EARLY PREGNANCY IN THE PIG

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(Received 10th January 1962)

Summary. Observations have been made on fifty-eight sows and gilts killed between 2 and 40 days after mating to study various aspects of early pregnancy, and to determine the incidence of embryonic loss, and the time and stage of development when this loss occurs.

The corpus luteum count was found to provide an accurate estimate of the number of ova ovulated. No difficulty was encountered in recovering all the eggs from the Fallopian tubes, but only 89% of the expected number were recovered from the uterus. In forty mated animals killed before the 10th day after the onset of oestrus, 95.5% of all eggs recovered were fertilized. The stages in cleavage of the eggs and the elongation of the blastocysts are described. Development was normal in all tubal eggs, but 22% of those recovered from the uterus between the 6th and 9th days were degenerating. Embryonic loss in thirteen pigs killed between the 13th and 18th days was 28.4%; the greater part of this loss occurred in two of the animals. The loss in a third group of five gilts killed between the 25th and 40th days was 34.8%, about one-half of which was contributed by one of the animals.

The uterus was found to elongate throughout the first 18 days of pregnancy and the elongation was most rapid between the 2nd and 6th days when a 50% increase in length occurred. Linear growth is correlated with increase in uterine weight. These facts are contrasted with those obtained for other species.

Many of the corpora lutea in animals killed before the 10th day become distended to form cysts. This condition was considered to be transient as few were found between the 10th and 18th days and none at later stages. A sudden increase in the size of the ‘normal’ corpus luteum takes place soon after the 6th day, at the time of blastocyst formation.

INTRODUCTION

By the 25th day of pregnancy in the pig, a discrepancy of 30 to 40% (see Hanly, 1961) exists between the numbers of embryos and corpora lutea; the latter are generally assumed to represent the number of ova ovulated. The greater part of this discrepancy is due to embryonic loss, but the stage in gestation at which death occurs has not been determined precisely. This investigation was undertaken to study the numerical relationship between corpora lutea and embryos.
at earlier stages, in animals in which the time of onset of oestrus was accurately known. It was extended to include some aspects of ovarian histology and measurements of the reproductive tract.

MATERIALS AND METHODS

Animals

Three groups of Large White pigs were obtained from different sources and at different times. The first group consisted of eleven gilts and ten primiparous sows bred in the Institute: they were killed between June and November, 1958. Groups 2 and 3 were purchased from pedigree breeders in the locality. Group 2 comprised three gilts from each of six litters; they were allocated to three sets of six, designated A1 to 6; B1 to 6 and C1 to 6, so that A1, B1 and C1 were litter sisters, and so on. All these animals were killed between January and June, 1960. Group 3 contained four litter sisters from each of six litters. These animals were allocated to sets, E, F, G and H, and again numbered so that E1, F1, G1 and H1 were litter sisters, and so on.

Mating

The onset of oestrus was determined by testing with a boar every 3 to 6 hr, and mating was allowed at first acceptance and again on the following day. Two animals (G3 and G5) refused a second service.

Time of slaughter

The animals were slaughtered in a commercial abattoir at selected intervals after the onset of oestrus. Throughout this paper, times given in hours or days refer to the interval between the onset of oestrus and the time of slaughter. Forty animals were killed between the 2nd and 9th days, thirteen between the 13th and 18th days, four between the 26th and 29th days and one on the 40th day. The reproductive tract was removed immediately after the animals had been bled, and the ovaries were placed on dry ice. The tract was freed from the mesometrium and mesosalpinx, and the Fallopian tubes and uterine horns were measured in the relaxed condition.

Recovery of eggs

After preliminary trials during which some eggs were undoubtedly lost, the following methods were adopted:

(a) Fallopian tube. The tube was removed with the tip of the uterine horn attached, and about 200 ml of Krebs-Ringer solution was forced through from the fimbrial end and collected in a beaker.

(b) Uterine horn. The horns were severed at the cervical end and about 1 litre of solution was poured through from the ovarian end and collected in a beaker. After about 5 min most of the flushing fluid was siphoned slowly into a second beaker and the remainder (about 50 ml) distributed between about ten small glass basins for examination under the dissecting microscope. The main volume of fluid was re-siphoned and the remainder re-examined. If after the second examination a number of ova equal to the number of corpora lutea had been found, no further flushing was considered necessary, but if not, the
was one-half between and the three pigs killed up to the 9th day after the onset of oestrus.

