POLYSpermIC FERTILIZATION FOLLOWING TUBAL SURGERY IN PIGS, WITH PARTICULAR REFERENCE TO THE RÔLE OF THE ISTHMUS

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Summary. Unilateral resection of the isthmus of the Fallopian tube followed by end-to-end anastomosis of the remaining portions was performed in twenty-one pigs, after which mating took place at known times relative to the onset of spontaneous oestrus. Eggs from both the anastomosed and control tubes were recovered at slaughter immediately following the end of oestrus.

Neither the surgical intervention nor the temporary presence of a tubal catheter influenced the duration of the oestrous cycle. Inhibition or retardation of ovulation found on the side of the anastomosis in three animals was presumed to be associated with the development of post-operative adhesions. As judged by the presence of spermatozoa in the zona pellucida of eggs recovered from thirteen animals, eleven of the tubes were patent above and below the anastomosis. It has been shown that fertilization and early cleavage of pig eggs can take place in the absence of the isthmus, and that the rate of cleavage is not modified by this situation. An increased number of spermatozoa was found on the eggs from the anastomosed tube which, together with the striking incidence of polyspermic eggs (32.4%), indicates that the isthmus normally limits passage of spermatozoa to the upper reaches of the Fallopian tube. Attention is drawn to the implications of these findings in human tubal surgery.

INTRODUCTION

The mammalian Fallopian tube has been widely studied from the point of view of its anatomy, histology and secretory function (for detailed review, see Hafez & Blandau, 1969) and more recently, methods for collecting and analysing the tubal fluids have been developed in several laboratory and domestic species (summarized by Restall, 1967; Hamner & Fox, 1969). However, as far as the processes of capacitation and fertilization are concerned, the respective contributions of the isthmic and ampullary portions have yet to be adequately defined,

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MATERIALS AND METHODS

Animals

Twenty-one Large White gilts, weighing from 86 to 143 kg, and aged between 6 and 9 months, were used in these experiments. They were penned and fed in groups of three to five in an indoor piggery, and tested for oestrus with a boar at 07.30 hours and 16.30 hours. None of the animals had previously been involved in experimental work: all were used during their first or second recorded oestrous cycles, the operation being performed during the first 10 days of the cycle.

Resection of the isthmus

Anaesthesia was induced by intravenous injection of thiopental sodium, and maintained by an oxygen–cyclopropane closed circuit system after endo-tracheal intubation. The reproductive tract was exposed by mid-ventral laparotomy, and the ovaries examined for the number of corpora lutea and large Graafian follicles. The Fallopian tube adjoining the ovary with the smaller number of corpora lutea was selected for isthmic resection, the contralateral tube remaining intact to serve as a control.

With the exception of the tube and a small portion of adjacent uterine horn, the tract was returned to the peritoneal cavity and the incision bordered with surgical cloths. Continuous moistening of this area was maintained with physiological saline in ten of the animals in order to minimize dehydration of the exposed tissue and possible development of adhesions. Resection of the isthmus and end-to-end anastomosis of the remaining portions of the Fallopian tube were achieved in the following steps.

1. Preventive haemostasis of the vascular arcade bordering the tube with ligatures of No. 0000 gut placed adjacent to the isthmus, the principal ligatures acting as points of traction (Text-fig. 1a).
2. Liberation of the ischaemic portion of the ligatured mesosalpinx.
3. Removal of the ischaemic at the level of the anoxic frontiers, transection being in the approximate region of the ampullary–isthmic junction and 0.5 cm proximal to the utero-tubal junction. Care was taken to preserve the integrity of the latter junction.
4. Verification of the haemostasis and positioning of further ligatures if necessary.
TEXT-FIG. 1. These figures illustrate the principal surgical steps involved in resection of the isthmus followed by end-to-end anastomosis of the remaining portions of the Fallopian tube.
(5) Introduction of a catheter into the remaining portions of the Fallopian tube (Text-fig. 1b). In the first fourteen animals, a Teflon catheter terminated by a flexible barb for anchorage in the uterine lumen was used, but this was abandoned in favour of a silicone rubber (silastic) tube passing through the utero-tubal junction, and fixed at its exit from the uterine wall by a ‘U’ knot tied with No. 0000 gut. A catheter was not used in the first animal in this series.

