Synthetic microspheres transferred to the rat oviduct on Day 1 of pregnancy mimic the transport of native ova

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Summary. Microspheres of various materials and diameters were transferred microsurgically to the rat oviduct on Day 1 of pregnancy and autopsies were done at various times thereafter up to Day 10 to assess the recovery and segmental distribution of microspheres and eggs in the genital tract or the viability of embryos. The number and distribution of eggs in the treated and control sides after unilateral transfers were not different on Day 4 and 5 and the number of embryos implanted on Day 10 was not significantly affected after bilateral transfers.

The segmental distribution of eggs and starch microspheres within the oviduct on Days 2, 3 or 4 showed that both are transported partly intermingled from ampulla to uterus. When microspheres of poly(DL-lactide-co-glycolide), starch, dextran or dextran blue were transferred, their distribution in the genital tract in the morning of Day 5 showed that poly(DL-lactide-co-glycolide) and dextran microspheres stayed longer in the oviduct while starch and dextran blue microspheres were transported to the uterus at the same time as the eggs. Transfer of starch microspheres of 40–60 µm to one oviduct and 180–200 µm in diameter to the opposite oviduct showed that distribution on one side was nearly identical to that of the other side from Days 2 to 5.

We conclude that the behaviour of synthetic surrogate ova in rats differs from that in rabbits. The rat oviduct does not change the rate of transport of native eggs following transfer of synthetic surrogate ova. Also, in the rat the composition of the surrogates has greater influence than their size on their time of passage to the uterus. Some surrogates mimic quite well the oviducal transport of embryos and can be used therefore to study this process in species in which the eggs are not coated with additional layers after ovulation.

Keywords: rat; oviduct; embryo transport; egg surrogate; microsphere

Introduction

The feasibility of designing a test to assess tubal transport in women by using surrogate ova rests to a large extent upon basic knowledge of the behaviour of spheroid particles in the oviduct of other species. Following the original work of Harper et al. (1960) the transfer of microspheres to the oviduct for studying their transport to the uterus as surrogate ova has been accomplished mostly in the rabbit. Pauerstein et al. (1975) showed that surgical transfer changes the transport of native eggs. In addition, in this species the eggs and foreign particles are progressively covered by a layer of mucin after entering the isthmus (Greenwald, 1958; Croxatto et al., 1973; Hodgson et al., 1976; Bourdage & Halbert, 1984). The external surfaces of native eggs and surrogates therefore become identical in quality. To the extent that surface properties could affect transport, the behaviour of surrogates in the rabbit may differ considerably from that in other species which lack mucin deposition. This is in fact the case in sheep (Bennett & Rowson, 1961).
We therefore attempted the transfer of ovum surrogates to the oviducts of rats for which normal transport and transport modified by several experimental conditions have been well characterized (Alden, 1942; Ortiz et al., 1979; Croxatto et al., 1982; Forcelledo et al., 1981; Villalón et al., 1982).

The objectives of this work were to determine: (1) the feasibility of transferring microspheres to the rat oviduct without affecting the transport and viability of native eggs; (2) whether synthetic surrogates and native eggs are transported at the same rate through the oviduct; and (3) whether the physical and chemical properties of these particles affect their oviducal transport.

Materials and Methods

Animals. Adult virgin Sprague-Dawley rats were kept in controlled conditions of temperature (21–24°C) and light (07:00-21:00 h). Water and pelleted food were supplied ad libitum. Vaginal smears were taken daily and pro-oestrous females were caged overnight with fertile males. The following day was designated Day 1 of pregnancy if spermatozoa were present in the vaginal smear.

Microspheres. The following materials and diameters were used. (1) Starch: usually 80–100 μm, except as specified (Lot DK850810, Pharmacia Uppsala, Sweden). (2) Poly(DL-lactide-co-glycolide) (Poly-LG): 80–100 μm (Lot AL565-105-4, Southern Research Institute, Birmingham, AL, U.S.A.). (3) Dextran 120–140 μm (Sephadex G100, Pharmacia, Sweden). (4) Dextran blue 120–140 μm (Density Marker Beads, DMB1, Lots EL12859 and HF26353, Pharmacia, Sweden).