**Examination of eggs**

Eggs were examined immediately after recovery. The presence of spermatozoa in the zona pellucida was adopted as a criterion of fertilization, and all eggs, except those which had a shrunkened vitellus, or cells of very unequal size, were regarded as undergoing normal development. No advantage was gained from the use of the phase-contrast microscope because of the large amount of fat in the pig egg. The eggs were fixed in 4% formol-saline solution, and in some cases all those from one animal were imbedded within a single block of fixed tissue. Sections were cut at 3µ and stained in haematoxylin and eosin.

**Dissection of uterus**

The uterus of animals killed between the 13th and 18th days after the onset of oestrus was dissected under 0·9% sodium chloride solution in a metal tank having a matt black inner surface and measuring 84 in. × 6 in. × 1½ in. The myometrium was cut longitudinally along the anti-mesometrial border; the cut edges were stretched and the mucosa was torn gently to expose the endometrium (see Pl. 1, Fig. 1). The blastocysts, which at this stage of development are greatly elongated, were then readily seen. Their location in the uterus was noted before they were freed from the folds of the mucosa. They were then floated out and measured and the position of the embryo on the embryonic sac was recorded. The stage of development of somites, amniotic folds and allantois was determined under the dissecting microscope.

**Examination of ovaries**

The ovaries of the first group of twenty-one animals were fixed in Bouin’s fluid; serial sections were cut and one in twenty mounted and stained with haematoxylin and eosin. The volume of each corpus luteum was calculated from three diameters. The normal and atretic vesicular follicles more than 0·5 mm diameter were counted and measured. The corpora lutea in the remaining groups were dissected in the fresh condition and weighed.

**RESULTS**

**TIME OF ONSET OF OESTRUS**

The records show that among the fifty-eight animals in which the onset of heat was determined as accurately as possible, forty were mated for the first time between 6 a.m. and 6 p.m. and eighteen between 6 p.m. and 6 a.m. About one-half (nineteen) of the former matings occurred between 9 a.m. and noon, and one-third (twelve) of the latter occurred between 9 p.m. and midnight. Only three mated between midnight and 6 a.m. No attempt was made to determine the time of ovulation, but it is generally agreed that it occurs 24 to 36 hr after the onset of oestrus.
RECOVERY OF EGGS

The data given in the top row of Table 1 show that the number of eggs recovered from the intact Fallopian tube by the method described above tallies exactly with that of the associated corpora lutea. The proportion of eggs recovered from the uterus was 89%. Only one egg was lost in each of eight of the sixteen animals from which the recovery of uterine eggs was incomplete, and in the remainder the losses varied from two to six eggs. Seventeen of the 507 eggs were damaged during collection; the zona pellucida had been ruptured and the vitelline contents extruded. There was no significant difference \((P>0.1)\) between the proportion of damaged eggs recovered from the Fallopian tubes \((5.6\%)\) and from the uterine horns \((2.3\%)\). As spermatozoa were embedded in all of them they were regarded as having been fertilized.

<table>
<thead>
<tr>
<th>Location of eggs</th>
<th>Time after onset of oestrus (hr)</th>
<th>No. animals</th>
<th>No. corpora lutea</th>
<th>No. eggs recovered</th>
<th>No. animals in which recovery was complete</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubal</td>
<td>48 to 82</td>
<td>9*</td>
<td>118</td>
<td>118</td>
<td>9</td>
</tr>
<tr>
<td>Tubal + uterine</td>
<td>65 to 107</td>
<td>5</td>
<td>70</td>
<td>63</td>
<td>2</td>
</tr>
<tr>
<td>Uterine</td>
<td>71 to 214</td>
<td>25</td>
<td>363</td>
<td>324</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>39</td>
<td>551</td>
<td>507</td>
<td>20</td>
</tr>
</tbody>
</table>

* Excluding one animal (Red 4) in which the Fallopian tubes were cut into segments before flushing.

