EARLY EMBRYOLOGY OF THE COW.

I. GASTRULA AND PRIMITIVE STEAK STAGES

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SUMMARY

Serial sections of 16 bovine embryos recovered from cows slaughtered at 16, 17, and 18 days after insemination were studied to establish suitable standards of normal development and to define the limits of normal variation encountered during early pregnancy. The 16-day-old blastocysts were engaged in the processes of gastrulation, and ranged in development from a hollow bilaminar sphere to an elongate chorionic vesicle with an elevated germinal disk. Their principal features were the remarkably rapid growth of the trophoblast and the differentiation of somatic and splanchnic mesoderm. Blastocysts recovered at 17 days were characterized by further growth, cephalocaudal elongation of the germinal disk, and differentiations of Hensen's node and the primitive streak establishing the longitudinal axis. In the 18th day of development, the blastocysts extended throughout the major portion of the gravid horn and the embryos displayed well-defined primitive node, primitive groove, and notochord development. Amniogenesis was initiated and, in two specimens, completed amniotic sacs were present. Two cases of probable embryonic death were encountered in which the tropheoblast persisted after loss and resorption of the embryonic area. The morphogenetic processes involved in gastrulation and primitive streak formation are considered in the light of reported high embryonic death during early pregnancy.

The literature pertaining to studies of the embryology of the cow is notably sparse. Hallman (11) studied the development of placentae from cows from 3 to 5 mo. pregnant, with special reference to placental pathology. Hammond (13) studied the relations between rate of development of the uterus, fetus, fetal fluids, and fetal membranes, and included brief descriptions and illustrations of a few fetal specimens recovered at the end of each lunar month of pregnancy. Hartman et al. (14) and Miller et al. (23) described a two-celled bovine egg recovered 48 hr. after mating and an unfertilized tubal ovum 72 hr. after mating. Yapp (31) presented data on 31 fetuses ranging from 41 to 277 days of pregnancy, emphasizing changes in body proportions during various periods of development. Kupfer (21) reported on a series of specimens with estimated ages from about 3 to between 15 and 17 wk. of age.

The most valuable and precise information on the embryology of the cow is provided by Winters et al. (30). Employing timed specimens, they presented data and illustrations on material ranging from one-day-old ova to the new-born calf. The scope of this undertaking made it necessary to base some observations

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upon single specimens; moreover, there are a number of omissions in the day-by-day descriptions of the embryological development.

Nichols (25) collected data on body weights and a number of body measurements from about 100 embryos and fetuses of Hereford cows, but precise knowledge of the stages of gestation was lacking. A study of the rate of development of the gravid uterus and its contents at various stages of pregnancy was made by Swett et al. (27), using data from 115 cows ranging from 14 to 276 days of gestation.

More recently, the development of the bovine ovum up to the stage of blastocyst formation has been reported by Hamilton and Laing (12). Chang (5) described blastocysts obtained from slaughterhouse material estimated to range from about 8 to 18 days of development. Greenstein and Foley (9, 10) reported briefly on the development of the bovine embryo between the late blastocyst stage and early organogenesis.

The recognition of early embryonic death as an important factor in bovine infertility (1, 3, 4, 6, 15, 16, 28, 29) has pointed up the need for more detailed, accurate information relative to normal embryonic development during the critical stages of germ layer formation, the development of body form, and the establishment of the organ systems. This paper is the first in a series describing the day-by-day development of the cow from the late blastocyst stage to the fully formed embryo.

MATERIAL AND METHODS

Reproductive tracts were recovered from dairy and beef cows and heifers slaughtered at the University abattoir. These cows represented culls from the University herds. Detailed records were available relative to ancestry, health, age, breeding history, and reproductive performance of all individuals. The cattle were closely watched for signs of estrus, and those that came into estrus in the morning were mated at that time and again in the afternoon; those coming into heat in the afternoon were bred that day, and again the following morning if still in estrus.

Since ovulation normally occurs from 10 to 15 hr. postestrum (2, 13, 24), in calculating the ages of the embryos in this study the day after the last breeding was considered as the first day of pregnancy and the date of slaughter as the final day.

