Morphologic stages of the equine embryo proper on days 17 to 40 after ovulation

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Objective—To describe the gross and histologic changes that develop in the equine embryo proper (ie, the portion of the embryo that becomes the fetus) from days 17 to 40 after ovulation and to compare the external features of equine embryos with those of porcine, ovine, and human embryos.

Sample Population—34 embryos collected from mixed-breed pony mares.

Procedure—External features for each embryo proper, including length, number of branchial arches, growth of appendages, face and head features, and body features, were examined, using a dissecting microscope, for embryos collected on days 17 to 40. Internal features were histologically examined by serially sectioning embryos collected on days 20 to 35.

Results—Number of embryos recovered for each day ranged from 1 to 5. The initial detection of features was not related closely to age; typically, the first attainment of a given body length or characteristic varied over a 3-day period among embryos. Similarly, the period during which individual characteristics for a given Carnegie stage were attained ranged from 3 to 6 days. Age at first appearance of a characteristic was greater for equine embryos than ages reported for ovine and porcine embryos but less than for human embryos. Indicators of age included number of pairs of branchial arches, all limb buds present, retinal pigmentation, and prominence of the pontine flexure.

Conclusions—No embryologic structures or changes were found that could be considered unique to equine embryos on days 17 to 40 after ovulation. (*Am J Vet Res* 2001;62:1358–1364)

In the equine species, the term embryo has been used to define the whole conceptus (fertilized or cleaved ovum, embryonic vesicle) and the embryo proper (portion that becomes the fetus or foal). In this report, the term embryo refers exclusively to the embryo proper, unless stated otherwise. Agreement on the use of the terms embryo and fetus in the equine species is not firm. In this report, based on a review of terminology,[1] the nominal day of transition for the first use of fetal terms will be 40 days after ovulation. Many morphologic and functional transitions occur near this time, including the beginning of intrinsic muscular activity (eg, movement of head), formation of an umbilical cord, and the invasion of chorionic cells into the endometrium.[1]

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Results of 2 detailed morphologic studies of equine embryos have been reported; each study used a single embryo. One study[2] was of an embryo obtained 21 days after the end of estrus, and the report included a review of studies from the 19th century. The large discrepancy in age of the embryo when based on day of breeding or a day during the long estrus (6 or 7 days) was discussed. The other study[3] was of an embryo of unknown age that was 12.5 mm in length. The age of the embryo was approximately 30 days after ovulation (day 30) on the basis of a comparison of the photographs and drawings to those in another report[1] and on embryo lengths obtained by use of ultrasonography.[4] Results of a study[5] that extended from 20 days after breeding to parturition have been reported, but the morphologic descriptions of the embryo proper were limited. Other reports[6-8] have dealt primarily with the placental membranes but included notes on the embryo proper, and another report[9] listed selected external features of the embryo proper. All of these reports used intervals of several days or more for obtaining specimens. Daily detailed descriptions of the entire conceptus have been made for days 1 to 6 and for primarily the outer investments of the conceptus until day 22.[10] A detailed description from many equine embryos estimated to be 21 to 49 days of age has been reported[11] but is limited to cardiogenesis with emphasis on the channeling of ventricular outflow. Other reports on specialized areas of equine embryos concern the embryonic vascularization of the pituitary gland[12,13] and the morphologic development and migratory pathway of putative primordial germ cells.[14] A systematic morphologic study of the equine embryo proper, with specimens obtained at frequent intervals and based on a reliable reference point for aging (eg, ovulation), apparently is lacking.

Earlier researchers concluded that vertebrate embryos of various species pass through nearly identical morphologic stages; however, more recent assessments have refuted this concept.[15] Nevertheless, information on the ages of embryos at similar morphologic stages among species is useful for comparative work, especially in designing experimental protocols. For example, knowledge of the embryonic stage for first appearance of primordial germ cells in a species may be useful in searching for the first appearance of such cells in another species. For this purpose, several staging systems have been developed, including the Theiler[16] and Carnegie[17] systems. The Carnegie staging system was developed from studies of human embryos and has been adapted to several species of primates, rodents, birds, and exotic animals and, among farm animals, to pigs and sheep.[18] The relationships of the features of equine embryos to the staging systems used for other species have not been reported.

The purpose of the study reported here was to describe the changes in external features that develop in equine embryos from days 17 to 40 days after ovulation and the histologic features that develop from days 20 to 35. External features were compared with those reported for pigs, sheep, and humans.

