DEVELOPMENT OF THE EGG OF THE COW UP TO THE STAGE OF BLASTOCYST FORMATION

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The first description of the early stages of the development of the cow egg was given by Hartman, Lewis, Miller & Swett (1931). In this they described the living unsegmented egg and the two-cell stage but gave no details of the sectioned eggs. A brief account of the early development was given by one of us (W.J.H.) at a meeting of the Anatomical Society in 1941. Winters, Green & Comstock (1942) described and illustrated various stages of pre-natal development of the cow but gave few details of the early stages. Reference is made in a table by Amoroso, Griffiths & Hamilton (1942) to the rate of cleavage of the cow egg compared with that of the goat, sheep and pig.

Amongst the ungulates a detailed description of the early development has been given by Assheton (1898*a*) and Heuser & Streeter (1929) for the pig, by Assheton (1898*b*) and Clark (1934) for the sheep, and by Amoroso *et al.* (1942) for the goat. The further relevant literature in early ungulate development has been reviewed by Hartman *et al.* (1931) and Amoroso *et al.* (1942). The present communication gives an account of the early development of the cow egg from the pre-ovulation stages until the formation of the blastocyst. The material was obtained from forty-six animals which were the subject of experimental work on bovine infertility.

MATERIAL AND METHODS

The ova were collected from twenty-eight heifers and eighteen cows of various ages and breeds. They comprise two follicular oocytes removed from ovarian follicles, twenty ova obtained from normal oestrus, twenty-one obtained after oestrus induced by expression of the corpus luteum in mid-cycle, two obtained following the injection of horse pituitary extract, and one obtained following the expression of the corpus luteum and injection of pregnant mare serum (Table 1).

The animals were artificially inseminated, usually at the termination of oestrus, and were slaughtered at various intervals from 22 to 190 hr. after the end of oestrus. The end of oestrus was determined to within 4 hr. by mating tests, or was taken as 72 hr. from the time of expression of the corpus luteum, it having been found that, after removal of the corpus luteum, animals come on heat with fair regularity after approximately 60 hr. and remain in season for not more than about 12 hr. Insemination was performed in most of the cows at the presumed end of oestrus, and it was concluded that if a fertilized egg was obtained the presumption had been correct. Insemination and killing of the animals were both timed to the nearest quarter of an hour. The uterus and uterine tubes were removed within 15 min. of slaughter.

The eggs were recovered by flushing the uterine tubes or uterus with Locke's fluid into small watch-glasses. The eggs after being isolated were transferred into several changes of Locke's fluid in order to separate them from small pieces of epithelial detritus from the tube wall; they were photographed in the living state with a Vicker's projection microscope at a magnification of $\times 480$. After being photographed they were fixed in either Bouin, Flemming or Susa fixative. Most of the eggs were then embedded by the agar technique, as described by Samuel (1944), and sectioned at $7\,\mu$. Serial photographs were made of the sectioned eggs, and from these photographs wax models were constructed. Details are given of the size of the eggs before and after fixation, and also of the volume of the egg from the unsegmented stage to the eight-cell stage in the living specimen.

Calculation of size of ova. All measurements were made on photographs of living ova.

Volume of zona pellucida. The ova were assumed to be spherical, and the volume was calculated from the formula $\frac{4}{3}\pi r^3$, deriving the radius from the average measured diameter.

Diameter and volume of unsegmented ovum. Two diameters at right angles were measured and the average taken. The ova were assumed to be spherical and the volume was calculated as above.

Volume of two-cell stage. As a first approximation it may be assumed that each blastomere is an ellipsoid and so its volume can be calculated from the formula $\frac{4}{3}\pi abc$, where a, b and c are the semi-axes. By measurement it is found that b and c are equal. The total volume was obtained by addition.

Volume of four-cell stage. Where four spheres are inscribed within another, the mean diameter of the inscribed spheres is 0·9 of the radius of the circumscribing sphere.

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Table 1. *Ages and stages of development of eggs examined*

Cow no.	Age of ovum from end of oestrus (hr.)	Stage of development as shown by sections	Remarks
295	—	—	Ovarian
305	—	—	Ovarian
H.14	22	Unfertilized	Normal oestrus
I.30	24	Unfertilized	Corpus luteum expressed
95	35¼	Unfertilized	Corpus luteum expressed
437	42	Unfertilized	Corpus luteum expressed
80	46¼	Unfertilized	Normal oestrus
561	—	Not sectioned. Normal oestrus	100 mg. horse pituitary
279	48½	Maturation spindle	Corpus luteum expressed
I.39	23	2nd maturation spindle	Corpus luteum expressed
305	40	Early central pronuclei	Corpus luteum expressed
418	44½	Late centra lpronuclei	Corpus luteum expressed
I.50	50	Early eccentric pronuclei	Corpus luteum expressed
723	30½	Eccentric pronuclei	Normal oestrus
623	51¾	Pronuclei	Corpus luteum expressed 200 mg. horse pituitary
I.34	24	Pronuclei	Corpus luteum expressed
481	40	Prophase, 1st cleavage spindle	Normal oestrus
I.26	45½	Prophase, 1st cleavage spindle	Corpus luteum expressed
H.24	40	Metaphase, 1st cleavage spindle	Normal oestrus
H.15	44½	Late cleavage spindle	Normal oestrus
		Two-cell stage	
H.10	40½	Vesicular nuclei	Normal oestrus
E.87	45¾	Vesicular nuclei	Corpus luteum expressed
A.35	45	Vesicular nuclei	Corpus luteum expressed
302	96 (in uterus)	Vesicular nuclei	Corpus luteum expressed
E.D.5	45¼	Two spindles	Normal oestrus
H.23	55½	Two spindles	Normal oestrus
H.32	70	Degenerating nuclei	Pregnant mare serum—corpus luteum expressed
I.35	47	Abnormal	Corpus luteum expressed
		Three-cell stage	
620	70	One spindle, two vesicular nuclei	Corpus luteum expressed
621	44	—	Corpus luteum expressed
		Four-cell stage	
154	Unknown	Vesicular nuclei	1500 i.u. pregnant mare serum
E.92	62¾	Vesicular nuclei	Normal oestrus
H.25	66¾	Vesicular nuclei	Normal oestrus
H.5	51¼	Vesicular nuclei	Normal oestrus
H.9	43¾	Vesicular nuclei	Normal oestrus
		Six-cell stage	
625	46¼	One nucleus with spindle	Corpus luteum expressed
		Seven-cell stage	
H.13	71	Vesicular nuclei	Normal oestrus
		Eight-cell stage	
H.20	62½	—	Normal oestrus
H.6	63½	—	Normal oestrus
H.22	65	Vesicular nuclei	Normal oestrus
E.91	71	—	Corpus luteum expressed
299	72½	—	Corpus luteum expressed
282	96 (in uterus)	—	Corpus luteum expressed
		Morula stage	
I.17	72 (in uterus)	19 cells	Corpus luteum expressed
I.25	141 (in uterus)	16 cells	Normal oestrus
29	144 (in uterus)	44–48 cells	Normal oestrus
		Blastocyst stage	
H.40	190 (in uterus)	—	Normal oestrus

The diameter of the interior of the zona pellucida (the circumscribing sphere) was measured in two directions and the average taken. The mean diameters of the inscribed spheres (the blastomeres) were calculated.

