Maternal recognition and maintenance of pregnancy in the mare

W. R. Allen

University of Cambridge, Department of Veterinary Medicine, Equine Fertility Unit, Mertoun Paddocks, Woodditton Road, Newmarket, Suffolk CB8 9BH, UK.

Abstract

This review highlights some of the unusual physiological features of pregnancy in equids. These include the relatively prolonged sojourn of the zygote in the mare's oviduct and the requirement for embryonic synthesis and release of prostaglandin E_2 (PGE_2) to stimulate oviducal relaxation and contractility for onward embryonic passage into the uterus. The conceptus remains spherical due to persistence of its glycoprotein blastocyst capsule, and it must move continually throughout the uterine horn between Days 6 and 17 (Day 0 = ovulation) in order to release its, as yet unidentified, maternal recognition of pregnancy signal onto a sufficient area of endometrium to suppress the normal cyclical release of endometrial prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and thereby establish luteostasis and the pregnancy state.

The trophoblast of the developing chorion differentiates into invasive and non-invasive components between Days 25 and 35. The former invades the maternal endometrium to form the short-lived endometrial cups, the hormonal product of which, eCG, supports progesterone production by stimulating the development of accessory corpora lutea. The non-invasive trophoblast develops an increasingly complex interdigitation with the luminal epithelium of the endometrium to form the diffuse epitheliochorial-chorioallantoic placenta that provides haemotrophic sustenance for the fetus until term. The placenta also synthesizes appreciable quantities of steroid hormones utilizing fetal gonad sources of C-19 androgens for aromatization to estrogens and a combination of maternal and fetal adrenal gland sources of pregnenolone for conversion to 5α-dihydroprogesterone and other 5α-reduced progestagens.

Keywords: horse, conceptus, endometrial cups, placental steroids

Introduction

Several features of pregnancy in the mare and other equids are unusual and differ markedly from equivalent events in other, well-studied large domestic animal species. From the slow passage of the equine embryo through the oviduct, to its free movement throughout the uterine lumen between Days 6 and 17 (Day 0 = ovulation), the remarkable measured pace of fetal membrane development and differentiation between Days 20 and 35, the strange division of the trophoblast into invasive and non-invasive components between Days 25 and 35, the development and death of the fetal endometrial cups between Days 38 and 120, the slow pace of attachment and interdigitation of the allantochorionic placenta between Days 40 and 150, to the even more puzzling production of relatively huge quantities of progestagenic and estrogenic steroid hormones by the feto-placental unit during the second half of gestation, to the final ultra-rapid and generally uncomplicated expulsion of the highly altricial foal at term (Day 320 to 360), the genus Equus stands out as being quite different from the rest. A summary of some of these events is depicted in Fig. 1.

Oviducal transport

Van Niekerk and Gerneke (1966) first drew attention to the differential transport of oocytes and embryos within the equine oviduct. Namely, the unfertilized oocyte travels only as far as the ampullary-isthmic junction where it lodges in the convoluted folds of oviducal mucosa and slowly degenerates there over many months (Flood *et al.*, 1979b). The zygote and early embryo, on the other hand, pass all the way down the oviduct to enter the uterus through the protruberant uterotubal papilla between 144 and 158 hours after ovulation (Webel *et al.*, 1977; Battut *et al.*, 1998). Thus, flushing the oviducts of mares *post mortem* typically yields multiple, degenerate oocytes that have accumulated from ovulations without fertilization from previous estrous cycles (Fig. 2a; Betteridge and Mitchell, 1974; David, 1975). Yet, the occurrence of fertilization after mating or insemination can result in the young embryo either bypassing the aggregated oocytes or dragging them with it as it enters the uterus (Onuma and Ohnami, 1975).

A series of elegant experiments involving both the *in vitro* culture of early stage embryos (Weber *et al.*, 1991b; Freeman *et al.*, 1992) and surgical implantation of hormone-secreting minipumps onto the mesosalphinx of mares at various times after ovulation (Weber *et al.,* 1991a; 1992; 1995) provided the definitive answer to the oviducal transport puzzle. These authors demonstrated convincingly that the embryo, but not the unfertilized oocyte, begins secreting appreciable quantities of prostaglandin E_2 (PGE_2) when it reaches the compact morula stage of

development on Day 5 (Fig. 2b). This embryonic hormone acts locally to relax the circular smooth musculature of the oviduct which then allows rapid onward movement and entry of the embryo into the uterus some 24 hours later. Thus, it is the stage-dependent development of the embryo's hormone-secreting capacity, not just a subtle change in embryonic size or configurational changes in its outermost coat, that brings about its passage from the oviduct into the uterus.

