

FOETAL AUTONOMY

A Ciba Foundation Symposium

Edited by

G. E. W. WOLSTENHOLME

and

MAEVE O'CONNOR



J. & A. CHURCHILL LTD.

104 GLOUCESTER PLACE, LONDON

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Symposium on Foetal Autonomy held 3rd–5th December 1968

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The Ciba Foundation



The Ciba Foundation was opened in 1949 to promote international cooperation in medical and chemical research. It owes its existence to the generosity of CIBA Ltd, Basle, who, recognizing the obstacles to scientific communication created by war, man's natural secretiveness, disciplinary divisions, academic prejudices, distance, and differences of language, decided to set up a philanthropic institution whose aim would be to overcome such barriers. London was chosen as its site for reasons dictated by the special advantages of English charitable trust law (ensuring the independence of its actions), as well as those of language and geography.

The Foundation's house at 41 Portland Place, London, has become well known to workers in many fields of science. Every year the Foundation organizes six to ten three-day symposia and three or four shorter study groups, all of which are published in book form. Many other scientific meetings are held, organized either by the Foundation or by other groups in need of a meeting place. Accommodation is also provided for scientists visiting London, whether or not they are attending a meeting in the house.

The Foundation's many activities are controlled by a small group of distinguished trustees. Within the general framework of biological science, interpreted in its broadest sense, these activities are well summed up by the motto of the Ciba Foundation: *Consociet Gentes*—let the peoples come together.

Preface

THE control exercised by the mammalian foetus over its own growth, development and security is now being studied by workers in so many different fields that, quite apart from its intrinsic interest, it seemed a particularly suitable topic for a Ciba Foundation symposium. In these symposia and in its shorter study groups the Foundation aims to bring together international and multidisciplinary groups whose interests cut across those of the established scientific and medical societies. In order that there may be plenty of scope for uninhibited discussion of the formal presentations the number attending is kept to about 25 people, and the Foundation tries to provide the best environment it can for a free and relaxed exchange of ideas.

The original stimulus for the meeting recorded in this book came from reports of the work of Dr. G. C. Liggins on the role of the foetal adrenal in determining the onset of parturition in the sheep. Dr. G. S. Dawes and Dr. R. V. Short assisted the Director of the Ciba Foundation by giving much time and careful thought to the planning of the meeting, and their enthusiastic support contributed greatly to its success. Dr. Dawes was an attentive and highly effective chairman whose interest and help, to grateful editors, continued long after the meeting ended.

CHAIRMAN'S OPENING REMARKS

DR. G. S. DAWES

AT an early stage in its life the mammalian embryo establishes contact with its mother, while concealing its own immunological identity, and induces in her the hormonal, metabolic, cardiovascular and respiratory changes associated with pregnancy. During foetal life it develops the homeostatic mechanisms necessary to maintain its internal environment as a foetus, and those additional mechanisms required for independent respiration and survival after birth. And finally there is evidence to suggest that the foetus itself normally initiates the process of parturition, thus liberating itself from the intrauterine environment which has protected it from cold and light, limited its tactile and auditory sensations, and provided it, through the placenta, with the means for growth and development. In these several ways it demonstrates its innate capacity for influencing its external and maintaining its internal environment—that is, its autonomy.

During development the mammalian organism passes through a number of critical periods—at implantation, during organogenesis, at the time of imprinting the sexual character on the nervous system, at birth, and after birth during the establishment of behavioural patterns—periods which pass never to return. Failure to make the transition at the right time is crippling or lethal. The mechanisms concerned necessitate the integration of many bodily functions, and are sophisticated, fascinating and difficult to unravel.

Work on these systems is in progress by investigators separated not just by distance but even more by discipline, who publish in the journals of very different learned societies, and who rarely meet together to exchange information of common interest. There still remains a large, almost unexplored territory between the embryologist and the foetal physiologist. The gap between the experimental immunologist and the obstetrician or paediatrician is only slightly less.

So one purpose of this symposium is to explore the gaps, and perhaps to spot the connexions between the different branches of the subject. We seek the integrated function of the whole foetus, the mechanisms which regulate it and their limits of tolerance, on which foetal autonomy depends. It is an ambitious task.

IMPLANTATION AND THE MATERNAL RECOGNITION OF PREGNANCY

R. V. SHORT

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THE maternal organism first becomes aware of the presence of an embryo in the uterus in diverse ways. In most mammals, this critical piece of information must be relayed to the mother at an early stage of gestation, and we will begin by considering in general terms both the nature of the message and the mode of its transmission. We shall then be in a position to investigate variations on the basic pattern, species by species.

One of the first outward and visible signs that an embryo has made its presence felt in the uterus is when the corpus luteum of the cycle becomes transformed into a corpus luteum of pregnancy, and oestrous or menstrual cycles cease to recur. Let us therefore examine this luteotropic action of the conceptus in a little more detail. Can the stimulus be initiated by the embryo before it has achieved an anatomical union with the endometrium? Is the stimulus itself mechanical in nature, giving rise to afferent neural stimuli to the hypothalamus, which in turn bring about the release of luteotropic hormone(s) from the anterior pituitary, or does the conceptus have a hormonal action, elaborating its own luteotropic substances? In those species in which the endometrium of the non-pregnant uterus seems to produce a luteolytic factor, how does the embryo act to neutralize this effect? These are some of the questions to which we must attempt to find the answers. Furthermore, it may be a mistake to concentrate all our attention on luteal maintenance as the first maternal premonition of a pregnancy; fundamental differences between the pregnant and non-pregnant animal may begin to become apparent soon after fertilization, and in a number of species the lifespan and secretory activity of the corpus luteum is unaffected by pregnancy. Undoubtedly much still lies outside our comprehension in this most fascinating area of investigation.

