THE FATE OF FERTILIZED EGGS TRANSFERRED TO THE UTERUS OR OVIDUCT DURING ADVANCING PSEUDOPREGNANCY IN THE RABBIT

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Summary. Morulae recovered from superovulated donors 60 hr *post coitum* were transferred to the uteri of recipients ('temporary' recipients) on Day 3, 5, 7, 9 or 11 of pseudopregnancy. The eggs were again recovered either 6 hr or 24 hr after transfer and, following microscopic examination, re-transferred to the uteri of synchronous recipients in order to test viability.

The proportion of eggs recovered from the temporary recipients, 73 to 97%, was little affected by the stage of pseudopregnancy, except for a slight decline on Day 11 compared with earlier stages. However, the reaction of the eggs, as judged after 24 hr, was markedly affected, varying from continued development (up to the early blastocyst stage) in the Day-3 or Day-5 uteri to degeneration in the most asynchronous uteri (Day 9 and Day 11).

The appearance of the eggs was strongly correlated with their ability to survive following re-transfer; practically no eggs (3 out of 113) exposed for 24 hr to the Day-9 or Day-11 uteri survived to term. The associated low rates of pregnancy (failure of implantation) indicated that the eggs perished early (already dead at transfer, or dying shortly afterwards), which was confirmed by autopsy 3 or 4 days after transfer.

The injurious effect of the progestational uterus on the morula appeared to develop gradually; it was detectable in some does on the 7th day and expressed itself fully by the 9th to 11th days of pseudopregnancy. No specifically harmful factor or deficiency has been identified in such uteri in which stages earlier than the morula are also affected.

Morulae transferred to the oviduct on the 11th day of pseudopregnancy continued to develop with the majority reaching the early blastocyst stage. After 24 or 48 hr under such conditions, 56% and 27% proved fully viable when re-transferred.

INTRODUCTION

Chang (1950), using rabbits, demonstrated by means of egg transfer that to establish pregnancy there is a need for synchrony between the egg and its environment, as related to the age of the corpora lutea. Thus, pregnancy only

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succeeded when the stage of egg or blastocyst development was not more than 2 days out of phase with the recipient's luteal stage. Subsequently, similar conditions have been found to apply in every other species so far investigated, including the mouse (McLaren & Michie, 1956; Doyle, Gates & Noyes, 1963), the rat (Noyes & Dickmann, 1960), sheep (Moore & Shelton, 1964a; Rowson & Moor, 1966), cow (Rowson, Moor & Lawson, 1969), ferret (Chang, 1969) and pig (Webel, Peters & Anderson, 1970). In qualification, it may be noted that, with few exceptions, research into synchronization requirements has been concentrated upon establishing the optimum 'donor-recipient' combinations, and degrees of asynchrony which might be expected to be incompatible with success have generally been avoided.

Comparatively little is known, therefore, of the fate of eggs transferred asynchronously, except for certain observations on the rabbit (Chang, 1950; Adams, 1965; 1968), rat (Dickmann & Noyes, 1960), mouse (Doyle *et al.*, 1963) and ferret (Chang, 1969). Observations on the rabbit and ferret reveal that eggs transferred asynchronously may develop for varying lengths of time, sometimes beyond implantation, but invariably fail before reaching foetal stages. Species variation exists concerning the reaction of the 'younger egg-older uterus' combination, i.e. recipient's luteal stage in advance of that of the donor. For example, in the ferret in the 4→8 and 8→13 day combinations, some eggs survived to implantation (Chang, 1969) whereas, in the rabbit, only 5% of 2½-day morulae could develop into blastocysts following transfer to the 4½-day uterus (Adams, 1968). Differences in the response of rat and mouse eggs to a 'more advanced' endometrium have been referred to by Doyle *et al.* (1963). The association of particular 'egg-endometrium' combinations may also be critical. For example, in the rat, Dickmann & Noyes (1960) observed that some 4-day eggs transferred to the Day-5 uterus, which is near the normal time of implantation, showed signs of degeneration after only 9 hr, which became marked after 12 hr. By re-transferring such eggs to a favourable environment, they established that survival was impaired, only 27% of eggs incubated for 11 to 12 hr surviving re-transfer compared with 58% of those incubated for 4 hr. Recently, Adams (1970a) reported that 60-hr rabbit morulae underwent degenerative changes within 6 to 24 hr following transfer to the uterus on the 9th day of pseudopregnancy. However, preliminary observations on the survival of the eggs indicated that more than 6-hr exposure was required to produce an irreversibly damaging effect.

