1990

Changes in morphology, cell number, cell size and cellular estrogen content of individual littermate pig conceptuses on days 9 to 13 of gestation

A. E. Pusateri
Iowa State University

Max F. Rothschild
Iowa State University, mfrothsc@iastate.edu

C. M. Warner
Iowa State University

S. P. Ford
Iowa State University

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Abstract
Days 9 to 12 of gestation in the pig are marked by a pronounced asynchrony among littermate embryos. Previously published studies have suggested that the most advanced embryos within a litter by d 12 synthesize greater amounts of estrogen. Embryonic estrogen secretion has been shown to advance endometrial secretions, which may adversely affect less-developed littermates. To date, however, no comprehensive study of the developmental pattern and synthetic activities of individual littermate embryos during this period has been conducted. Litters were collected from Yorkshire gilts on d 9 (n = 11), 11 (n = 10), 12 (n = 5) and 13 (n = 8). Size (greatest diameter), DNA content (cell number), protein:DNA ratio and estrone (E1) and estradiol-17 beta (E2) content were determined for each embryo. Embryo sizes (mm greatest diameter) were (x +/- SEM) 1 +/- .1, 5.6 +/- .7, 41.2 +/- 11.7 and 405.7 +/- 16.7 on d 9, 11, 12 and 13, respectively. The daily variation in embryo size, expressed as CV was 82% on d 9, 145% on d 11, 206% on d 12 and 46% on d 13. DNA per embryo increased progressively from d 9 to 13, whereas the protein:DNA ratio declined. Content of E1 and E2 per embryonic cell was greatest on d 11 and d 12 before declining markedly on d 13. Cell number and embryo size were correlated positively in embryos 1 to 7 mm (P less than .01) and embryos greater than 100 mm (P less than .01) but not in embryos 8 to 100 mm.

Keywords
Pigs, Embryos, Morphology, DNA, Estrogens

CHANGES IN MORPHOLOGY, CELL NUMBER, CELL SIZE AND CELLULAR ESTROGEN CONTENT OF INDIVIDUAL LITTERMATE PIG CONCEPTUSES ON DAYS 9 TO 13 OF GESTATION[1,2]

A. E. Pusateri[3,5], M. F. Rothschild[3], C. M. Warner[4,6] and S. P. Ford[3,7]

Iowa State University, Ames 50011

ABSTRACT

Days 9 to 12 of gestation in the pig are marked by a pronounced asynchrony among littermate embryos. Previously published studies have suggested that the most advanced embryos within a litter by d 12 synthesize greater amounts of estrogen. Embryonic estrogen secretion has been shown to advance endometrial secretions, which may adversely affect less-developed littermates. To date, however, no comprehensive study of the developmental pattern and synthetic activities of individual littermate embryos during this period has been conducted. Litters were collected from Yorkshire gilts on d 9 (n = 11), 11 (n = 10), 12 (n = 5) and 13 (n = 8). Size (greatest diameter), DNA content (cell number), protein:DNA ratio and estrone (E_1) and estradiol-17β (E_2) content were determined for each embryo. Embryo sizes (mm greatest diameter) were ($\bar{x} \pm$ SEM) 1 ± .1, 5.6 ± .7, 41.2 ± 11.7 and 405.7 ± 16.7 on d 9, 11, 12 and 13, respectively. The daily variation in embryo size, expressed as CV was 82% on d 9, 145% on d 11, 206% on d 12 and 46% on d 13. DNA per embryo increased progressively from d 9 to 13, whereas the protein:DNA ratio declined. Content of E_1 and E_2 per embryonic cell was greatest on d 11 and d 12 before declining markedly on d 13. Cell number and embryo size were correlated positively in embryos 1 to 7 mm ($P < .01$) and embryos >100 mm ($P < .01$) but not in embryos 8 to 100 mm. Initial rapid elongation appears to result both from a redistribution of embryonic cells and an increase in cell number. These data suggest that embryonic diversity and differential estrogen synthesis among littermate embryos is marked on d 11 and d 12.
(Key Words: Pigs, Embryos, Morphology, DNA, Estrogens.)

