Chronological and Cytological Details of Fertilization and Early Embryonic Development in the Domestic Pig, *Sus scrofa*

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ABSTRACT The sequence of cytological events from sperm penetration of the oocyte until emergence of the blastocyst from the zona pellucida was studied in 1441 eggs from 134 animals in which the time of ovulation had been controlled precisely by gonadotrophin injection. Observations were also made on the rate of egg passage through the Fallopian tubes, on the process of denudation, and on the increase in numbers of spermatozoa associated with the zona pellucida.

Eggs may be penetrated and activated within three hours of mating or insemination close to the time of induced ovulation. A decondensation and swelling of the chromatin is seen very soon after incorporation of the sperm head into the vitellus, and central apposition of the pronuclei occurs three to five hours later. The male pronucleus is slightly larger than the female, and a portion of the flagellum is frequently closely associated with it until late syngamy. Cleaved embryos can be recovered within 14 to 16 hours of sperm penetration, but the two-celled stage lasts only six to eight hours compared with 20 to 24 hours for the four-celled stage. Embryos enter the uterus at the latter stage approximately 46 hours after ovulation. Morulae of 16 to 32 cells can occasionally be observed late on the third day of development, and blastocysts are present on the fifth day. However, the zona pellucida is not shed until the sixth day, after which the trophoblast commences the massive elongation characteristic of this ungulate blastocyst.

Although the domestic pig, *Sus scrofa*, was much used during the nineteenth century as a model for the study of mammalian embryology, the first major reference to the early stages of development of the pig egg did not appear until the publication of Assheton (1898). Some 30 years later, a more comprehensive study by Heuser and Streeter ('29) recorded cytological details on various stages of the early embryo, and also the timing of cleavage as related to the time of mating. These findings were supplemented by the report of Green and Winters ('46), but none of these studies attempted a systematic examination of the embryo up to and including the formation of blastocysts.

Details of the processes of sperm penetration and pronuclear development in pig eggs were not published until the work of Pitkjanen ('55), Thibault ('59) and Hancock ('61). In these descriptions, which were quite limited in extent, the stages of development were related only to an estimate of the time of ovulation or mating, which in turn was based on determination of the onset of oestrus. The accuracy of this approach, therefore, depended upon the frequency and care with which the detection of oestrus was undertaken. The more recently favoured method of controlling the time of ovulation by injection of human chorionic gonadotrophin (HCG) enabled Dziuk and Polge ('62) to examine fertility in pigs in relation to the age of the gametes. Induction of ovulation has also been employed in experiments concerning several aspects of fertilization in pigs (Polge and Dziuk, '65; Baker, Dziuk and Norton, '67; Hunter, '67a,b; Baker and

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Coggins, '68; Day and Polge, '68; Hunter and Dziuk, '68; Hunter, '72a,b,c; Hunter, '73; Hunter and Hall, '73).

In the present study, mating or insemination of gilts at precise times relative to induced ovulation has permitted an analysis of the sequence of events from sperm penetration into the egg until formation of the blastocyst. A description of pre-implantation development in this polytocous species which presents a significant level of embryonic loss (Hanly, '61; Perry and Rowlands, '62) is important. The information should also be valuable in order to recover eggs at specific stages of development for use in culture experiments or in transplantation studies.

MATERIALS AND METHODS

These have been described in detail in previous reports (Hunter, '67a,b; '72a,b). The essential points can be summarized as follows.

Purebred Large White animals, or their Landrace or Essex crosses, were used in this work. They were aged between six and nine months, and weighed 80 to 145 kg. All gilts were checked for oestrus at least once daily in the presence of a mature boar, and those having oestrous cycles within the range of 19 to 21 days were injected intramuscularly during late pro-oestrus with 500 i.u. HCG in 4–5 ml physiological saline. Ovulation occurred approximately 42 hours after this injection (Hunter, '67c). Boars of established fertility were used for mating or semen collection; in the latter case, the semen was instilled fresh and undiluted via the cervix in volumes ranging from 80–120 ml.

Animals were slaughtered at known intervals after semen deposition and ovulation, following which eggs were recovered, treated and examined by phase-contrast microscopy. Details of whole-mount preparations of eggs (Chang, '52) were recorded graphically and photomicrographs taken of suitable specimens both before and after staining (Dziuk and Polge, '65). The observations that follow are based on 1441 eggs recovered from 134 animals studied under these defined experimental conditions (table 1).

