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Differential transport of fertilized and unfertilized eggs

Transporte diferencial de huevos fertilizados y no fertilizados

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Oviductal transport of fertilized and unfertilized ova in rodents occurs normally in association with different physiological conditions. To assess if each kind of egg exerts a different influence on its transport, the number and distribution of both types of ova in the genital tract were compared at appropriate intervals in animals in the same physiological condition. This was achieved by artificial insemination of animals with dead or live spermatozoa. A marked difference in the oviductal phase of transport of embryos and unfertilized ova was observed in mouse and hamster, whereas in the rat, the influence of the ova was better recognized in the uterine phase. This suggested that in the rat only the uterus can recognize ovum signals, or that only advanced preimplantation embryos can produced them. The rat oviduct sensitivity was tested by transferring to it fertilized or unfertilized hamster ova while the influence of embryo development was assessed by transferring rat embryos of different developmental stages to the rat oviduct on day one of pregnancy. Fertilized hamster eggs and more developed rat embryos advanced the arrival of native and foster ova into the uterus. It is concluded that: The rat oviduct, like the hamster and mouse oviduct, has built-in mechanisms that allow differential transport of embryos with different biological properties. The properties relevant to this differential transport appear earlier in hamster and mouse than in rat embryos. Some embryos might provide a local signal that makes the oviduct or the utero-tubal juction advance or delay their passage to the uterus. Further experiments are needed to disclose the nature of the egg signals as well as the mechanisms that allow the genital tract to recognize them.

INTRODUCTION

Fertilized and unfertilized ova are transported at different rates in bats and horses (1, 2, 3), suggesting that the embryo may regulate its transport to the site of implantation. In both species, unfertilized ova are usually retained in the oviduct and only developing embryos are transported into the uterus. In all other mammals so far studied, both types of eggs enter the uterus. Notwithstanding, it is conceivable that they enter the uterus at different times. We have examined this possibility in rodents (4, 5, 6, 7), and this report will focus on our results in rat, hamster and mouse.

The transport of fertilized ova in rodents normally occurs in pregnant animals whereas the transport of unfertilized ova is restricted to pseudopregnant and cycling animals. The time course of ovum transport through the genital tract differs significantly in pregnant, pseudopregnant and cyclic animals. This may be attributed not only to differences in the ova but also to differences in estradiol and progesterone serum levels which differ from one condition to another since the first days after ovulation (8, 9). Therefore, the influence of ova on their transport through the genital tract, must be determined by comparing the transport of biologically different ova in animals under the same physiological condition. With this purpose we have used two experimental models:

In the first, females in the same condition were inseminated with fertile or infertile spermatozoa (4, 5). With this model, we have examined the influence of different kinds of ova on their transport through the genital tract in rat, hamster

and mouse. In some cases, this comparison was possible in the same animal, since after inseminating fertile spermatozoa into one uterine horn, and infertile ones into the contralateral horn, embryos were transported by one side and unfertilized ova by the other.

In our second model, we transferred ova from the oviduct of one animal into the oviduct of another (6, 7). According to the condition of the donor, ova at different developmental stages, or even from different species, could be transported simultaneously by the same oviduct.

Using these models we have examined the influence of different kinds of ova on their transport through the genital tract or the conditions under which the oviduct recognizes the presence of a developing embryo.

MATERIAL AND METHODS

Animals: The animals were kept in an animal house with food and water ad libitum, at temperatures ranging between 21 and 24°C Rats and hamsters had lights on from 07.00 to 21.00 h; and mice from 07.00 h to 18.00 h.

Rats: Adult Sprague Dawley rats were used. Males were 4 to 5 months old, weighed 350 to 400 g, and were of proven fertility. Females were selected among those having at least two regular cycles of 4 days. The regularity of the cycles was verified by daily vaginal smears. The day of estrus was considered as day one of the cycle. To obtain pregnant females, they were housed overnight with males, on the day of proestrus. On the following day, mating was verified by the presence of spermatozoa in the vaginal smear. This was considered as day 1 of pregnancy.

Hamsters: Adult golden hamsters were used. Males were 4 to 5 months old, weighed 150 g, and were of proven fertility. Females were selected among those having at least two regular 4-day cycles. Regularity was verified by examining the vaginal discharge during the morning of the day of estrus, considered as day one of the cycle. To obtain pseudopregnant females, they were mated with vasectomized males, in the evening of day 4 of the cycle. The following day was considered as day one of pseudopregnancy. Under these conditions pseudopregnancy was obtained in over 95% of the females.