Microsyringe. Microspheres were transferrred to the oviducal lumen with a microsyringe designed for this purpose. The syringe was made of two glass capillary tubes one of which fits closely inside the other: the wider tube was 76 mm long, 1-20 mm i.d. and 1-60 mm o.d. and the equivalent measurements for the smaller tube were 68 mm, 0-74 mm and 1-14 mm (Chase Instruments Corp., Poultnay, VT, U.S.A.). These tubes were heated over a flame and pulled to the shape of a Pasteur pipette. The thinner segment of the wider tube was cut at a point in which the internal diameter was about 80, 140 or 200 μm. These diameters were chosen according to the size of the microspheres to ensure that they could only fit in a single line and with a minimal volume of fluid. The tube with the smaller diameter was used as a plunger. Its thinner segment was cut at a point selected to fit well within the point of the barrel and at the same time to exceed in length its matching barrel. A piece of masking tape was attached to its thicker portion to serve as a stop when the plunger was pushed to deliver the microspheres. The position of this stop was adjusted to allow the tip of the plunger to protrude 100–200 μm.

The tips were cut with the aid of a diamond under a microscope furnished with a micrometer scale in the eye piece and were heat polished.

Transfer technique. Transfers were done under a surgical microscope (OPMI 6-SDFC, Zeiss, West Germany) on Day 1 of pregnancy. Each oviduct and ovary were exposed through flank incisions done under pentobarbital anaesthesia (20 mg/kg i.p.). Blood vessels in the periovular sac were cauterized with an electric coagulator (Codman CMC-1: Codman and Shurtleff Inc., Randolph, MA, U.S.A.) before the sac was cut open to expose the fimbria. The microsyringe, previously filled with 6 microspheres, was introduced through the ostium 1–2 mm into the infundibulum where the microspheres were released by pushing the plunger as illustrated in Fig. 1. The periovular sac was replaced around the ovary, the organs were returned to the peritoneal cavity and muscle and skin were sutured. Six

Fig. 1. Schematic illustration of the technique used to deliver microspheres to the lumen of the infundibulum of the rat oviduct after opening the periovular sac. The size and proportions shown do not reflect accurately the actual dimensions detailed in ‘Materials and Methods’.
Transport of surrogate ova by the rat oviduct

microspheres were transferred because this number provides sufficient data points and, together with the normal ovulation number, does not exceed the highest number of ova carried by one oviduct when the number ovulated per side is extremely asymmetric.

**Assessment of transport.** Animals were killed with an overdose of ether at the times indicated below. Oviducts and uterus were removed free of fat tissue and were flushed separately. In separating the oviduct from uterus care was exercised to leave the entire interstitial segment attached to the oviduct. In some experiments the oviducts were divided into 3 segments: ampulla, distal isthmus and proximal isthmus (proximal = closer to the uterus). Cuts were done at the ampullary–isthmic junction and mid-isthmus after the oviduct was straightened by gentle dissection under the operating microscope. A fourth segment (mostly interstitial segment) was obtained, as indicated, by cutting one-quarter of a loop away from the external junction of the isthmus with the uterus. Each of these segments was flushed separately with saline (0.9% w/v NaCl) and the flushings were examined under low-power magnification to assess the number of microspheres and eggs recovered. Since starch microspheres become less visible after entering the uterus, probably due to enzymic degradation (Lindberg *et al.*, 1984), they were counted after adding a drop of Lugol solution to the flushings to turn them violet.

**Statistical analysis.** Differences in the number of eggs or microspheres between sides or between groups were analysed by the Wilcoxon one- or two-sample test (Conover, 1980) as indicated in the ‘Results’. Differences in their distribution along several segments were analysed by the $\chi^2$ test. Probability values $<0.05$ were considered statistically significant.

**Results**

**Experiment 1: does the procedure affect transport of native eggs?**

Fourteen rats received 6 poly-LG microspheres in one oviduct on Day 1 of pregnancy. The contralateral oviduct was left undisturbed. Seven rats were killed on Day 4 and 7 on Day 5 to determine the number of eggs in the oviducts and uterine horns and to check for the presence of microspheres.

The numbers of eggs (mean ± s.e.) in the treated and control sides were 5·3 ± 0·8 and 6·1 ± 1·3 on Day 4 and 5·7 ± 1·1 and 5·1 ± 1·0 on Day 5 respectively. All eggs were recovered from the oviduct on Day 4 and almost all (95–97%) from the uterus on Day 5. Microspheres were recovered from the treated side in all animals. There was no statistically significant difference between the treated and the control side in the number of eggs or in their distribution on Days 4 or 5 (Wilcoxon one-sample test).