SPECIFIC CONSIDERATIONS

(1) *Women*

Some of the major events of early pregnancy are summarized diagrammatically in Fig. 1. Immediately after the last menstruation, during the follicular or proliferative phase of the cycle, blood progesterone and urinary pregnanediol values are very low, whilst the excretion of oestrone and oestriol is slowly rising (Loraine and Bell, 1966; Neill *et al.*, 1967). At the presumed time of ovulation, when urinary oestrone and oestriol excretion is maximal, there is a sudden pronounced increase in the blood luteinizing hormone (LH) concentration, lasting about a day (Midgley and Jaffe, 1966; Ross, Odell and Rayford, 1967; Neill *et al.*, 1967); the levels then return once more to the base-line values found during the proliferative phase.

After ovulation, the corpus luteum develops rapidly and within about six days it is secreting maximal amounts of progesterone (Loraine and Bell, 1966; Neill *et al.*, 1967). It is this post-ovulatory secretion of progesterone, and the attendant production of its thermogenic metabolite, pregnanediol, that is responsible for the post-ovulatory shift in the basal body temperature (Kappas *et al.*, 1960).

The fertilized egg burrows under the endometrium (interstitial implantation) about six days after ovulation (Boyd and Hamilton, 1952); unfortunately, no figures are available on the blood hormone levels at this critical early stage of pregnancy. However, in a classical study Brown, Klopper and Loraine (1958) measured the urinary excretion of oestrogens, gonadotropins and pregnanediol during the menstrual cycle of a woman who conceived to a single artificial insemination; Brown (1956) and Loraine and Bell (1966) have also investigated the hormone excretion patterns in other patients during the cycles in which they conceived. From such studies, and from our knowledge of the changes in the blood progesterone and urinary pregnanediol levels at the end of a normal menstrual cycle (Loraine and Bell, 1966; Neill *et al.*, 1967), it is possible to build up a picture of events during those critical days during which maternal endocrine mechanisms first seem to become aware of and adapt to the presence of a conceptus in the uterus.

The interstitial implantation of the embryo, on the sixth day after ovulation, is followed three to four days later by the first appearance of chorionic gonadotropin (HCG) in the maternal urine, and it can be detected in blood by about the 15th day after ovulation (Goldstein *et al.*, 1968). Thus HCG is produced some five days before the corpus luteum would stop secreting progesterone if the woman were not pregnant. It has therefore

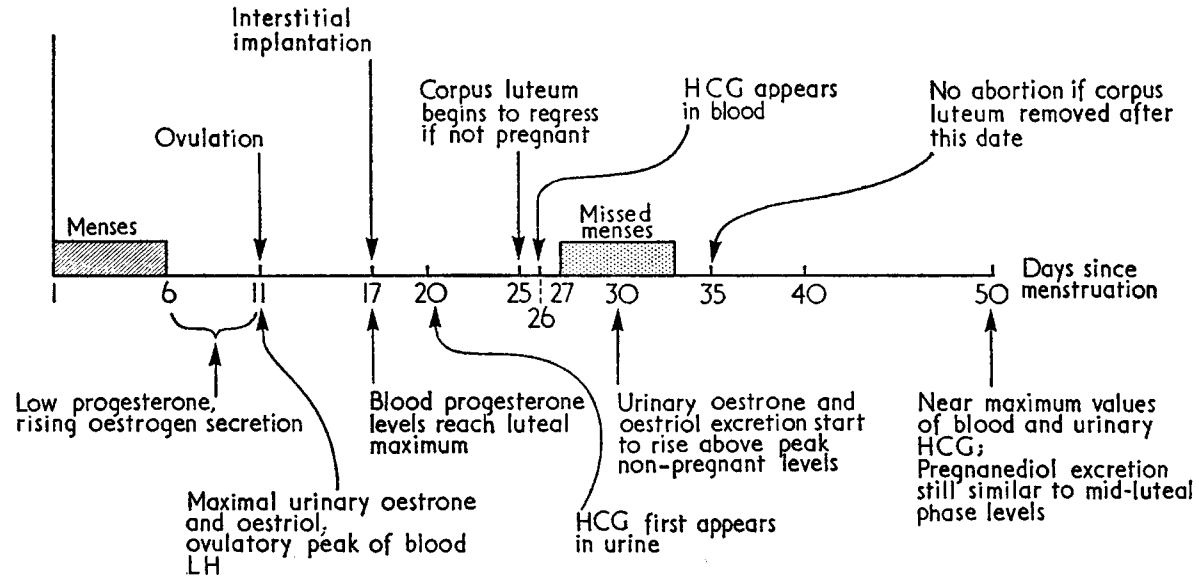


FIG. 1. Summary of the events of early pregnancy in a woman. First day of menstruation = Day 1. Duration of gestation 40 weeks from last menstrual period. HCG: human chorionic gonadotropin. LH: luteinizing hormone.

been assumed that this rising HCG excretion is the cause of luteal maintenance.

In addition to HCG, it appears that the placenta is capable of synthesizing progesterone at a very early stage of gestation, since the corpus luteum of pregnancy can be enucleated as early as the 35th day after the last menstrual period without producing an abortion; there are even claims that ovariectomy may safely be performed at an earlier date than this (Tulsky and Koff, 1957). The placenta also seems to be making significant amounts of oestrogen by the time of the first missed menstrual period (Brown, Klopper and Loraine, 1958).

Let us consider for a moment the postulated luteotropic role of HCG. Brown and Bradbury (1947), de Watteville (1949), Bradbury, Brown and Gray (1950) and Segaloff, Sternberg and Gaskill (1951) have all succeeded in postponing menstruation by administering HCG to women during the menstrual cycle, and recent experiments have shown that LH or HCG is capable of stimulating progesterone synthesis by slices of human corpora lutea *in vitro* (Savard, Marsh and Rice, 1965). But although the theory that HCG is the foetal luteotropin certainly explains the facts, we need a great deal more evidence before we can regard it as proved.