Materials and Methods

Twenty-four pregnant mixed-breed pony mares 5 to 15 years of age were used in 48 embryo-collection attempts. The ovaries were scanned by use of transrectal ultrasonography every 24 hours as described.[4] Ovulation (day 0) was recorded as occurring on the day of the examination during which the preovulatory-sized follicle was gone and a collapsed follicle or corpus luteum was detected. Mares were bred every 2 days, beginning when the largest follicle was 30 mm in diameter. Mares were not bred on or after day 0 because of reported effects of breeding on embryo size and survival.[4] Only mares determined to be pregnant by use of ultrasonography on the day of embryo collection (days 17, 18, 19, 20, 22, 24, 26, 28, 30, 35, 37 or 40) were used. The conceptus was collected transcervically by uterine lavage. Physiologic saline (0.9% NaCl) solution (500 to 2,000 ml) was infused into the uterus by gravity flow through a rubber tube, and the uterine horns and uterine body were flushed. The discharging flushing fluid was collected with an open receptacle held beneath the vulva and not through the tube used for saline solution infusion.

Measurement of the greatest length of each embryo and specific structures and count of somites were done after fixation. It is customary to fix embryos before measurements are made, because the original Carnegie system used

Table 1—Summary of data for equine embryos collected on various days after ovulation

Day	n	Length* (mm)	Branchial arches†	Appendages†	Face and head†	Body features†
17	2	3.5 4.1	None	None	Cranial end distinguishable (2)	10, 11 somites Cardiac prominence (2) Midneural tube closing (2)
18	3	3.7 4.5 4.8	I (2) I and II (1)	None	Optic eminence (1) Otic placodes (2)	15, 17, 23 somites Cranial neural tube closed (1)
19	3	4.5 5.0 5.0	I (1) I and II (2)	None	Optic eminence (3) Otic placodes (3)	17, 24, 25 somites Under folding at ends (3) Cranial neural tube closed (1) C-shaped embryos (1)
20	3	4.5 5.2 5.2	I and II (1) I–III (2)	FL buds (2) Tail bud (2)	Optic vesicle (3) Otic cup (2)	Future midbrain detectable (3) C-shaped embryos (3) Neural tube closed cranially and open caudally (3)
22	2	5.6 5.8	I–III (2)	FL buds (2) HL represented by low ridges (2) Tail bud (3)	Lens placodes (2) Otic cups(2) Nasal placodes (1)	Caudal neural tube starting to close (2)
24	3	5.8 6.4 6.6	IV (2) Mand and max processes (3)	FL and HL buds (3) Tail defined (3)	Lens pits (2) Otic cups (1) Otic vesicles (2) Nasal placodes (2) Nasal pits (1)	35, 35, 39 somites Cervical flexure angular (3)
26	5	7.0 7.6 8.7 9.3 9.6	IV (5) Max borders defined (5) Mouth slit (4)	Tail curled (5) FL buds 1.5 mm HL buds 0.9 mm	Lens pits (5) Otic vesicles (5) Nasal pits (5) Medial and lateral nasal processes (3)	Brain components defined (5) Pontine flexure (4) Genital tubercle (3)
28	3	9.6 10.1 10.8	Mand processes fusing (3) Mouth slit (3)	FL facing midline, paddle-shaped (3)	Lens vesicles (2) Retinal pigmentation (2) Nasolacrimal groove (2) Medial and lateral nasal processes (3)	Umbilical hernia (3) Pontine flexure (3) Genital tubercle (3)
30	3	11.0 14.3 14.3	Max & nasal processes fusing (3) Mouth open (1)	FL 3.0–3.2 mm, footpad forming (2) HL 2.3–2.9 mm	Lens vesicles (3) Retinal pigmentation (3) Early auricular hillocks (2) Nasolacrimal groove (3)	Cervical flexure less prominent (3) Somites visible only caudally (3) Genital tubercle enlarged (3) Midbrain loop (3)
35	3	15.5 16.8 18.5	Mouth open (3) Tongue (1)	FL 4.0–5.3 mm, footpad (3) HL 4.0–5.0 mm, footpad forming (2)	Lower eyelid forming (1) Auricular hillocks (3) Lens (3)	Flattened ellipsoid shape (3) Pontine flexure slit (3)
37	1	18.2	Tongue	FL 5.3 mm, notched, footplate, elbow joint HL 3.7 mm, footpad Limbs soliped	Lens Lower eyelids forming Pinna beginning to develop Developed nostrils	Pontine flexure closed Genital tubercle enlarged No vertebral segmentation
40	3	22.3 22.3 23.7	Tongue (3)	FL 6.6–7.5 mm, bent elbow joint (3), notched footplate (2) HL 6.5–7.2 mm Limbs soliped (3)	Upper and lower eyelids (3) Pinna distinctive flaps (3) Developed nostrils (3)	Pontine flexure closed (3) Ribcage prominent (2) Bilobed forebrain separated from midbrain (3) Genital tubercle migrated cranially (1)