The present work was designed to extend these observations by evaluating the survival of 60-hr morulae after short-term exposure to uteri during early (synchronous) to advanced (asynchronous) pseudopregnancy. The results show clearly that less than 24 hr exposure of the fertilized egg to the advanced progestational uterus is incompatible with its survival.

Additionally, certain questions raised during the course of the work, e.g. the egg's fate in the oviduct during pseudopregnancy, have also been investigated.

MATERIAL AND METHODS

A total of 187 sexually mature rabbits, 'Strain A' (Albino) or Dutch stock from our own colony, were used; forty-two (Strain A) to provide eggs, 144 to act

as recipients, including forty (Strain A) in a temporary capacity, and one (Strain A) to provide endometrial tissue and uterine flushings. Recent data on live weight and ovulation rate of our Strain A and Dutch stock are available (Adams, 1970b).

Donors

Superovulation was induced by treatment with a horse anterior pituitary preparation, prepared according to the method described by Moore & Shelton (1964b) and given subcutaneously twice daily for 3 days (total dose 12 mg) as described by Pincus (1940). Twelve hours after the final priming injection, the does were mated with two fertile males and given 25 i.u. HCG (Lutormone, Burroughs Wellcome). Egg recovery was carried out either 30 hr (three does) or 60 hr later by flushing each oviduct with 2 ml 0.9% NaCl solution either at autopsy (thirty does), or *in vivo* (twelve does) as described earlier (Adams, 1953). The eggs were then examined under a zoom binocular microscope ($\times 20$ to $\times 80$), and their stage of development was recorded before rejecting any that were considered abnormal.

Recipients: egg transfer, recovery and re-transfer

Thirty does were mated with a vasectomized male to induce ovulation and pseudopregnancy. On the 3rd, 5th, 7th, 9th or 11th day after mating, ten to twenty freshly recovered morulae were transferred to each uterine horn, as described earlier (Adams, 1962). Either 6 or 24 hr later, the does were killed by the rapid intravenous injection of 1.5 ml Expiral (Pentobarbitone sodium, 20 mg/ml; Abbott Laboratories Ltd). The jugular veins were severed for the purpose of exsanguination. After about 5 min, the whole genital tract was removed and placed on filter paper, which facilitates dissection. The uterine horns were divided near the cervixes and separated from the oviducts, before trimming away the fat and connective tissue along the mesometrial border. Finally, a slit about 1.5 cm long was made through the cervix in order to prevent curling of the horns which otherwise occurs when pressure is applied during flushing. Each horn was flushed with 10 ml physiological saline introduced through a No. 15 hypodermic needle placed in the lumen near the utero-tubal junction. Whenever fewer eggs were recovered than had been transferred, flushing was repeated. The eggs were counted and separated from the native one-cell eggs, and their condition was noted. A small volume of homologous plasma was added to protect the eggs pending re-transfer to the uteri of eighty-five synchronous, pseudopregnant recipients, in which ovulation had been induced by mating with a vasectomized male approximately 72 hr previously. Each recipient received a total of five to eight eggs. After 7 or 8 days, the recipients were palpated in order to determine whether they were pregnant. Litter size was recorded at term.

In order to obtain information on the immediate fate of the re-transferred eggs, a supplementary experiment was performed. Morulae were transferred to the uterus of a recipient on the 10th day of pseudopregnancy, recovered after 24 hr, and re-transferred to three recipients, which were killed 3 or 4 days later. The uteri were prepared and flushed, as already described.

