4, prior to the sharp increase to maximum concentrations afterwards.

During the first stage of pregnancy, $PGF_{2\alpha}$ showed a rhythmic pattern with rather constant levels on Day 3 and on Day 4. Oviductal PGE_2 levels

resembled those observed for plasma oestradiol, with much higher concentrations detected during the night periods.
Treatment with oestradiol caused a sharp increase of oviductal prostaglandin concentrations. Both $PGF_{2\alpha}$ and PGE_2 doubled in the case of cyclic hamsters while during pregnancy the administration of oestradiol was even more effective. The basal levels of PGE_2 increased approximately 7-9 times and those of $PGF_{2\alpha}$ about 3-4 times in pregnant animals.
The combined treatment of pregnant hamsters with indomethacin followed by oestradiol administration always resulted in significantly lower prostaglandin tissue concentrations as compared to the levels induced by a single oestradiol injection: By indomethacin treatment, $PGF_{2\alpha}$ decreased 7-8 times and PGE_2 levels were reduced approximately 2-4 times.
The oral administration of indomethacin to pregnant animals caused a significant decline (about 2-4 times) of the endogenous tissue concentrations of prostaglandins. $PGF_{2\alpha}$ levels decreased more markedly than those of PGE_2.
The subcutaneous administration of $PGF_{2\alpha}$ had no effect on ovum transport. Intraperitoneal injections of the same dosages of $PGF_{2\alpha}$ significantly accelerated this process. When $PGF_{2\alpha}$ was given on Day 2, the effect on ovum transport was much stronger than after treatment on Day 1 post coitum.
Termination of pregnancy was observed in 6 out of 36 hamsters after subcutaneous injection of $PGF_{2\alpha}$ on Day 1 or Day 2 of pregnancy. When equal dosages of the drug were administered by intraperitoneal injection on one of these days, pregnancy terminated in approximately 40 % of the cases. In 6 out of 10 groups of treated animals a significantly lower number of embryos was observed on Day 6 of pregnancy.
The subcutaneous administration of oestradiol prior to ovulation caused a complete arrest of oviductal ova on Day 3. Combined treatment with the oral administration of indomethacin and oestradiol did not alter the pattern of tube-locking induced by oestrogens alone.
Finally, indomethacin administration in the evenings of three consecutive days did not change the pattern of normal ovum transport.

From these results it was concluded that:
1. There exists a close relationship between the patterns of plasma oestradiol, $PGF_{2\alpha}$ and PGE_2 in oviductal tissue of cyclic hamsters and of hamsters in the first stage of pregnancy.
2. The endogenous tissue levels of prostaglandins in the oviducts of ham-

sters are stimulated by the administration of oestradiol and this stimulating effect is counteracted by simultaneous treatment with indomethacin.
3. $PGF_{2\alpha}$ affects ovum transport and pregnancy when injected intraperitoneally on Days 1 or 2 post coitum.

STEROID HORMONEN, PROSTAGLANDINEN EN OVUM TRANSPORT; EEN STUDIE BIJ DE GOUDHAMSTER, MESOCRICETUS AURATUS (WATERHOUSE)

SAMENVATTING EN CONCLUSIES

De hypothese, dat oestrogenen het transport van ova door het oviduct beïnvloeden via veranderingen in de weefselconcentraties van prostaglandinen (Hoofdstuk I) is gebaseerd op de bekende effecten van oestrogenen op het ovum transport en de mogelijke rol van prostaglandinen in dit proces (Hoofdstuk II). Voor het toetsen van deze hypothese werd de goudhamster als proefdier gekozen (Hoofdstuk III). Eerst werden enkele fysiologische processen van de vierdaagse cyclus en van de eerste vier dagen van de zwangerschap bestudeerd. Tijdens de cyclus werd het interval en het tijdstip van ovulatie vastgesteld en het patroon van het normaal verlopende ovum transport gedurende de vroege zwangerschap op gedetailleerde wijze geregistreerd. De beide processen werden gerelateerd aan de patronen van de plasmaspiegels voor oestradiol en progesteron welke transversaal op frequente, gedefiniëerde tijdstippen van de gehele cyclus en gedurende het eerste stadium van de zwangerschap werden bepaald (Hoofdstuk IV). De plasmaspiegels van de twee steroid hormonen werden gemeten met behulp van specifieke radioimmunologische bepalingstechnieken (Hoofdstuk III.) Vervolgens werden op frequente, gedefiniëerde tijdstippen gedurende deze beide perioden de concentraties van de prostaglandinen $F_{2\alpha}$ en E_2 in de oviducten van hamsters bepaald. De invloed van exogeen toegediend oestradiol op de concentraties van de twee prostaglandinen in oviductweefsel werd getest in cyclische en in zwangere dieren. Bovendien werd het effect van indomethacine op de door oestradiol geïnduceerde prostaglandinespiegels in het oviductweefsel bestudeerd, evenals de invloed van indomethacine op de endogene prostaglandineconcentraties in de oviducten van zwangere hamsters (Hoofdstuk V). Tenslotte werden de effecten van prostaglandine $F_{2\alpha}$, van indomethacine en van oestradiol op het ovum transport en op de zwangerschap onderzocht om in het kader van de bovengenoemde hypothese een beter inzicht te verkrijgen in de rol van de prostaglandinen in het proces van ovum transport (Hoofdstuk VI).

Onder de gehanteerde laboratoriumcondities (kunstlicht van 05.00 – 19.00 uur) ovuleert de goudhamster tussen 00.00 en 04.00 uur op Dag 1. De helft van alle Graafse follikels was om 02.00 uur gesprongen. Dit moment werd aangenomen als ovulatietijdstip.

