Equine Pregnancy: Physical Interactions Between the Uterus and Conceptus

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The equine species have evolved a series of effective, often unique, interrelated, dynamic, physical interactions between the uterus and conceptus. Included are embryo mobility, fixation, and orientation, embryo reduction, formation of endometrial cups, fetal mobility, fetal presentation, uterine horn closures, encasement of the fetal hind limbs by a uterine horn, and special mechanisms of uterine-fetal rotations during parturition. The morphologic and physiologic aspects of these events are described. Author's address: University of Wisconsin, Animal Health and Biomedical Sciences, 1655 Linden Drive, Madison, WI 53706. E-mail: ojg@ahabs.wisc.edu

1. Introduction

This report considers the equine intrauterine conceptus from the time of entry from the oviduct as a blastocyst to expulsion as a foal. A series of events involving physical interactions between the uterus and conceptus will be highlighted, and the associated morphologic and physiologic aspects will be considered. Most of the events were discovered and characterized by transrectal ultrasonic imaging and transcervical endoscopic viewing. Certain aspects of the phenomena have been reviewed for embryos,[1–3] fetuses,[4] and both stages.[5,6]

The term embryo is well engrained and will be used in reference to the entire early conceptus.[5] The terms embryonic vesicle and embryo proper will also be used when needed to emphasize or distinguish between the entire conceptus and the forerunner of the fetus, respectively. The embryo terms will be used only to Day 39 (Day 0=ovulation). From Day 40 to parturition, the terms fetus or fetal stage will be used. The choice of Day 40 as the transition day facilitates categorizing and discussing the phenomena highlighted in this paper. The beginning of umbilical cord formation, completion of replacement of the yolk sac with the allantoic sac, and the beginning of fetal activity (head nods) are on approximately Day 40. Equine theriogenologists and biologists should be deliberate in using the terms embryo and fetus. For example, the terms embryo mobility versus fetal mobility and embryo reduction versus fetal reduction involve distinctly different mechanisms for embryos versus fetuses.

2. The Intrauterine Embryo

A. Origin of Embryonic Placental Layers

Equine theriogenologists should not consider the three germ layers of the embryonic placenta—ectoderm, mesoderm, and endoderm—as esoteric terms used only by embryologists. Transrectal ultrasonography has exposed veterinarians to embryonic placental membranes that formerly received little practical consideration. Knowledge of the layers of the yolk-sac wall and the membranes associated with transition from yolk sac to allantoic sac is required for full interpretation of the ultrasonic

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images of singletons, differentiating singletons from twins, distinguishing between the membranes of impinging uterine cysts and embryonic vesicles, and comprehending today's postulates on the mechanisms of embryo mobility, fixation, orientation, and twin reduction. An embryonic layer that generally is not taught in embryology courses is the capsule, which among farm animals develops only in the equine species. Research on the anatomy of the embryonic placenta is needed because of importance to the equine clinician and scientist. The following discussion of the temporal and spatial interrelationships of the three embryonic layers is based on a small number of embryonic vesicles[5] and should be considered provisional.

The origin of the three germ layers and their incorporation into the yolk-sac wall is diagrammed for Days 9–15 (**Fig. 1**). Diagrams for subsequent embryonic placental development are incorporated into the figures for the various physiologic phenomena.

The embryo becomes a blastocyst by the time or soon after it enters a uterine horn on Day 6.[7] The term blastocyst is used when a central cavity forms, and the inner cell mass is established at one pole, as shown for Day 9.[5] The inner cell mass will form the embryonic disc and eventually will develop into the embryo proper, fetus, and foal. The membrane surrounding the blastocyst cavity is a single layer of

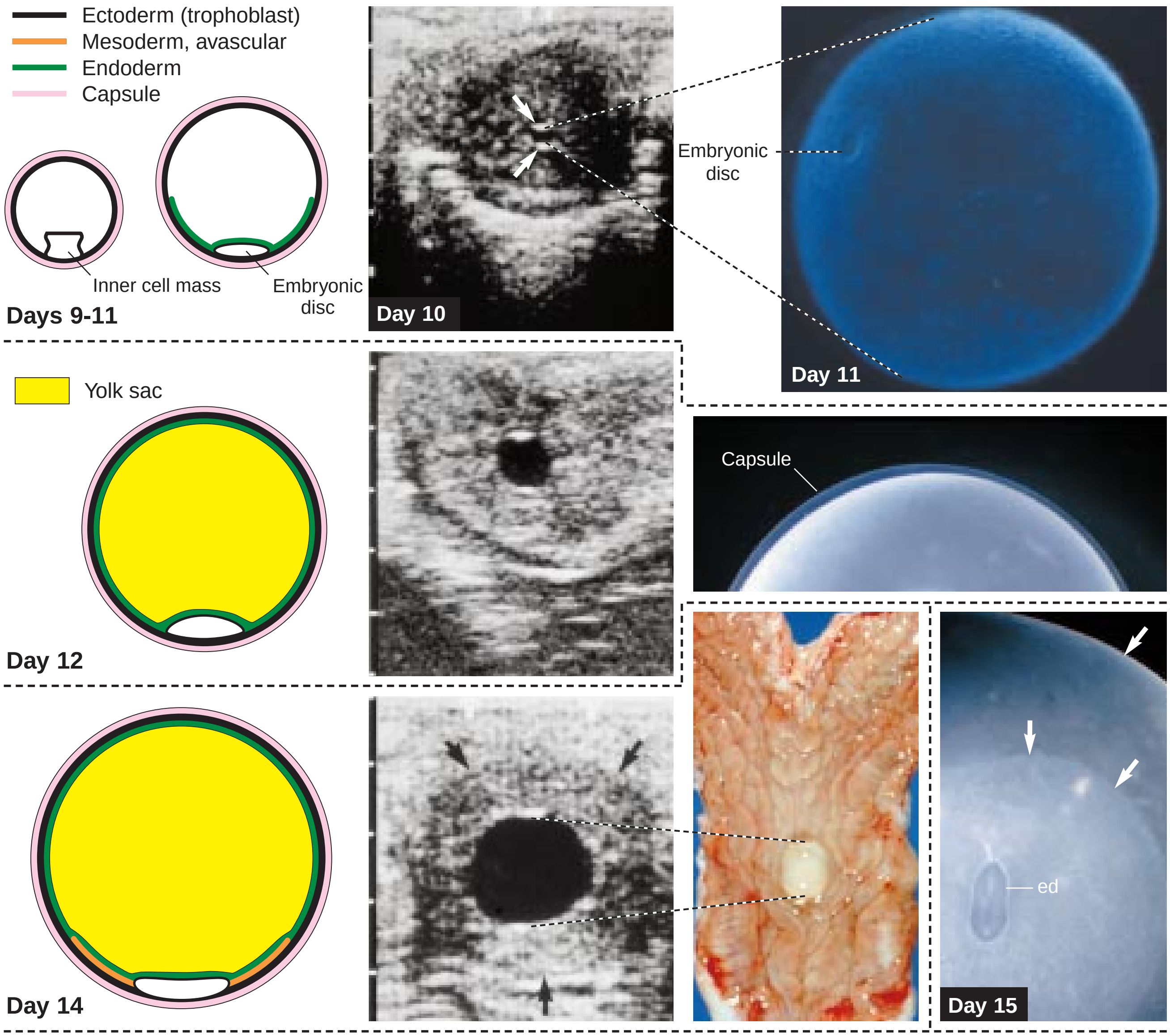


Fig. 1. Diagrams, sonograms, and photographs of the embryo for Days 9–15. The arrows indicate the following: Day-10 sonogram—specular reflections; Day-14 sonogram—periphery of cross-section of the uterine horn; Day-15 specimen—edge of the invading mesoderm (two arrows) and yolk-sac wall (one arrow). The Day-12 specimen shows the capsule after contraction of the blastocyst wall. Note the circular embryonic disc at Day 11 and the elongated disc at Day 15. The head is at the lower end of the Day-15 disc. In these and other sonograms, the graduated scale to the left is in centimeters. ed, embryonic disc.

ectodermal cells called the trophoblast, which continues as the absorptive placental contact with the endometrium throughout pregnancy.

The capsule of an equine blastocyst develops between the trophoblast and zona pellucida.[8] Within a day or so after the blastocyst enters the uterus, the zona pellucida is shed and the capsule becomes the outermost layer. The capsule is a thin (e.g., 3 μm) but tough, mucinous (anti-adhesive[9]) layer of glycoproteins. As the blastocyst expands, the capsule thickens at least until Day 11.[8] The capsule assumes considerable elasticity and resiliency and is a supportive wrapping around a delicate package during embryo mobility (Section 2B), fixation (Section 2C), and orientation (Section 2D). It seems unlikely that these phenomena would have evolved in the absence of the capsule. The capsule disappears by approximately Day 21,[10] suggesting that its role is complete soon after orientation. The popping sensation sometimes experienced by practitioners during elimination of a twin embryo by digital compression[11] is attributable to rupture of the capsule.

Conversion of the single-layered wall of the blastocyst to a two-layered structure occurs upon encirclement of the blastocyst cavity by a single layer of endodermal cells. Although not studied critically, encirclement is completed by approximately Day 12.[5] The resulting primitive placental vesicle can be called a yolk sac, although some authors prefer to continue to call the conceptus a blastocyst until it fixes to the uterus.[10] The endoderm of the yolk sac is continuous with the endoderm of the primitive gut (forerunner of digestive system) of the embryo proper. Therefore, whatever the yolk sac absorbs from the intrauterine environment becomes available to the embryo proper. Beginning growth of the mesoderm from the embryonic disc into the area between the trophoblast and the yolk-sac endoderm is shown in the diagram for Day 14 and by a specimen for Day 15. The invasion of the mesoderm results in a three-layered wall for increasingly greater proportions of the yolk sac, as shown on the diagrams of subsequent figures. The mesoderm differentiates into supportive connective tissue and blood vessels.

Practitioners using an intrarectal 5-MHz ultrasound transducer can view embryos by Days 9–11 when they are 3–5 mm.[6] Only about 5–10% are detectable on Day 9, and then only those that are on the upper end of the Day-9 range; 98% are detectable by Day 11. The vesicles are spherical and produce a black (anechoic) circumscribed image. The spherical shape is maintained with a progressive increase in diameter over Days 9–16. The anechoic area seen by ultrasonography represents the fluid of a blastocyst (Days 9–11) or a yolk sac (Days 12–16). Although invasion by the mesoderm begins by Day 14, the germ layers and capsule are too thin to image individually. Bright white (echoic) spots often are present on the images of the upper and lower surfaces of the blastocyst or yolk-sac parallel to the transducer. These ultrasonically generated specular reflections[12] are an aid in locating the early vesicle. For example, the Day-10 blastocyst in the sonogram may have been missed without the specular reflections. A photograph of a Day-14 conceptus is shown in the uterine body after exposure by an incision along the dorsal uterine midline. The Day-14 vesicle is spherical even though it is freestanding (i.e., not submerged in fluid), indicating that it is a turgid structure. The uterine folds are arranged longitudinally, which may favor embryo mobility.

B. Embryo Mobility and Luteal Maintenance

Uterine ligation studies suggest that the embryo first reaches the uterine body on Day 8[13] and then begins an intrauterine mobility phase that continues until Days 15–17.[14,15] During the embryo-mobility phase, regardless of the side of ovulation, the embryo can be anywhere in the uterine lumen from the tips of either horn to the cervix (**Fig. 2**). When first detected (Days 9-11) the embryo is frequently (60%) found in the uterine body.[14] Thereafter, the frequency of entries into the uterine horns increases as the embryo enters the phase of maximum mobility. The embryo enters every part of the uterine lumen and moves from one horn to another 10 to 20 times per day. Practitioners must be especially aware of the unique embryo mobility characteristic

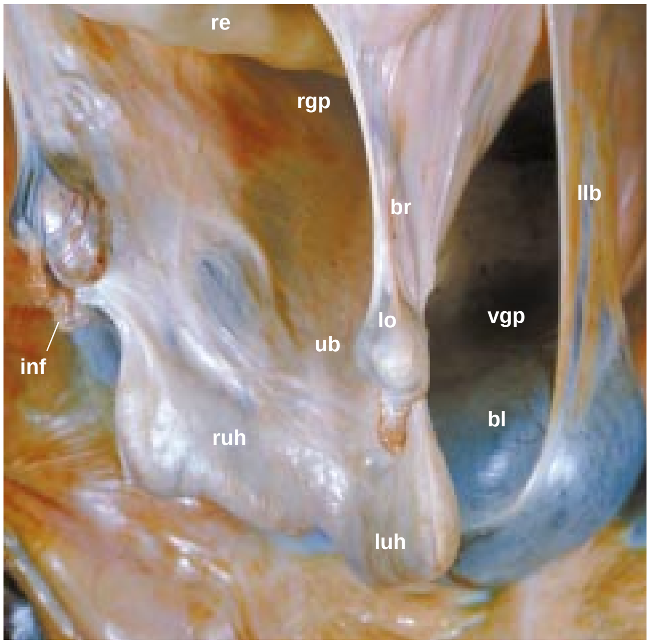


Fig. 2. Frontal-oblique view of suspended reproductive tract after removal of other abdominal viscera.

bl = bladder
br = broad ligament
inf = infundibulum
llb = lateral ligament of bladder
lo = left ovary
luh = left uterine horn
re = rectum
rgp = rectogenital pouch
ruh = right uterine horn
ub = uterine body
vgp = vesicogenital pouch

in this species so that the entire expanse of the uterine lumen is searched during pregnancy diagnosis. Furthermore, the mobility phenomenon is an important aspect of differentiating embryos and cysts and finding and manually eliminating a member of a twin set.

The embryo is subjected to considerable pressure by uterine contractions during embryo mobility. So much so, that the Day 13 or 14 vesicle may undergo periodic compressions (e.g., every 5–14 seconds).[1] During compression, one dimension of the previously spherical vesicle can become twice as great as the other (**Fig. 3**). The compression phenomenon can be seen ultrasonically when the vesicle is viewed in a longitudinal section of the uterus (e.g., uterine body with a linear-array transducer). The widest dimension of the compressed vesicle is in the longitudinal direction of the uterine lumen. Presumably, resiliency and elasticity of the capsule allow uterine-induced distortions of the yolk-sac wall, but provide enough resistance against uterine contractions so that the embryo is moved along the uterine lumen.

Data illustrating embryo mobility in a mare at Day 13[16] and the extent of embryo mobility[14] and uterine contractility[17] on various days are shown (**Fig. 4A,B**). The propulsive force for embryo mobility is uterine contractions as indicated by the following: (1) changes in the extent of uterine contractions, as assessed ultrasonically,[17–20] parallel changes in the extent of mobility (**Fig. 4B**); (2) mobility decreases when uterine contractions are inhibited experimentally[21]; (3) contractions are greater in parts of the uterus exposed to the embryo, based on uterine ligation studies (**Fig. 4C**),[13] indicating that the embryo produces a myometrial stimulant that results in embryo mobility[1]; and (4) the extent of uterine contractility diminishes after the embryo leaves an area.[13] In conclusion, the extensive embryo mobility is attributable to uterine contractions and is favored by the spherical form of the vesicle, the turgidity and anti-adhesive quality of the vesicle resulting from the capsule (Section 2A), and the longitudinal arrangement of the uterine folds (**Fig. 1**).

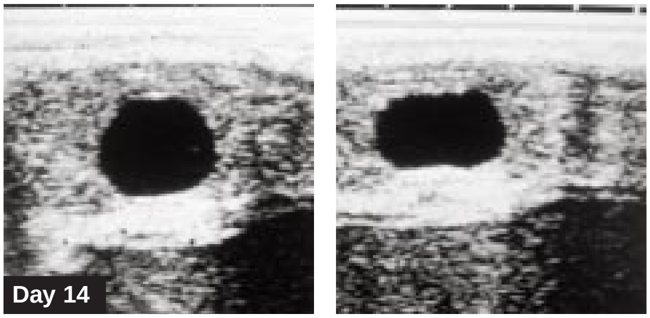


Figure 3. Expansion and contraction of the embryonic vesicle during embryo mobility, as viewed in a longitudinal section of the uterus.

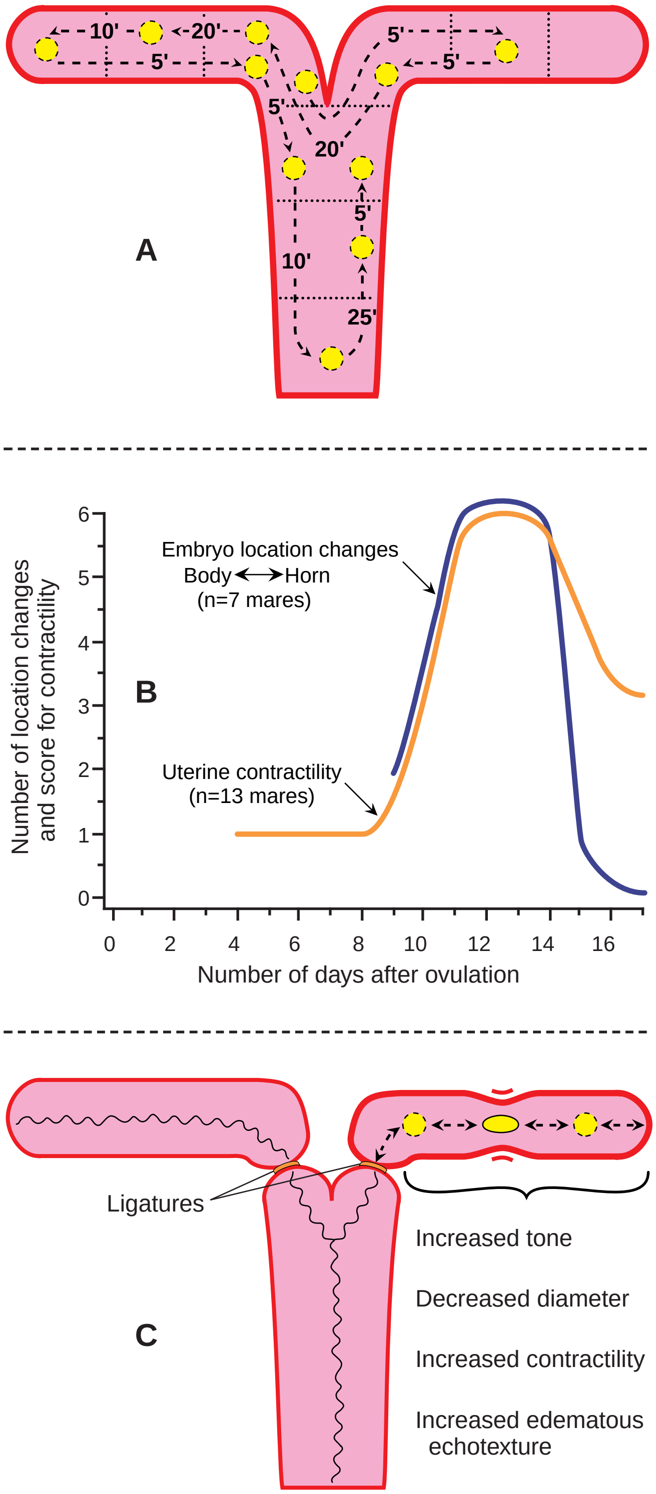
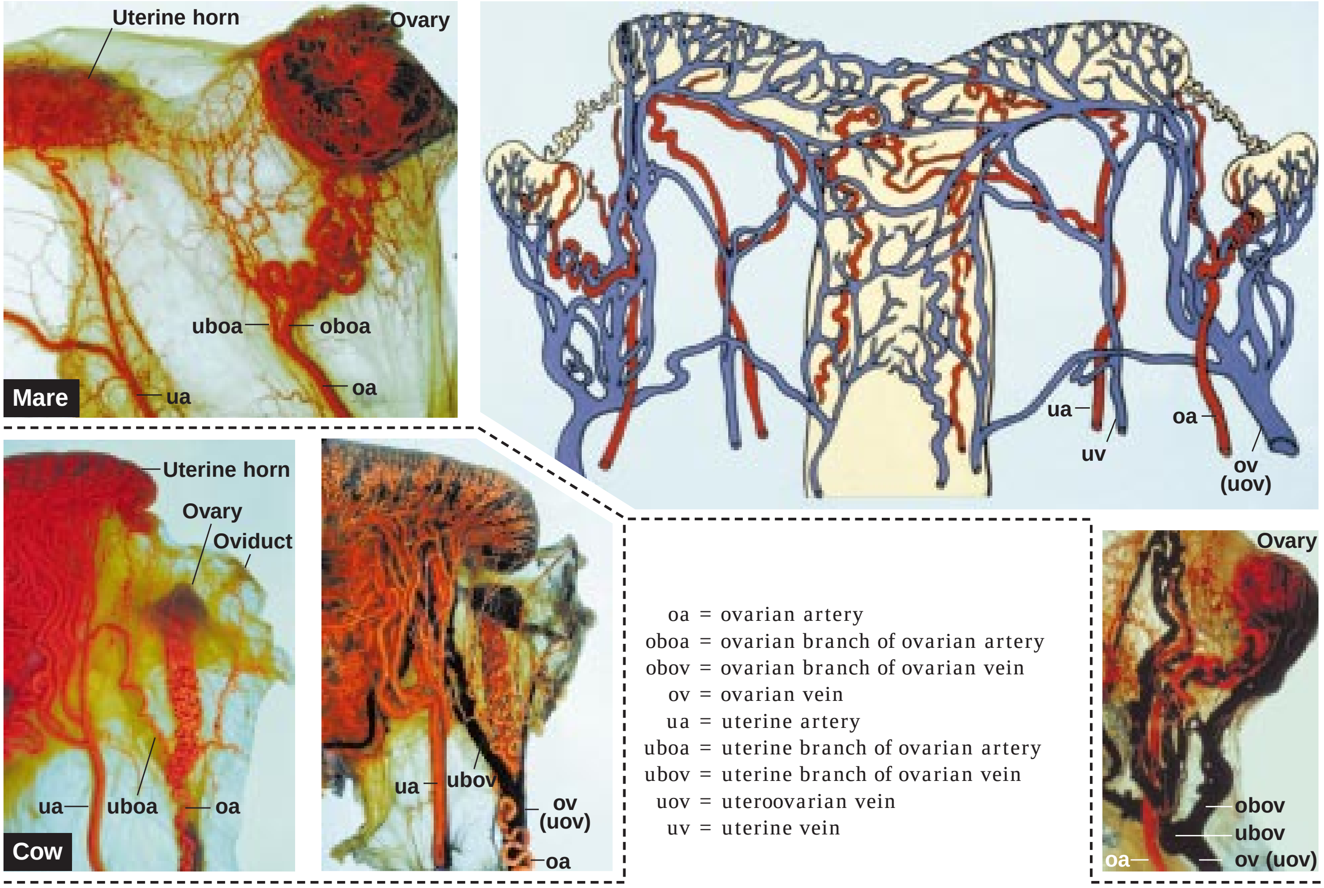


Fig.4. (A) Results of a two-hour mobility trial in a single mare on Day 13. Embryo location was assigned to one of nine arbitrary uterine segments every 5 minutes. The number of minutes required for the embryo to move from one uterine segment to another is shown. (B) Summary of reported data on scores for the extent of uterine contractility and embryo mobility as indicated by the number of location changes between the uterine body and a horn during a two-hour mobility trial. (C) Local effects of the embryo on the uterus as shown by restricting the embryo to one uterine horn.