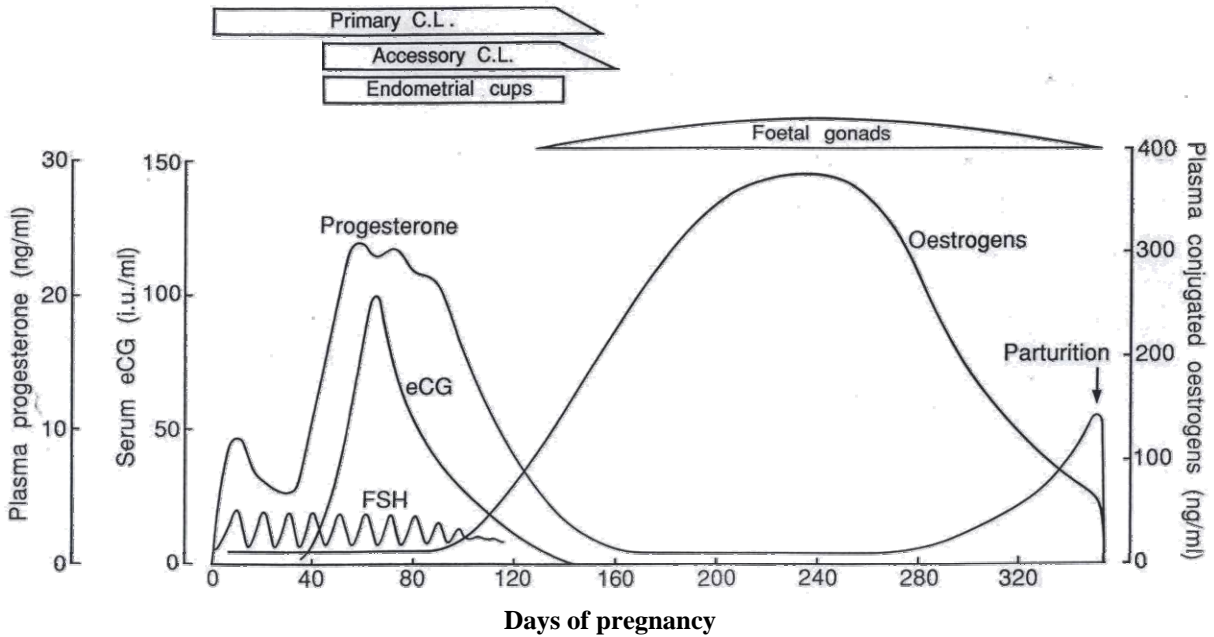**Events of pregnancy in the mare**

Figure 1. Diagrammatic summary of significant developmental and endocrinological changes in the mare during pregnancy (b).
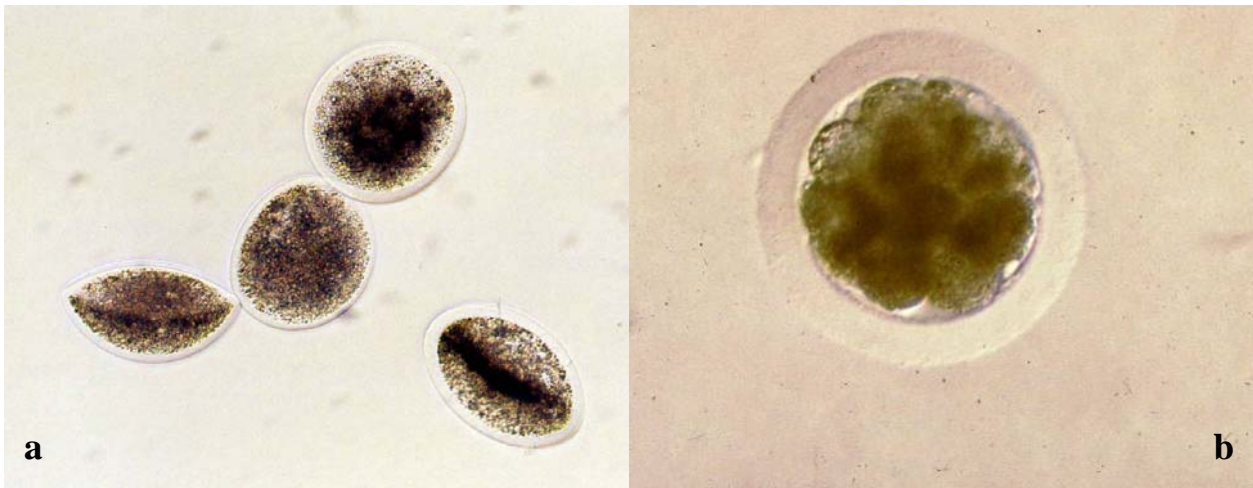

Figure 2. Degenerate oocytes recovered when flushing one oviduct of a 7-year-old, cycling Thoroughbred mare (a; 320x) and a Day 5, compact horse morula (b) that would be secreting PGE_2 to relax the circular, and contract the longitudinal, smooth musculature of the oviduct and thereby ensure its onward passage into the uterus 24 hours later (456x).

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Maternal recognition of pregnancy

Short (1969) coined the phrase "maternal recognition of pregnancy" when he compared the different strategies employed by the common domestic animal species to ensure continuation of the life and secretory function of the CL beyond its normal cyclical lifespan and thereby maintain the uterus in the correct progestational state to support the pregnancy. The mare provides a distinct and apparently unique contrast to the pig and the wide range of ruminant species in the manner in which its embryo transmits the maternal recognition of pregnancy factor during early gestation. Enveloped in a tough and closely-fitting glycocalyx capsule between Days 6.5 and 22 (Betteridge, 1989), the equine embryo is unable to rearrange and elongate its trophectoderm between Days 10 and 14 like its porcine and ruminant counterparts to bring the trophoblast into close contact with a sizeable area of endometrium in the gravid uterine horn (Perry *et al.,* 1973; Wooding, 1982). Instead, the equine conceptus remains spherical and completely unattached (Fig. 3a), and it moves continually throughout the uterine lumen, propelled by strong peristaltic contractions of the myometrium (Ginther, 1983a; b; 1985) that are induced by rhythmical releases of $PGF2\alpha$ and PGE2 by the embryo itself (Stout and Allen, 2001a). This unusual process of prostaglandin-driven conceptus mobility in the mare (Fig. 3b) persists until Day 17 when a sudden, spasm-like increase in myometrial tone "fixes" the conceptus at the site of eventual implantation at the base of one of the uterine horns (van Niekerk, 1965; Ginther, 1983a).

The nature of the signal by which the equine embryo biochemically "informs" the mare of its presence in her uterus and achieves the necessary luteostasis for pregnancy maintenance remains a mystery to the present day. Unlike ruminants (Lamming *et al.*, 1995), the equine conceptus does not produce any interferon-like protein molecules with luteostatic properties (Baker *et al.*, 1991). However, like the young pig embryo (Perry *et al.*, 1973), it does begin to secrete appreciable quantities of estrogens from as early as Day 10 (Zavy *et al.*, 1979; Flood *et al.*, 1979a; Heap *et al.*, 1982). It has frequently been speculated that, like the pregnant pig in which the embryonic estrogens achieve luteostasis by re-directing the flow of endometrial $PGF2\alpha$ away from the uterine venous drainage (Bazer and Thatcher, 1977), estrogens secreted by the equine embryo may likewise constitute the maternal recognition of pregnancy signal in the mare. However, the many experiments undertaken to date to prove or disprove this theory have all given equivocal results (Stout and Allen, 2001b), and a definitive answer is still awaited.
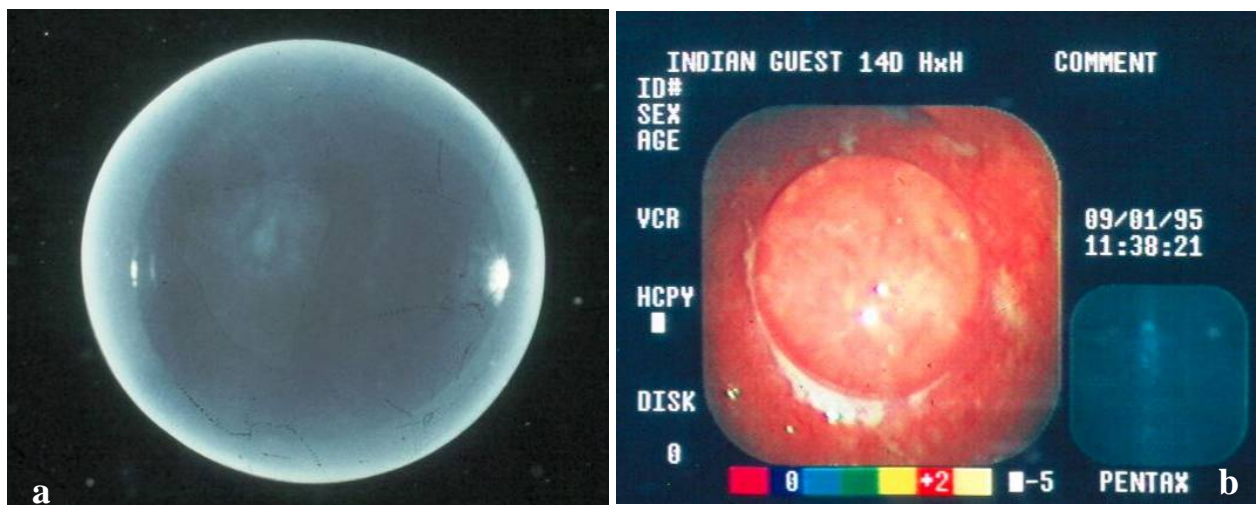

Figure 3. A free-living, Day 14 horse conceptus (a) with its pre-embryonic primitive streak visible near the center of the spherical structure and a Day 14 conceptus (b) *in utero* viewed with a video endoscope. Note the accumulated endometrial gland secretions ("uterine milk") around the unattached embryonic sac.

Embryogenesis and fetal membrane development

In addition to providing strength to the expanding blastocyst enabling it to withstand the endometrial contractions which propel it throughout the uterus between Days 6 and 17 (Betteridge, 1989), the blastocyst capsule also regulates the supply of nutrients to the embryo. Due to its negative electrostatic charge and unusual glycocalyx configuration (Oriol *et al.*, 1993b), the outer surface of the capsule is a site for adherence of other proteins. It therefore accumulates a range of proteins (Stewart *et al.*, 1995) and other components of endometrial gland secretions ("uterine milk", Fig. 4; Amoroso, 1952) on its outer surface as the conceptus moves through the uterus resulting in a doubling or more in weight of the capsule between hatching of the

blastocyst on Days 7 to 8 and immobilization of the conceptus around Day 17 (Oriol *et al.*, 1993a).

The capsule begins to disintegrate around Day 21, presumably as a consequence of proteolytic enzymes secreted by the trophoblast and/or luminal epithelium of the endometrium (Denker *et al.*, 1987). This allows areolae-like tufts of trophoblast cells to develop on the external surface of the non-vascularised choriovitelline membrane that protrude into the mouths of the endometrial glands for more efficient imbibition of the gland secretions and to provide physical adherence of the conceptus to the endometrium. The nutritional importance of these temporary areolae to the equine embryo (Amoroso, 1952) is shown by the high rate (~70%; Ginther, 1985; Morris and Allen, 2002) of

spontaneous death and resorption of one of unicornuate twin conceptuses between Days 20 and 28 when the absorptive bilaminar choriovitelline portion of one conceptus abuts against its co-twin conceptus rather than to the nutritionally provident endometrium.