**Experiment 2: does the procedure affect the viability of eggs?**

Six starch microspheres were transferred to each oviduct of 11 rats on Day 1. Rats were killed on Day 10 to count the implantation sites. A group of 12 pregnant rats was left undisturbed as controls.

There were 12·5 ± 0·6 implanted embryos on Day 10. A slightly higher number of implantations (14·3 ± 0·5) was found in the control group but the difference did not reach statistical significance (Wilcoxon two-sample test).

**Experiment 3: do microspheres and native eggs reach the uterus at the same time if transfer of the former is performed on Day 1?**

Six starch microspheres were transferred to each oviduct of 16 animals on Day 1. Eight rats were autopsied at 15:00–16:00 h on Day 4 and 8 at 10:00–10:30 h on Day 5 to determine the numbers of microspheres and eggs in the oviducts and uteri.

As shown in Fig. 2, all microspheres and eggs were in the oviduct on Day 4 whereas the majority of both had reached the uterus by the morning on Day 5. There was no statistically significant difference between the distribution of eggs and microspheres on either day.

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Experiment 4: are microspheres and native eggs transported together through the oviduct?

Six starch microspheres were transferred to each oviduct of 12 rats on Day 1. Four rats were killed on Day 2, 4 on Day 3 and 4 on Day 4. To assess the segmental distribution of microspheres and eggs, oviducts of rats killed on Day 2 were divided in three segments and oviducts of the other 2 groups into 4 segments.

As shown in Fig. 3, at all times microspheres and eggs were more numerous in one of the segments and they became progressively closer to the uterus from Day 2 to Day 4. At each time microspheres and eggs had a very similar distribution profile although that of the spheres was slightly more shifted towards the ovary. This difference was statistically significant on Day 3 ($\chi^2$ test). Microspheres and eggs were found in a single segment in nearly half of the oviducts which had been divided into 4 segments and they were never found spread in more than 2 segments. The segment containing most microspheres and eggs did not coincide in all oviducts and so the tendency of microspheres and eggs to be found in a single segment was partly hidden in the group averages shown in Fig. 3.

These results were further analysed to determine how microspheres and eggs were distributed between two contiguous segments when the cut that was done to divide the segments also divided microspheres and/or eggs into two subgroups. Pairs of contiguous segments that contained only microspheres in one and only eggs in the other were not found. All pairs of contiguous segments that had microspheres and eggs in both ($n = 7$) or microspheres + eggs in one and microspheres or eggs in the other ($n = 11$) were included to analyse the distribution of microspheres and eggs across the distal and proximal sides of each dividing cut. While microspheres were almost equally distributed between proximal and distal sides (53 and 46 respectively) there were twice as many eggs in the proximal than in the distal sides (90 and 39 respectively). This difference was statistically significant ($\chi^2$ test).

Experiment 5: does the composition of microspheres affect their transport through the oviduct?

Four groups of 12 rats each received microspheres of starch, poly-LG, dextran or dextran blue. Six microspheres of the same type were transferred to each oviduct. The numbers of microspheres and eggs in oviducts and uteri were determined at 10:00 h on Day 5.
Fig. 3. Distribution of native eggs (solid bars) and microspheres (open bars) along the oviducal segments on Days 2, 3 and 4 of pregnancy in the rat. Six starch microspheres were transferred to the infundibulum of each oviduct on Day 1 of pregnancy. Values are based on a mean recovery of >13 eggs per rat and >80% of the transferred surrogates. Values are mean ± s.e.m. for 8 oviducts.

Fig. 4. Percentage distribution of native eggs (solid bars) and different microspheres (open bars) on the morning of Day 5 of pregnancy in the rat. Six microspheres of each type were transferred to the infundibulum of each oviduct on Day 1 of pregnancy. Values are based on a mean recovery of >11 eggs per rat and >71% of transferred surrogates. Values are mean ± s.e.m. for 12 rats. Comparisons were done with the Wilcoxon two-sample test.