De plasmaspiegels van oestradiol gedurende de cyclus waren laag rond de ovulatie en veranderden nauwelijks tot 03.00 – 05.00 uur op Dag 3. Aanzienlijk hogere concentraties werden gemeten tussen 21.00 – 23.00 uur op Dag 3. Een continue daling tot 12.00 uur op Dag 4 ging vooraf aan de scherpe stijging naar piekwaarden die preovulatoir tussen 15.00 – 16.00 uur werden gemeten.

De progesteronconcentraties fluctueerden op Dag 1 en Dag 2. Minimumwaarden werden gemeten op Dag 3 en in de ochtend van Dag 4. Een onverhoeds sterke stijging werd waargenomen tussen 15.00 – 16.00 uur van Dag 4, gevolgd door maximum preovulatoire progesteronconcentraties die voortduurden tot middernacht van dezelfde dag.

De oestradiolspiegels die werden gemeten in de eerste vier dagen van de zwangerschap waren 's avonds en 's nachts steeds aanzienlijk hoger dan de waarden van de tijdstippen overdag. Voor progesteron werden in deze periode van de zwangerschap slechts matige schommelingen tussen 6.7 en 12.9 ng/ml waargenomen.

Het ovum transport werd bestudeerd door het berekenen van de tijd die 50 % van de eicellen nodig had om de diverse segmenten van het oviduct te doorlopen. Kort na ovulatie bereikten de ova het distale gedeelte van de ampulla. De overgang van de ampulla naar isthmus werd 12 uur na de ovulatie gepasseerd door 50 % van het aantal eicellen. De isthmus was 36 uur later ook geheel doorlopen terwijl $58^1/_2$ uur na ovulatie hetzelfde percentage ova in de uterus was aangekomen. De helft van het aantal eicellen was 77 uur na de ovulatie geïmplanteerd en het hele proces was voltooid binnen 87 uur.

De waarnemingen omtrent de contractiliteit van het oviduct in vitro toonden aan, dat zichtbare peristaltische contracties afwezig waren op diverse tijdstippen vóór de ovulatie. In de periovulatoire periode werden sterke abovariële contracties en relaxaties van de verwijde ampulla waargenomen, die eindigden bij de overgang van de ampulla naar isthmus. Op latere tijdstippen werden contracties hoofdzakelijk waargenomen in die segmenten van het oviduct waar de ova zich bevonden.

De prostaglandinespiegels in de oviducten van cyclische hamsters waren laag op Dag 2. $PGF_{2\alpha}$ verdubbelde in de avond van Dag 2 en een tweevoudige stijging werd waargenomen tussen 21.00 – 23.00 uur van Dag 3. De verhoogde spiegel bleef constant totdat tussen 19.00 en 20.00 uur op Dag 4 een zeer sterke stijging optrad naar maximumwaarden, die aanhielden gedurende de hele nacht tot in de ochtend van Dag 1. De PGE_2 concentraties vertoonden

een tamelijk overeenkomstig cyclisch patroon In tegenstelling tot $PGF_{2\alpha}$ liet het patroon voor PGE_2 bovendien ook nog minimumwaarden zien in de ochtend en middag van Dag 3 en Dag 4, voorafgaand aan de scherpe stijging naar maximum concentraties daarna

In het eerste stadium van de zwangerschap vertoonde $PGF_{2\alpha}$ een ritmisch patroon met tamelijk constante spiegels op Dag 3 en Dag 4 De waarden van PGE_2 in het oviduct vertoonden overeenkomst met de waarnemingen aan plasma oestradiol, waarbij eveneens veel hogere concentraties in de nachtelijke perioden werden gemeten

De behandeling met oestradiol veroorzaakte een sterke stijging van de prostaglandineconcentraties in het oviduct Zowel de concentraties van $PGF_{2\alpha}$ als van PGE_2 verdubbelden indien oestradiol tijdens de cyclus werd toegediend, terwijl tijdens de zwangerschap de toediening van oestradiol zelfs nog veel effectiever was De basale waarden van PGE_2 namen ongeveer 7-9 maal toe en de spiegels van $PGF_{2\alpha}$ stegen circa 3-4 maal in zwangere dieren

De gecombineerde behandeling van zwangere hamsters met indomethacine, gevolgd door oestradiol resulteerde steeds in significant lagere weefselconcentraties van prostaglandinen, vergeleken met de spiegels die konden worden geïnduceerd met behulp van een enkelvoudige oestradiolinjectie: Door de indomethacinebehandeling daalden de $PGF_{2\alpha}$ concentraties met een faktor 7-8 en de PGE_2 spiegels werden ca 2-4 maal lager

De orale toediening van indomethacine aan zwangere dieren veroorzaakte eveneens een significante daling (circa 2-4 maal) van de endogene weefselconcentraties van de prostaglandinen $PGF_{2\alpha}$ spiegels daalden beduidend sterker dan de waarden van PGE_2

De subcutane toediening van $PGF_{2\alpha}$ had geen effect op het ovum transport. Intraperitoneale injecties met dezelfde $PGF_{2\alpha}$ doseringen versnelden dit proces aanmerkelijk Het effect op het ovum transport was het meest uitgesproken, indien $PGF_{2\alpha}$ op Dag 2 post coitum werd toegediend.