RESULTS

1. General observations

(a) Location of eggs

Eggs can be recovered from deep in the ampulla of the Fallopian tube within 30 to 45 minutes of completion of ovulation. As is the case for several other mammalian species, particularly the rabbit (Greenwald, '61; Harper, '61; Boling, '69), the eggs reach the ampullary-isthmic junction in a very short interval, a proportion of them arriving in this region in less than an hour. Although the present observations on egg transport, and those in other reports on pigs (Andersen, '27; Alanko, '65; Oxenreider and Day, '65) have been made at autopsy and are therefore open to criticism, the rapid rate of descent through the ampulla has been confirmed in surgical studies (unpublished results).

Most of the tubal sojourn is represented by the subsequent passage of eggs from the ampullary-isthmic junction through the isthmus, occupying a period of approximately 44 to 45 hours. This timing appears to be similar for both fertilized and unfertilized eggs. The developing embryos or degenerating unfertilized eggs pass through the region of the utero-tubal junction and enter the proximal portion of the uterine horn about 46 hours after ovulation. They remain in the upper half of the horns for the next two to three days, after which a more complete distribution of embryos throughout the uterine cornua commences.

(b) Dispersal of cells of cumulus oophorus

Shortly after completion of ovulation, the eggs are assembled in the ampulla invested with cells of the cumulus oophorus. In animals that have not been mated or inseminated, the follicular elements about individual eggs tend to aggregate to form a compact cumulus plug, enmeshed within which the eggs traverse the length of the ampulla. If, however, mating or insemination has taken place some hours before ovulation so that a population of spermatozoa is already established in the Fallopian tubes, a cumulus plug encompassing several eggs is rarely assembled. Instead, the process of denudation is accelerated, and
### TABLE 1
**Summary of data on 1441 eggs recovered from the Fallopian tubes or uterine cornua of 134 animals during the first six days of embryonic development**

<table>
<thead>
<tr>
<th>Interval from ovulation to autopsy</th>
<th>Number of</th>
<th>Range of developmental stages observed</th>
<th>Location of eggs in reproductive tract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Animals yielding eggs</td>
<td>Corpora lutea</td>
<td>Eggs recovered (and percentage)</td>
</tr>
<tr>
<td>days 1</td>
<td>76</td>
<td>886</td>
<td>762 (86.0)</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>208</td>
<td>187 (89.9)</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>151</td>
<td>133 (88.1)</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>181</td>
<td>160 (88.4)</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>87</td>
<td>72 (82.8)</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>168</td>
<td>127 (75.6)</td>
</tr>
<tr>
<td>Total</td>
<td>134</td>
<td>1681</td>
<td>1441 (85.7)</td>
</tr>
</tbody>
</table>

1 Number of eggs expressed as a percentage of the total number of corpora lutea counted at autopsy.
the dispersal of cumulus cells follows a fairly consistent pattern. Dissolution of each individual cumulus oophorus is rapid (plate 1, figs. 1 to 4), the investments occasionally being reduced to cells of the corona radiata in less than 50 minutes of ovulation. In fact, as judged from preparations flushed following pre-ovulatory mating, denudation of most eggs can be virtually completed (plate 1, figs. 5, 6) within an hour or two of ovulation.

It is important to note that sperm penetration of the zona pellucida, and activation of the second meiotic division, may have occurred before the eggs are fully divested of cells of the corona radiata. But evidence from ultrastructural studies (Szollosi and Hunter, '73) indicates that liberation of these cells depends largely on withdrawal of their processes from the substance of the zona pellucida, a cellular change which may not be hastened significantly by the presence of spermatozoa.

(c) Numbers of spermatozoa on eggs

Because of the problem of distinguishing between spermatozoa attached to the zona pellucida and those commencing to enter it, the figures that follow refer to the total number of spermatozoa both on and in the zona (see plate 2, figs. 7 to 12). They give some indication of the numbers of spermatozoa entering the tubes and reaching the eggs, but because many factors influence the rate and quantitative efficiency of sperm transport, they will rarely prove reliable for predicting sperm numbers on eggs at given intervals after mating.