In his initial studies, Bradbury succeeded in postponing menstruation in six patients treated with daily doses of 2500–20000 i.u. HCG during the secretory phase of the cycle. However, it seems more significant that in three of these women, two of whom were on the highest dosage schedule, menstruation recurred *during the course of treatment*, and the other three all menstruated on the day that treatment was discontinued. In six other trials, where treatment was started in the follicular phase of the cycle and continued into the luteal phase, menstruation was only postponed in one case (Bradbury, Brown and Gray, 1950). De Watteville's study (1949) is even more interesting; four women in the luteal phase of the cycle were treated for seven to ten days with 20000 i.u. HCG daily, and the time of menstruation was postponed in some of them. But at the end of the treatment period, an ovariectomy was performed and in three patients both ovaries were examined; in all three, *two* corpora lutea were present (de Watteville, 1949). Similarly, Segaloff and co-workers (1951) gave 10000 i.u. HCG daily to six women, four of whom were laparotomized (presumably for hysterectomy) on days 29, 30, 37 and 40 of the cycle, and once again two corpora lutea were found in one case. Thus one could equally well postulate that menstruation was postponed, not because of any intrinsic luteotropic action of the HCG, but because in large doses it induces the formation of new corpora lutea which in turn inhibit menstruation. In

order to settle this point, it will be necessary to carry out further studies on the fate of individually marked corpora lutea after HCG treatment. As for the claim that HCG is steroidogenic, stimulating progesterone production by the corpus luteum, one has only to look at the blood progesterone and urinary pregnanediol values in women during the luteal phase of the cycle and in early pregnancy to see that this cannot be true of the *in vivo* situation. Blood LH levels are minimal at the time of maximal activity of the cyclical corpus luteum, and blood progesterone and urinary pregnanediol values do not increase above non-pregnant values during the first eight weeks after the last menstrual period, at a time when the HCG excretion is at its very highest (Loraine and Bell, 1966; Neill *et al.*, 1967).

If HCG is not the luteotropic hormone, how *does* the human corpus luteum know that the uterus is pregnant? It seems that we should be looking for a luteotropin that acts by prolonging the functional life of the corpus luteum, rather than by increasing its secretory activity. Prolactin acts in this fashion in the ewe (Short, 1967; Denamur, 1968), and even though prolactin preparations of animal origin are apparently inactive as luteotropins in women (Bradbury, Brown and Gray, 1950), nobody seems to have investigated the action of human placental lactogen (see Josimovich, Kosor and Mintz, 1969).

The widespread use of the intrauterine contraceptive device allows us to state with some assurance that mere distension of the uterine lumen does not have a significant effect on luteal lifespan in women (Marston and Kelly, 1966); hence we cannot invoke mechanical stimulation as a likely mechanism for the maternal recognition of pregnancy. It is possible that the non-pregnant endometrium produces a lytic substance that brings about the cyclical regression of the corpus luteum in the absence of a pregnancy; Andreoli (1965) claims to have prolonged the life of the human corpus luteum by hysterectomy performed early in the luteal phase of the cycle. However, the results of S. Markham (personal communication) suggest that cyclical ovarian activity is not disturbed after removal of the uterus. This whole subject requires detailed re-investigation.

The foregoing arguments will have been sufficient to convince the reader that our ideas on the hormonal mechanisms for the initiation of pregnancy in women are still in their formative stage. And yet, if we are to control human populations, one of the things we need to control is the human corpus luteum. If its regression at the end of the cycle is an active process under the control of a lytic hormone, we should surely be able to emulate the process pharmacologically. If the maintenance of a corpus luteum of pregnancy is dependent on the production by the developing conceptus of

a tropic hormone of large molecular weight, then the possibilities of passive or active immunization of the mother against the foetal hormone suggest themselves as logical points of attack. But attempts to induce early abortions by interfering with luteal activity may be confounded by the early stage at which the placenta itself is able to produce sufficient progesterone to maintain the pregnancy.

(2) *Rhesus monkey*

The principal events of early pregnancy are shown diagrammatically in Fig. 2. All studies of rhesus monkeys are hampered by the fact that one seldom knows exactly when ovulation takes place; hence the temporal relationships shown in Fig. 2 can only be regarded as approximate. Ovulation occurs on about day 13 of the cycle, and may be preceded by a rise in the peripheral blood progesterone level (Johansson, Neill and Knobil, 1968). However, Betteridge, Kelly and Marston (1969) contend that a number of the pre-ovulatory follicles illustrated by Johansson's group are in fact post-ovulatory follicles undergoing early luteinization, so that this blood progesterone rise may not be pre-ovulatory at all.

After fertilization, the developing morula enters the uterus within about three or four days (Marston, Kelly and Eckstein, 1969), at a time when the corpus luteum would be secreting maximal amounts of progesterone in the non-pregnant animal (Neill, Johansson and Knobil, 1967, 1969). The blastocyst first becomes attached to the uterine epithelium about nine days after ovulation (Boyd and Hamilton, 1952), and the following day the cyto- and syncytiotrophoblast begin to proliferate, with a concomitant brief rise in the peripheral blood progesterone level lasting about five days (Neill, Johansson and Knobil, 1969). If the animal is not pregnant, the blood progesterone levels begin to decline at about this time (Neill, Johansson and Knobil, 1967). Gonadotropic activity, in the form of monkey chorionic gonadotropin (MCG), is first detectable in the maternal urine about 12 days after ovulation (Arslan, Meyer and Wolf, 1967), and it is interesting to note that the blood progesterone levels are beginning to fall at a time when the MCG levels are rising (Tullner and Hertz, 1966a; Arslan, Meyer and Wolf, 1967).