Zwangerschapsafbreking werd waargenomen in 6 van de 36 hamsters na subcutane injectie van $PGF_{2\alpha}$ op Dag 1 of Dag 2 van de zwangerschap Indien dezelfde doseringen van deze verbinding werden toegediend door middel van een intraperitoneale injectie op een van deze dagen, dan werd de zwangerschap in circa 40 % van de dieren beëindigd In 6 van de 10 groepen behandelde dieren werden significant lagere aantallen embryos waargenomen op Dag 6 van de zwangerschap

De subcutane toediening van oestradiol voor de ovulatie veroorzaakte een complete stilstand van eicellen in het oviduct zoals werd waargenomen op Dag 3 De gecombineerde behandeling van oraal toegediend indomethacine en oestradiol veroorzaakte echter geen enkele verandering in de stilstand van

het ovum transport die werd geïnduceerd door de toegediende oestrogenen. Tenslotte werd gevonden dat indomethacine na toediening op de avonden van drie opeenvolgende dagen het patroon voor het normaal verlopende ovum transport niet veranderde.

Uit deze resultaten werd geconcludeerd dat:

1. Er een nauwe relatie bestaat tussen de patronen van plasma oestradiol en van $PGF_{2\alpha}$ en PGE_2 in het oviductweefsel van cyclische hamsters en van hamsters in het eerste stadium van de zwangerschap.
2. De endogene weefselspiegels van prostaglandinen in de oviducten van hamsters worden gestimuleerd door toediening van oestradiol. Dit stimulerende effect wordt opgeheven door gelijktijdige behandeling met indomethacine.
3. $PGF_{2\alpha}$ beïnvloedt het ovum transport en de zwangerschap na intraperitoneale injectie van deze stof op Dag 1 of Dag 2 post coitum.

REFERENCES

Alden R H. (1942) The periovarial sac in the albino rat *Anat. Rec. 83*, 421-435.

Alleva J.J. and Umberger E.J. (1966). Evidence for neural control of the release of pituitary ovulating hormone in the golden Syrian hamster. *Endocrinology 78*, 1125-1129.

Andersen D (1927) The rate of passage of the mammalian ovum through various portions of the Fallopian tube. *Amer. J. Physiol 82*, 557-569

Auletta F.J., Caldwell B V., van Wagenen G and Morris J M. (1972) Effects of postovulatory estrogen on progesterone and prostaglandin F levels in the monkey. *Contraception 6*, 411-421.

Austin C.R. (1955) Ovulation, fertilization, and early cleavage in the hamster (Mesocricetus auratus) *J. roy micr. Soc 73*, 141-154.

Banik U K and Pincus G. (1964). Estrogens and transport of ova in the rat. *Proc. Soc. exp. Biol. Med 116*, 1032-1034

Baranczuk R J. and Greenwald G.S (1972). Daily changes in estrone (E_1) and estradiol (E_2) in the cycling hamster as measured by radioimmunoassay *Biol. Reprod. 7*, 137.

Baranczuk R. and Greenwald G.S. (1973). Peripheral levels of estrogen in the cyclic hamster. *Endocrinology 92*, 805-812.

Bartke A., Merrill A.P. and Baker C.F. (1972). Effects of prostaglandin $F_{2\alpha}$ on pseudopregnancy and pregnancy in mice. *Fertil and Steril 23*, 543-547.

Bast J D and Greenwald G.S (1974). Serum profiles of follicle-stimulating hormone, luteinizing hormone and prolactin during the estrous cycle of the hamster *Endocrinology 94*, 1295-1299

Bastiaans L A. (1973). *Effects of some oestrogenic and progestational substances on ovum transport and pregnancy in the golden hamster, Mesocricetus auratus (Waterhouse)*. Ph D Thesis, Nijmegen

Bennett J.P. (1969). The effect of drugs on egg transport. *Adv. Biosci. 4*, 165-180.

Bennett J.P (1976). Drug regulation of egg transport. In. Harper M.J K., Pauerstein C.J., Adams C.E., Coutinho E.M , Croxatto H.B. and Paton D M , eds. *Ovum transport and fertility regulation*, pp 441-465 Scriptor, Copenhagen.

Betteridge K.J , Flood P F and Mitchell D. (1976) Possible role of the embryo in the control of oviductal transport in mares. In Harper M J.K , Pauerstein C J., Adams C E., Coutinho E M., Croxatto H B and Paton D M , eds. *Ovum transport and fertility regulation*, pp. 381-389 Scriptor, Copenhagen.

Bex F J and Goldman B D (1975). Serum gonadotropins and follicular development in the Syrian hamster *Endocrinology 96*, 928-933.

Black D.L. and Asdell S.A. (1959). Mechanism controlling entry of ova into rabbit uterus. *Amer. J Physiol. 197*, 1275-1278.

Blaha G C and Leavitt W W (1974). Ovarian steroid dehydrogenase histochemistry and circulating progesterone in aged golden hamsters during the oestrous cycle and pregnancy. *Biol. Reprod. 11*, 153-161

Blandau J.R (1961) Biology of eggs and implantation. In: Young W.C., ed. *Sex and internal secretions*, pp. 797-882. The Williams and Wilkins Co., Baltimore

Blandau R J and Boling J L (1973) An experimental approach to the study of egg transport through the oviduct of mammals In Segal S J , Crozier R , Corfman P A and Condliffe P G , eds *The regulation of mammalian reproduction* pp 400-415 C C Thomas, Springfield

Blandau R J and Verdugo P (1976) An overview of gamete transport Comparative aspects In Harper M J K , Pauerstein C J , Adams C E , Coutinho E M , Croxatto H B and Paton D M , eds *Ovum transport and fertility regulation*, pp 138-146 Scriptor, Copenhagen

Blatchley F R , Donovan B T , Poyser N L , Horton E W , Thompson C J and Los M (1971) Identification of prostaglandin $F_{2\alpha}$ in the utero-ovarian blood of the guinea-pig after treatment with oestrogen *Nature (Lond) 230*, 243 244

Blatchley F R , Donovan B T , Horton E W and Poyser N L (1972) The release of prostaglandins and progestin into the utero-ovarian venous blood of guinea-pigs during the oestrous cycle and following oestrogen treatment *J Physiol (Lond) 223*, 69 88