Specimens were flushed from the uterine horns with physiological saline for observation under the dissecting microscope. They were then photographed, or fixed immediately in a modified Bouin's fluid, followed by standard dehydration, clearing, and Tissuemat infiltration. The embryos were serially sectioned at 8 or 10 microns and stained with hematoxylin-eosin or a modified Mallory triple stain.

Observations. Sixteen timed embryos were available for study. A list of the embryos and their principal characteristics is presented (Table 1).

Sixteen-day embryos. Six embryos were recovered from animals slaughtered 16 days after breeding. The gross appearance of the specimens varied consider-
TABLE 1

<table>
<thead>
<tr>
<th>Pregnant Animal (No.)</th>
<th>Bilaminar vesicle (mm.)</th>
<th>Stage of embryonic development</th>
<th>Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE-20 1.5</td>
<td>Bilaminar hollow sphere. Embryonic disk area distinct from trophoblast.</td>
<td>1</td>
<td>4, 5, 6, 7</td>
</tr>
<tr>
<td>NE-93* 6</td>
<td>Early elongation of blastocyst. Dense spherical disk (0.4 mm.) elevated to surface.</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>NE-69* 29</td>
<td>Marked growth and folding of trophoblast wall. Embryonic disk unchanged.</td>
<td>3, 8, 9, 10, 11</td>
<td></td>
</tr>
<tr>
<td>NE-16 60</td>
<td>Slender vesicle (1.65 mm. in diameter). Disk occupies middle portion.</td>
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<tr>
<td>NE-132 75</td>
<td>Cephalocaudal elongation of disk (0.45 by 0.6 mm.). Proliferation of mesoderm.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE-22 90</td>
<td>Embryonic failure. Trophoblast undergoing degeneration.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE-50 70</td>
<td>Embryonic failure. Persistent chorionic development.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE-61* 125</td>
<td>Primary germ layers well-established. Extension of extraembryonic mesoderm.</td>
<td>12, 13, 14, 15</td>
<td></td>
</tr>
<tr>
<td>NE-148 135</td>
<td>Oval disk (0.4 by 0.75 mm.) with caudal primitive groove. Possible early amniongenesis.</td>
<td>16, 17</td>
<td></td>
</tr>
<tr>
<td>NE-39 150</td>
<td>Longitudinal axis clearly evident. Undercutting of embryonic area.</td>
<td>18, 19</td>
<td></td>
</tr>
<tr>
<td>NE-55 240</td>
<td>Pronounced Hensen’s node and mesodermal delamination.</td>
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<td></td>
</tr>
<tr>
<td>NE-49* 100</td>
<td>Thickened ectoderm, well-formed primitive streak. Disk 0.56 by 0.72 mm.</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>NE-85 175</td>
<td>Distinct extraembryonic coelom. Suggestion of primitive amniotic folds.</td>
<td>21, 22, 23</td>
<td></td>
</tr>
<tr>
<td>NE-90 Length of gravid horn 225</td>
<td>Marked primitive groove. Extensive extraembryonic coelom.</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>NE-131 Invading non-gravid horn</td>
<td>Well-developed amnion, notochordal mass, and lateral mesoderm.</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

*a Five or more services required for conception.

*Previous abortion.

ably, particularly with regard to the elongation of the chorionic vesicles, ranging from 1.5 to 90 mm. in length, with a mean length of 43.6 mm. The diameters of the vesicles were relatively constant at approximately 1.5 mm. The embryonic disks were similar in all specimens, being nearly spherical, with an approximate diameter of 0.4 mm.

Specimen NE-20 appeared as a minute, bilaminar, hollow sphere with only faint evidence of an embryonic disk on its surface. Unfortunately, this specimen was lost in processing. A somewhat larger blastocyst was recovered from NE-93, as illustrated (Figure 1). The embryonic disk was imperceptible in saline solution but, with the addition of fixative, became evident as an opaque spherical structure.