Nevertheless, the mean number of spermatozoa about the small proportion of eggs with some sperm recovered two hours after insemination was 5.0 (range 1 to 14), increasing to 9.3 (range 1 to 80) at three hours after insemination (see Hunter and Dziuk, '68). In a later study, the mean number of spermatozoa associated with the zona pellucida increased from 6.8 (range 1 to 25) at three hours to 109.2 (range 1 to 466) some six hours after natural mating at the time of ovulation (Hunter, '72a). By the time the two-celled stage is reached, 200 or more spermatozoa may have penetrated the zona (plate 2, fig. 8), and this number can increase substantially during the tubal descent of the embryo.

These figures indicate that under conditions of mating or insemination close to the time of ovulation, a progressive increase in sperm numbers associated with the egg is occurring during the interval from activation of the secondary oocyte until passage of the developing embryo into the uterus. At the latter stage, sperm numbers in the zona may be of the order of several hundred, and on occasions exceed 400 to 500 (Hunter and Léglise, '71). After the embryos enter the uterus, spermatozoa competent to penetrate the zona are no longer available in the physiological situation, such cells having been engulfed by polymorphonuclear leucocytes. Accordingly, there is rarely a significant increase in zona sperm after the four-celled stage is reached.

2. Developmental details

(a) Fertilization

The timing of sperm penetration into the zona pellucida and activation of the second meiotic division have been treated extensively in two previous publications (Hunter and Dziuk, '68; Hunter, '72a), as has the timing of pronuclear development up to the stage of syngamy. In summary, these observations indicated that 24 to 36% of the eggs may be activated within three hours of mating or insemination near the time of ovulation, and that the proportion increased to 72% some five hours after mating. The interval needed for activation of all the eggs in this situation would probably be between six and eight hours after mating in most animals.

The requirements for capacitation of boar spermatozoa were also considered in this earlier work. Under conditions of induced ovulation, it was noted that a population of fully capacitated spermatozoa could be available in the Fallopian tubes of oestrous pigs within two to three hours of semen deposition by way of the cervix.

Because the time of sperm penetration into eggs can be estimated with some accuracy, the developmental stages described below are related to this baseline. The moment of activation of the secondary oocyte would, in any case, seem the most
appropriate stage from which to chronicle its development (table 2).

(b) Cytological features

A decondensation and swelling of the chromatin is seen very soon after incorporation of the sperm head into the vitellus although, at the level of the light microscope, pronuclear membrane formation cannot be detected at this early stage (plate 3, fig. 13). However, nucleoli appear rapidly within the transforming male structure (plate 3, fig. 14), and a membrane can be distinguished as the pronucleus commences maturation and central migration. Upon clearing the stained preparations, the sperm mid-piece and tail can frequently be demonstrated until the stage of late syngamy. These structures were usually closely associated with the male pronucleus and, when the egg was orientated appropriately, the fertilizing sperm tail was seen to be split at the rostral end of the mid-piece. Meanwhile, the female chromatin, having completed the reduction division and extruded the second polar body (plate 3, fig. 15), commences a parallel series of changes, although these are slightly retarded with respect to those of the male. This delay is reflected in the size of the pronuclei, the male structure being marginally the larger of the two (plate 3, fig. 16).

During migration of the pronuclei, a reorganisation of the ooplasm is frequently noted, particularly around the nuclear elements, and ultrastructural evidence for a sperm aster is accumulating (Szollosi and Hunter, '73). The peripheral region of the vitellus is usually finely textured at this early pronuclear stage, whereas large globular material predominates in the medulla, although not immediately around the pronuclei. Differences in the distribution of chromatin in the male and female pronuclei become visible during migration, and are pronounced at the commencement of syngamy when a heavy asymmetric con-

| TABLE 2 |
| Chronology of development of the pig embryo during the first six days. The figures represent the calculated time intervals from sperm penetration of the oocyte until appearance of a given stage |

<table>
<thead>
<tr>
<th>Stage of embryonic development</th>
<th>Approximate interval from activation (^{1}) until developmental stage</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance of pronuclei</td>
<td>1.5–2 hours</td>
<td>Rapid formation of pronuclear membranes</td>
</tr>
<tr>
<td>Apposition of pronuclei</td>
<td>5–6</td>
<td>Phase of relatively long duration</td>
</tr>
<tr>
<td>First mitotic metaphase</td>
<td>12–13</td>
<td>Restoration of diploid condition</td>
</tr>
<tr>
<td>2-cells</td>
<td>14–16</td>
<td>2-celled stage of short duration (6–8 hours)</td>
</tr>
<tr>
<td>3 to 4 cells</td>
<td>20–24</td>
<td>Relatively long 4-celled stage (20–24 hours)</td>
</tr>
<tr>
<td>5 to 8 cells</td>
<td>46–52</td>
<td>Asynchronous cleavage becomes prominent</td>
</tr>
<tr>
<td>9 to 16 cells</td>
<td>64–(^{2})</td>
<td>Range in development found within animals</td>
</tr>
<tr>
<td>&gt; 16 cells (Morulae)</td>
<td>70–(^{2})</td>
<td>Mitotic figures frequently visible</td>
</tr>
<tr>
<td>&gt; 32 cells (Intact blastocysts)</td>
<td>92–(^{2})</td>
<td>Blastocoele and inner cell mass become conspicuous</td>
</tr>
<tr>
<td>Zona — free blastocysts</td>
<td>120 – &gt;</td>
<td>&gt; 200 cells may have formed by hatching</td>
</tr>
</tbody>
</table>