Blatchley F R , Maule Walker F M and Poyser N L (1975a) Utero-ovarian venous plasma levels of prostaglandin $F_{2\alpha}$, progesterone and oestradiol in non-pregnant and early pregnant guinea-pigs *J Endocr 64*, 12P

Blatchley F R , Maule Walker F M and Poyser N L (1975b) Progesterone, prostaglandin $F_{2\alpha}$, and oestradiol in the utero-ovarian venous plasma of non-pregnant and early, unilaterally pregnant guinea-pigs *J Endocr 67*, 225-229

Boling J L (1969) Endocrinology of oviductal musculature In Hafez E S E and Blandau R J , eds *The mammalian oviduct*, pp 163-181 The University of Chicago Press, Chicago

Boling J L (1971) Endocrinology of the oviduct In Sherman A I , ed *Pathways to conception*, pp 3 - 17 C C Thomas, Springfield

Boling J L and Blandau R J (1971a) The role of estrogens in egg transport through the ampullae of oviducts of castrate rabbits *Fertil and Steril 22*, 544-551

Boling J L and Blandau R J (1971b) Egg transport through the ampullae of the oviducts of rabbits under various experimental conditions *Biol Reprod 4*, 174-184

Bosu W T K , Holmdahl T H Johansson E D B and Gemzell C (1972) Peripheral plasma levels of oestrogens, progesterone and 17α-hydroxyprogesterone during menstrual cycle of the rhesus monkey *Acta endocr (Kbh) 71*, 755-764

Burdick H O and Pincus G (1935) The effect of oestrin injections upon the developing ova of mice and rabbits *Amer J Physiol 111*, 201-208

Burdick H O and Vedder H (1941) The effect of stilbestrol in early pregnancy *Endocrinology 28*, 629-632

Burdick H O and Whitney R (1937) Acceleration of the rate of passage of fertilized ova through the Fallopian tubes of mice by massive injections of an estrogenic substance *Endocrinology 21*, 637-643

Burdick H O and Whitney R (1938) Fate of ova accelerated in their rate of passage through the Fallopian tubes of mice by massive injections of Progynon-B *Endocrinology 22*, 631-638

Burdick H O , Whitney R and Emerson B (1942) Observations on the transport of tubal ova *Endocrinology 31*, 100-108

Burdick H O , Whitney R and Pincus G (1937) The fate of mouse ova tube locked by injections of oestrogenic substances *Anat Rec 67*, 513-516

Chang M C (1966) Effects of oral administration of medroxyprogesterone acetate and ethinyl estradiol on the transportation and development of rabbit eggs *Endocrinology 79*, 939-948

Chang M C (1967) Effects of progesterone and related compounds on fertilization, transportation and development of rabbit eggs *Endocrinology 81*, 1251-1260

Chang M C (1976) Estrogen, progesterone and egg transport -overview and identification of problems In Harper M J K , Pauerstein C J , Adams C E , Coutinho E M , Croxatto H B and Paton D M , eds *Ovum transport and fertility regulation*, pp 473-484 Scriptor, Copenhagen

Chang M C and Harper M J K (1966) Effects of ethinyl estradiol on egg transport and development in the rabbit *Endocrinology 78*, 860-872

Chang M C and Hunt D M (1970) Effects of various progestins and estrogen on the gamete transport and fertilization in the rabbit *Fertil and Steril 21*, 683-686

Chang M C and Hunt D M (1972) Effect of prostaglandin $F_{2\alpha}$ on the early pregnancy of rabbits *Nature (Lond) 236*, 120-121

Chang M C and Pickworth S (1969) Egg transfer in the laboratory animal In Hafez E S E and Blandau R J , eds *The mammalian oviduct*, pp 389-405 The University of Chicago Press, Chicago

Chang M C , Hunt D M and Polge C (1973) Effects of prostaglandins (PGs) on sperm and egg transport in the rabbit *Adv Biosci 9*, 805-810

Cox R I , Thorburn G D , Currie W B , Restall B J and Schneider W (1973) Prostaglandin F group (PGF), progesterone, and estrogen concentrations in the uteroovarian venous plasma of the conscious ewe during the estrous cycle *Adv Biosci 9*, 625-630

Croxatto H B and Ortiz M -E S (1975) Egg transport in the Fallopian tube *Gynecol Invest 6*, 215-225

Deanesly R (1938) The reproductive cycle of the golden hamster (Cricetus auratus) *Proc zool Soc Lond 108*, 31-37

Dreisbach R H (1959) The effects of steroid sex hormones on pregnant rats *J Endocr 18*, 271-277

Earthy M , Bishop C and Flack J D (1975) Progesterone and prostaglandin F concentration in utero-ovarian venous plasma of cyclic guinea-pigs *J Endocr 64*, 11P 12P

Edgren R A and Shipley G C (1961) A quantitative study of the termination of pregnancy in rats with estrone *Fertil and Steril 12*, 178 181

El-Banna A A , Sacher B and Schilling E (1976) Effect of indomethacin on egg transport and pregnancy in the rabbit *J Reprod Fertil 46* 375-378

Elder M G Myatt L and Chaudhuri G (1977) The role of prostaglandins in the spontaneous motility of the Fallopian tube *Fertil and Steril 28* 86 90

Ellinger J V and Kirton K T (1974) Ovum transport in rabbits injected with prostaglandin E_1 and $F_{2\alpha}$ *Biol Reprod 11*, 93 96

Ferin M , Tempone A , Zimmering P E and Vande Wiele R L (1969) Effect of antibodies to 17ß-estradiol and progesterone on the estrous cycle of the rat *Endocrinology 85*, 1070-1078

Fox R. R. and Laird C. W. (1970). Sexual cycles. In: Hafez E. S. E., ed. *Reproduction and breeding techniques for laboratory animals*, pp. 107-122. Lea and Febiger, Philadelphia.