FERTILIZATION RATE

The number of eggs ovulated (= number of corpora lutea) in these fifty-eight animals ranged from nine to twenty-two (average = 14.9). In only one of these animals (G3) were all the eggs unfertilized. This animal was a gilt in which the period of oestrus at the time of mating was unusually brief and it was only served once. The criterion of fertilization which was used was applicable only to the forty animals which were slaughtered up to 9 days after oestrus. All the eggs that were recovered were considered to have been fertilized in thirty-five of these animals. The remainder included the one mentioned above (G3), one in which fertilization occurred in only one uterine horn, one in which there were two unfertilized eggs and two in which there was one. Of the 514 eggs examined 491 (95.5%) were fertilized; nineteen of the twenty-three unfertilized eggs occurred in only two of the forty animals.

CLEAVAGE AND LOCATION OF FERTILIZED EGGS

The data concerning the cleavage of eggs in animals killed up to the 9th day are summarized in Table 2. The first cleavage had already occurred in sixty-nine and the second had been completed in forty of the eighty-two eggs recovered during the 3rd day after the onset of oestrus (18 to 42 hr after ovulation). The
**Table 2**

ANALYSIS OF LOCATION, FERTILIZATION AND CLEAVAGE OF EGGS RECOVERED FROM FORTY MATED PIGS KILLED BETWEEN THE 2nd AND 9th DAYS AFTER THE ONSET OF OESTRUS

<table>
<thead>
<tr>
<th>Time after onset of oestrus (hr)</th>
<th>No. animals</th>
<th>Location of eggs (No. animals)</th>
<th>No. fertilized eggs</th>
<th>No. unfertilized eggs</th>
<th>Site of recovery</th>
<th>Stage in cleavage of normal eggs (No. cells. M = Morula, Bl = Blastocyst)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Tubal only</td>
<td>Tubal/uterine</td>
<td>Uterine only</td>
<td>Normal</td>
<td>Degenerating</td>
</tr>
<tr>
<td>48 to 71</td>
<td>8</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>82</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>72 to 95</td>
<td>9</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>110</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>96 to 119</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>56</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120 to 143</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>42</td>
<td>23</td>
</tr>
<tr>
<td>144 to 167</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>71</td>
<td>27</td>
</tr>
<tr>
<td>168 to 191</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>51</td>
<td>1</td>
</tr>
<tr>
<td>192 to 215</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>40</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>423</strong></td>
<td><strong>17</strong></td>
</tr>
</tbody>
</table>

Fertilization rate = 95.5%.
second division, however, is shown not to have been completed in all eggs for another 2 days (66 to 90 hr after ovulation) and the third division, which had already begun in one animal (A1) killed early in the 3rd day, had not been completed in all of them until the 7th day. This variation is largely attributable to individual differences in the time of ovulation relative to the onset of oestrus. Eggs in the four-celled stage were recovered from almost all animals killed up to 120 hr after the onset of oestrus. The grouped data in Table 2 suggest that cleavage is more advanced in eggs recovered from the uterus than in those located in the Fallopian tubes within the same 24-hr period after the onset of oestrus. This suggestion is not borne out by the records of individual animals, or by the data obtained from the five animals in which eggs were recovered from both regions of the tract and in which, therefore, variation between animals in the time of ovulation during oestrus is eliminated. The total numbers of tubal and uterine eggs at differing stages of cleavage in these five animals was as follows:

<table>
<thead>
<tr>
<th></th>
<th>4-cell</th>
<th>5-cell</th>
<th>6-cell</th>
<th>7-cell</th>
<th>8-cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubal</td>
<td>15</td>
<td>6</td>
<td>12</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Uterine</td>
<td>9</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

Blastocysts were first seen on the 6th day and all fertilized eggs had reached this stage before the 8th day. All sixty-two blastocysts recovered during the 8th and 9th days (168 and 214 hr; see Table 2) had begun to increase in size and had lost the zona pellucida. They were still spherical, the largest being 4 mm in diameter.