*Greatest length on days 17 to 19; crown rump length on days 20 to 40. †No. in parentheses indicates the no. of embryos with that feature.
FL = Forelimb. HL = Hind limb. Mand = Mandibular. Max = Maxillary.

for characterizing human embryos was formulated on the basis of observations of embryos that were fixed immediately after they were obtained.[18] A stereomicroscope was used to measure greatest length in a straight line from the cranial to the caudal most aspect of the embryo without attempting to straighten the embryo or follow a curvature. By day 20, embryos developed a ventral curvature at the head and tail, and greatest length measurements were then identical to the crown-rump length or the sitting height.[19] The external features were examined, using a dissecting microscope. Terms of external features are described in a recent review.[20] The internal features were examined by serially sectioning embryos collected from days 20 to 35. Each conceptus was prepared for histologic study as described.[20] Embryos and extra-embryonic membranes were fixed in 4% paraformaldehyde for 12 to 24 hours and washed in 70% ethanol. The fixed embryos were separated from the membranes, processed through a graded ethanol series, and embedded in paraffin wax. Blocks were serially sectioned every 10 µm in the transverse or sagittal planes and stained with H&E.

Ages of equine embryos at which each feature was first obtained and stage of the Carnegie system were determined. In addition, ages of equine embryos at which each Carnegie stage was achieved were compared with reported ages for humans, porcine, and ovine embryos.[18]

Table 2—Diagnostic features reported for Carnegie stages[18] and age of equine embryos for each Carnegie stage

Carnegie system		Age of individual equine embryos (d)
Stage	Features	
10	4 to 12 pairs of somites	17, 17
	Neural folds begin to fuse	17, 17,
	Otic placode	18, 18, 19, 19, 19
11	13 to 20 pairs of somites	18, 18, 19
	Cranial neuropore closed	18, 19, 20, 20, 20
12	21 to 29 pairs of somites	18, 19, 19
	Caudal neural tube closed	22, 22
	C-shaped embryo	19, 20, 20, 20
	3 branchial arches	20, 20, 22, 22
	Beginning forelimb buds	20, 20, 22, 22
13	4 limb buds	24, 24, 24
	Max and mand processes	24, 24, 24
	Otic vesicle	24, 24, 26, 26, 26, 26, 26
	Nasal placode	22, 24, 24
	Tail bud	20, 20, 22, 22
14	Cervical flexure	24, 24, 24
	Lens pit	24, 26, 26, 26, 26, 26
	Definitive tail	24, 24, 24
15	Forelimb footpad	30, 30, 35, 35, 35
	Nasal pit	24, 24, 26, 26, 26, 26, 26
	Closed lens vesicle	28, 28, 30, 30, 30
	Early auricular hillocks	30, 30
	Umbilical hernia	28, 28, 28
16	Retinal pigment	28, 28, 30, 30, 30
	Hind limb footpad	35, 35, 37
	Medial and lateral nasal processes	26, 26, 26, 28, 28, 28
17	Distinct auricular hillocks	35, 35, 35
	Nasolacrimal groove	28, 28, 30, 30, 30
18	Notched hand plate	37, 40, 40
	Elbow joint	37, 40, 40, 40
	Beginning eyelids	35, 37, 40, 40, 40
19	Limbs extend nearly straight forward	37
	Head begins to be raised beyond a right angle	40, 40, 40
	Continued fusion of auricular hillocks	37, 40, 40, 40
20	Arms increased in length and bent at elbow joint	40, 40, 40

Results

Embryos were recovered during 34 of 48 (71%) collection attempts (**Table 1**). The regression line best characterizing the mean change in the length of embryo and an indication of the age for the first appearance of some features was determined (**Fig 1**). In addition, comparisons between characteristics of equine embryos and those of the Carnegie stages and of other species were tabulated (**Tables 2 and 3**). Photographs of embryos and external features were also obtained (**Fig 2**).