J. Anim. Sci. 1990. 68:3727–3735

Introduction

In pigs, increased variability in developmental stage among preimplantation littermate embryos has been linked to increased embryonic mortality by d 30 (Pope et al., 1986). Between d 11 and 13 of gestation, pig blastocysts progress from 8- to 10-mm spheres to filamentous forms up to 1 m long (Geisert et al., 1982a). At this time, embryos of all developmental stages may be found within the same uterine horn (Anderson, 1978), and estrogen is elevated in the uterine luminal fluid, seemingly as a result of blastocyst estrogen synthesis (Perry et al., 1976) associated with blastocyst expansion (Ford et al.,

[1]Journal Paper J-13712 of the Iowa Agric. and Home Econ. Exp. Sta., Ames. Proj. 2443, 2444 and 2825; supported in part by USDA grant 85-CRCR-1-1783 and a grant from the Iowa State Univ. Biotechnology Council.

[2]The authors thank Carole Hertz and Lane Christenson for their technical expertise, and Donna Johnston for typing this manuscript.

[3]Dept. of Anim. Sci.

[4]Dept. of Biochem. and Biophysics.

[5]Present address: Pittman-Moore, Terre Haute, IN 47808.

[6]Present address: Dept. of Biol., Northeastern Univ., Boston, MA 02115.

[7]Reprint requests: S. P. Ford, Dept. of Anim. Sci., 11 Kildee Hall, Iowa State Univ.

Received October 5, 1989.

Accepted March 30, 1990.

1982; Geisert et al., 1982b; Fischer et al., 1985). Estrogen alters the uterine environment by triggering the synchronous release of endometrial secretory products (Geisert et al., 1982b, 1987; Young et al., 1987).

Embryo transfer experiments have revealed that more advanced embryos appear to have a detrimental effect on their less-developed littermates (Pope et al., 1982b, 1986; Wilde et al., 1988). It is hypothesized that more advanced embryos within a litter elongate first and release estrogen; estrogen advances the uterine secretory environment to a point incompatible with the survival of the least-developed embryos, which thus are eliminated from the litter (Pope and First, 1985). To determine whether embryonic asynchrony is sufficient for embryonic loss to occur in this way and to more closely document the developmental patterns of individual littermate pig embryos, this study was conducted using Yorkshire sows and gilts from which embryos were collected on d 9, 11, 12 and 13 of gestation.

Materials and Methods

Embryo Collection and Handling

Animals. Purebred Yorkshire sows and gilts were housed in outside pens and checked for estrus daily, between 0800 and 0900, with a mature boar. Females exhibiting estrous cycles of normal duration (18 to 22 d) were mated at the time of estrus and daily thereafter until the end of estrus. Sows and gilts were uniformly distributed for embryo collection at surgery on d 9, 11 and 12 of gestation and at slaughter on d 13 (first day of estrus = d 0).

Surgical Embryo Collection. Feed was withheld for 24 h before surgery. Anesthesia was induced with sodium thiamylal (Surital; 1 g/animal)[8] and maintained with a mixture of oxygen and halothane (fluothane)[9] as previously described (Magness and Ford, 1982). The reproductive tract was exposed through a midventral incision. Corpora lutea (CL) were counted, and the number was recorded. Embryos were recovered from each uterine horn with a single flush of 20 ml of Eagle's Minimum Essential Medium (MEM)[10] injected into the uterine lumen by using a sterile 25-ml glass syringe with an 18-gauge, 3.8-cm needle inserted through the uterine wall at the base of each uterine horn. The flushing medium was gently massaged toward the utero-tubal junction, where flushings were collected into a sterile petri dish through a funnel-tipped glass tube inserted through a small (approximately 1-cm) incision in the uterine wall and firmly secured in the uterine lumen with a surgical silk ligature surrounding the overlying perimetrium.

Collection of Day-13 Embryos at Slaughter. The uterus of each animal was excised at slaughter and immediately placed on ice. The uterine horns were dissected free of the mesometrium, and each horn was bisected at its junction with the uterine body. Each uterine horn then was submerged in a long pan containing distilled H_2O, and the horn was opened longitudinally along the mesometrial border. Embryos then were visualized and individually separated, using a magnifying lens and fine forceps, and placed immediately in phosphate buffered saline (PBS; pH 7.4; 33 m*M* NaH_2PO_4, 35 m*M* Na_2HPO_4, and 125.9 m*M* NaCl). The length of each embryo was measured with the aid of a dissecting microscope. It took approximately 20 to 30 min to remove the embryos from each uterine horn.