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\(^{1}\) Activation indicating morphological evidence of resumption of the second meiotic division.

\(^{2}\) Difficult to determine because of range in cell numbers and the problem of counting greater than 16 cells with accuracy.
densation develops on the membrane of the female pronucleus opposing the male structure (plate 3, figs. 17, 18). This stage of pronuclear apposition may be reached within two to three hours of sperm penetration, but five to six hours probably represent more characteristic values.

Shortly after the central apposition of the two pronuclei, there is a further condensation of chromatin, the pronuclear membranes undergo dissolution (plate 3, fig. 19), and the two haploid groups of prophase chromosomes can be distinguished (plate 3, fig. 20). These are rapidly assembled at metaphase of the first mitotic division (plate 3, fig. 21), restoration of the diploid condition being accomplished approximately 12 to 14 hours after sperm penetration. The cleavage division follows almost immediately (plate 3, figs. 22, 23), although partition of the cytoplasm is rarely completed before membrane formation about the daughter nuclei has commenced. Remnants of the mitotic spindle, including its mid-body, may be found between the two blastomeres (plate 3, fig. 24).

Two-celled embryos (plate 4, fig. 25) can be recovered within 14 to 16 hours of sperm penetration. Both polar bodies are essentially intact at this stage, and tend to position themselves at the end of the cleavage furrow. The peripheral cytoplasm in the two-celled stage still lacks the larger vitelline globules (plate 4, fig. 25) as does the perinuclear region. The second cleavage division proceeds after a fairly short interval in the two-celled condition (6 to 8 hours), and can be observed within 20 to 24 hours of activation. This second division is usually synchronous, mitotic spindles being visible in both blastomeres at about the same time, but three-celled embryos are not uncommon. It is in the relatively long-lasting four-celled stage (plate 4, figs. 26, 27) that most embryos pass through the utero-tubal junction about 46 hours after ovulation. A few hours later, the formation of eight-celled embryos commences, but a progressive degree of asynchrony is found in this and subsequent divisions. The polar bodies are no longer conspicuous and, by the time the 16-celled stage is reached (plate 4, fig. 28), it is difficult to count the number of blastomeres in the unstained preparation.

The approximate timing of the ensuing mitotic divisions is presented in table 2, morulae of 16 to 32 cells occasionally being observed late on the third day of development. Such embryos are still located in the proximal half of the uterine horns. Formation of the blastocyst (plate 4, figs. 29, 30) is witnessed by the fifth day, and the inner cell mass becomes particularly prominent on the sixth day (plate 2, fig. 12). The embryo is still encompassed by the zona pellucida at this stage, although the latter shows signs of thinning (plate 2, fig. 12), and cell numbers may have reached some 150 to 200. The zona is shed later on the sixth day, and the hatched blastocyst which frequently exhibits properties of adhesiveness but is still unattached to the endometrium, enlarges rapidly. Considerable variation in size between blastocysts from the same uterus is soon apparent. These differences are magnified progressively in seven- and eight-day blastocysts, during which time the spherical form is lost and a wrinkled appearance develops. The subsequent massive elongation of the embryonic membranes and associated differentiation of the embryo have been considered in detail elsewhere (Weyssse, 1894; Assheton, 1898; Heuser, 27; Heuser and Streeter, '29) and will not be commented on here, except to indicate that it is during this process of elongation that intra-uterine migration of the pig blastocyst commences (Corner, '21a, '23b). The trophoblast is extremely fragile and susceptible to mechanical damage at this time, but successful transplantation experiments have been achieved using seven- and eight-day embryos (Hunter, Polge and Rowson, '67; Polge, '72).