The greatest and least radius of each blastomere was measured. The average of these and the mean radius was taken and the volume of each blastomere calculated from the formula $\frac{4}{3}\pi r^3$ using the average obtained as r. The total volume was obtained by addition.

Volume of eight-cell stage. The greatest and the least radius of each blastomere was measured. The average of these two was taken as the radius in each case, and the volume calculated from the formula $\frac{4}{3}\pi r^3$. The total volume was obtained by addition.

TIME OF OVULATION

Ovulation in cattle is known to occur usually after the end of oestrus. An average time of 27 hr. from the onset of oestrus is given by Hammond (1927). Calculating from this and his average figure of 17 hr. for the duration of oestrus, the average time of ovulation is 10 hr. post-oestrum. Most of the animals from which these results were obtained were heifers. Gerasimova (1938) found in 89·1 % of cases in cows that ovulation took place between 20 and 32 hr. from the beginning of heat, never earlier than 16 hr. or later than 38 hr., the average being 27 hr. 50 min. At the same time she states that the length of oestrus varied from 6 to 20 hr. and averaged 12½ hr. Therefore, ovulation occurred about 15½ hr. after the end of heat. Brewster, May & Cole (1940) found that ovulation occurred in fifty-three cows 13·57 ± 0·63 hr. post-oestrum, with a range of 2–26 hr. Nalbandov & Casida (1942) found that ovulation occurred, on the average, 14·16 hr. post-oestrum. The latter workers found also that in two out of seventy-two oestral periods studied in twenty-two cows ovulation was delayed for from 47 to 104 hr.

There is thus general agreement that ovulation occurs at from about 10 to 15½ hr. post-oestrum, the shorter time in heifers, and the longer in cows.

OBSERVATIONS

Ovarian oocyte

Two oocytes, with their surrounding corona radiata cells, were removed from large ovarian follicles and after fixation were sectioned.

The first oocyte was recovered from an almost mature follicle of a cow (no. 295); after sectioning it was found to have a mean diameter of 94 μ.

The second oocyte (Pl. 1, fig. 1) was recovered from a heifer (no. 305) that had been in oestrus 40 hr. previously; after sectioning, this oocyte had a mean diameter of 79 μ. An unsegmented egg at the pronuclear stage was also recovered from the uterine tube of this animal.

In each of these oocytes the cytoplasm was finely granular in the centre of the egg, but more coarsely granular at the periphery. In each there was a large vesicular nucleus with a distinct nuclear membrane and scattered nuclear chromatin.

Unsegmented ovum

In our material there are eighteen unsegmented eggs, some of which are unfertilized while others are at various pronuclear and spindle stages (see Table 1).

The times of ovulation, as already stated, range from 10 to 15½ hr. after the termination of oestrus. Six eggs were obtained from cows which had ovulated after a normal oestrous cycle; eleven eggs were procured from animals in which oestrus had been induced by the expression of the corpus luteum of the previous cycle; in one animal of the latter group horse pituitary extract was administered (see Table 1).

Single-cell stage

The living ovum

Owing to the presence of a large number of fatty globules in the vitellus it is not possible by an examination of the living egg to state whether or not the egg has been fertilized. In one specimen only (no. I.30), obtained 24 hr. post-oestrum, were corona radiata cells found attached to the zona pellucida. This specimen, as subsequent histological examination showed, was in the early stages of degeneration, as many scattered vacuoles were found in the cytoplasm and the chromatin material was no longer recognizable.

The zona pellucida appears as a homogeneous and structureless membrane with an average thickness of 16·5 μ. In it, as in the eggs from most of the animals that had been inseminated, many sperms were seen while the egg was alive, and their presence was confirmed by subsequent examination of the sections.

In the recently ovulated unfertilized egg the vitellus completely fills the zonal cavity (Pl. 1, fig. 2). In all of the fertilized eggs there is a distinct perivitelline space and hence the volume of the vitellus is less than that of the zonal cavity (Table 2). The presence of the space, however, is not a criterion of fertilization since a space is also present in degenerating eggs. Polar bodies are only occasionally seen in the perivitelline space of living eggs (Pl. 1, fig. 3). The vitellus is composed of a large number of fine, highly refractile granules evenly distributed in the cytoplasm, there being no evidence of polarity in the normal egg. In many eggs, which subsequent histological examination showed to be degenerating, there was a crescentic area of varying extent that was devoid of granules.

The fixed ovum

Agar whole mounts

After embedding in agar, and dehydration, many of the eggs were photographed and examined as whole mounts in methyl benzoate. It will be seen by comparing Pl. 1, figs. 3 and 4 that the egg has undergone a considerable shrinkage. The fatty material has been dissolved by the alcohol and the cytoplasm has assumed a granular appearance. In many of the whole mounts it was possible to recognize the nuclear structure of the egg (Pl. 1, fig. 4).

Sectioned material

Two eggs showing a maturation spindle were recovered from cows (nos. I.39 and 279) at 23 and 48½ hr. post-oestrum. The corpus luteum of the previous cycle in each animal was expressed 60 hr. previous to the onset of oestrus. Both animals were artificially inseminated at the end of oestrus but the eggs were unfertilized.

As will be seen from Table 1 there are several eggs at the pronuclear stage. The youngest pronuclear stage (I.34) was obtained 24 hr., the oldest (no. 623) 51¾ hr. post-oestrum. This last egg (no. 623) showed degenerative changes and it seems probable that it would not have developed further. Typical eccentrically placed pronuclei are seen in Pl. 1, figs. 5 and 6. This egg was obtained from a cow (no. 723) 30½ hr. post-oestrum. Centrally placed pronuclei obtained from a cow (no. 418) 44½ hr. post-oestrum are shown in Pl. 2, fig. 7; at this stage the pronuclei are large and vesicular. In none of

Table 2. *Volume of vitellus and zonal cavity in living unsegmented and segmented eggs*

Cow no.	External diameter of zona pellucida (μ)	External volume of zona pellucida (cu. μ)	Internal diameter of zona pellucida (μ)	Internal volume of zona pellucida (cu. μ)	Thickness of zona pellucida (μ)	Diameter of vitellus (μ)	Volume of vitellus (cu. μ)
437	168·7	2,515,000	137·5	1,361,000	15·6	112·5	745,500
443	170·8	2,610,000	137·5	1,361,000	16·65	118·8	878,300
279	181·25	3,118,000	148·0	1,697,000	16·625	131·25	1,183,500
I.50	172·9	2,708,000	141·66	1,489,000	15·62	120·8	984,600
481	172·9	2,708,000	131·25	1,183,500	20·825	116·6	830,400
I.26	160·0	2,145,000	135·4	1,300,000	13·3	116·6	830,400
H.24	168·7	2,515,000	137·5	1,361,000	15·6	120·8	984,600
			Two-cell stage				
I.35	164·6	2,335,000	133·33	1,242,000	15·635	—	—
E.87	181·25	3,118,000	150·0	1,768,000	15·625	—	1,855,000
A.35	170·8	2,610,000	143·75	1,556,000	13·525	—	644,000
302	166·66	2,424,000	137·5	1,361,000	14·58	—	1,127,000
E.D.5	175·0	2,806,000	139·6	1,424,000	17·7	—	661,000
			Three-cell stage				
620	168·7	2,515,000	139·6	1,424,000	14·6	—	1,044,000
			Four-cell stage				
H.5	177·0	2,903,000	143·75	1,556,000	16·625	—	743,000
H.9	162·5	2,249,000	139·6	1,424,000	11·45	—	714,000
H.25	175·0	2,806,000	139·6	1,424,000	17·7	—	—
			Six-cell stage				
625	158·33	2,078,000	129·16	1,128,000	14·535	—	—
			Seven-cell stage				
H.13	164·6	2,335,000	135·4	1,300,000	14·6	—	—
			Eight-cell stage				
H.20	160·0	2,145,000	135·4	1,300,000	12·3	—	—
H.6	160·0	2,145,000	139·6	1,424,000	10·2	—	680,000
H.22	164·6	2,335,000	141·66	1,489,000	11·47	—	—
E.91	177·0	2,903,000	148·0	1,697,000	14·5	—	—
299	168·7	2,515,000	141·66	1,489,000	13·52	—	803,000
282	170·8	2,610,000	141·66	1,489,000	14·57	—	—
			Morula stage				
I.17	168·7	2,515,000	139·6	1,424,000	14·55	—	—
I.25	168·7	2,515,000	143·75	1,556,000	12·475	—	—
29	164·6	2,335,000	137·5	1,361,000	13·55	—	—
			Blastocyst stage				
H.40	168·7	2,515,000	137·5	1,361,000	15·6	—	—