By about the 21st day after ovulation the placenta can apparently produce sufficient progesterone to compensate for the removal of the ovaries (Tullner and Hertz, 1966b) and hypophysectomy does not necessarily result in an abortion if performed after day 29 (Smith, 1954). Not only does ovariectomy fail to produce an abortion, but it fails to abolish the second transitory rise in the blood progesterone level that normally occurs at about

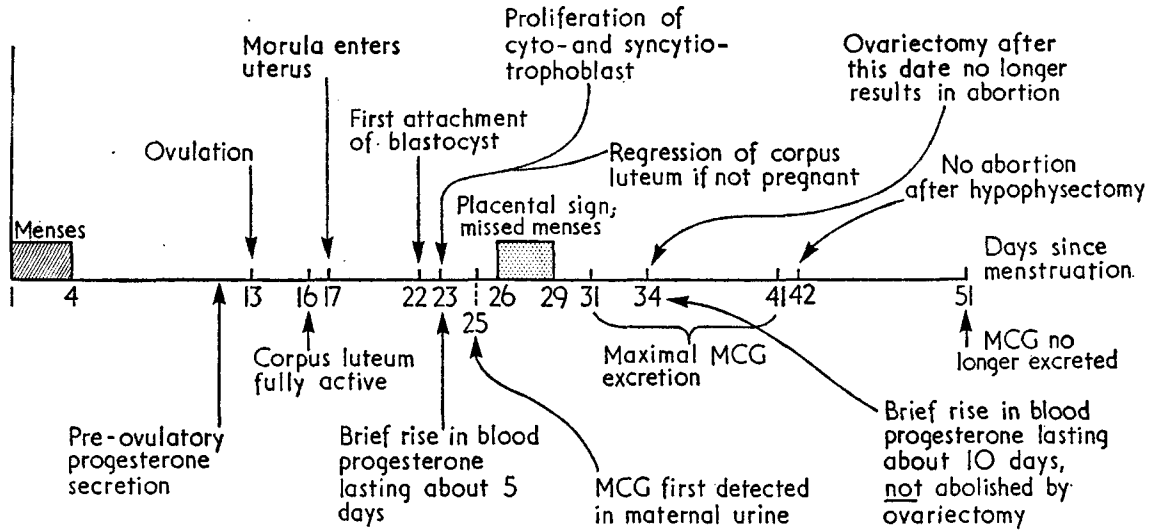


FIG. 2. Summary of the events of early pregnancy in a rhesus monkey. First day of menstruation = Day 1. Mean duration of gestation = 164 days from ovulation. The "placental sign" is the appearance of blood in the vagina often seen in pregnant monkeys at around the time of the first missed menses; however, its appearance and duration are extremely variable. MCG: monkey chorionic gonadotropin.

this time (Neill, Johansson and Knobil, 1969). Gonadotropic activity eventually disappears from the urine by about the 38th day after ovulation (Arslan, Meyer and Wolf, 1967), and the blood and placental levels of oestrogen and progesterone fail to show the spectacular increases during the latter half of gestation that are such a feature of human pregnancy (Short and Eckstein, 1961; Neill, Johansson and Knobil, 1969).

Thus it can be seen that there are a number of major qualitative and quantitative differences between the course of pregnancy in women and rhesus monkeys; not only does the type of implantation and the form of the placenta differ, but the timing of events and the amounts of hormones produced are also completely different.

If we knew little of the nature of the initial luteotropic stimulus in women, we know even less about it in the rhesus monkey. The generally held view, based on the studies of Hisaw (1944) and Bryans (1951), is that chorionic gonadotropin is luteotropic whereas prolactin is not. But whilst it is true that Hisaw and Bryans were both able to postpone menstruation by administering daily doses of 330 to 1 000 i.u. HCG to monkeys in the luteal phase of the cycle, no attempt was made to mark the corpus luteum present at the beginning of treatment. Only by doing this would one be able to tell whether menstruation was postponed because of luteal maintenance, or merely because of the new corpora lutea that had been produced by the gonadotropin treatment. In Hisaw's study there was definite evidence of fresh ovulations having occurred in some animals during the course of HCG treatment; it also seems significant that all his animals menstruated whilst still being injected with HCG.

Fig. 2 also shows reasons to doubt the view that MCG is the luteotropic hormone in monkeys; it can be seen that the corpus luteum decides whether or not to regress two days before MCG first makes its appearance in the maternal urine.

As far as other factors controlling the corpus luteum are concerned, there is no evidence that mechanical distension of the uterus, for example with an intrauterine contraceptive device, has any effect at all (Marston and Kelly, 1966; Kelly, Marston and Eckstein, 1969). Similarly, Burford and Diddle (1936) and Van Wageningen and Catchpole (1941) could find no evidence that hysterectomy prolonged luteal life. This experiment should certainly be repeated, and the fate of a marked corpus luteum present at the time of hysterectomy should be followed by serial blood progesterone determinations.

We must therefore conclude that in the rhesus monkey the evidence, such as it is, suggests that the developing conceptus must have a luteotropic function within about a day of implantation. This could be mediated by MCG,

but a great deal more experimental evidence is needed before one can form an opinion on this point.

(3) *Mare*

The events of early pregnancy in the mare are summarized diagrammatically in Fig. 3, and in many ways the mare is the most fascinating of all the species.