In the absence of an embryo, the equine uterus produces a potent luteolysin (prostaglandin F2α)[22,23] that travels to the ovary through the systemic circulation, in contrast with the more well-known local or unilateral route in farm ruminants.[24] The indications (reviewed[5,24,25]) of a systemic route for uterine-induced luteolysis in mares versus a local route in cattle are listed (**Table 1**). In farm species, each ovary and the major portion of the uterine tissue on that side is drained by a common vein (uteroovarian vein; **Fig. 5**). In species with a local pathway (e.g., cattle), the ovarian artery is tortuous and is in close apposition to the wall of the uteroovarian vein. In a species with a systemic pathway (horses) the ovarian artery does not contact the uteroovarian vein. The presence or absence of a local utero-ovarian pathway in a given species is attributable to these differences in vascular anatomy.

Table 1. Indicators that the Pathway from Uterus to Ovaries for Uterine-induced Luteolysis is Systemic in Mares and Local in Cows

Item	Mares	Cows
1. Partial hysterectomy	No relationship between side of removal and luteal maintenance [26]	Luteal maintenance only when ipsilateral uterine horn is removed [27]
2. Intrauterine device [24]	Stimulates luteal regression regardless of side relationships	Stimulates luteal regression only if ipsilateral to corpus luteum
3. Route of PGF2α administration for inducing luteolysis [24,28,29]	No differential effect between the IU and IM routes	IU route much more effective than IM route
4. Minimal luteolytic dose of PGF2α [24]	Low (e.g., 5 mg)	High (e.g., 25 mg)
5. Relationship of ovarian artery to uteroovarian vein [25]	Artery and vein not in apposition	Artery tortuous and closely applied to vein



oa = ovarian artery
oboa = ovarian branch of ovarian artery
obov = ovarian branch of ovarian vein
ov = ovarian vein
ua = uterine artery
uboa = uterine branch of ovarian artery
ubov = uterine branch of ovarian vein
uov = uteroovarian vein
uv = uterine vein

Fig.5. A drawing (upper-right) and photographs of cleared specimens after injection of latex into arteries (red) and veins (blue).

Before the identity of the uterine luteolysin, comparisons of the uteroovarian vascular anatomy of mares (no local pathway) with that of the other farm species provided the earliest suggestion that the pathway between uterus and ovaries was venoarterial (reviewed[24,25]). Experimental vascular anastomoses were used to test the hypothesis of a unilateral venoarterial pathway without assumptions on the identity of the luteolysin. For example, in unilaterally hysterectomized ewes, the uterine vein or ovarian artery from the intact side was anastomosed to the corresponding vessel on the hysterectomized side. The anastomoses allowed luteal regression on the hysterectomized side, whereas luteal maintenance occurred if blood was not shunted from the intact side. Surgical anastomoses between ovarian arteries in ewes with the donor artery originating from various levels of the vascular pedicle delineated the functional area of venoarterial transfer as shown (**Fig. 6**).

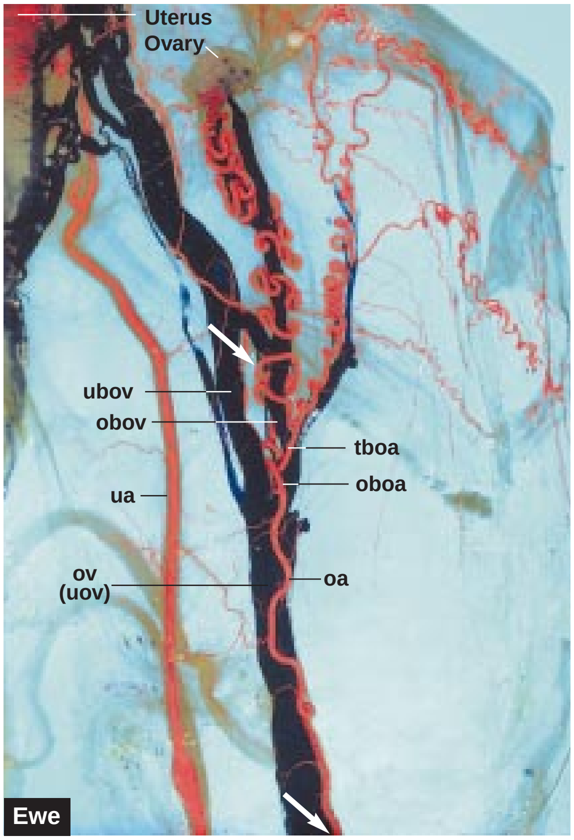


Fig. 6. Specimen of the uteroovarian vascular stem. The two arrows delineate the area of venoarterial transfer of substances between uterine venous blood and ovarian arterial blood. Note the anastomosis (above upper arrow) carrying uterine venous blood into the obov in the convoluted area of the oboa.

oa = ovarian artery
oboa = ovarian branch of ovarian artery
obov = ovarian branch of ovarian vein
ov = ovarian vein
tboa = tubal branch of ovarian artery
ua = uterine artery
ubov = uterine branch of ovarian vein
uov = uteroovarian vein

In the area of apposition between the two vessels, the vessel walls are thin and the connective tissue of the external layers forms a single stratum. Most likely passage of the luteolytic substance through the walls of the vein and artery occurs passively.

When an embryo is present, luteolysis must be blocked (first luteal response to pregnancy[5]) because the corpus luteum through its hormone, progesterone, is vital to embryo development.[30] Evolutionary pressures apparently directed strategies for embryo survival that were compatible with the systemic uteroovarian pathway for uterine-induced luteolysis in mares versus the unilateral pathway in other farm species. The mobility phenomenon in mares allows the embryo to contact all parts of the uterine lining. In this manner, the relatively small spherical embryo is able to block luteolysis despite the relatively large uterus. Restricting the conceptus to a small portion of the uterus by uterine ligation prevents direct contact between the embryo and the remaining portion of the uterus and apparently results in complete or partial luteolysis,[13,31] although more study is needed.[13] It appears that embryo mobility evolved for blocking all of the uterus in mares, whereas expansion of the trophoblast in the uterine horn on the side of the corpus luteum evolved in cattle (**Fig. 7**).

In addition to the reduction in exposure of the corpus luteum to PGF2α, illustrated in the diagrams, there are indications that the conceptus produces a substance that acts as an antiluteolysin at the ovarian level. Experimental anastomoses of uterine veins or ovarian arteries in pregnant sheep and cattle have demonstrated that the venous drainage of the gravid horn contains such a substance. (reviewed[24]) Studies in ewes indicate that prostaglandin E2 (PGE2) is a candidate for the antiluteolysin. In mares, exogenous PGE2 affects uterine contractility and tone during the time of embryo blockage of uterine-induced luteolysis (Section 2C); a role as an antiluteolysin needs to be studied.

C. Fixation

Fixation is defined as the cessation of embryo mobility.[32] Discovery of the phenomena of embryo mobility and fixation is a research milestone because it provided rationale for hypotheses on the following perplexing phenomena: (1) occurrence of fixation almost always in the caudal portion of one of the uterine horns[32]; (2) lack of agreement between side of ovulation and side of fixation[32]; (3) more frequent embryo fixation in postpartum mares in the most involuted horn[33,34]; (4) greater incidence of unilateral than bilateral fixation in mares with twins, especially when the vesicles are of unequal size (Section 6A); and (5) ability of a small conceptus to block the uterine luteolytic mechanism throughout a relatively large uterus (Section 2B).

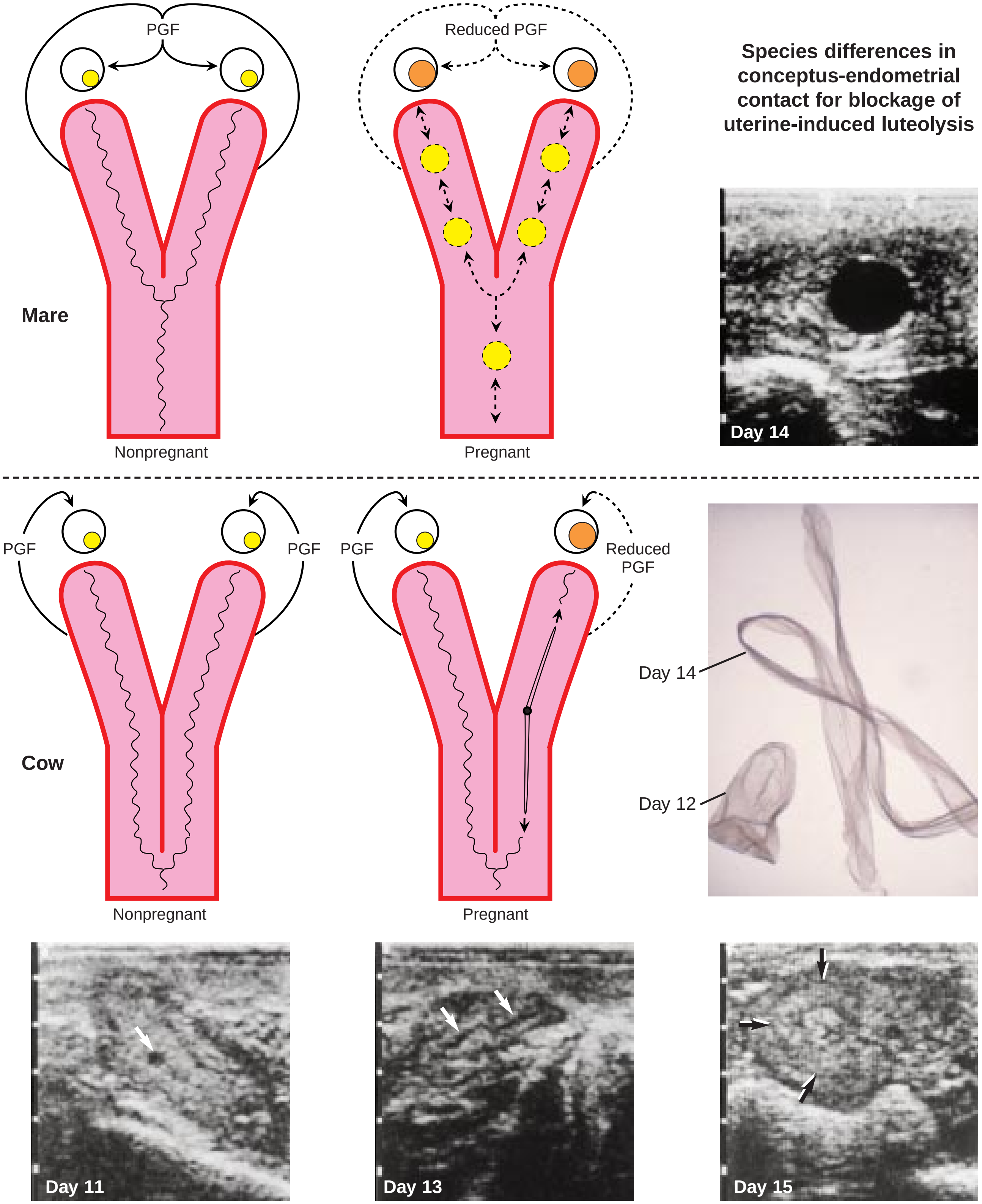


Fig. 7. Embryo-endometrial contact associated with the blockage of uterine-induced luteolysis by the embryo (luteal response to pregnancy). For illustration, the diagrams depict a single embryo and a corpus luteum (regressing=yellow; maintained=orange) in each ovary. The mare has a systemic uteroovarian pathway; the spherical and mobile embryo covers all parts of the uterus and thereby prevents luteal exposure to PGF2α during the critical time (Days 11–15), regardless of luteal location. The cow has a unilateral pathway, and the expanding conceptus during the luteal response to pregnancy covers only the ipsilateral horn. The expansion of the bovine conceptus between days is shown by the sonograms (arrows) and specimens.

On the day of fixation, the vesicle is still spherical as shown by ultrasound studies.[6] At this time, less than half of the yolk-sac wall has developed a third layer (mesoderm), and blood vessels have begun to develop in the mesoderm near the embryo proper (**Fig. 8A**).[5] A cavity, the exocoelom, forms within the mesoderm near the embryo proper, dividing the mesoderm into two layers. Folds of ectoderm and mesoderm begin to pass over the embryo proper and will give rise to the amnion. The membrane consisting of ectoderm and mesoderm is called the chorion and its future is discussed in Section 2E.

Fixation occurs on mean Days 15 in ponies and 16 in horses (**Fig. 8B**).[6] Fixation usually occurs near a flexure in the caudal portion of one of the uterine horns. It has been postulated[32] that fixation occurs at this site, despite continuing uterine contractions,[17–19] because the flexure is the greatest

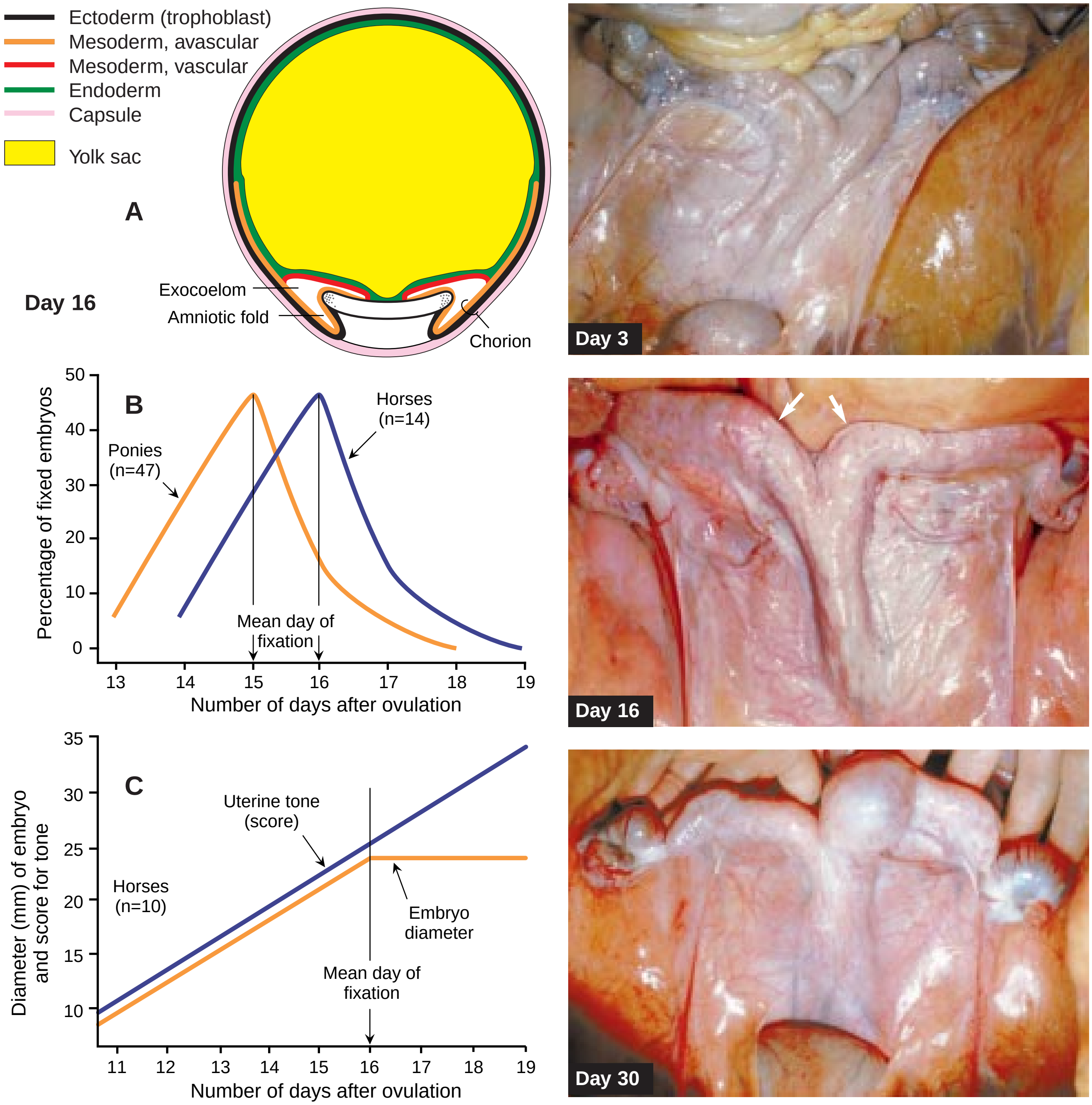


Fig. 8. (A) Diagram of embryo on the day of fixation. (B) Summary of data on frequency of fixation on various days in ponies and horses. (C) Summary of data on temporal association between changing uterine tone and embryo diameter. The photographs depict changes in uterine tone. The increasing uterine tone and embryo diameter are believed to result in the cessation of mobility (fixation) at a flexure (arrows, Day 16) in a caudal uterine horn. Tone increases and confines the embryonal bulge as shown for Day 30.

intraluminal impediment to continued embryo mobility. The photographs show the flexures in the caudal portion of the horns for Day 16 and the corresponding location of the embryonal bulge for Day 30. The uterus rides upon and intermingles with other viscera to a variable extent,[5] and has a T-shape when lying on other viscera.

The equine uterus is flaccid during and immediately after estrus as shown for Day 3, increases in tone until mid-diestrus, decreases in tone until Day 10 or 11, and then, if the mare is pregnant, gradually increases in tone and becomes turgid[20] as shown for Day 16. The increase in uterine tone is associated with a decrease in uterine diameter[34–36] and continues until Days 25–30 as shown for Day 30.[20,37,38] The gradually increasing uterine tone and decreasing uterine diameter together with increasing conceptus diameter[6] apparently combine to result in fixation of the conceptus (**Fig. 8C**).[32] This hypothesis is compatible with the following: (1) earlier fixation and greater uterine tone in young mares than in old mares[39]; (2) higher frequency of fixation in the most involuted horn postpartum[33,34]; (3) the larger the embryo on Day 14, the sooner fixation occurs[18]; (4) fixation when the diameter of the conceptus is similar to the distance between the inner opposite walls of the myometrium of the turgid horns[18]; and (5) fixation a day later in horses than in ponies (**Fig. 8B**).[6] In regard to point 5, the embryo is similar in diameter between the two mare types,[6] but the uterine horns are larger in diameter in horses.[35] Fixation may be aided by a reduction in the slipperiness of the capsule[9] (Section 2B).

The conceptus distributes a tone-stimulating substance during embryo mobility; uterine ligation studies demonstrated that tone was greater in uterine horns exposed to the embryo (**Fig. 4C**).[13] Estradiol may contribute to tone stimulation as indicated by the following: (1) beginning on Day 12, the conceptus produces estrogens in increasing amounts in proportion to its increasing diameter[40]; (2) small doses of exogenous estradiol increased uterine tone in progesterone-primed anestrous mares[37]; (3) exogenous estradiol caused earlier fixation and tended to increase uterine tone[41]; and (4) the confined conceptus locally stimulates estrus-like edematous echotexture of the endometrium.[13] In regard to point 4, moderate edematous changes in the endometrium in early pregnancy do not necessarily indicate impending abortion. Results of a recent study[42] suggest that PGE2, produced by the embryo,[43] plays a role in both stimulating uterine contractions and increasing uterine tone during the embryo-mobility phase. A continuing progesterone source is necessary for embryo mobility and fixation.[30] When luteolysis occurs after fixation, the uterus loses its turgidity, and the embryo leaves the site of fixation. Sometimes the embryonic heart is still beating after the embryo leaves the fixation site; embryonic death, however, is imminent.[44]

To summarize Sections 2A–D (**Fig. 9**), the encapsulated embryo stimulates myometrial contractions and thereby travels throughout the uterus, especially on Days 11–15. The mobile embryo systemically blocks uterine-induced luteolysis and at the same time distributes a substance that gradually stimulates increasing uterine tone. By the time the blockage of luteolysis is complete (mean Day 16), the vesicle has grown and uterine tone has increased (decreased uterine diameter) to the point that fixation occurs in the area of the greatest impediment to mobility (flexure in a caudal horn). This is an exquisite series of events that should pique the curiosity of evolutionists, as well as veterinarians.