Transfer of uterine flushings or endometrial tissue to the uterus during early pregnancy

In the preceding experiment, the medium in which the morulae were re-transferred was to a small extent contaminated with fluid and/or tissues from the uterus of the temporary recipient. It was, therefore, necessary to determine whether such contamination might itself exert a harmful effect, either directly or indirectly, on the early embryo. This was tested by depositing, as in egg transfer, either endometrial tissue or uterine flushings, obtained on the 11th day of pseudopregnancy, into the uterine lumen of four does which had been mated with fertile males 72 hr previously.

Transfer of two- to four-cell eggs to the advanced progestational uterus

Eggs at the two- to four-cell stage were recovered from three superovulated donors 30 hr *post coitum* (*p.c.*) and transferred to the uterine horns of three recipients on the 9th (one doe) or 11th days of pseudopregnancy. Autopsy was performed either 24 hr (two does) or 48 hr later to permit examination of the eggs.

The fate of 'alien' 60-hr morulae and native one-cell eggs in the progestational uterus and oviduct

In this experiment, one oviduct was ligated near the utero-tubal junction 18 hr after the intravenous injection of 25 i.u. HCG. Subsequently, on the 9th day of pseudopregnancy, 60-hr morulae were transferred to the uterine horn ipsilateral to the ligated oviduct and to the contralateral oviduct which was then also ligated. The recipients were killed 2 or 3 days later to permit examination of the transferred and native eggs.

Transfer of 60-hr morulae to the oviduct on the 11th day of pseudopregnancy

Morulae recovered 60 hr *p.c.* were transferred to the oviducts of four does on the 11th day of pseudopregnancy. The oviducts were ligated near the utero-tubal junction to prevent the eggs entering the uterus. Either 24 hr or 48 hr after transfer, the oviducts were excised at autopsy and flushed with 2 ml 0.9% NaCl solution. After microscopic examination, the eggs were re-transferred to twelve recipients, which had been rendered pseudopregnant by mating with a vasectomized male 3 or 4 days previously.

RESULTS

Donors

A total of 1552 eggs (mean 36.9 ± 2.7 , range 8 to 94) was recovered, including 1386 eggs 60 hr *p.c.* and 166 eggs 30 hr *p.c.* In all but five does, in which recovery was poor, there was good agreement between the estimated number of ovulations (exact counts are impossible when superovulation is intense) and eggs recovered. Seventy-eight eggs (5.0%) were unfertilized, three appeared abnormal and four others showed retarded cleavage. Of the eggs recovered 60 hr *p.c.*, all but fifty-two (3.75%) had developed normally into morulae.

Recovery of eggs transferred to temporary recipients

Details of the total numbers of eggs transferred and recovered relative to the

TABLE 1
THE RECOVERY OF FERTILIZED EGGS EITHER 6 OR 24 HR AFTER TRANSFER TO THE UTERI OF
'TEMPORARY' RECIPIENTS 3 TO 11 DAYS PSEUDOPREGNANT

Days pseudo- pregnant	6 hr				24 hr			
	No. of recipients	No. of eggs		Egg recovery (%)	No. of recipients	No. of eggs		Egg recovery (%)
		Transferred	Recovered			Transferred	Recovered	
3	1	38	32	84.2	1	45	41	91.1
5	4	141	114	80.8	3	96	86	89.6
7	4	128	109	85.2	2	71	69	97.2
9	5	171	151	88.3	3	105	93	88.6
11	4	140	102	72.9	3	99	82	82.8
Total	18	618	508	82.2	12	416	371	89.2

stage of pseudopregnancy of the temporary recipients are given in Table 1. The proportion of eggs recovered varied from 73 to 88% and from 83 to 97% in the 6-hr and 24-hr groups respectively. The higher rate of egg recovery after 24 hr is attributed to the fact that more time was available for their location early in the day (24-hr group) than in the late afternoon when the 6-hr group was killed. Overall, the mean numbers of eggs transferred and recovered were 34.5 ± 1.6 (seventeen to fifty-two) and 29.3 ± 1.5 (fourteen to forty-seven) respectively. The proportion of eggs recovered appeared to be little influenced by the stage of pseudopregnancy, except for a drop of 8 to 15% on Day 11 compared with earlier stages.