Ovulation occurs about 24 hours before the end of behavioural oestrus, and the effects of an incipient pregnancy begin to make themselves apparent almost at once. It has been convincingly demonstrated by van Niekerk and Gerneke (1966) that unfertilized eggs remain in the Fallopian tubes for several months, where they slowly degenerate; it is only the fertilized egg that ever passes on down into the uterus. Such an amazing discovery is as inexplicable as it is unexpected, but it does serve to emphasize the fact that a number of pregnancy-detecting mechanisms may be at work long before the corpus luteum is called upon to make a decision as to whether to regress or remain active.

We do not know the time taken for tubal transport of the fertilized egg in the mare, but it is probably around four to six days. The corpus luteum seems to regress on about day 12 in the non-pregnant animal, as judged by serial blood progesterone determinations (Allen, Smith and Short, unpublished observations), and rectal palpation at around this time indicates differences between the pregnant and non-pregnant uterus: the pregnant uterus maintains its tone and thickness, whereas the non-pregnant uterus becomes flaccid and thin-walled (van Niekerk, 1965*a,b*). Curiously, even though the corpus luteum is maintained as a result of a pregnancy, the ensuing period of oestrus is not completely suppressed. Many pregnant mares show signs of increased follicular activity in the ovaries in the period between the 17th and 30th days of gestation (van Niekerk, 1965*a*; Bain, 1967) and about 10 per cent may actually exhibit behavioural oestrus at this time.

By about the 21st day of pregnancy the blastocyst, which is still lying free within the uterine lumen, measures 7 cm × 6.5 cm (van Niekerk, 1965*b*); at this stage the allantois is only just beginning to form, and the sinus terminalis, which marks the division between bilaminar and trilaminar omphalopleur, is equatorial in position (see Fig. 4). It is interesting that the vertical axis of the blastocyst is parallel to the long axis of the uterine lumen (see Fig. 5).

After the 21st day of pregnancy, the sinus terminalis moves down the blastocyst to reach the abembryonic pole; at the same time the allantois

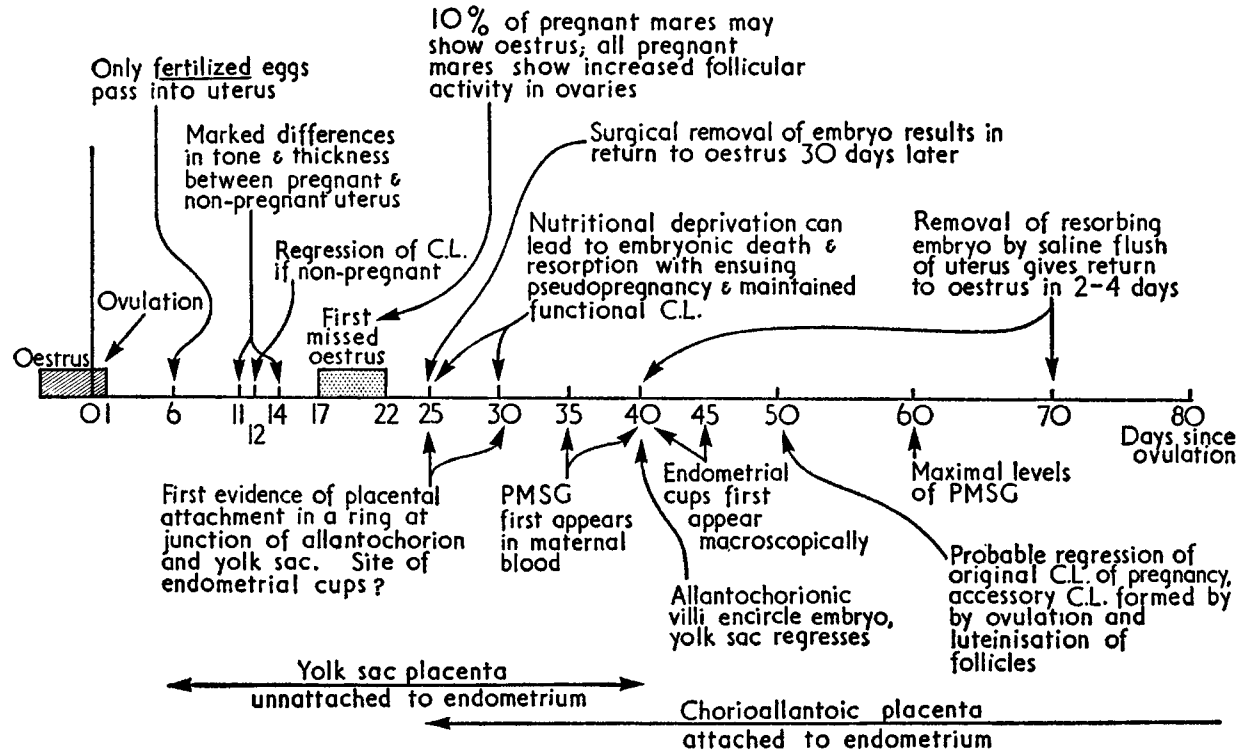


FIG. 3. Summary of the events of early pregnancy in a mare. Day of ovulation = Day 0. Duration of gestation about 11 months. C.L.: corpus luteum. PMSG: pregnant mare's serum gonadotropin.

begins to grow down from the embryonic pole, converting the yolk sac placenta into a true allantochorionic placenta. The first anatomical attachment of this new placenta to the endometrium probably occurs in a band around the circumference of the conceptus at the front of the advancing allantochorion. The large villous projections formed in this area begin to interdigitate with the endometrium by about days 25 to 30; by day 40 the allantois has reached the abembryonic pole, thus effectively obliterating the yolk sac placenta, and the entire allantochorion is now covered with fine villi (van Niekerk, 1965*b*). Since placentation in the horse is of the diffuse, epitheliochorial type, there is never any erosion of maternal or foetal tissue layers, and anatomically it may be extremely difficult to decide at exactly which point in time attachment of the embryo does take place.