ELONGATED BLASTOCYSTS

The material described in this section comprises thirteen animals killed between the 13th and 18th days, in which the blastocysts had already elongated. A portion of one of these blastocysts (from C5, 13 days) is shown in situ in Pl. 1, Fig. 1. The mucosa is greatly corrugated, and slightly hyperaemic along the line of the mesometrium. The blastocyst follows the mucosal folds, and therefore occupies only a short length of the uterus compared with its own length when extended. Thus the blastocysts of C5, as shown in Table 3, occupied an average distance of 32-9 cm of uterus, whereas their average length when extended was 121 cm. One of these blastocysts after its removal from the uterus is shown in Pl. 1, Fig. 2 and the position of its embryo is indicated.

The disposition of the blastocysts within the uterine horns of ten of these animals is shown diagrammatically in Text-fig. 1; two other animals had to be omitted because of incomplete data, and a third one (C3) because it contained no embryos. In animals in which most or all embryos have survived, the blastocysts occupy almost the entire length of the uterine horns. There is rarely any measurable overlap between adjacent blastocysts but their ends sometimes touch (as in C5) and occasionally adhere. A sac with 'twin' embryos was noticed in two animals (F4 and C2). The blastocysts were of very variable length in individual animals and, in general, they were longer when there were fewer of them in a uterine horn. The range of embryonic development in these animals is given in Table 3; the amniotic folds were widely open (Pl. 2, Fig. 3)
Fig. 1. Portion of uterine horn opened longitudinally to show part of elongated blastocyst in situ, 13 days (actual size).

Fig. 2. An elongated blastocyst from the same animal. The position of the embryo is indicated by an arrow (actual size).
Fig. 3. An embryo from gilt F4 (14 days) (×21).

Fig. 4. Section of ovary (4 days) showing six ‘normal’ corpora lutea and several normal and few atretic follicles (×3·3).

Fig. 5. Section of ovary (9 days) showing one normal and four distended corpora lutea. All the larger follicles are atretic (×2·9).

(Facing p. 181)
### Table 3

**Embryonic Development in Thirteen Mated Gilts between the 13TH and 18TH Days after the Onset of Oestrus**

<table>
<thead>
<tr>
<th>Gilts</th>
<th>Time after onset of Oestrus (days)</th>
<th>No. corpora lutea</th>
<th>Embryo</th>
<th>Embryonic Sacs (cm)</th>
<th>Length of uterine horns (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>Stage in development</td>
<td>Average length in situ</td>
<td>Average length (extended)</td>
</tr>
<tr>
<td>C5</td>
<td>13</td>
<td>17</td>
<td>10</td>
<td>0 to 4 somites</td>
<td>32.9</td>
</tr>
<tr>
<td>F4</td>
<td>14</td>
<td>15</td>
<td>16</td>
<td>Pre-somite</td>
<td>18.1</td>
</tr>
<tr>
<td>C2</td>
<td>15</td>
<td>18</td>
<td>17</td>
<td>6 to 10 somites</td>
<td>20.6</td>
</tr>
<tr>
<td>F2</td>
<td>15</td>
<td>10</td>
<td>9</td>
<td>8 to 10 &quot;</td>
<td>33.2</td>
</tr>
<tr>
<td>H5</td>
<td>15</td>
<td>16</td>
<td>14</td>
<td>Pre-somite</td>
<td>19.6</td>
</tr>
<tr>
<td>F5</td>
<td>15</td>
<td>11</td>
<td>10</td>
<td>3 to 9 somites</td>
<td>23.8</td>
</tr>
<tr>
<td>C6</td>
<td>15</td>
<td>18</td>
<td>17*</td>
<td>4 to 6 &quot;</td>
<td>25.4</td>
</tr>
<tr>
<td>H2</td>
<td>16</td>
<td>12</td>
<td>11</td>
<td>12 &quot;</td>
<td>25.4</td>
</tr>
<tr>
<td>C1</td>
<td>16</td>
<td>13</td>
<td>13</td>
<td>5 to 6 &quot;</td>
<td>25.4</td>
</tr>
<tr>
<td>C6</td>
<td>16</td>
<td>21</td>
<td>0</td>
<td>&quot;</td>
<td>-</td>
</tr>
<tr>
<td>H1</td>
<td>16</td>
<td>18</td>
<td>3</td>
<td>3 to 5 somites</td>
<td>25.4</td>
</tr>
<tr>
<td>D2</td>
<td>17</td>
<td>11</td>
<td>10</td>
<td>5 to 6 &quot;</td>
<td>28.1</td>
</tr>
<tr>
<td>H6</td>
<td>18</td>
<td>14</td>
<td>10</td>
<td>25 &quot;</td>
<td>26.5</td>
</tr>
<tr>
<td>Total</td>
<td>194</td>
<td>139</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Includes one degenerating embryo.
and somites had not formed, in two of them. Closure of these folds seems to occur when the embryos contain five to six pairs of somites. Embryonic development was considerably more advanced in the animal (H2) killed on the 18th day, and a rudimentary blood-vascular system was visible. The embryonic sacs were distended as compared with those of early specimens, especially in the vicinity of the embryo. They were slightly adherent to the endometrium over the whole surface of the trophoblast.