(6) Traction and suture of the opposing ends of the tube, an important preliminary being the establishment of an X-point on the borders of the sectioned mesosalpinx (Text-figs. 1c and 1d). The anastomosis could only be accomplished by triangulation with difficulty and was unduly traumatic. A continuous overlapping suture was therefore made, using silk and a vascular atraumatic needle (Robert and Carrière, Paris), particular care being taken to cover the junction with peritoneum.

(7) Trans-peritoneal passage of a nylon or cotton thread attached to the silastic catheter at its ‘fimbrial’ end, and brought out at the groin. Attachment was by means of a capucin knot, its size being sufficient to facilitate removal of the catheter after healing of the anastomosis.

(8) Retraction of the exposed line 4 to 8 days later, and verification of the integrity of the withdrawn catheter.

Post-operative care

Animals were isolated for 6 to 8 days following the operation to avoid the risk of the indwelling catheter being accidently displaced or withdrawn. Notwithstanding this precaution, the catheter could not be located in one animal in this series, thereby necessitating a second laparotomy, when it was recovered from the abdominal cavity. The Fallopian tube was perfused with physiological saline at this time and was found to be patent.

Mating procedure

Oestrus and ovulation were allowed to occur spontaneously. At either the first, second or third post-operative oestrus, animals were mated twice to one of three Large White boars of known fertility, mating taking place at 17.00 hours on the 1st day of oestrus and again at 08.00 hours the following morning.

Autopsy

Slaughter was arranged as soon as possible after the end of oestrus and took place on the 3rd or 4th day of the new cycle in all instances. Animals were stunned electrically in the Institute’s abattoir, and the reproductive tract removed between 3 and 5 min later. A careful examination was made for the presence of surgical adhesions between the tract and the intestines or body wall, and for the disposition of the fimbria in relation to the ovary on the side of the resection.

After counting the number of recent ovulations and large follicles, the Fallopian tubes were dissected free from their ligaments, separated from the uterus just caudal to the utero-tubal junction, and perfused with 20 ml physiological saline. Distension of the operated tube invariably followed injection by way of the fimbria, since the anastomosis between the relatively thin-walled
ampulla and muscular portion of the tube adjacent to the utero-tubal junction appeared to be acting as a valve when under pressure. In the majority of animals, the tube was therefore perfused towards the fimbria, a blunted needle being introduced through the tip of the uterine horn to within 2 to 3 mm of the anastomosis for this purpose. The recovered eggs were examined under a binocular microscope (×40) for their stage of development and the presence of spermatozoa in the zona pellucida. They were fixed for approximately 24 hr in Bouin-Holland’s solution, included in agar, dehydrated with alcohol, and embedded in paraffin (Thibault, 1949). The blocks were sectioned serially at 8 to 10 µ and stained with Regaud’s haematoxylin, the contents of each tube being treated separately. The preparations were then examined under oil immersion (×100) using a Zeiss microscope, particular attention being paid to all nuclear structures, and the number of spermatozoa on and in the zona pellucida was recorded.

RESULTS

One of the animals which underwent surgery for resection of the isthmus developed an abscess on the left fore-leg soon after the operation and was slaughtered. This gilt had not returned to oestrus, and was eliminated from the experiment. The remaining twenty animals were mated and were included in the data.

Occurrence and duration of oestrus

The oestrous cycle during which the operation was performed varied in length from 20 to 22 days in sixteen animals, cycles of 19, 23, 23 and 26 days, respectively, being recorded in the remaining four. Because the majority of animals were operated on during their first recorded oestrous cycles, no comparison of pre- and post-operative cycle lengths could be made but, with the exception of the cycle of 26 days, neither the operation nor the temporary presence of the indwelling catheter appeared to have influenced the onset of oestrus. Likewise, the duration of the post-operative oestrus, which varied between 2 and 3 days, was apparently uninfluenced by the surgical intervention.

Incidence of ovulation

One animal had not ovulated when it was killed at 16.15 hours on the 3rd day of oestrus, although large pre-ovulatatory Graafian follicles were present on both ovaries. A second animal exhibited peritonitis with extensive adhesions between the reproductive tract and the body wall, these presumably having inhibited or retarded the process of ovulation. Fifteen of the remaining eighteen animals had newly-formed corpora lutea on both ovaries when examined at autopsy.

In three instances, failure of ovulation on the side of the anastomosis was clearly associated with the presence of post-operative adhesions between the ovary and adjacent tissues of the reproductive tract, or between the intestines and the uterine horn, doubtless arising from dehydration during the course of the surgical intervention. Nevertheless, large follicles (8 to 12 mm in diameter)
had developed on these ovaries, and there was no evidence of any compensatory increase in the number of follicles on the control ovary. Ovulations were present on both ovaries in all ten animals in which the operation was performed under conditions of continuous saline irrigation, and in these, the extent of fimbrial and tubal adhesions was much reduced.