Handling of Embryos. From the time of collection, each embryo was treated individually. The morphologic characteristics of each blastocyst were recorded. The diameter (spherical) or length and width (ovoid, tubular, filamentous) of each embryo was measured to the nearest .5 mm. Embryonic size was defined as the greatest diameter or length, and each embryo then was rinsed in PBS. Each embryo then was lyophilized, brought to a known volume in PBS with EDTA (2 m*M*) and 2 *M* NaCl, sonicated to form a homogeneous solution and frozen at –88°C until it was assayed. DNA and protein contents were determined for each embryo. In addition, content of estrone (E_1) and estradiol-17β (E_2) of each embryo in four litters was determined on d 11, 12 and 13.

Grouping of Embryos. In addition to grouping by day of collection, embryos were assigned to developmental groups on the basis of their size (greatest diameter) and morphological characteristics. The four developmental

[8]Parke-Davis Laboratories, Morris Plaines, NJ.
[9]Ayerst Laboratories, New York.
[10]Sigma Chemical Company, St. Louis, MO.

groups were defined as follows: embryos ≥1 mm and ≤7.5 mm, spherical; embryos ≥8 mm and ≤12 mm, transitional; embryos ≥12.5 mm and ≤100 mm, elongating; and embryos >100 mm, filamentous. The range of sizes assigned to the transitional group was based on our finding that 8 mm was the smallest diameter at which the ovoid structure was observed and that embryos smaller than 12 mm were never dumbbell-shaped. The separation between the elongating group and the filamentous group at 100 mm was based on the findings of Geisert et al. (1982b), who determined that the initial rapid stage of elongation was from 9 or 10 mm to 100 mm.

Assay Methodology

DNA Assay. Embryonic DNA was quantitated by using the methods of LaBarca and Paigen (1980) with the following modifications. To maximize fluorescence, 2 *M* NaCl was included in the assay buffer. Assay standards were prepared from calf thymus DNA in the assay buffer and the standards were sonicated by using the same protocol that was used for the embryos. A standard pool of pig splenocytes containing 1.4×10^6 cells (determination based on 5.0 pg DNA per cell [Vendrely and Vendrely, 1949]) per milliliter of assay buffer was sonicated and included in each assay to estimate assay variation. The pool was diluted to fall near the center of the standard curve. The sensitivity of the assay was 5×10^{-3} μg DNA/ml and the interassay and intra-assay CV were 6.25% and 1.74%, respectively.

Protein Determination. The protein content of each embryo was determined spectrophotometrically by using an acidic solution of Coomassie Brilliant Blue G-250 (Bio-Rad Protein Assay)[11] and measuring the absorbance at 595 nm. Assay standards were prepared from bovine serum albumin, which was sonicated the same way as the embryos. A pool of sonicated pig splenocytes containing 33.3 μg protein/ml was diluted to fall near the center of the standard curve and included in each assay to determine variation. The assay sensitivity was 1 μg protein/ml, and the interassay and intraassay CV were 9.43% and 6.37%, respectively.

[11]Bio-Rad Chemical Division, Richmond, CA.

Estrogen Radioimmunoassay. Estrone and E_2 content of each embryo was determined by radioimmunoassay as previously described and validated in this laboratory (Magness and Ford, 1982). A pool of homogenized porcine embryos was utilized in each assay to estimate variation. The pool contained 328 ± 80 pg of E_1 and 781 ± 116 pg of E_2 per milliliter and was diluted to fall near the center of the respective standard curves. The interassay CV for E_1 and E_2 were 14.4% and 15.1%, respectively. Intra-assay CV were 10.3% for E_1 and 6.1% for E_2.

Statistical Analyses. Data were analyzed by using least squares analysis of variance (SAS, 1985). The statistical model included effects of day of collection and litter within day or developmental group of the embryo. Preplanned comparisons were made using the PDIFF procedure.