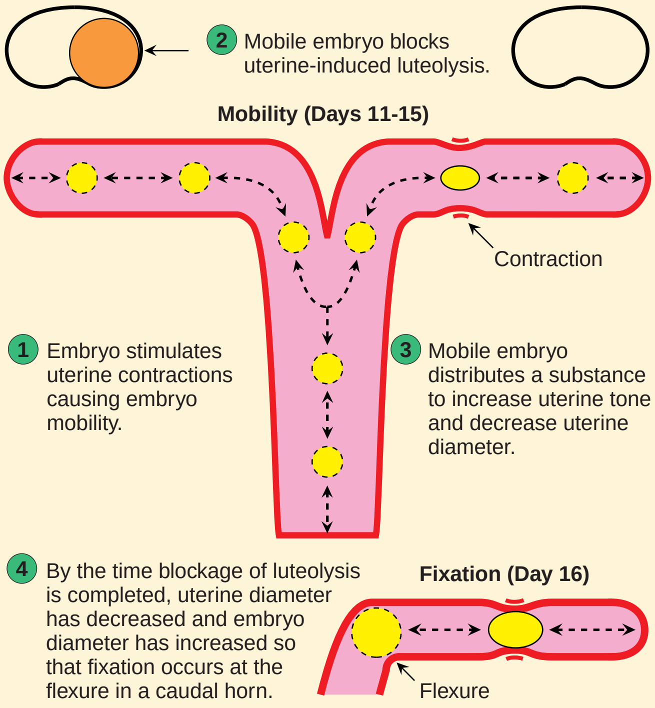


Fig. 9. Summary of events associated with embryo mobility, first luteal response to pregnancy, and embryo fixation.

D. Orientation

Orientation is defined as rotation of the embryonic vesicle so that the embryo proper is on the ventral aspect of the yolk sac.[32] The importance of orientation at this time will become clear in the discussions on the transition between yolk sac and allantoic sac (Section 2E) and formation of the umbilical cord (Section 3A). The preceding diagrams (**Figs. 1,8**) depicted the embryonic disc on the ventral surface of the yolk sac. However, the mobile prefixation vesicle probably rotates, and the embryonic disc can be anywhere on the circumference at this time. In this regard, simulated embryonic vesicles tend to roll when responding to uterine contractions.[1]

The yolk sac is three-layered and vascularized at the embryonic pole and two-layered at the opposite pole (**Fig. 10**).[5] The islands of blood cells that developed in the mesoderm of the three-layered portion have coalesced to form a continuous vascular network. The yolk sac does not contain stored food as in a bird egg, but with vascularization it becomes an efficient purveyor of nutritive material from the uterus to the rapidly developing embryo proper. The amniotic cavity results from a union of the two folds of chorion that pass over the embryo as shown. Closure of the amniotic folds is completed by Day 20,[10] just before loss of the capsule.

Between Days 16 (**Fig. 8)** and 18 (**Fig. 10**), much (>50%) of the yolk-sac wall is still two-layered (no mesoderm)[5] as shown for Day 17 (**Fig. 11**). The mesoderm of the remaining three-layered wall differentiates into connective tissue and a vascular network and is much stronger than the two single-cell layers of the two-layered portion. The difference in strength between the two portions of the yolk-sac wall and the relative expanse of the two portions have been utilized to develop postulates on embryo orientation and embryo reduction in mares with twins (Section 6A). The exposed Day-18 conceptus (**Fig. 10**) does not maintain a spherical shape as it did at Day 14. The sonogram shows that in cross sections of the uterine horn the vesicles tend toward a guitar-pick or irregular shape. The apex of the vesicle is orientated dorsally, and the smooth, rounded base is orientated ventrally. The irregular shapes after fixation are normal and should not be taken as a sign of abnormal development and a harbinger of embryo loss. The shape change is attributable to uterine turgidity and thickening of the dorsal uterine wall, especially on each side of the mesometrial attachment. The vesicle becomes more spherical when an inhibitor of uterine tone is given on Day 19.[45] Because of the uterine turgidity, the vesicle does not enlarge on Days 18–26 when viewed in a cross section of the horn (**Fig. 12**). During this time, a compensatory increase in conceptus length occurs along the longitudinal uterine lumen and accommodates the increasing growth of the conceptus (**Fig. 13**).[6]

Uterine contractions continue after fixation,[17–20] and may play a role in orientating the embryonic

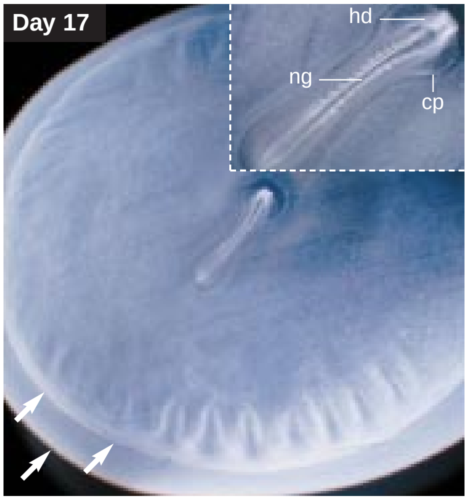


Fig. 11. The peripheries of mesoderm (two arrows) and yolk-sac wall (one arrow) are indicated. The embryo proper (inset) has 11 pairs of somites. cp, cardiac prominence; hd, head; ng, neural groove.

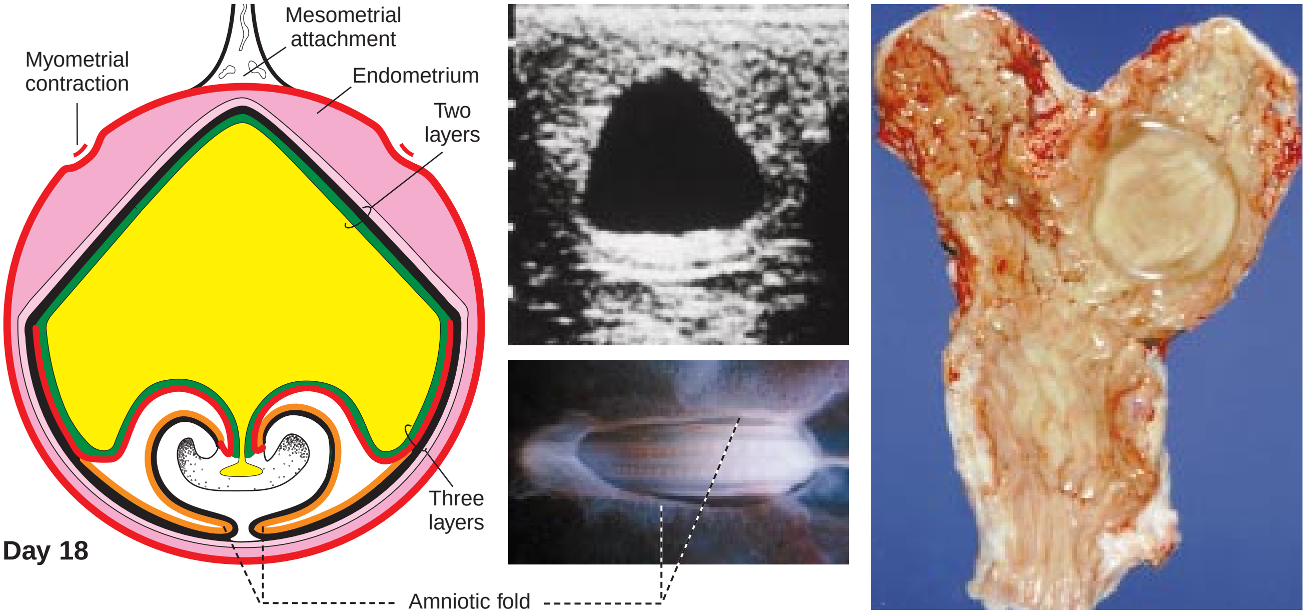


Fig. 10. Diagram, sonogram, and photographs of the embryo on Day 18. The embryo proper is not yet ultrasonically detectable. Thin versus thick portions of the vesicle wall, the continuation of uterine contractions, and disproportional hypertrophy of endometrial folds are believed to result in rotation of the vesicle after fixation. The amniotic folds have not yet closed. For color key, see Fig. 8.

vesicle. Continuous ultrasonic viewing indicates that shape of the fixed embryo is continually altered by uterine contractions (**Fig. 14**).[6] It has been postulated[32] that orientation occurs between the time of fixation and the appearance of the irregular shapes. Three factors are believed[1,32] to interact during orientation: (1) thick (three-layered) and thin (two-layered) portions of the yolk-sac wall; (2) asymmetrical encroachment from thickening of the upper turgid uterine wall on each side of the mesometrial attachment (**Fig. 15**); and (3) the massaging action of uterine contractions. The interaction of these factors results in the thickest portion of the yolk-sac wall (embryonic pole) rotating to a ventral position (antimesometrial). It is not clear, however, whether the localized thickening of the endometrium adjacent to the thin-walled portion of the yolk sac (**Fig. 10**) occurs before, during, or after orientation; the cause of the thickening has not been determined. The orientation hypothesis is compatible with six reported cases of disorientation associated with a flaccid uterus or the presence of twin vesicles at the postulated time of orientation.[1,6] Once fixation and orientation are established, increasing horn turgidity, cranial and caudal to the vesicle (**Fig. 8**), prevents the vesicle from dislodging longitudinally in the uterine lumen. The orientated position, as viewed in cross-section, apparently is aided by adhesiveness and cross-ridging of the endometrial folds.[10] Later, after loss of the capsule on Day 21 (Section 2A), a ridge of trophoblast indents the endometrium and may further anchor the vesicle and help prevent rotation.[10]

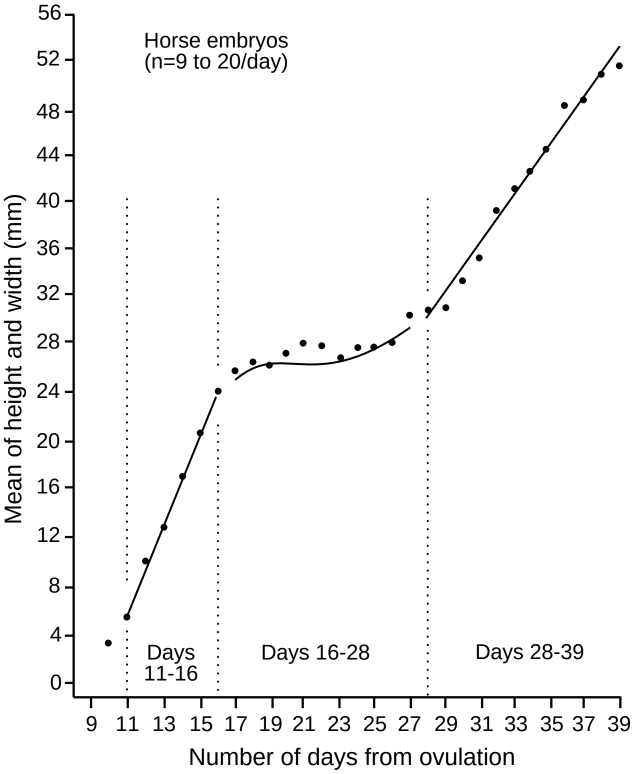


Fig. 12. Cross-sectional growth profile of the embryonic vesicle.

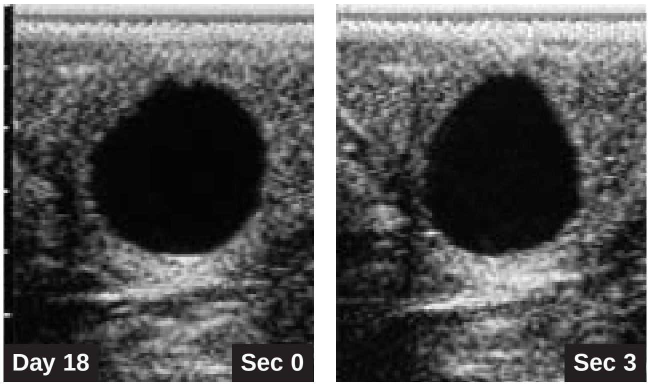


Fig. 14. Example of uterine massage of the vesicle on Day 18. The vesicle changed shape when viewed continuously. The two sonograms were taken at an interval of 3 seconds.

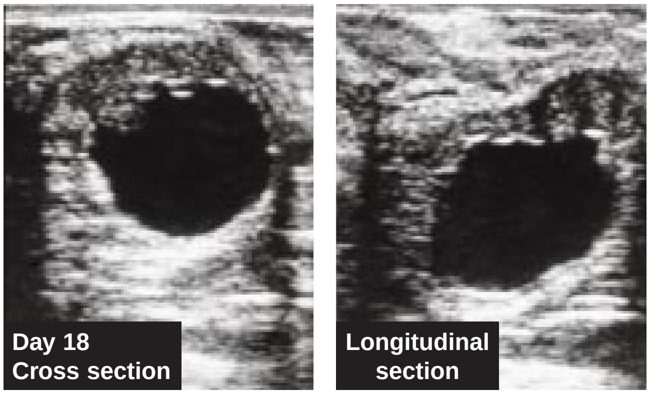


Fig. 13. Cross and longitudinal sections of an embryonic vesicle. The longitudinal section shows that expansion occurs along the uterine lumen and accounts for the plateau in cross-sectional expansion on Days 18–26 that is shown in Fig. 12.

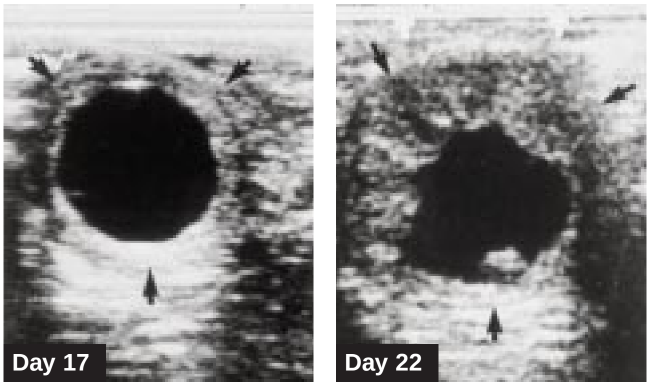


Fig. 15. Cross-sectional views showing the disproportional hypertrophy of the dorsal endometrial folds after Day 17. The outer limits of the uterine wall are delineated by arrows.

E. Transition from Yolk Sac to Allantoic Sac

By Day 21, the amniotic cavity is completely formed (**Fig 16**). The allantois has begun to emerge from the hindgut as shown in the diagram and photograph of a Day-21 embryo proper. The allantois grows into the exocoelom.[5] The allantois becomes prominent by Day 24, as shown in the sonogram and photograph. In the sonogram, the allantoic membrane and the closely apposed yolk-sac membrane appear as a thin echoic line suspending the embryo proper.

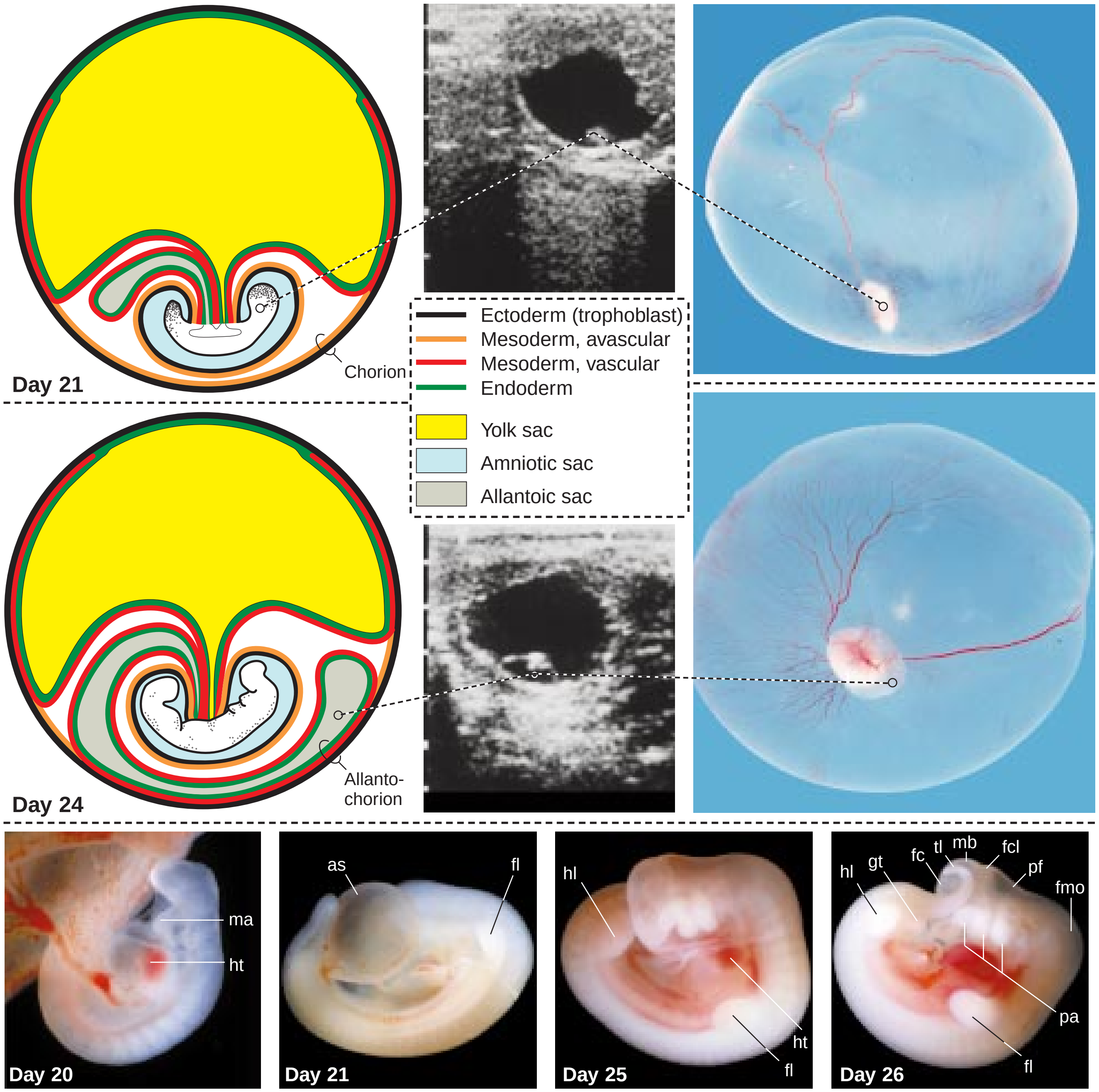


Fig. 16. In the photographs of the embryonic vesicles at Days 21 and 24, the vesicles were submerged and tilted slightly to expose the embryonic pole. The broken line for Day 21 connects the embryo propers among illustrations, and the line for Day 24 connects the allantoic sacs. Note the emergence and position assumed by the allantois. Lengthening of the limb buds between Days 25 and 26 is apparent. The genital tubercle (forerunner of clitoris and penis) is already visible at Day 26 and by Day 60 will have migrated to a position that will allow ultrasonic diagnosis of fetal gender.

as = allantoic sac
fc = future cerebrum
fcl = future cerebellum
fl = forelimb
fmo = future medulla oblongata
gt = genital tubercle
hl = hind limb
ht = heart
ma = mandibular arch
mb = mid brain
pa = pharyngeal arches
pf = pontine flexure
tl = tail

During Days 30 to 36, the size of the allantoic sac continues to increase and the embryo proper moves farther away from the vesicle floor (**Fig. 17**). The nonvascular portion of the yolk-sac wall opposite to the embryo proper becomes a relatively small area by Day 30 but continues to be detectable even in the terminal placenta. The area of chorion between the allantois (below) and the yolk sac (above) is the location of the chorionic girdle (forerunner of endometrial cups; Section 5). The mesoderm in this area remains avascular. The growing allantois forms a sac under the amnion and embryo proper, lifting them from the floor

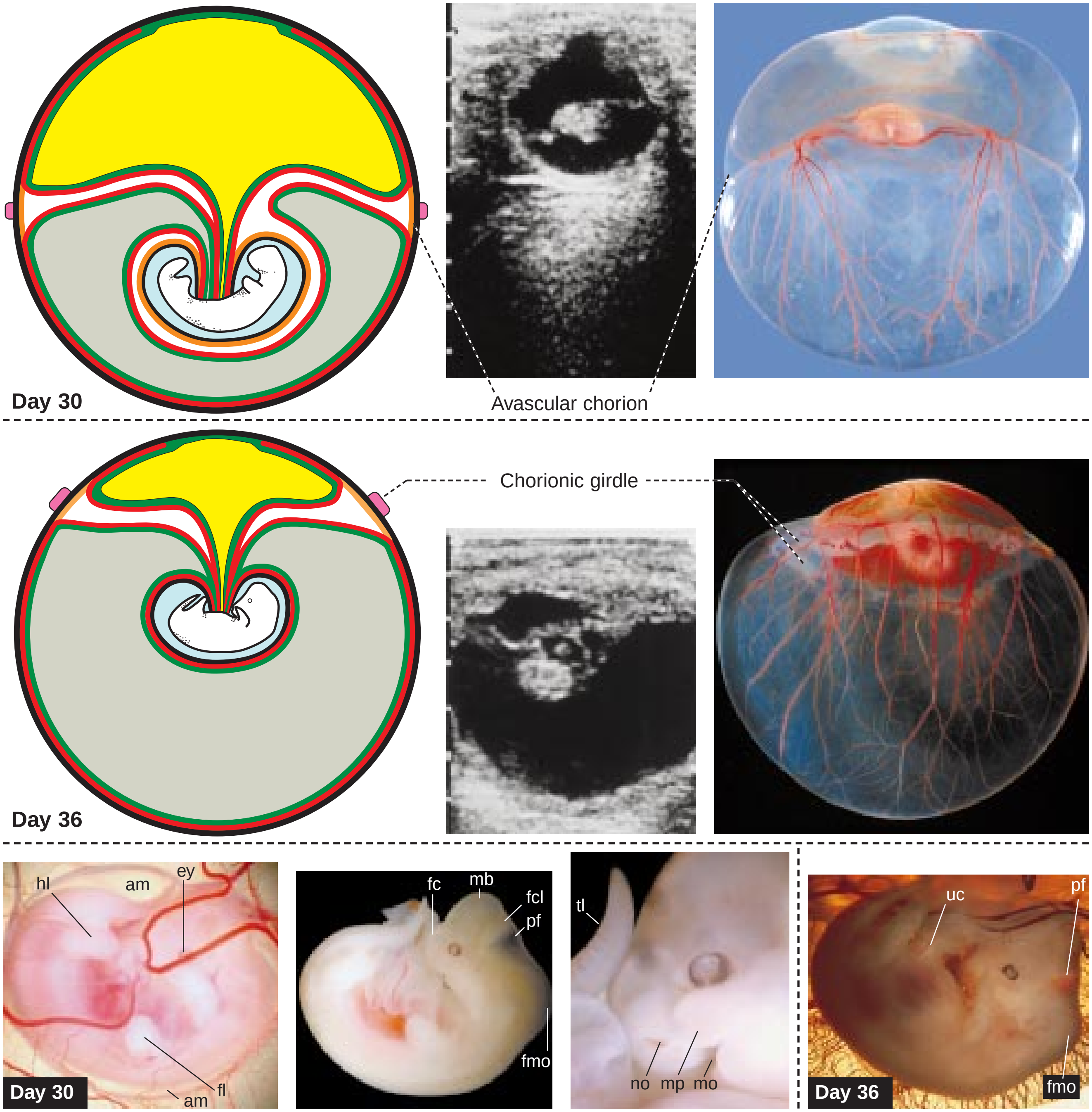


Fig. 17. The broken lines connect the avascular chorion and the chorionic girdle between the diagrams and specimens. These structures are not directly detectable on the sonograms. The far side of the girdle shows through the translucent allantoic sac in the Day-36 vesicle. Compare photographs of developing embryo propers for Days 17–36 (Figs. 1, 10, 11, 16, 17). For color key, see Fig. 16.

am = amnion
ey = eye
fc = future cerebrum
fcl = future cerebellum
fl = forelimb
fmo = future medulla oblongata
hl = hind limb
mb = mid-brain
mo = mouth
mp = maxillary process
no = nose
pf = pontine flexure
tl = tail
uc = umbilical cord

of the vesicle. The union of the chorion and the allantois first shown for Day 24 (**Fig. 16**) is the beginning of the allantochorionic placenta for maternal-fetal exchange during the entire fetal stage. Blood vessels develop in the mesoderm of the allantoic portion and vascularize the allantochorion and the amniochorion. As shown in the figures for Days 21–36, the allantoic sac becomes progressively larger than the yolk sac, so that the embryo proper and the echoic membrane separating the two sacs move toward the dorsal aspect of the vesicle. The embryo proper is an echoic nodule on the separating line between the two placental sacs.[6] The separating membrane tends toward the horizontal, whereas the apposed walls of twin embryonic vesicles tend toward the vertical, thus serving as an aid in differentiating a singleton from twins.