The condition of the eggs at recovery varied both according to the stage of pseudopregnancy and to the duration of exposure. In the synchronous or near synchronous combinations, some further development occurred during 24 hr but under the most asynchronous conditions the eggs became abnormal. There was progressive swelling of the area bounded by the zona pellucida, which itself became very thin, and shrinkage of the blastomeres into a small clump. In some cases involving '9 to 11-day' recipients, even 6 hr exposure led to morphological changes in the blastomeres. Examples of the changes undergone by the eggs are illustrated in Plate 1.

TABLE 2
DEVELOPMENT OF EGGS RE-TRANSFERRED TO SYNCHRONIZED RECIPIENTS
FOLLOWING EXPOSURE FOR 6 HR TO THE UTERUS OF TEMPORARY RECIPIENTS
3 TO 11 DAYS PREGNANT

Day of pseudo-pregnancy eggs transferred to temporary recipient	No. of recipients	Recipients with implants		No. of eggs transferred	Young born	
		No.	%		No.	%
3	3	3	100	20	9	45
5	9	6	67	66	25	38
7	11	9	81	87	38	44
9	10	8	80	71	19	27
11	13	7	54	96	31	32

No problem was involved in separating the transferred morulae from the native one-cell eggs which were regularly found in the flushings. Based on the counts of corpora lutea, 61% of the native eggs expected (155/255) were recovered in twenty-three does. The morphology of degenerating one-cell eggs has been described recently (Adams, 1970c).

Developmental potential of 'morulae' re-transferred to synchronized recipients following exposure to the uterine horns of temporary recipients 3 to 11 days pseudopregnant

After 6 hr exposure. Details of the numbers of recipients and of eggs transferred, pregnancy rate (proportion of recipients with implants), and young born are given in Table 2. Except for the '11-day' group, pregnancy rate was not significantly affected by the eggs' previous treatment, averaging 79%, which is only slightly below the mean of 89%, recorded for single transfers by the same

technique (Adams, 1962). The proportion of eggs developing to term varied from 27 to 45%, being somewhat lower in the case of eggs exposed to '9- and 11-day' uteri, than for those exposed to '3- to 7-day' uteri (30% versus 42%). The performance of the eggs exposed briefly to the 11-day uterus suggests that they tended to behave as a group, i.e. either none (all affected adversely) or a high proportion survived. This probably reflects variation in the uterine environment provided by individual temporary recipients, though variations in 'egg sensitivity' may also have contributed.

After 24 hr exposure. In this series, the influence of the temporary recipient was pronounced both regarding pregnancy rate and survival of eggs, as Table 3

TABLE 3
DEVELOPMENT OF EGGS RE-TRANSFERRED TO SYNCHRONIZED RECIPIENTS
FOLLOWING EXPOSURE FOR 24 HR TO THE UTERUS OF TEMPORARY RECIPIENTS
3 TO 11 DAYS PSEUDOPREGNANT

Day of pseudo-pregnancy eggs transferred to temporary recipient	No. of recipients	Recipients with implants		No. of eggs transferred	Young born	
		No.	%		No.	%
3	6	5	83	42	18	43
5	10	10*	100	83	39	47
7	7	3	43	50	12	24
9	8	2*	25	48	1	2
11	8	1	12	65	2	3

* One recipient subsequently failed to produce young.

shows. Thus, whereas temporary transfer to Day-3 or Day-5 recipients was compatible with a very high pregnancy rate (fifteen/sixteen) and satisfactory survival to term (43 to 47%), very few eggs (only three out of 113) survived being exposed to the uterus on Days 9 or 11 of pseudopregnancy. The intermediate result obtained with the Day-7 group indicates that the injurious effect of the uterine environment developed progressively.