Diameter was not accurately determined for embryos <1 mm in diameter. Because of this, and because their estrogen content could not be determined, these embryos were included only in analyses comparing DNA content, protein content and protein:DNA ratio. When analysis was based on day of collection, embryos <1 mm in diameter were included in data from the appropriate day. These embryos were treated as a separate group when analysis was based on developmental group.

Results

A total of 440 embryos from 34 litters resulting from the matings of 8 boars, 3 sows, and 28 gilts was collected. Two litters were collected from three of the gilts, but on different days of gestation. Eleven litters were collected on d 9, 10 on d 11, 5 on d 12 and 8 on d 13. The percentage of embryos recovered (number of embryos/number of corpora lutea × 100%) was 83.6 ± 2.3% and was not affected by day. On d 9 of gestation, all embryos collected were spherical in shape (Table 1). On d 11, embryos ranged in size from 1 mm to 82 mm, on d 12, the range was from 1 mm to 494 mm and on d 13, the vast majority (96.8%) were filamentous. Embryo sizes (mm greatest diameter) were 1 ± .1, 5.6 ± .7, 41.2 ± 11.7 and 405.7 ± 16.7 on d 9, 11, 12 and 13, respectively. Variation in developmental stages of embryos was greatest on d 11 and 12. One gilt on d 11 and one gilt on d 12 exhibited the entire range of embryo sizes for that day

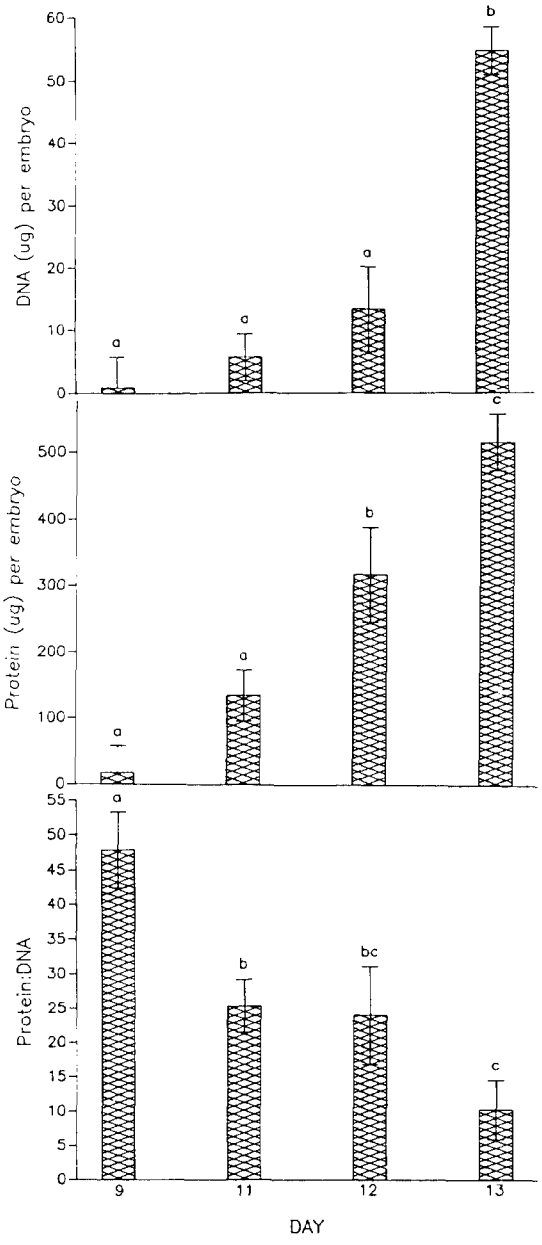

Figure 1. Content of DNA (top panel), protein (middle panel), and the protein:DNA ratio (bottom panel) of pig embryos collected on d 9 (n = 96), 11 (n = 136), 12 (n = 52) and 13 (n = 109) of pregnancy. Least squares means ± SEM; within a panel, bars with different superscripts differ ($P < .01$).

within its litter. Across litters, the CV of embryonic diameter was 82.4% on d 9, 145.2% on d 11, 206.5% on d 12 and just 46.0% on d 13.