Goldman B. D. and Porter J. C. (1970). Serum LH levels in intact and castrated golden hamsters. *Endocrinology 87*, 676-679.

Greenwald G. S. (1961a). A study of the transport of ova through the rabbit oviduct. *Fertil. and Steril. 12*, 80-95.

Greenwald G. S. (1961b). The anti-fertility effects in pregnant rats of a single injection of estradiol cyclopentylpropionate. *Endocrinology 69*, 1068-1073.

Greenwald G. S. (1961c). Quantitative study of follicular development in the ovary of the intact or unilaterally ovariectomized hamster. *J. Reprod. Fertil. 2*, 351-361.

Greenwald G. S. (1967). Species differences in egg transport in response to exogenous estrogen. *Anat. Rec. 157*, 163-172.

Greenwald G. S. (1971). Preovulatory changes in ovulating hormone in the cyclic hamster. *Endocrinology 88*, 671-677.

Greenwald G. S. (1976). Effects of estrogen and progesterone on egg transport: Summary and conclusions. In: Harper M. J. K., Pauerstein C. J., Adams C. E., Coutinho E. M., Croxatto H. B. and Paton D. M., eds. *Ovum transport and fertility regulation*, pp. 539-543. Scriptor, Copenhagen.

Gutknecht G. D., Cornette J. C. and Pharris B. B. (1969). Antifertility properties of prostaglandin $F_{2\alpha}$. *Biol. Reprod. 1*, 367-371.

Gutknecht G. D., Wyngarden L. J. and Pharriss B. B. (1971). The effect of prostaglandin $F_{2\alpha}$ on ovarian and plasma progesterone levels in the pregnant hamster. *Proc. Soc. exp. Biol. Med. 136*, 1151-1157.

Hafez E. S. E. (1970). Female reproductive organs. In: Hafez E. S. E., ed. *Reproduction and breeding techniques for laboratory animals*, pp. 74-106. Lea and Febiger, Philadelphia.

Hafez E. S. E. (1973). Gamete transport. In: Hafez E. S. E. and Evans T. N., eds. *Human reproduction conception and contraception*, pp. 85-118. Harper and Row, New York.

Hafez E. S. E. (1976). Anatomical and physiological mechanisms of ovum transport. In: Harper M. J. K., Pauerstein C. J., Adams C. E., Coutinho E. M., Croxatto H. B. and Paton D. M., eds. *Ovum transport and fertility regulation*, pp. 361-374. Scriptor, Copenhagen.

Ham E. A., Cirillo V. J., Zanetti M. E. and Kuehl Jr. F. A. (1975). Estrogen-directed synthesis of specific prostaglandins in uterus. *Proc. Nat. Acad. Sci. USA 72*, 1420-1424.

Haour F. and Saxena B. B. (1974). Detection of a gonadotropin in rabbit blastocyst before implantation. *Science 185*, 444-445.

Harper M. J. K. (1961a). The mechanisms involved in the movement of newly ovulated eggs through the ampulla of the rabbit Fallopian tube. *J. Reprod. Fertil. 2*, 522-524.

Harper M. J. K. (1961b). Egg movement through the ampullar region of the Fallopian tube of the rabbit. *Proc. IVth Int. Congr. Anim. Reprod.*, pp. 375-380. Drukkerij Trio, The Hague.

Harper M. J. K. (1964). The effects of constant doses of oestrogen and progesterone

on the transport of artificial eggs through the reproductive tract of ovariectomized rabbits *J Endocr 30*, 1-19

Harper M J K (1965a) Transport of eggs in cumulus through the ampulla of the rabbit oviduct in relation to day of pseudopregnancy *Endocrinology 77*, 114-123

Harper M J K (1965b) The effects of decreasing doses of oestrogen and increasing doses of progesterone on the transport of artificial eggs through the reproductive tract of ovariectomized rabbits *J Endocr 31*, 217-226

Harper M J K (1966) Hormonal control of transport of eggs in cumulus through the ampulla of the rabbit oviduct *Endocrinology 78* 568-574

Harper M J K , Pauerstein C J , Adams C E , Coutinho E M , Croxatto H B and Paton D M , eds (1976) *Ovum transport and fertility regulation* Scriptor, Copenhagen

Harrington F E (1964) Effect of estradiol benzoate on ova transport in superovulated immature mice *Endocrinology 75*, 461-463

Harvey E B , Yanagimachi R and Chang M C (1961) Onset of estrus and ovulation in the golden hamster *J exp Zool 146*, 231-235

Heilman R D and Strainer S M (1976) Dose-response effects of prostaglandin $F_{2\alpha}$ on the rabbit oviduct Reproductive endocrine influence and tachyphylaxis *Prostaglandins 12*, 127-137

Hilliard J and Eaton Jr L W (1971) Estradiol-17β, progesterone and 20α-hydroxypregn-4-en-3-one in rabbit ovarian venous plasma II From mating through implantation *Endocrinology 89*, 522-527

Hodgson B J (1976) Effects of indomethacin and ICI 46, 474 administered during ovum transport on fertility in rabbits *Biol Reprod 14*, 451-457

Hodgson B J and Eddy C A (1975) The autonomic nervous system and its relationship to tubal ovum transport -A reappraisal *Gynecol Invest 6*, 162-185

Hori T , Ide M and Miyake T (1969) Pituitary regulation of preovulatory estrogen secretion in the rat *Endocr jap 16*, 351-360