In only two animals did the number of ovulations on the side of the anastomosis exceed that on the control side. The number of ovulations for fifteen ovaries on the side of the anastomosis varied from two to sixteen, with a mean of 5·2. The range for eighteen ovaries on the control side was from four to eleven, with a mean of 7·4.

**Egg recovery**

Details of the proportion of eggs recovered are given in Tables 1 and 2. A total of 165 eggs was obtained from eighteen animals which, on the basis of the number of corpora lutea counted at autopsy, represented an overall recovery of 77·8% of the eggs. The control tube yielded a total of 124 eggs in seventeen of the eighteen animals with ovulations on this side; failure to recover eggs in one

<table>
<thead>
<tr>
<th>Table 1</th>
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<tr>
<td>THE INCIDENCE OF OVULATION AND FERTILIZATION IN TWENTY PIGS IN WHICH THE ISTHMS OF ONE OF THE FALLOPIAN TUBES HAD BEEN REMOVED AND THE REMAINING PORTIONS ANASTOMOSED</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Intact control side</th>
<th>Contralateral anastomosed side</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>%</td>
</tr>
<tr>
<td>Ovulating</td>
<td>18</td>
</tr>
<tr>
<td>Yielding tubal eggs</td>
<td>17</td>
</tr>
<tr>
<td>With fertilized eggs</td>
<td>16</td>
</tr>
<tr>
<td>With polyspermic eggs</td>
<td>1</td>
</tr>
</tbody>
</table>

instance may have been associated with the presence of fimbrial adhesions on the control ovary. A total of forty-one eggs was recovered from thirteen of the anastomosed tubes, but not a single egg was recovered in the two remaining instances. The proportion of eggs obtained from the control tubes (93%) was significantly higher than that found on the opposite side (53%). Some deleterious effect of the operation on the mechanism of fimbrial function and egg pick-up may have been responsible for the lower recovery, but loss of eggs is considered more likely to have occurred during the procedure of tubal perfusion. Accelerated transport of eggs was not an explanation of this discrepancy, for in no instance were eggs located in the uterus.

**Tubal patency**

As judged by the recovery of cleaved or single-cell eggs with spermatozoa in the zona pellucida from the side of the resection, eleven of the thirteen Fallopian tubes yielding eggs were patent above and below the anastomosis (Table 1).
Since fluid distension of the tubes was not found, the anastomosis was presumably permeable to the increased secretion which occurs during oestrus. Although ten unfertilized eggs were flushed from the control tube in one instance, this situation was unlikely to have been influenced by the operation. In eleven pigs slaughtered during the second cycle after surgical intervention, the absence of any eggs from the previous ovulation in the anastomosed tube was taken to indicate the patency of the tube to egg passage.

**Fertilization and early cleavage**

The proportion of eggs recovered that were fertilized did not differ significantly between the two sides of the tract, being 82·8% for the anastomosed tube and 90·3% for the control tube. Excluding the one animal yielding unfertilized eggs, all the control eggs were fertilized. Failure of fertilization in two anastomosed tubes was apparently due to a failure of sperm transport, since spermatozoa were not observed on or about eggs in these instances. Whether the anastomosis was at fault, or whether these represented spontaneous conditions of unilateral fertilization, could not be determined.

A striking finding was that 32·4% of thirty-four fertilized eggs from the anastomosed tube had undergone polyspermic fertilization, compared with 0·9% of the 114 control eggs. Polyspermic eggs were found in the anastomosed tube of five animals, whereas only a single control egg was polyspermic (Tables 1 and 2). All the eggs recovered from three of these five anastomosed tubes were polyspermic, two of three eggs in the fourth tube were polyspermic, but only one of nine eggs in the fifth tube showed this condition.

With the exception of pronuclear eggs recovered from both tubes of an animal killed at 09.15 hours on the 3rd day of oestrus, all the fertilized eggs were completely denuded of cumulus and corona cells when examined immediately upon flushing. No overall dissimilarity in the timing of egg development was noted between the two sides of the tract, stages from pronuclear to six-cells being represented, although one of the anastomosed tubes contained a normal eight-cell egg.