DISCUSSION

These observations indicate that the rate of development of the pig embryo from sperm entry into the vitellus until blastocyst liberation from the zona pellucida can be defined with some accuracy when the time of ovulation is known. It is not productive, however, to make a close comparison between these data and those for other domestic species since the technique of controlling the moment of ovula-
tion has been restricted largely to the pig. Nevertheless, a number of features of the above study should be highlighted, particularly the length of time required for certain cleavage stages. Whilst the interval between oocyte activation and completion of the first mitotic division occupies about 14 to 16 hours, this must be contrasted with the two-celled stage of six to eight hours. The brief duration of the two-celled condition has been noted by Polge (’66), and would indicate a rapid phase of synthetic activity prior to the second mitotic division. On the other hand, the four-celled stage lasts approximately 24 hours, and none of the ensuing mitotic divisions before blastocyst formation appear to be achieved in intervals as short as six to eight hours.

Asynchrony in the apparent rate of development between the embryos of an individual animal can be detected by the first cleavage, and is thought to be associated principally with variation in the time of sperm penetration (Thibault, ’67; Hunter, ’72a). Variability of this nature does not become greatly magnified during further development for, even though several cleavage stages may be recovered from the same uterine horn, the blastocysts of a given animal leave the zona pellucida within a relatively short interval on the sixth day. It is only after hatching has occurred that marked differences in size appear during the massive expansion and elongation of this ungulate blastocyst. But it is not yet known whether these differences in the growth of trophoblast are indicative of transient variation in metabolic rate, or whether they are directly correlated with the ultimate viability of the embryo. This question is currently being examined by means of culture and transplant studies.

Judging from the figures recorded in this work, transport of relatively high numbers of spermatozoa to the site of fertilization can occur if gilts are mated or inseminated shortly before ovulation. In fact, more than one sperm may have attached to the zona pellucida by the time of activation, although there is strong circumstantial evidence that simultaneous penetration through the zona would lead to the polyspermic condition in pig eggs (Hunter and Léglise, ’71; Hunter, ’72b, ’73). Sperm transport to the Fallopian tubes may be accelerated during this mid to late phase of oestrus (Du Mesnil du Buisson and Dauzier, ’55), an effect that would be mediated through increased contractile activity of the female reproductive tract. The possibility that capacitation may also be enhanced in the induced ovulation situation has been discussed (Hunter, ’72a). These considerations aside, the very large numbers of spermatozoa that gain the isthmus during tubal passage of the eggs are well illustrated by the increase in spermatozoa in the zonae of two- and four-celled eggs. Whereas the block to polyspermy continues to operate at the level of the inner zona, the outer region of this membrane still permits penetration and accordingly accumulates spermatozoa during the descent of the cleaving zygote to the uterus.

In this connection, perhaps the most striking feature of the pig zona is its structural resilience, even when penetrated by 200 or more spermatozoa. In spite of the eroding influence of the pathways digested by such spermatozoa, the zona fully encompasses the embryo until the sixth day. Thus, the block to polyspermy established in the innermost region of the zona very soon after penetration of the vitellus appears to be remarkably stable and long lasting. In the development of this protective mechanism against polyspermy, an action of the cortical granule material (Austin and Braden, ’56; Szollosi, ’62, ’67; Barros and Yanagimachi, ’71; Gwatkin, Williams, Hartmann and Kniazuk, ’73) on the molecular configuration of the inner zona to render it structurally impenetrable remains attractive, but the precise nature of the change requires clarification.

The general pattern of pronuclear formation and development resembles that described for many mammalian species (Austin and Walton, ’60; Austin, ’61; Blandau, ’61). The present observations suggest that a difference in size of the two pronuclei is a constant feature in eggs of the domestic pig. The male pronucleus is slightly larger, this discrepancy presumably arising because the sperm head chromatin commences its decondensation and swelling whilst the activated egg has still
to complete its reduction division. Whether or not this asynchrony is associated with the distribution of chromatin in the swelling pronuclei has yet to be resolved. But the asymmetric arrangement of chromatin on the membranous portion of the female pronucleus opposing the male has been noted in previous studies (Thibault, '59; Hancock, '61; Hunter, '72a), and is a characteristic feature that appears during the phase of DNA duplication and is seen until syngamy. It is therefore conspicuous during pronuclear movement, and could be a response to the systems that regulate migration of these organelles.

