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PERSISTENCE AND PARTHENOGENETIC CLEAVAGE OF TUBAL OVA IN THE MARE*

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ABSTRACT

As many as ten different ova in all stages of cytolysis were encountered in the uterine tubes of mares. As the follicles of the mare were consistently found to be mon-ovular, it is concluded that unfertilized ova do not, as a rule, pass out of the uterine tubes, but, contrary to all accepted data on the migration of ova, may remain there up to seven and a half months or longer. During this time they undergo gradual disintegration characterized by the following order of changes: deutoplasmic condensation, deutoplasmic extrusion, cytoplasmolysis, deutoplasmic fragmentation and comminution of yolk granules. The final stage identified is a fluid-filled, collapsed vesicle (zona vesicle) surrounded by the zona pellucida only.

Immediately after ovulation the ovum was found to be without a corona radiata but enclosed in a large, irregular gelatinous mass of follicular origin. This is considered to be the reason why newly ovulated ova are so often missed. This mass becomes separated from the ovum within the second day after ovulation. Its significance in fertilization is speculated upon.

Some unfertilized ova are able to undergo parthenogenetic cleavage. This hampers the identification of early cleavage stages of fertilized ova.

Ovulation takes place after the first meiotic division. Sperm entrance stimulates the second meiotic division, as in most mammals.

INTRODUCTION

It is generally accepted that in all mammals the ova, whether fertilized or not, migrate through the uterine tubes to the uterus, where they either disintegrate or are extruded through the vagina. The normal tubal sojourn of fertilized and unfertilized ova has been established as varying from three to seven days, depending on the species (Zuckerman, 1962, p. 477). By contrast, only Long & Evans (1922) noted that tubal eggs of unmated rats never reach the uterus.

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In most mammals unfertilized eggs are able to undergo a few initial parthenogenetic divisions (Parkes, 1960, p. 385; Witschii, 1956, p. 57). Similar divisions may be stimulated experimentally by treatment with hypo- or hypertonic solutions or low temperatures. In one case, which could not be repeated, artificially induced parthenogenesis resulted in the birth of viable rabbits (Pincus & Enzmann, cited by Parkes, 1960, p. 389).

In the hamster as many as 80 per cent of tubal eggs may undergo spontaneous activation (Parkes, 1960, p. 386). If one considers the various possible mechanisms for " regulation to diploidy ", complete spontaneous parthenogenesis may occur. The chromosome number in such cases would be quite normal (Parkes, 1960, p. 386).

Parthenogenesis has never been taken into consideration in any description of equine ova. Hamilton & Day (1945) are the only authors who have described early cleavage stages of the ova in this species. They found three degenerating eggs, two of which were in the uterine tubes but failed to see the significance. Amoroso, Griffiths & Hamilton (1939) found a single, unsegmented tubal ovum with evident signs of degeneration and regarded it as having been in the tube for some time after ovulation. Day (1939) also found an unsegmented ovum about 95 hours after ovulation. In the light of this investigation it must have been much older.

Materials and Methods

The observations forming the basis of the present study were made on twenty mares slaughtered during experimental studies on pregnancy and embryonic resorption undertaken by Van Niekerk (1965).

The mares were of the light farm type, four to eleven years old. With two exceptions they were either maiden mares or had been barren for at least twelve months. All the mares were teased every morning by an active, vigorous stallion. Stallions used for service were tested for fertility. The ovaries were palpated per rectum at least once and in some cases up to three times a day. Mares and stallions were kept strictly apart.

Of the twenty mares ten were served and ten not. They were slaughtered at periods from a few hours up to four months after ovulation.

Initially, in five cases, only the uterine tube associated with the ovulating ovary was examined. Of the remaining fifteen mares both uterine tubes were dissected immediately after slaughter and flushed with normal saline. These flushings were examined by means of a stereoscopic dissecting microscope. The ova were mounted on drop centre slides in dilute glycerine, coloured slightly with safranin or cresylecht violet. The preparations were sealed with paraffin wax. Measurements of some of the ova were made using an ocular and stage micrometer. Microphotographs were taken before and after mounting and the best selected.

Follicular fluid and saline flushings of six follicles were collected and examined separately for ova (Mares No. 5513, 5502).

RESULTS

(A) *Tubal sojourn of ova*

In the first five tubes examined (mares No. 4153, 4625, 4460, 5479 and 4619) one to six ova were recovered from the distal half (Table 1). Daily rectal palpation of the ovaries before ovulation correlated with subsequent post mortem examination revealed that during each heat period only a single follicle had ovulated resulting in the formation of only one corpus luteum. The only exception to this rule was found in mare 5501 which had ovulated from one follicle in each ovary. This was due, however, to the intravenous injection of 4000 IU of " Lutormone " on the morning of the first ovulation.

Only a single ovum was found in six follicles examined. Whenever more than one ovum was found in a tube, they were always in different stages of degeneration. A single ovum was usually found for each corpus luteum or corpus albicans present in the ovaries (seen to best advantage in mares 5720 and 4410). These observations established beyond doubt that only a single ovum is set free at each ovulation and that normally only one follicle ovulated during each heat period.

The above observations raised the question of persistence of unfertilized ova in the uterine tubes. To confirm this, 15 additional mares were slaughtered at intervals from a few hours to ± 4 months after the last ovulation. In these cases both uterine tubes were examined for ova. When the number of ova recovered was correlated with their ovulation dates and state of degeneration it became possible to estimate their ages as given in Table 1.

TABLE 1.—*Number and approximate ages (in days) of ova found in uterine tubes*

Mare No.	Left tube	Age	Right tube	Age
4619	1	± 60	not exam.	
4153	6	2, 20, 37, 120–150	not exam.	
4625	2	5, 29	not exam.	
4460	not exam.		2	1½, 45 +
5479	2	5,30	not exam.	
5505	1	50	1	68 +
5510	4	99, 117, 137, 157	3	137, ± 150, ± 150
5506	0		3	1, 20, 40
5511	1	?	6	?
5598	0		1	54
5751	2	14, 120–150	4	35, ?
5748	2	27, 70–80	2	8, 50
2897	1	± 120	3	2–4 hrs., 19, 58
5513	0		1	± 90
5502	4	44, 63, 150 & 170	4	90, 105 ?
5501	4	1½, 22, 107, 147	6	1, 46, 63, 129, 150, 180–225
5708	0		2	± 105, ± 120
4410	4	71, 95, 115, 135 +	2	115 +, 135 +
5720	0		1	84 +
4124	2	7 ?, ± 210	2	6, 28

Measurements (in μ) of some of the ova recovered are given in Table 2.

TABLE 2.—*Measurements (in μ) of some ova recovered (Z.P. = zona pellucida)*

Mare No.	Left tube	Z.P.	Right tube	Z.P.
5505	135 × 129	12	144 × 123	12
5510	1. 132 × 126 2. 132 × 108	12 12	1. 168 × 151 2. 174 × 162 3. 168 × 162	5·6 8·4 5·6
5751	1. 132 × 132 2. 132 × 132	15 6	1. 150 × 138 2. 138 × 138 3. 141 × 141 4. 141 × 117	12 12 12 9
5501	1. 137 × 126 2. 137 × 129 3. 151 × 118 4. 126 × 112	11·2 14 11·2 8·4	1. 140 × 132 2. 146 × 129 3. 132 × 132 4. 132 × 132 5. 140 × 112 6. 137 × 137	14 14 14 14 5·6 11·2
4410	1. 138 × 129 2. 147 × 120 3. 174 × 129 4. 159 × 129	9 12 12 9	1. 150 × 129 2. 156 × 129	12 9
4124	1. 150 × 144 2. 150 × 150	12 15	1. 160 × 160 2. 150 × 144	18 12
5720			144 × 129	7

(B) *Gelatinous masses*

Globular, gelatinous bodies, attached to a central larger mass by short strands simulating a bunch of grapes, were consistently observed in all uterine tubes from which ova were recovered (Fig. 27). None was found in the left tube of mare 5720, from whose corresponding ovary no ovulation had yet occurred. In tubes with only old ova (mare No. 5708) these masses were more compressed and had no pedicles. Gelatinous masses were often found to block the tubo-uteral junctions (e.g. in mare 5513).

In the initial stages of this investigation these masses were disregarded and considered to be masses of tubal secretions and desquamated epithelium. Toward the end of this investigation a recently ovulated ovum (Fig. 19 and 20) was seen to be embedded in a similar, but less condensed, mass. It was also completely transparent sticky and elastic, and contained blood specks and corona radiata cells. The ovum was separated from it by means of two dissecting needles. A similar mass was seen being detached from a 4-cell cleavage stage (Fig 8) $\pm$ 30 hours after ovulation whereas a morula stage (Fig. 7) and a 8–16-cell cleavage stage (Fig 2) were devoid of a gelatinous mass. These findings are not sufficiently extensive to warrant hard and fast conclusions, but the opinion is advanced that this phenomenon occurs regularly.

No evidence for tubal secretion of the masses was found. On the other hand the inner surface of the newly ovulated follicle (mare No. 2897) was clearly seen to be covered by gelatinous material, indicating a follicular origin for this gelatinous mass.

As the significance of these gelatinous masses was only realized towards the end of this investigation, no observations could be made to determine their role in fertilization.

It must be emphasized that many masses not containing ova were seen.

(C) *Maturation and degeneration of ova*

Apart from an atretic ovum, already oval in shape and in the process of losing its corona radiata, flushing of follicles revealed the following ova which are regarded as representing the normal process of maturation:—

(*a*) An almost mature ovum surrounded by its corona radiata and without a perivitelline space (Plate 1, A).

(*b*) An ovum just undergoing its first meiotic division (Plate 1, C). The polar body was seen as a hillock with segmentation furrows forming at its base. A perivitelline space was present with scattered fine granules (probably microvilli). Half of the corona radiata had been desquamated.

In the newly ovulated ovum (Plate 1, D) a very distinct vitelline membrane, no corona radiata, a polar body and a perivitelline space could be discerned. The zona pellucida was tightly enclosed in a transparent, sticky gelatinous mass. Yolk granules were so large and numerous that the nuclei of the ova were never identified.

Examination of the tubal ova of known or estimated age enabled us to arrange them in a series, representing typical stages of degeneration. (No sections were cut, thus no comment can be made on the state of the nucleus. It is also impossible at this stage to determine the moment of death of the ovum.) The morphological changes involved in degeneration do not all progress at the same rate nor does every ovum undergo all the selected, apparently typical stages. These changes involve the following:—

(*a*) *Deutoplasmic condensation* (Plate 1, E): The cytoplasm is condensed to a compact mass surrounded by a large perivitelline space filled with extruded fluid. This state may exist for about 25 days. One polar body in unfertilized ova and two or three in fertilized ova are normally present.

They may persist for some time or become degenerate even before, or at ovulation leaving some granular material.

(*b*) *Deutoplasmic extrusions*: These arise as protrusions and are eventually extruded into the perivitelline space (Plate 2, G, H & I) starting at about 20 days after ovulation (sometimes earlier). The extrusions become more numerous (up to 50 days +) and eventually break up leaving scattered granules. This is the beginning of the next stage.

(*c*) *Deutoplasmic fragmentation*: Initiated already in formation of extrusions it eventually results in a complete breaking up of the entire deutoplasm (Plate 2, J, K & L). It is complete around 60 days after ovulation. Discrepancies in the rate of breaking up occur as is seen in the ova depicted on Plate 2: the ovum in Fig. L (70 days) is younger than that in Fig. K (115 days) but more fragmented.