The amniotic folds had not yet closed over any of the 3 embryos collected on day 19. By day 20, the amnion was completely closed over all 3 embryos, and the yolk sac was extensively vascularized. There were prominent vitelline vessels and a sinus terminalis. For the first time, heart contractions were prominent in the newly removed embryos. Blood was clearly circulating throughout the embryos and yolk sac. The allantois was emerging cranial to the tail bud. Internally, there was no definite midgut. Only a small portion of a hindgut could be defined at the caudal end, which gave origin to the

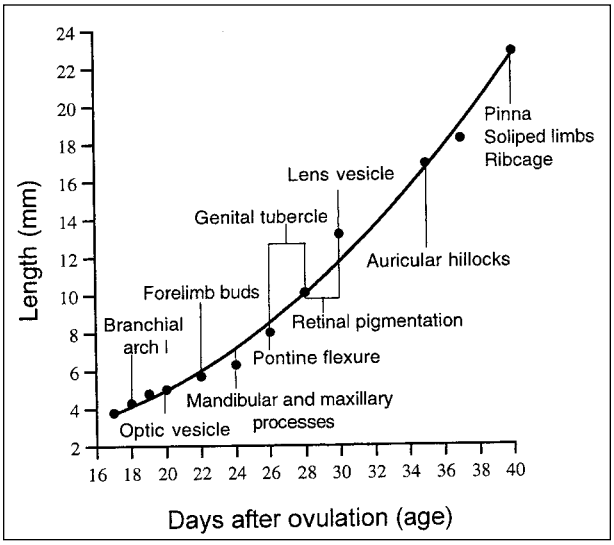

Figure 1—Regression line best characterizing the mean lengths of equine embryos and the mean age of first appearances of features.

Table 3—Comparison of embryo age* for each Carnegie stage among species with various gestation lengths†

Carnegie stage	Human (266 d) ‡	Porcine (114 d)‡	Ovine (150 d)‡	Equine (340 d)‡
10	22	15	16	17–19
11	24	16	16–17	18–20
12	21–29	17	18–19	18–22
13	28–32	18	19–20	20–26
14	31–35	19	20–21	24–26
15	35–38	20–21	22	24–35
16	37–42	21–22	23	26–37
17	42–44	23	24–25	28–35
18	44–48	24	25–26	35–40
19	48–51	25–26	27–28	37–40
20	51–53	27–28	29–30	40

*No. of days for each stage was the estimated no. of days after ovulation for humans, days after breeding for pigs and sheep, and known days after ovulation for horses. †Data for human, porcine, and ovine embryos were obtained from a review article.[18] ‡Mean length of gestation.