By Day 21, the embryo, with its primitive beating heart, can be discerned at one pole of the still-spherical conceptus (Fig. 5a; van Niekerk and Allen, 1975), and the allantois is just appearing as an outpouching of its hind gut (Ewart, 1915). The latter grows rapidly to surround the embryo and fuse with the outer chorion so that by Day 28, the resulting allantochorion, with its well-vascularised allantoic mesoderm, occupies about one-third of the total volume of the conceptus (Fig. 5b; Van Niekerk and Allen, 1975).
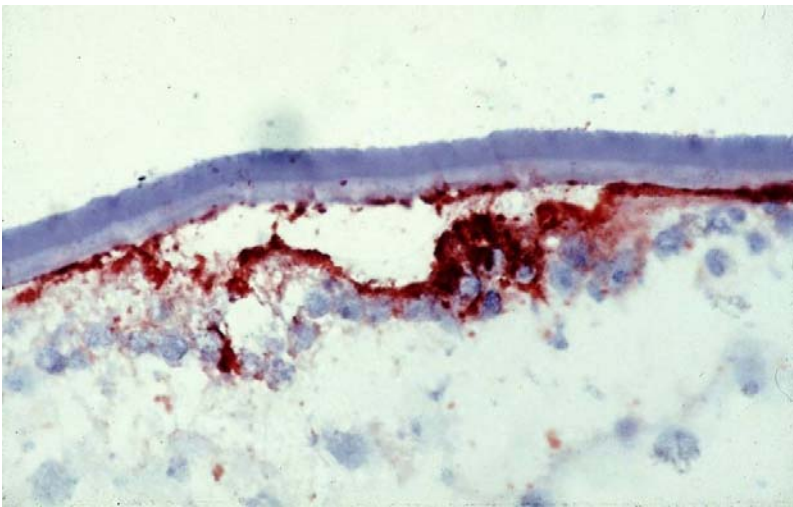

Figure 4. A histological section of part of a Day 16 horse embryo (456x). The trophectoderm is stained with lectin (red coloration). The investing blastocyst capsule is clearly bilaminar with the darker blue outer layer composed of attached endometrial gland secretions (photograph kindly supplied by Dr Julio Oriol).
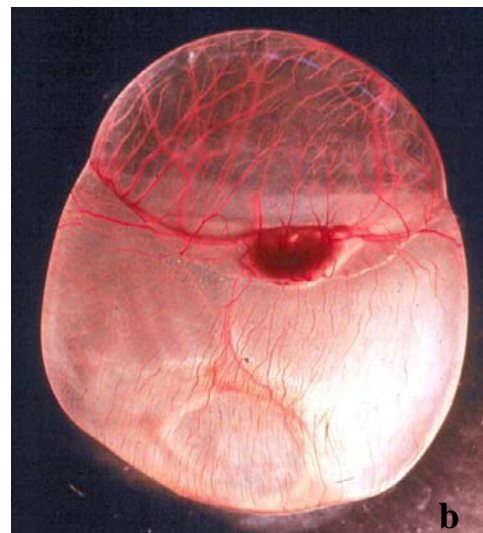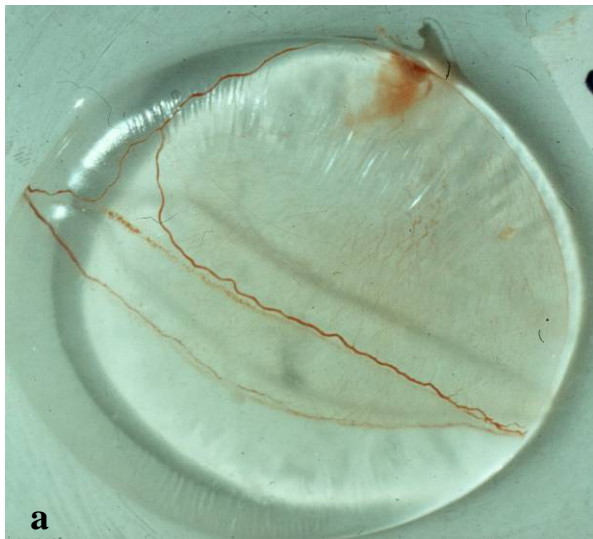

Figure 5. A 21-day horse conceptus (a) showing the primitive embryo situated at one pole of the embryonic vesicle. The vitelline artery terminates in the sinus terminalis in the equatorial region, and the allantois is just beginning to develop as an outpouching of the embryonic hind gut. The blastocyst capsule (not visible) is beginning to disintegrate at this stage. By Day 28, the embryo (b) is more prominent and the sinus terminalis and associated mesodermal vasculature has almost reached the abembryonic pole. The well-vascularized allantois is enlarging above the embryo. The invasive chorionic girdle is beginning to develop at the junction of the enlarging allantois and regressing yolk sac.

Endometrial cup formation

A unique feature of pregnancy in equids, which may be considered as a sort of developmental holding operation to stave off endocrinological and immunological disaster while the fetus attaches its diffuse, non-invasive, allantochorionic placenta to the endometrium, is the formation of endometrial cups. This first manifests itself between Days 38 and 40 as a series of small, pale, slightly raised plaques on the surface of the endometrium arranged in a circle around the conceptus at the base of the gravid uterine horn. The cups enlarge and become saucer-shaped over the next 20 to 30 days (Fig. 6a). Between Days 70 and 100, the cups begin to regress. They become increasingly pale and "cheesy" in appearance and release a yellow, sticky exocrine secretion that accumulates in the central crater-like depression on the luminal surface of the cup (Fig. 6b). Eventually, between Days 100 and 140, the necrotic cups and their adhered secretion (Fig. 6c) are sloughed off the surface of the endometrium where they indent into the surface of the allantochorion to form a series of pedunculated structures, known as allantochorionic pouches, which hang in the allantoic cavity during the remainder of gestation (Fig. 6d; Clegg *et al.*, 1954; Amoroso, 1955).
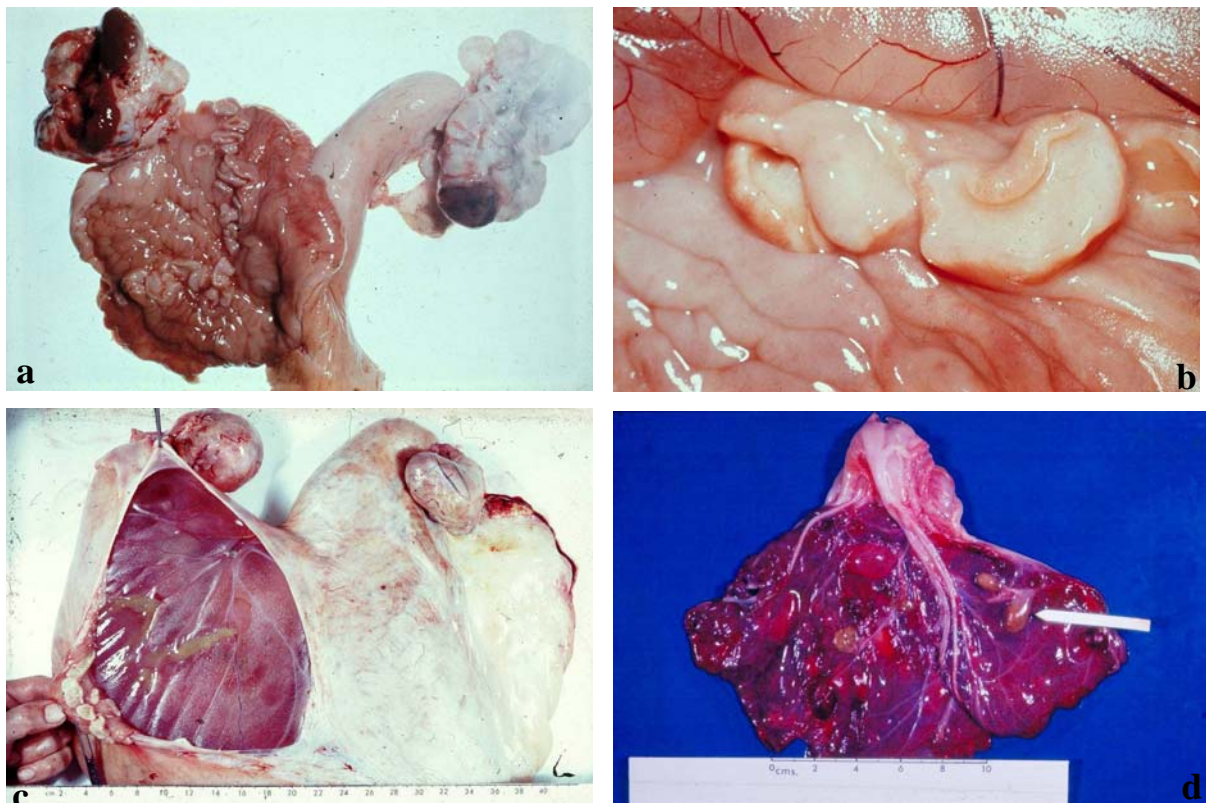
Figure 6. Photographs of endometrial cups in the gravid uterine horns of mares from which conceptuses have been removed at various times during gestation. At 65 days the cups are seen as circle of ulcer-like endometrial protuberances (a). By 85 days the central depression of each cup has become pale and cheesy in appearance due to degeneration and death of the fetal cup cells (b). Note the cup on the right of the photograph is filled with a gold-colored exocrine secretion that is rich in eCG activity. By Day 98, the endometrial cups (c) are pale and degenerating. The eCG-rich exocrine cup secretion is adhered to the surface of the underlying allantochorion. Pedunculated allantochorionic pouches (d; indicated by arrows) are formed on the allantoic surface of the term placenta by indentation of the dehisced endometrial cups into the maternal surface of the allantochorion much earlier in gestation.