(*d*) *Comminution of the yolk granules* takes place very gradually. As soon as they are fine enough, they can be seen passing through the pores in the zona pellucida (Plate 3, N, Q & R). The yolk granules therefore gradually decrease in number as they become finer (Plate 2, K & L, Plate 3, M–R). This tendency eventually results in a vesicle surrounded only by the zona pellucida: it is termed a " zona vesicle ". (Plate 3, R).

It was observed that after a tubal sojourn of about 3 months the degenerate ova lose their globular shape and may become disc-shaped or collapsed with a tri-radiate form. When mounted, the latter collapsed form inbibe fluid and assumes a triangular shape (Plate 3, P).

In mounted ova it was seen that the dye could gain entrance into the ovum within about 20 minutes; thus relatively rapid passage through the zona pellucida is possible.

(D) *Early cleavage stages recovered*

During the initial part of the study of served mares, when we were as yet unaware of the lengthy tubal sojourn of equine ova in the tubes, only three early cleavage stages were actually found; two were apparently already undergoing degeneration (Fig. 2 & 8) while only the third (4625, Fig. 7) appeared healthy. No spermheads were encountered in the zona pellucida nor in the perivitelline space of any of these ova The details are recorded in Table 3.

TABLE 3.—*Examples of ova undergoing early cleavage*

Mare No.	Age	Cell-stage	Ages given by Hamilton & Day (1945)
4460	30 hr.	4-cell	30–36 hr.
4153	2 days	8–16 cell	not given
4625	5 days	32–64 cell	98 hr. (Morula)

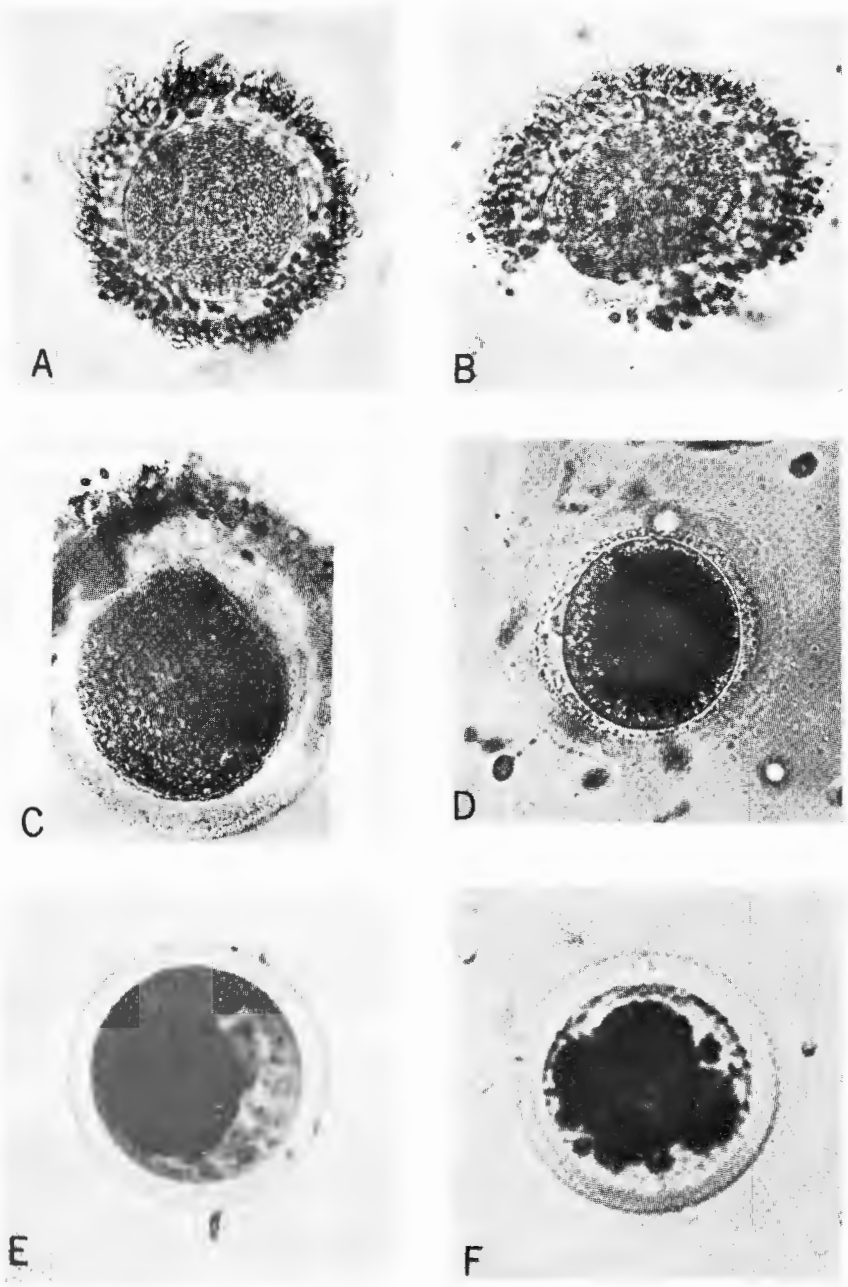

PLATE 1.—A. An almost mature ovum, complete with corona radiata, flushed from a follicle, 3 × 3 × 2½ cm. B. A disc-shaped ovum with incomplete corona radiata flushed from an atretic follicle, 2 × 1¾ cm. C. An ovum, with the first polar body being formed as a hillock with a basal cleavage furrow, flushed from a pre-ovulatory follicle, 5½ × 5⅝ × 3½ cm. The corona radiata was partly desquamated thereby revealing the porous surface of the zona pellucida. D. An ovum, 2 to 4 hr after ovulation revealing one polar body, a distinct vitelline membrane and a zona pellucida surrounded by a transparent jelly mass. E. Deutoplasmic condensation with a large perivitelline space as seen 24 hours after ovulation. F. A four-cell parthenogenetic cleavage stage with some small deutoplasmic extrusions

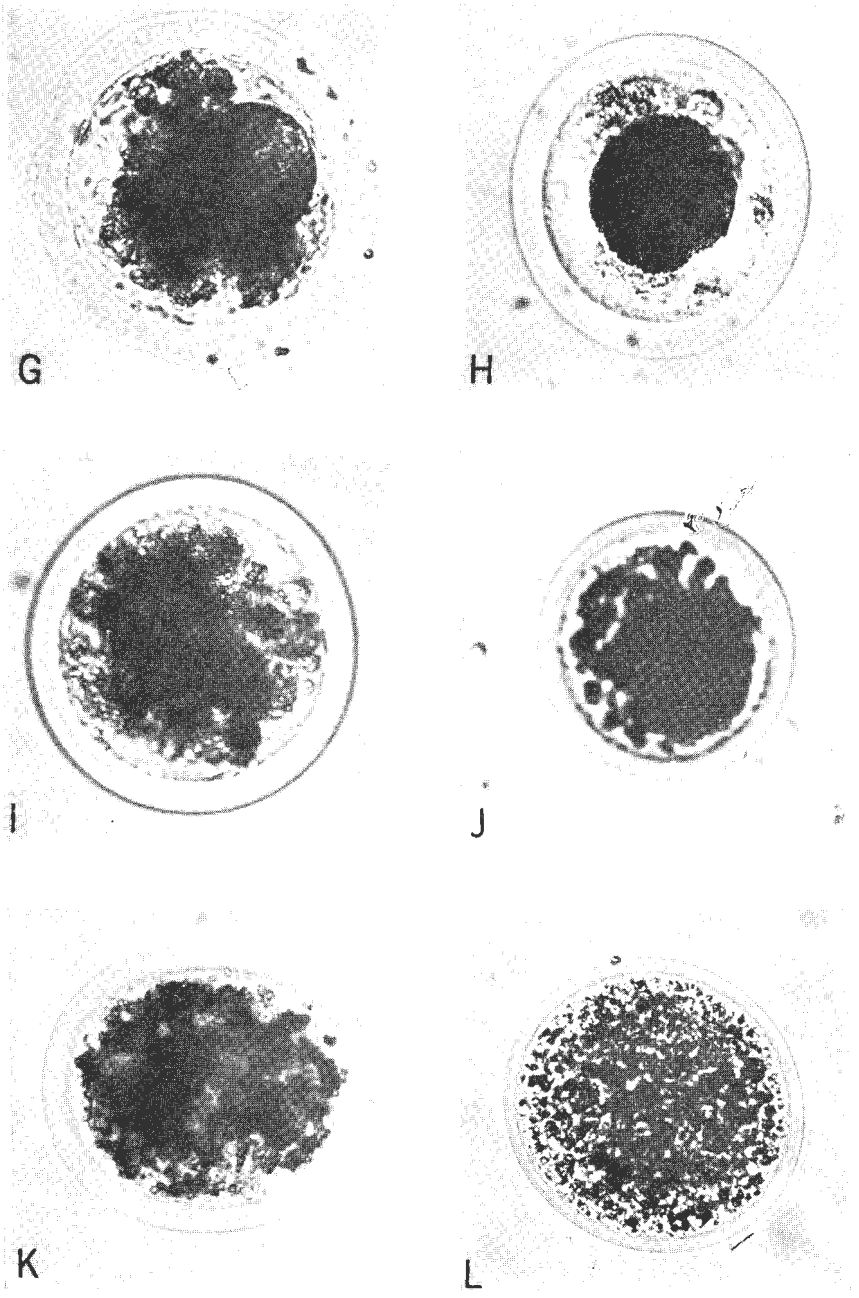

PLATE 2.—G. Six days after ovulation with extrusions well advanced. H. An eight day old, two-cell parthenogenetic cleavage stage with cleavage furrow only visible on careful focussing. Slight extrusions visible but deutoplasm condensation distinctly revealed. I. Fragmentation well advanced even in a 28-day old ovum. J. After 40 days fragmentation may not yet be complete. K. Even after 115 days fragmentation may not have advanced very much further than that seen at 40 days. L. Complete fragmentation with obliteration of the perivitelline space seen in this ovum already at 70 days