3. The Fetal Stage

A. Early Development of the Fetus and Umbilical Cord

By the beginning of the fetal stage (Day 40), the amnion is vascularized (**Fig. 18**, fetal-amniotic unit,

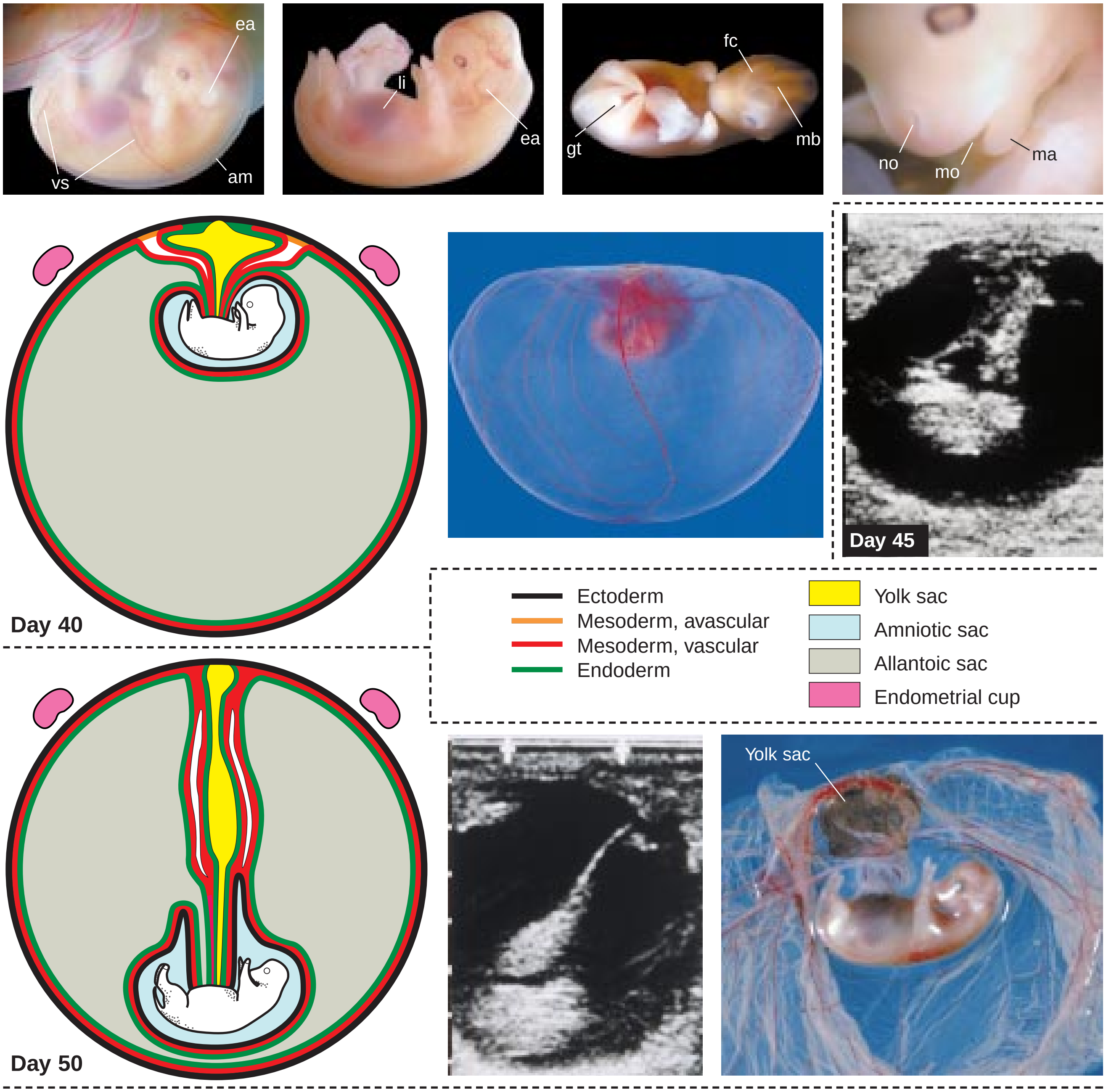


Fig. 18. Fetus and conceptus at the beginning of the fetal stage.

am = amnion
ea = ear
fc = future cerebrum
gt = genital tubercle
li =liver
ma =mandible
mb = mid-brain
mo = mouth
no = nose
vs = vessels in amnion

upper left) and the pontine flexure of the embryo stage (**Figs. 16,17**) is gone (**Fig. 18**, second fetus in upper row). The fetus has arrived at the pole opposite the pole of the orientated embryo proper (Section 2D). Replacement of the yolk-sac placenta by the allantochorionic placenta is nearly complete. The membranes and associated vessels separating the yolk sac and allantoic sac are coming together and will form the umbilical cord. The umbilical cord lengthens so that the fetal-amniotic unit reaches the lower wall or ventral aspect of the allantoic sac by Day 48,[46] as shown in the Day-50 diagram and sonogram. The yolk sac has been incorporated into the umbilical cord. The illustrations demonstrate why the umbilical cord attaches to the upper surface of the allantochorion. Furthermore, the site of the allantochorionic attachment of the umbilical cord throughout pregnancy identifies the horn and site where embryo fixation occurred. Thus, the horn of fixation will be identified later in this report as the umbilical-cord horn.

To recapitulate Sections 2D–E and 3A, the fixed embryo undergoes orientation on Days 17–18 so that the embryo proper is at the floor of the yolk sac. The allantoic sac emerges from the embryo proper a few days later. The allantois lifts the embryo proper from the floor of the conceptus and assumes a larger and larger proportion of conceptus volume at the expense of the yolk sac. The embryo proper arrives at the ceiling of the conceptus at the end of the embryo stage. The area of apposition of the allantoic and yolk sacs forms a vascularized membrane that comes together from all sides and originates the umbilical cord. It is as though the sequential events beginning with orientation on Days 17–18 were designed to ensure that the umbilical cord attaches to the ceiling of the allantoic sac. Therefore, for the remainder of pregnancy the fetus will not be resting upon the allantoic attachment of the umbilical cord.

B. Fetal Activity

Fetal head nods were first detected at mean Day 40.[4,6] The nods can be observed by ultrasound with prolonged and steady observation of a longitudinal section of the fetus. The extent of fetal activity gradually increases to detectable limb movements by mean Day 46. By the time the fetus reaches the floor of the allantoic sac (mean Day 48), whole-body activity is sufficient to raise the fetus a few millimeters off of the allantoic floor. In continuous 5-minute transrectal ultrasonic scans each week in 10 mares from Day 44 to term, intrinsic fetal activity increased in intensity and duration over Months 2–4; percentage of time a fetus was active during a 5-minute continuous examination during Months 2, 3, and 4 was 22, 42, and 56%, respectively. Some fetal activity was detected during every examination with an average of 2.5 minutes of detected activity for each examination. These results indicate that fetal demise should be suspected when no activity is detected during 5 minutes even when the heart is not accessible, but more documentation is needed. It is not known whether the transrectal ultrasound examinations had a stimulating effect on fetal activity, but ballottement was avoided.

C. Allantoic-fluid Shifts

The fluid-filled allantochorionic sac reaches the cervix by mean Day 50, the tip of the horn of fixation by Day 60, and involves the entire uterus by Day 65.[46] Filling of the uterine lumen by the allantoic fluid of the conceptus is not a constant, gradual process like the filling of a balloon. Instead, the filling alternately proceeds and recedes; a uterine horn can be distended by the allantoic fluid within the allantochorionic membrane at a given examination but not at the next examination (**Fig. 19**).

Transient uterine constrictions can involve an entire horn or a narrow segment (e.g., 10 mm). The transient nature of uterine constrictions is highlighted by their development or disappearance during a 5-minute examination. Constrictions cause

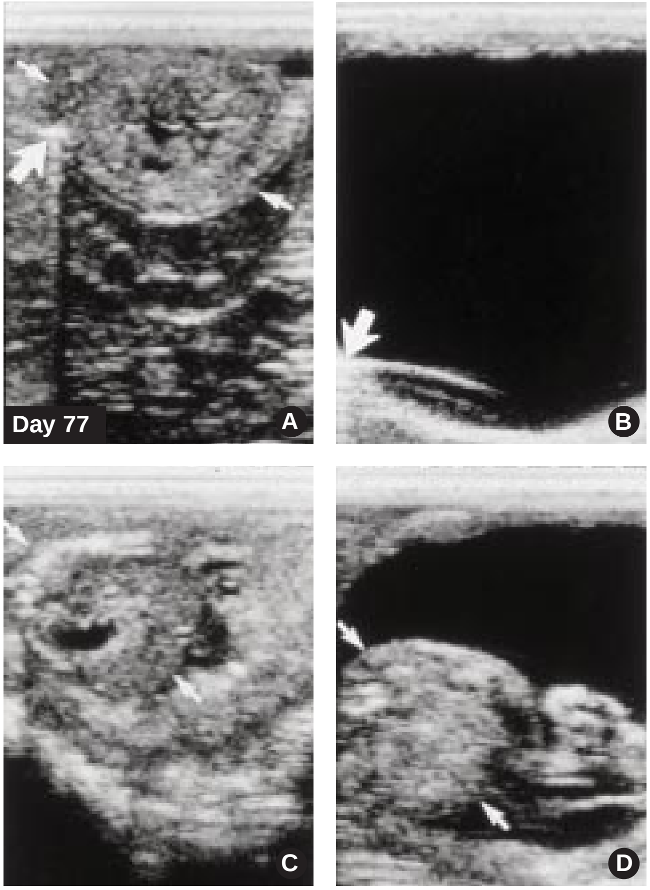


Fig. 19. (A) Cross section of the middle of the nonfetal horn (small arrows) with a limited quantity of allantoic fluid and (B) 22 minutes later with much fluid. The large arrow points to a steel ball which was surgically attached to the uterine horn to identify a specific location. (C) Walls of the fetal horn in close apposition to the fetus (arrows) and (D) 6 minutes later after filling with fluid.

the allantoic-fluid shifts by expelling the fluid to other areas of the conceptus (**Fig. 20**). During continuous ultrasonic viewing,[47] alternate maximal dilation and complete constriction (no visible lumen) of the entrances into the uterine horns (**Fig. 21**) occurred an average of 1.3 times/horn/hour. The extent of retreat of the allantochorionic membrane along with the expulsion of allantoic fluid from a previously invaded area has not been demonstrated directly. However, some interdigitation of villi occurs by Day 50,[10] and therefore it is unlikely that the membranes follow the shifts in fluid. The constrictions result from myometrial activity, as indicated by decreased frequency of constrictions and increased frequency of dilations when a myometrial inhibitor is given.[47] The constrictions can temporarily impinge upon fetal parts, as shown. The frequent opening and closing of uterine segments during conceptus expansion and formation of transient uterine constrictions after expansion emphasize the dynamic nature of uterine constrictions and the resulting allantoic-fluid shifts.

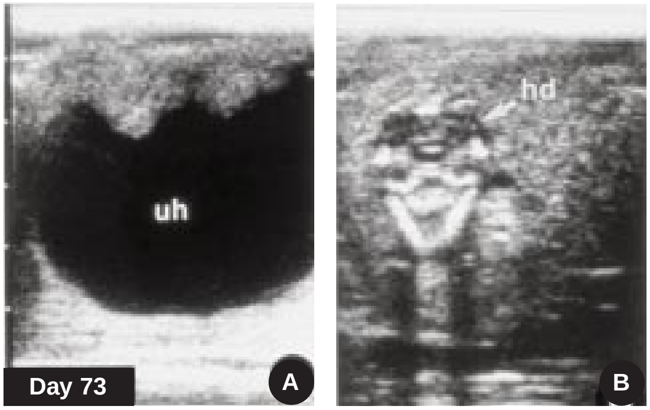


Fig. 20. (A) Sonogram showing a fluid-filled dilation of the allantoic sac in the cranial uterine horn (uh). (B) A transient constriction of the uterine wall upon the fetal head (hd) caudal to the dilation.

D. Fetal Mobility

During Months 2–4, the length of the umbilical cord and the volume-ratio of allantoic fluid to fetal mass increases considerably. The aquatic fetus moves about within the relatively large pool, tethered only by a long lifeline—the umbilical cord (**Fig. 22A**). Bouts of fetal activity, such as neck arching and limb thrusts, propel the fetus off the inner wall of the allantochorion and into the pool of allantoic fluid (**Fig. 23**). Sometimes the fetal-amniotic unit is enclosed in a uterine horn and then appears to be forced through a constriction into a dilated portion (**Fig. 21**). The fetus changes direction, recumbency, and location with great vigor and seemingly practices the neuromuscular coordination that it will need during independent life, including those required for limb action, head shaking, nursing, and chewing. For example, the fetus changed direction (presentation) a mean of

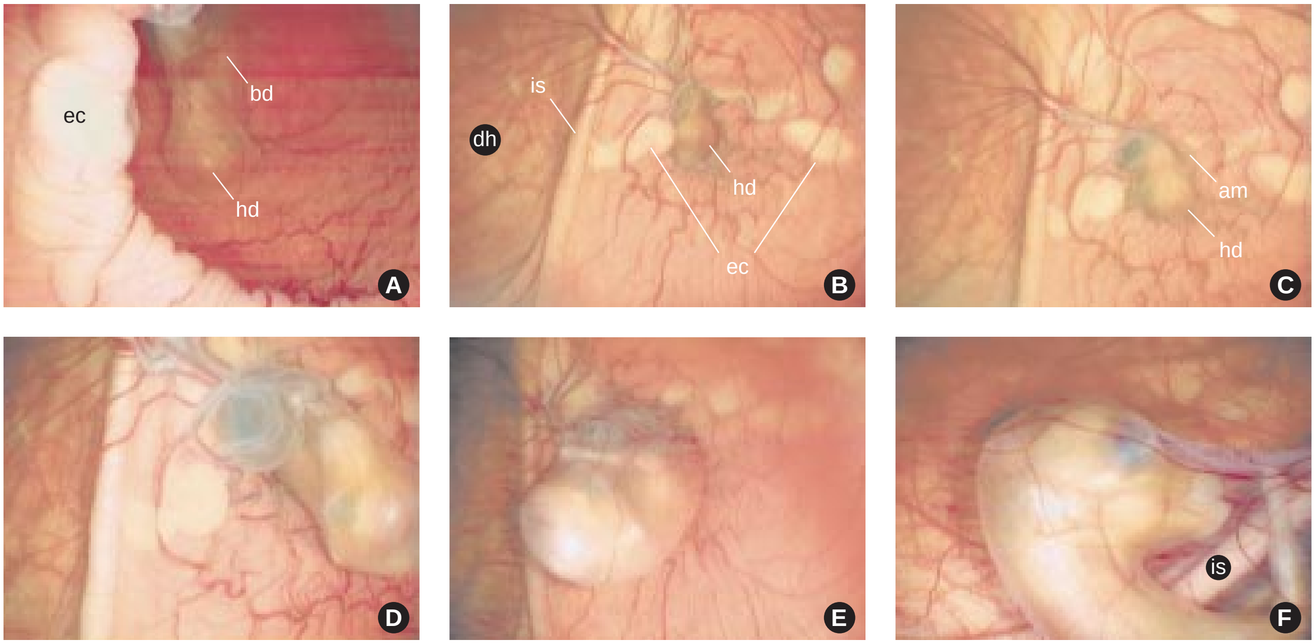


Fig. 21. Sequential transcervical videoendoscopic images at Day 55, illustrating fetal mobility and uterine-horn dilations and constrictions. The time interval from images A to F was 6.5 minutes. (A) Cranial portion of fetus deep in the partially dilated right horn. (B) Constriction in caudal portion of the right horn over the fetal head. (C) Amnion and head emerging through the constriction. (D) Head and neck have emerged. (E) One-half of fetus has emerged. (F) Neck and right forelimb resting on the septum between horns; entire fetus is in the uterine body. am, amnion; bd, body of fetus; dh, dilated left horn entrance; ec, endometrial cup in caudal portion of right horn; hd, head; is, intercornual septum.

five times/hour during Month 3.[48] Because of rapid fetal enlargement, the relative volume of the placental pool decreases over Months 4–9, and the meanderings of the fetus diminish, but the in-place activity remains (Section 3B).

The extensive mobility of the fetus within the allantoic sac was discovered by transcervical endoscopy (**Fig. 21**)[49] and confirmed by transrectal ultrasonography.[48] Fetal mobility is sometimes attributable to extraneous movements (mare, intestine), but mobility depends primarily on the fetus[47]; injections of succinylcholine, a neuromuscular blocker, into the fetus reduced the number of changes in location and presentation by five-fold. The phenomena of mobility and activity complicate attempts to ultrasonically examine the fetus, such as during a search for twins or the diagnosis of fetal gender.

E. Fetal Presentation and Horn Closures

The terms cranial and caudal presentations of the fetus indicate that the cranial and caudal aspects of the fetal torso are directed toward the cervix. The equine fetus at term is almost always (>99%) in cranial presentation.[34,50] However, an equal likelihood of cranial and caudal presentations occurs during Months 2–5[51] and is attributable to the extensive fetal mobility. Thus, a mechanism exists for the final selection of cranial presentation. In this regard, both uterine horns close by Month 8, expelling the placental fluids into the area of the conceptus housed by the uterine body. The allantochorionic membrane is retained in the horn after placental fluid is expelled, as indicated by ultrasonic imaging of placental vessels.