Although the cups develop entirely within the maternal endometrium and show no physical attachment to the overlying allantochorion, they are, in fact, fetal in origin. Between Days 25 and 35, a discrete, annulate portion of the outermost chorion of the developing conceptus becomes pale and thickened in appearance due to rapid multiplication of the trophoblast cells in that region (Fig. 7a). Then, between Days 35 and 38, the chorionic girdle peels off the fetal membranes and adheres to the overlying endometrium (Fig. 7b) to enable the specialized, invasive girdle cells to penetrate the luminal epithelium and move deeper into the endometrial stroma (Fig. 7c; Allen and Moor, 1972; Allen *et al.*, 1973). Here, the invaded cells become sensile, enlarge greatly, acquire a second nucleus and bunch tightly together to form the endometrial cup (Fig. 7d).

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By Day 50 to 60, each cup consists of a densely packed mass of large, epithelial, binucleated trophoblast cells that are interspersed with occasional blood vessels and the dilated fundic portions of the endometrial glands, the apical regions of which were destroyed during the initial invasion by the chorionic girdle cells (Fig. 8a). Appreciable numbers of maternal leucocytes, including lymphocytes, plasma cells, and eosinophils, accumulate in the endometrial stroma at the periphery of each cup to form a distinct immunological barrier between maternal and fetal tissues. Beyond about Day 80, the large cells near the luminal surface of the cup begin to degenerate and slough off, thereby re-establishing patency of the blocked ducts of the distended endometrial glands. Coincidentally, the accumulated maternal leucocytes begin to invade the peripheral regions of the cup and destroy the fetal cup cells (Fig. 8b). Eventually, the lump of necrotic tissue and attached coagulum is sloughed off the surface of the endometrium in a manner akin to rejection and sloughing of a foreign skin graft (Allen, 1975; Antczak and Allen, 1984).

The large fetal endometrial cup cells are the source of the high concentrations of equine chorionic gonadotropin (eCG) that circulate in the blood of pregnant equids between Days 40 and 120 of gestation (Cole and Hart, 1930; Cole and Goss, 1943; Allen, 1969). This high molecular weight (72 KDa) glycoprotein (Gospodorowicz, 1972) expresses both FSH- and LH-like biological activities in a ratio of approximately 1.4: 1 (Stewart *et al.*, 1976), and it ovulates or luteinizes (without ovulation) dominant ovarian follicles that develop during the first half of pregnancy by continuation of surges in pituitary FSH concentrations in maternal blood (Evans and Irvine, 1975; Urwin and Allen, 1982). Thus, secondary corpora lutea begin to form from the time of first appearance of eCG and this, in turn, results in rises in maternal serum progesterone concentrations (Fig. 9). The accessory luteal structures increase in number and persist until mid-gestation by which time the placenta is mature enough to take over the production of progesterone and other progestagens entirely and maintain the pregnancy without any further help from the maternal ovaries (Holtan *et al.*, 1979).
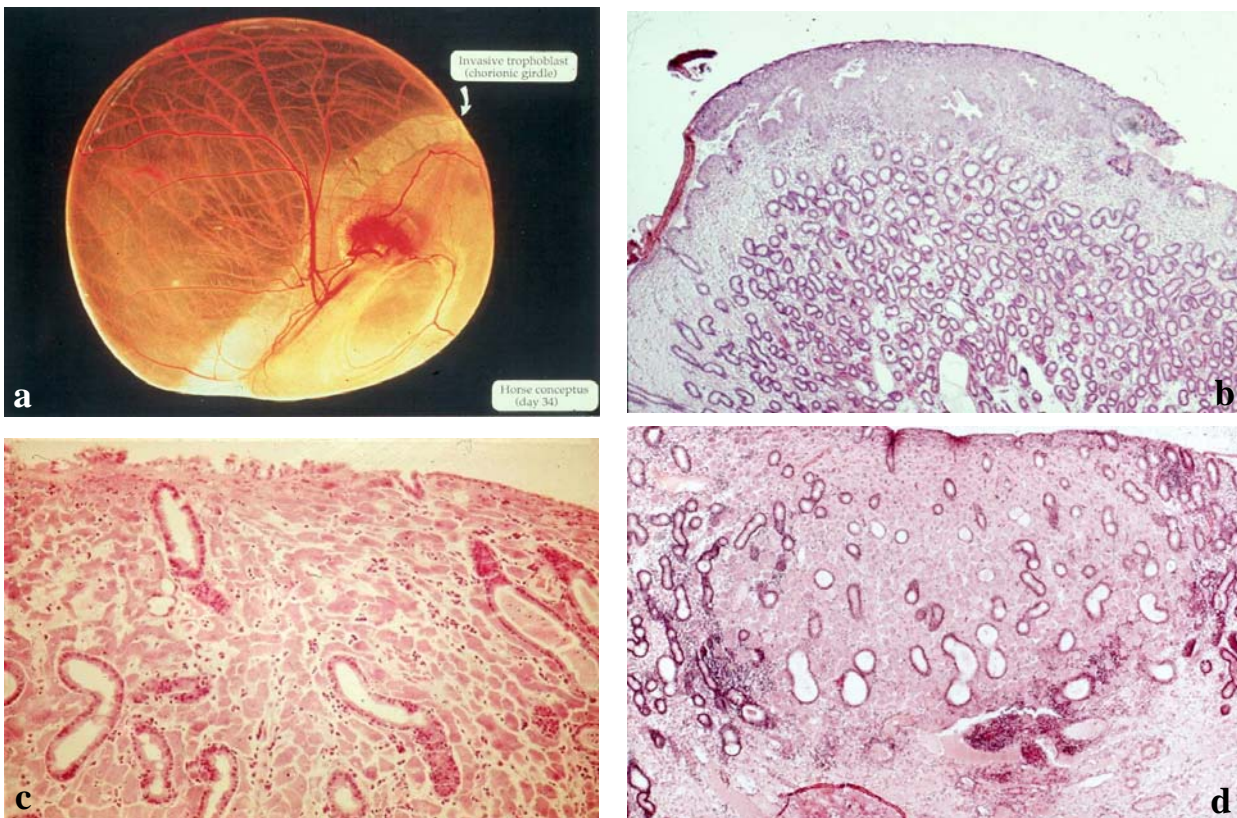