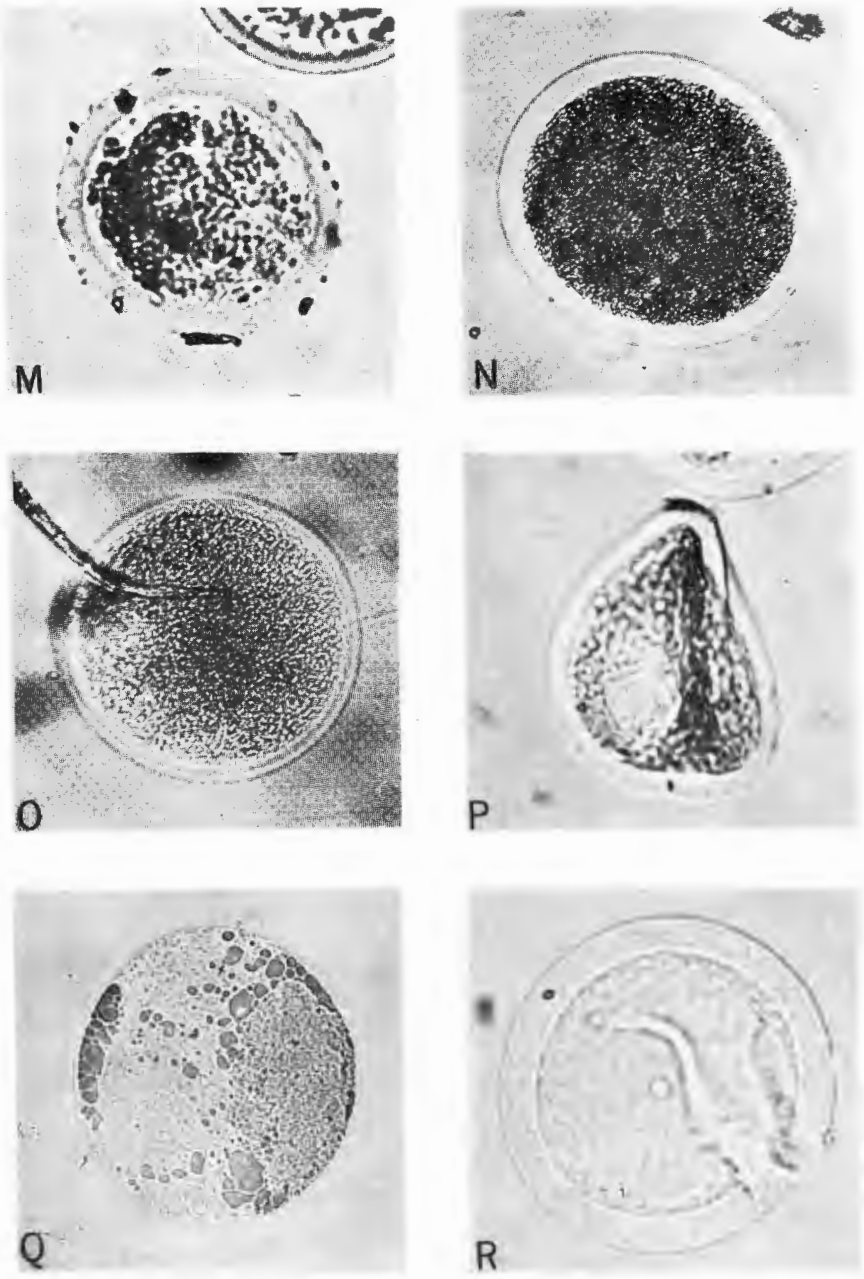

PLATE 3.—M. Comminution of granules and their extrusion through the porous zona pellucida has resulted in a diminished number of granules left after 105 days. N. Comminution well advanced without any apparent removal of fine granules—137 days old. O. Comminution practically complete with passage of finer granules through pores becoming apparent only after 150 days in this case. P. A typical tri-radiate, collapsed ovum of about 170 days with only a few granules left. Q. A disc-shaped ovum of about 180 days with advanced comminution of granules. R. A typical " zona vesicle " with the last of the yolk granules in a very fine state seen passing through the porous zona pellucida—about 210 days old. The zona pellucida ruptured on mounting

(E) *Parthenogenetic divisions*

Various examples of parthenogenetic cleavage, mostly in degenerate ova, were encountered (see Table 4). None of these ova had been fertilized.

TABLE 4.—*Examples of ova in parthenogenetic cleavage*

Fig. 12 (Fig. F. Plate 1)	24 hr old	4-cell stage
Fig. 18	8 days old	2-cell stage (Indistinct)
Fig. 12	20 days old	8-cell stage (Indistinct)
Fig. 30	22 days old	2-cell stage
Fig. 14	35 days old	4-cell stage
Fig. 4	37 days old	4-cell stage
Fig. 29	63 days old	2-cell stage?

It is possible that evidence of parthenogenetic cleavage may be erased by advanced degeneration. Without regard to this possibility, 9 per cent of all unfertilized ova had undergone early stages of parthenogenesis.

DISCUSSION

The persistence of ova in the uterine tubes, their parthenogenetic divisions and the encasing of recently ovulated ova in a gelatinous mass present features never considered before in the reproductive physiology of the mare. With the exception of parthenogenesis, these features represent a unique species difference and are best discussed under the following subdivisions:—

(*a*) *Sojourn of ova in the uterine tubes*

Although polyovulation and even poly-ovular follicles were initially considered as possible explanations for supernumerary tubal ova, the observations made are sufficiently convincing to conclude that normally during one heat period only ons follicle ovulates, that only one ovum is set free at a time and that only one corpue luteum and eventually one corpus albicans result thereform. This is in general agreement with the literature, in which the mare is always described as mon-ovular. Contrary to all former opinions, persistence of unfertilized ova in the uterine tubes in the mare is now an established fact.

The question arises: what causes fertilized ova to descend into the uterus while unfertilized ones remain in the tubes, and—with three exceptions [mare No. 2897 (Fig. 19 & 21) and mare No. 5501 (Fig. 29)]—always in the isthmus region? Muscular contraction, mainly in the isthmus, and ciliary action, mainly in the ampulla and infundibular regions, are the accepted mechanisms in ovum transport (Zuckerman, 1962, p. 477). Sufficient evidence, however, seems to be accumulating (Zuckerman, 1962, p. 478) that such transport is primarily affected by ovarian hormones, the exact nature of which may differ in various species.

Harper (1964), in an experimental study in the rabbit, used goldplated spheres to simulate eggs and came to the following conclusion:— " that the amount of oestrogen present is critical in ensuring rapid transport through the ampulla, but that a proper balance of oestrogen and progesterone must be maintained to ensure retention of spheres or eggs in the isthmus until correct time for entry into the uterus ". In the mare it must be different. Her mechanism for controlling the transport of ova must be selective in its effect as it ensures the transport of fertilized ova (maximum sojourn 4 to 6 days, Bone, *et al.*, 1963, p. 611) but causes retention of unfertilized

degenerate ova for varying periods up to at least $7\frac{1}{2}$ months (mares No. 5501 and 4124). At present we have no definite answer to this problem. One can only speculate upon the differential effects of fertilized and unfertilized ova upon ovarian hormone secretion. At one stage it was thought that the persistence of the gelatinous envelope might provide a mechanical advantage, but this idea had to be abandoned in the light of subsequent observations (see below).

(*b*) *Role of gelatinous masses in the uterine tube* (Fig. 27)

Initially, as has probably been the case in all previous investigations, these masses were considered as insignificant, condensed, tubal mucoid secretions or even desquamated cellular masses and no attention was paid to them until it was realized that at least some contain freshly ovulated ova. Hamilton & Day (1945) found that in the mare tubal eggs have " a thin coat of material resembling the albumin of the rabbit egg." Nothing similar was ever encountered in this study.

All available evidence points to these masses being formed in the follicle, probably by granulosa cells at or just before ovulation. In any case an infundibular origin for the masses does not appear logical because they are soon discarded in the ampulla. From the observations it is also concluded that the ovum loses its corona radiata cells before ovulation and becomes surrounded by the gelatinous mass at ovulation. Some time during the second day the ovum is separated from this mass probably due to condensation thereof.

It is extremely unfortunate that their real significance, namely that they can envelop recently ovulated ova (Fig. 19 & 20) only came to light at the end of this investigation. Such ova must have been missed and gaps have thereby been left which will have to be filled by further investigation.

The role, if any, which these sticky gelatinous masses may play in fertilization also needs some consideration. It may, for instance, act as a mechanism for attracting or accumulating sperm to ensure fertilization. Although no special attention was paid to them initially, they were never seen to contain any sperm as would be expected if they had any special attraction for sperm.

These masses could act as a mechanical barrier against the entrance of additional sperm similar to the albuminous layer of the rabbit ovum (Hartman, 1963, p. 289). In view of the postulated follicular origin of these masses, in contradistinction to the tubal origin of the albuminous layer of the rabbit ovum, deposited after fertilization, such a role as mechanical barrier is difficult to visualise. As the mare's ovum remains viable (i.e. " fertilizable ") only for two to four hours after ovulation (Nelsen, 1953, p. 83), the gelatinous mass must still surround the ovum during this time, apparently only being discarded on the second day. In this case it could only act as a mechanical block against sperm entry if fertilization took place before ovulation as happens in the tenrecs of Madagascar (Parkes, 1960, p. 318). Another alternative would be to regard the gelatinous mass as having a selective action, allowing entry of a single sperm and subsequently repelling all others, i.e. it must then undergo a change similar to the zona reaction (Parkes, 1960, p. 336).

A further possible function that can be ascribed to the gelatinous mass, due to its stickiness, is that of ensuring infundibular retention of the ovum after ovulation. Ciliary action would then effect its entrance into the uterine tube.

As stated the idea was entertained that the gelatinous mass was retained in the case of fertilized ova and hence could facilitate their selective transport to the uterus, but this had to be abandoned in view of subsequent experience.

Until further evidence accumulates as to whether the finding of a newly ovulated ovum in a gelatinous mass is a mere coincidence or an essential species phenomenon one may merely speculate upon its ultimate significance.

(*c*) *Fertilization of the ovum*

From our observations it is clear that the first meiotic division takes place before ovulation (Fig. 28) and that newly ovulated eggs therefore possess one polar body (Fig. 19 & 20). Sperm entry into the ovum must stimulate completion of the second meiotic division. Hamilton & Day (1945) differed from us in finding no polar bodies in recently ovulated ova. It may well be that, unaware of the phenomenon of persistence of tubal ova, these authors were dealing mainly with ova in early degenerative stages.

As no spermatozoa were ever encountered in the perivitelline space or zona pellucida in any of the ova studied, or were seen in any of the photographs published by Hamilton & Day (1945), it is uncertain whether the phenomena of vitelline block and/or zona reaction (Parkes, 1960, p. 336) play a role in the mare.

In practice conception is extremely rare if a mare is served after ovulation. Van Niekerk's (1965) observations on a large number of mares indicate that only a maximum of 5 per cent becomes pregnant under such conditions. The short viability of the ovum, as well as the question of capacitation of spermatozoa (Hartman, 1963, p. 289), are certainly some of the factors responsible. In most mammals the sperm must be present in the uterine tube for some hours before they are able to penetrate the zona pellucida. If service is allowed at ovulation or shortly after, the sperm may not be capacitated and will only be able to fertilize some hours later by which time parthenogenetic divisions or degeneration may have set in. At its best, delayed fertilization may occur and only in a small percentage of cases.

Blandau & Young (1939) found that in guinea pigs delayed fertilization produced lower conception rate and an increase in the number of abnormal pregnancies terminated by death of the embryo and abortion. Although no direct evidence for a similar state of affairs was found in the mare, some foetal resorptions could be the direct result of a delayed fertilization aggravated by nutritional deficiencies.

(*d*) *Degeneration of ova*

The lengthy sojourn of unfertilized ova in the uterine tubes of the mare enables one to study their degeneration, something never previously considered. It is not surprising that no literature (Parkes, 1960, p. 316) could be found on this aspect while many workers report that in the rabbit the ova soon pass into the uterus—a fact confirmed by us.

When morphological changes in the ova are correlated with ovulation dates, a general degenerative trend becomes apparent (see Plates 1 to 3). Although condensation of deutoplasm is one of the early phenomena encountered in tubal ova it is also considered as a normal process which is undergone by most fertilized mammalian ova (Balinsky, 1960), preparatory to the first cleavage. It is therefore difficult to determine the stage at which degeneration sets in. Various authors (Hamilton & Day, 1945; Starck, 1959, p. 42) state that some mammalian ova, especially those rich in lipoids normally give rise to protrusions of deutoplasm into the perivitelline space. These may scatter single yolk granules into the perivitelline space, to be taken up again later by the dividing cells. This phenomenon is termed deutoplasmolysis. Although deutoplasmolysis is considered a strictly normal process, we consider the term rather ill-chosen on semantic grounds, as there is no evidence of lysis of yolk granules. Deutoplasmic extrusion would have been a more appropriate and descrip-

tive term, and hence it has been used in this context throughout. In view of the confusion that may have occurred in distinguishing viable from degenerating ova, it is uncertain whether deutoplasmolysis, as it is commonly called, occurs as a normal process in equines.