![Diagram indicating position of embryos and extent of trophoblastic sacs in the uterus of ten gilts 14 to 18 days after the onset of oestrus.](image)

**Text-fig. 1.** Diagram indicating position of embryos and extent of trophoblastic sacs in the uterus of ten gilts 14 to 18 days after the onset of oestrus. +, position of embryo; + +, point of contact between tips of two adjacent blastocysts; ±, slight overlap and adhesion of tips of two adjacent blastocysts; T, twin embryos in a single trophoblastic sac.

**EMBRYONIC MORTALITY**

*In animals killed up to the 9th day*

Table 2 indicates that all the fertilized eggs recovered before the 6th day appeared to be developing normally, but a considerable number of those recovered during the next 3 days were degenerating. Eggs were described as fertilized but degenerating when there were spermatozoa embedded in the zona pellucida and the vitellus was fragmented, or shrunken and misshapen, and much darker in colour than is normal. They were found in ten of the seventeen animals killed between 130 and 214 hr after the onset of oestrus, and they comprise fifty-one of the 212 fertilized eggs (22\%) recovered from these animals. In only one animal (F4) were all the eggs (thirteen) abnormal in appearance.

*In animals killed between 13 and 18 days*

The discrepancy between the total number of corpora lutea (194) and developing embryos (139) in these thirteen animals (see Table 3) represents a loss of 28·4\%. The greater part of this loss occurred in two of the animals; one (C3) had twenty-one corpora lutea and no embryos, and the other (H1) had eighteen corpora lutea and three surviving embryos. Fragments of trophoblastic tissue were found in both of these animals. Ten complete although friable trophoblastic sacs, one with a degenerating embryo and nine without embryos, were recovered from the animals in this group. The recovery of empty sacs and fragments of trophoblastic tissue points to the recent death of some of the embryos, in all probability after the elongation of the blastocysts.
Early pregnancy in the pig

In animals killed between 26 and 40 days

The proportion of foetuses surviving in five animals killed during this period is shown in Table 4. In addition to the sixty viable foetuses, there were four dead embryos and thirteen trophoblastic sacs without embryos. The overall loss may be shown to be 34.8% of which nearly one-half is contributed by one of the animals.

<table>
<thead>
<tr>
<th>No. gills</th>
<th>Time after onset of oestrus (days)</th>
<th>No. corpora lutea</th>
<th>No. normal embryos</th>
<th>No. dead embryos</th>
<th>No. sacs without embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td>H3</td>
<td>26</td>
<td>21</td>
<td>18</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>D6</td>
<td>27</td>
<td>22</td>
<td>16</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>D5</td>
<td>27</td>
<td>15</td>
<td>12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D1</td>
<td>29</td>
<td>17</td>
<td>3</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>G6</td>
<td>40</td>
<td>17</td>
<td>11</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>92</td>
<td>60</td>
<td>4</td>
<td>13</td>
</tr>
</tbody>
</table>

In animals of the same litter

The twelve litters of gilts obtained from outside sources were allocated in such a way that members of the same litter were distributed among groups killed at different times after mating. The variation in embryonic mortality between animals from different litters was not significant when allowance was made for the very wide variation between members of the same litter.