During the time of closure of both horns, the fetus is confined to the uterine body, which has become a large chamber, free of the recesses of open uterine horns (**Fig. 22B**). The chamber contains all of the placental fluid because the horns are closed. As a result, the stage is prepared for the fetus to undergo the maneuver that must be successfully completed to favor normal birth—the fetus positions itself so that its front is toward the mare's cervix (cranial presentation). It has been hypothesized[4,6] that the fetus reaches a neurologic age at this time which allows the intricate mechanisms in the internal ear to respond to a maternal directional signal; the maternal signal is the incline of the floor of the allantochorionic sac or ventral uterine body wall upward toward the cervix. During Months 6 and 7, the mean

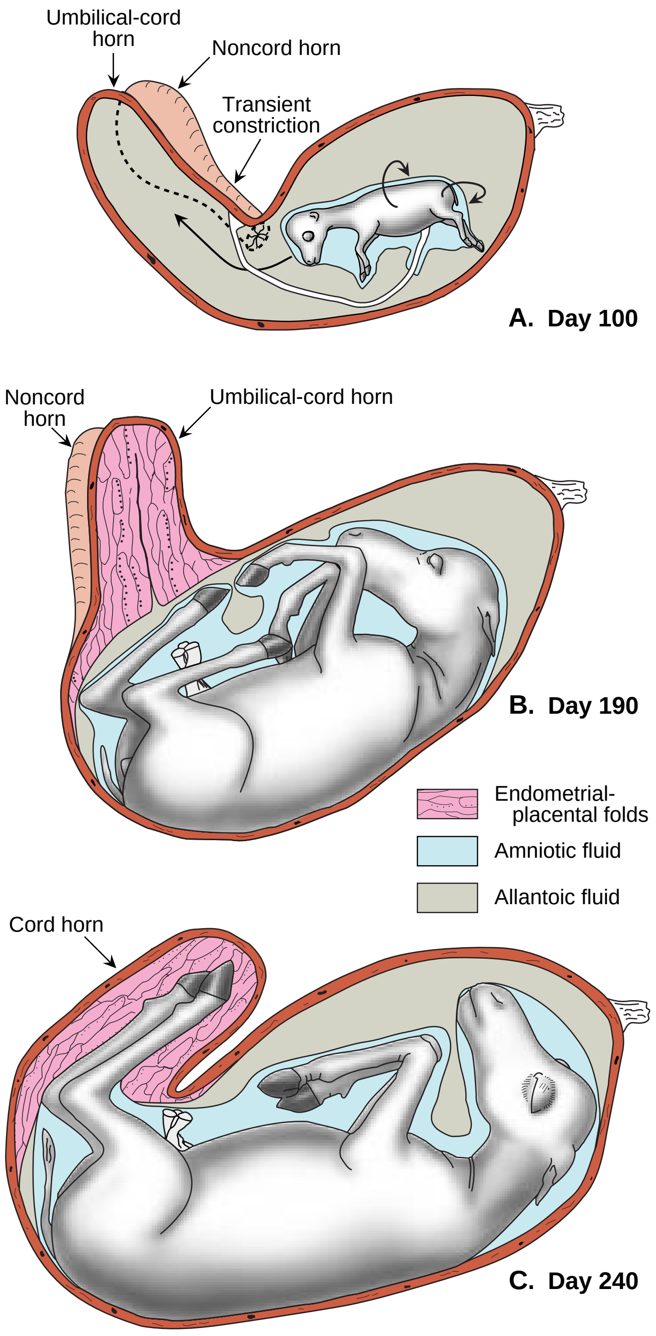


Fig. 22. Changes in fetal and uterine relationships. (A) Mobile fetus, transient uterine constriction, and shifts in allantoic fluid. (B) Fetus finds cranial presentation while confined to the uterine body by closed horns. (C) Hind limbs encased in a uterine horn; placental membranes are close to the limbs and are not shown.

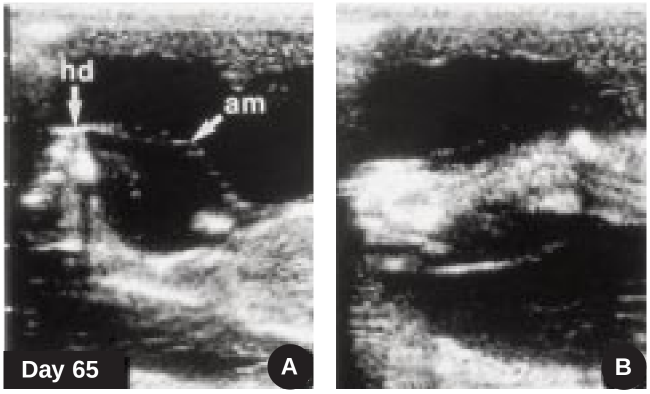


Fig. 23. (A) Fetus lying on the allantoic floor. (B) Vigorous arch of the neck, propels the fetal body into the allantoic fluid. hd, head; am, amnion.

incline of the floor of the uterine body in 46 ultrasound examinations was 40°; an incline was present in all examinations in 18 mares.[4] Comparative support for the incline hypothesis comes from a study in cattle in which final cranial presentation occurs at a similar developmental stage[52]; the fetal torso was in the perpendicular portion of the curled uterine horn at the time of establishment of cranial presentation.

Fetal mobility begins to slow after Month 4 (decreasing percentage of presentation changes between and within examinations), followed by an increased likelihood of cranial presentations after Month 5 (**Fig. 24**).[51] Percentage of cranial presentations continued to increase so that, with few exceptions, all of 10 fetuses were cranial at all weekly examinations during Months 9 to 11.

The percentage of examinations with closed uterine horns is shown (**Fig. 24**). Complete reclosure of both horns occurred in all mares. Closure was increasingly observed after Month 3, and both horns were closed by Month 8. Permanent reclosure (placental-fluid height, <5 mm) of the full lengths of uterine horns after having been dilated by conceptus fluid was discovered during ultrasound studies (**Fig. 25**).[46]

F. Encasement of Hind Limbs by the Cord Horn

After final cranial presentation, both fetal hind limbs enter a closed horn and become encased by it (**Fig. 22C**).[53] In 30 mares, all limb entries were into the umbilical-cord horn; the noncord horn remained closed and the placental membranes remained empty of fluid and fetal parts. The hind limbs begin to enter the closed cord horn during Months 7 and 8 (**Fig. 24**). The limbs can enter the horn only when the fetus is in dorsal recumbency because by this stage the horn meets the body at an acute angle (Section 3H). Initially, retraction of limbs after entry into the caudal and middle segments of the horn was common (31% incidence). Gradually the limbs progressed further into the horn (**Fig. 24**), with the hooves reaching the horn tip by Month 10 (98% of examinations). Sonograms of the closed horns are shown (**Fig. 25**). Limb retractions were not detected during Months 9-11, even though the limbs remained active, sometimes with piston-like thrusts. After partial retraction, the hocks would bump into the uterine-body wall near the horn base (**Fig. 22C**), and apparently this was the impediment that prevented re-entry of the limbs into the uterine body.

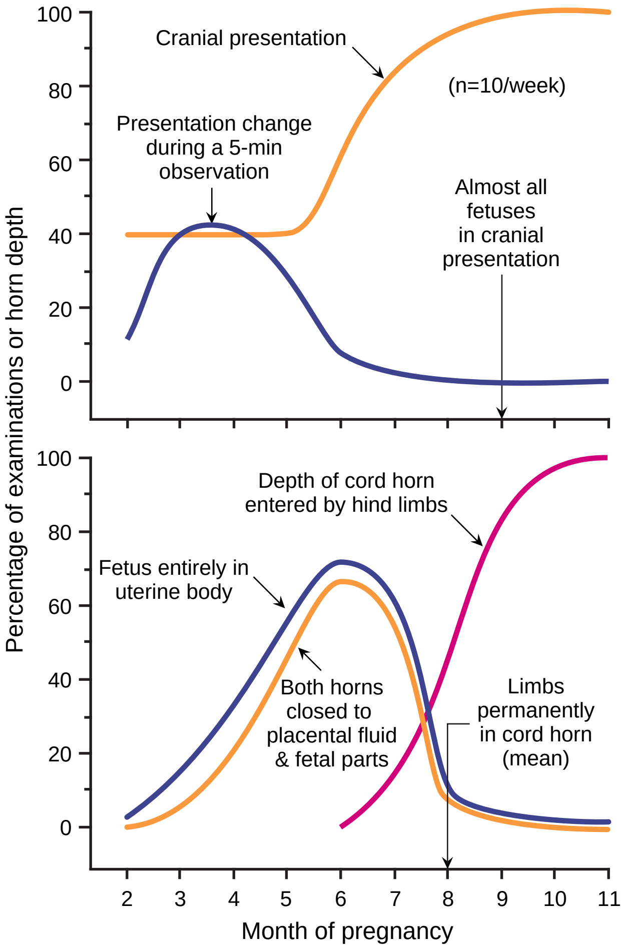


Fig. 24. Summary of data on monthly frequencies in mobility (presentation changes during a 5-minute scan), cranial presentation, and closure of uterine horns with confinement of the fetus to the uterine body and percentage of the length (depth) of the uterine horn entered by the hind limbs.

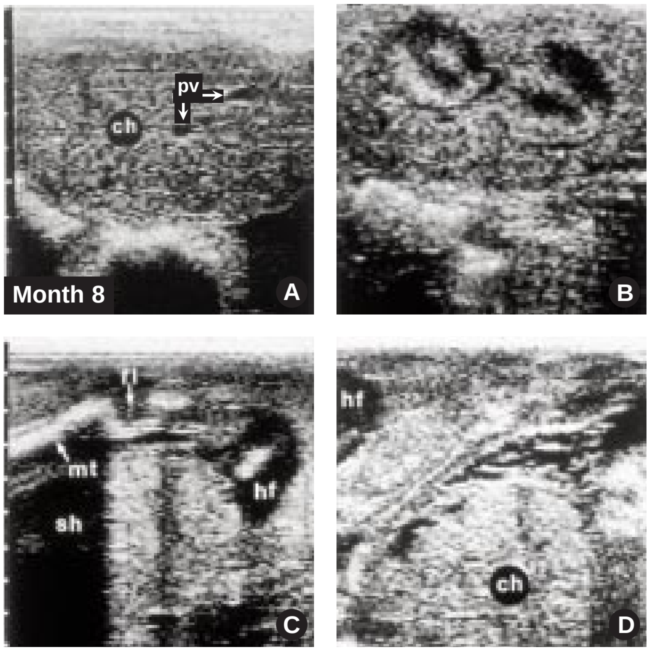


Fig. 25. Relationships between the hind limbs and cornual wall, showing the close endometrial-limb contact at Month 8. (A) A cross section of the nonlimb closed horn. (B) A cross section at the hooves showing the close contact with the endometrium. (C) Longitudinal section of a distal limb. (D) Partial overlapping of the closed nonlimb horn by the limb horn. ch, closed nonlimb horn; fl, fetlock; hf, hoof; mt, metatarsal bone; pv, placental vessels; sh, shadow of bone.

The apposition between the cornual endometrial-placental folds and the hind limbs was close during Months 7 and 8 so that intervening placental fluid >5 mm in height was not detected between the limbs and folds (**Fig. 25**).[53] During Months 9-11, the limb horn flattens and the walls become thinner and some placental fluid may re-enter the uterine horn from the uterine body (**Fig. 26**). Small pockets of fluid, >5 mm in height, between the hind limbs and cornual wall were detected in 0, 10, 25, and 63% of examinations during Months 8–11, respectively, indicating some readmittance of placental fluid.

The entrapment of the hind limbs in a horn maintains the caudal portion of the fetus on its back (**Fig. 22C**). The fetus cannot normally return to a potentially disastrous caudal presentation, despite the relatively large uterine body. Maintenance of the caudal half of the fetal torso in dorsal recumbency and entrapment of the limbs may serve to protect the umbilical cord from fetal weight and entanglement with the vigorous hind limbs. The trapping of the hind limbs in a uterine horn until parturition is an apparent species adaptation to the large uterine body and the long umbilical cord and fetal appendages. A similar trapping mechanism is not present in cattle,[52] which have a small uterine body.

Although the nonlimb horn does not accept fetal parts, the horn is probably essential for full fetal development, especially in late pregnancy. Deep prominent folds (rugae) develop in the endometrium of the nonlimb horn, providing much surface area for placental exchange (**Fig. 27,28**). The contribution of the closed nonlimb horn to placental exchange is indicated by the large blood vessels seen by ultrasound (**Fig. 25A**). The large endometrial folds extend on to the uterine-body wall at the base of each horn, but the remaining uterine-body wall and some of the caudal portion of the limb horn is stretched and thinner and does not provide for nearly as much exchange per unit area (**Figs. 27,28**). Thus, the nonlimb horn has been utilized for a major allotment of placental exchange by not subjecting it to expansion and stretching. Therefore, the postpartum former nonlimb horn involutes more rapidly and is prepared to fix (Section 2C) and accommodate a new embryo shortly after parturition—another clever, evolutionary adaptation in this species. These provisions contribute to the mare's natural ability to foal each spring, despite an 11-month gestation.

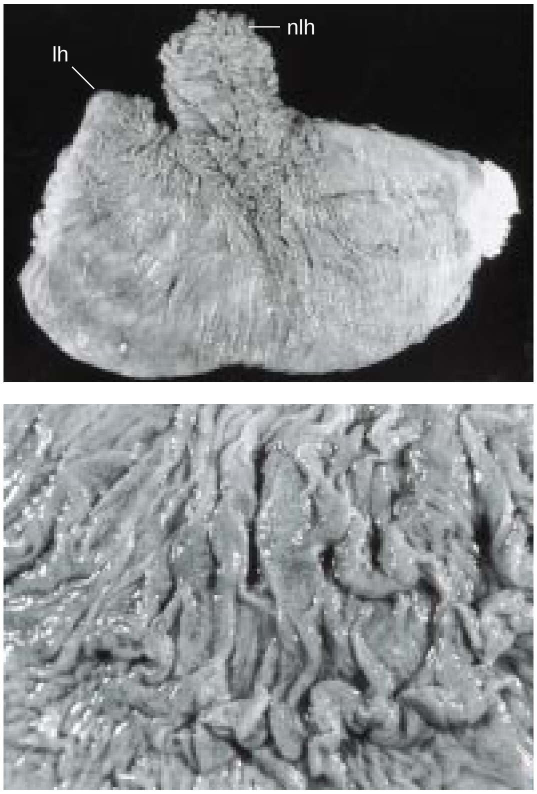


Fig. 27. Inner surface of an inverted uterus from late pregnancy showing rugae distribution. The close-up is from the nonlimb horn. Note the crypts (small black dots on the rugae) that contained the placental microcotyledons. lh, limb horn; nlh, nonlimb horn.

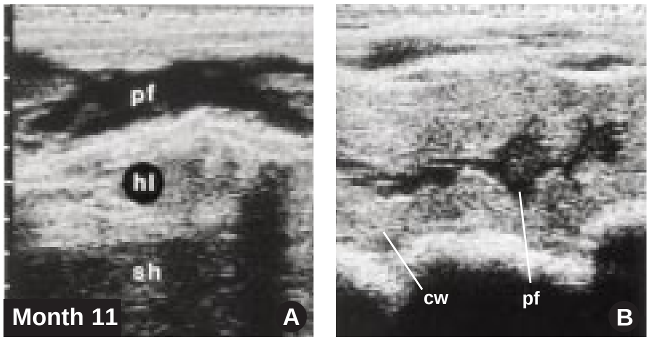


Fig. 26. Limb horn at Month 11, illustrating the re-entry of placental fluid into the horn. (A) Fetlock area of hind limb with placental fluid between it and the cornual wall. Shadowing beneath bone is extensive. (B) Longitudinal section to the side of the fetal limbs. cw, cornual wall; hl, hind limb; pf, placental fluid; sh, shadowing.

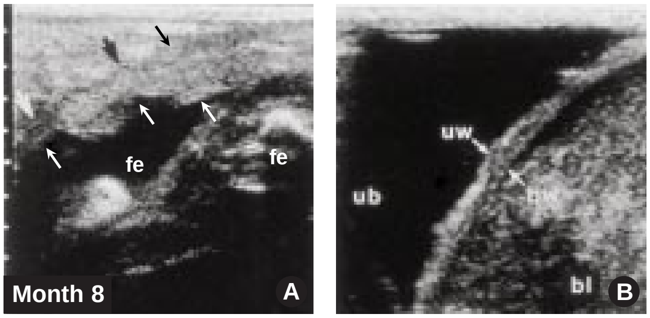


Fig. 28. Thick and thin portions of uterine-body wall. (A) Longitudinal section at the attachment of a horn to the dome-like cranial portion of the uterine body, showing the outer and inner edges of the uterine rugae between arrows. (B) Allantoic fluid in uterine body and the thin uterine-body and bladder walls. bl, bladder; bw, bladder wall; fe, fetus in uterine body; ub, uterine body; uw, uterine-body wall.

The sequential events described in Sections 3B–3F begin with the development of intrinsic fetal activity and end with the fetus in cranial presentation and dorsal recumbency maintained by encasement and entrapment of the hind limbs in a uterine horn. The intrinsic fetal activity includes whole-body movements by the time the umbilical cord lengthens and allows the fetus to reach the floor of the allantoic sac. With increasing length of the umbilical cord and an increasing proportion of placental fluid, the vigorous aquatic fetus, tethered by the umbilical cord, propels itself into the placental pool. The resulting fetal mobility increases during Months 2–4 and decreases during Months 4–9 in association with an increase and decrease, respectively, in the relative size of the pool. By Month 8, both uterine horns close, the cornual-placental fluid is added to the uterine-body placental fluid, and the fetus is entrapped in a recess-free chamber with maximal fluid availability. Apparently, the intricate mechanisms of the fetal internal ears reach a stage of development at this time that allows the fetus to respond to the incline of the floor of the uterine body, and the fetus is thereby directed toward final cranial presentation. Next, when the fetus is in dorsal recumbency, the active hind limbs thrust their way into the umbilical-cord horn. Once the limbs are encased by the horn, the fetus cannot normally return to a potentially disastrous caudal presentation.

G. Dynamics of the Uterus and Fetus during the Last Month

The changing axial or long-axis rotational positions of the cranial and caudal portions of the fetal torso and the uterine body during the last month of pregnancy will be described in reference to the face of a clock. The clock will be visualized suspended over the withers of a standing mare with the clock facing an operator standing behind the mare. For this report, the clock approach is more suitable than the traditional method of describing fetal positions[54] because axial rotation of the uterus will be included. The fetal and uterine points that will be related to the clock face are the fetal withers (cranial portion of torso), fetal tailhead (caudal portion), and the dorsal, outer surface of the uterine body between the attachments of the left and right broad ligaments (**Fig. 29**). For simplification, 12, 3, 6, and 9 o'clock will include an hour on each side. For example, when the cranial torso is upright (fetal withers toward the maternal spine), the withers will be at 12 o'clock; when the cranial torso is lying on its side (lateral position), the withers will be at 3 or 9 o'clock.

The axial position of the cranial half of the fetus was estimated by transrectal ultrasonography and palpation in 23 mares after Day 330, based on location of sternum or limbs.[4,6] The withers was at 6 o'clock (dorsal recumbency; 61%) or at either 3 or 9 o'clock (lateral recumbency; 39%). Regardless of the rotational position of the cranial torso, both forelimbs and head/neck were usually flexed (78% of fetuses), and in the remainder, at least one limb or the head was extended. Thus, about 50% (61% x 78%) were in the base position (**Fig. 29C**). The changing positions of the cranial fetus were studied in seven mares every 10 minutes for four times just before the induction of parturition.[55] The withers rotated from 12 o'clock to 3 or 9 o'clock or vice versa a mean of 2.2 times out of a possible three times in four of seven fetuses. Both forelimbs and the neck were flexed in all fetuses (**Fig. 29C**), except that extensions occurred (**Fig. 29B**) one or two times in two of seven fetuses. These findings illustrate the extensive axial-rotational ability and limb/head activity of the cranial one-half of the fetus in late pregnancy and the folly of assuming that examinations before parturition will establish the postures (forelimbs and head) and rotational position of the torso (excluding presentation) that will be present at the onset of parturition.

Examination through a flank laparotomy indicated that the uterus was at 12 o'clock in 10 of 10 mares examined on Days 227 to 295 (before the last month) and at 12 o'clock (five mares), 9 o'clock (five mares), or 3 o'clock (one mare) on Days 321 to 340 (last month) of gestation.[55] Thus, a 90° rotation to 9 or 3 o'clock was common for the caudal as well as the cranial portion of the equine fetus during the last month (**Fig. 29E**); before the last month, only the cranial portion rotated (**Fig. 29D**). During the last month, the uterine body, which contains all of the fetus except the distal half of the hind limbs (Section 3F), rests upon the lower maternal abdominal wall. As a result, tension is removed from the broad ligaments and the uterus is able to rotate to the extent allowed by the ligaments. When the uterus rotates, as it frequently does, to 3 or 9 o'clock, the right and left broad ligaments become taut and the two ovaries are brought close together. Occasional transient uterine counterclockwise rotations to 8 o'clock were observed and are apparently normal. Presumably, exaggerated uterine rotations beyond 6 o'clock can result in pathologic uterine torsions.