the stages in our collection is it possible to state which is the male and which is the female pronucleus.

First cleavage spindles (Pl. 2, fig. 8) were obtained at 40 hr. in cows (nos. 481 and H. 24) at 44½ hr. (H. 15) and at 45½ hr. (I. 26) post-oestrum; the youngest was obtained after normal oestrus and the oldest after expression of the corpus luteum.

Two-cell stage

There are eight eggs at this stage of development. A two-cell stage with vesicular nuclei was recovered from the uterine tube 40½ hr. post-oestrum (H. 10). This is the earliest time at which a two-cell stage was found. The latest time at which a normal two-cell stage was recovered following normal oestrus was 55½ hr. (no. H. 23). Each blastomere in this egg shows a spindle of division and these are arranged approximately at right angles to each other. A two-cell stage was recovered 96 hr. after oestrus induced by the expression of the corpus luteum. This egg was undergoing degenerative changes, many vacuoles being present in the cytoplasm.

In the normal living egg (Pl. 2, fig. 9), in the agar preparation (Pl. 2, fig. 10), and in the histological specimen (Pl. 2, fig. 11), the cytoplasm is similar in appearance to that of the unsegmented egg (Pl. 1, figs. 3, 4 and Pl. 2, fig. 8). The blastomeres at the two-cell stage are similar in appearance but show slight differences in their volume (see Table 3).

Three-cell stage

There are two eggs at this stage, one at 44 hr. and the other at 70 hr. post-oestrum. In each cow the corpus luteum of the previous cycle had been expressed. One of the cells in each of the specimens was much larger than the other cells. In the 70 hr. ovum (no. 620) the large cell showed a spindle of division.

Four-cell stage

Five eggs at this stage of development were obtained from five cows at times ranging from 43¾ to 66¾ hr. post-oestrum. The eggs, with one exception (no. 154), were obtained following a normal oestrous period. Egg 154 was obtained from an animal which received 1500 i.u. of pregnant mare serum 1 day before the corpus luteum was expressed. The animal was slaughtered 4 days after the expression of the corpus luteum; the precise time of mating was not known. Cow E. 92 was inseminated 13½ hr. post-oestrum, whilst the other three cows were inseminated at the end of oestrus.

The blastomeres in all of the eggs were arranged in pairs so as to form a regular cross, i.e. in a tetrahedral formation (Pl. 2, fig. 12 and Pl. 3, fig. 13). Except for slight differences in size the blastomeres both in the living egg and after sectioning were similar in appearance. The cytoplasm (Pl. 3, fig. 14) was essentially similar to that of the unsegmented egg. The nuclei of all the blastomeres were in the

Table 3. *Calculated volumes of the blastomeres of living eggs from two- to eight-cell stage*

Cow no.	Volumes of individual blastomeres (living ova) (cu. μ)								Total volume (cu. μ)
				Two-cell stage					
A. 35	339,000	305,000							644,000
	53%	47%							
E.D. 5	334,000	327,000							661,000
	51%	49%							
E. 87	1,023,000	832,000							1,855,000
	55%	45%							
302	666,000	461,000							1,127,000
	59%	41%							
				Three-cell stage					
620	524,000	260,000	260,000						1,044,000
	50%	25%	25%						
				Four-cell stage					
H. 9	239,000	187,000	144,000	144,000					714,000
	34%	26%	20%	20%					
H. 5	268,000	187,000	144,000	144,000					743,000
	36%	26%	19%	19%					
				Eight-cell stage					
299	144,000	108,000	105,000	102,000	92,000	87,000	87,000	78,000	803,000
	18%	13·5%	13%	12·5%	11·5%	11%	11%	9·5%	
H. 6	102,000	92,000	87,000	87,000	82,000	82,000	74,000	74,000	680,000
	15%	13·4%	12·8%	12·8%	12%	12%	11%	11%	

resting state. The measurements of the volumes of the cells are given in Table 3.

Six-, seven- and eight-cell stages

At these stages of development there are eight specimens which were obtained from eight cows.

Egg 625 is at the six-cell stage and was recovered 46½ hr. post-oestrum following the expression of the corpus luteum. Two of the cells, one with a spindle and the other with a resting nucleus, are much larger than the other four cells. The disposition of the cells was such that accurate measurements of them could not be made in the living specimen.

Egg H.13 is at the seven-cell stage and it was recovered 71 hr. after the termination of a normal oestrus. In this egg one cell is larger than the others (Pl. 3, fig. 15). All the nuclei are in the resting condition.

The remaining six eggs, at the eight-cell stage, were recovered from six cows at times ranging from 62½ to 96 hr. post-oestrum. Three of the eggs (nos. H.20, H.6 and H.22) were recovered following normal oestrus; the three other eggs (nos. 282, 299 and E.91) were recovered from cows after the expression of the corpus luteum. All the eggs, with the exception of no. 282, were from the uterine tube; this latter egg was recovered from the uterus at 96 hr. post-oestrum.

The cells at the eight-cell stage are approximately spherical in shape and are surprisingly uniform in size (Pl. 3, figs. 16, 17, and Table 3). The blastomeres are closely packed in the zonal space and are arranged as two groups of four cells, which interdigitate with one another. Many sperms are still present in the zona pellucida. In the sectional material the cytoplasm was similar to that of earlier stages of development (Pl. 3, figs. 18, 19). There is as yet no microscopical evidence of differentiation among the cells.

Morula stage

There are three eggs at this stage of development.

A morula with nineteen cells was recovered from the uterine tube of a cow (no. I.17) 72 hr. post-oestrum following the expression of the corpus luteum. In the living state the blastomeres were seen to be approximately of equal size (Pl. 4, fig. 20) and this was confirmed when the sectioned egg was examined. The egg when cut was present in seventeen sections of 8 μ giving it a maximum diameter of 126 μ. One blastomere is centrally placed, the other cells being arranged around it (Pl. 4, figs. 21, 22). In the fixed and sectioned egg all the blastomeres have resting nuclei and the cytoplasm of the cells shows no qualitative differences.