Figure 7. Photographs of early endometrial cups and their formation: a) 34-day horse conceptus showing the pale, thickened chorionic girdle that has formed at the junction of the enlarging, well-vascularized allantois and the yellow-colored regressing yolk sac; b) histological section of the mature fetal chorionic girdle attached to the surface of the endometrium allowing the girdle cells to begin invading the maternal tissue (40x); c) section of a young endometrial cup at Day 40 of gestation showing the newly invaded and rapidly transforming chorionic girdle cells streaming out into the endometrial stroma (100x); d) section of an endometrial cup at Day 50 (x 40). The cup (d) consists of a densely packed mass of large eCG-secreting fetal cup cells interspersed with the dilated fundic portions of endometrial glands. Maternal leucocytes are beginning to accumulate at the periphery of the cup tissue.
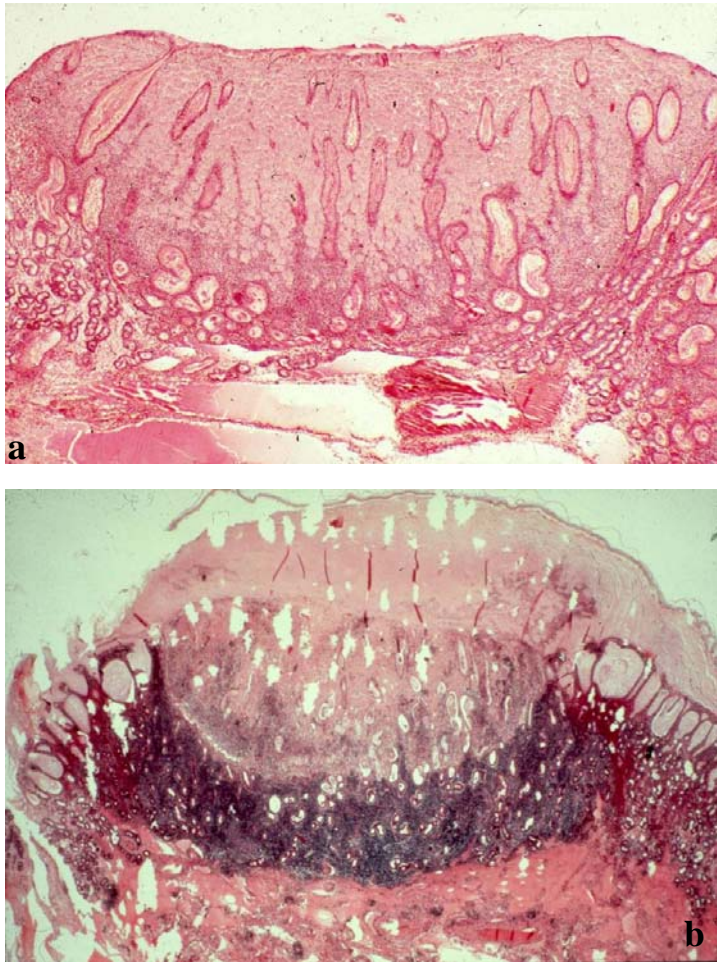
Figure 8. Histological sections of endometrial cups at 65 (a) and 110 days (b) of gestation (40x). In section A, note the increasing distension of the fundic endometrial glands within the cup and the growing accumulation of maternal leucocytes around the fetal cup tissue tending to wall it off from the adjacent normal endometrium. In section B, the whole of the cup tissue is necrotic and is covered by a coagulation of admixed exocrine gland secretion and necrotic cup cells. An extremely dense wall of maternal lymphocytes and other leucocytes surrounds the dead cup.
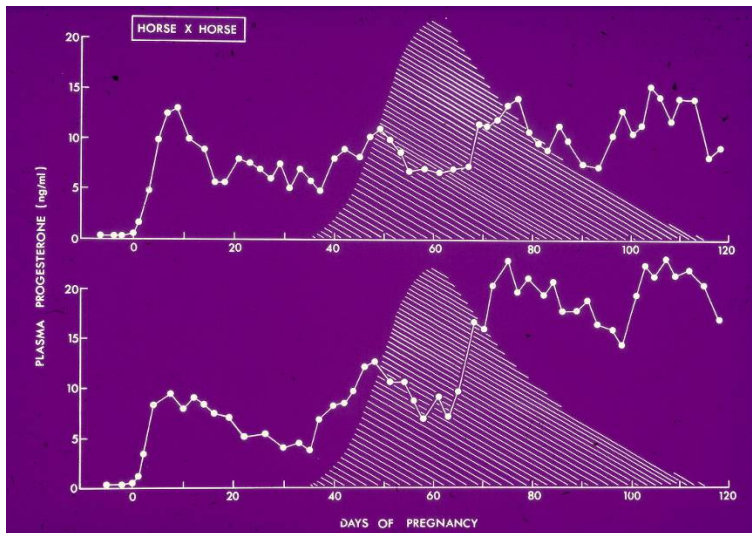

Figure 9. Peripheral plasma progesterone profiles of two mares during the first 120 days of gestation. The period of eCG-secretion (hatched) is represented diagrammatically. Note the sequential rises in progestagen concentrations in both mares after Day 38 with the development of each secondary corpus luteum.

Placentation

Commencing around Day 40 to 42 and within only a day or two after invasion of the maternal endometrium by the chorionic girdle cells that will initiate endometrial cup formation, the non-invasive trophoblast cells of the allantochorion at last begin to establish a microvillous attachment to the luminal epithelial cells of the endometrium (Fig. 10a; Samuel *et al.*, 1975). The allantochorion starts to elongate steadily, and at the feto-maternal interface, blunt finger-like villi of allantochorion interdigitate with thin, more elongated and branching outgrowths from the surface of the endometrium. (Fig. 10b). Beyond Day 60, both the maternal and fetal components of this steadily deepening interdigitation begin to become extensively branched (Fig. 10c) so that, by around Day 120, the fundamental haemotrophic exchange unit of the allantochorionic placenta, the microplacentome, is well formed, and these maternofetal interdigitatory structures cover the entire maternal surface of the diffuse placenta (Samuel *et al.,* 1975; Bracher *et al.*, 1996). Branching and sub-branching of both the fetal and maternal villi of the microplacentome and of the fetal and maternal capillaries within them (Fig. 10d) continues throughout gestation to maximize the total area of contact between the fetal and maternal epithelial layers for haemotrophic exchange of nutrients, gasses, and waste products. Furthermore, the process is aided by the marked indentation of the capillaries into the base of these epithelial layers on both the fetal and maternal sides of the interface (Samuel *et al.*, 1976)