It is assumed that the underlying cause of deutoplasmic fragmentation is cytoplasmolysis. Whether this is caused by the liberation of hydrolytic enzymes from lysosomes at death of the ovum is doubtful because lysosomes have been reported to be absent in the early cleavage stages (Dalcq *et al.*, 1963, p. 260) and therefore also in the ovum.

Comminution of deutoplasmic granules takes place possibly as a result of Brownian movement, which in all ova examined was exceptionally active. Again, actual lysis of the granules does not appear to take place. This view is supported by the observation that rabbit ova, left for almost two months in normal saline containing a bactericide (Seclomycin), underwent no autolytic effects except marked deutoplasmic condensation and in some, early deutoplasmic fragmentation. The perivitelline space in each was extensive. When left in saline alone they were attacked by bacteria and underwent lysis. In such instances the bacteria penetrated the pores of the zona pellucida and could be seen protruding from the surface like the spokes of a helm. It is also almost certain that very few, if any, cytolytic enzymes would be found within the naturally sterile lumen of the uterine tube. The ova also do not act as foreign bodies and therefore do not stimulate migration of phagocytes. It is therefore not surprising that degeneration of the ova in a sterile medium free from lytic enzymes takes place so gradually.

Attention must be drawn to three features when considering degeneration of ova in general. Firstly, there may be a distinct variation in size of yolk granules in ova of different individuals; secondly, the process of fragmentation and comminution of granules may proceed at different rates (Plate 2, K & L). This is seen particularly if all the present ova are arranged in a series of increasing age and not according to morphologically typical stages as illustrated (Plates 1 to 3). Thirdly, we have not lost sight of the fact that very rarely degenerate ova do slip through to the uterus. Although we have not examined the uterus for degenerate ova, Hamilton & Day (1945) report the finding in the uterus of one degenerate ovum in which the perivitelline space was already obliterated.

How long the final product of degeneration, viz. a " zona vesicle ", may persist in the uterine tube is not known. It clearly illustrates the efficiency of the zona pellucida as an ideal protective membrane for the ovum.

(*e*) *Parthenogenetic division of ova*

It has been established beyond doubt that parthenogenetic division definitely takes place in some unfertilized ova of mares. It is quite common in unfertilized ova of vertebrates, including mammals, but this is the first record of its occurrence in the horse. As early as in 1863 it was first commented on by Pflüger (cited by Bacsich & Wyburn, 1945). A substantial volume of literature has accumulated since then (Bacsich *et al.*, 1945). Kampmeier (1929), Long & Evans (1922) and Clark (1923) consider parthenogenetic cleavage stages " in the majority of cases but manifestation of degeneration and not homologous to segmentation in the fertilised egg " (cited by Bacsich, & Wyburn, 1945).

In the light of this investigation it appears as if some of the cleavage stages illustrated and sectioned by Hamilton & Day (1945) fit the description of degenerating and parthenogenetically dividing ova. A reinvestigation of early cleavage stages of fertilized ova is therefore indicated. This is all the more imperative in view of the fact that segmented ova have been regarded as definite proof of fertilization having taken place.

Conclusions

For the first time a lengthy sojourn for unfertilized ova in the uterine tubes has been established in the mare. They may persist for as long as $7\frac{1}{2}$ months in the uterine tube.

An ovum shortly after ovulation was found to be surrounded by a gelatinous mass of follicular origin. Its significance is not yet clear.

During their sojourn in the uterine tubes ova undergo various stages of degeneration characterized consecutively by deutoplasmic condensation, formation of deutoplasmic extrusions, fragmentation as a result of cytoplasmolysis and eventually comminution of yolk granules to a state where their escape from the ovum gives rise to fluidfilled " zona vesicles ". It has been generally accepted that the first two stages also occur in normal, fertilized ova. Whether this holds true for the mare's ova, is doubtful.

About 9 per cent of unfertilized ova are able to undergo parthenogenetic divisions, a feature severely complicating the identification of early healthy cleavage stages.

Due to limitations in the investigation the real significance of the gelatinous masses in fertilization was not established. These masses, whether they envelop ova or not are constantly being recovered, after flushing, from tubes containing ova.

Follicles were found to be mon-ovular. Polyovulation was excluded by regular rectal palpations and by noting the number of corpora lutea present. Supernumerary ova were always in different degenerative stages.

Ovulation was found to occur soon after the first meiotic division. A single polar body is therefore found in newly ovulated unfertilized eggs. Sperm entry stimulates formation of additional polar bodies as in most mammals.

Acknowledgements

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REFERENCES

AMOROSO, E. C., GRIFFITHS, W. B. & HAMILTON, W. J., 1939. A living tubal ovum of the horse. *Vet. Rec.*, 51, 168.

BACSICH, P. & WYBURN, G. M., 1945. Parthenogenesis of atretic ova in the rodent ovary. *J. Anat.* 79, 177–179.

BALINSKY, B. I., 1960. An Introduction to Embryology. Philadelphia: W. B. Saunders & Company.

LANDAU, R. J. & YOUNG, W. C., 1939. The effects of delayed fertilization on the development of the guinea pig ovum. *Amer. J. Anat.*, 64, 303–329.

BONE, J. F., CATCOTT, E. J., GABEL, A. A., JOHNSON, L. E. & RILEY, W. F. Jr., 1963. Equine Medicine and Surgery. California: *American Veterinary Publications*, inc.

DALCQ, A. M., *in* DE REUCK, A. V. S. & CAMERON, M. P., 1963. Lysosomes, Ciba Foundation Symposium. London: J. & A. Churchill, Ltd.

DANIEL, J. C., 1964. Some effects of steroids on cleavage of rabbit eggs in vitro. *Endocrinology*, 75, 706–710.

DAY, F. T., 1939. *J. Agric. Sci.*, 29, 459.

HAMILTON, W. J. & DAY, F. T., 1945. Cleavage stages of the ova of the horse, with notes on ovulation. *J. Anat.*, 79, 127–130.

HARPER, M. J. K., 1964. The effects of constant doses of oestrogen and progesterone on the transport of artificial eggs through the reproductive tract of ovariectomized rabbits. *J. Endocrinology*, 30, 1–19.

HARTMAN, C. G., 1963. Mechanisms concerned with conception. London: Pergamon Press.

LONG, J. A. & EVANS, H. M., 1922. The oestrous cycle in the rat and its associated phenomena. Memoirs Univ. California, 6, 1–111.

NELSEN, O. E., 1953. Comparative embryology of the Vertebrates. New York: The Blakiston Company.

PARKES, A. S., 1960. Marhall's Physiology of Reproduction. Vol. I: Part II. Longmans.

PARKES, A. S., 1952. Marshall's Physiology of Reproduction. Vol. II. London: Longmans, Green & Co.

STARCK, D., 1959. Ontogenie und Entwicklungsphysiologie der Säugetiere. Berlin: Walter de Gruyter & Co.

VAN NIEKERK, C. H., 1965. I. Early clinical diagnoses of Pregnancy in Mares. II. Early embryonic resorption in mares. *J. S. Afr. Vet. Med. Ass.*, 36, 53–69.

WITSCHI, EMIL, 1956. Development of Vertebrates. London: W. B. Saunders Company.

ZUCKERMAN, SIR SOLLY, 1962. The Ovary. Vol. I, New York: Academic Press.

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APPENDIX

Mare, 4619

Observation period: 1.2.64 to 25. 3. 64.

Slaughter date: 25.3.64.

Period, last ovulation to slaughter: 2 days.

Heat periods	Service dates	Ovulation dates		Pregnancy
		Left ovary	Right ovary	
1–5.2.64	4.2.64	5.2.64	Small and inactive	—
21–26.2.64	22.2.64	24.2.64		—
19–24.3.64	21.3.64	23.3.64		+?

Description of ova recovered:—

Left uterine tube.—One ovum, (Fig. 1) oval in shape, showed deutoplasmic fragmentation. It was considered bo be ± 2 months old as ova of 5.2.64 and 24.2.64 were most probably fertilized but early resorption must have occurred in the uterus as no pregnancy resulted. The newly ovulated ovum of 23.3.64 was not found.

Right uterine tube.—Not examined.

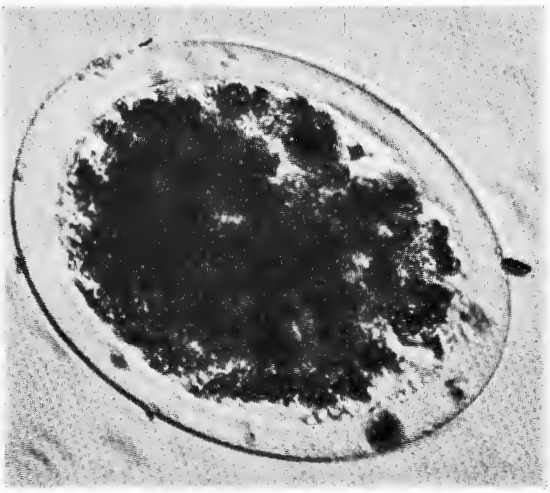

Fig. 1

Mare, 4153

Observation period: 1.2.64 to 27.3.64.

Slaughter date: 31.3.64.

Period, last ovulation to slaughter: 2 days.

Heat periods	Service dates	Ovulation dates		Pregnancy
		Left ovary	Right ovary	
16–20.2.64	—	20.2.64	Ovary small and inactive	—
8–11.3.64	—	9.3.64		—
26–30.3.64	27.3.64	29.3.64		+

Description of ova recovered:—

Left uterine tube.—Six ova recovered, one proximally and five distally.

1. The ovum recovered proximally (Fig. 2) was in a 8 to 16-cell stage and was unfortunately photographed after the zona pellucida had ruptured during mounting. No spermheads could be detected in the zona pellucida. The separate blastomeres were distinct. According to the ovulation date this cleavage stage is two days old—therefore younger than the five-cell stage (50 to 56 hr) of Hamilton & Day (1945). No gelatinous mass was found around it. Deutoplasmic extrusions were present. Although fertilized, it was considered to be in the initial stages of degeneration.

2. The ovum in Fig. 3 showed deutoplasmic condensation and extrusions into the perivitelline space. According to its stage of degeneration and ovulation date 9.3.64, it was 20 days old.

3. The ovum in Fig. 4 was 37 days old (ovulation date 20.2.64). It had evidently reached a four-cell parthenogenetic cleavage stage before cytoplasmolysis and deutoplasmic fragmentation set in. Some swelling occurred after mounting.

4. The ovum in Fig. 5 reveals a late stage of deutoplasmic fragmentation and comminution and therefore must be at least 5 to 6 months old.

5 & 6. These ova were not mounted.

Right uterine tube.—Not examined.

Mare, 4625

Observation period: 1.2.64 to 8.4.64.

Slaughter date: 8.4.64.

Period, last ovulation to slaughter: 5 days.