GROWTH OF UTERINE HORNS AND FALLOPIAN TUBES IN EARLY PREGNANCY

The data given in Table 2 show that the uterus increases in length throughout the first 18 days after mating. The elongation is rapid between the 2nd and 6th days, and represents an increase of 50%. The average length of uterine horn in thirteen animals killed on the 13th to 18th days was 360 cm compared with 190 cm in nine others killed during the 3rd day, when all the eggs were in the Fallopian tubes. The length of the uterus was found not to be related to the number of corpora lutea, or the number of embryos surviving, at any of the stages examined. The length and weight of the uterine horns were known for thirty-two of the animals and were found to be significantly correlated \( r = 0.74 \). The linear regression of uterine length in cm \( y \) on weight in g \( x \) is given by the formula \( y = 0.339x + 102.5 \).

The length of the Fallopian tubes of these animals is given in the upper part of Text-fig. 2. There was a significant increase \( (P < 0.05) \) in tubal length with time in sixteen animals killed before 100 hr. A closer correlation \( (r = 0.643; \)
\( t = 3.10; P, 0.02 - 0.01 \) was observed in ten of these animals in which all the eggs were within the Fallopian tubes. The physiological significance of this observation is problematical.

![Text-fig. 2. Growth of Fallopian tubes and uterine horns during the first 20 days of pregnancy. \( \circ \), tubal eggs only; \( \Theta \), tubal and uterine eggs; \( \bullet \), uterine eggs or blastocysts only.]

THE OVARIES

Corpus luteum

A high proportion of the ovaries of the first twenty-one animals that were examined were conspicuous on account of the variable size, colour and consistency of the corpora lutea, caused by different degrees of cystic distension. This condition has been described by Perry & Pomeroy (1956), who showed that it was incompatible with normal embryonic development beyond the 18th day. In the present work, it is studied in relation to embryonic survival before this time. A detailed account of the histology of the corpus luteum of pregnancy in the pig was given by Corner (1915). Sections through two ovaries, one in which the corpora lutea are of the normal type, and the other in which most of them are distended, are shown in Pl. 2, Figs. 4 and 5. The rupture point was visible externally for about 4 days in normal corpora lutea, and for longer in some of the cystic ones where a small amount of luteal tissue was extruded. The volume of the luteal tissue in the cystic corpora lutea could not be measured with sufficient accuracy to confirm the visual impression that it was similar to that in the normal ones. The luteal tissue was, however, histologically similar in all, and the individual luteal cells were of similar size in normal and cystic corpora lutea at comparable stages of gestation.

There was considerable variation in the size of individual corpora lutea in
many of the animals, in addition to that associated with cystic distension. In a number of animals, the largest normal corpus luteum was twice the weight or volume of the smallest. Very occasionally, one of the corpora lutea was much smaller or larger than all the others in the set, but histologically similar to them and apparently formed at the same time. Throughout the series of ovaries examined histologically no corpus luteum was found with a retained egg.

The average size of the corpora lutea increases suddenly about the 6th day after the onset of oestrus, i.e. when the embryos reach the blastocyst stage. Thus, the mean volume of ‘normal’ corpora lutea was 60 mm³ in four animals killed during the 5th day when some of the eggs were still in the four-cell stage, compared with 140 mm³ in three animals killed on the 7th, 8th and 9th days respectively, in which all the embryos had reached the blastocyst stage. There is evidently some further luteal growth after the 9th day, since the mean fresh weight (329 mg) of the corpora lutea in eleven animals killed on the 6th to 9th days was significantly less (P<0.05) than that (419 mg) in eleven animals killed on the 13th to 18th days after the onset of oestrus. There was a very wide variation in weight of the corpora lutea both between and within animals of this series and among other sows killed at later stages of pregnancy in other work in this laboratory. The average weight of the corpora lutea, excluding those with some degree of cystic distension, was apparently not related to the origin of the animal (since litter sisters varied as widely as unrelated animals) or to the number of corpora lutea, or to the presence or absence of cystic corpora lutea in the same ovaries.

Follicles

All vesicular follicles more than 0.5 mm in diameter were counted and measured in the serial sections of the ovaries of each of the twenty-one animals referred to above, killed 2 to 9 days after the onset of oestrus, and in one killed at 16 days. An oocyte was located in each of the follicles measured. No anovular or polyovular follicles were encountered.