In addition to the extensive development of the microplacentomes and their haemotrophic exchange mechanisms, the long, multibranched endometrial glands remain functional throughout gestation and release their protein-rich exocrine secretions into well defined spaces, termed areolae, between adjoining microplacentomes (Amoroso, 1952; Steven and Samuel, 1975, Steven, 1982). The trophoblast overlying the gland openings becomes pseudostratified (Fig. 10d) as an adaptation for absorbing uterine gland secretions. This second, histotrophic form of nutrition in the pregnant mare no doubt plays an important role in helping meet the demands of the rapidly growing fetus, especially during the last 1 to 2 months of gestation when the glands become more densely arranged in the endometrium (Fig. 10d; Samuel *et al.*, 1977).
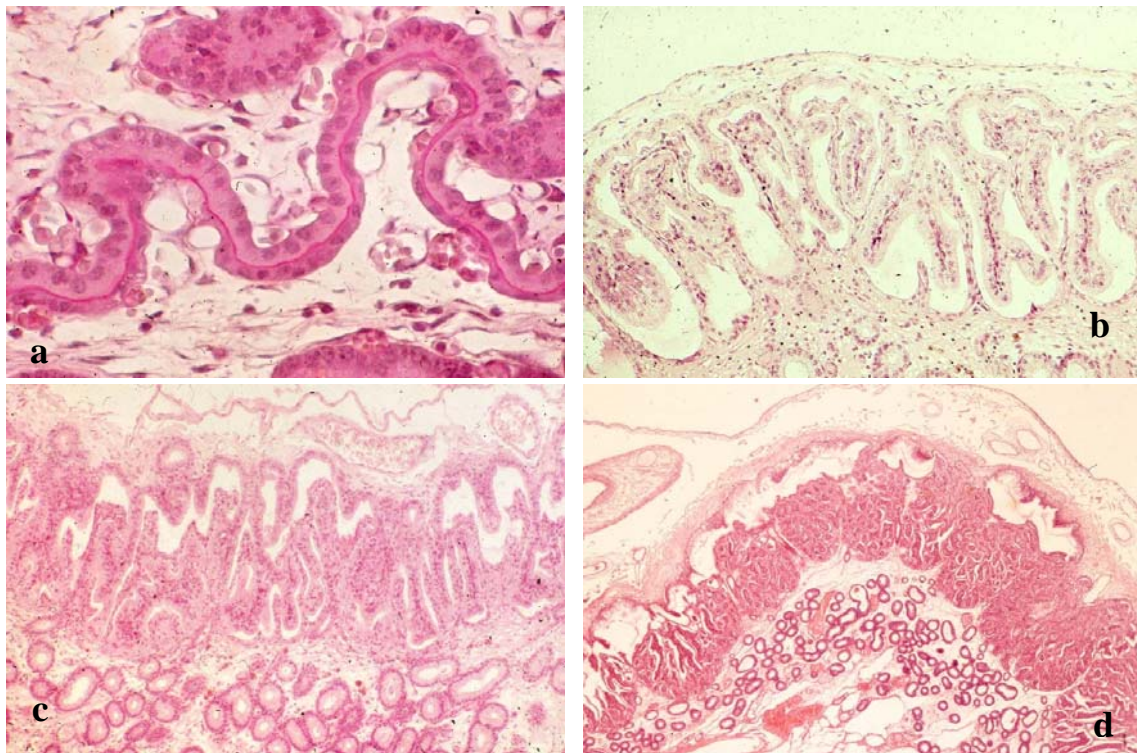

Figure 10. Histological sections of the placental interface at the following stages of gestation in the mare: a) Day 43 - the trophoblast and luminal epithelium of the endometrium are attached by a stable microvillous border (dark pink stain), and blunt, finger-like villi of allantochorion are protruding into the surface of the endometrium (456x); b) Day 60 - fixation shrinkage shows the interdigitation of elongating allantochorionic villi with corresponding upgrowths of endometrium (320x); c) Day 85 - interdigitating allantochorionic villi and accommodating endometrial up growths are becoming branched and sub-branched to form microplacentomes that increase markedly the surface area of contact between maternal and fetal endometrial layers (320x); d) Day 309 - individual microplacentomes are now well developed, and darkly stained absorptive areolae of pseudostratified trophoblast cells are seen at irregular intervals on the basal trophoblast layer between adjacent microplacentomes (40x).

Placental compromise

A healthy and extensive microcotyledonary placenta attached to a healthy and fully functional endometrium is a prerequisite for a successful pregnancy in the mare (Fig. 11a; Steven and Samuel, 1975). Any diminution in this vast area of intimate feto-maternal contact will lead to a certain degree of runting and weakness in the newborn foal (Cotterill *et al*., 1991; Wilsher and Allen, 2003) or may cause embryonic death early in gestation or abortion later in pregnancy (Bracher *et al*., 1996). Twinning is a classical example of this phenomenon since the allantochorions of the two conceptuses compete for the limited surface area of endometrium to which they may attach. Occasionally, in the case of bicornuate twins, each allantochorion attaches to roughly one-half of the uterus and pregnancy can proceed to term with the birth of live, but smaller than normal, foals (Jeffcott and Whitwell, 1973). More commonly, twins are unicornuate (Ginther, 1985; Morris and Allen, 2002) and one conceptus is pushed forward to occupy just the anterior portion of the gravid uterine horn (Fig. 11b). The disadvantaged fetus can survive this unequal situation for the first half of gestation, but beyond about 7 months, its nutritional demands begin to exceed the total nutrient and gas exchange capabilities of its much smaller placenta resulting in death and abortion of both fetuses (Fig. 11c; Jeffcott and Whitwell, 1973).

A second defect, endometrosis, likewise reduces the total area of placental exchange available to the growing equine fetus and originates on the maternal side of the feto-maternal interface. The age-related, degenerative changes in the mare's endometrium that are collectively termed endometrosis (Kenney, 1993; personal communication) include: i) untoward accumulations of mononuclear cells in the endometrial stroma, ii) degeneration and dysfunction of the endometrial glands by the deposition of fibrous tissue around them to form "gland nests", iii) degeneration and occlusion of endometrial blood vessels, and iv) generalized stromal fibrosis combined with myometrial atony that causes stasis of lymph flow and development of distended lymphatic lacunae within the stromal tissue and lymph-filled endometrial cysts which protrude into the uterine lumen (Fig.12a and b; Kenney, 1975; 1978; Ricketts, 1975; Bracher *et al*., 1992; Schoon *et al*., 1997). These adverse changes in endometrial structure and function limit, to varying extents, the size, complexity and function of the individual microplacentomes that develop on the maternal surface of the placenta, together with the density of these structures per unit area of tissue (Fig. 12c and d). In turn, the efficiency of total placental exchange is reduced thereby leading to intrauterine growth retardation that results in either abortion of a cachectic fetus or stunted growth of the newborn foal (Bracher *et al*., 1996; Wilsher and Allen, 2003).

A third and particularly interesting illustration of the effects of reduced placental exchange in the mare was first highlighted by Walton and Hammond (1938) when they used artificial insemination to reciprocally cross Shire horses with Shetland ponies. Of the two foals born, the one gestated by the Shetland mother was only half the weight of its half sibling born from the Shire mare as a result of the much smaller maternal uterus of the Shetland mare. Furthermore, the great difference in size between the two reciprocal, between-breed crosses persisted into adult life. In a follow-up experiment, Tischner and Klimczak (1989) used embryo transfer to create 3 pairs of sex-matched, full-sibling Konik pony foals whereby one of each pair was transferred, as a blastocyst, to the uterus of a larger, draft-type recipient mare while the sibling was gestated in the uterus of its genetic pony mother. All 3 transferred foals were born larger and grew faster while nursing on their larger surrogate mothers. At 4 years of age, two of the 3 foals born to the draft-type mares were still taller and heavier than their sibling counterparts (Tischner and Klimczak, 1989).