Mice: Adult animals of the Swiss Rockefeller strain were used. Males were 4 to 5 months old, weighed 30 to 35 g and were of proven fertility. Females were 2 months old and weighed 20-25 g.

Techniques

Obtention of spermatozoa: Spermatozoa were obtained from the cauda epididymis of adult males, killed by decapitation or cervical dislocation. They were collected in sterile saline at 37°C. 0.9% NaCl was used for rat spermatozoa, TALP (10) for hamster, and PB-1 (11) for mice. To obtain dead spermatozoa, an aliquot of each sample was frozen and thawed.

Insemination: For insemination, rats were anesthetized with ether, and hamster and mice with 0.5 ml/kg, i.m. Hypnorm (fluanisone 10 mg plus phentanylcitrate 0.315 mg/ml. Janssen Pharmaceutica / Beerse / Belgium) supplemented with ether. The uteri were exposed through medioventral incisions and the sperm suspension was injected into the upper third of each horn. Sham-operated females received the corresponding saline in each horn (100 μl for mice, and 200 μl for rats and hamsters).

Ova recovery from donors: Animals were decapitated, the oviducts were removed free of fat tissues, and flushed with sterile saline at 37°C containing 0.4 mg/ml bovine serum albumin. BMOC-2 (12) was used for rat embryos and PB-1 for hamster embryos. Flushings were examined under low power magnification to assess the general condition and development of the eggs recovered. One-cell embryos with two pronuclei and free of follicular cells were selected from rats and hamsters on day one of pregnancy (P1). Embryos of 2, 3 and 4 cells were selected from rats on day 3 of pregnancy (P3) and eggs of 1 cell also free of follicular

cells from hamster on day one of the cycle (C1). Only ova of normal appearance were included.

Transfer technique: Ova were transferred to the oviductal lumen using the same microsyringe system and procedure previously described (13). Transfers were made under a surgical microscope (OPMI 6-SDFC, Zeiss, Germany) in the evening of P1. The oviduct and the ovary were exposed through flank incisions made under sodium pentobarbital anesthesia (20 mg/kg, i.p.) Blood vessels in the periovarial sac were cauterized with an electric coagulator (Codman CMC-1, Codman and Shurleff, Inc., Randolph, MA) before the sac was cut open to expose the fimbria. The microsyringe, previously filled with 10 embryos of the same condition, was introduced through the ostium 1-2 mm down the infundibulum, where the embryos were released. The microsyringe was examined under low magnification to verify if there were remains of crushed ova. Some females had to be discarded after verifying that the microsyringe contained remains of crushed ova and zonae pellucidae. We choose to transfer 10 embryos in each side to provide sufficient data points. The periovarial sac was replaced around the ovary, the organs were returned to the peritoneal cavity, and muscles and skin were sutured.

Assessment of transport: Rats were killed with an overdose of ether, and hamster and mice by cervical dislocation. Oviducts and uteri were removed free of fat tissue. On separating oviduct from uterus, care was taken to leave the intersticial segment attached to the oviduct. Oviducts and uterus were flushed separately with saline (0.9% NaCl) and the flushings were examined under low-power magnification to assess the number and condition of the eggs recovered from each segment of the genital tract. In experiments where hamster eggs were transferred to the rat oviduct, these were readily distinguished from the native eggs. On the other hand, when rat embryos were transferred to the oviduct, transferred eggs could be distinguished from the native ones when the former were in a more advanced developmental stage.

Statistical analysis: Differences between groups in the total number of eggs recovered from the genital tract, in the percentage of eggs recovered from the oviduct and in the number of embryos were analyzed by the Kruskal-Wallis test (14). Comparisons were made with an analysis of variance applied to data or transformed percentages.

RESULTS AND DISCUSSION

Influence of egg condition on egg transport in rat, hamster and mouse

It was previously shown that the influence of the egg condition in pseudopregnant rat, was slight and more evident in the uterus than in the oviduct (4) while, in cycling hamster, the egg condition markedly influenced its oviductal and uterine transport (5). These observations disclosed possible differences between both species or the physiological condition, therefore the transport of biologically different ova was assessed in other physiological conditions in rats and hamsters.