**Table 2**

<table>
<thead>
<tr>
<th>CONDITION OF THE EGGS RECOVERED FROM THE INTACT AND RESECTED FALLOPIAN TUBES OF EIGHTEEN PIGS IN WHICH THE ISTMUS OF ONE OF THE TUBES HAD BEEN SURGICALLY REMOVED AND THE REMAINING PORTIONS ANASTOMOSED</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intact control tube</strong></td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td><strong>No. of eggs</strong></td>
</tr>
<tr>
<td>Ovulated</td>
</tr>
<tr>
<td>Recovered</td>
</tr>
<tr>
<td>Fertilized</td>
</tr>
<tr>
<td>Polyspermic</td>
</tr>
</tbody>
</table>

was at fault, or whether these represented spontaneous conditions of unilateral fertilization, could not be determined.
### Table 3
COMPARISON OF THE NUMBER OF SPERMATOZOA IN AND ABOUT THE ZONA PELLUCIDA OF FERTILIZED EGGS RECOVERED FROM INTACT AND RESECTED FALLOPIAN TUBES OF TEN PIGS

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Stage of egg development</th>
<th>Intact control tube</th>
<th>Tube without isthmus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of eggs examined</td>
<td>No. of polyspermic eggs</td>
</tr>
<tr>
<td>850</td>
<td>Pronuclear</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>9032</td>
<td>Pronuclear</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>9056</td>
<td>Pronuclear</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>9101</td>
<td>Pronuclear</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>9037</td>
<td>Pronuclear</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>9030</td>
<td>2 to 4 cells</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>817</td>
<td>2 to 4 cells</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>9045</td>
<td>2 to 6 cells</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>9055</td>
<td>2 to 4 cells</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>9073</td>
<td>2 to 8 cells</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>71</td>
<td>0</td>
</tr>
</tbody>
</table>

* The sections of two of these eggs were poor, preventing an accurate count of the number of spermatozoa.
Numbers of spermatozoa

An indication of sperm transport to both the control and anastomosed Fallopian tubes was obtained from counts of the spermatozoa attached to the eggs. Because of the difficulty of determining with certainty whether the sperm heads were on the surface of, or had actually penetrated the zona pellucida, a summarized figure has been given for the number of spermatozoa in and about the eggs. Table 3 presents these findings for the ten animals yielding fertilized eggs from both Fallopian tubes.

The mean number of spermatozoa on the seventy-one eggs from the control side was 221, with a range of 1 to 628. For thirty-two eggs examined from the anastomosed tube, the mean number of spermatozoa was 344, with a range of 13 to 916. In seven animals, a greater number of spermatozoa was present on the eggs recovered from the anastomosed tube. Likewise, in four of the five animals yielding polyspermic eggs, significantly more spermatozoa were present on the eggs from the anastomosed tube (Table 3). Within individual animals, however, the polyspermic eggs did not always have more spermatozoa on the zona pellucida than normally fertilized eggs recovered from the same Fallopian tube. Similarly, normally fertilized eggs from the control tube possessed, in a number of instances, a higher mean number of spermatozoa than the polyspermic eggs recovered from the other side of the tract.

Concerning differences in the mean number of spermatozoa about pronuclear as compared with cleaved eggs, two interesting and complementary findings emerge. First, the mean number of spermatozoa on pronuclear eggs from the anastomosed tube (360) was more than twice the figure for the control tube (153). Secondly, whereas the mean number of spermatozoa on the cleaved eggs from the control tube had increased to almost twice that for pronuclear eggs (290 versus 153), there was no such increase in this figure for the anastomosed tube (329 versus 360). Taken together, these findings indicate that in the absence of the isthmus, a significantly higher number of spermatozoa reached the ampulla soon after mating, a point that is further emphasized by comparing the number of spermatozoa on pronuclear eggs from the anastomosed tube (360) with the number on cleaved eggs from the control tube (290).

Cytological details of polyspermic eggs

Nine of eleven polyspermic eggs were recovered from the anastomosed tube at the pronuclear stage of fertilization, and contained from one to five (mean 2.3) discrete, well-formed, accessory male pronuclei. These pronuclei were of approximately the same size as the presumptive male and female pronuclei undergoing syngamy although, in two of the eggs, the accessory male pronuclei were distinctly larger. Chromatin condensation was discernible at the interface of the membranes of the two pronuclei undergoing syngamy. Fusion of more than two pronuclei was not observed; in fact, the accessory pronuclei were not in contact with other nuclear structures, and were usually disposed towards the periphery in the opposite hemisphere of the egg.