A second morula of sixteen cells was recovered from the uterine cavity of a cow (no. I.25) killed 141 hr. after normal oestrus. In the living egg (Pl. 4, fig. 23) slight differences in the size of the cells could be readily appreciated. The egg, which was cut at 7 μ, was present in fifteen sections giving it a diameter of 105 μ. A single blastomere is again centrally placed as in the previous morula. In the fixed and sectioned egg all the cells have resting nuclei and are essentially similar in appearance. The differences in size and form of the blastomeres at the morula stage as described by Amoroso *et al.* (1942) for the goat are not manifest in the egg of the cow.

The third morula was obtained from the uterine cavity of a cow (no. 29) killed 144 hr. after normal oestrus. In the living state, owing to the number of cells, the differences in the size of the blastomeres could not be readily appreciated. Irregularly arranged intercellular spaces, the forerunners of the blastocyst cavity, are present between the outer layer of cells and those in the interior of the egg (Pl. 4, figs. 24, 25).

The morula stages at our disposal give no indication as to how the centrally placed cell comes to occupy its position.

Blastocyst stage

An egg at this stage was recovered from the uterine cavity of a cow (no. H.40) killed 190 hr. after normal oestrus. The appearance of the egg in the living state is shown in Pl. 5, fig. 26. It will be seen that the cells have shrunk from the inner aspect of the zona pellucida leaving an extensive perivitelline space. After fixation and clearing the blastocyst cavity can readily be recognized (Pl. 5, fig. 27).

On section the blastocyst is found to consist of an outer layer of cuboidal or irregularly polygonal cells and an inner mass; the latter is in close relationship with the cells which cover it. There is, as yet, no visible difference between the covering cells and the inner cell mass, nor is there any segregation of the cells of the inner mass into embryonic ectoderm and endoderm (Pl. 5, figs. 28–30).

COMMENTS AND COMPARISONS

Zona pellucida

The zona pellucida of the cow egg has an appearance similar to that of other mammalian eggs. It persists at least until the blastocyst stage at 190 hr. post-oestrum.

Cumulus cells

In contrast to many mammalian eggs, e.g. guinea-pig (Squier, 1932), rat (Gilchrist & Pincus, 1932), and ferret (Hamilton, 1934), the cumulus cells in the cow disappear soon after ovulation. In only one specimen, recovered 24 hr. post-oestrum, were a few cumulus cells attached to the zona pellucida.

An unfertilized egg recovered as early as 22 hr. post-oestrum showed no cumulus cells. The examination of eggs removed from large ovarian follicles shows that there is a well-organized layer of cumulus cells. Webster (1921) refers to cumulus cells as being present in the large follicles. The cells persist for not more than 9–14 hr. after ovulation and in most eggs for even a shorter period.

Of the ungulates so far examined, cumulus cells, soon after ovulation, are absent on the eggs of the sheep (Assheton, 1898*a*; McKenzie & Allen, 1933; Clark, 1934; and McKenzie & Terrill, 1937), the cow (Hartman *el al.* 1931; and Evans & Miller, 1935), the pig (Assheton, 1898*b*; Corner & Amsbaugh, 1917; and Heuser & Streeter, 1929), the horse (Amoroso *et al.* 1939; and Hamilton & Day, 1945), and the goat (Amoroso *et al.* 1942). Whether these ungulates show a marked loosening of the cumulus cells such as is described by Lewis & Hartman (1941) in the pre-ovulatory stages in the monkey, we have no evidence to present. We can offer no explanation for the rapid disappearance of the cells, but suggest that it may be a characteristic of the ungulates.

There can be little doubt that the presence of living spermatozoa may assist in the separation of the cells, but that they are not essential is shown by the fact that the cells are absent in unfertilized as well as in fertilized eggs. From the large number of spermatozoa found in the zona pellucida of many fertilized eggs, it appears that large numbers of them surround the ovum in its passage down the uterine tube. Pincus (1930) and Pincus & Enzmann (1932) have shown that in the rabbit and rat respectively when eggs with attached cumulus cells are placed *in vitro* with living sperm suspensions the cells are rapidly dispersed, but this does not occur in sperm-free media. Yamane (1930) believes that the dispersion is due to a proteolytic enzyme in the spermatozoa. For details of the effect of sperm suspensions on maturation, etc., the work of Pincus & Enzmann (1935) should be consulted.

Cytoplasm of the ovum

There are striking differences in the appearances of living eggs of different mammals; even among the eggs of a single genus, differences are apparent. In some, e.g. the cow, the cytoplasm of the egg is made up of many small discrete fatty globules which are closely packed together. In others, e.g. the mouse and the golden hamster, the fatty globules are absent.

The nucleus cannot be seen in the living egg of the former group, whereas such structures as the nucleus and even the sperm head and tail may be easily recognized in the latter. Amongst the ungulates, the eggs of the sheep and goat are strikingly similar in appearance and contain much less fatty material than those of the cow, whilst the eggs of the pig and horse contain much more fatty material. There is no evidence of polarity in the cow egg and in this respect it is similar to the eggs of the sheep, goat, pig and horse.

Location of the eggs

No attempt has been made to determine the precise location of the eggs during their passage through the uterine tube. The times at which the different stages were recovered are shown in Table 4.

The earliest time at which an egg was found in the uterus was 96 hr. post-oestrum—a two-cell stage from cow 302 and an eight-cell stage from cow 282. For the eight-cell stage the time of ovulation was estimated to be between 10 and 15½ hr. post-oestrum, which means that the time taken for the tubal journey of this egg was from 80½ to 86 hr. The time interval between insemination and the entry of the egg into the uterus is given in Table 4 for a number of mammals. This table, however, does not give a true estimation of the time taken for the tubal transit since in some forms (e.g. mouse) ovulation occurs any time during oestrus, in others (e.g. rabbit) shortly after copulation, while in others (e.g. ferret) not until 31½–53½ hr. after copulation. If due allowance is made for this variation in the times of ovulation in relation to oestrus and insemination, the tubal journey takes about 3–3½ days in most mammals with the exception of the opossum, as estimated by Hartman (1939).

Volume of the vitellus

A review of the size of the mammalian egg was given by Hartman (1929). At that time relatively few accurate observations had been made of the size of the living egg, hence estimations of the size of many eggs were deduced from calculations made on fixed specimens.

Since that date a number of papers (Gregory, 1930; Gilchrist & Pincus, 1932; Squier, 1932; Lewis & Hartman, 1933; Clark, 1934; Lewis & Wright, 1935; and Amoroso *et al.* 1942) have appeared. These publications give measurements of the living eggs of different species. Many of the authors have given the diameters of the zona pellucida and vitellus only, whilst a few have calculated the volume of the egg during cleavage. The vitellus of the cow egg has a diameter which is approximately 1½ times greater than that of the mouse and a volume that is approximately 4 times greater than that of the mouse. Lewis & Wright (1935) point out that 'the difference in volume is far more striking and probably more significant'.