Rat: To determine whether the egg influences its transport in cycling rats in the same way as in pseudopregnant ones (4) the number, condition, and distribution of eggs in the genital tract were determined at different times after ovulation in cycling rats previously inseminated with fertile or infertile spermatozoa. Proestrous rats were alloted to different groups: 1) intact; 2) sham-operated; 3) inseminated with infertile spermatozoa; 4) inseminated with fertile spermatozoa; 5) inseminated with infertile spermatozoa and re-operated in the morning of the third day to place a ligature in each uterine horn; 6) inseminated with infertile spermatozoa in one uterine horn and with fertile spermatozoa in the contralateral horn. Rats were inseminated in the night of proestrous (23.00 to 24.00 h) approximately 6 h before ovulation, and were autopsied on day 4 of the cycle (next proestrus).

Sixty to one hundred percent of ova recovered from the genital tract of females inseminated with fertile spermatozoa, were

morulae of normal aspect, whereas all ova recovered from females inseminated with infertile spermatozoa were cytolized or fragmented resembling those recovered from intact or sham-operated females.

Fig. 1. shows the number of eggs recovered from oviduct and uterus at 09:30 and 14:30 h of day 4 of the cycle from groups 1, 2, 3, and 4. At 09:30 h no difference was observed among groups. In all of them, most eggs were found in the oviducts; although the total number of eggs was higher in animals inseminated with fertile spermatozoa, this difference was not significant. At 14:30 h, the number of ova recovered from the oviduct had decreased and this was accompanied by a proportional increase of eggs recovered from uterus in animals inseminated with fertile spermatozoa. In this group, the mean number of ova recovered from uterus was significantly higher than in animals inseminated with infertile spermatozoa, indicating that embryos remained in the uterus for a longer time than unfertilized ova.

The mean number of ova recovered from the genital tract in rats whose uterine horns had been ligated after insemination with infertile spermatozoa (group 5) was significantly higher than those without ligated uterine horns (Fig. 2), indicating that the decrease in the number of non-fertilized eggs is due to an expulsion from the genital tract.

Fig. 3. shows the number of ova recovered from oviduct and uterus at 14:30 h on day 4 of the cycle in group 6. The mean number of ova recovered from the genital tract was significantly higher on the side inseminated with fertile spermatozoa, indicating that while unfertilized ova had been expulsed on one side of the genital tract, embryos had been retained in the contralateral side. These results show that in cycling rats –like in pseudopregnant ones– the embryo affects more evidently the uterine than the oviductal phase of transport.

Hamster: To determine whether the egg has an influence on its transport in pseudopregnant hamster as in cycling females (5), the number, condition and distribution of

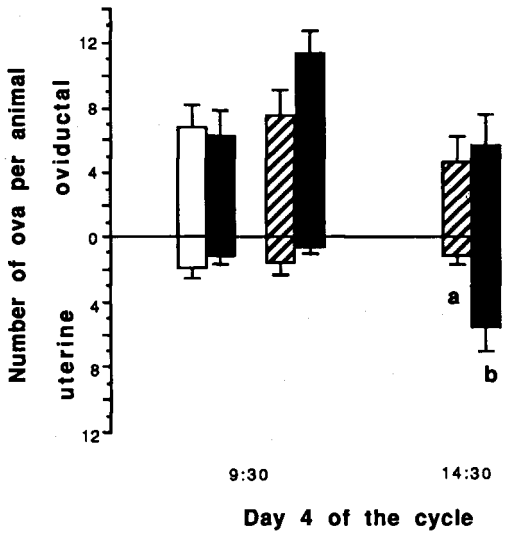

Fig. 1: Number of ova recovered from the genital tract at 09:30 and 14:30 h on day 4 of the cycle, from rats. Undisturbed (Open bar) sham-inseminated (Shadowed bar) inseminated with infertile spermatozoa (Hatched bars) inseminated with fertile spermatozoa (Solid bars). Day 1 = day of ovulation. Each bar represents the mean ± SEM of 8 animals. Note a statistically significant decrease ($a \neq b$ $p < 0{,}01$) in uterine ova recovered at 14:30 h in the group inseminated with infertile spermatozoa.