A survey of the literature reveals some discrepancy in the duration reported for the descent of pig eggs through the Fallopian tubes. Assheton (1898) correctly inferred that entry into the uterus occurred about two days after ovulation, but the erroneous figure of three days has been quoted extensively (Corner, '21b; Andersen, '27; Nalbandov, '64). By controlling the time of ovulation, it has been established that both fertilized and unfertilized pig eggs pass through the utero-tubal junction approximately 46 to 48 hours after release from the ovary. This is 24 to 48 hours sooner than embryos enter the uterus of many common laboratory and domestic species (Blandau, '61, '69; Thibault, '72). The precocious passage of pig eggs is reflected in the development of the embryo, which reaches the uterus at the four-celled stage. Administration of HCG to regulate the time of ovulation did not apparently disturb the endocrine environment sufficiently to hasten the rate of tubal descent. In a recent study on the output of ovarian steroids following injection of 500 i.u. HCG, the concentration of oestrogens and progesterone in peripheral blood was not increased significantly by this treatment (Hunter, Hall, Cook and Taylor, '72). In any event, the figure of 46 to 48 hours for the tubal sojourn agrees well with the estimates of Pomeroy ('55) and Oxenreider and Day ('65) in studies not employing HCG.

This paper has dealt with certain aspects of the sequence of events during the process of normal fertilization and early development. But it should not be overlooked that a proportion of eggs may fail to reach the stage of hatched blastocysts (Corner, '23a), and also that abnormalities of fertilization are relatively frequent in eggs of the domestic pig, especially under situations of delayed mating or insemination (Pitkjanen, '55; Hancock, '59; Thibault, '59; Dziuk and Polge, '62; Hunter '67a). In the latter case, these may contribute significantly to early embryonic loss. A clearer definition of modifications at the cellular level that predispose abnormalities of fertilization would be of fundamental importance and might have valuable consequences if an appropriate corrective therapy could be imposed.

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LITERATURE CITED


——— 1969 Gamete transport — comparative


Szollosi, D. 1962 Cortical granules: a general


PLATE 1
EXPLANATION OF FIGURES

Whole-mount preparations of eggs freshly recovered from the Fallopian tubes of pigs within two to three hours of ovulation, and photographed under a phase-contrast microscope. The sequence shows various stages during denudation of the oocyte, which involves loss of the cumulus oophorus and corona radiata. All figures × C. 360.

1–2 The eggs are still encompassed by cells of the corona radiata together with other elements of the granulosa. The cytoplasm is dense and opaque due to its content of yolky material, and a perivitelline space cannot be detected.

3–4 These preparations show a progressive loss of corona cells associated with dissolution of the hyaluronic acid cement substance, and withdrawal of the corona cell processes from the zona pellucida.

5–6 Complete denudation has been achieved in the upper portion of the Fallopian tube. The small number of cells on the oocyte in figure 5 are no longer attached to the zona pellucida. Note that the perivitelline space is still barely conspicuous.
PLATE 2
EXPLANATION OF FIGURES

Whole-mount preparations of pig eggs freshly recovered from the Fallopian tubes or proximal half of the uterine horns. The preparations were photographed in physiological saline under a phase-contrast microscope, and illustrate stages of embryonic development found in the first six days post ovulation. All figures × C. 440.

7 A pronucleate egg that contains nineteen spermatozoa in the zona pellucida, and is completely denuded of all cells of the corona radiata. Note that the fluid-filled perivitelline space is already conspicuous, and that a re-arrangement of the vitelline globules has taken place.

8 A two-celled embryo recovered from the Fallopian tube approximately 18 hours after ovulation. The blastomeres are of similar size. More than 80 spermatozoa have penetrated the zona pellucida of this egg.

9 A four-celled embryo recovered from the Fallopian tube some 41 hours after ovulation. Note the very large number of spermatozoa embedded in the zona pellucida (> 200), and also that the second cleavage division has resulted in apparently similar blastomeres.

10 An eight-celled embryo recovered from the upper uterine horn some 55 hours after ovulation. The third cleavage division appears to have been closely synchronous, and once again all blastomeres are of similar size and morphology.

11 Morula containing between 16 and 32 cells of varying size recovered from the upper uterine horn approximately 73 hours after ovulation. Note the massive number of spermatozoa associated with the zona pellucida, and the apparent thickening of this membrane.