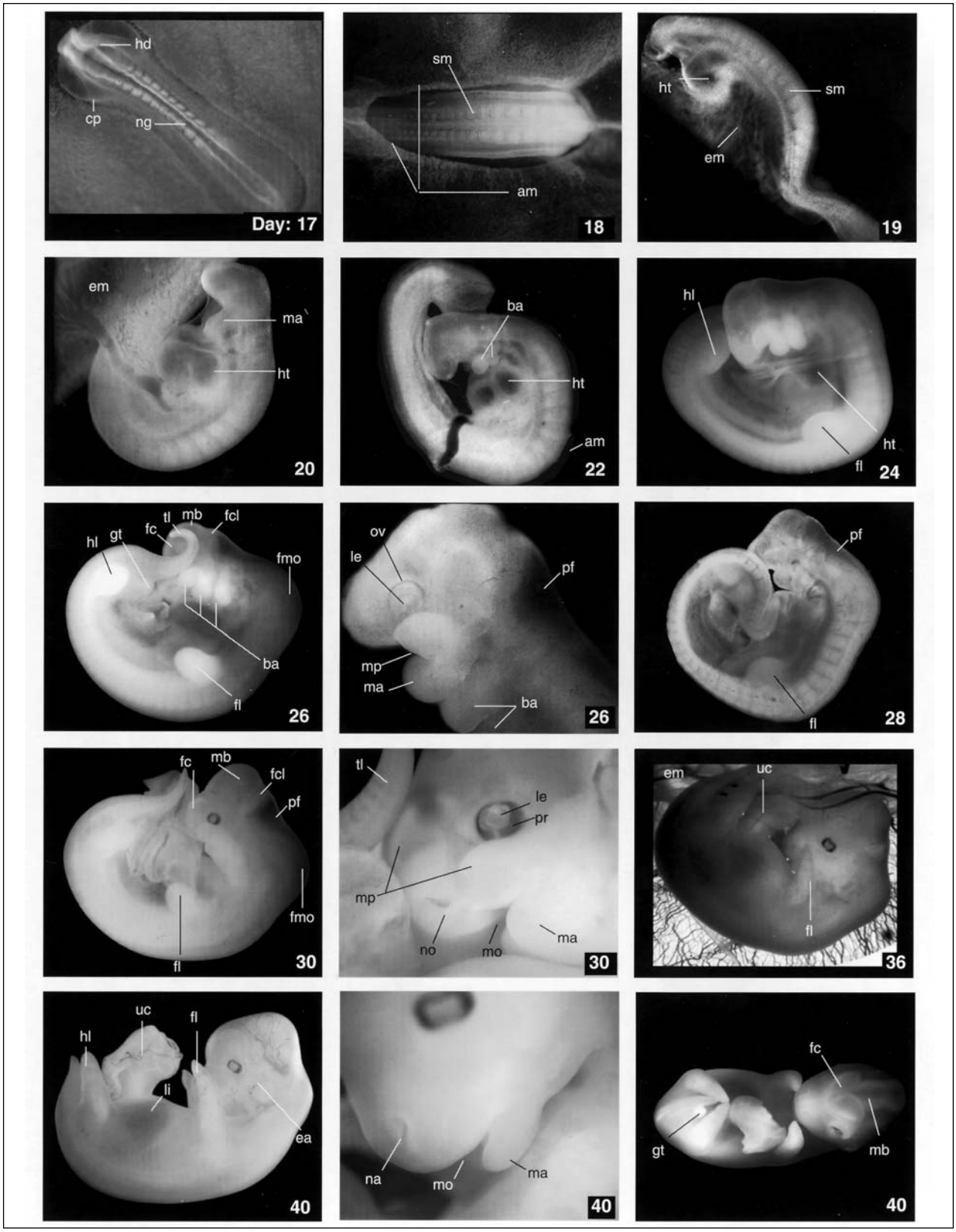

Figure 2—Photographs of the developing embryo proper illustrating external features that develop between 17 and 40 days after ovulation. Age of embryo is indicated in the lower right corner of each photograph. Length is given for embryos in Table 1. am = Amnion. ba = Branchial arches. cp = Cardiac prominence. ea = Ear. em = Embryonic membrane. fc = Future cerebrum. fcl = Future cerebellum. fl = Forelimb. fmo = Future medulla oblongata. gt = Genital tubercle. hd = Head. hl = Hind limb. ht = Heart. le = Lens. li = Liver. ma = Mandibular process. mb = Midbrain. mp = Maxillary process. mo = Mouth. na = Nasal aperture. ng = Neural groove. no = Nose. ov = Optic vesicle. pf = Pontine flexure. pr = Pigmented retina. sm = Somites. tl = Tail. uc = Umbilical cord.

allantois. At day 22, the midgut was definitive, and the pronephric duct and nephric vesicle (or primordium) contained small tubules. At day 24, the lung buds, thyroid diverticulum, and pancreatic bud were identified. The urogenital ridges were found on either side of the dorsal mesentery of the hindgut and were most pronounced in the midtrunk region, where they protruded slightly into the dorsal part of the peritoneal cavity. The nephric ducts were canalized along much of their length and extended caudally toward the region of the cloaca. A few mesonephric glomeruli were identified. At day 26, the optic cups, liver, and oral pharynx were observed. There were large numbers of mesonephric vesicles and glomeruli in the lateral parts of the urogenital ridges. The mesonephric ducts were patent along their entire length but had not yet opened into the cloaca.