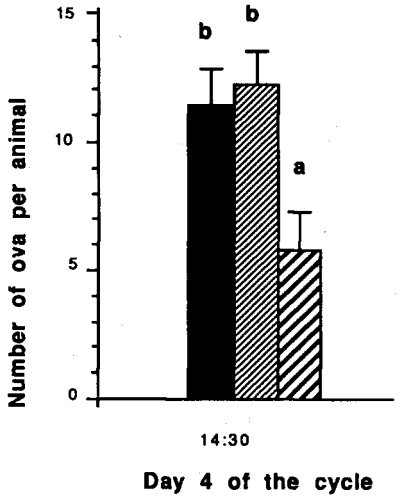

Fig. 2: Mean number of ova recovered from the genital tract on Day 4 of the cycle, from rats. Inseminated with fertile spermatozoa (Solid bar) inseminated with infertile spermatozoa and with uterine horns ligated after insemination (Light hatched bar), inseminated with infertile spermatozoa (Hatched bar). Each bar represents the mean ± SEM of 8 animals. Note statistically significant difference ($a \neq b$ $p < 0.05$) between ligated and nonligated animals inseminated with infertile spermatozoa.

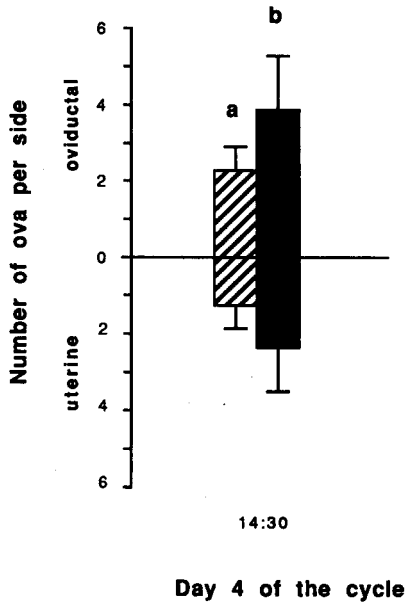

Fig. 3: Mean number of ova recovered from either side of the genital tract of 8 rats at 14:30 h on day 4 of the cycle. Side inseminated with infertile spermatozoa (Hatched bar). Side inseminated with fertile spermatozoa (Solid bar). Note statistically significant decrease in the number of ova recovered ($a \neq b$ $p < 0.05$) in the side inseminated with infertile spermatozoa.

eggs in the genital tract was determined at different times after ovulation in pseudopregnant hamster inseminated with fertile or infertile spermatozoa. Pseudopregnant animals were allocated to different groups: 1) intact; 2) sham-operated; and 3) inseminated with fertile spermatozoa in one horn and with infertile spermatozoa in the contralateral. Females were inseminated at 06:00 h on day 1 of pseudopregnancy. 1 or 2 h after ovulation. Animals of groups 1 and 2 were killed at 17:00 h and those of group 3 at 17:00 h, 20:00 h and 24:00 h on day 3 of pseudopregnancy. The total number of ova recovered from the genital tract, and the number of ova from each side were similar in all groups (Table 1).

In females of group 3, most of the eggs recovered from the side inseminated with fertile spermatozoa were embryos of normal aspect, whereas those recovered from the side inseminated with infertile spermatozoa were non-fertilized oocytes, resembling those recovered from intact or sham-operated females. The proportion of embryos was: 81%, 87%, 93% and 98% in animals autopsied at 17:00 h, 20:00 h, 22:00 h and 24:00 h respectively.

The distribution of ova between oviduct and uterus in sham-operated females (group 2) was different from that of intact females (group 1). In intact females most of the eggs were recovered from the uterus while in sham-operated females most ova were in the oviduct, indicating that the insemination procedure delayed the passage of ova from oviduct to uterus. It is therefore necessary in this species to compare the distribution of different ova in both sides of the same animal, as shown in Fig. 4 for group 3. The percentage of eggs recovered from the uterus was progressively higher with time, particularly in the side inseminated with fertile spermatozoa.

TABLE 1

Number of ova recovered from the hamster genital tract at different times on day 3 of pseudopregnancy

Groups	Time of autopsy	N° of animals	N° of ova (x ± SEM) Total	Side 1*	Side 2*
Undisturbed	17:00	12	9.4 ± 0.9	4.6 ± 0.6	4.8 ± 0.6
Sham Ins.	17:00	10	10.6 ± 1.1	6.2 ± 0.8	4.4 ± 0.9
Inseminated	17:00	13	11.9 ± 0.7	5.2 ± 0.7	6.8 ± 6.6
Inseminated	20:00	10	12.2 ± 1.6	6.8 ± 1.5	5.4 ± 0.6
Inseminated	22:00	14	10.8 ± 0.7	4.9 ± 0.5	6.0 ± 0.6
Inseminated	24:00	8	13.8 ± 0.6	6.6 ± 1.0	7.3 ± 0.8

* In inseminated groups, Side 1 had been inseminated with spermatozoa rendered infertile by freezing and thawing and Side 2 with fertile spermatozoa.