Heat periods	Service dates	Ovulation dates		Pregnancy
		Left ovary	Right ovary	
9–13.3.64	—	10.3.64	Ovary small and inactive	—
None during Feb.				
28.3–4.4.64	2.4.64	3.4.64		+

Description of ova recovered:—

Left uterine tube.—Two ova recovered.

1. A typical morula (Fig. 7), 5 days old and probably in the 32 to 64-cell stage. The zona pellucida had contracted slightly during mounting. This cleavage stage appeared quite healthy and there was no reason to suspect any degeneration. Three distinct polar bodies were present and therefore fertilization must have taken place. There was no indication of deutoplasmic extrusions or spermheads in the zona pellucida.

2. This ovum showed slight cytoplasmolysis, with yolk granules fairly large and the perivitelline space distinct. It was 29 days old (ovulation date 10.3.64). The zona pellucida contracted during mounting (Fig. 6).

Right uterine tube.—Not examined.

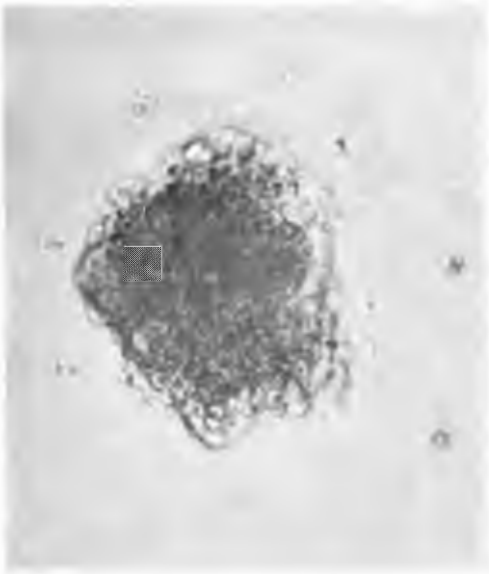

Fig. 2

Fig. 3

Fig. 4

Fig. 5

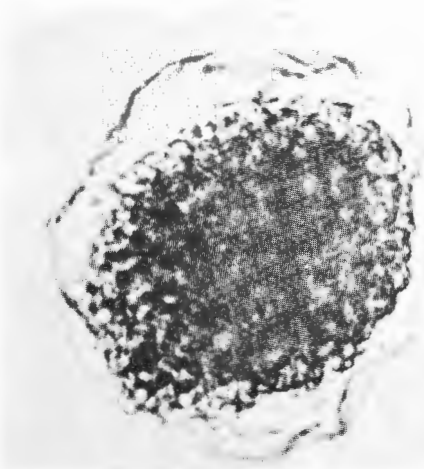

Fig. 6

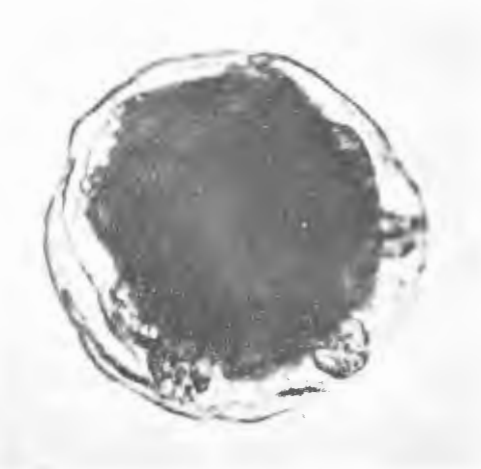

Fig. 7

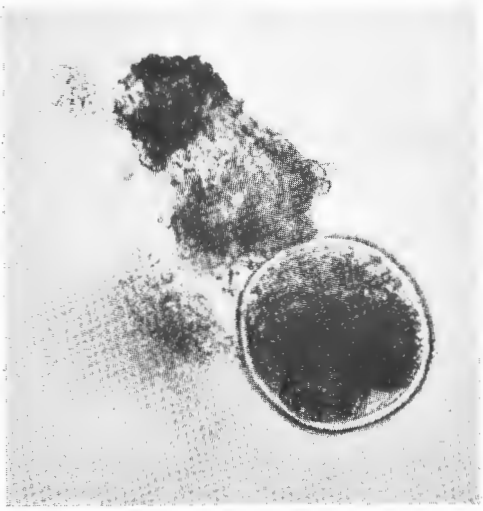

Fig.8

Mare, 4460

Observation period: 25.2.64 to 20.4.64.

Slaughter date: 20.4.64.

Period, last ovulation to slaughter: ± 30 hours.

Heat periods	Service dates	Ovulation dates		Pregnancy
		Left ovary	Right ovary	
9–21.3.64	—	—	—	—
12–20.4.64	19.4.64 (after ovulation)	—	19.4.64	+

Description of ova recorded:—

Left uterine tube.—Not examined.

Right uterine tube.—Two ova recovered.

1. In a four-cell stage (Fig.8) one cell of which showed deutoplasmic fragmentation. It is most likely that this ovum represented the newly ovulated one but was apparently undergoing degeneration already. Its zona pellucida was exceptionally thin with no spermheads present. It appeared to have just separated from its surrounding gelatinous mass. Due to service after ovulation it is most likely that this was a case of delayed fertilization resulting in degeneration (see " Discussion ").

2. This ovum was oval, with fine granules and no perivitelline space. According to ovulation date this ovum was at least six weeks old but according to advanced deutoplasmic fragmentation probably much older.

Mare, 5479

Observation period: 1.3.64 to 20.4.64.

Slaughter date: 20.4.64.

Period, last ovulation to slaughter: 5 days.

Heat periods	Service dates	Ovulation dates		Pregnancy
		Left ovary	Right ovary	
18–22.3.64	—	21.3.64	—	—
6–19.4.64	—	15.4.64	—	—

Description of ova recorded:—

Left uterine tube.—Two ova recovered, not mounted.

1. One was round, revealing distinct deutoplasmic condensation with a large perivitelline space. Deutoplasmic extrusions were hardly evident. This ovum was five days old without any signs of a surrounding gelatinous mass.

2. This ovum was oval, showed complete cytoplasmolysis and a perivitelline space was therefore absent. Fragmentation was fairly advanced and therefore it may have been ovulated prior to 21.3.64 at which date it would only have been 30 days.

Right uterine tube.—Not examined.

Mare, 5505

Observation period: 1.12.64 to 7.4.65.

Slaughter date: 7.4.65.

Period, last ovulation to slaughter: 30 days.

Heat periods	Service dates	Ovulation dates		Pregnancy
		Left ovary	Right ovary	
7–13.12.64	—	—	—	—
22.12–15.1.65	—	—	—	—
28.12–31.1.65	—	—	31.1.65	—
15–18.2.65	—	16.2.65	—	—
4–9.3.65	7.3.65	8.3.65	—	+ Embryo 30 days

Description of ova recorded:—

Left uterine tube.—One ovum found distally, 50 days old.

Right uterine tube.—One ovum found distally. It revealed advanced fragmentation and according to ovulation date (31.1.65) was at least 68 days old.

These ova were not immediately mounted nor photographed but left in formol-saline. When mounted a month later they were still completely intact.

Mare, 5510

Observation period: 1.12.64 to 14.4.65.

Slaughter date: 14.4.65.

Period, last ovulation to slaughter: 28 days.

Heat periods	Service dates	Ovulation dates		Pregnancy
		Left ovary	Right ovary	
7–23.12.64	—	19.12.64	—	—
30.12–9.1.65	—	6.1.65	—	—
18–22.1.65	21.1.65	—	22.1.65	+ Resorption of Embryo
12–18.3.65	16.3.65	—	17.3.65	+ Embryo 28 days

Description of ova recovered:—

Left uterine tube.—Four ova recovered distally.

1. Eight-cell parthenogenetic cleavage stage but partly fragmented; 99 days old according to ovulation date.

2. More advanced fragmentation, 117 days old according to ovulation date.

3. & 4. ± 137 and ± 157 days old according to ovulation dates. About half the granules of these two ova after staining with Sudan IV gave a positive reaction for lipoids.

Right uterine tube.—Three ova recovered distally. (Fig. 9, 10, 11).

1. The youngest (Fig. 9) according to the ovulation date was 137 days or older. Fragmentation was complete but granules still rather coarse.

2. The granules of this egg were all fine, evenly distributed with no perivitelline space. Age ± 150 days (Fig. 10).

3. The oldest (Fig. 11) was disc-shaped with fine granules and must have been about 150 + days old.

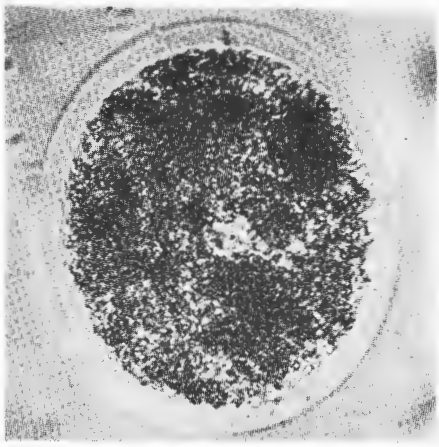

Fig. 9

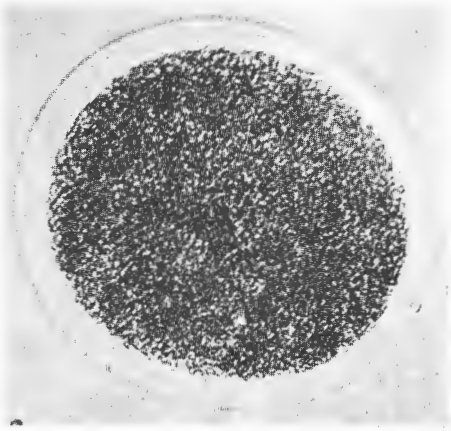

Fig. 10

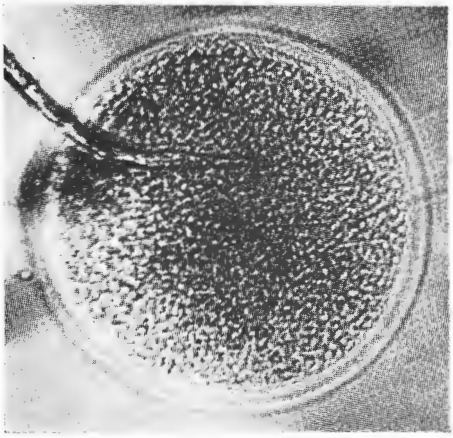

Fig. 11

Mare, 5506

Observation period: 1.2.65 to 1.4.65.

Slaughter date: 1.4.65.

Period, last ovulation to slaughter: 24 hours.

Heat periods	Service dates	Ovulation dates		Pregnancy
		Left ovary	Right ovary	
9–13.3.65	—	—	12.3.65	—
28.3–1.4.65	—	—	31.3.65	—

Description of ova recovered:—

Left uterine tube.—No ova recovered.

Right uterine tube.—Three ova recovered distally (Fig. 12).

1. Four-cell parthenogenetic cleavage stage with deutoplasmic extrusions—less than 24 hours oid.

2. Eight-cell parthenogenetic cleavage stage, was 20 days old (ovulation date 12.3.65) and revealed deutoplasmic extrusions.