The sharp decline in pregnancy rate recorded with eggs exposed to 7-, 9- and 11-day uteri points to the increasing failure of complete batches of eggs to implant. Further evidence of early failure was obtained in the recipients killed 3 or 4 days after receiving twenty-six morulae which had spent 24 hr in a '10-day' uterus. These yielded only seven blastocysts, including one that was degenerate, two that were abnormally small and four normal ones. Failure to recover nineteen of the twenty-six morulae, indicates that they perished shortly after transfer.

The data presented in Tables 2 and 3 on pregnancy rate and embryo survival are illustrated in Text-figs. 1 and 2.

The effect of progestational uterine flushings or endometrial tissue on early pregnancy

The uterine horns receiving flushings or scrapings yielded ten and seven young respectively, whilst the corresponding sets of ovaries each bore eleven corpora lutea. By comparison with the control untreated horns, there was no evidence that either treatment affected embryo survival. Thus, the harmful

effect of the advanced progestational endometrium on the egg appears to be wholly direct, and any additional indirect action on the endometrium of the recipient test animal can be discounted.

The fate of two- to four-cell eggs in the advanced progestational uterus

At autopsy, twenty out of twenty-seven, thirty-one out of forty and thirty-nine out of forty-five of the transferred eggs were recovered. None of the eggs had developed further, and all showed degenerative changes, including expansion and thinning of the zona pellucida and lysis of the blastomeres (see Pl. 1, Figs. 1 and 2).

TEXT-FIG. 1. The proportion of recipients becoming pregnant (pregnancy rate) after receiving eggs which had spent either 6 hr (●) or 24 hr (○) in the uteri of pseudopregnant rabbits (temporary recipients).

The fate of 'alien' 60-hr morulae and native one-cell eggs in the progestational uterus and oviduct

Ten out of the eleven native eggs expected to be present in the two ligated oviducts were recovered, but none of the ten expected in the uterine horns was found. Of the fifteen morulae transferred to the oviducts, twelve were recovered and these had developed into early blastocysts, whereas the sixteen morulae

EXPLANATION OF PLATE 1

FIG. 1. Two-cell egg recovered 24 hr *p.c.* $\times 385$.

FIG. 2. The result of transferring a two-cell egg to the uterus of a recipient on the 11th day of pseudopregnancy. Recovery 24 hr after transfer. $\times 175$.

FIGS. 3 to 8. Sixty-hr morulae (Pl. 2, Fig. 13) were transferred to the uteri of pseudopregnant recipients as follows:

FIGS. 3 and 4. On Day 7 and recovered after 24 or 48 hr respectively. $\times 145$. Note that development occurred, with blastocoele formation taking place between 24 and 48 hr after transfer. However, this egg is atypical in comparison with the stages normally observed 72 to 96 hr *p.c.*

FIGS. 5 and 6. On Day 9 and recovered after 6 and 24 hr respectively. $\times 145$ and $\times 125$.

FIGS. 7 and 8. On Day 9 and recovered after 6 hr and 48 hr respectively. $\times 155$ and $\times 125$. Note the variation between the morulae depicted in Figs. 5 and 7, which is attributed to differences in the uterine environments provided by the two recipients.

PLATE I

(Facing p. 106)

PLATE 2

(Facing p. 107)

transferred to the uterus, of which fifteen were recovered, had undergone degeneration. Representative groups of native and transferred eggs are shown in Plate 2.

The fate of 60-hr morulae transferred to the oviduct on the 11th day of pseudopregnancy

Seventy-four of the ninety eggs transferred were recovered from the ligated oviducts, the recovery rate being near maximum in three of the four does. Among the forty-one eggs recovered 24 hr after transfer, only four remained at the morula stage, the majority having developed into early blastocysts, six of which had undergone some expansion with formation of the inner cell mass.