Figures 4 to 7 illustrate representative 8-micron sections of specimen NE-93. The germinal disk has become completely elevated to the surface, with only an occasional vestige of overlying trophoblast or Rauber’s membrane (Figure 6) remaining as detached cells. The endoderm has separated from the germinal
disk area and forms a complete lining for the blastocyst. Mitotic figures are seen frequently in the germinal disk and trophoblast, indicating a period of rapid growth and elongation.

Specimen NE-69 is shown in Figure 2. Although the chorionic vesicle was considerably longer than in the previous specimen, sections through the germinal disk indicated that the embryonic area did not differ significantly from NE-93. There was, however, a marked growth and folding of the wall of the trophoblast. The disk remained essentially spherical with a diameter of 0.35 mm. and was located in the mid-portion of the vesicle. There was no evidence of overlying trophoblast cells.

In Specimen NE-16 the trophoblast had elongated considerably, and the width of the chorion had increased to approximately 1.65 mm.

Figure 3 illustrates the prominent, raised, oval germinal disk of Specimen NE-132. The embryonic area was located approximately midway along the slender (1.5-mm. diameter) vesicle. Figures 8 to 11 are representative 10-micron sections taken at various levels through the germinal disk. This specimen shows a striking advancement over the specimen in Figures 4 to 7. The ectodermal disk is well-formed, and the mesoderm has separated to form the extraembryonic somatopleure, splanchnopleure, and coelom. Undercutting of the embryonic area indicates the formation of the future body stalk.

Although NE-22, when recovered, appeared to be the most advanced specimen of this 16-day-old group by virtue of its length, the embryonic disk was not discovered either upon gross examination or in tissue sections. Large numbers of pyknotic nuclei indicated that the trophoblast was undergoing degeneration, and it is reasonable to assume that the embryonic tissue proper had disappeared sometime prior to slaughter.

Seventeen-day embryos. Five embryos recovered 17 days after breeding were available for detailed study. The variation in appearance among the specimens was not as pronounced as it was on the previous day. The length of the chorion ranged from 70 to 240 mm., with a mean length of 144 mm. In all cases, the vesicle was somewhat enlarged in the central region, tapering toward the extremities. The embryo occupied a position in the approximate mid-portion of the vesicle, and at this stage there was a consistent tendency toward elongation of the germinal disk to assume an oval rather than a circular shape.

PLATE 1

1. Entire 16-day-old blastocyst NE-93 and millimeter rule photographed in saline. Arrow points to opaque embryonic disk. 6.2×.

2 and 3. Portions of elongate 16-day-old blastocysts, NE-69 and NE-132, showing characteristic raised germ disks on surface of trophoblast. 33× and 30×.

4, 5, 6, and 7. Transverse 8-micron sections of NE-93 (Figure 1) showing separation of endoderm layer from germ disk. Notice frequency of mitotic figures. Vestige of overlying trophoblast may be seen in Figure 6. 125×.

8, 9, 10, and 11. Transverse 10-micron sections of NE-132 (Figure 3). Note undercutting of embryonic area, the presence of a definitive yolk sac, and the delamination of mesoderm to form splanchnopleure, somatopleure, and extraembryonic coelom. 125×.
The embryonic area of the chorionic vesicle in specimen NE-50 was not positively identified either grossly or after serial sectioning. On the basis of microscopic examination of the tissue sections, it may be concluded that this specimen represents a case of persistent chorionic development in spite of prior embryonic failure. This conclusion is supported also by the relatively retarded length of the vesicle.

Specimen NE-61, whose germinal disk measured 0.6 by 0.8 mm., was located in the mid-portion of the vesicle. Sectioned at 8 microns, 98 sections were obtained. Aside from an over-all increase in the size of the disk, the appearance of the primary germ layers is essentially the same as in Specimen NE-132.