At day 28, the ureter and first pharyngeal pouch were identified. The urogenital ridges were more prominent than in earlier embryos, and numerous mesonephric vesicles opened into the mesonephric ducts. The mesonephric ducts passed caudally and made contact with the wall of the cloaca in the region of the urogenital sinus. Well-differentiated mesonephric glomeruli were identified. Ureteric buds were first seen at this age. They were represented as diverticula from the mesonephric ducts just proximal to the area of contact with the urogenital sinus. At day 30, an olfactory lobe and Rathke's pouch were identified. The mesonephroi were fully formed, and early metanephroi were observed. The urogenital ridges extended from the level of the forelimbs to the pelvic area. The mesonephros was situated on the lateral aspect of the urogenital ridge, and the gonadal primordium was located on the medial aspect. The urogenital mesentery was broad. Within the mesonephros, the tubules were prominent. Glomeruli and the mesonephric ducts were prominent. The ends of the ureteric buds were dilated, and these regions were surrounded by the metanephric blastema. At day 35, development of skeletal structures had begun, and ossification was especially noticeable in the limbs. The mesonephroi contained tubular tissue and glomeruli and appeared to be regressing. The urogenital mesentery was narrower than in earlier embryos.

Discussion

Although the embryo recovery procedure we used was simple, it was adequate for our objective. The mares were confirmed pregnant by use of ultrasonography on the day of collection. Each recovered conceptus was intact without rupture of the placental membranes in 20 of the 34 (61%) successful collections. The embryos with ruptured membranes were not damaged in a way that interfered with study of the targeted features. Loss of embryos can be attributed to severe damage during the flushing procedure. It was expected that even the youngest intact conceptus (day 17) would be recognizable in the flushing medium (expected diameter of the conceptus at day 17 is 22 mm[4]). When a conceptus was not found, the remnants either were not recognized on macroscopic inspection of the flushing fluid or were retained and not recognized during ultrasonographic inspection of the reproductive tract. An unsuccessful collection attempt was not attributed to a misdiagnosis of pregnancy, because the ultrasound technology used is extremely accurate.[4] Confusing a uterine cyst for a conceptus was precluded by the history of findings on ultrasonographic examination that was used for this research herd. There was a clear partitioning of the collection data into days 17 to 24 (28 attempts) and days 26 to 40 (20 attempts). The percentage of recovered embryos and the percentage of recovered embryos with unruptured placental membranes were lower for days 17 to 24 (48 and 33%, respectively) than for days 26 to 40 (90 and 83%). The resiliency of the embryos on days 26 to 40 is attributable to the strengthening of much of the conceptus wall by the enlarging allantoic sac.[4]

The study began on day 17 or at the beginning of intrauterine fixation of the previously mobile embryonic vesicle.[1] This day was chosen because it precedes the migration of the primordial germ cells, a subject of interest in our laboratory.[13] Age of specimens was calculated on the basis of findings on ultrasonographic examinations that were performed every day for detection of ovulation. Thus, specimens collected on a given day varied in age by approximately 24 hours, accounting for at least part of the morphologic variation among embryos. In addition, embryos of the same species develop at variable rates after fertilization.[18] The stated day of gestation reported for other species often was imprecise, because the day of ovulation was unknown. In sheep, the age of embryo is often calculated on the basis of the day of estrus or breeding.[21] In humans, the timing of gestation is complicated by the unreliability of menstrual cycle data and, particularly, by the lack of reliable signs that would indicate ovulation.[22]

A systematic morphologic study of equine embryos from commonly obtained specimens was justified because of the dearth of information for this species. No embryologic structures or changes were detected either internally (histologically) or externally that can be considered unique to equine embryos. The histologic aspects, therefore, were not discussed. The features visible from the surface were used for species comparisons and for staging criteria[18] and are, therefore, discussed here in detail.

The embryo lengthened in a curvilinear fashion with a slower growth rate during the first half of the study. Length seemed to have limitations as a predictor of age. For example, lengths of 4.5 mm were found on days 18, 19, and 20, and embryo lengths ranged from 7.0 to 9.6 mm on day 26. This is more than can be accounted for by the variation of 24 hours in the day of ovulation. The range of lengths within a day was usually less than 1 mm (mean range, 0.6 mm) on days 17 to 24, whereas mean range was 2.3 mm on days 26 to 40. The period of characterization (days 17 to 40) provides some missing information on length, because a previous study[5] of the lengths of removed embryos was done without knowledge of the day of ovulation and did not begin until 24 days after breeding. According to measurements made by use of ultrasonography,[4] the size of the equine embryo proper and embryonic vesicle is not affected by maternal type, despite the large differences in body size (eg, ponies vs horses). Inspection of growth profiles obtained by use of ultrasonography indicates that lengths of pony and horse embryos are about 6 mm

on day 24, 12 mm on day 30, and 23 mm on day 40.[4] Thus, our length measurements, made after fixation of removed specimens, were close to those obtained by use of transrectal ultrasonography.