In the present investigation we have calculated, in the living egg, the volume of the vitellus in the unsegmented egg, and of the blastomeres up to the eight-cell stage in the living egg and also in the fixed specimen. The examination of the living specimen

Table 4. *Stages of development in hours of different species*

Type	Observer	Estimated time of ovulation	1-cell	2-cell	3–4-cell	5–8-cell	9–16-cell	Morula	Blastocyst	Time and stage at which egg reaches uterus	Total gestation period (days)
Ungulate:											
Cow	Present series	10–15·5 hr. post-oestrum	23–51·75	40·5–55·5	44–65·75	46·25–96	71–141	144	190	96 (8–16-cell)	284
Cow	Hartman *et al.*	—	—	48	—	—	—	—	—	—	—
Cow	Winters *et al.*	Post-oestrum	34	50–62	—	62–64	110	134	182	110 (16-cell)	284
Goat	Amoroso *et al.*	—	30·5	30·5–48	60	85	98	120–140	158	98 (10–13-cell)	150
Sheep	Clark	Late in oestrus	0–38·75	38–39	42	44	65–77	96	113·5–138·75	77–96 (16-cell)	150
Pig	Heuser & Streeter	—	0–51	51–66	66–72	90–110	—	110–114	114	75 (4-cell)	112
Carnivora:											
Ferret	Hamilton	31·5 hr. after copulation	31·5–53·5	—	64–72	64–116·5	74–120	120–146	146–264	120–140 (up to 32-cell)	42
Primate:											
Macacus rhesus	Lewis & Hartman	Determined by bimanual palpation	—	0–24	24–36	36–48	48–72	72–96	—	96 (16-cell)	164
Rodent:											
Mouse	Lewis & Wright	Any time during oestrus	0–24	24–38	38–50	50–64	60–70	68–80	74–82	72 (morula)	19–21
Mouse	Sobotta	—	0–24	24–38	50	60	70	—	—	—	—
Rat	Huber	—	0–24	42–70	63–73	89	89–96	—	—	—	—
Rat	Gilchrist & Pincus	As early as 8·5 hr. after copulation	8·5–27	27–44	60–85	71–95	—	—	—	—	22
Rat	Macdonald & Long	—	12–20 (15)	37–61 (45)	57–85 (65)	64–87 (79)	84–92 (90)	—	105–109 (107)	—	—
Rabbit	Gregory	—	Up to 22	22–26	26–32	32–40	40–47	47–68	68–76	70 (blastocyst)	30–32
Rabbit	Assheton	—	12–24	24–28	28	—	—	48	75–96	—	—
Rabbit	Pincus	10 hr. after copulation	11–21	21–24	—	—	—	—	—	—	—
Guinea-pig	Squier	After copulation	3–30	30–35	30–75	80	—	100–115	115–140	80–85 (8-cell)	63

is not vitiated by the different amount of shrinkage which occurs in fixed material (Table 6). We have compared (see Table 5) the volumes so obtained with those given by other investigators or estimated by us from photographs of living eggs or from data given in the articles quoted. Data and comparisons of a similar nature derived from models and other calculations have been given by other investigators for fixed eggs. It has not, however, been possible to estimate the volume of the different blastomeres beyond the eight-cell stage as the cells overlap in the photographs.

Cleavage

The differences in the rates of cleavage of the eggs of different species have been commented upon by many investigators and are set out in tabular form (Table 4). There are also variations in the time at which cytological differentiation becomes manifest amongst the blastomeres, and in the relative sizes of the blastomeres in different species during the early cleavage stages (see Amoroso *et al.* 1942 for literature).

In the cow egg there are not the striking differences in the size of blastomeres during the early stages of cleavage nor is there the early segregation of the blastomeres into larger, more slowly dividing cells, and smaller, more actively dividing cells, as described by Heuser & Streeter (1929) in the pig and to a lesser extent by Amoroso *et al.* (1942) in the goat. In the morula stages the blastomeres show only slight differences in their size and are cytologically similar. This relative lateness of differentiation in the egg of the cow may be associated with the longer gestation period compared with that of the pig and goat (see Table 4).

Table 5. *Volumes of vitellus in different species*

Type	Observer	Average internal diameter of zona pellucida (μ)	Volume of ovarian ovum (cu.μ)	Average diameter of vitellus (μ)	Average volume of vitellus in cu.μ: 1-cell	2-cell	4-cell	8-cell	Morula
Ungulate:									
Cow	Present series	138·4	—	120	919,500	1,071,750	729,000	741,500	—
Cow	Hartman *et al.*	143	1,537,000	120	907,200	—	—	—	—
		—	1,298,000	—	—	679,000	—	—	—
		135	1,600,000	—	—	—	—	—	—
Goat	Amoroso *et al.*	145·3	1,615,000	132	1,204,000	—	—	—	—
Sheep	Calculated from Clark	147	1,663,000	—	1,056,000	919,000	712,200	615,700	—
Pig	Heuser & Streeter	130	1,150,400	111	716,090	—	—	—	—
Primate: (Macacus rhesus)	Lewis & Hartman	125–143	1,000,000–1,633,000	140 (3 eggs)	549,000 (3 eggs)	562,000	527,000	546,000	—
Rodent:									
Mouse	Lewis & Wright	87·8	254,000	71·6	192,000	158,000	162,000	138,000	219,000
Ferret	Hamilton	153	1,876,000	124	1,003,000	881,249	842,000	737,000	—

Table 6. *Volumes of vitellus in living and fixed ova*

Cow no.	Volume of vitellus of living ovum (cu.μ)	Volume of vitellus of fixed ovum (cu.μ)	Loss during fixation %
A.35	644,000	430,000	33
E.D.5	661,000	430,000	36
H.9	714,000	460,000	35
H.5	743,000	570,000	24
299	803,000	290,000	64
H.20	680,000	360,000	47
H.40	—	80,000	—

SUMMARY

1. An account is given of the cleavage stages of the egg of the cow.

2. The rate of cleavage is discussed and compared with that of other mammalian eggs.

3. The diameters of the different eggs and of the volumes of the blastomeres during cleavage as far as the eight-cell stage are given.

4. The shrinkage undergone by the eggs as the result of fixation is estimated.

5. The time taken by the egg to traverse the uterine tube is 96 hr.

6. Histological differentiation is not apparent in the late morula and early blastocyst stage.

It is a pleasure to record our indebtedness to those who have given of their services in many different ways. We wish to thank Dr J. S. Paterson, of the Institute of Animal Pathology, Cambridge, for giving us access to some of the animals used in this investigation. We are indebted to Mr W. S. Mansfield, Director of the University Farm, Cambridge, for providing accommodation for some of the animals. Part of the work was carried out by one of us (J.A.L.) while holding the Miss Aleen Cust

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Photograph of a section of an oocyte recovered from a large ovarian follicle of a heifer (no. 305) killed 72 hr. post-oestrum. × *c.* 416.

Fig. 2. Photograph of a living recently ovulated unfertilized ovum recovered from a cow (no. 107). × *c.* 280.

Fig. 3. Photograph of a living fertilized ovum recovered from a cow (no. 481) killed 40 hr. post-oestrum. A polar body is visible. In the zona pellucida there are many sperms. × *c.* 384.

Fig. 4. Photograph of unsegmented ovum, after fixation and clearing, recovered from a cow (no. 126) killed 45·5 hr. post-oestrum. × *c.* 384.

Figs. 5, 6. Photographs of two adjacent sections of a zygote with eccentrically placed pronuclei. The cow (no. 723) was killed 30·5 hr. post-oestrum. × *c.* 416.