Figure 12 illustrates the gross appearance of Specimen NE-148. Sixty-eight sections were obtained at 10 microns and these revealed a well-developed mesoderm between the ectodermal plate and the yolk-sac endoderm. The lining of the cavity of the yolk-sac is composed of small, round cells with only a narrow rim of cytoplasm surrounding the oval, vesicular nuclei (Figures 13 and 14). The convex dorsal surface of the disk is rounded and smooth, except for evidence of a caudal primitive groove (Figure 15). The area of proliferation marking Hensen’s node also may be seen in this photograph. The ectodermal cells with numerous mitotic figures rest on a basement membrane with the tall columnar cells gradually becoming cuboidal at the lateral borders of the disk. An abrupt transition to flat ectodermal cells is seen in the lateral body folds. There are indications that the embryonic disk has settled somewhat into the blastocyst, so that primitive amniotic folds appear to be growing centripetally over the embryo. If this interpretation is correct, this specimen is the most advanced of the group in terms of amnion formation.

The gross appearance of Specimen NE-39 illustrates the progressive cephalo-caudal elongation of the disk, characteristic of this stage. Figures 16 and 17 represent sagittal sections through the embryonic area. Although the primary germ layers are well-established, there is no evidence of amnionogenesis.

The chorionic vesicle of Specimen NE-55 extended the full length of the gravid horn. With the exception of NE-50, all other specimens in this age group occupied about two-thirds of the horn. At 10 microns, 105 serial sections were obtained, two of which are shown (Figures 18 and 19). In comparing these sections with those of NE-148 (Figures 13, 14, and 15), a marked similarity is noted.

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PLATE 2

12. Mid-portion of 17-day-old blastocyst, NE-148, illustrating gross appearance of oval germinal disk and wrinkled trophoblast. 29×.

13, 14, and 15. Transverse 10-micron sections through NE-148. Numerous mitotic figures are seen among the tall columnar ectodermal cells of the disk. Evidence of a primitive groove in the caudal region is seen in Figure 15. 125×.

16 and 17. Representative sagittal sections of NE-39 illustrating cephalo-caudal elongation of germ disk and well-defined primary germ layers. 125×.

18 and 19. Transverse 10-micron sections of NE-55. Note clear delineation of primary germ layers and well-defined separation of the mesoderm. The section in Figure 19 passes through the area of proliferation recognized as Hensen’s node. 125×.
The former, however, appears to have progressed further, particularly with reference to a more pronounced Hensen's node and a more definitive mesoderm. *Eighteen-day embryos.* Observations were made on five embryos recovered 18 days after the last breeding. In this age group, Specimen NE-49 appeared the least advanced. The embryonic disk (Figure 20) yielded 45 sections at 10 microns. These sections indicate that morphogenesis has not progressed appreciably beyond the stages previously described for 17-day embryos. The embryo displays a thickened ectoderm, a well-formed primitive streak and mesoderm, and continued extension of extraembryonic endoderm.

The gross appearance of Specimen NE-85 is seen (Figure 21). The embryo was located in the somewhat expanded mid-portion of the elongate, narrow chorionic vesicle. The embryonic disk measured 0.36 by 0.6 mm. after fixation, and 51 sections at 10 microns were obtained. Figures 22 and 23 are representative transverse sections through the embryo and vesicle, which show clearly the lateral portions of the mesoderm partially filling the area between the trophoderm and yolk sac. The mesoderm has undergone delamination to form extraembryonic coelom. In the region where the heart will develop, this formation foreshadows the early appearance of pericardial coelom. As noted earlier in the 17-day-old Specimen NE-148, there is a suggestion of primitive amniotic folds. However, somatic mesoderm is not associated with the folded trophoderm.

A definite primitive groove was grossly discernible in Specimen NE-90. This was confirmed in sections (Figure 24) with 56 somewhat oblique serial sections obtained at 10 microns. The continued elongation of the chorionic vesicle to fill the gravid horn and the progressive extension of the limits of the extraembryonic coelom placed this embryo in a stage of development beyond those previously described. Evidence for the upward bending of somatopleure at the periphery of the embryonic plate was entirely lacking, however.