TEXT-FIG. 2. The proportion of eggs developing to term in recipients to which they were transferred after spending either 6 hr (●) or 24 hr (○) in the uteri of pseudopregnant rabbits (temporary recipients).

EXPLANATION OF PLATE 2

FIG. 9. Group of unfertilized eggs recovered from the ligated oviduct 11 days *p.c.* $\times 55$.

FIG. 10. Group of unfertilized eggs recovered from the uterus 9 days *p.c.* $\times 30$.

FIG. 11. Group of degenerating early blastocysts recovered 48 hr after transferring 60-hr morulae to the ligated oviduct on Day 9 of pseudopregnancy. $\times 60$.

FIG. 12. Group of degenerating morulae recovered 72 hr after transferring 60-hr morulae to the uterus on Day 9 of pseudopregnancy. $\times 60$.

FIG. 13. Morula recovered 60 hr *p.c.* $\times 275$.

FIG. 14. An early blastocyst recovered 48 hr after transfer of 60-hr morulae to the ligated oviduct on Day 9. $\times 140$.

FIG. 15. An early blastocyst that developed from a 60-hr morula transferred to a Day-9 uterus for 6 hr and then re-transferred to a synchronous recipient for 24 hr. $\times 110$.

FIG. 16. A degenerate morula recovered 48 hr after transfer of a 60-hr morula to the uterus on Day 9. $\times 225$.

In the group recovered after 48 hr, there were nine morulae and twenty-four early blastocysts, including sixteen that had expanded a little. A few of the latter had 'collapsed' so that the zona pellucida presented a crinkled appearance.

Following re-transfer, twenty-two out of thirty-nine (56%) and nine out of thirty-three (27%) developed to term after spending 24 and 48 hr respectively in the ligated oviduct. This was in marked contrast to the fate of the eggs transferred to the uterus.

DISCUSSION

Previous observations on a variety of mammals have shown that when eggs are transferred asynchronously, i.e. >2 days out of phase, pregnancy will not succeed. In the rabbit at least, it appears that, as the degree of asynchrony increases, egg development fails earlier. An extreme position was reached in the present experiments when the eggs were subjected to a markedly asynchronous environment, i.e. the recipient up to 8½ days in advance of the donor. Under these conditions not only was egg development arrested but severe morphological changes of a degenerative nature took place well within 24 hr. Moreover, it was established by means of re-transfer to a favourable (i.e. synchronous) uterus that the majority of the eggs were defunct and only very few were capable of developing to term. The hostile reaction of the advanced progestational uterus, which expressed itself fully between Days 9 and 12, i.e. after the normal time of implantation, appears not to develop suddenly since the survival of eggs exposed for 24 hr to the Day-7 uterus was intermediate in comparison with the Day-3 and Day-5 groups. That Day 7 represents a transitional phase is also evident from the variation observed in the morphology of eggs recovered after 6 hr (see Pl. 1, Figs. 5 and 7). Previously, a deleterious uterine effect has been reported to occur in the rat on the 5th day of pseudopregnancy (Dickmann & Noyes, 1960). In the mouse, 'ova younger than the uterus' will 'often cease cleaving or begin to fragment before the time of implantation, and unlike the rat ova, cannot be recovered—damaged or otherwise—after the period of normal implantation' (Doyle *et al.*, 1963). The eggs apparently disappear during the evening of Day 4. It would be of interest to know whether, in the rat and mouse, the 'hostile phase' is limited to the time of implantation or whether it persists throughout pseudopregnancy.

In general, the proportion of eggs recovered (recovery rate) from the temporary recipients on Days 4 to 12 of pseudopregnancy was high, but a decline was evident in the 'Day-11' group. In the rat, a significant drop in the recovery rate of '4-day' eggs transferred to the '5-day uterus' was noted, from 73% 4 to 6 hr after transfer to 14% at 17 to 28 hr, suggesting that the eggs were being destroyed (Dickmann & Noyes, 1960). The persistence of the rabbit eggs in spite of severe morphological change may be due to protection afforded by the mucin layer.