It is tempting to suppose that it is the initial equatorial band of placental tissue which provides the stimulus for the development of the maternal endometrial cups. These are specialized areas of maternal decidual tissue and hypertrophied endometrial glands which first become apparent macroscopically on about day 40-45 (Clegg, Boda and Cole, 1954; Amoroso, 1955; King, 1965), and which begin to secrete measurable amounts of pregnant mare's serum gonadotropin (PMSG) into the maternal circulation by day 35-40 (Allen, 1969).

The endometrial cups are not apparently formed at predetermined sites in the uterus, since they cannot be seen in the non-pregnant animal. They develop in a circle near the junction of the pregnant horn with the body of the uterus, and if a mare is carrying twins in opposite horns of the uterus two sets of endometrial cups will develop; however, if both embryos are in one horn of the uterus, there may only be one set of cups (Rowlands, 1949).

The function of the PMSG* is problematical; the concentration of PMSG in the serum reaches maximal values around the 60th to 65th day of gestation (Allen, 1969), and the combined follicle-stimulating and luteinizing activities of this gonadotropin cause the formation of varying numbers of accessory corpora lutea in the maternal ovaries, which arise either by ovulation or by the luteinization of unruptured follicles (Cole, Howell and Hart, 1931; Amoroso, Hancock and Rowlands, 1948). These new corpora lutea may have an important function to perform, since it is believed that the initial corpus luteum of ovulation regresses by about the 40th to 50th day of gestation (Cole, Howell and Hart, 1931) and a second crop of corpora lutea may be required to maintain the supply of progesterone until such time as the placenta can take over. Progesterone can be detected in the placenta by the third month (Short, 1958), the accessory corpora lutea have

*See also p. 31.

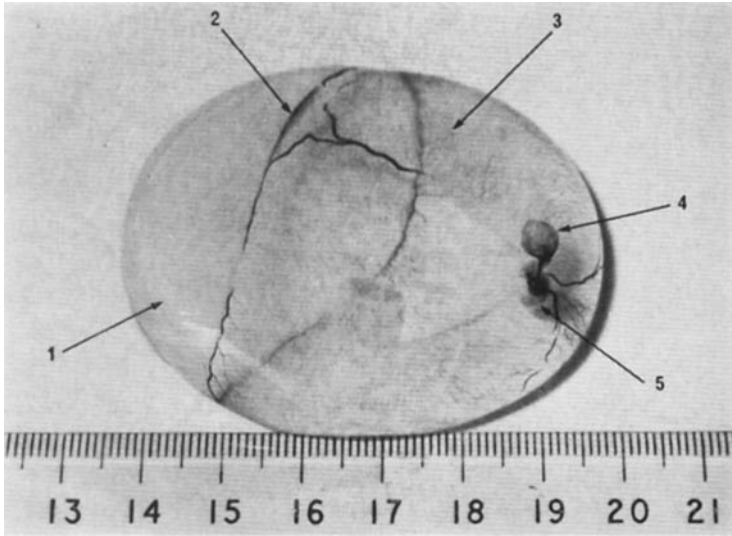


FIG. 4. Early horse embryo, approximately 25 days old, showing 1: bilaminar omphalopleur; 2: sinus terminalis; 3: trilaminar omphalopleur; 4: developing allantois; 5: embryo. Scale in cm.

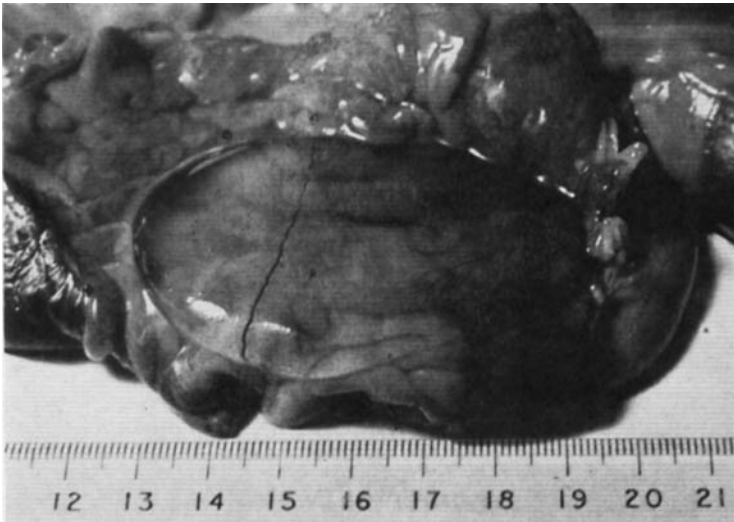


FIG. 5. Same embryo as in Fig. 4 *in situ*; lying free within the uterine lumen with its vertical axis parallel to the long axis of the uterus. Sinus terminalis clearly visible.

regressed in the maternal ovaries by the fifth month (Cole, Howell and Hart, 1931), and ovariectomy no longer results in abortion if performed after day 167 (Amoroso and Finn, 1962). During the later months of pregnancy, the placenta produces large amounts of progesterone; this seems to be largely metabolized by the uterus before it can enter the maternal circulation, since progesterone virtually disappears from the maternal blood during the second half of pregnancy (Short, 1957, 1959).

The endometrial cups are unique in being an example of hormonally active maternal decidual tissue; they seem to be formed in response to the antigenic stimulus of a conceptus. If a mare is mated to a jack donkey instead of a stallion, so that she is carrying a mule embryo, the concentrations of PMSG in her serum are much lower than if she were carrying a horse embryo (Bielanski, Ewy and Pigoniowa, 1956; Clegg *et al.*, 1962; Allen, 1969). Recent attempts to induce the formation of endometrial cups by a purely mechanical stimulus, in the form of a rubber balloon inserted in the uterus at oestrus, were unsuccessful. Distension of the uterus in this way did not even result in a significant prolongation of the oestrous cycle (King, 1965).