Horton E W and Marley P B (1969) An investigation of the possible effects of prostaglandins E_1, $F_{2\alpha}$ and $F_{2\beta}$ on pregnancy in mice and rabbits *Brit J Pharmacol 36*, 188P-189P

Hotchkiss J , Atkinson L E and Knobil E (1971) Time course of serum estrogen and luteinizing hormone (LH) concentrations during the menstrual cycle of the rhesus monkey *Endocrinology 89*, 177 183

Howe G R and Black D L (1973) Autonomic nervous system and oviduct function in the rabbit I Hormones and contraction *J Reprod Fertil 33* 425-430

Humphrey K W (1968) Observations on transport of ova in the oviduct of the mouse *J Endocr 40*, 267-273

Kirton K T , Niswender G G , Midgley Jr A R , Jaffe R B and Forbes A D (1970) Serum luteinizing hormone and progesterone concentration during the menstrual cycle of the rhesus monkey *J clin Endocr 30*, 105-110

Koester H (1969) Tubal secretion and egg development *Adv Biosci 4* 181-198

Koester H (1970) Ovum transport In Gibian H and Plotz E J , eds *Mammalian reproduction*, pp 189-228 Springer-Verlag, Berlin

Labhsetwar A P (1971) Luteolysis and ovulation induced by prostaglandin $F_{2\alpha}$ in the hamster *Nature (Lond) 230*, 528-529

Labhsetwar A P (1972a) Prostaglandin E_2 Analysis of effects on pregnancy and corpus luteum in hamsters and rats *Acta endocr (Kbh) Suppl 170*, 1-32
Labhsetwar A P (1972b) Effects of prostaglandin $F_{2\alpha}$ on some reproductive processes of hamsters and rats *J Endocr 53* 201-213
Labhsetwar A P (1972c) Luteolytic and ovulation-inducing properties of prostaglandin $F_{2\alpha}$ in pregnant mice *J Reprod Fertil 28*, 451 452
Labhsetwar A P (1973) Prostaglandin E_1 Studies on antifertility and luteolytic effects in hamsters and rats *Biol Reprod 8* 103-111
Labhsetwar A P (1974) Prostaglandins and the reproductive cycle *Fed Proc 33*, 61-77
Labhsetwar A P Joshi H S and Watson D (1973) Temporal relationship between estradiol, estrone and progesterone secretion in the ovarian venous blood and LH in the peripheral plasma of cyclic hamsters *Biol Reprod 8* 321-326
Lamothe P , Bousquet D and Guay P (1977) Cyclic variation of F prostaglandins in the uterine fluids of the cow *J Reprod Fertil 50*, 381-382
Leavitt W W and Blaha G C (1970) Circulating progesterone levels in the golden hamster during the estrous cycle, pregnancy, and lactation *Biol Reprod 3*, 353-361
Leavitt W W , Kline D L and Blaha G C (1970) Plasma progesterone and cortisol levels in the hamster during the estrous cycle and pregnancy *Fed Proc 29*, 705Abs
Liu T C , Cho C , Chu L C , Chen L R , Cheng K T , Sun I , Chiang K T and Ma T N (1974) Effect of prostaglandins $F_{2\alpha}$ and E_2 on fertilized ovum transport in the mice *Acta zool Sin 20*, 1-7
Lukaszewska J H and Greenwald G S (1970) Progesterone levels in the cyclic and pregnant hamster *Endocrinology 86*, 1-9

Magalhaes H (1970) Hamsters In Hafez E S E , ed *Reproduction and breeding techniques for laboratory animals*, pp 258-272 Lea and Febiger, Philadelphia
Maia H , Salinas L A Fernandez E O and Pauerstein C J (1977) Pharmacologic modification of the time course of ovum transport in guinea pigs *Fertil and Steril 28*, 1361-1364
Mastroianni Jr L and Komins J (1975) Capacitation, ovum maturation, fertilization and preimplantation development in the oviduct *Gynecol Invest 6* 226-233
Mattos C E R de and Coutinho E M (1971) Effect of the ovarian hormones on tubal motility of the rabbit *Endocrinology 89* 912-917
Mishell Jr D R , Nakamura R M , Crosignani P G , Stone S , Kharma K , Nagata Y and Thorneycroft I H (1971) Serum gonadotropin and steroid pattern during the normal menstrual cycle *Amer J Obstet Gynec 111* 60-65
Moawad A H , Hedqvist P and Kim M H (1976) Correlation of plasma estrogens and progesterone levels with the in vitro adrenergic responses in the isthmus of the human oviduct In Harper M J K Pauerstein C J , Adams C E , Coutinho E M , Croxatto H B and Paton D M , eds *Ovum transport and fertility regulation*, pp 276 292 Scriptor, Copenhagen
Monroe S E , Atkinson L E and Knobil E (1970) Patterns of circulating luteinizing hormone and their relation to plasma progesterone levels during the menstrual cycle of the rhesus monkey *Endocrinology 87* 453-455

Norman R L and Greenwald G S (1971) Effect of phenobarbital, hypophysectomy and X-irradiation on preovulatory progesterone levels in the cyclic hamster *Endocrinology 89* 598-605

Nutting E F (1969) Antifertility activity of prostaglandin E_2 in hamsters, rabbits and rats *Proc Soc Study Reprod 2nd Meet* p 1

Nutting E F and Cammarata P S (1969) Effects of prostaglandins on fertility in female rats *Nature (Lond) 222,* 287 288

Oeri H U (1960) Der Bau des Eileiters beim Goldhamster (Mesocricetus auratus) *Z Saugetierkd 25,* 52-65

Ogra S S , Kirton K T , Tomasi T B and Lippes J (1974) Prostaglandins in the human fallopian tube *Fertil and Steril 25* 250 255