The harmful effect of the advanced progestational uterus was not confined to the 60-hr morula stage; early cleavage stages proved equally susceptible to damage, as did unfertilized eggs. In fact, it appears that the accelerated degeneration of unfertilized eggs that occurs in the uterus from Day 7 onwards,

is due to changes in the uterine environment and not primarily to ageing (Adams, 1970c). On the other hand, 6-day blastocysts are better adapted to survive, since more than 50% implanted following transfer to the 9-day uterus (Chang, 1950). In the rat, the uterine environment suddenly changes on the 5th day, 'becoming detrimental to "younger" ova, yet stimulating 5-day blastocysts. . . , thus starting the process of implantation' (Dickmann & Noyes, 1960). Further confirmation of the deleterious effect of progesterone on the morula stage comes from a recent series of experiments by Dickmann (1970). In rats that were spayed on Day 2 and then treated with progesterone for 2, 3, 4, 5 or 6 days before receiving morulae, he found that 49%, 38%, 13%, 2.5% and 2%, respectively, of the morulae developed into fetuses.

So far, the uterine factor(s) responsible for causing the eggs to degenerate has not been identified. Histologically, the proliferative response of the rabbit endometrium to progesterone in pseudopregnancy is well defined, but information is scarce on the concomitant changes in its secretions. Chang (1969) reasoned that the death of ferret embryos in the 'older' uterus was due to 'certain deficiencies of endometrial activities as influenced by the age of corpora lutea'. In the rabbit, a specific uterine protein, called 'blastokinin', which is believed to facilitate expansion of the early blastocyst, reaches its maximum level approximately 5 days *p.c.* and then declines to near zero by the 9th day (Krishnan & Daniel, 1967). However, for various reasons, it is considered most unlikely that a deficiency of blastokinin is responsible for the degeneration of the eggs. Rabbit morulae are not only well preserved morphologically but may also develop to the early blastocyst stage in the absence of any (progestational) uterine protein, e.g. *in vitro* (Adams, 1970d), in the ligated oviduct of oestrous does (unpublished data) and in the uterus of does spayed for 3 weeks (Adams, 1968). Rather, the facts point to the existence of a deleterious factor or factors than to a deficiency of a vital substance. So far, attempts to demonstrate a similar action *in vitro*, by culturing eggs in the presence of either uterine flushings or endometrial scrapings obtained on Day 10 have proved negative, but this may only indicate that the effect is not reproducible under the particular experimental conditions employed rather than that such a factor is entirely absent. To induce degeneration of the egg may require a constant action such as may occur *in vivo*. Alternatively, the effect may be more physical in character; in fact, the appearance of the eggs is suggestive of an osmotic effect. Under the influence of progesterone, it is known that uterine secretion becomes increasingly scanty and viscous (Lutwak-Mann, Bournsnel & Bennett, 1960). On the other hand, swelling and disintegration of the zona pellucida has also been reported to occur when newly ovulated eggs were placed in the uterus of oestrous rabbits, in which fluid may be relatively abundant, or in uterine fluid *in vitro* (Chang, 1968).

It is noteworthy that whilst the advanced progestational uterus is hostile to the cleaving egg, the adjacent oviduct permits further development, at least up to the very early blastocyst stage. Whereas practically no 60-hr morulae survived 24 hr exposure to the 11-day uterus, similar transfers to the ligated tube were associated with 57% survival to term after re-transfer. Even after a further 24 hr exposure, the survival rate was still 27%. These figures are

remarkably close to those previously reported for the survival of similar morulae after culture *in vitro*, namely 61% and 33% after 24 hr and 48 hr respectively (Adams, 1970d). A marked divergence in the reaction of the tube and uterus under the influence of progesterone has also been observed in connection with the process of sperm capacitation (Chang, 1958; Bedford, 1970).

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