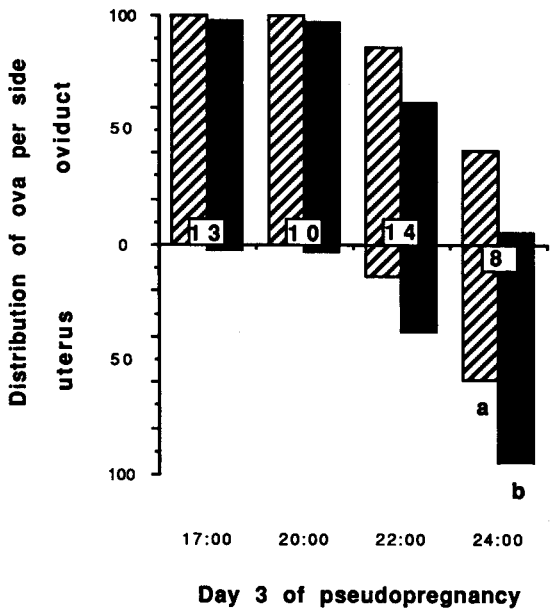

Fig. 4: Time of passage of fertilized (Solid bars) and unfertilized oocytes (Hatched bars) into the uterus in pseudopregnant hamsters. Animals were inseminated with fertile spermatozoa in one uterine horn and with infertile spermatozoa in the opposite horn so that fertilized and unfertilized oocytes were transported in different sides of the genital tract. The percentage of ova recovered from each segment at the specified times is indicated. Figures at the foot of each bar indicate the number of animals autopsied at each time of day 3 of pseudopregnancy. Day 1 = day of ovulation and insemination. Note a statistically significant difference ($a \neq b$ $p < 0.05$) between sides, in the proportion of eggs recovered from oviduct and uterus at 24:00 h.

This difference was significant at 24:00 h, indicating that in pseudopregnant females the embryos enter the uterus before unfertilized eggs. Further analysis of egg distribution at 22:00 h on the side inseminated with fertile spermatozoa showed that the majority of ova found in the uterus were advanced morulae whereas most unfertilized eggs and less developed embryos were still in the oviduct.

These results showed that in pseudopregnant as well as in cycling females the influence of the egg is recognized as an earlier passage of embryos to the uterus in comparison with unfertilized eggs. However the difference was less evident in pseudopregnant than in cycling females. This may be explained by the delay in ovum transport caused by sham operation and/or insemination in the morning of day 1 of pseudopregnancy, an effect not observed in cycling animals inseminated in the night of day 4 before ovulation.

In conclusion, in rat and hamster the influence of the ovum on its transport through the genital tract is noticeable in cycling and pseudopregnant animals but differs between the two species.

Mouse: A possible influence of ova on their transport was explored in the mouse by comparing the transport of fertilized and unfertilized ova in mice inseminated with fertile or infertile spermatozoa.

In the cycling mouse, vaginal smears do not allow an accurate prediction of the time of ovulation, hence ovulation was induced by injecting (i.p.) 5 IU PMS followed by 5 IU HCG 45 h later. Injection of HCG at 09:00 h was considered as time zero of the experiment. Treated mice were allocated to 4 groups: 1) intact; 2) sham operated; 3) inseminated with infertile spermatozoa; 4) inseminated with fertile spermatozoa. Females were inseminated between 8 and 10 h after HCG injection, and autopsied 54 and 60 h afer HCG, to determine the number and condition of the ova in the genital tract.

The number of ova recovered from the genital tract was similar among goups, the means ± SEM ranged from 21.3 ± 3.5 to 31.1 ± 6.4.

In females inseminated with fertile spermatozoa, most of the ova were 2 to 4-cell embryos at 54 h, and 4 to 6-cell embryos at 60 h. The proportion of embryos with normal appearance was 78% and 84% at 54 and 60 h respectively. In intact females, with sham operation or inseminated with infertile spermatozoa, the ova recovered were cytolized or fragmented.

As shown in Fig. 5, at 54 h, 40% of the ova were recovered from the uterus in intact, sham operated females, and in females inseminated with infertile spermatozoa while, in those inseminated with fertile spermatozoa, 100% of the ova were still in the oviduct. At 60 h, when 50% of the ova in females inseminated with infertile spermatozoa were in the uterus, only 15% of the ova had reached that segment of the genital tract in females inseminated with fertile spermatozoa.