Although the limbs are encased in a uterine horn, the intrinsic activity of the hind limbs is equal to the activity of the forelimbs; both were active 30% of the time during continuous ultrasonic viewing in one study.[4] Occasionally, the hind limbs extend so that the hooves and horn tip are pushed caudally to the area of the fetal head or eye (**Fig. 30**) and sometimes the hind hooves are the caudal-most portion of the fetus.[53] Instances were recorded in which the hind hooves moved above and past the cervix to within 6 inches (15 cm) of the vulvar labia in ponies; the thin vaginal wall was imaged next to the cornual wall (**Fig. 30D**). Thus, during manual intravaginal prepartum examinations, fetal hooves in the vaginal fornix or dorsal to the vaginal wall for a fetus in cranial presentation may be from either the hind limbs or forelimbs. This statement, although initially startling and seemingly a contradiction for cranial presentation, can be comprehended by study of the drawing (**Fig. 29A**). In addition to extension of

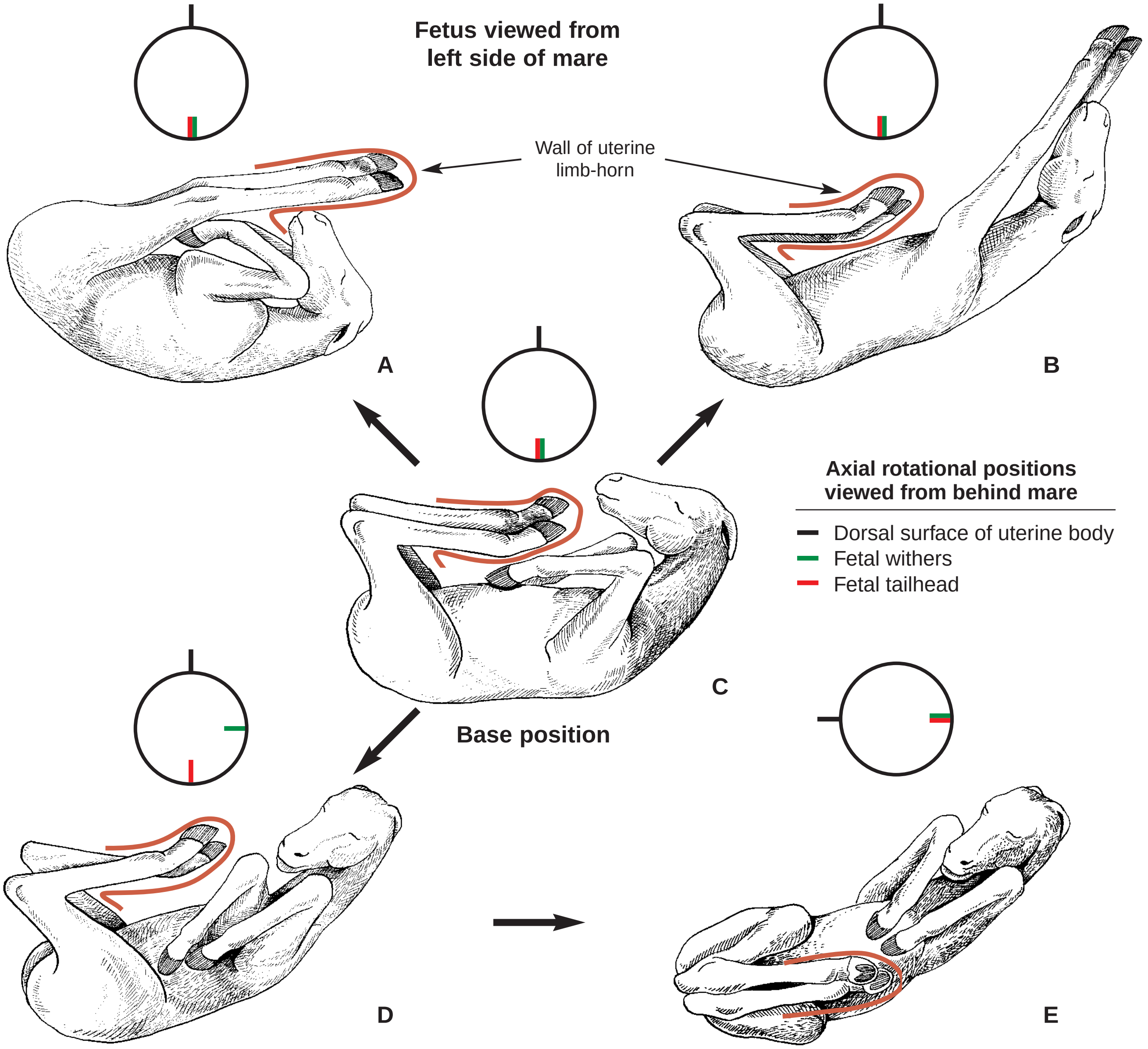


Fig. 29. Positions assumed by the fetus and uterus during the last month of pregnancy, viewed from the mare's left side. The red line represents the horn that contains the hind limbs. The markers on the circles indicate the positions of the dorsal surface of the uterine body, fetal withers, and fetal tailhead as viewed by an operator standing behind the mare. The positions (A–E) are described in the text.

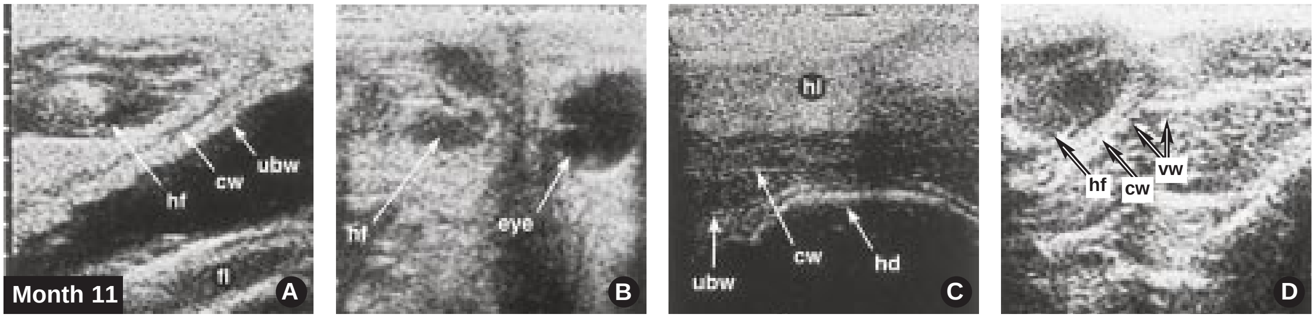


Fig. 30. Dynamics of hind limbs within the uterine horn during the last month of pregnancy. (A) Hoof at tip of horn showing cornual and body walls. (B) Hind hoof in horn near the fetal eye in the uterine body. (C) Hind limbs have extended so that the limb horn is above the fetal head. (D) Hind limbs have further extended so that the vaginal wall is next to the limb horn. cw, cornual wall; fl, forelimb; hd, head; hf, hoof; hl, hind limb; ubw, uterine body wall; vw, vaginal wall.

the limb joints, the fetal rump may elevate to force the rear hooves well into the pelvis. Hind-limb extension may explain a recent case history of a rupture at a horn tip.[56] In this regard, vigorous piston-like thrusts of the hind limbs in the uterine horn have been observed by ultrasound.[53]

At term, the volume of allantoic fluid (about 10 liters) and amniotic fluid (about 3.5 liters)[50] is small compared to the fetal mass. In the drawing of the cranial half of the fetus (**Fig. 31**), there does not appear to be enough room for extension of the head and forelimbs. However, much of the fetal maneuverability is attributable to the prepartum flaccidity of the uterine wall. Increased flaccidity of the uterine wall is suggested by the flattening and thinning of the limb horn during the last month (Section 3F). The cranial half of the fetus maneuvers and rotates within the uterine body and is not dependent on the long-axis rotational position of the uterine body. In contrast, the rotational position of the caudal half of the fetus is associated with the rotational position of the close-fitting uterus because the hind limbs are trapped in a uterine horn. The uterine wall covers the adjacent caudal fetal parts like a glove, including the hind limbs. The tailhead position can be expected to be approximately 180° from the dorsal surface of the uterine body.

H. Shaping of the Uterus

The orientation of the uterine horns to the uterine body changes dramatically as pregnancy advances.[53] Initially, the horns point cranially and dorsally (**Fig. 22A**). As the conceptus enlarges, the uterus sinks further into the intestinal viscera and the horns gradually assume a perpendicular orientation. The horns are approximately perpendicular to the upper-cranial aspect of the uterine body by Months 6 and 7 (**Fig. 22B**). As the fetus enlarges, the dome-like cranial portion of the uterine body and adjacent caudal limb horn move further cranially, and the dorsal surface of the horns (mesometrial attachment) move increasingly closer to the dorsal surface of the uterine body as shown (**Figs. 22C,31,32**). Thus, by

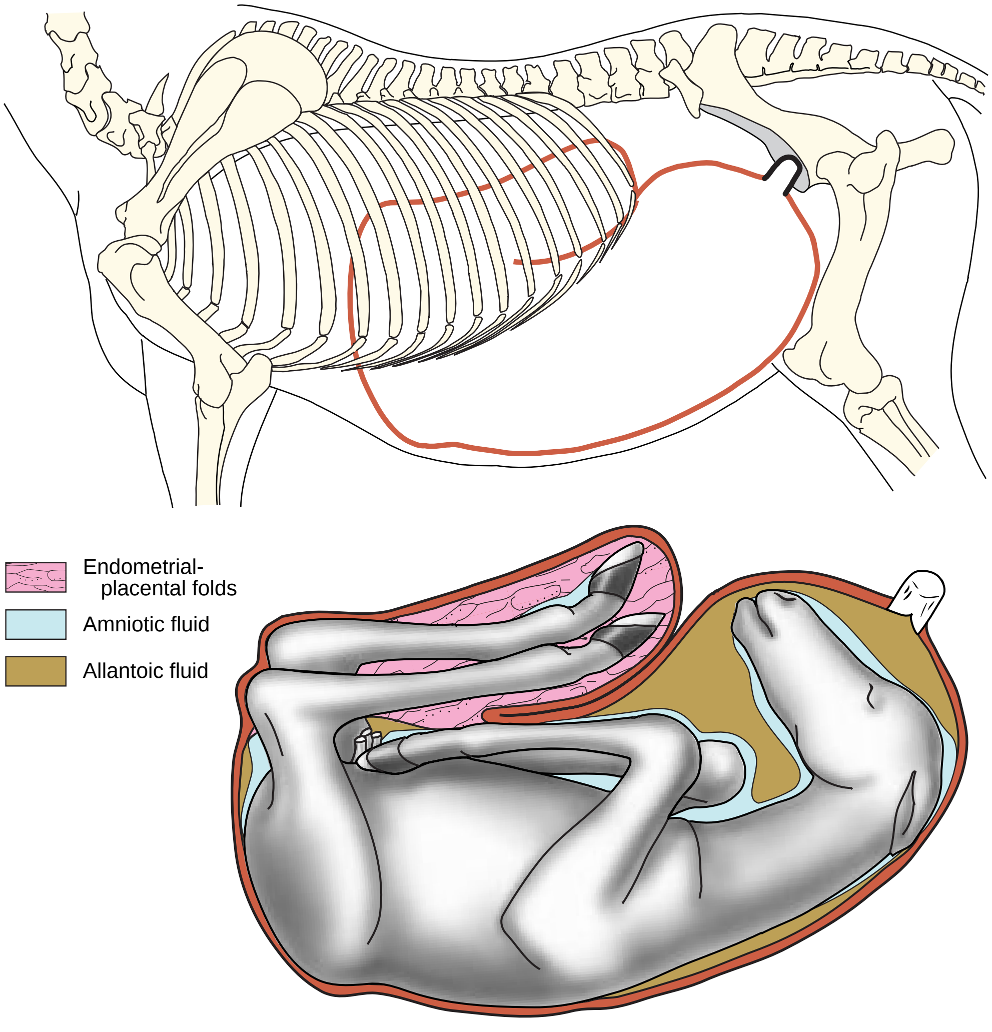


Fig.31. Relationship of the gravid uterus just before parturition to the mare's body and relationship of the fetus to the uterus.

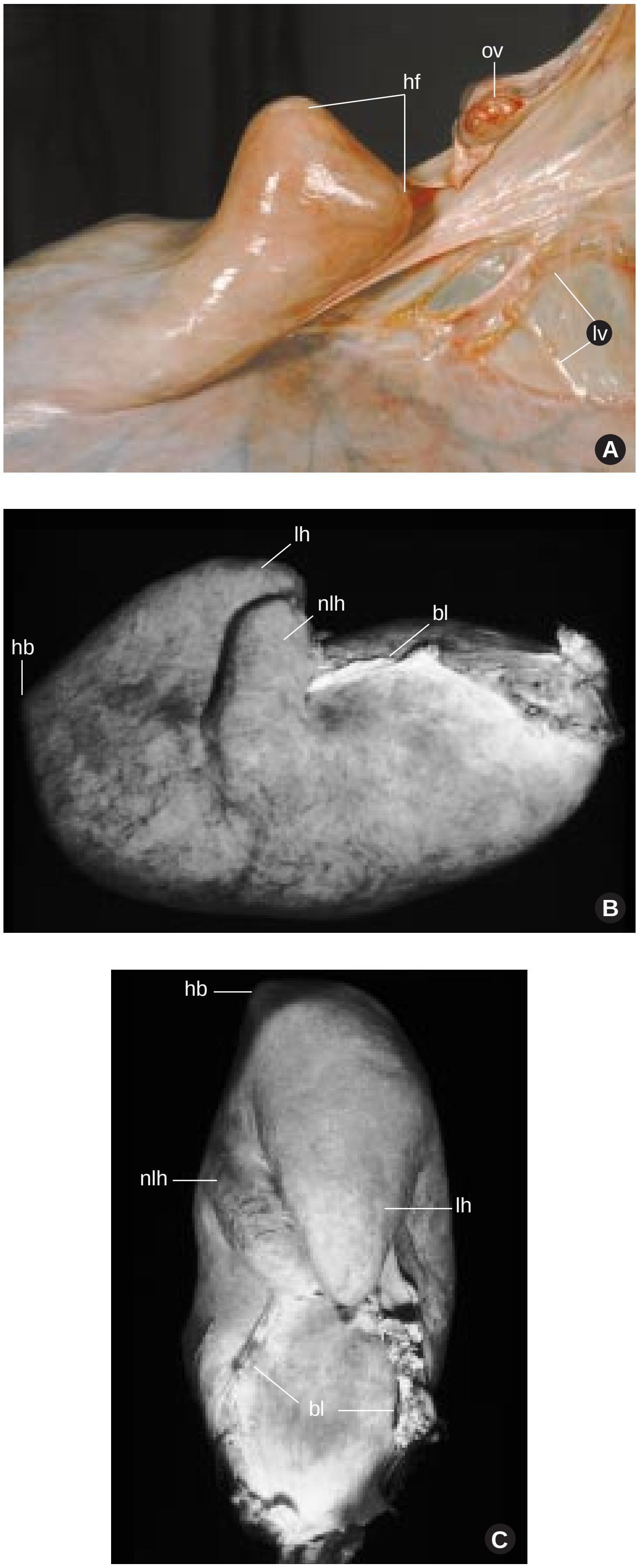


Fig. 32. Gravid uterus of Month 10. (A) Suspended uterus and stretched broad ligament. Left (B) and dorsal (C) aspects showing line of attachment of broad ligament, hock bulge, limb horn, and nonlimb horn. bl, broad ligament; hb, hock bulge; hf, hoof location; lh, limb horn; lv, lymph vessels; nlh, nonlimb horn; ov, ovary.

Month 11 the mesometrial surface of the limb horn is often in close apposition with the mesometrial surface of the uterine body; the horn lays on the surface of the uterine body with the tip of the horn directed toward the cervix. The tips of the horns and ovaries are attached by relatively short ligaments, accounting for the change in orientation of the horns as the uterine-body enlarges. The weight of the uterus and conceptus is removed from the broad ligaments as the gravid uterus assumes the support of the ventral abdominal wall. The two loosely attached horns often are in close apposition to the dorsal surface of the uterine body. The limb horn may partly overlap the nonlimb horn.[46] This arrangement often can be seen, ultrasonically (**Fig. 25D**).

In summary (Sections 3G–H), as the gravid uterus enlarges, the lower surface of the uterine body moves toward the ventral abdominal wall, the cranial dome of the uterus moves further cranially, and the tips of the horns increasingly point back toward the cervix. The cranial portion of the fetus is able to rotate to a lateral position within the uterine body. Before the last month of pregnancy, the broad ligaments minimize rotation of the uterus, and therefore the caudal portion usually does not rotate; the hind-limb horn controls the rotational position of the caudal fetus. However, when the uterine body reaches the maternal abdominal floor, the broad ligaments loosen, the horns lie freely upon the uterine body, and the cranial uterus and its contained caudal fetal torso and hind limbs are free to rotate. Therefore, during the last month the caudal portion of the fetus, as well as the cranial portion, sometimes rotates to a lateral position. The forelimbs and hind limbs remain active during the last month. The head and forelimbs sometimes extend and the hind limbs periodically extend above and beyond the cervix well into the pelvis. The most frequent fetal position in the last month is dorsal recumbency (withers and tailhead at 6 o'clock) with flexed forelimbs and head.

4. Uterine and Fetal Rotations during Parturition

The changing positions of the fetal skeleton during parturition have been studied by sequential X-rays.[57] This approach provided the first account of fetal rotation during parturition, but did not provide information on uterine rotation and was done before knowledge on the close encasement of the hind limbs by a uterine horn. We have, therefore, investigated uterine and fetal rotation in ponies during oxytocin-induced parturition.[55] Several monitoring techniques were used at various times, including ultrasonic imaging and transrectal, transvaginal, and intravaginal palpation. Occasionally, palpation through a flank laparotomy was used, especially to monitor the relationship between the initial uterine tone changes and extensions of the head and forelimbs and exiting of the hind limbs from the uterine horn.

The interval from oxytocin injection to completion of parturition was not altered significantly by the monitoring procedures, compared to six contemporary unexamined mares (mean ±standard error for interval from treatment to fetal expulsion, 38 ±8 and 46 ±8 minutes, respectively). The interval in both groups was highly variable (range, 14 to 62 minutes and 24 to 80 minutes, respectively). Much of the variation in duration of parturition among mares appeared attributable to the interval between oxytocin injection and passage of the hooves through the cervix (range, 6 to 25 minutes). Monitoring parturition events did not appear to affect the ease of birth or the health of the mare and foal. However, in two examined mares an unruptured allantochorion appeared at the labia (**Fig. 33)**.

The face of a clock will be used to describe the changing rotational positions during parturition. The withers, tailhead, and the dorsal uterine body will be related to the clock face as described (Section 3G). The clock positions will be recorded as though the operator was standing directly behind the mare looking at a clock over the mare's withers and as though the mare remained standing throughout parturition.

The relationship of the uterus and fetus to the mare's left side just before parturition is shown (**Fig. 31**). The most common fetal position at the time of oxytocin injection was dorsal recumbency (withers and tailhead at 6 o'clock) and flexed forelimbs and head (base position; **Figs. 29C,31,34A**). The hind limbs are encased by the horn so that the caudal junction of the uterine horn and uterine body is at the middle of the cannon (metatarsal) bones. The first detected event after the oxytocin injection was a generalized increase in digitally assessed uterine tone. In three mares with continuous initial tone monitoring, an increase was detected in a mean of 58 seconds after injection. Tone continued to increase for 10 to 20 minutes. The increasing tone was associated with extension of fetal head and forelimbs so that the nose and hooves were in the vicinity of the cervix (**Fig. 34B**). Extension occurred in a mean of 15 minutes, except in two fetuses in which the head and limbs were already extended at the time of oxytocin treatment and one fetus in which extensions occurred a few minutes after treatment. Head extension occurred a few minutes before limb extension. The presence of placental fluid in the uterine body likely facilitates extension and activity of the head and forelimbs, even while the tone of the uterine wall is increasing.

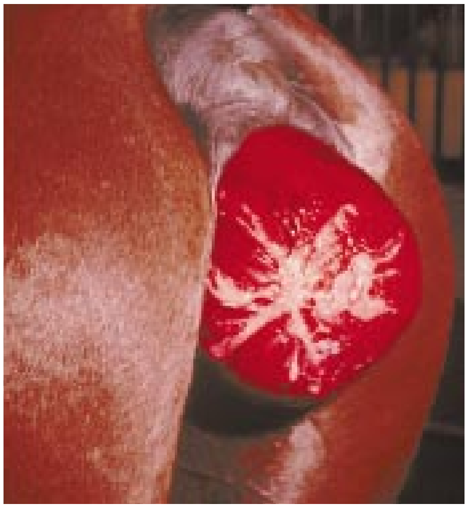


Fig. 33. Emergence of the allantochorion through the labia; the allantochorion failed to rupture internally. The cervical star (avillous area) is prominent. *Courtesy of D.C. Sharp*.

It seems likely that the increasing uterine tone was perceived by the fetus and was the signal for head and limb extension, thereby initiating the cascade of physical uterine and fetal events associated with parturition. The head usually moved to lateral recumbency during or soon after extension, as shown (**Fig. 34B**). The forelimbs and head did not return to a flexed posture after full extension; however, they were quite active during and after extension, and in a few fetuses near continuous or intermittent to-and-fro motion of the forelimbs occurred until the head was passing through the vulva. Probably as a result of the increasing uterine tone, the tip of the hind-limb horn sometimes moved from a relaxed position slightly toward the side of the uterine body to a more central position. Extension of the hind limbs occurred periodically (e.g., two times/10 minutes), especially during the time of head and forelimb extension. The tips of the hind limbs sometimes reached well into the vaginal area (Section 3F).

Rotation of the fetus during parturition was counterclockwise in seven of eight mares (**Fig. 34**), which statistically differs from 50:50. In this regard, five of six uterine rotations during the last month were counterclockwise (Section 3G). Perhaps adjacent intestinal viscera interfere with uterine rotation more effectively on the right side. Variation was considerable, but a presumed representative pattern of rotational events and uterine-fetal relationships during parturition are shown (**Fig. 34C–F**) for six of eight fetuses that initially were in the base position (**Fig. 34A**) at the time of treatment.

The rupture of the allantochorion with fluid expulsion did not occur until the fetlocks or knees were at the level of the external cervical opening. Thus, any lubricating qualities of the discharged fluid were applied to the vaginal wall as the fetal head was entering the vagina. As the nose reached the vulva, the cranial half of the torso rotated from 6 o'clock to 3 o'clock (**Fig. 34C**). Some to-and-fro rotational movements of the cranial fetus continued to occur, and slight to-and-fro rotational movements of the tailhead and uterus also began about this time. The hind limbs remained at the same depth within the uterine horn and therefore close apposition between the fetal rump and the cranial dome of the uterine body was maintained. That is, the uterus was carried along with the fetus or the fetus was expelled as the uterus contracted. The hind-limb horn was still closely applied to the central-dorsal surface of the uterine body.