3. This ovum was at least 40 days old and revealed considerable fragmentation.

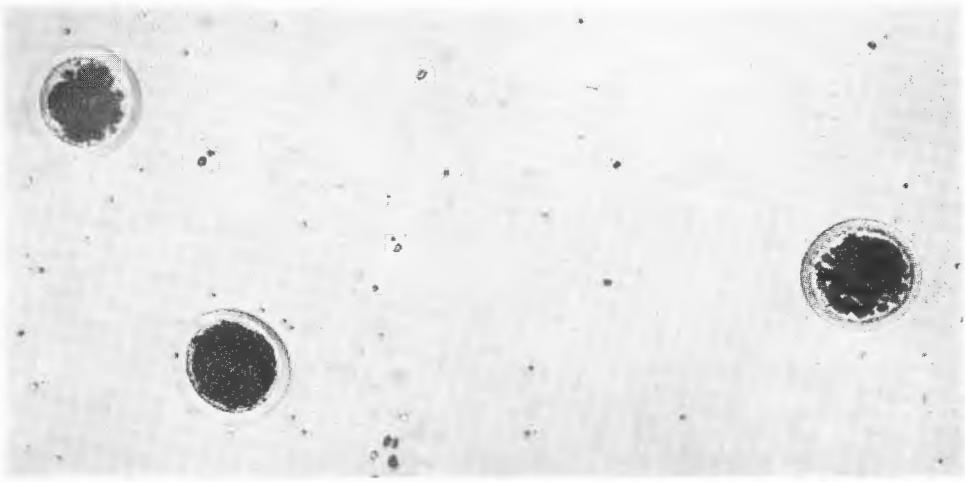

Fig. 12

Mare, 5511

Observation period: 18.1.65 to 7.4.65.

Slaughter date: 7.4.65.

Period, last ovulation to slaughter: 38 days.

Heat periods	Service dates	Ovulation dates		Pregnancy
		Left ovary	Right ovary	
28–30.1.65	—	—	30.1.65	—
26–28.2.65	27.2.65	—	28.2.65	+ Embryo 38 days

Description of ova recovered:—

Left uterine tube.—One ovum recovered distally.

Right uterine tube.—Six ova were found distally, all in various stages of degeneration. These were not photographed but left in formol-saline. A month later all were still intact but the zona pellucida had become so sticky that handling was impossible.

Mare, 5598

Observation period: 30.1.65 to 5.4.65.

Slaughter date: 5.4.65; Foaling date: 30.1.65.

Period, last ovulation to slaughter: 40 days.

Heat periods	Service dates	Ovulation dates		Pregnancy
		Left ovary	Right ovary	
8–11.2.65	—	—	10.2.65	—
21–24.2.65	22.2.65	23.2.65	—	+ Embryo resorbed

Description of ova recovered:—

Left uterine tube.—No ova recovered.

Right uterine tube.—One ovum, 54 days old. Two distinct and two indistinct cells could be discerned. It was considered to be in a four-cell parthenogenetic cleavage stage but already showing fragmentation.

Mare, 5751

Observation period: 16.2.65 to 9.4.65.

Slaughter date: 9.4.65.

Period, last ovulation to slaughter: 14 days.

Heat periods	Service dates	Ovulation dates		Pregnancy
		Left ovary	Right ovary	
1–6.3.65	—	—	5.3.65	—
23–27.3.65	—	26.3.65	—	—

Description of ova recovered:—

Left uterine tube.—Two ova recovered distally (Fig. 13).

1. Fourteen days old, revealed a distinct vitelline membrane, a large perivitelline space and early deutoplasmic extrusions.

2. Three to four months old, advanced fragmentation of deutoplasm.

Right uterine tube.—Four ova recovered distally.

1. Thirty-five days old (ovulation date 5.3.65), in a four-cell parthenogenetic cleavage stage (Fig. 14) revealing early fragmentation. A faint vitelline membrane was still visible on one side. These three ova 2, 3 and 4 (not photographed) showed various stages of more advanced fragmentation.

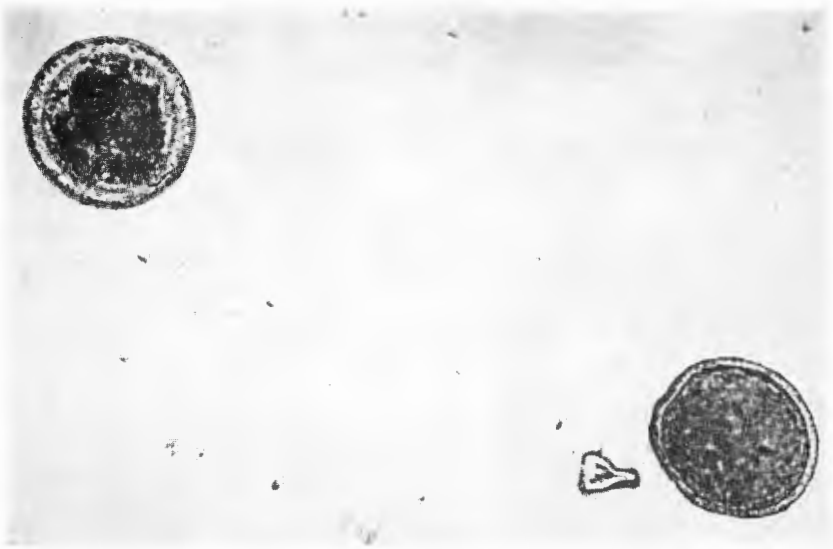

Fig. 13

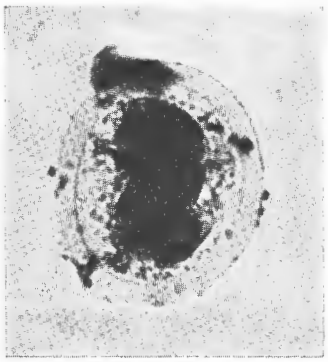

Fig. 14

Mare, 5748

Observation period: 16.2.65 to 2.4.65.
Slaughter date: 2.4.65.
Period, last ovulation to slaughter: 8 days.

Heat periods	Service dates	Ovulation dates		Pregnancy
		Left ovary	Right ovary	
26.2–7.3.65	—	6.3.65	—	—
23–26.3.65	—	—	25.3.65	—

Description of ova recovered:—

Left uterine tube.—Two ova recovered distally.

1. Twenty-seven days old (Fig. 15) with deutoplasmic extrusions.

2. Between 70 and 80 days old (Fig. 16), complete fragmentation of deutoplasm but comminution of granules had only commenced.

Right uterine tube.—Two ova recovered distally.

1. Eight days old, two-cell parthenogenetic cleavage stage, visible only on careful focussing and not in Fig. 17. Deutoplasmic condensation distinct while extrusions were commencing.

2. About 50 days old, fragmentation of deutoplasm (Fig. 18).

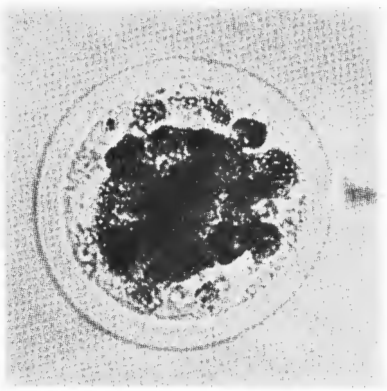

Fig. 15

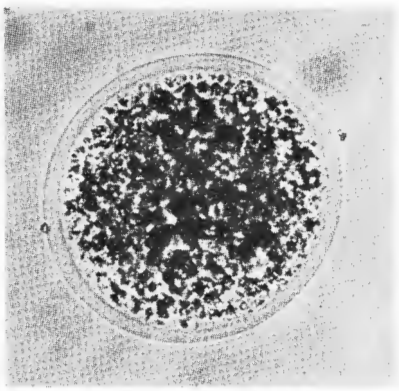

Fig. 16

Fig. 17

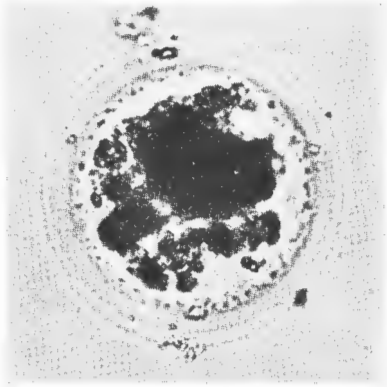

Fig. 18

Mare, 2897

Observation period: 1.12.64 to 27.4.65.

Slaughter date: 27.4.65.

Period, last ovulation to slaughter: 2–4 hours.

Heat periods	Service dates	Ovulation dates		Pregnancy
		Left ovary	Right ovary	
3–8.12.64	—	6.12.64	—	—
21–25.12.64	—	24.12.64	—	—
4–11.1.65	—	—	9.1.65	—
22–27.1.65	26.1.65	26.1.65	—	—
8–12.2.65	10.2.65	11.2.65	—	—
24–28.2.65	—	—	28.2.65	—
15–20.3.65	17.3.65	—	18.3.65	—
3–9.4.65	—	—	8.4.65	—
21–27.4.65	—	—	27.4.65	—

Description of ova recovered:—

Left uterine tube.—One ovum recovered distally. It had a tri-radiate collapsed shape with advanced comminution of granules (Fig. 22, right). The ova ovulated on 26.1.65 and 11.2.65 were probably fertilized but must have been resorbed in the uterus. The above ovum therefore had been ovulated on 24.12.64 or earlier, so its minimum age was at least four months.

Right uterine tube.—Two ova were recovered proximally and one distally.

1. This ovum (Fig. 19, 20) was embedded in a gelatinous mass which was completely transparent and was also found to be sticky and elastic (Fig. 20, phase-contrast). It was separated from the original larger mass (resembling that seen in Fig. 27 but less condensed) by using two dissecting needles. This larger mass contained specks of blood and some cells amongst which corona radiata cells were identified. A first cleavage furrow could be discerned only on careful focussing. Its most characteristic features, however, were the presence of a very distinct polar body and a very distinct vitelline membrane with only a small perivitelline space. The fine granules seen on the inside of the zona pellucida were caused either by the cytoplasmic projections of corona radiata cells or by the pores in the zona pellucida. This ovum was 2 to 4 hours old.

2. The other ovum found proximally (Fig. 21) showed a shrunken cytoplasm with a thinner vitelline membrane which was absent in some places. The perivitelline space was slightly larger than that of the previous ovum but still presented a fine granulation. This ovum, before the discovery of the previous one, was considered to be the newly ovulated one but according to the ovulation date it was 19 days old (ovulation date 8.4.65)

3. The ovum found distally (Fig. 22, left) presented advanced fragmentation with comminution of granules just commencing. Its age (ovulation date 28.2.65) was judged to be at least 58 days as the ovum ovulated on 18.3.65 was probably fertilized and resorbed in the uterus.