One of the classical techniques of endocrinology is to remove the endocrine gland and study the deficiency symptoms produced. It is surprising that this line of investigation has not been applied more often to the problem of the endocrine role of the developing conceptus. If we suspect the embryo of having a luteotropic role, then its removal should result in rapid luteal regression and a return to oestrus. Van Niekerk (1965c) has provided some fascinating evidence along these lines in the mare. He noticed that if animals were subjected to a severe nutritional deprivation between days 25 and 30 of gestation, then death and resorption of the embryo rapidly supervened. However, these animals then entered a state of pseudopregnancy; the corpus luteum remained functional, as judged by its post-mortem appearance, and the mares did not return to oestrus. In three instances van Niekerk was able to terminate the period of pseudopregnancy by flushing the resorbing embryos out of the uterus with a large volume of saline; treated animals returned to oestrus within two to four days. Allen (personal communication) has been able to show that even surgical removal of the entire embryo from mares on days 25 and 34 of gestation only results in a return to oestrus 20 to 30 days later. If total removal of the embryo does not cause immediate luteal regression, we must therefore think again about the luteotropic activity of the conceptus.

King (1965) has recorded the presence of well-developed endometrial cup tissue in the uteri of a number of non-pregnant Grevy's zebras with

active or regressing corpora lutea in their ovaries. In view of van Niekerk's and Allen's findings, it now seems probable that these zebras were in a state of pseudopregnancy following the death and resorption of an embryo, and King's observations do not invalidate the concept that endometrial cup tissue is only formed in the uterus in response to a pregnancy.

To attempt to summarize the complex endocrine events at the beginning of gestation in the mare is to emphasize once more our complete lack of understanding of the whole subject. We have no hypothesis to explain the differential tubal transport of fertilized and unfertilized eggs. We do not know how the unimplanted blastocyst can cause maintenance of the corpus luteum, except that the effect is unlikely to be a mechanical one. We do not know whether there is normally any lytic influence of the uterus on the corpus luteum in the mare, which the blastocyst might be able to counteract. It is not clear why a specific area of trophoblast should induce the formation of hormonally active maternal decidual tissue, nor why the genotype of the foetus should have such an important influence on the amount of gonadotropin this tissue produces. And finally, we cannot understand why death or removal of the embryo does not result in immediate luteal regression and a return to oestrus. The pregnant mare, that has already contributed so much to the development of endocrinology, obviously still has much more to offer.

(4) *Sow*

The events of early pregnancy in the sow are summarized diagrammatically in Fig. 6.

After ovulation, which occurs around the middle of the two-day period of behavioural oestrus, the fertilized eggs pass down the Fallopian tube to reach the uterus at the 4- to 8-cell stage 48 hours later (Perry and Rowlands, 1962). Studies in progress, in which embryos have been flushed out of the uterus at various stages of gestation, indicate that there has been no maternal recognition of pregnancy at least until day 7; the length of the oestrous cycle is not prolonged if embryos are removed before that date (Moor, 1968*a*). However, pregnancy has apparently been recognized by day 11; creation of a sterile uterine horn by flushing embryos out at this stage allows pregnancy to continue in the other horn, whereas if the flushing is done on days 3 or 9, the pregnancy fails (Dhindsa and Dziuk, 1968).

The embryos have spaced themselves out along the uterine horns by day 12, and then on day 13 the embryonic sacs undergo a spectacular elongation, becoming thin, fragile ribbons of tissue 55-191 cm in length when fully extended, but closely following the corrugated folds of the endometrium

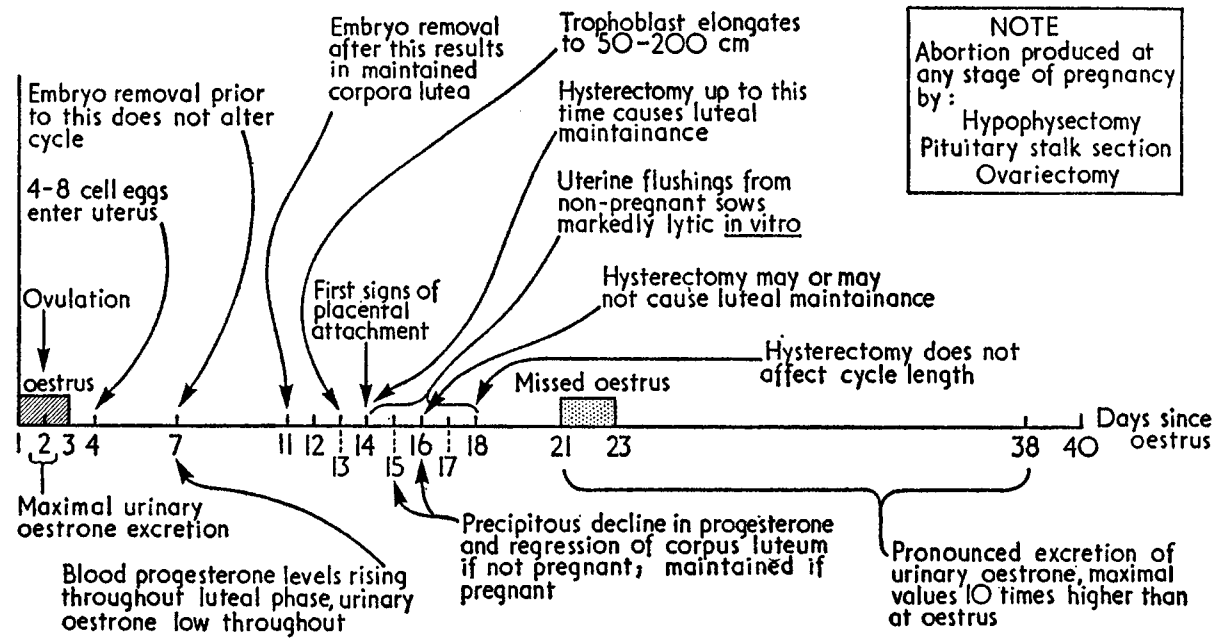


Fig. 6. Summary of the events of early pregnancy in a sow. Day of onset of oestrous behaviour = Day 1. Duration of gestation approximately 113 days.