The DNA per embryo (Figure 1, top panel) increased ($P < .01$) from d 9 to 13. Protein per embryo (Figure 1, middle panel) followed a pattern similar to that of DNA, increasing between d 9 and 13 ($P < .01$). The ratio of protein to DNA decreased between d 9 and 13 (Figure 1, bottom panel; $P < .01$). This ratio decreased between d 9 and 11 ($P < .01$), did not change between d 11 and 12, but again tended to decrease between d 12 and 13 ($P = .11$).

On the basis of preliminary observations, we determined that the estrogen content of d-9 embryos fell below the sensitivity of our radioimmunoassay. Accordingly, determinations of estrogen content were not made on these embryos. Total embryonic content of E_2 differed by day ($P < .10$), as did the content of E_1 ($P < .10$). Estradiol-17β (E_2) content did not differ between d 11 and 12 (745 ± 408 pg vs 1,572 ± 404 pg, respectively), but decreased ($P < .05$) by d 13 (162 ± 321 pg). The E_1 content of the embryos followed a pattern similar to that of E_2, with embryos containing 895 ± 387 pg, 1,553 ± 383 pg and 257 ± 305 pg E_1 on d 11, 12 and 13, respectively.

Differences in estrogen content were more dramatic when expressed on a per-cell basis. The content of E_2 per million embryonic cells (based on 5 pg DNA/cell) was not different between d 11 and 12 but decreased sharply by d 13 (Figure 2; $P < .05$). As with E_2 content, E_1 content per million embryonic cells was similar on d 11 and 12, before decreasing markedly ($P < .01$) on d 13. The mean CV within litter for embryonic E_2 content was 38 ± 13% on d 11, it increased to 84 ± 13% on d 12 ($P < .05$), and it reached its highest level on d 13 (105 ± 12%; $P < .01$). The mean CV within litter for embryonic E_1 content did not differ by day and were 49 ± 16%, 62 ± 16%, and 97 ± 14% on d 11, 12 and 13, respectively.

Comparison of embryos by developmental group showed that DNA per embryo increased ($P < .01$) as embryos progressed from the spherical to the filamentous form and that protein per embryo also increased ($P < .01$; Table 2). For both DNA and protein, average content increased with each progressive developmental group, except that there were no differences between the transitional and elongating groups. Protein:DNA ratio decreased significantly ($P < .01$) as embryos progressed from <1 mm spheres to the filamentous form. Protein:DNA ratio decreased ($P < .01$) be-

TABLE 1. PERCENTAGE OF PIG EMBRYOS OF EACH DEVELOPMENTAL GROUP COLLECTED ON EACH DAY OF EARLY GESTATION

Day of gestation	Developmental groups				
	<1 mm	Spherical	Transitional	Elongating	Filamentous
9	37.8	62.2	0	0	0
11	0	84.5	7.4	8.1	0
12	0	45.3	13.2	28.3	13.2
13	0	.8[a]	0	2.4	96.8

[a]Represents a single embryo.

tween the embryos <1 mm (ratio = 67 ± 4) in diameter and embryos in the spherical group (ratio = 30 ± 2). This ratio did not change significantly between spherical and transitional embryos (ratio = 25 ± 6) or between transitional and elongating embryos (ratio = 20 ± 5); however, the ratio of protein:DNA in elongating embryos tended to be smaller than those of spherical embryos ($P < .10$). The value in filamentous embryos (protein:DNA ratio = 11 ± 2) was smaller than that in any other group observed ($P < .01$).

On a whole-embryo basis, embryonic E_2 content increased ($P < .01$) from 507 ± 145 pg to 1,544 ± 267 pg between the spherical and transitional groups, remained elevated in the elongating group (2,051 ± 209 pg), then decreased markedly ($P < .01$) to only 356 ± 118 pg in the filamentous group. Embryonic E_1 content followed the same pattern of increase and decline, averaging 642 ± 127 pg, 1,760 ± 231 pg, 2,062 ± 181 pg and 404 ± 102 pg in the spherical, transitional, elongating and filamentous groups, respectively. On a per-cell basis, as depicted in Figure 3, the E_2 content of embryos increased ($P < .05$) from 355 ± 50 pg per million cells in spherical embryos to 575 ± 94 pg in transitional embryos. The content per million embryonic cells did not change with the progression from transitional to elongating, but it dropped markedly ($P < .01$) from 619 ± 72 pg in elongating embryos to only 90 ± 41 pg in the filamentous embryos. The content of E_1 per million cells followed a similar pattern, with an increase from 448 ± 44 pg in spherical to 617 ± 82 pg in transitional embryos ($P < .10$); there was no difference in content between the transitional and elongating embryos but a large ($P < .01$) decrease from 619 ± 62 pg in elongating embryos to 84 ± 36 pg in filamentous embryos.