PLATE 2

Fig. 7. Photograph of a section of a zygote with centrally placed pronuclei recovered from a cow (no. 418) killed 44·5 hr. post-oestrum. × *c.* 512.

Fig. 8. Photograph of a section of a zygote with first cleavage spindle recovered from a cow (no. 481) killed 40 hr. post-oestrum (cp. with Pl. 1, fig. 4). × *c.* 416.

Fig. 9. Photograph of a living two-cell stage recovered from a cow (no. E.D.5) killed 45·5 hr. post-oestrum. × *c.* 384.

Fig. 10. Photograph of a two-cell stage, after fixation and clearing, recovered from a cow (no. H.23) killed 55·5 hr. post-oestrum. × *c.* 384.

Fig. 11. Photograph of a section of the same two-cell stage as shown in fig. 10. The nuclei are at the prophase. × *c.* 416.

Fig. 12. Photograph of a living four-cell stage recovered from a cow (no. H.9) killed 43·75 hr. post-oestrum. × *c.* 384.

PLATE 3

Fig. 13. Photograph of a four-cell stage after fixation and clearing recovered from a cow (no. H.5) killed 51·5 hr. post-oestrum. × *c.* 416.

Fig. 14. Photograph of a section of the same four-cell stage as shown on fig. 13. Many sperm heads are seen in the zona pellucida. × *c.* 416.

Fig. 15. Photograph of a seven-cell stage, after fixation and clearing, recovered from a cow (no. H.13) killed 71 hr. post-oestrum. × c. 384.

Fig. 16. Photograph of a living eight-cell stage recovered from a cow (no. H.20) killed 62·5 hr. post-oestrum. × c. 384.

Fig. 17. Photograph of the same egg as in fig. 16 after fixation and clearing. × c. 384.

Figs. 18, 19. Photographs of the fifth and ninth sections of the same egg as in figs. 16 and 17. × c. 416.

PLATE 4

Fig. 20. Photograph of a living morular stage (19 cells) recovered from a cow (no. I.17) killed 72 hr. post-oestrum. × c. 384.

Figs. 21, 22. Photographs of the tenth and eleventh sections of the same egg as in fig. 20. × c. 416.

Fig. 23. Photograph of a living morular stage (16 cells) recovered from a cow (no. I.25) killed 141 hr. post-oestrum. × c. 384.

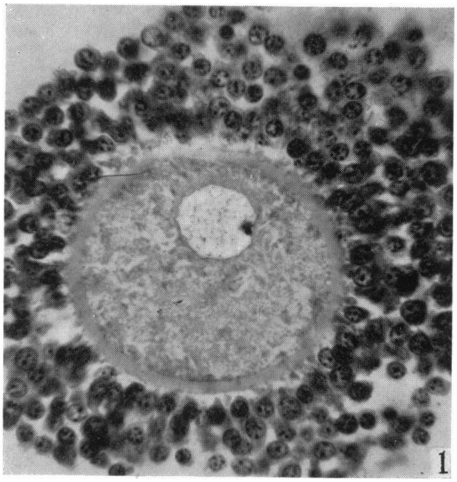
Figs. 24, 25. Photographs of the eighth and ninth sections of an egg recovered from a cow (no. 29) killed 144 hr. post-oestrum. × c. 416.

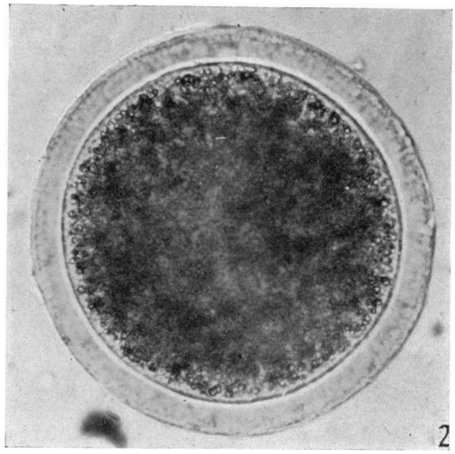
PLATE 5

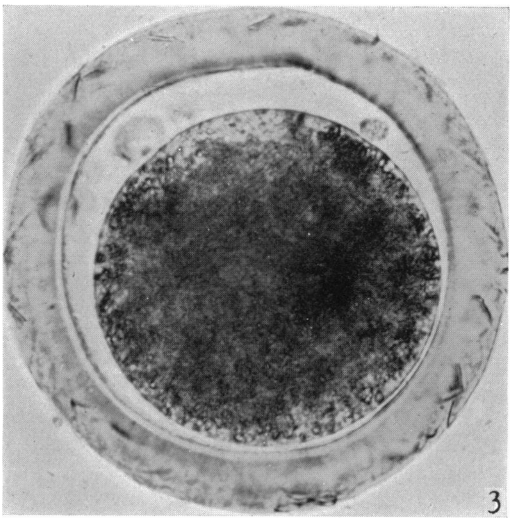
Fig. 26. Photograph of a living blastocyst recovered from a cow (no. H.40) killed 190 hr. post-oestrum. × c. 384.

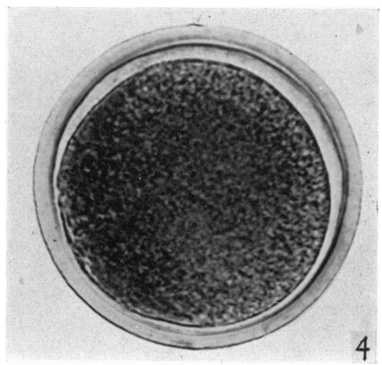
Fig. 27. Photograph of the same blastocyst as in fig. 26 after fixation and clearing. The blastocyst cavity can be recognized. × c. 384.

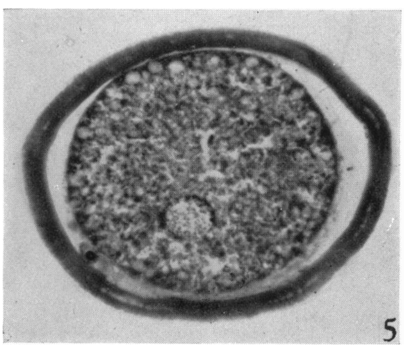
Figs. 28–30. Photographs of the eighth, ninth and tenth sections of the blastocyst shown in fig. 26. × c. 416.