so that they have an effective length *in situ* of about 33 cm (Perry and Rowlands, 1962). The first signs of placental attachment can be seen on day 14 after this elongation has occurred (Perry, personal communication). Although placentation is of the diffuse, epitheliochorial type, as in the horse, the pig never develops a functional yolk sac placenta.

The corpora lutea of the cycle stop secreting progesterone abruptly on day 15–16 if the animal is not pregnant, but continue secreting at a constant rate if the sow is pregnant, or if it is hysterectomized (Masuda *et al.*, 1967; Stabenfeldt *et al.*, 1969). It is interesting to note that if a non-pregnant sow is hysterectomized at any time until day 14, the corpora lutea will behave like corpora lutea of pregnancy; on day 16, the results are variable, and on day 18, hysterectomy can no longer arrest luteal regression (Anderson, Butcher and Melampy, 1963).

The first real clue as to how the corpora lutea know that there are embryos in the uterus came from the experiments of du Mesnil du Buisson (1961). He showed that if one uterine horn was surgically isolated from the rest of the uterus, it did not alter the length of the oestrous cycle. However, if such animals were mated, they could seldom maintain a pregnancy. This appeared to be because the non-pregnant uterine horn caused the regression of the corpora lutea in both ovaries, and hence death of the embryos in the pregnant horn. If the non-pregnant horn was removed before the 14th day, then the corpora lutea in both ovaries survived and the pregnancy was allowed to continue. When the amount of non-pregnant uterine tissue was progressively reduced, a stage could be reached when luteal regression was confined to the ovary on the non-pregnant side and the pregnancy was maintained by the surviving corpora lutea in the other ovary. Du Mesnil's findings have since been amply confirmed by others, and the evidence has been neatly summarized by Moor (1968a).

It is experiments of this nature in cows, sheep, pigs and guinea pigs that have led to the concept of a uterine luteolytic hormone. Thus it seems probable that in these species the corpus luteum of the cycle has a lifespan potentially as long as the corpus luteum of pregnancy, but that it is prevented from living out its life because it is "murdered" by some luteolytic agent coming from the uterus. This acts initially by a local mechanism, having its greatest effect on the adjacent ovary, but there may also be some spill-over to give a generalized systemic effect (Short, 1967). Such a viewpoint has been generally accepted by many workers for some time (see reviews by Melampy and Anderson, 1968; Moor, 1968a; Hawk, 1968); however, it is still hotly denied by others (Nalbandov and Cook, 1968). This scepticism is desirable until somebody can demonstrate the nature of the

luteolytic substance, and the route whereby it reaches the ovary from the uterus without the intervention of the systemic circulation.

Perhaps the closest we have come to the isolation of a luteolytic substance is the demonstration by Schomberg (1967) that uterine flushings collected from sows at days 14 to 18 of the cycle rapidly destroy pig luteal cells growing in tissue culture, whereas flushings at other stages of the cycle do not show this effect. The active principle appears to be thermolabile and non-dialysable, but shows only limited organ and species specificity.

If we can for the moment accept this concept of a uterine luteolytic hormone that is responsible for cutting short the life of the corpus luteum in the non-pregnant animal, then we must re-examine the whole question of how the corpus luteum knows that there is an embryo in the uterus. In the pig, it is more attractive to postulate that the embryos are anti-luteolytic, rather than directly luteotropic. Pituitary stalk section or hypophysectomy are followed by luteal regression and abortion at all stages of pregnancy (du Mesnil du Buisson *et al.*, 1964; du Mesnil du Buisson, 1966; Anderson *et al.*, 1967), suggesting that pig placentae never have appreciable luteotropic activity in their own right. The placentae also seem to be incapable of manufacturing sufficient progesterone to maintain pregnancy after ovariectomy, regardless of when it is performed (Short, 1956; du Mesnil du Buisson and Dauzier, 1957); however, they can manufacture large amounts of oestrogen (Lunaas, 1962; Rombauts, 1962; Raeside, 1963), which may interfere with the luteolytic effect of the uterus during the pregnancy.

If the embryos are essentially anti-luteolytic, then the reason for the marked similarity between corpora lutea of hysterectomy and of pregnancy is at once apparent. However, hysterectomy can save the life of the corpora lutea of the cycle if performed as late as day 14-16, but embryo removal experiments suggest that embryos have already had a significant effect on the corpora lutea by day 11 (Dhindsa and Dziuk, 1968). The luteolytic activity of the endometrium also explains why a sow is normally unable to maintain a pregnancy unless there are four or more embryos present in the uterus (Polge, Rowson and Chang, 1966); but if a greater part of the endometrium is removed, then one embryo alone is quite sufficient (du Mesnil du Buisson and Rombauts, 1963).

If we reject the concept of an anti-luteolytic action of the conceptus, what alternatives are there? It seems unlikely that the embryo could maintain the corpus luteum by mere mechanical stimulation of the endometrium, since various intrauterine foreign bodies have been shown to have no effect on oestrous cycle length in sows (Anderson, 1962; Gerrits and Hawk, 1966; Hawk, 1968). If a luteotropic stimulus were required to maintain the