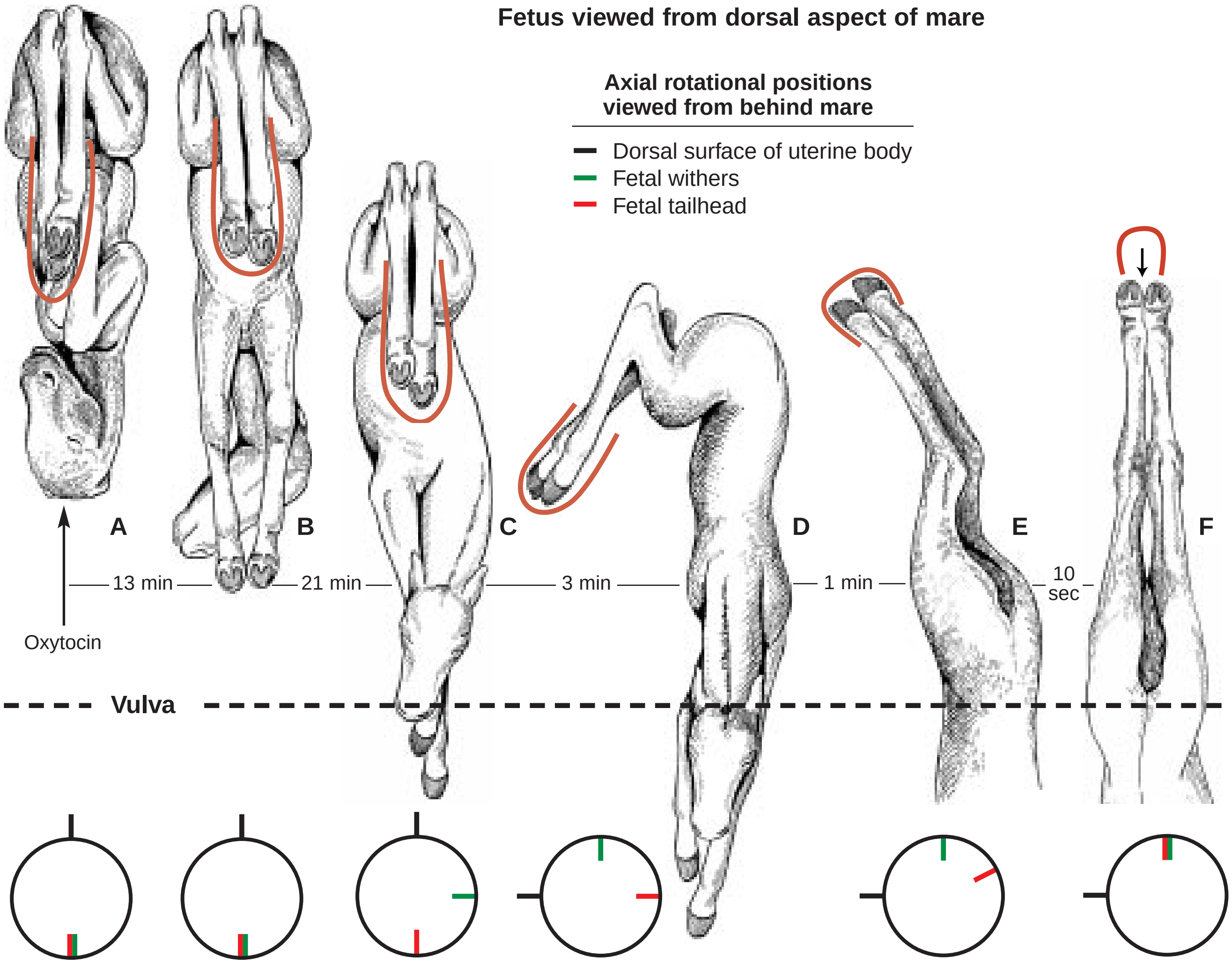


Fig. 34. Representative rotational positions of fetal parts during parturition, viewed from dorsal aspect of mare. The axial rotational position is shown on the circles for the dorsal surface of uterus, fetal withers, and fetal tailhead viewed from behind the mare. The relationship of the hind-limb horn to the limbs is shown by a red line representing the cornual wall. Representative time intervals between positions are shown. The various positions (A–F) are described in the text.

The withers continued to rotate counterclockwise and reached about 12 o'clock when the head was through the vulva (**Fig. 34D**). At this time, the uterus rotated to 9 o'clock and the tailhead to 3 o'clock. The hind-limb uterine horn moved away from the surface of the uterine body to an angle of about 45°. By this time, the uterine horn had contracted so that only about 25% of the two cannon bones were encased. The tip of the horn maintained contact with the tip of the hind hooves until near the end of parturition, except during to-and-fro activity of the limbs. The cranial dome of the uterine body continued to move caudally with the fetal rump.

When the fetal abdomen was leaving the vulva, the hind limbs extended back, sometimes in a snapping motion (**Fig. 34E**), apparently because the stifles impinged upon the pelvic brim. As a result, the hind-limb uterine horn pointed toward the mare's head so that the front surface of the limbs was toward 9 o'clock. The limbs were still encased by the horn to the level of the fetlock. As the fetus moved forward, the tailhead was still at 3 o'clock and the uterus at 9 o'clock.

As the hips began to leave the vulva, the tailhead quickly rotated to 12 o'clock and the hind limbs rotated accordingly (**Fig. 34F**). The interval from **E** to **F** was only about 10 seconds and involved the final expulsion of the hind limbs. The backward extension of the hind limbs (**Fig. 34E**) and their rotation (**Fig. 34F**) sometimes occurred as one continuous motion, depending, apparently, on the speed of discharge of the caudal half of the fetus. The uterus contracted and helped expel the fetus or was carried caudally by the hook-like posture of the flexed hind limbs in a horn (**Fig. 34A–D**). Therefore, immediately after fetal expulsion, the cranial aspect of the uterine body was only about 12 inches (30 cm) from the cervix or near the flank (**Fig. 31**).

To summarize Section 4, parturition was induced with oxytocin while monitoring the uterus and fetus. A detectable increase in uterine tone began in 1 minute and continued to increase for 10 to 20 minutes. The increasing tone appeared to elicit extension of the forelimbs and head. The cranial half of the fetus rotated to lateral recumbency (withers at 3 o'clock) about the time the nose reached the vulva. When the head was through the vulva, the withers reached 12 o'clock, and the caudal half of the fetus reached lateral recumbency (tailhead at 3 o'clock) with the hind limbs still in the uterine horn. When the fetal abdomen was leaving the vulva, the hind limbs extended, pointing toward the mare's head. The hind limbs were still encased by the horn to the level of the fetlock. As the hips left the vulva, the tailhead quickly rotated to 12 o'clock and the hind limbs rotated accordingly. The hind hooves left the horn just before complete expulsion of the foal.

5. Endometrial Cups

Formation of the endometrial cups at the beginning of the fetal stage involves restructuring of intermingling placental and maternal cells. The cups represent intricate microphysical and chemical interactions between the uterus and conceptus. A band of specialized trophoblastic cells (chorionic girdle) develops over Days 28-35[5,10] and encircles the conceptus in the avascular area of the chorion at the junction of the yolk sac and allantoic sac (Section 2E; **Fig. 35**).[58] The mature chorionic girdle at Days 35 or 36 is a 9-mm wide band that attaches to the endometrial epithelium. A small proportion[59] of the girdle cells successfully invades the maternal tissue to form the endocrine cells of the cups. The morphogenesis of the cups, as described by English workers,[60–62] is depicted (**Fig. 35**). Girdle-cell invasion involves amoeboid-like phagocytosis and digestion of the epithelial cells of the uterus, followed by migration into the endo-metrium. The differentiated cup cells produce equine chorionic gonadotropin (eCG). The process denudes the endometrial epithelium, but the epithelium regenerates quickly, leaving the cup cells of fetal origin dependent on the support of maternal tissue.

The cups generally form a longitudinal ring (as opposed to a cross-sectional ring) or horseshoe-like configuration along the lateral and medial walls of the caudal umbilical-cord horn.[5] The location of fixation (Section 2C), success of orientation (Section 2D), and resulting dorsal allantoic attachment of the umbilical cord (Section 3A) relate to the location and shape of the circle of cups with the umbilical attachment to the center of the circle. The irregular shape and sizes (a few millimeters to several centimeters) of individual cups is attributable to variation in girdle-cell contact across the irregular topography of the endometrial folds. Similar weight of the cups (approximately 10 g) in pony and horse mares[5] is attributable to a similar diameter of the conceptus at Day 36 (Section 2C) and accounts, at least in part, for the higher eCG concentrations in ponies because of the smaller blood volume.

Each cup represents a discreet package of adjacent cells with different genotypes—maternal and fetal. They are in effect, fetal allografts.[63,64] The fetal antigens are foreign to the mare, resulting in an influx of immune lymphocytes that is the most striking known example in any species of a cell-mediated immune response to pregnancy. In this regard, the immunologic aspects of the endometrial cups may be the most intensively researched aspect of mare reproductive biology. The lymphocytes have the potential of destroying the foreign tissue. It appears that the cups initially defend against the lymphocytic attack, but eventually the defensive mechanism is overcome and apparent lymphocyte-mediated demise of the cups begins.[65] The entire cup is slowly detached, beginning on about Day 80, and the degenerating cup either lies free between the endometrium and allantochorion or becomes enclosed in a fold of allantochorionic tissue (allantochorionic pouch; **Fig. 35**).[66] Some of the allantochorionic pouches are broadly attached to the inner surface of the allantochorion and some are pedunculated and dangle into the allantoic fluid. Pouches, as long as 2 cm, can sometimes be found attached to the terminal discharged placenta.

The cups produce prodigious quantities of eCG—the quantity circulating in the mare's blood at a given moment (e.g., 2 million units)[5] can stimulate the ovaries of 1,000 cows or 100,000 rabbits. At the approximate time that eCG appears in the blood, the primary corpus luteum begins to resurge in size,[67,68] increase its productivity of progesterone, and initiate the production of estradiol[69] (second luteal response to pregnancy[5]). Formation of supplementary corpora lutea is also closely and temporally related to circulating eCG concentration[68] (third luteal response to pregnancy[5]). The luteal source of progesterone and estrogen from eCG stimulation declines as the fetoplacental source inclines, suggesting that a role of eCG is to stimulate a temporary source of these steroids until the fetoplacental source is adequate. Another suggested role of the cups is to prepare immunologically the uterine environment for the later intricate and intimate microcotyledonary attachment of the allantochorionic placenta to the uterine lining.[63] An undesirable aspect of the cups from the practitioner's viewpoint is their unaltered eCG-producing capacity after loss of the conceptus.[5] The continued flood of eCG into the maternal system interferes with the re-establishment of timely ovarian cyclicity. After abortion, the cups may be ultrasonically detectable and eventually become calcified.[5]

Gradual attachment of the vast surface of the trophoblast to the uterus is another intricate physical interaction between the uterus and conceptus. Vivid descriptions of the morphogenesis of the placental-exchange units—the microcotyledons—have been published[70–73] and will not be reviewed here.

Formation and loss of endometrial cups

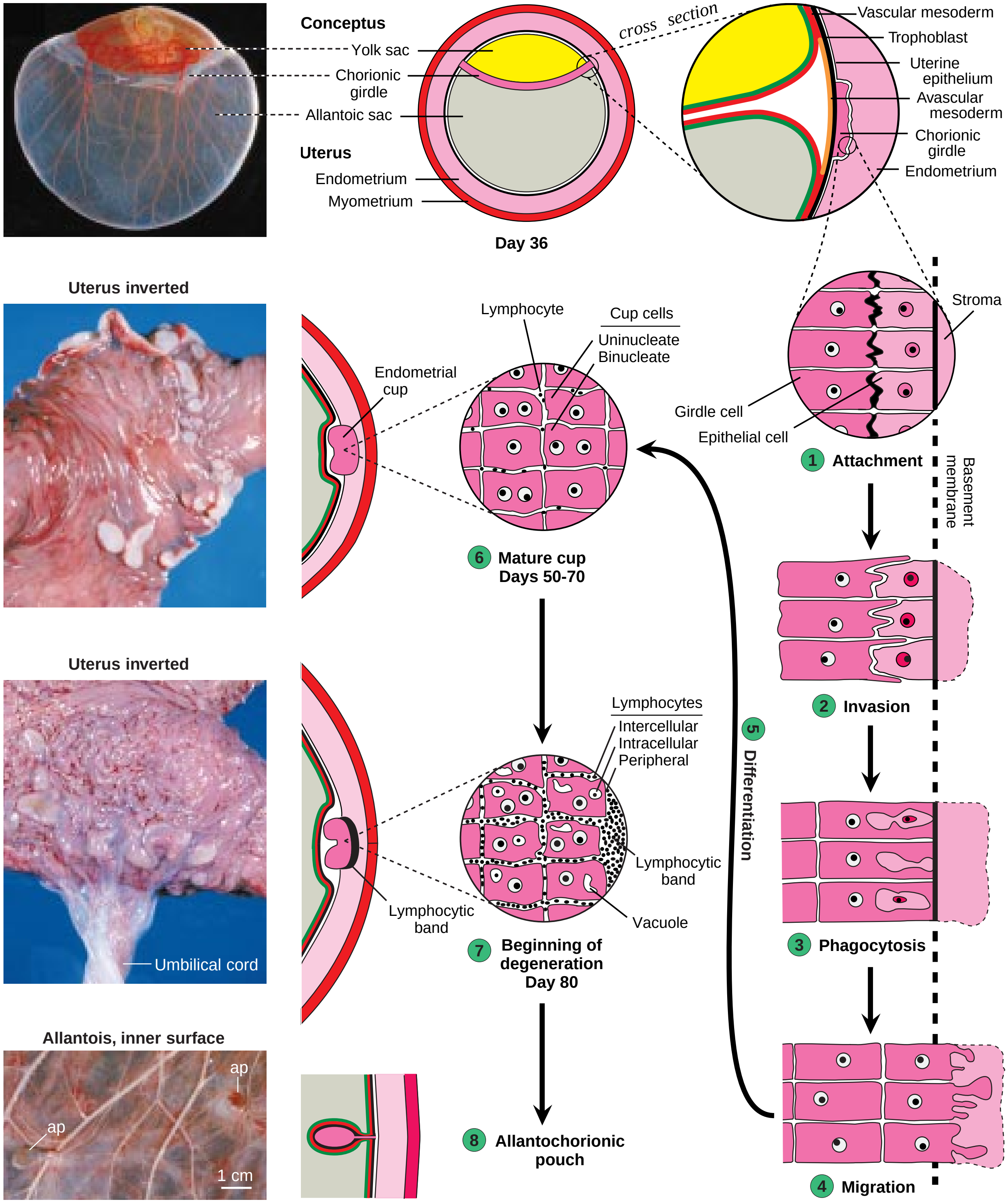


Fig. 35. Diagram and photographs depicting the formation of endometrial cups from cells of the chorionic girdle and the eventual degeneration and sloughing of cups with the formation of allantochorionic pouches. The photograph of the Day-36 embryonic vesicle shows the opposite side of the vesicle shown in Fig. 17. The photograph of the inner surface of the allantois shows a pedunculated allantochorionic pouch (left; ap) and a nonpedunculated pouch (right). Sequential events are numbered chronologically as shown with the green dots.

In summary (Section 5), the equine endometrial cups are unique structures originating from an intermingling of cells of fetal and maternal origin. The hormone-producing component originates from a band of cells that encircle the embryonic vesicle and invade the endometrium at about Day 36. The cells produce eCG—a potent ovarian stimulant when injected into other species. Because of the mixing of fetal and maternal cells, the endometrial cups are intriguing immunologic entities. They attract many lymphocytes but initially are able to defend against them. By Day 80 or so, the defensive mechanism is overcome and the cups begin to degenerate. The role of the prodigious quantities of eCG excreted into the mare's system is not clear. It is known that eCG rejuvenates the primary corpus luteum, and the resulting increased and extended production of progesterone and estrogen may serve as a transient source of these needed steroids until fetoplacental production is adequate.

6. Twins

A. Development and Reduction of Twin Embryos

Discussions of the anatomy of the embryonic vesicle and the phenomena of embryo mobility, fixation, orientation, and transition to an allantoic placenta (Section 2) set the stage for an understanding of twin development and elimination of one member of a twin set (embryo reduction). The prefixation growth rate and mobility pattern for each embryo of a twin set are similar to those for singletons, except the pair move together slightly more often than what would be expected by chance.[74]

Embryo reduction apparently does not occur before Day 11[75] or during the embryo mobility phase[74]; the reduction mechanism, described below, occurs only when the two embryos fix in close apposition in one uterine horn. When twins were similar in diameter (difference of 0–3 mm), the incidence of fixation in one horn was 62% (**Fig. 36**).[2] When twins were dissimilar in diameter (≥4 mm; equivalent to greater than one day's growth), the incidence of fixation in one horn was considerably higher (85%). Apparently, fixation of the larger vesicle serves as an impediment to continued mobility of the smaller vesicle. The incidence of reduction for twins in one horn was 73% when the vesicles were similar in diameter but increased to 100% (all of 22 sets) when dissimilar in diameter (**Fig. 36**). Furthermore, the greater the diameter dissimilarity, the sooner embryo reduction occurred.

Embryo reduction is highly efficient and unidirectional and does not involve detectable competition between members. The diameter of the survivor is not diminished, and its survival is not jeopardized. Ultrasonic study of twin-embryo morphology[76] has led to the deprivation hypothesis[77] for embryo reduction. The deprivation hypothesis states that the vesicle which is eliminated has a critical portion of its vascularized wall (three-layered; Section 2D) covered by the nonvascularized wall (two-layered) of the adjacent survivor. The doomed vesicle is deprived of adequate embryonal-maternal exchange and, therefore, regresses. The greater the proportion of the vascularized wall that is blocked, the sooner embryo reduction occurs.

It appears that the likelihood of reduction and the time required for reduction are established immediately after fixation when a singleton would be undergoing orientation. If twins are present, the uterine component of the orientation mechanism (Section 2D) massages the two vesicles against each other; variations in the final relationship between the two vesicles determines the time course for the reduction mechanism and whether both embryos will survive. The vascularized areas depicted by the colored lines on the sonograms (**Figs. 37–39**) are presumptive and are based on the deprivation hypothesis; the red and yellow colors indicate uncompromised and compromised portions of a vascularized wall, respectively.

Reduction is most acute when the vesicles are dissimilar in size; the vascularized wall of the smaller vesicle impinges well into the thin wall of the other, and, because of its relatively small size, most or all of its vascularized wall is denied endometrial contact (**Fig. 37**). The deprived smaller vesicle, therefore, undergoes early and rapid (e.g., 1 day) regression. When vesicles are similar in diameter, smaller portions of the vascularized wall of a vesicle will be compromised, resulting in its later reduction (**Fig. 38**) or

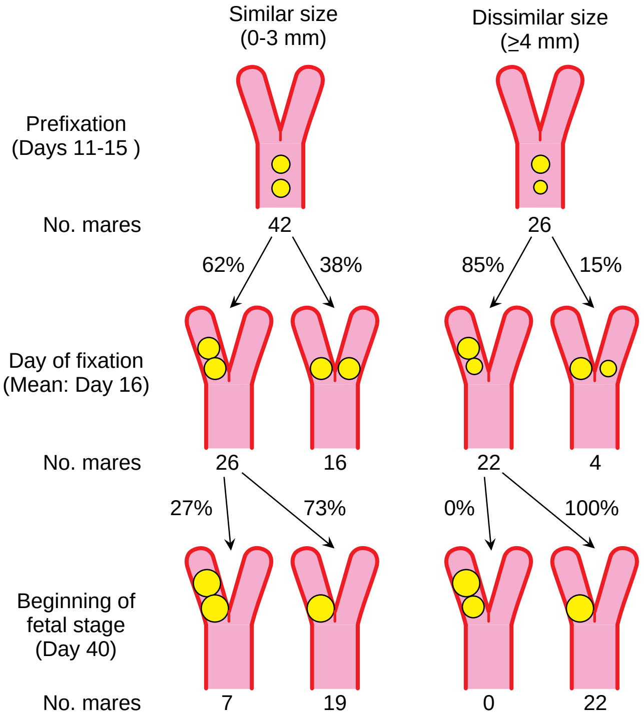


Fig. 36. Effects of similarity versus dissimilarity in diameter of twin embryonic vesicles on the frequency of fixation of both embryos in one horn and on the frequency of embryo reduction.

survival of both vesicles (**Fig. 39**). When the reduction is completed early (<Day 20), the survivor is usually orientated similar to singletons, perhaps because there was time for orientation of the survivor to occur after reduction. Disorientation of the survivor is especially likely when reduction is completed late in the embryo stage (>Day 20).

The deprivation hypothesis is consistent with the following observations made by transrectal ultrasonic imaging[3,77]: (1) embryo reduction occurs only after the two vesicles become fixed in close apposition in one horn; (2) reduction just after fixation is frequent and rapid; (3) the process is efficient and unidirectional so that the survivor is similar in size to a singleton, and the incidence of loss of both vesicles is similar to that of singletons; (4) unequal diameters favor a higher incidence of reduction, earlier reduction, and a shorter interval from initiation to completion of reduction; (5) the survivor is sometimes disorientated, as indicated by location of the embryo proper and subsequent attachment of the umbilical cord at the ventral hemisphere of the conceptus; and (6) the site of reduction is at the thin two-layered yolk-sac wall of the survivor.

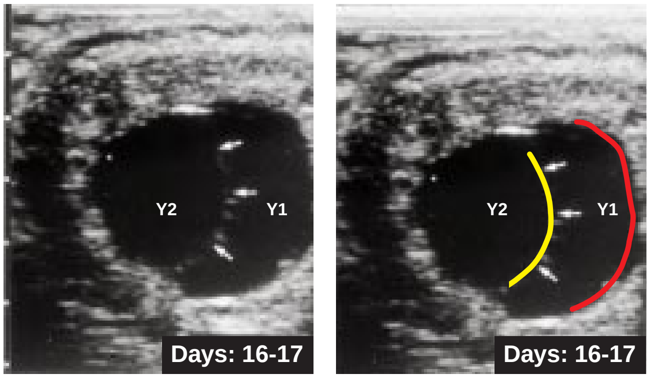


Fig. 37. Embryo reduction in twin embryos dissimilar in diameter by an equivalent of 1 day. During the uterine massage associated with the orientation mechanism of a singleton, the thick vascularized area of the yolk-sac of the smaller vesicle (arrows) is forced into the thin avascular wall of the larger vesicle. As a result, the smaller vesicle dies rapidly because of blockage of its vascular supply (depicted by the yellow line). The vascularity of the larger vessel is not compromised (depicted by the red line). Y1, yolk sac of thriving vesicle; Y2, yolk sac of doomed vesicle.