12 Blastocyst recovered from the upper portion of the uterine horn 117 hours after ovulation. The inner cell mass and fluid-filled blastocoele are both conspicuous. Note that the zona pellucida still encompasses the embryo, although it is considerably thinner than in the preceding stages, and that a large number of sperm heads can still be distinguished in this membrane.
Whole-mount preparations of pig eggs recovered from the Fallopian tubes and photographed under a phase-contrast microscope at various stages of fertilization. The preparations were fixed for 24 hours in 25% acetic alcohol, and stained with 0.5% orcein in 45% acetic acid. The zona pellucida is gradually removed by this staining procedure.

13 Oocyte shortly after penetration and activation, showing the fertilizing sperm head and a portion of the flagellum in the vitellus. The nucleo-protein has commenced swelling and decondensation, but the structure is still recognizable as a sperm head rather than as a pronucleus. × C. 940.

14 An early stage in the formation of a male pronucleus. The structure is still located peripherally but a nuclear membrane now encloses the re-arranged chromatin, and many small nucleoli are associated with the inner surface of this membrane. × C. 940.

15 Formation of the second polar body in a recently-penetrated egg, the chromatin being arranged at late telophase of the meiotic division. Rotation of the metaphase spindle does not precede this division. The chromatin of the neighbouring first polar body appears as a compact and darkly-staining mass. × C. 750.

16 Centrally-arranged pronuclei shortly before apposition; the male pronucleus is somewhat larger. There is a pronounced condensation of chromatin on the opposing face of the female pronucleus. Note that the first and second polar bodies are still closely associated with the vitellus. × C. 445.

17 Centrally-positioned pronuclei at syngamy. Although the nuclear chromatin is still largely dispersed, the characteristic condensation can be seen in the female pronucleus on its interface with the male structure. × C. 675.

18 Another pair of pronuclei to show the heavy asymmetric condensation of chromatin in the female. The sperm mid-piece and a portion of the flagellum are still closely associated with the male pronucleus. × C. 750.

19 At a late stage of syngamy, the pronuclear membranes undergo dissolution. The nuclear structures become difficult to see at this time, but a prophase arrangement of chromosomes can soon be detected. × C. 675.

20 The two haploid groups of prophase chromosomes shortly before their organization on the mitotic spindle. The pronuclear membranes can no longer be distinguished by light microscopy. × C. 750.

21 Restoration of the diploid condition. The chromosomes have contracted and thickened, and are now arranged at the first mitotic metaphase. × C. 675.

22 Late anaphase of the first mitotic division. Not all the chromosomes are yet arranged at the poles of the spindle. × C. 540.

23 Telophase of the first mitotic division. The chromosomes now appear as compact lumps of chromatin at the poles of the spindle. Division of the cytoplasm is imminent. × C. 675.

24 A two-celled embryo shortly after completion of the first cleavage division. The daughter nuclei are fully membrane-bounded. Elements of the mitotic spindle, particularly its mid-body, can be seen between the blastomeres. × C. 540.
Whole-mount preparations of pig embryos recovered from the Fallopian tubes or uterus at various stages of development during the first six days post ovulation. The preparations were fixed for 24 hours in 25% acetic alcohol, stained with 0.5% orcein in 45% acetic acid, and examined under a phase-contrast microscope. The zona pellucida has been removed by the 45% acetic acid.

25 A two-celled embryo in which the blastomeres appear of unequal size due to the orientation of this preparation. Compare the fine granulation of the peripheral cytoplasm with the inner concentration of vitelline globules. × C. 290.

26 A four-celled embryo in which only three blastomeres and their nuclei are in the plane of focus. The cells are of similar size, and again show the characteristic differences between the peripheral and medullary cytoplasm. × C. 330.

27 Another four-celled embryo with all four blastomeres, but not all nuclei, in the same focal plane. The nuclei are large and mature, but no suggestion of asynchronous cleavage can be detected morphologically. × C. 330.

28 A morula of approximately 16-cells in which nucleoli are conspicuous within several large nuclei. The embryo is still essentially spherical, but shows a minor degree of deutoplasmolysis. × C. 290.

29 A blastocyst containing between 32 and 64 cells. Note the concentric arrangement of the large mature nuclei, indicating that formation of the blastocoele is imminent or has just occurred. The cell boundaries are no longer distinguishable. × C. 450.

30 A blastocyst recovered shortly before the time of hatching, and containing more than 100 cells. Concentric rings of nuclei and mitotic figures can be detected in this preparation. × C. 450.