When Specimen NE-37 was flushed from the horn of the uterus, examination of its entire length failed to produce a positive identification of the embryonic area. Representative portions of the central region were preserved, however, and in subsequent sectioning the embryo was located. Complete serial sections were not available for study, because the embryo had suffered some damage in

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**PLATE 3**

20 and 21. Gross appearance of 18-day-old germ disks of NE-49 and NE-85. Note progressive tendency for elongation of embryonic area. Chorionic vesicle remains slender, as seen in Figure 21. 25× and 15×.

22 and 23. Transverse sections of NE-85 (Figure 21) through the entire blastocyst in the embryonic area. Note the complete endodermal lining of the blastocyst, the lateral extensions of mesoderm, and the suggestion of early amnionogenesis. 100×.

24. Oblique section through the primitive groove of NE-90. Note well-defined yolk sac and mesodermal elements. 125×.

25. A near-sagittal section of NE-37. Prominent features are the completed amnion, Hensen's node, primitive groove, and mass of notochordal cells. 100×.

26 and 27. Transverse sections of NE-131. The embryo is seen to be completely enclosed within the amniotic sac. Figure 26 is a section passing through Hensen's node; whereas, Figure 27 shows the thickening of the lateral mesoderm. 125×.
processing. The plane of the sections also passed somewhat obliquely through the longitudinal axis. The most notable feature of this embryo (as seen in Figure 25) is a well-defined, complete amniotic sac enfolding the developing embryo. Anterior to the thickened area of Hensen’s node and the primitive groove is seen the rod-shaped mass of notochordal cells occupying the space between the ectoderm and endoderm.

Additional evidence for possible amnion formation during the 18th day of development was provided by Specimen NE-131. The embryonic area measured approximately 0.4 by 0.65 mm. after fixation, and a total of 74 transverse sections of 8 microns was obtained. This specimen was the most advanced in the series, judging by the microscopic appearance of the embryo and the extension of the chorionic vesicle into the nongravid horn. Figure 26 is a section through Hensen’s node showing the nodal area as a center of proliferation for a median band of cells, which seemingly merge with the hypoblast below. Fusion of the amniotic folds above the mid-dorsal region of the embryo completes the amniotic sac, and the embryo in its amnion lies free in the surrounding trophoderm. The thickened lateral mesoderm seen in this figure (and in Figure 27) foreshadows the emergence of the epimeric mesoderm and the future somatic mesoderm of the trunk area.

**DISCUSSION**

The determination of the exact age of recovered embryos is very difficult in most species, because of a lack of precise knowledge of the actual time of ovulation and the time of fertilization. The copulation age generally employed is, at best, an approximation and accounts for many of the discrepancies found in the literature. Thus, Winters et al. (30) dated the specimens from 6 P.M. of the day the cow was mated until the time of slaughter, and described a 13-day, 14-hr. embryo that was very similar to the 16-day-old specimen NE-93 of the present series. Chang (5) described similar embryos recovered from slaughterhouse material and, based on the description of Winters et al. (30) and Hamilton and Laing (12), estimated their ages at from 13 to 14 days.

Similarly, the variation in development encountered within a single day among the specimens described in the present paper emphasizes the shortcomings of copulation-age estimates. This lack of precise information relative to the age of specimens makes it difficult to evaluate the influence of other factors, such as individuality, age, and breed differences.

Whereas the ultimate aim of descriptive embryology is to characterize the successive and progressive developmental events as a continuum, the scarcity of accurately timed bovine material necessitates the establishment of standards within the limits of normal variation encountered using an arbitrary time-scale. The stage of embryonic development under discussion is one of extremely rapid growth and differentiation. In view of the heavy losses reported for repeat-breeders during the early stages of pregnancy, and the current interest in the causes of early embryonic mortality, suitable standards of normal bovine development expressed in terms of copulation age are needed.
The bilaminar condition of the growing germ disk described for the youngest specimen of the 16-day group is approximately comparable to the human embryo at the end of the second week (17, 18), to that of the pig at about 8 or 9 days of age (19), and to the sheep at from 10 to 11 days (8). In the human, however, the amnion is established precociously by cavitation at from 7 to 8 days; whereas, in these other species, a more leisurely dorsal folding of the somatopleure occurs.