Orsini M W (1961) The external vaginal phenomena characterizing the stages of the estrous cycle, pregnancy, pseudopregnancy, lactation, and the anestrous hamster, Mesocricetus auratus Waterhouse *Proc Anim Care Panel 11,* 193-206

Pauerstein C J , ed (1974) *The Fallopian tube A reappraisal* Lea and Febiger, Philadelphia

Pauerstein C J , ed (1975a) Seminar on tubal physiology and biochemistry *Gynecol Invest 6* 101-264

Pauerstein C J (1975b) Clinical implications of oviductal physiology and biochemistry *Gynecol Invest 6,* 253 264

Pauerstein C J , Anderson V , Chatkoff M L and Hodgson B J (1974) Effect of estrogen and progesterone on the time-course of tubal ovum transport in rabbits *Amer J Obstet Gynec 120,* 229-308

Piper P and Vane J (1971) The release of prostaglandins from lung and other tissues *Ann N Y Acad Sci 180,* 363-385

Piper P J , Vane J R and Wyllie J H (1970) Inactivation of prostaglandins by the lungs *Nature (Lond) 225,* 600-604

Poyser N L (1972) Production of prostaglandins by the guinea-pig uterus *J Endocr 54,* 147-159

Poyser N L (1973) The formation of prostaglandins by the guinea-pig uterus and the effect of indomethacin *Adv Biosci 9,* 631 634

Ridley K and Greenwald G S (1975) Progesterone levels measured every two hours in the cyclic hamster *Proc Soc exp Biol Med 149,* 10-12

Saidapur S K and Greenwald G S (1978) Peripheral blood and ovarian levels of sex steroids in the cyclic hamster *Biol Reprod 18* 401-408

Saksena S K and Harper M J K (1972a) Levels of F prostaglandin (PGF) in uterine tissue during the estrous cycle of hamster effects of estradiol and progesterone *Prostaglandins 2,* 405-411

Saksena S K and Harper M J K (1972b) Level of F prostaglandin (PGF) in peripheral plasma and uterine tissue of cyclic rats *Prostaglandins 2,* 511-517

Saksena S K and Harper M J K (1975) Relationship between concentration of prostaglandin F (PGF) in the oviduct and egg transport in rabbits *Biol Reprod 13,* 68-76

Saksena S K , Watson D T , Lau I F and Shaikh A A (1974) Peripheral plasma

levels of E and F series prostaglandins during pseudopregnancy in the rat *Prostaglandins 5*, 557 565

Seegal R F and Goldman B D (1975) Effects of photoperiod on cyclicity and serum gonadotropins in the Syrian hamster *Biol Reprod 12*, 223-231

Shaikh A A (1972) Estrone estradiol, progesterone and 17α-hydroxyprogesterone in the ovarian venous plasma during the estrous cycle of the hamster *Endocrinology 91*, 1136-1140

Shaikh A A and Saksena S K (1973) Cyclic changes in uterine venous and peripheral plasma levels of F prostaglandins correlated with peripheral progesterone levels in the golden hamster *Adv Biosci 9* 635-639

Shaikh A A , Birchall K and Saksena S K (1973) Steroids in the ovarian venous plasma and F prostaglandins in the peripheral plasma during pseudopregnancy and days 1-9 of pregnancy in the golden hamster *Prostaglandins 4* 17-30

Smith M G (1926) On the interruption of pregnancy in the rat by the injection of ovarian follicular extract *Bull Johns Hopk Hosp '39*, 203-214

Snedecor G W and Cochran W G (1967) *Statistical methods*, 6th ed Iowa State University Press, Ames

Soderwall A L , Kent H A , Turbyfill C L and Britenbaker A L (1960) Variation in gestation length and litter size of the golden hamster, Mesocricetus auratus *J Geront 15*, 246-248

Spilman C H (1974) Oviduct response to prostaglandins Influence of estradiol and progesterone *Prostaglandins 7*, 465-472

Spilman C H (1976) Prostaglandins, oviductal motility and egg transport In Harper M J K , Pauerstein C J , Adams C E , Coutinho E M , Croxatto H B and Paton D M , eds *Ovum transport and fertility regulation*, pp 197 211 Scriptor, Copenhagen

Spilman C H and Harper M J K (1972) Effect of prostaglandins on oviduct motility in conscious rabbits *Biol Reprod 7*, 106

Spilman C H and Harper M J K (1973) Effect of prostaglandins on oviduct motility in oestrous rabbits *Biol Reprod 9* 36-45

Spilman C H and Harper M J K (1974) Comparison of the effects of adrenergic drugs and prostaglandins on rabbit oviduct motility *Biol Reprod 10*, 549 554

Spilman C H and Harper M J K (1975) Effects of prostaglandins on oviductal motility and egg transport *Gynecol Invest 6*, 186-205

Squier R R (1932) The living egg and early stages of its development in the guinea pig *Contr Embryol Carneg Instn 23* 223 250

Thomas C M G , Corbey R S and Rolland R (1977) Assessment of unconjugated oestradiol and progesterone serum levels throughout pregnancy in normal women and in women with hyperprolactinaemia, who conceived after bromocriptine treatment *Acta endocr (Kbh) 86*, 405-414

Thomas C M G , van den Berg R J , de Koning Gans H J and Lequin R M (1978a) Radioimmunoassays for prostaglandins I Technical validation of prostaglandin $F_{2\alpha}$ measurements in human plasma using Sephadex G-25 gelfiltration *Prostaglandins 15*, 839-847

Thomas C M G van den Berg R J , de Koning Gans H J and Lequin R M (1978b) Radioimmunoassays for prostaglandins II Measurement of prostaglandin