Embryo size (maximum diameter) was correlated with DNA content in the spherical ($r = .90$; $P < .01$) and filamentous ($r = .43$; $P < .01$) groups, whereas during the transitional and elongating stages, no significant correlations were observed between size and DNA content. The cellular content of both E_1 and E_2 were positively correlated with embryo size in the spherical group ($r = .30$; $P < .05$; and r =

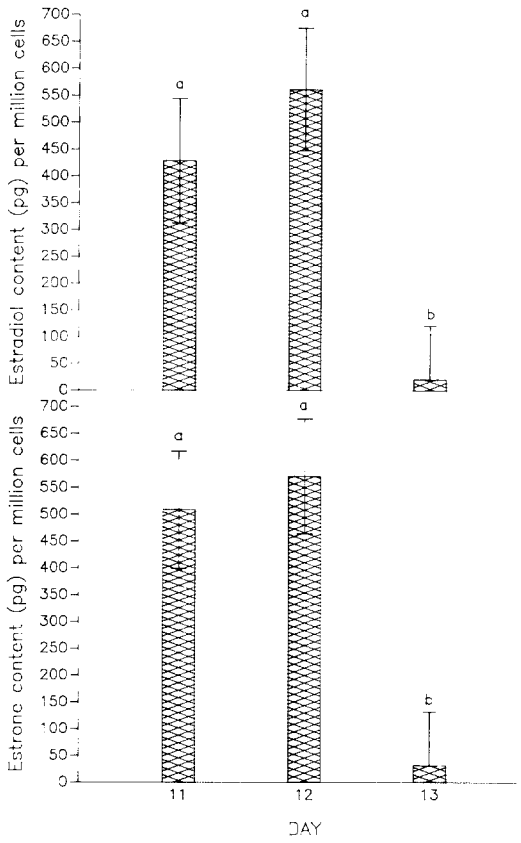

Figure 2. Estradiol-17β (top panel) and estrone (bottom panel) content per million cells in pig embryos collected on d 11, 12 and 13 of pregnancy. Least squares means ± SEM; bars with different superscripts differ ($P < .05$).

TABLE 2. DNA AND PROTEIN CONTENT (μg) IN PIG EMBRYOS IN DIFFERENT DEVELOPMENTAL GROUPS[a]

	Embryo developmental group				
	<1 mm	Spherical	Transitional	Elongating	Filamentous
DNA content	.26[bf] ± 2.32 n = 47	3.18[b] ± 1.08 n = 218	13.36[c] ± 3.86 n = 17	16.88[c] ± 2.95 n = 29	51.71[d] ± 1.43 n = 124
Protein content	8[b,g] ± 28 n = 32	81[c] ± 11 n = 203	303[d] ± 37 n = 18	324[d] ± 30 n = 28	507[e] ± 15 n = 112

[a]Means ± standard errors.

[b,c,d,e]Least squares means ± SEM with different superscripts within a row differ ($P < .05$).

[f]Median value: 1.5

[g]Median value: 40.

.27; $P < .05$, respectively), were not significantly correlated with size in the transitional and elongating groups and were negatively correlated with embryo length for E_2 (r = –.30; $P < .01$) and for E_1 (r = –.32; $P < .01$) in the filamentous group.

Discussion

These data are the first to quantify and compare the size, protein, DNA and estrogen content of individual littermate pig embryos during the preimplantation period. Variation in the developmental stages of littermate embryos was less on d 9 and 13 than on d 11 and 12. Day-12 embryos showed the most variation; a range of embryo sizes from 1 to 494 mm was observed and all developmental stages were represented. This finding of a large littermate variation on d 12 agrees with those of others (Anderson, 1978; Ford et al., 1982; Geisert et al., 1982a) and is consistent with the hypothesis that embryonic asynchrony in the preimplantation period may be a factor contributing to embryonic mortality.