A further egg, classified as polyspermic, had reached telophase of the first cleavage division, and contained a supplementary spermatozoon with its head arranged on the surface of the vitelline membrane, and the sperm tail in the
perivitelline space. Engulfment of the sperm head did not appear to have begun, neither did this structure show any enlargement when compared with spermatozoa present on the zona pellucida. The eleventh egg considered as polyspermic had reached the four-cell stage. A swollen sperm head with adjoining mid-piece was present in one of the blastomeres.

Ten of the eleven polyspermic eggs possessed first and second polar bodies. The single polar body present on the remaining egg was judged to be the second, after taking into account the small and compact arrangement of the chromatin, and comparing this structure with both polar bodies on neighbouring eggs.

The single polyspermic egg recovered from the control tube possessed first and second polar bodies, two pronuclei at syngamy, and four discrete accessory male pronuclei of approximately similar size and structure.

Sperm mid-pieces and tails were seldom identified in the pronuclear eggs, presumably because of the procedure of sectioning the eggs at 8 to 10 µ, with the result that only a transverse section of the tail would have been apparent in most instances. However, in two cases of polyspermy, a large segment of the sperm mid-piece and tail could be distinguished and this was closely associated with a male pronucleus.

DISCUSSION

The results of these experiments demonstrate that fertilization and early cleavage of pig eggs can take place in a Fallopian tube from which the isthmus has been removed by a surgical procedure, and that the rate of cleavage does not differ between the two tubes under these conditions. Any biochemical contribution to the processes of capacitation and fertilization by the isthmus must, therefore, remain questionable. But evidence from studies in vivo and in vitro suggests strongly that the secretions essential for the fertilization and early development of the egg are furnished by the ampulla rather than the isthmus, the former having been shown in a number of species to be the most active secretory region of the entire oviduct (e.g. for the ewe: Hadek, 1955; Restall, 1966; Restall & Wales, 1968). Furthermore, Whittingham (1968) has reported that mouse zygotes degenerated when cultured within the explanted isthmus, whereas the explanted ampullar region of the tube was able to support development, and he commented that the paucity or absence of secretory cells in the mouse isthmus could explain this situation.

A comparison of the numbers of spermatozoa about the eggs from the sectioned and control tubes indicates that the isthmus performs a vital physiological function in limiting the passage of spermatozoa to the site of fertilization. In the absence of the isthmus, the numbers of spermatozoa on the eggs from the two tubes differed as much as ten-fold in some animals, this disparity frequently being more pronounced at the pronuclear stage of development. The precise mechanism whereby the isthmus regulates sperm ascent is not clear, but its powerful muscular ability and the extensive longitudinal folding of the mucosa must certainly be invoked to explain the enormous reduction in sperm numbers between those present at the utero-tubal junction of the pig (du Mesnil du Buisson & Dauzier, 1955a; Rigby, 1964, 1966) and those reaching the site of
fertilization in the ampulla (du Mesnil du Buisson & Dauzier, 1955b; Hunter & Dziuk, 1968). Quite apart from this function as a barrier against migration of an excessive number of spermatozoa to the ampulla, it seems that the isthmus can also be envisaged as a reservoir from which vigorous spermatozoa are continuously and selectively released during the period of oestrus. In the ewe, Quinlivan & Robinson (1969) have suggested that the isthmus rather than the utero-tubal junction constitutes an obstacle to the free progression of spermatozoa. They further considered that the isthmus might act as a reservoir for the ampulla in the same manner as does the cervix, and possibly the vagina, for the uterus.

The striking incidence of polyspermic eggs recovered from the anastomosed tube can be viewed as a sequel to the much increased number of spermatozoa reaching the vicinity of the eggs at the moment of fertilization. Failure to demonstrate a consistent correlation between the incidence of polyspermy and the number of spermatozoa about the eggs is understandable, for the quantitative factor that would influence the occurrence of this anomaly is the number of spermatozoa reaching the zona pellucida at the time of fertilization, rather than the number attaching subsequently. Concerning the finding of polyspermy, the criticism might be raised that the control tube should have been catheterized in the same way and for a similar length of time as the anastomosed tube. Moreover, it could be argued that, whereas the utero-tubal junction had not been subjected to surgical interference, the indwelling catheter may have damaged the tubal mucosa and contributed to abnormal function of this region, which has been reported to act as a site of sperm storage in this species (du Mesnil du Buisson & Dauzier, 1955a; Pitkjanen, 1961; Rigby, 1964, 1966). Although these points may be valid, it should be recalled that, in the first pig (No. 850) in which tubal anastomosis was performed, cannulation was not used to ensure patency of the tube following surgery, and yet all three eggs recovered from the resected tube of this animal were highly polyspermic.