An extension of the Tischner and Klimczak (1989) experiment was undertaken by Allen *et al*. (2002) when between-breed embryo transfer was carried out using large Thoroughbred (TB) mares with a mean (± S.D.) body weight of 589 ± 49 kg and smaller pony (P) mares (325 ± 52 kg). Four types of pregnancies were established: i) TB foals in P mares in which the fetus could be expected to suffer intrauterine cramping and nutritional restriction (n=8), ii) P-in-TB mares in which the fetus would encounter a relatively "luxurious" *in utero* existence (n=7), and both iii) TB-in-TB (n=7) and iv) P-in-P (n=7) controls. As expected, the TB-in-TB foals were heaviest at birth, and the P-in-P foals were lightest while the P-in-Tb ("luxurious") foals were born heavier than their TB-in-P ("deprived") counterparts. These differences in mean birthweight between the 4 types of pregnancy were paralleled closely by equivalent differences in the mean weight, volume, and total surface area of the placentae (Allen *et al*., 2002). The size differences between the foals persisted into adulthood (Allen *et al*., 2004). Thus, as illustrated convincingly by all three scenarios described in this section (twins, endometrosis, and between-breed embryo transfer), the intrauterine development of the fetus and the resulting size of the foal at birth are closely dependent upon the microscopic structure and total area of utero-placental exchange that is, in turn, dependent upon the surface area and health of the endometrium that, in turn, is dependent upon the size and age of the mare (Wilsher and Allen, 2003).

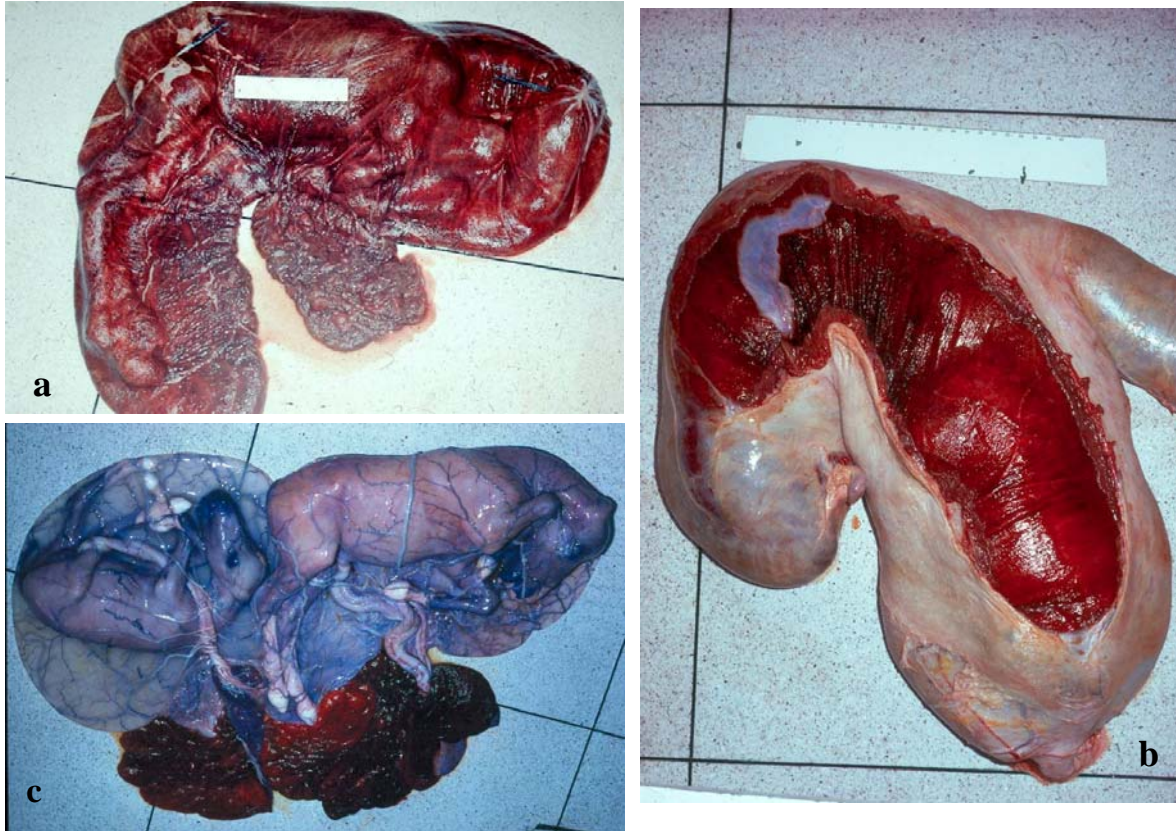Anim. Reprod., v.2, n.4, p.209-223, Oct./Dec. 2005

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Figure 11. Photographs of singleton and twin pregnancies in the mare: a) intact singleton pregnancy at Day 260 of gestation showing the considerable area of normal allantochorion covered with a dense array of microcotyledons; b) twin conceptuses at approximately Day 250 - the placenta of the larger twin occupies the base of the gravid horn, the body, and the whole of the non-gravid horn of the uterus whereas that of the smaller, disadvantaged twin occupies only the anterior half of the gravid horn - microcotyledons do not develop where the two placentae abut against each other; c) Day 250, twin fetuses after removal from the uterus - the advantaged twin with the much larger area of attached placenta weighs over twice as much as its disadvantaged co-twin that had been pushed up to the tip of the gravid uterine horn.

Fetoplacental steroidogenesis

Another unusual phenomenon of equine pregnancy is the enormous growth and subsequent regression of the fetal gonads during the second half of gestation (Cole *et al.*, 1933). Commencing around Day 80 in both male and female fetuses, the gonads begin to enlarge steadily until they reach a maximum size and combined weight of approximately 350 g between Days 230 and 260. At this time, they occupy as much as one-third of the total volume of the abdominal cavity of the fetus and have the appearance of a kidney much more than a future differentiated gonad. To the naked eye, the ovaries are distinguishable from the testes by a pale layer of primordial follicles in the cortical region of the organ. Histologically, the gonads of both sexes consist of densely packed masses of large, epithelioid interstitial cells supported within a fibrous tissue framework through which numerous blood and lymph vessels run. Germinal regions are limited to a narrow cortical zone in the fetal ovaries and scattered cords throughout the interstitial tissue in the fetal testes (Hay and Allen, 1975; Merchant-Larios, 1979). The gonads begin to shrink after Day 270 and have reduced in size and appearance by term (Day 336) to the small, relatively insignificant structures one anticipates of neonatal gonads in other large domestic animals.

Estrogens

In close parallel with the enlargement and regression of the fetal gonads, maternal serum estrogen concentrations rise steadily to reach peak values as high as 400 ng/ml between Days 190 and 250 and decline thereafter to baseline values at term. They include both the common phenolic estrogens, estrone and estradiol, and the equine-unique ring B-unsaturated estrogens,

equilin and equilenin (Gaudry and Glen, 1959; Cox, 1975; Raeside and Liptrap, 1975).

A series of elegant fetal infusion experiments in the late 1960's established that estrogens present in the pregnant mare are produced by placental aromatization of large quantities of dihydroandrosterone (DHA), dihydroepiandrosterone (DHEA), and unusual 3ß-hydroxylated C-19, DHEA-like precursors, all of which are secreted by the interstitial cells of the enlarged fetal gonads (Bhavnani et al., 1969; 1971; Bhavnani and Short, 1973; Tait et al., 1983). Production of these equine placental estrogens is similar to the production of large quantities of estrogens, including the pregnancy-specific estriol, found in pregnant women resulting from placental aromatization of DHA and DHEA secreted by the enlarged fetal adrenal glands (Diczfalusy, 1969).

The biological significance of the estrogens secreted by the equine feto-placental unit are not well understood although they may play a role in regulating both uterine and placental blood flow, as has been shown to be the case in the pregnant ewe (Resnik et al., 1974; Pupkin et al., 1975), and in the synthesis and storage of prostaglandin precursors in the endometrium and myometrium. Pashen and Allen (1979) demonstrated that mares carrying foals that underwent bilateral fetal gonadectomy between Days 197 and 253 showed an immediate fall in plasma estrogen concentrations which did not occur in 3 sham gonadectomized controls. The gonadectomized animals were born lighter and had less muscle development than the sham-gonadectomized controls. Although parturition commenced spontaneously 70 to 97 days after removal of the fetal gonads, all the births were characterized by weak myometrial contractions, lower than normal releases of PGF2α into maternal blood, and retained placentae (Pashen and Allen, 1979).