As shown in Fig. 4, greater proportions of poly-LG and dextran microspheres had not reached the uterus on Day 5 compared to native eggs (Wilcoxon two-sample test). The distribution of starch and dextran blue microspheres was not different from that of the eggs.
Six starch microspheres of 40–60 μm diameter were transferred to one oviduct of 42 rats and 6 starch microspheres of 180–200 μm diameter to the contralateral oviduct. The numbers of microspheres and eggs in oviducts and uteri were determined at 10:00 h on Days 2, 4 and 5, using one-third of the animals at each time.

As shown in Fig. 5, besides an early loss of small microspheres there was no difference between large and small microspheres in their distribution along the genital tract from Day 2 to Day 5. The percentage recoveries of the large and small microspheres fluctuated between 88 and 100% and 51 and 62% respectively. The recovery and distribution of eggs (not shown) was nearly identical with that observed with the same class of microspheres in the previous experiments. In an attempt to account for 40% of smaller spheres already lost on Day 2, 6 small microspheres were transferred on Day 1 to each oviduct of 6 rats which were killed 3 h later. At this time 30% of the microspheres had been lost.

Discussion

This study demonstrates that the transfer of microspheres to the rat oviduct is feasible and adequate for studying the egg transport function of this organ since it does not disturb the process and the surrogates travel at a pace similar to the eggs. In addition, it shows that a substance layered by the oviduct on the external surface of ova and microspheres, as occurs in rabbits and other species (Blandau, 1969), is not necessary to mimic ovum transport by foreign particles.

The transfer procedure affected neither the number nor the distribution of eggs recovered on Days 4 and 5 of pregnancy, when the treated side was compared with the control side. Furthermore the distribution of native eggs on Days 4 and 5 in rats subjected to unilateral or bilateral transfer is in agreement with previous findings in undisturbed animals in the same laboratory (Forcelledo et al., 1981).

Embryo viability was not significantly affected, at least until Day 10 of pregnancy, by the transfer procedure or by the presence of starch microspheres in the vicinity of the eggs.

Experiments 3 and 4 showed that starch microspheres reach the uterus, at about the same time as the eggs, after being transported together along the oviduct. Microspheres and embryos were found intermingled in discrete oviducal segments, supporting the assertion of Alden (1942) that the
mechanical activity of the oviduct does not spread the luminal content over more than two loops. The segment containing the microspheres and eggs became closer to the uterus as a function of time. Although the eggs and microspheres travelled together, they were not homogeneously intermingled: there was a tendency for eggs to travel in front and microspheres in the rear of the group. This analysis is confined in the present work to starch microspheres which arrived in the uterus with no significant delay with respect to the eggs.

The composition of the microspheres had a small, but significant, effect on the time they reached the uterus. None travelled faster than the eggs and poly-LG and dextran microspheres entered the uterus with a significant delay compared to the eggs. This could mean that the oviduct or part of it functions differently in response to different surfaces or materials or that the same propulsive forces act less efficiently upon microspheres with certain surface characteristics. The first possibility is less likely since the rate of transport of native eggs was not changed by the presence of any of these 4 types of microspheres.

Varying the size of starch microspheres did not affect their distribution between oviduct and uterus from Day 2 to the morning of Day 5, indicating that the two sizes compared reached the uterus at a similar time. The lower recovery of smaller microspheres is due to an unaccounted for loss restricted to a very early period after transfer.

In contrast with the rabbit, the rat oviduct did not exhibit differential transport of microspheres according to their size (Croxatto et al., 1973; Hodgson et al., 1976). Doubling the diameter of surrogate ova (i.e. from 100 to 200 μm) caused significant changes in their rate of transport through the rabbit oviduct whereas in the rat a 3–4-fold increase (40–60 to 180–200 μm) made no difference in their time of appearance in the uterus. The physiological implication of this species difference is not clear. We believe that it reflects differences in the relative participation of ciliary, smooth muscle and secretory activities in the propulsive mechanisms and in the resistance to the movements of the luminal content.

The lack of effect of the transfer procedure or of the surrogates on the time course of transport of native embryos plus the fact that some microspheres, notably dextran blue and starch, mimic embryo transport quite well, makes this technique suitable for newer analysis of the transport function of the oviduct, particularly in the absence of native ova. The results reported warrant the testing of this technique in other mammals in which the eggs remain without oviducal coverings after ovulation.

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References


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