A simple hypothesis to explain the occurrence of polyspermy in pigs under a variety of experimental situations can now be proposed. The progressive increase in this condition following delayed mating or insemination (Hancock, 1959; Thibault, 1959; Hunter, 1967a) may suggest, on the basis of the above results, an effect of progesterone secretion by the developing corpora lutea on the musculature of the isthmus and utero-tubal junction, and the same effect may account for the very high incidence of polyspermy demonstrated in eggs induced to ovulate during the luteal phase of the cycle (Hunter, 1967b), or when injections of progesterone are given shortly before ovulation (Day & Polge, 1968). The influence of progesterone, whether endogenous or exogenous, may be to induce relaxation of the isthmic musculature and mucosa of the utero-tubal junction, allowing passage of many more spermatozoa to the site of fertilization, with a concomitant increase in the risk of polyspermic fertilization. This explanation is not intended to be exclusive for, as has frequently been suggested, the integrity of the egg and the efficiency of its block to polyspermy would also be expected to diminish with post-ovulatory ageing. Consideration must, of course, be given to the fact that monospermic fertilization may follow insemination of an unreasonably large population of ejaculated spermatozoa.
directly into the Fallopian tube. But such a finding can be reconciled with the present hypothesis on the basis that capacitation of suitable individual spermatozoa within such a large population would not occur simultaneously, especially when all the components of the seminal plasma are present, with the result that the eggs may be exposed to relatively few competent spermatozoa before the block to polyspermy becomes complete.

Since the pronuclei within polyspermic eggs had all achieved similar stages of development and swollen cytoplasmic sperm heads were not found in pronuclear eggs, it can be inferred that the male elements entered these eggs at approximately the same time before the zona reaction was complete. Failure to observe syngamy of more than the presumptive female and a single male pronucleus, even in eggs containing four or five accessory male pronuclei, suggests that either the cell organelles that facilitate conjugation of the pronuclei no longer function in this rôle after syngamy has been attained, or that there is an active repulsion of accessory male structures by the conjugating pronuclei. If the latter were true, however, it would be difficult to explain the occasional formation of triploids in this manner, a subject which has been comprehensively reviewed by Bomsel-Helmreich (1965). In any case, the fate of these accessory pronuclei could not be determined from the present data, but Austin (1965) has written that formation of supernumerary pronuclei leads apparently inevitably to abnormal embryonic development.

Our observations on pronuclear development in polyspermic eggs contrast with the findings in eggs induced to ovulate during the luteal phase in the pig, in which both unswollen sperm heads and incompletely developed male pronuclei were found in the cytoplasm of polyspermic eggs (Hunter, 1967b). It was envisaged that a cytoplasmic factor necessary for the metamorphosis of the sperm head into a pronucleus was present in only a limited quantity under these conditions, a theme previously developed by Austin & Braden (1955) after their finding of pronuclei that were distinctly smaller than usual in polyspermic rat and mouse eggs. Failure of cytoplasmic sperm heads to undergo swelling and transformation into male pronuclei has also been reported for the pig by Polge & Dziuk (1965) in eggs that they termed immature, but following polyspermic penetration of apparently mature eggs under conditions of progesterone dominance (Day & Polge, 1968), pronuclear formation usually took place.

Patency of the tubes to sperm passage was demonstrable in a high proportion of animals after resection of the isthmus and end-to-end anastomosis. However, egg transport through the remaining portions of the Fallopian tube and the probability of subsequent implantation may be altered after this operation since the rate of passage along the tube is undoubtedly influenced by the isthmus. The descent of the egg may therefore be considerably faster than usual, although no evidence of premature arrival of eggs in the uterus was found in this study. Conversely, transport of the egg through the scar region of the anastomosis may be impeded, even though the tube may be patent to fluid passage. As far as attempts to alleviate infertility in women with occluded Fallopian tubes are concerned, it is clearly of particular interest to know whether eggs can successfully negotiate an anastomosed section of the tube, for if the incidence of trans-
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Port into the uterus is low, then the risk of ectopic pregnancy should make any such intervention unjustifiable. Consideration must also be given to the possibility of an abnormal fertilization occurring in these circumstances.

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