During the early period of somite formation, human,[23] murine,[16] and ovine[21] embryos have lordosis (dorsal concavity) of the body. Equine embryos had lordosis at day 17 (stage 10). Also at this time, fusion of the midneural tube was in progress. Similarly, the midneural tube begins to fuse in ovine embryos 16 days after breeding.[21] The fusion of the neural folds in equine embryos extended rostrally to the region of the midbrain by days 18 to 20, but the neuropore was closed in the more advanced specimens. A dorsal convex curvature of the body became distinct and included the thoracolumbar region at day 20. The embryos, therefore, had a characteristic C-shape. These results were similar to reports that equine embryos had a C-shape at day 18[7] and day 20.[6] Similarly, ovine embryos attained the C-shape by 21 days after breeding.[21]

The genital tubercle was evident in equine embryos at day 26 and enlarged through day 40. The tubercle first formed between the caudal portion of the hind limb buds. In 1 of 3 day-40 embryos, the genital tubercle had migrated to a more cranial location. In a previous report,[5] the genital tubercle had migrated cranially in males and caudally in females 45 days after breeding. Apparently, the genital tubercle begins to change location in a manner that suggests sex near the nominal beginning of the fetal stage. The migration of the genital tubercle toward the umbilicus or tail is used for ultrasonographic diagnosis of fetal sex.[4] In addition to the genital tubercle, the pontine flexure appeared in the cephalic region on day 26 and was in the form of an equilateral triangle when viewed from the side. The flexure was slit-like on day 35 and disappeared by day 37. Similar descriptions of the pontine flexure have been reported on the basis of the number of days after breeding.[5] The prominence of the pontine flexure should be a useful aid for estimating age during days 26 to 37. A physiologic umbilical hernia was first evident on day 28. The body wall surrounding the hernia was thin, so that loops of the midgut were visible within the sac by day 30. An increased width of the trunk region reflected the growth of the organs and muscular plate at the nominal beginning of the fetal stage (day 40).

Branchial arch I was detected at a similar age as in ovine embryos[21] but earlier than in human embryos.[23] The branchial groove between arches II and III was distinctive by day 20. First detection of arches I and IV on days 18 and 24, respectively, in equine embryos compares with days 24 and 28 in human embryos. By the time of the appearance of arch IV (day 24), branchial arch I divided into the mandibular and maxillary processes. In a previous study,[2] all 4 branchial arches were described in an equine embryo obtained 21 days after breeding, which is earlier than in our study. Branchial arches III and IV began to regress into the cervical sinus as early as day 26. Then, the medial components of the bilateral mandibular processes from branchial arch I merged midline and formed the point of the lower jaw at day 28. Similarly, results of a previous report[3] indicated that the mandibular processes were completely joined in a 12.5-mm-length embryo (about day 30). By day 30, the lateral nasal processes merged with the superficial region of the maxillary processes from branchial arch I over the region of the nasolacrimal groove, and the medial nasal processes and the maxillary processes fused to form part of the upper jaw. In contrast, it has been reported that the maxillary and medial nasal processes were not fused on day 34.[6] In ovine embryos, the mandibular process fused by 24 days, and the medial nasal processes and maxillary process fused by 26 days after breeding.[21] The equine olfactory apparatus first became visible on days 22 and 24 as a pair of thickened ectodermal nasal placodes located on the frontal aspect of the head. Then the nasal placodes began to recede from the surface and depressed to form nasal pits. The nasolacrimal groove formed between the maxillary process and lateral nasal process. The processes began to grow as the nasal pits continued to deepen, so that the nostrils were formed approximately at the end of the embryo stage. Similar descriptions of the nasal pits have been reported on the basis of the number of days after breeding.[5]