The growth of spherical blastocysts from less than 1 mm in diameter to 8 mm in diameter appears to be due to rapid cellular hyperplasia. This explains the positive correlation (r = .90; $P < .01$) between embryo diameter and embryonic DNA content in spherical embryos. These data confirm those of Geisert et al. (1982a), who reached the same conclusion concerning pig embryos up to about 10 mm in diameter on the basis of mitotic index and DNA content.

On the basis of the rapidity with which pig blastocysts exhibit structural change and elongate, Perry (1981) suggested that elongation is by deformation rather than by cell division. Geisert et al. (1982a) found that DNA content did not increase and that mitotic activity was low in pig blastocysts during the initial stages

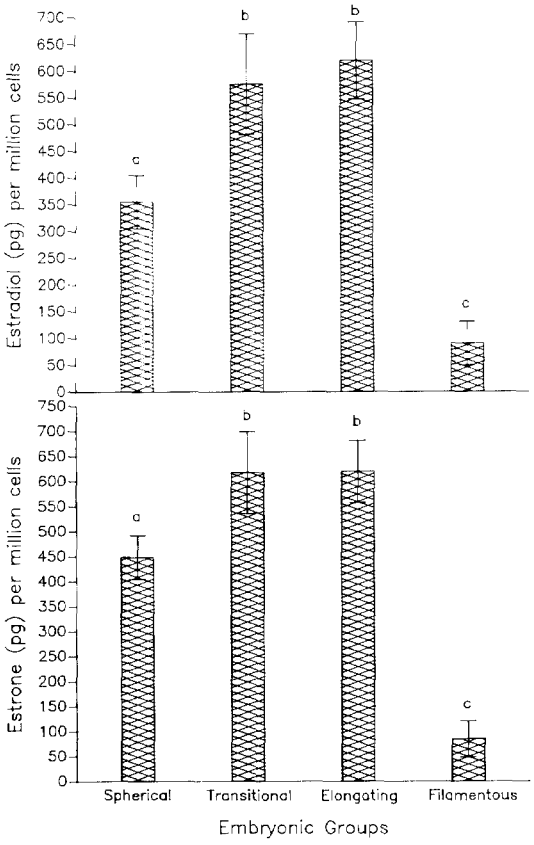

Figure 3. Estradiol-17β (top panel) and estrone (bottom panel) content per million cells in pig embryos in different development groups. Least squares means ± SEM; bars with different superscripts differ ($P < .05$).

of elongation (10 to 330 mm). They also reported the formation of an elongation zone in ovoid and tubular blastocysts and concluded that the initial stages of elongation were achieved by cell migration and restructuring. Data from the present study lend additional support to the concept of embryo restructuring during this period and more closely pinpoint the period during which mitosis is of lesser importance in the elongation process. From the time embryos first become ovoid until they are filamentous forms approximately 100-mm long, mitosis is not the primary contributor to the increased length of the embryo. This is based on the findings that DNA content and maximum diameter (or length) were not correlated in the transitional or elongating groups and that DNA content was not different between the transitional and elongating groups. At lengths above 100 mm, mitosis does appear to be closely related to size increases because DNA content and length were once again positively correlated ($r = .43$; $P < .01$).

Embryonic protein synthetic activity (Godkin et al., 1982) and the cellular ultrastructure of embryonic cells (Geisert et al., 1982a) change during the period of gestation studied. The observed changes in embryonic protein: DNA ratio may reflect these events. Perhaps the decreasing protein:DNA ratio is due, at least in part, to a reduction in average embryonic cell size (Baserga, 1985). Viewed in this way, the changes in cell size agree well with the pattern of mitosis indicated by embryonic DNA contents: decreasing cell size and rapid mitosis as spherical embryos expand to about 8 mm, unchanging cell size and slow mitosis as embryos change structure and begin elongating up to about 100 mm, and decreasing cell size with rapid mitosis as embryos elongate past 100 mm. It should be noted that any implications of these data for cellular events must be interpreted on a whole-embryo basis, because whole embryos were used for all determinations in this study and no differentiation can be made between the activities or sizes of different cell types within embryos.