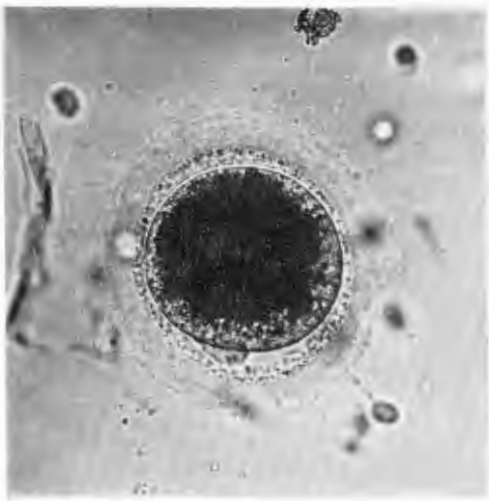

Fig. 19

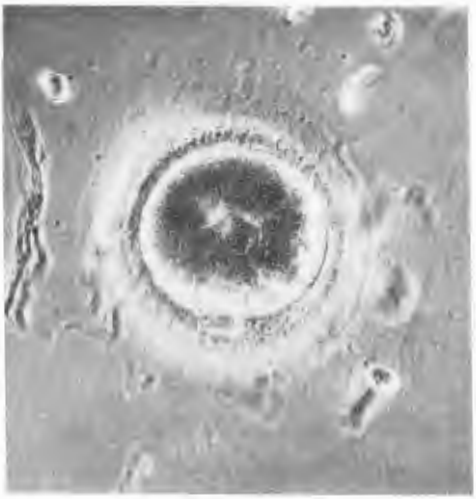

Fig. 20

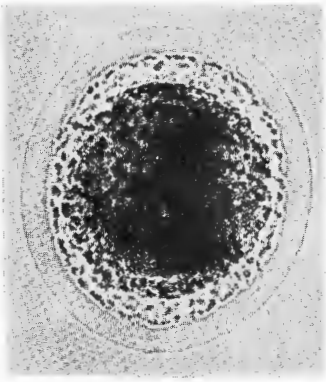

Fig. 21

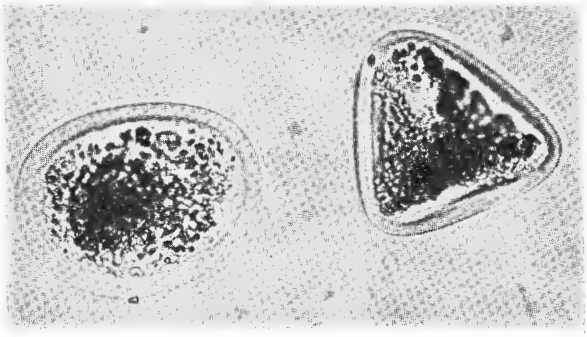

Fig. 22

Observation period: 25.3.65 to 29.4.65.

Slaughter date: 29.4.65; Foaling date: 23.1.64.

Period, last ovulation to slaughter: 21 days.

Heat periods	Service dates	Ovulation dates		Pregnancy
		Left ovary	Right ovary	
1–9.4.65	7.4.65	—	8.4.65	+ Embryo 21 days

Description of ova recovered:—

Left uterine tube.—No ova recovered.

Right uterine tube.—One ovum recovered distally. Its granules were fine with no perivitelline space and a thin zona pellucida. It was considered to be at least ± three months old.

Follicle 3 × 3 × 2·5 cm—1 ovum (Fig. 23).

Follicle 2 × 1·75 cm — 1 ovum.

Atretic follicle 2 × 1·75 cm — 1 ovum, corona radiata incomplete and ovum was disc-shaped and not globular as usual (Fig. 24).

Mare, 5502

Observation period: 1.12.64 to 28.4.65.

Slaughter date: 28.4.65.

Period, last ovulation to slaughter: 17 days.

Heat periods	Service dates	Ovulation dates		Pregnancy
		Left ovary	Right ovary	
12–21.12.64	—	20.12.64	—	—
2–15.1.65	—	—	13.1.65	—
22–26.1.65	—	—	24.1.65	—
18–22.2.65	—	23.2.65	—	—
12–16.3.65	—	15.3.65	—	—
1–12.4.65	10.4.65	11.4.65	—	+ Embryo 17 days

Description of ova recovered:—

Left uterine tube.—Four ova recovered distally (Fig. 25). Their ages according to ovulation dates were 44 days (15.3.65), 63 days (25.2.65), 150 days (20.12.64) and 170 days at least. Three were disc-shaped while one was tri-radiate and collapsed. The size of the granules simplified arrangement in this order.

Right uterine tube.—Four ova were recovered distally (Fig. 26). Two were globular, one was disc-shaped and one tri-radiate and collapsed. Their ages according to ovulation dates were 90 days (24.1.65) and 105 days (13.1.65) while the remaining two were older than 150 days. Again the the size of the granules simplified arrangement in a series according to the stage of degeneration.

In a large pre-ovulatory follicle, 5·5 × 3·8 × 3·5 cm, a single ovum in the early stage of forming the first polar body was found. The polar body is shown distinctly as a hillock with a basal cleavage furrow (Fig. 28). A partly desquamated corona radiata, probably due to the saline flushing of the follicle to obtain the ovum, was present.

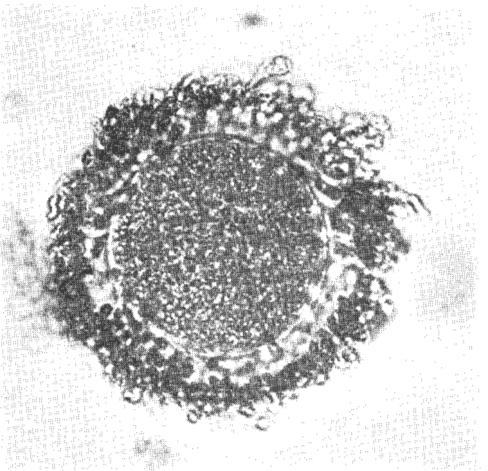

Fig. 23

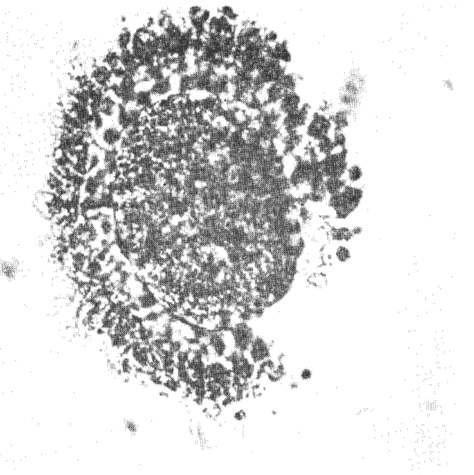

Fig. 24

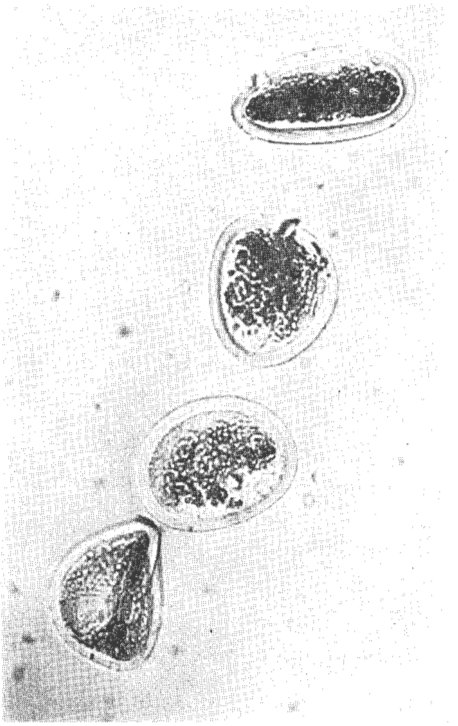

Fig. 25

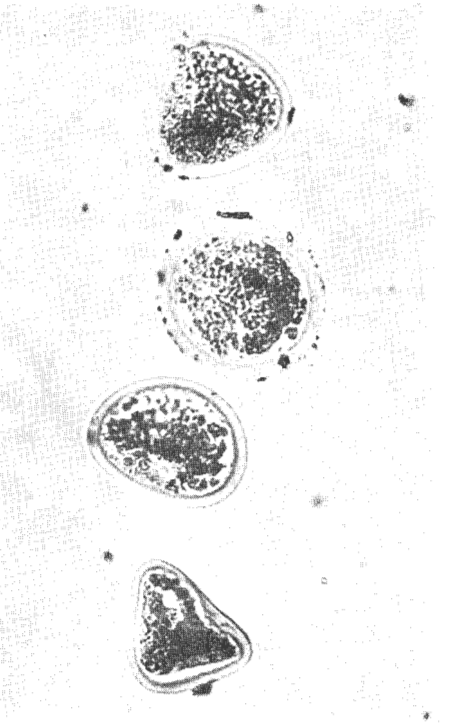

Fig. 26

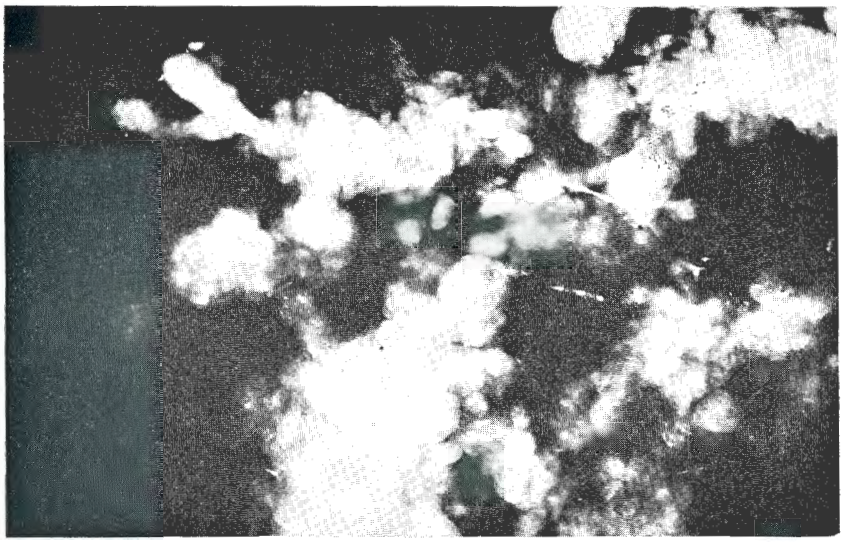

Fig. 27

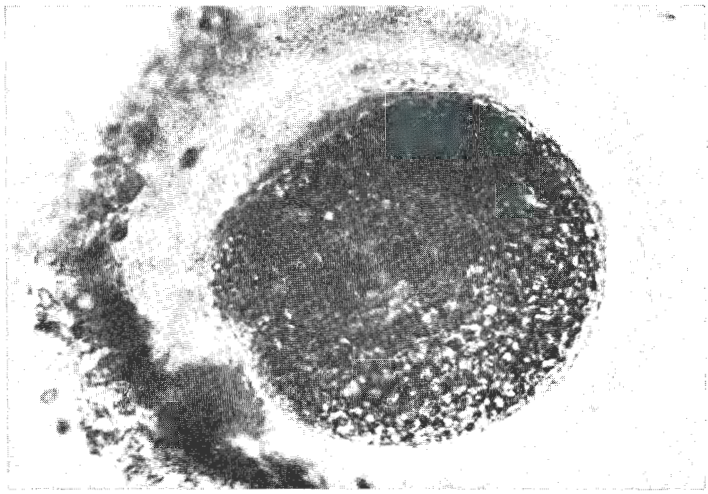

Fig. 28

7219177–15

Mare, 5501

Observation period: 1.12.64 to 23.4.65.

Slaughter date: 23.4.65.

Period, last ovulation to slaughter: 1½ days after first and one day after second ovulation.

Heat periods	Service dates	Ovulation dates		Pregnancy
		Left ovary	Right ovary	
	—	—	15.12.64	—
	—	6.1.65	—	—
	—	—	19.2.65	—
	—	—	8.3.65	—
	—	1.4.65	—	—
	—	21.4.65	—	—
	—	—	22.4.65	—

This mare received 4,000 IU of " Lutormone " intravenously on the morning of 21.4.65.

Description of ova recovered:—

Left uterine tube.—Four ova (Fig. 30) recovered distally.