The more advanced specimens within the 16-day group are actively engaged in the gastrulation process. The growth and elongation of the blastocyst occurs soon after the endoderm has been established as a definite layer. The variation in the length of the chorionic vesicles noted in these specimens is often encountered within a single litter in the pig and attests to the rapidity of growth at this stage. Hawk et al. (16), reporting on 31 bovine embryos recovered at 16 days, noted that the extraembryonic membranes varied in length from 2 to 225 mm., with a mean length of 95 mm. The disk itself lengthens cephalocaudally, converting the disk from a spherical to an oval shape. Both formative mesoderm and extraembryonic mesoderm make their appearance at this time, with the latter giving rise to the mesoderm of the extraembryonic somatopleure, splanchnopleure, and coelom. These features agree closely with the description by Winters et al. (30) of a 16-day, 14-hr.-old embryo.

The 17-day-old group is characterized by a continuing elongation of the blastocyst and local germ disk differentiation to establish the longitudinal axis of the embryo and the primitive streak. This stage of development is comparable to the 11- or 12-day-old pig, and the 13-day-old sheep, embryo. As might be expected, there is some overlapping in development when individual specimens are compared with the more advanced of the 16-day-old embryos, on the one hand, and the less developed of the 18-day-old specimens, on the other.

The most notable features of the 18-day-old group are the formation of the primitive groove, the extension of the boundaries of the mesoderm, the formation of the primitive node, the emergence of the notochord and, in some instances, the establishment of a definite amniotic sac. Although the successive steps in amnion formation are not clearly defined in these specimens, it is reasonable to assume a dorsal projection and fusion of anterior, lateral, and posterior amniotic folds similar to those found in the sheep and pig. With the attainment of the amnion and primitive streak stage, the 18-day-old cow embryo approximates the human embryo of the same age (20), a 13- to 14-day-old sheep (8), and a 13-day-old pig (26). A suggestion that amnion formation in the cow is more precocious than in the pig derives from the contention of Heuser and Streeter (19), that in the pig the embryonic area remains entirely exposed until after the primitive streak and neural folds are formed.

In the present series of 16 specimens, there are two instances of possible embryonic failure. Studies on the nature of reproductive failures in repeat-breeder cows have produced estimates that from 54 to 65% of all fertilized ova in such cows die before 34 days (3, 4, 28, 29). In a similar study with a group of 80 repeat-breeder cows, Hawk et al. (16) estimated embryonic mortality be-
between 16 and 34 days to be 51.7%, and suggested that the majority of the deaths occurred before 25 days.

It is undoubtedly significant that this period of high embryonic death coincides with these critical phases of morphogenesis during which the primary germinal tissues are established. These orderly, successive, integrated processes are dependent upon successful completion of each preceding step and a proper environment. Disturbing factors that in any way upset these normal interrelationships would preclude the successful continuation of the development of body form.

The expansion of the extraembryonic endoderm and the rapid growth of the trophoblast suggest that this period is a critical one in the nutrition of the embryo. The relatively late implantation (33 to 36 days) of the bovine embryo (7, 22) necessitates such a structural adaptation for the absorption of nutrients from the uterine environment after its own intrinsic energy sources have been exhausted. The functional state of the endometrium, in turn, depends upon maintenance of the corpus luteum, which at this same period in a normal estrous cycle would be undergoing degenerative processes. The stimulus for the continuation of a functional corpus luteum of pregnancy undoubtedly arises in the embryo and is probably of trophoblastic origin. Any consideration of possible causes of embryonic death, therefore, must take into account the complex interrelationships of the embryo, the uterus, and the gonads.

Then too, the numerous, intricate processes involved in gastrulation and primitive streak formation provide many opportunities for constitutional defects or fortuitous errors in development to express themselves in embryonic failures. These factors, in addition to the possibilities of endocrine and environmental causation of embryonic pathology, make accurate diagnosis relative to the nature and cause of individual failures virtually impossible with our present knowledge. A prime requisite for a final understanding of the problem is sufficient comprehensive information concerning the normal early embryology of the species to facilitate the recognition of abnormal and retarded development.

REFERENCES