The follicles were classified as normal or atretic; the degree of degeneration in the latter varied from pycnosis and slight separation of the cells of the membrana granulosa to complete disintegration of the membrane. One animal, killed 48 hr after the onset of oestrus, had not ovulated. The average volume of twelve pre-ovulatory follicles in this animal was 138 mm³; the ovaries contained no other normal, and very few atretic, follicles larger than 2.0 mm³. Approximately one-half of sixty follicles measuring about 1.0 mm³ in this animal were atretic. Another animal killed at 48 hr had ovulated; it had a similar number of normal follicles of about 1.0 mm³, but there were fewer atretic ones. An animal killed at 60 hr had fifty-seven normal and eight atretic follicles of 1.0 mm³ or less, and no others. The gradual increase in the number of slightly larger follicles in animals killed between 72 and 96 hr suggests progressive growth of the smaller vesicular follicles, and the appearance of new ones (Pl. 2, Fig. 4). Atretic follicles up to 1.0 mm³ were found in all the ovaries during this period (the 4th day after the onset of oestrus). Some of the animals had follicles of 15 to 20 mm³ by 100 hr, and there was no atresia of any but minute follicles in the fourteen animals killed during the 4th to 7th days. The
data derived from the remaining two animals, killed on the 8th and 9th days, suggest that another 'wave' of atresia overtakes the growing follicles during this period, when the blastocysts lose the zona pellucida and begin to enlarge. Our histological material does not extend beyond this stage except for one animal (C3) killed on the 16th day. No embryos survived in this animal at the time of slaughter, although remnants of trophoblastic sacs were found in the uterus and the corpora lutea had not noticeably regressed. The ovaries contained thirteen follicles greater than 20 mm³ which perhaps would have matured and ovulated within a few days. The atresia of about half of the smaller follicles may also have been comparable with that seen in the immediately pre-ovulatory stage, rather than a characteristic phenomenon of pregnancy 15 days after ovulation.

Within the first 9 days of pregnancy, the size and condition of the follicles appear to be totally independent of the occurrence or distribution of cystic corpora lutea.

DISCUSSION

There would seem to be little doubt that in the pig the corpus luteum count is a reliable measure of the number of ova ovulated. The evidence for this statement lies in the recovery from the intact Fallopian tube of a number of eggs equal to the number of corpora lutea in the corresponding ovary, and the absence of polyovular or anovular follicles in any of the ovaries examined histologically. In one animal, the number of embryos (sixteen) exceeded by one the number of corpora lutea (see F4, Text-fig. 1). Two of these embryos were found in one trophoblastic sac, but it was not possible to determine whether these 'twins' were monozygotic or whether two separate sacs had fused during development. In another animal (C2) where two embryos occupied the same sac, the total number of embryos equalled the number of corpora lutea.

It would seem that the discrepancy between the numbers of corpora lutea and recovered eggs, in animals killed during and after the passage of eggs into the uterus, must be due to our failing to flush all the eggs out of the uterus or to our losing them in the large volume of fluid that was used. It is probable that the chances of recovering all the eggs would depend on the degree to which the reproductive tract can be distended during the process of flushing so as to expose all the epithelial surface to the flow of saline solution. The Fallopian tubes present no difficulty but the uterine epithelium is so extremely folded that the whole of its surface cannot be exposed even when the horn is so distended with fluid as to be in danger of rupture.

The results, coupled with those of Hancock (1957) and of others quoted by him, indicate that in the pig a very high proportion of the ova that are ovulated become fertilized. The distribution of unfertilized ova in individual animals is very irregular. Thus, Hancock showed that of 9% of eggs which were unfertilized the majority (fourteen out of nineteen) were recovered from only one of fifteen sows, and in our results nineteen of twenty-three unfertilized eggs were recovered from only two of forty animals.