The forerunners of the eye and ear were first evident at day 18 or 19. The optic eminence developed in the forebrain, and an indication of invagination of the otic placodes was also seen. Invagination of the optic placodes constitutes the optic vesicle[20] and was seen at day 20. Similar descriptions of the optic vesicle have been reported on the basis of the number of days after breeding.[5] At day 20, the otic cups were almost closed but not detached, and 2 days later a complete sheath, the lens placodes, covered the optic vesicle. By day 26, the lens had various degrees of indentation to form the lens pits, and the otic vesicles were closed. Then, the optic vesicle flattened and became concave to form the optic cup, while the lens continued to invaginate and formed the lens vesicle. The lens was completely formed by day 35. In contrast, in another report,[5] the ocular lens was formed 30 days after breeding. In equine embryos, retinal pigmentation appeared in the external layer of the optic cup. This would be a good indicator that an embryo is at least 28 or 30 days of age, because it was readily identified and was first detected in 2 of 3 day-28 embryos and 3 of 3 day-30 embryos. The pigmentation was obvious through the remainder of the study. Three nodular masses of mesenchyme (auricular hillocks) appended along each side of the first branchial groove by day 30. Similarly, an auricular hillock was detected as a depression surrounded by an embossed edge in a 12.5-mm-length embryo.[3] The auricular hillocks enlarged asymmetrically. They coalesced to form a recognizable external ear or pinna, which formed a distinctive flap by day 40. The pinna of equine embryos was described in a previous report.[5] The eyelids and pinna were observed in ovine embryos 27 days after breeding.[21]

The forelimb buds began to bulge from the body wall at day 20. Two days later, the forelimb buds had definite ridges on the surface, and the hind limb buds were beginning to form. At day 26, the forelimbs were rounded projected appendages that curved toward the midline, but the hind limbs did not curve toward the midline until day 28. In an earlier report[5], recognizable legs had started to replace limb buds in equine embryos by 28

days after breeding. At day 30, the tip of the forelimbs began to thicken and form footpads with 2 distinct regions, but the hind limbs did not yet have signs of footpads. By days 37 and 40, the forelimbs were regionally subdivided into a footplate, forearm, elbow, and shoulder region. The forelimbs were straight with an elbow joint and with slight irregularities representing apparent rudiments of digits. By day 40, all limbs were bent toward the midline, and the tips were hoof-shaped (soliped). Tapered feet have been reported at 36 days after breeding.[5] Another report[3] described forelimbs in a 12.5-mm-length embryo (about day 30) as trilobite at the distal end, which is earlier than detected in our study. Sheep develop trilobite forelimbs by 25 days after breeding.[21]

The age of development of the equine staging features differed from other species. The Carnegie staging system was developed for human embryos. It has been stated that embryonic stages are based on the apparent morphologic state of development and are not directly dependent on either chronologic age or on size.[19] It was further stated that the individual differences between embryos were less apparent and less important for staging than the general appearance of the developing embryos. Despite the longer gestational length in the horse (340 days), compared with humans (266 days), human embryos appear to develop more slowly than equine embryos.[19] During the early somite-forming stages 10 to 13, equine embryos were 5 to 8 days ahead of development of human embryos. However, during stages 15 and 16 while features of the limbs were beginning to develop, equine embryos developed at a similar age as for human embryos. The forelimb footpad developed in both species around day 35, and the hind limb footpads developed in both species around day 37. Most of the other features of equine embryos continued to develop more rapidly than in human embryos. By stage 20, the lag period for human versus equine development was more than 10 days.

Developing features of the early somite-forming stages of porcine, ovine, and equine embryos are similar. However, development of equine embryos was 3 to 5 days slower than the development of porcine embryos for most structures examined. These results are consistent with the statement that the development of pony embryos lagged by about 4 days behind pig embryos.[24] Two equine embryos, one 13 mm (about day 30) and one 18 mm (about day 37) in length, corresponded to porcine embryos of approximately 8 mm (about days 20 to 21) and 20 mm (about days 27 to 28) in length.[25] Compared with ovine embryos, development of equine embryos lagged by about 2 to 4 days during stages 10 to 13 (ages: equine embryo, days 17 to 24; ovine, days 16 to 21). However, during stages 15, 16, and 17, the limbs, which are a critical external feature for Carnegie staging, took even longer to develop in the horse. The limbs developed about 16 days later than in the sheep. The sheep's forelimbs were extended and had developed an elbow joint by 24 days after breeding,[21] whereas the horse's elbow joints did not develop until day 40. The difference in developmental time between horses and sheep may be related to the relatively longer and more complex limbs in adult horses. The development of the external ear and eyelids in the horse followed about a 10-day prolonged developmental pattern, compared with sheep. These differences between horse and sheep embryos emphasize that timespan comparisons among species can be markedly influenced by the structures under consideration.

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