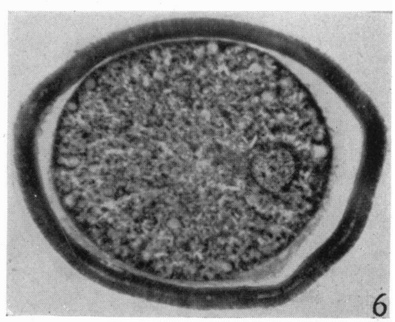




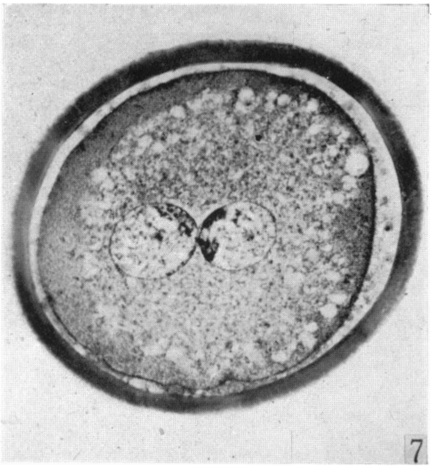








HAMILTON AND LAING—DEVELOPMENT OF THE EGG OF THE COW UP TO THE STAGE OF BLASTOCYST FORMATION



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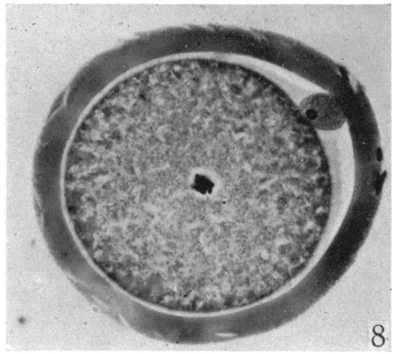

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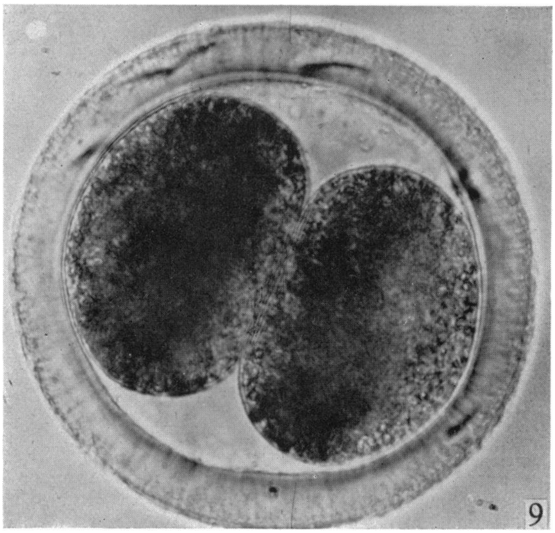

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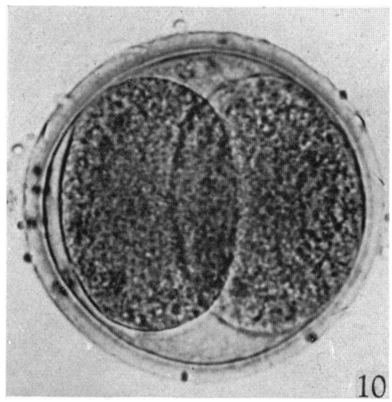

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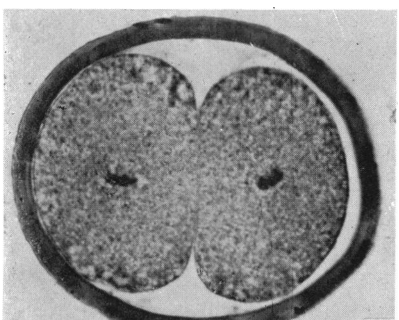

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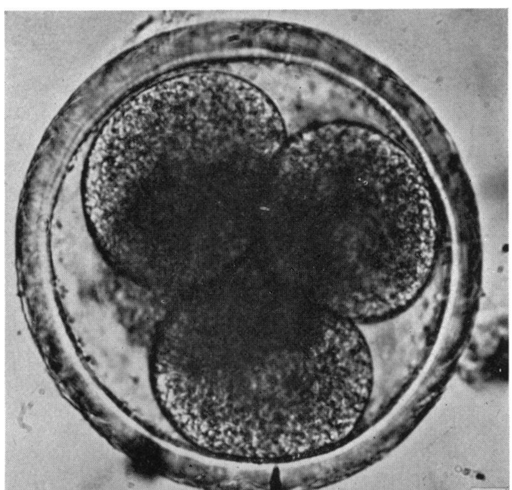

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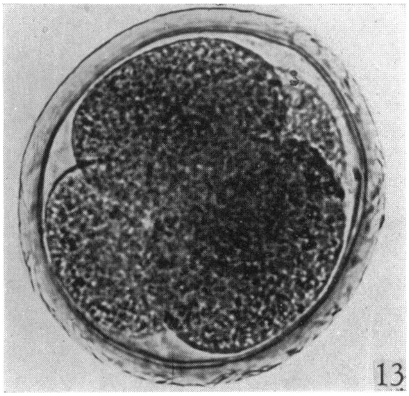
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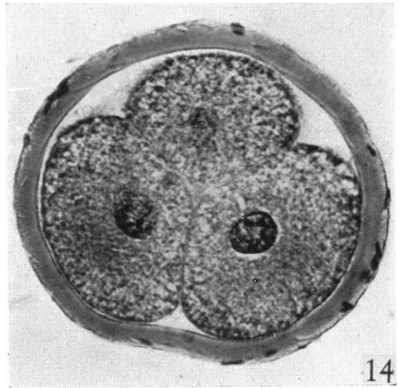
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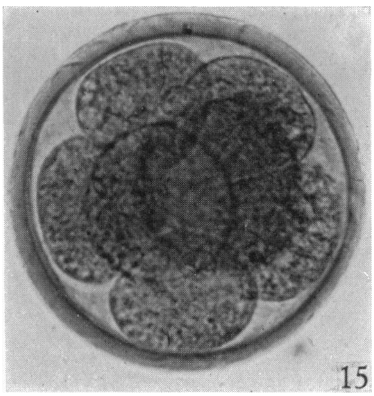
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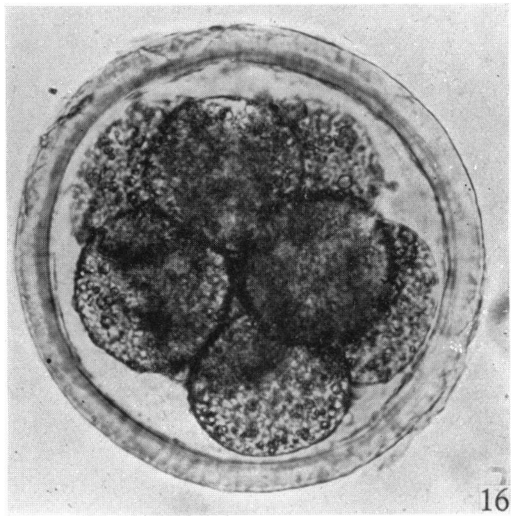
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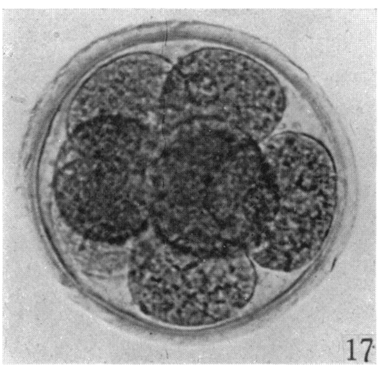
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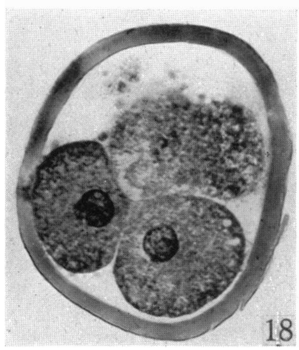
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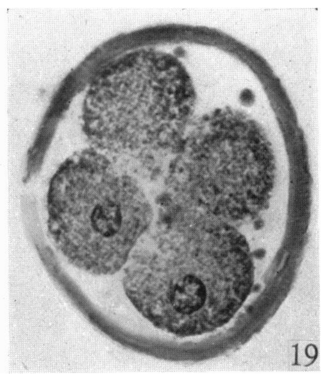
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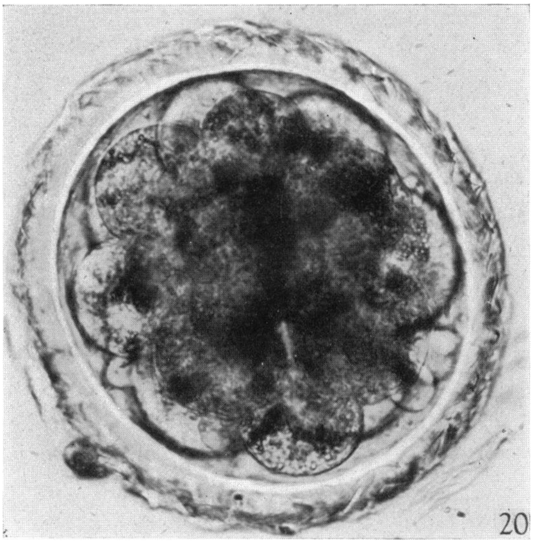
HAMILTON AND LAING—DEVELOPMENT OF THE EGG OF THE COW UP TO THE STAGE OF BLASTOCYST FORMATION