With the assumption that embryonic estrogen content is a reflection of synthesis, data from this experiment indicate that the onset of estrogen synthesis is related very closely to structural changes and the initial stages of elongation of pig blastocysts. The pattern of the onset of estrogen synthesis is consistent with the hypothesis that estrogen synthesis is initiated by some embryos within a litter before others and that this estrogen may be a factor in embryonic mortality. Embryonic estrogen content was elevated on d 11 and 12 of pregnancy but declined by d 13. The within-litter CV for estradiol and estrone illustrate the great variation inherent among littermate pig embryos during this period. When compared on the basis of developmental groups, embryonic estrogen contents suggest that estrogen synthesis increased as embryos progressed from spherical to transitional, remained elevated during the initial period of elongation and then declined as embryos expanded to lengths greater than 100 mm. These data are consistent with those of Pope (1988), who reported that on d 12 of gestation in pigs, those embryos within a litter that were changing structure and elongating synthesized more E_2 in culture than spherical embryos. This pattern of embryonic synthesis would account for the results of Geisert et al. (1982b), who found that estrogen content in uterine flushings were elevated on d 12 of pregnancy but declined by d 14.

The apparent rapid decline in estrogen synthetic activity as elongation progresses may be due, all or in part, to cessation of synthesis in previously synthetically active cells. This is supported by the preliminary finding that the levels of 17α hydroxylase in pig embryonic tissue declines markedly between d 12 and 13 (personal communication, A. J. Conley, Southwest Texas Medical Center). Because content of estrogen was decreased in filamentous embryos, it seems that a cessation of synthesis must play some role. Considering the findings of Bate and King (1988) that there are differences in estrogen synthetic activity of trophectoderm from different regions of d-14 pig blastocysts, perhaps another contributor to the estrogenic decline may be an increase in the relative number of cells in the less synthetically active regions due to the increased mitotic activity in filamentous blastocysts.

There were no differences in proportion of embryos recovered between the days studied, but approximately 17% were not recovered, indicating a possible loss occurring before d 9. Thus, embryos destined to be lost during the late preimplantation period may not be eliminated until after d 13. This is in agreement with the findings of Pope et al. (1986), who

found that the greater embryonic mortality associated with increased variability among littermate embryos did not occur by d 13.

Critical events occurring during the period from d 11 to 13 include the equidistant spacing of embryos through the uterus (Dhindsa et al., 1967; Dziuk, 1968, 1985) and the initiation of attachment (Crombie, 1970; Dantzer, 1985). Estrogen appears to be very important in embryonic spacing (Pope et al., 1982a) and its release by embryos is closely associated with the time of attachment (Ford et al., 1982; Geisert et al., 1982b; Fischer et al., 1985).

Morgan et al. (1987) and Gries et al. (1989) have shown that embryos subjected to an estrogen-altered uterine environment on d 9 and 10 elongate normally but do not attach. Instead, they degenerate and die by d 16 to 18 of pregnancy. From this observation, probably the embryos adversely affected by their more advanced littermates do not space normally and fail to attach. As a result, these embryos, although they continue to develop for a time, ultimately might die and disintegrate by d 16 to 18 of pregnancy.

Although it reduces litter size from the theoretical maximum, embryonic variation may afford a reproductive benefit to the pig. Many environmental factors may interact differently with each pregnancy to affect embryonic survival (Hanly, 1961; Dziuk, 1987). Biological variation in the maternal system, whether environmentally induced or due to inherent differences between females, may result in variable uterine situations: d 12 in one sow during one pregnancy may not necessarily be equivalent to d 12 in another sow or in the same sow during another pregnancy. Embryonic developmental variability may function to ensure that, in most situations, some embryos within a litter will be at an appropriate stage of development during the period immediately preceding attachment to allow pregnancy to continue.

Implications

The greatest developmental diversity among littermate embryos occurs on d 11 and 12, in close association with the initial stages of elongation and the onset of embryonic estrogen synthesis. Our data support the hypothesis that, on d 11 and 12 of pregnancy, the more developed embryos within a litter preferentially secrete estrogen, which has been shown previously to alter the uterine environment.

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