Progestagens

Feto-placental production of progesterone and other C-21 progestagens in the pregnant mare differs significantly from estrogens. As demonstrated by the original maternal ovariectomy studies of Holtan et al. (1979), the placenta begins to produce appreciable quantities of progestagens from around Day 70, and it utilizes primarily maternal rather than fetal sources of cholesterol for this purpose, as illustrated by the elegant umbilical cord infusion studies of Bhavnani et al. (1969, 1971) and later confirmed by the fetal gonadectomy experiment of Pashen and Allen (1979). Moreover,

throughout the second half of gestation, a range of 5α-reduced metabolites of progesterone, most notably dihydroprogesterone (5α-DHP) and 20α-dihydroxy-5-pregnan-3-one (20α-5P), predominate in the maternal circulation, and progesterone itself is barely detectable (Short, 1959; Holtan et al., 1991). Furthermore, a definite rise in maternal plasma progestagen concentrations, which is more pronounced in Thoroughbred than Pony mares (Hamon et al., 1991), occurs during the last 4 to 6 weeks of gestation (Holtan et al., 1979; 1991; Pashen and Allen, 1979) and is followed by a sharp decline in values only during the immediate preparturient period (Haluska and Currie, 1988).

The source of this unusual, late-gestation rise in plasma progestagen concentrations in pregnant mares and its biological significance in fetal maturation and parturition has aroused much speculation and investigation over the past two decades (see Rossdale and Silver, 1982; Rossdale et al., 1992). This subject was brilliantly reviewed by Thorburn (1991; 1993) who speculated, as was subsequently shown by Chavatte et al. (1997), that the pregnenelone required for this additional progestagen synthesis by the placenta is secreted by the steadily enlarging fetal adrenal gland. A lack of 17α-hydroxylase activity in the adrenal cortex at this stage prevents pregnenelone from being metabolized to cortisol, and it instead passes to the placenta where it is converted into 5α-reduced pregnanes due to the persisting high levels of 5α-reductase enzyme in placental tissue. These products then enter the fetal and maternal circulations (Chavatte et al., 1997)

Thus, the feto-placental production of steroid hormones in the pregnant mare is riddled with unusual and apparently equine-specific oddities compared to other large domestic species. For estrogen synthesis, the fetal gonads and placenta together form a highly productive unit. The necessity of this unit is clearly greatest during mid-gestation and the products of which include the unusual, although still biologically active, Ring ß-unsaturated equilin and equilenin. For progesterone synthesis on the other hand, the placenta utilizes initially maternal and later fetal sources of pregnenelone for its high rate of hormone production. However, for some biologically inexplicable reason, it rapidly converts almost all the progesterone it produces into a range of 5α-reduced metabolites, only one of which, 5α DHP, is likely to be biologically active.
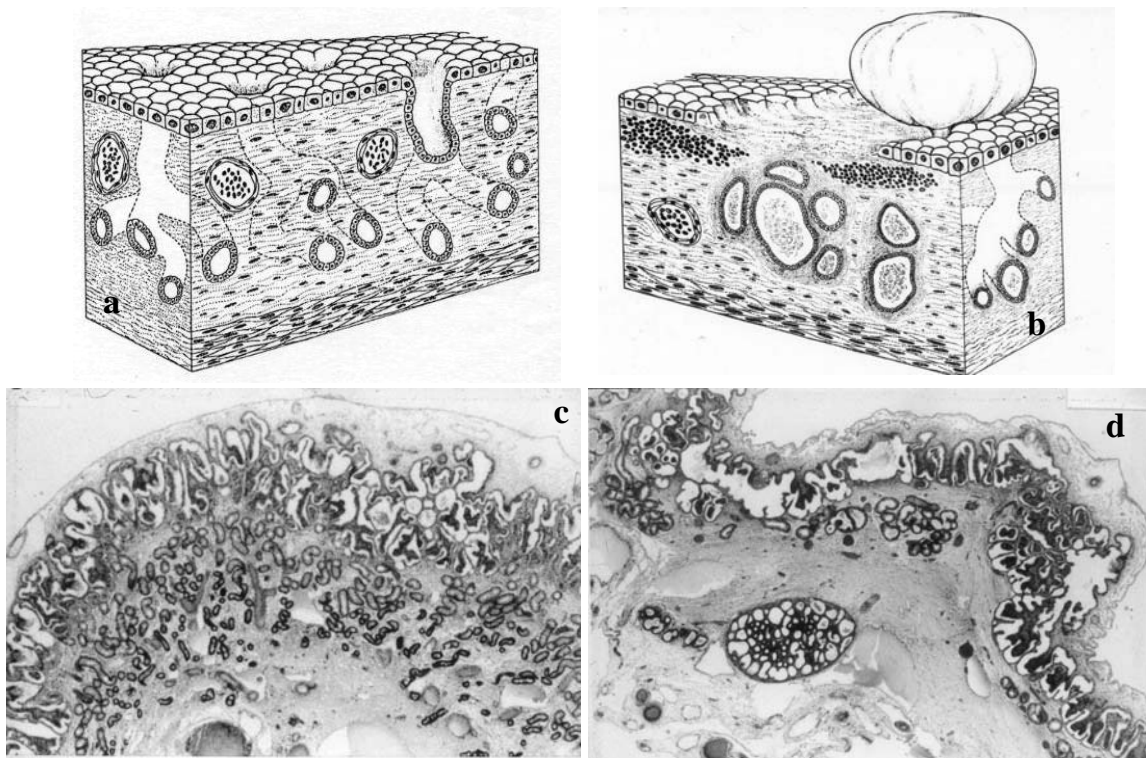
Figure 12. a) Diagrammatic representation of normal, healthy horse endometrium showing an intact lumenal epithelium invaginating frequently to form a regular and dense distribution of secretory glands in the stroma; b) Representation of the endometrium of an older mare showing age-related degenerative changes (endometrosis). Portions of the lumenal epithelium are eroded, accumulations of mononuclear cells persist in the endometrial stroma and layers of fibrous tissue are laid down around the basal portions of endometrial glands to form dilated non-functional "gland nests". Fibrous deposition also blocks lymph drainage channels leading to the formation of endometrial cysts which protrude into the uterine lumen; c) Low power histological section of the placental interface in a young healthy mare at day 120 of gestation. Note the regular and dense arrangement of microplacentomes on the endometrial surface and the dense arrangement of normal endometrial glands in the stroma (x 40); d) Section of the placental interface in an aged endometrotic mare, also at 120 days of gestation. Note the thickened allantochorionic villi and minimal upgrowth response from the endometrium to form microplacentomes and the sizeable areas that are completely devoid of microplacentomes. Notice also the paucity of endometrial glands and the "nesting" of the few glands that are present (x 40). The fetus in this mare weighed half as much as that in the healthy young mare shown in (c). From Bracher *et al*. (1996).

Conclusions

As described in the previous pages, a number of events in equine pregnancy appear to be unique to the genus. Perhaps the most striking of these is the strange, growth factor-driven differentiation of the annulate, invasive chorionic girdle portion of the placenta at the end of the first month of gestation and its vigorous injection of specialized, gonadotropin-secreting trophoblast cells deep into the maternal endometrium. The antigenic challenge of this uterine penetration to the maternal immune system is considerable as attested by the rapid development of high titers of paternal-specific lymphocytotoxic antibody in almost all maiden horse pregnancies that arise within 10 to 20 days after endometrial cup development and persist throughout gestation (Antczak *et al.*, 1984) and the vigorous and ultimately successful, cell-mediated reaction mounted against the invading endometrial cup cells (Allen, 1975). It seems such a foolhardy thing for the fetus to do when the endocrinological benefits of the move are questionable but the immunological risk is so great. Perhaps it is necessary to initiate attachment and interdigitation of the allantochorion and endometrium to form the definitive placenta to support fetal growth throughout gestation. But whatever the underlying *raison d'être*, the strategy is clearly successful since the great majority of equine pregnancies proceed safely to term.

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