Unfortunately, none of our animals was killed during the period when the
embryonic sacs were elongating. However, when fully elongated, the blastocysts have been shown rarely to over-lap each other. This fact provides indirect evidence in support of Patten’s (1948) statement that ‘spacing of the embryos apparently takes place before the elongation of the blastocysts occurs’. Their growth from about 4 mm on the 9th day to 110 cm, as an average length on the 16th day represents about a 300-fold elongation in one direction accompanied by a relatively slight attenuation. There follows a slight distension after elongation is complete, all of which is accomplished before the establishment of a functional blood-vascular system in the embryo. The significance of this elongation at this early stage in embryonic development remains obscure.

A heavy embryonic mortality has frequently been reported to have occurred in the pig by the 25th day of pregnancy. By this time the mortality in our animals was 35% which is similar to that given by a number of authors (see Casida, 1956). Table 2 shows that the major part of the mortality could be accounted for by the degeneration of fertilized ova between the 9th and 16th days of pregnancy, about the time of the dissolution of the zona pellucida. It has been shown that the apparent concentration of this degeneration into the 6th and 7th days was not related to the source from which the animals were obtained. It is possible that some of the ova, identified as degenerating, were in fact viable. If this were the case, the mortality among fertilized eggs up to the 9th day must be very small. However, the discrepancy between the numbers of corpora lutea and normal embryos in animals killed between the 13th and 18th days was 28% and only a small proportion of this loss could be accounted for by the recovery of trophoblastic sacs without embryos. The bulk of the loss observed in this group, therefore, had evidently occurred at an earlier stage in pregnancy. The only reason to doubt that it occurred during the 6th to 9th days was the fact that almost all the degenerating eggs which were identified were recovered from animals killed during the first half of this period.

The elongation of the uterine horns, which is correlated with increase in weight of uterine tissue, and which occurs in the very early stages in pregnancy in the pig, does not seem to follow the pattern of uterine growth during gestation as described by Reynolds (1949, 1959) for some other species. This pattern, based mainly on the rabbit and rat, indicates the existence of three phases in the adaptation of the uterus to accommodate the products of conception. The first, which occurs before implantation, is the period of progestational proliferation and involves hyperplasia without causing elongation or gain in weight. The second phase is one of hypertrophy and occurs after implantation when the weight of the uterus without that of its contents becomes progressively greater until the time of transition of the conceptus from a spheroid to a cylindrical shape (Reynolds, 1946). This change initiates the third phase when the uterus becomes greatly stretched but at the same time its rate of growth becomes reduced. A similar pattern has been described for the cow (Hammond, 1927) and man (Ivy, 1942), although in these species the third phase is not accompanied by a change in shape of the conceptus such as occurs in the rat and rabbit. Our own observations on the pig cover only the period of pregnancy corresponding to that of the first of the above phases but they show that uterine growth (length) is very rapid during the first few days of pregnancy, even before

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the eggs have reached the uterus. During this period a linear correlation
\((r = 0.74)\) was observed between weight and length of uterus. Pomeroy (1960)
remarked that uterine weight increased at about a constant rate throughout
pregnancy in the pig, but elongation was more rapid during the early stages.
Our results agree with his in this respect, but do not confirm his observations on
uterine weight. Both sets of results indicate that the pig does not conform to the
generalization made by Reynolds that uterine growth is dependent upon a
stimulus received at implantation. In fact, it is not possible to deduce from
these results whether uterine growth is caused by mating and the presence of
unimplanted eggs and blastocysts in the reproductive tract, or by the presence of
active corpora lutea in the ovaries, as no information is available about the
growth of the uterus in the sow during the di-oestrous cycle.

A perplexing feature of the ovaries of these sows during early pregnancy was
the cystic condition of some of the corpora lutea: there was no evidence that
the condition led to the termination of pregnancy. The first group of twenty-one
animals, which were all killed before the 10th day, included fourteen with
cystic corpora lutea. Among the remaining groups with a total of thirty-seven
animals, nineteen were killed before the 10th and eighteen after the 13th day;
six of the former and only one of the latter animals had one or more corpora
lutea in this condition. As far as they go, therefore, these data suggest that the
cystic condition is transient and that the corpus luteum reverts to a compact
structure before the 18th day. There is nothing to suggest that they should be
regarded as being ‘abnormal’ in the pathological sense.

REFERENCES