E_2 and the 13, 14-dihydro-15-keto metabolites of the E and F series Description of a reliable technique with a universal applicability *Prostaglandins 15*, 849 855
Thorneycroft I H , Mishell Jr D R , Stone S C , Kharma K M and Nakamura R M (1971) The relation of serum 17-hydroxyprogesterone and estradiol-17β levels during the human menstrual cycle *Amer J Obstet Gynec 111*, 947-951

Valenzuela G and Harper M J K (1976) Effect of estrogen on activity of prostaglandin synthetase in rabbit oviduct *Prostaglandins 12*, 535-549
Valenzuela G , Ross H D , Hodgson B J , Harper M J K and Pauerstein C J (1977) Effect of inhibitors of prostaglandin synthesis and metabolism on ovum transport in the rabbit *Fertil and Steril 28*, 992-997
Vane J R (1971) Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs *Nature New Biol 231*, 232-235
Van Niekerk C H (1976) Retention of unfertilized ova in the oviducts of mares In Harper M J K , Pauerstein C J , Adams C E , Coutinho E M , Croxatto H B and Paton D M , eds *Ovum transport and fertility regulation*, pp 375-380 Scriptor, Copenhagen
Vastik-Fernandez J , Gimeno M F , Lima F and Gimeno A L (1975) Spontaneous motility and distribution of prostaglandins in different segments of human Fallopian tubes *Amer J Obstet Gynec 122*, 663-668

Wakeling A E and Spilman C H (1973) Prostaglandin specific binding in the rabbit oviduct *Prostaglandins 4*, 405-414
Ward M C (1946) A study of the estrous cycle and the breeding of the golden hamster, Cricetus auratus *Anat Rec 94*, 139-161
Whitney R and Burdick H O (1936) Tube-locking by oestrogenic substances *Endocrinology 20*, 643 647
Wide L and Hobson B (1977) Presence of chorionic gonadotrophin and free alpha- and beta-subunits in placental extracts of the rat, mouse and hamster *Acta endocr (Kbh) Suppl 212*, 31
Wimsatt W A and Waldo C M (1945) The normal occurrence of a peritoneal opening in the bursa ovarii of the mouse *Anat Rec 93*, 47-57
Wu C H , Blasco L , Flickinger G L and Mikhail G (1977) Ovarian function in the preovulatory rabbit *Biol Reprod 17*, 304-308

Yanagimachi R and Sato A (1968) Effects of a single oral administration of ethinyl estradiol on early pregnancy in the mouse *Fertil and Steril 19*, 787-801

CURRICULUM VITAE

De schrijver van dit proefschrift werd op 17 juni 1945 geboren te Heerlen. Na het behalen van het eindexamen HBS-B aan het St. Bernardinuscollege te Heerlen werd in 1963 begonnen met de studie chemie aan de Rheinisch-Westfälische Technische Hochschule te Aken, West-Duitsland. Het doctoraal examen werd afgelegd in 1970. Sinds februari 1971 is hij als wetenschappelijk medewerker verbonden aan de Kliniek voor Gynaecologie en Obstetrie van het St. Radboudziekenhuis te Nijmegen.

I

De weefselconcentraties van de prostaglandinen $F_{2\alpha}$ en E_2 in het oviduct van de goudhamster vertonen gedurende de cyclus en de eerste vier dagen van de zwangerschap een duidelijk patroon.

dit proefschrift

II

Er bestaat een duidelijke relatie tussen de plasmaspiegels van oestradiol en de weefselconcentraties van de prostaglandinen $F_{2\alpha}$ en E_2 in het oviduct.

dit proefschrift

III

Toediening van de prostaglandine synthetaseremmer indomethacine verlaagt de oviductconcentraties van de prostaglandinen $F_{2\alpha}$ en E_2.

dit proefschrift

IV

Door toediening van oestradiol worden de concentraties van de prostaglandinen $F_{2\alpha}$ en E_2 in het oviductweefsel duidelijk gestimuleerd.

dit proefschrift

V

Indomethacine onderdrukt de door oestradiol geïnduceerde stijging van prostaglandinespiegels in het oviduct.

dit proefschrift

VI

Het effect van prostaglandine $F_{2\alpha}$ op het ovumtransport bij de goudhamster is afhankelijk van de wijze van toediening.

dit proefschrift

VII

Het deelnemen aan programma's voor externe kwaliteitscontrole is om diverse redenen waardevol. Het uitsluitend relateren van een bepalingsuitkomst aan het groepsgemiddelde vormt echter geen garantie voor de kwaliteit van de bepaling.

VIII

Het is spijtig te moeten vaststellen dat er ten behoeve van een verdergaande automatisering in de klinische chemie concessies worden gedaan aan de verworvenheden van de analytische chemie.

IX

Specificaties van toegepaste bindingsanalysesystemen zijn onontbeerlijk in geval van doorverwijzing. De referentiewaarden voor de normale populatie dienen te worden opgegeven.

X

Het is opmerkelijk dat de normen die internationaal worden gehanteerd ten aanzien van dankbetuigingen in wetenschappelijke publicaties, aanzienlijk verschillen van de richtlijnen die worden aangegeven in artikel 23 van de Promotieregeling van de Katholieke Universiteit te Nijmegen.

XI

Dat de tuba niet noodzakelijk is bij het tot stand komen van een zwangerschap bij de mens moet niet resulteren in een verminderde belangstelling voor de functie van dit orgaan.

XII

Het opvolgen van de aanbeveling om ook in Nederland tabaksoorten te onderzoeken op de aanwezigheid van natuurlijke radionucliden en de accumulatie hiervan in het lichaam, zal niet leiden tot een verandering in de opvatting dat roken schadelijk is voor de gezondheid.

A. Faanhof
Chem. Weekblad 21, m345, 1978

C. M. G. Thomas

Nijmegen, 1 december 1978