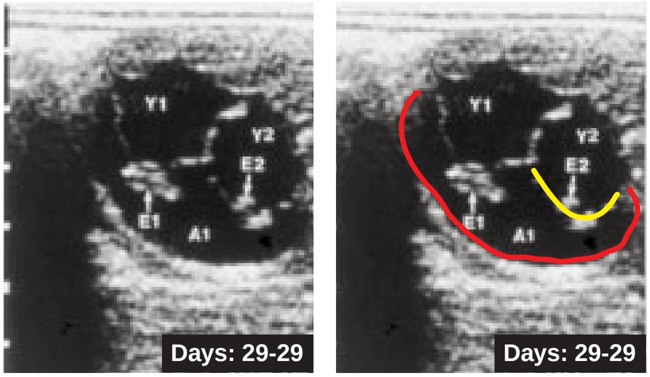


Fig. 38. Twin embryos similar in age with one vesicle undergoing reduction. The major allantoic vasculature of the doomed vesicle is blocked (yellow line), whereas the major vasculature of the survivor is not (red line). A1, A2, Y1, Y2, E1, E2, allantoic and yolk sacs and embryo propers of the thriving (1) and doomed (2) vesicles.

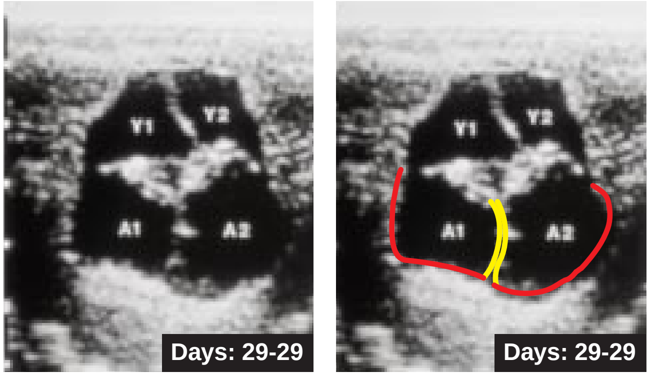


Fig. 39. Twin embryos similar in age with both vesicles surviving. Apparently each allantoic wall (red lines) has adequate exposure to the uterine lumen. The blocked areas (yellow lines) are smaller and apparently do not interfere. A1, A2, Y1, Y2, allantoic and yolk sacs of the two thriving vesicles.

B. Fetal Reduction and Loss of Both Fetal Twins

Twin embryos that do not undergo embryo reduction (**Fig. 36)** enter the fetal stage intact. Unlike the common occurrence of reduction during the embryo stage, with the survivor unharmed, both members of fetal-twin sets are likely to be lost or one or both are born weak and undersized. For example, only 13% of foals were born alive from 130 mares with twins on Day 42.[78] In a study[79] of 15 mares with a conceptus in each horn on Day 40, both fetuses died in 10 mares (67%), both survived in one mare (7%), and one died and one survived (fetal reduction) in four mares (26%). Death of both fetuses occurred during Month 2 (Days 40-60; seven mares), Month 3 (one mare), or after Month 7 (two mares). Two fetal reductions occurred early (Month 2) and the surviving foals seemed normal, whereas the remaining two reductions occurred late (Month 11) and the survivors were undersized.

The first day of apposition of the two allantochorions was studied in seven of the 15 mares by daily examination.[79] Apposition occurred on mean Day 46, and fetal reduction (two mares) or loss of both fetuses (two mares) occurred 1 or 2 days later. These limited results support the conclusion[80] that death or stunting of one or both twin fetuses results from competition, resulting from interference with vessel function in the area of apposition of the two allantochorions. That is, twin fetuses are subjected to two-directional competition (both are affected), whereas twin embryos undergo one-directional deprivation (only one is affected). However, if fetal reduction occurs immediately after apposition of the allantochorions, there may be no detectable negative effect on the survivor.

The relationship between the two sets of hind limbs and their encasement by uterine horns, as described for singletons (Section 3F), has not been determined. The placental exchange that would occur if one horn does not contain hind limbs may be a positive factor in survival considerations because of the considerable placental exchange in the noncord horn (Section 3F). Specific information on the outcome of fetal twins when both fixed in one horn is not available.

The area of apposition of the two allantochorions is useful for ultrasonic diagnosis of twins (**Fig. 40**).[79] The double membrane in the area of apposition can be called the twin membrane because of its diagnostic potential even in late pregnancy when twins might otherwise be missed. Thickness of the twin membrane was about 3 mm at Month 7 and gradually increased to 15 mm near the end of pregnancy. The two-layered composition of the membrane (one layer for each allantochorion) became distinct at Months 6 and 8 with a 5.0-MHz and 3.5-MHz transducer, respectively; the two layers were separated by a hypoechoic layer.

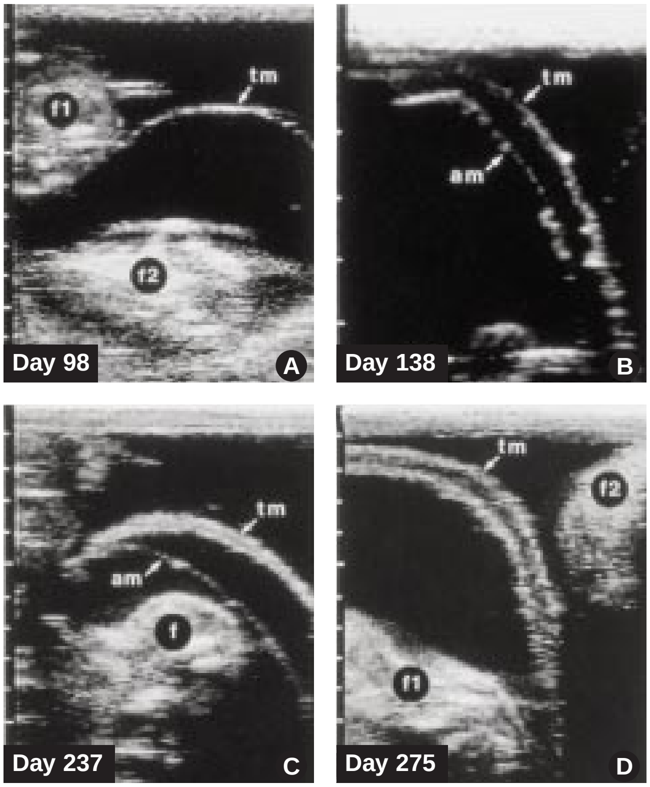


Fig. 40. Twin membrane (apposed layers of the two allantochorions) at various days. A 5.0-MHz transducer was used for image B and a 3.5-MHz transducer for images A, C, D. With the 3.5-MHz transducer, a two-walled appearance of the twin membrane was beginning at Day 237 (C) and was distinct at Day 275 (D). am, amnion; f, f1, f2, fetuses; tm, twin membrane.

In conclusion (Section 6), embryo reduction occurs when the two embryos are fixed in apposition in the same uterine horn. The occurrence, rapidity, and time of reduction depends upon the proportion of the vascularized wall of the doomed embryo that is covered by the nonvascularized wall of the survivor (deprivation hypothesis). When the mobile vesicles differ in diameter by ≥4 mm, unilateral fixation is more likely and embryo reduction occurs more frequently and rapidly. The reduction process is highly efficient and unidirectional. During the fetal stage, successful fetal reduction may occur soon after the two allantochorions touch. Thereafter, fetal loss or stunting involves both fetuses to a variable extent and is bidirectional. The apposed area of the two chorions is useful for diagnosing the presence of twins.

7. Fetal Hoof Pads

Foals are born with prominent cartilage-like or hard gel-like pads or processes on the ground surface of the four hooves (**Fig. 41**). A scientific report on the origin, nature, and function of these intriguing structures was not found. The pads are noted herein with the speculation that they provide protection for the uterine wall and placenta, especially for the vascularized amnion, against the vigorous activity of the four limbs. Many of the hoof pads in newborn ponies are in the form of prominent processes as shown (**Fig. 41**). In some foals (incidence not established) the pads have a more solid form as shown in the drawing (**Fig. 31**). The pads are shed soon after the foal begins to walk; in the example (**Fig 41**), some of the material was shed in the form of nearly complete processes

Fig 41. (A,B,C) Various views of the processes of foot pads of a newborn foal before standing and (D) 30 minutes after standing.

by 30 minutes. These structures raise questions concerning their time and mechanism of origin and their composition. They are shed rapidly after birth, yet withstand impact from limb thrusts against the amnion before birth; stretching of the amnion in response to limb movements is observed commonly by videoendoscopy. Basic and applied research on these structures is needed. Evaluation of the pads may provide information on foal maturity at birth and subsequently on walking activity.

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References

1. Ginther OJ. Dynamic physical interactions between equine embryo and uterus. *Equine Vet J Suppl* 1985;3:41–47.
2. Ginther OJ. Twin embryos in mares I: from ovulation to fixation. *Equine Vet J* 1989;21:166–170.
3. Ginther OJ. Twin embryos in mares II: post fixation embryo reduction. *Equine Vet J* 1989;21:171–174.
4. Ginther OJ. Equine physical utero-fetal interactions: A challenge and a wonder for the practitioner. *J Equine Vet Sci* 1994;14:313–318.
5. Ginther OJ. *Reproductive biology of the mare: basic and applied aspects*. 2nd ed. Cross Plains, Wisconsin: Equiservices, 1992.
6. Ginther OJ. *Ultrasonic imaging and animal reproduction: Book 2. Horses*. Cross Plains, WI: Equiservices Publishing, 1995.
7. Betteridge KJ, Eaglesome MD, Mitchell D, Flood PF, Beriault R. Development of horse embryos up to twenty-two days after ovulation: observations on fresh specimens. *J Anat* 1982;135:191–209.
8. Betteridge KJ. The structure and function of the equine capsule in relation to embryo manipulation and transfer. *Equine Vet J Suppl* 1989;8:92–100.
9. Chu JWK, Sharom FJ, Oriol JG, Betteridge KJ, Cleaver BD, Sharp DC. Biochemical changes in the equine capsule following prostaglandin-induced pregnancy failure. *Mol Reprod Devel* 1997;46:286-295.
10. Enders AC, Liu IKM. Lodgement of the equine blastocyst in the uterus from fixation through endometrial cup formation. *J Reprod Fert Suppl* 1991;44:427–438.
11. Ginther OJ. The twinning problem: From breeding to day 16, in *Proceedings*. Am Assoc Equine Pract 1983;11–26.
12. Ginther OJ. *Ultrasonic imaging and animal reproduction: Book 1. Fundamentals*. Cross Plains, WI: Equiservices Publishing, 1995.
13. Griffin PG, Ginther OJ. Effects of the embryo on uterine morphology and function in mares. *Anim Reprod Sci* 1993;31:311–329.
14. Leith GS, Ginther OJ. Characterization of intrauterine mobility of the early conceptus. *Theriogenology* 1984;22:401–408.
15. Ginther OJ. Mobility of the early equine conceptus. *Theriogenology* 1983;19:603–611.
16. Ginther OJ. Intrauterine movement of the early conceptus in barren and postpartum mares. *Theriogenology* 1984;21:633–643.
17. Cross DT, Ginther OJ. Uterine contractions in nonpregnant and early pregnant mares and jennies as determined by ultrasonography. *J Anim Sci* 1988; 66:250–254.
18. Gastal MO, Gastal EL, Kot K, Ginther OJ. Factors related to the time of fixation of the conceptus in mares. *Theriogenology* 1996;46:1171–1180.
19. Griffin PG, Ginther OJ. Uterine contractile activity in mares during the estrous cycle and early pregnancy. *Theriogenology* 1990;34:47–56.
20. Bonafos LD, Carnevale EM, Smith CA, Ginther OJ. Development of uterine tone in nonbred and pregnant mares. *Theriogenology* 1994;42:1247–1255.
21. Leith GS, Ginther OJ. Mobility of the conceptus and uterine contractions in the mare. *Theriogenology* 1985;22:401–408.
22. Douglas RH, Ginther OJ. Concentration of prostaglandins F in uterine venous plasma of anesthetized mares during the estrous cycle and early pregnancy. *Prostaglandins* 1976;11:251–260.
23. Douglas RH, Ginther OJ. Effect of prostaglandin F2a on length of diestrus in mares. *Prostaglandins* 1972;2:265–268.
24. Ginther OJ. Local versus systemic uteroovarian relationships in farm animals. *Acta Vet Scand Suppl* 1981;77:103–115.
25. Ginther OJ. Comparative anatomy of uteroovarian vasculature. *Vet Scope* 1976;20:3–17.
26. Ginther OJ, First NL. Maintenance of the corpus luteum in hysterectomized mares. *Am J Vet Res* 1971;32:1687–1691.
27. Ginther OJ, Woody CO, Mahajan S, Janakiraman K, Casida LE. Effect of oxytocin administration on the oestrous cycle of unilaterally hysterectomized heifers. *J Reprod Fert* 1967;14:225–229.
28. Douglas RH, Ginther OJ. Route of prostaglandin F2a injection and luteolysis in mares. *Proc Soc Exper Biol Med* 1975;148:263–269.
29. Douglas RH, Del Campo MR, Ginther OJ. Luteolysis following carotid or ovarian arterial injection of prostaglandin F2a in mares. *Biol Reprod* 1976;14:473-477.
30. Kastelic JP, Adams GP, Ginther OJ. Role of progesterone in the mobility, fixation, orientation and maintenance of the equine conceptus. *Theriogenology* 1987;27:655–663.
31. McDowell KJ, Sharp DC, Grubaugh W, Thatcher WW, Wilcox CJ. Restricted conceptus mobility results in failure of pregnancy maintenance in mares. *Biol Reprod* 1988;39:340–348.
32. Ginther OJ. Fixation and orientation of the early equine conceptus. *Theriogenology* 1983;19:613–623.
33. Feo JC. Contralateral implantation in mares mated during post partum oestrus. *Vet Rec* 1980;106:368, Abstract.

34. Griffin PG, Ginther OJ. Uterine morphology and function in postpartum mares. *J Equine Vet Sci* 1991;11:330–339.
35. Bergfelt DR, Ginther OJ. Ovarian, uterine and embryo dynamics in horses versus ponies. *J Equine Vet Sci* 1996; 16:66–72.
36. Griffin PG, Ginther OJ. Dynamics of uterine diameter and endometrial morphology during the estrous cycle and early pregnancy in mares. *Anim Reprod Sci* 1991;25:133–142.
37. Hayes KEN, Ginther OJ. Role of progesterone and estrogen in development of uterine tone in mares. *Theriogenology* 1986;25:581–590.
38. Van Niekerk CH. I. Early clinical diagnosis of pregnancy in mares. *J So Afr Vet. Med Assoc* 1965;36:53–58.
39. Carnevale EM, Ginther OJ. Relationships of age to uterine function and reproductive efficiency in mares. *Theriogenology* 1992;37:1101–1115.
40. Zavy MT, Mayer R, Vernon MW, Bazer FW, Sharp DC. An investigation of the uterine luminal environment of non-pregnant and pregnant pony mares. *J Reprod Fert Suppl* 1979;27:403–411.
41. Bessent C, Cross DT, Ginther OJ. Effect of exogenous estradiol on the mobility and fixation of the early equine conceptus. *Anim Reprod Sci* 1988;16:159–167.
42. Gastal MO, Gastal EL, Torres CAA, Ginther OJ. Transvaginal intrauterine injections in mares: effect of prostaglandin E2. *Theriogenology* 1998;49:258. Abstract.
43. Watson ED, Sertich PL. Prostaglandin production by horse embryos and the effect of co-culture of embryos with endometrium from pregnant mares. *J Reprod Fert* 1989;87:331–336.
44. Ginther OJ. Embryonic loss in mares: Nature of loss after experimental induction by ovariectomy or prostaglandin F2α. *Theriogenology* 1985;24:87–98.
45. Gastal MO, Gastal EL, Torres CAA, Ginther OJ. Effect of oxytocin, prostaglandin F2α, and clenbuterol on uterine dynamics in mares. *Theriogenology* 1998. In press.
46. Ginther OJ. Equine fetal kinetics: Allantoic-fluid shifts and uterine-horn closures. *Theriogenology* 1993;40:241–256.
47. Griffin PG, Ginther OJ. Role of the uterus in allantoic fluid shifts and fetal mobility in mares. *Anim Reprod Sci* 1993;31:301–310.
48. Griffin PG, Ginther OJ. Uterine and fetal dynamics during early pregnancy in mares. *Am J Vet Res* 1991;52:298–306.
49. Ginther OJ, Adams GP. Equine fetal mobility as observed by video-imaging endoscopy. *Compendium* 1989;10:1275–1280.
50. Rossdale PD, Ricketts SW. *Equine stud farm medicine.* 2nd ed. Baltimore: Williams and Wilkins Co., 1980.
51. Ginther OJ, Griffin PG. Equine fetal kinetics: Presentation and location. *Theriogenology* 1993;40:1–11.
52. Ginther OJ. *Ultrasonic imaging and animal reproduction: Book 3. Cattle.* Cross Plains, WI: Equiservices Publishing, 1998.
53. Ginther OJ, Williams D, Curran S. Equine fetal kinetics: Entry and retention of fetal hind limbs in a uterine horn. *Theriogenology* 1994;41:795–807.
54. Roberts SJ. *Veterinary obstetrics and genital diseases.* Woodstock, Vermont: S. J. Roberts, 1986.
55. Ginther OJ, Curran S. Uterine and fetal rotation during parturition in mares. Unpublished.
56. Dascanio JJ, Ball BA, Hendrickson DA. Uterine tear without a corresponding placental lesion in a mare. *J Am Vet Med Assoc* 1993;202:419–420.
57. Jeffcott LB, Rossdale PD. A radiographic study of the fetus in late pregnancy and during foaling. *J Reprod Fert Suppl* 1979;27:563–569.
58. Stewart F, Lennard SN, Allen WR. Mechanisms controlling formation of the equine chorionic girdle. *Biol Reprod Mono* 1995;1:151–159.
59. Enders AC, Liu IKM. Trophoblast-uterine interactions during equine chorionic girdle cell maturation, migration, and transformation. *Am J Anat* 1991;192:366–381.
60. Allen WR, Hamilton DW, Moor RM. The origin of equine endometrial cups. II. Invasion of the endometrium by trophoblast. *Anat Rec* 1973;177:485–501.
61. Allen WR, Moor RM. The origin of the equine endometrial cups. I. Production of PMSG by fetal trophoblast cells. *J Repro Fert* 1972;29:313–316.
62. Hamilton DW, Allen WR, Moor RM. The origin of equine endometrial cups. III. Light and electron microscopic study of fully developed equine endometrial cups. *Anat Rec* 1973;177:503–518.
63. Lunn P, Vagnoni KE, Ginther OJ. The equine immune response to endometrial cups. *J Reprod Immunol* 1997;34:203–216.
64. Antczak DF, Allen WR. Maternal immunological recognition of pregnancy in equids. *J Reprod Fert Suppl* 1989;37:69–78.
65. Allen WR. Immunological aspects of the endometrial cup reaction and the effect of xenogeneic pregnancy in horses and donkeys. *J Reprod Fert Suppl* 1982;31:57–94.
66. Clegg MT, Boda JM, Cole HH. The endometrial cups and allantochorionic pouches in the mare with emphasis on the source of equine gonadotrophin. *Endocrinology* 1954;54:448–463.
67. Bergfelt DR, Pierson RA, Ginther OJ. Resurgence of the primary corpus luteum during pregnancy in the mare. *Anim Reprod Sci* 1989;21:261–270.
68. Squires EL, Garcia MC, Ginther OJ. Effects of pregnancy and hysterectomy on the ovaries of pony mares. *J Anim Sci* 1974;38:823–830.
69. Daels DF, DeMoraes JJ, Stabenfeldt GH, Hughes JP, Lasley BL. The corpus luteum: source of oestrogen during early pregnancy in the mare. *J Reprod Fert Suppl* 1991;44:501–508.
70. Samuel CA, Allen WR, Steven DH. Studies on the equine placenta. II. Ultrastructure of the placental barrier. *J Reprod Fert* 1976;48:257–264.
71. Samuel CA, Allen WR, Steven DH. Studies on the equine placenta. III. Ultrastructure of the uterine glands and the overlaying trophoblast. *J Reprod Fert* 1977;51:433–437.
72. Steven DH, Samuel CA. Anatomy of the placental barrier in the mare. *J Reprod Fert Suppl* 1975;23:579–582.
73. Björkman N. *An atlas of placental fine structure.* Baltimore: Williams and Wilkins Co., 1970.
74. Ginther OJ. Mobility of twin embryonic vesicles in mares. *Theriogenology* 1984;22:83–95.
75. Ginther OJ, Bergfelt DR. Embryo reduction before Day 11 in mares with twin conceptuses. *J Anim Sci* 1988;66:1727–1731.
76. Ginther OJ. Postfixation embryo reduction in unilateral and bilateral twins in mares. *Theriogenology* 1984;22:213–223.
77. Ginther OJ. The nature of embryo reduction in mares with twin conceptuses: Deprivation hypothesis. *Am J Vet Res* 1989;50:45–53.
78. Pascoe RR. Methods for the treatment of twin pregnancy in the mare. *Equine Vet J* 1983;15:40–42.
79. Ginther OJ, Griffin PG. Natural outcome and ultrasonic identification of equine fetal twins. *Theriogenology* 1994;41:1193–1199.
80. Jeffcott LB, Whitwell KE. Twinning as a cause of foetal and neonatal loss in the Thoroughbred mare. *J Comp Path* 1973;83:91-106.