1. The youngest ovum was 1½ days old (ovulation date 21.4.65), revealed a condensed cytoplasm with a single polar body and distinct vitelline membrane. Some slight deutoplasmic extrusions were seen.

2. The two-cell parthenogenetic stage was 22 days old (ovulation 1.4.65) and presented more deutoplasmic extrusions.

3. & 4. According to ovulation dates these two were 107 days and 147 days at least. The one with the thinner zona pellucida and finer granulation was the older.

Right uterine tube.—Six ova (Fig. 29) were recovered, only one of which was found proximally.

1. The ovum found proximally had no gelatinous mass around it, showed quite a number of deutoplasmic protrusions and had a condensed cytoplasm with a very distinct vitelline membrane (not distinct in photograph due to dark staining). It was 24 hours old.

2. Forty-six days old (ovulated 8.3.65) with condensed cytoplasm and a few deutoplasm extrusions.

3. Sixty-three days old (ovulated 19.2.65). It showed a few large yolk masses.

4. One hundred and twenty-nine days old (ovulated 15.12.65), with granular masses distinct.

5. Collapsed, fine granules and at least five months old.

6. Visible as a clear vesicle surrounded by the zona pellucida—zona vesicle. The dark cells seen in the photograph are ciliated cells from the uterine tube which adhered to the surface of the zona pellucida and could not be removed once it was mounted.

If three weeks are taken as the average interovulatory period, the oldest of these ten ova should be at least 7½ months.

These ten ova were slightly stained with cresylecht violet before photography and therefore appear slightly darker than normal.

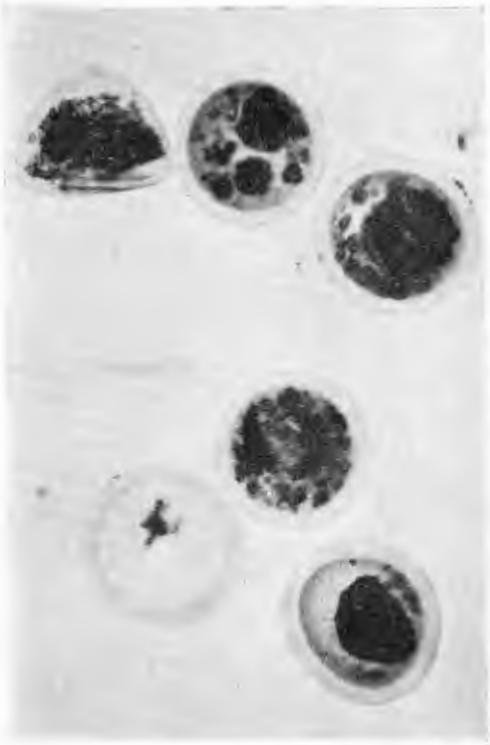

Fig. 29

Fig. 30

Mare, 5708

Observation period: 31.1.65 to 11.5.65.

Slaughter date: 11.5.65.

Period, last ovulation to slaughter: ± 4 months.

Heat periods	Service dates	Ovulation dates		Pregnancy
		Left ovary	Right ovary	
4–11.1.65	—	—	—	—
18–20.1.65	—	—	—	—
Anoestrus—				

Description of ova recovered:—

Left uterine tube.—No ova recovered.

Right uterine tube.—Two ova recovered (Fig. 31), both tri-radiate collapsed, finely granulated and no perivitelline space. According to ovulation dates one ovum was four months and the other 3½ months old.

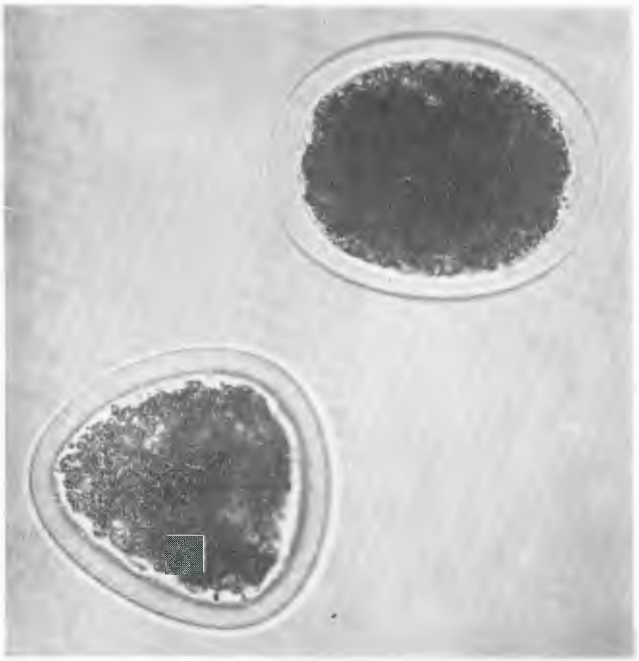

Fig. 31

Mare, 4124

Observation period: 2.1.65 to 21.5.65.

Slaughter date: 21.5.65.

Period last ovulation to slaughter: 7 and 6 days.

Heat periods	Service dates	Ovulation dates		Pregnancy
		Left ovary	Right ovary	
6–9.1.65	—	not examined		—
28.1–2.2.65	—	not examined		—
29.3–2.4.65	—	—	1.4.65	—
19–25.4.65	—	—	24.4.65	—
12–16.5.65	—	14.5.65	15.5.65	—

Description of ova recovered:—

Left uterine tube.—Two ova recovered.

1. Seven days old, ovulation date 14.5.65, cytoplasm was very granular and filled whole cavity, granules fairly large. Appeared too old for seven days (Fig. 32).

2. Typical zona vesicle, no granules were visible, only very fine ones in zona pellucida (Fig. 33).

Right uterine tube.—Two ova recovered (Fig. 34).

1. One was six days old with condensed cytoplasm, slight deutoplasmic protrusions, a distinct vitelline membrane and large perivitelline space (Fig. 34, right).

2. The other was 28 days old, fragmentation well advanced and a perivitelline space still present.

Mare, 5720

Observation period: 1.3.65 to 24.5.65.

Slaughter date: 24.5.65.

Period, last ovulation to slaughter: over 84 days.

Age of mare: 18 months old.

Heat periods.—Nil during observation period.

Ovulation.—If age is taken into consideration only one heat period was possible. Only one corpus albicans was found in right ovary and only one ovum in right uterine tube (Fig. 35). It was over 84 days old, triangular and revealed complete fragmentation of its cytoplasm; condensed gelatinous masses were present in right tube.

Left uterine tube.—No ova, no corpora albicanti nor any gelatinous masses.

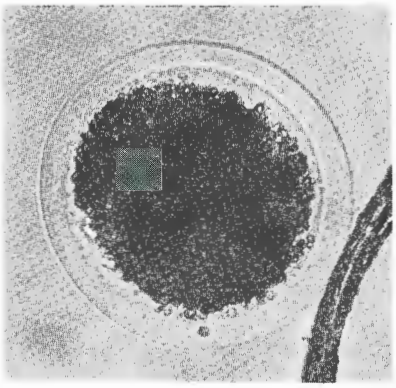

Fig. 32

Fig. 33

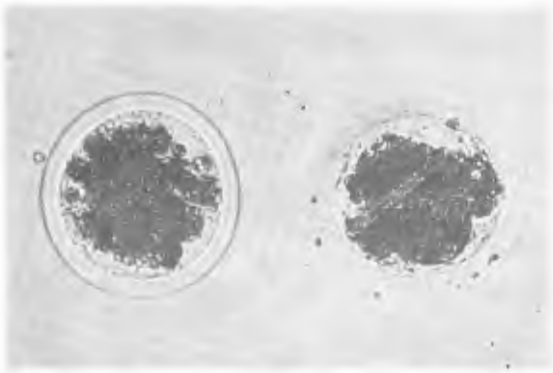

Fig. 34

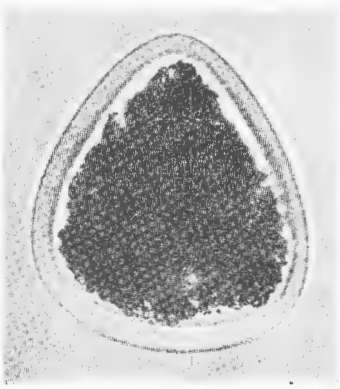

Fig. 35

7219177–16

Fig. 36

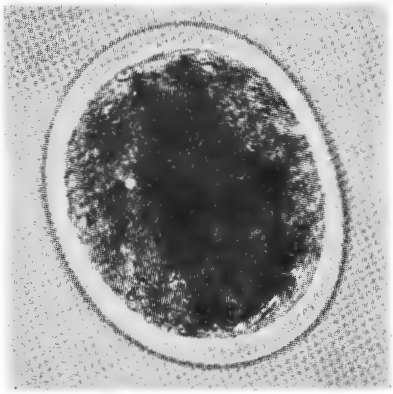

Fig. 37

Fig. 38

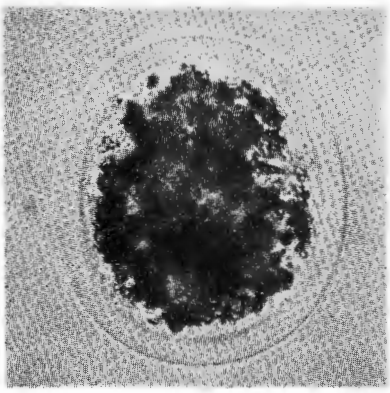

Fig. 39

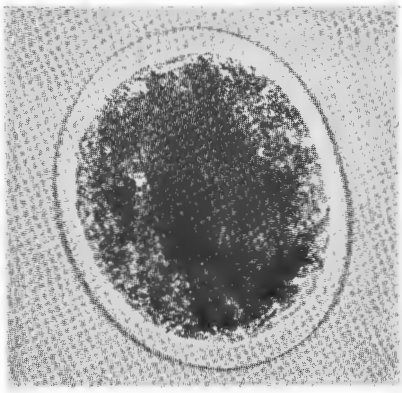

Fig. 40

Mare, 4410

Observation period: 2.1.65 to 24.5.65.

Slaughter date: 24.5.65.

Period, last ovulation to slaughter: 71 days.

Heat periods	Service dates	Ovulation dates		Pregnancy
		Left ovary	Right ovary	
5–11.1.65	—	not examined		—
25–31.1.65	—	not examined		—
15–19.2.65	16.2.65	18.2.65	inactive	—
11–15.3.65	—	14.3.65	inactive	—
Anoestrus				

Description of ova recovered:—

Left uterine tube.—Four ova recovered distally—only four corpora albicanti in left ovary.

1. According to ovulation date 71 days old, oval, granules still fine and evenly distributed, filling whole cavity.

2. According to ovulation date 95 days old, oval, granules tend to be clumped, some finer than others and tend to be disappearing from cavity by migration of finer granules through zona pellucida Fig. 36).

3. Oval, at least 115 days, probably older depending on whether ovulation occurred before 18.2.65 and on which side. Granules clumped, scattered and when compressed fill about half the cavity (Fig. 37).

4. Triangular collapsed, granules heaped on one side, some very fine and leaving cavity by migration through zona pellucida; 135 + days old (Fig. 38).

Right uterine tube.—Two ova recovered—only two corpora albicanti found in right ovary.

1. This ovum very much resembled 2 above. It was at least 115 days or older (Fig. 39).

2. The other ovum had granules slightly clumped, some were finer than others. It resembled 3 above but was at least 135 days old (Fig. 40).