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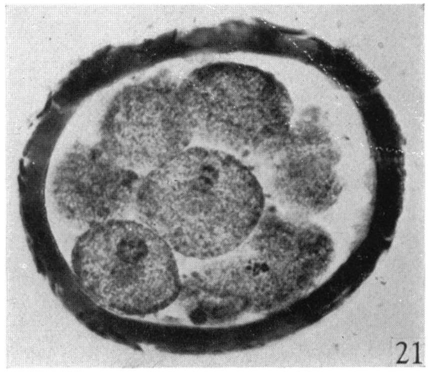

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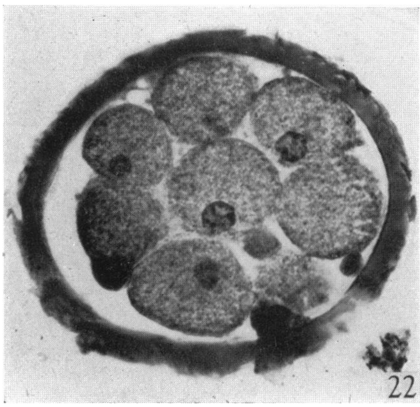

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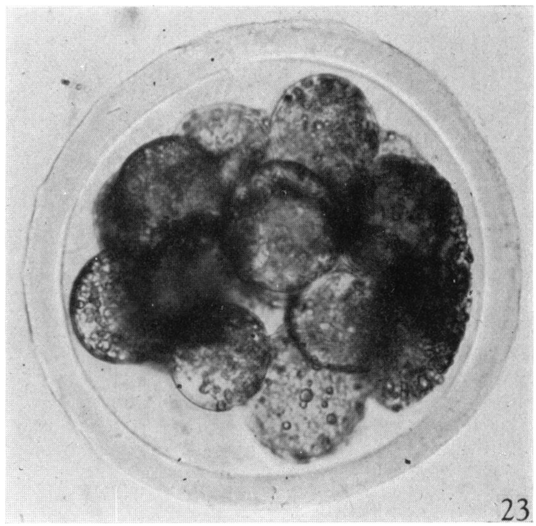

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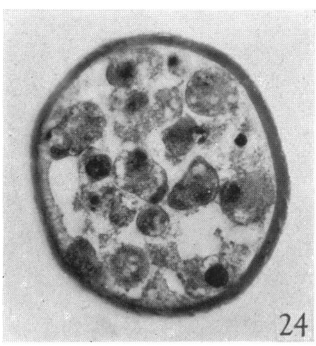

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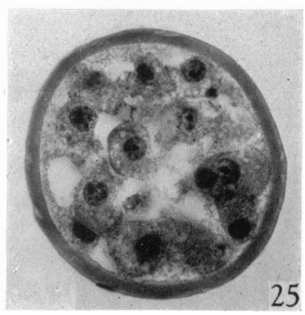

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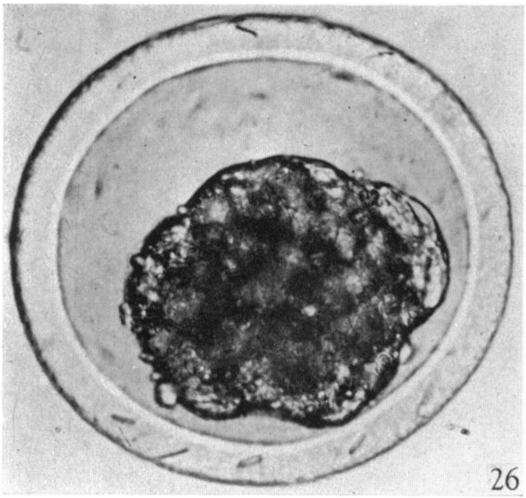

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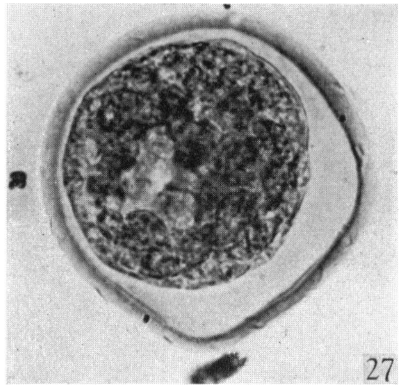

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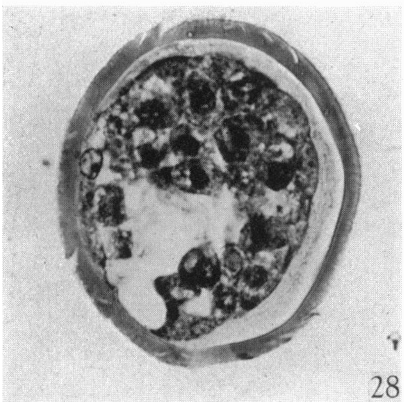

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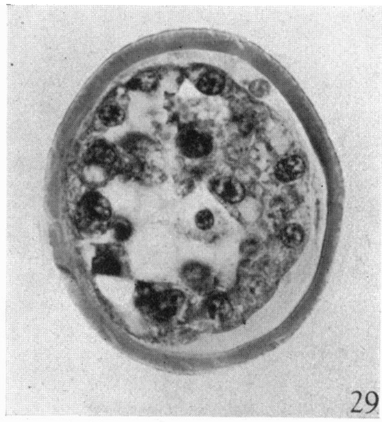

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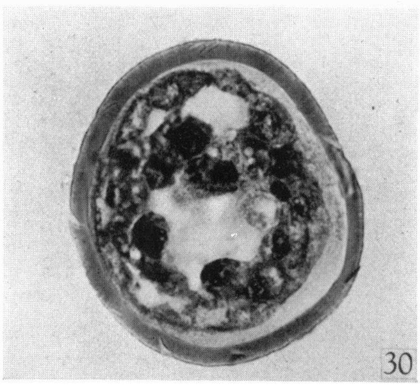

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HAMILTON AND LAING—DEVELOPMENT OF THE EGG OF THE COW UP TO THE STAGE OF BLASTOCYST FORMATION