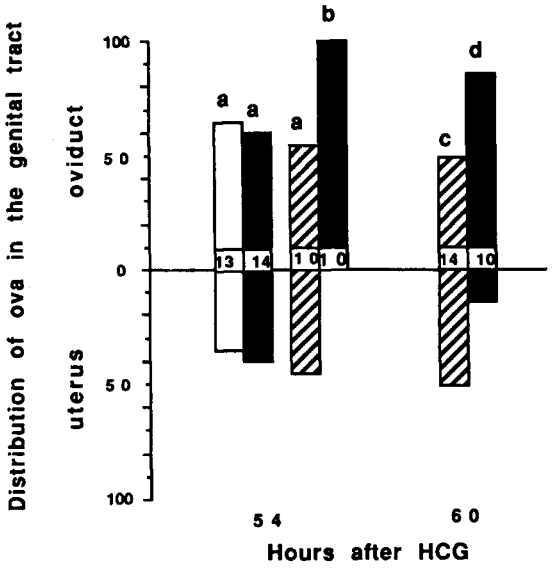

Fig. 5: Proportion of ova recovered from the oviduct and uterus at 54 and 60 h after HCG, from mice. Undisturbed animals (Open bar), sham-inseminated (Shadowed bar), inseminated with infertile spermatozoa (Hatched bars), inseminated with fertile spermatozoa (Solid bars). Animals were inseminated between 8 and 10 h after HCG injection. Figures at the foot of each bar indicate the number of animals autopsied at specified intervals after HCG. Note a statistically significant difference in the distribution of ova in the genital tract ($a \neq b$ $p < 0.01$) and ($c \neq d$ $p < 0.005$) between animals inseminated with fertile spermatozoa and the other groups.

Thus, at 54 h, the distribution of ova between oviduct and uterus was similar in groups transporting unfertilized ova. In these groups the same proportion of ova had entered the uterus, indicating that neither surgery nor insemination with infertile spermatozoa had modified the transport of non fertilized ova. The distribution of ova in group 4 was different from that of the other groups both at 54 and 60 h. The difference was recognized as a delay in the passage of the embryos into the uterus.

It is concluded that the mouse embryo also exerts an influence on its oviductal transport, recognized as a later passage into the uterus in comparison with unfertilized ova.

Influence of hamster ova and of embryo development on oviductal transport in the rat

The rat oviduct sensitivity to embryonic signals was examined by transferring fertilized or unfertilized hamster ova to the rat oviduct (7) and the influence of embryonic developmental stage on oviductal transport was examined by transferring rat embryos in different developmental stages to the rat oviduct (6).

To find out whether the influence of hamster ova on its oviductal transport is also exerted in the rat oviduct, hamster ova were transferred to the rat oviduct. The donors were hamster on day 1 of the cycle or pregnancy and the recipients were rats on day 1 of pregnancy allocated at random to 4 groups: 1) intact; 2) sham-operated; 3) recipients of unfertilized eggs recovered from hamsters on day 1 of the cycle; 4) recipients of fertilized eggs recovered from hamsters on day 1 of pregnancy. Transfers were made in the evening of day 1 of pregnancy and autopsies were practised at 15:00 h on day 4. Intact, sham operated or recipients of unfertilized hamster ova, had 100% or most of the eggs in the oviduct whereas recipients of fertilized hamster eggs, had at least 50% of the ova in the uterus (Fig. 6). These results suggest that the rat oviduct is sensitive to the influence of hamster ova on their transport.

To investigate the possible influence of advanced rat embryos on their oviductal transport, pronuclear ova or 4-cell embryos were transferred to the rat oviduct. The donors were rats on day 1 or 3 of pregnancy and the recipients were rats on day 1 of pregnancy allocated at random to 4 groups: 1) intact; 2) sham-operated; 3) recipients of fertilized eggs at the pronuclear stage recovered from animals on day 1 of pregnancy; 4) recipients of 4-cell embryos recovered from animals on day 3 of pregnancy. Transfers were made in the evening of day 1 of pregnancy and autopsies were practised at 15:00 h on day 4. Intact, sham operated or recipients of fertilized eggs at the pronuclear stage, had 100% or most of the eggs in the oviduct whereas recipients of 4-cell embryos, had at least 30% of the ova in the uterus (Fig. 7). These results, suggest that the more developed embryos provide local signals that drive the oviduct or the utero-tubal

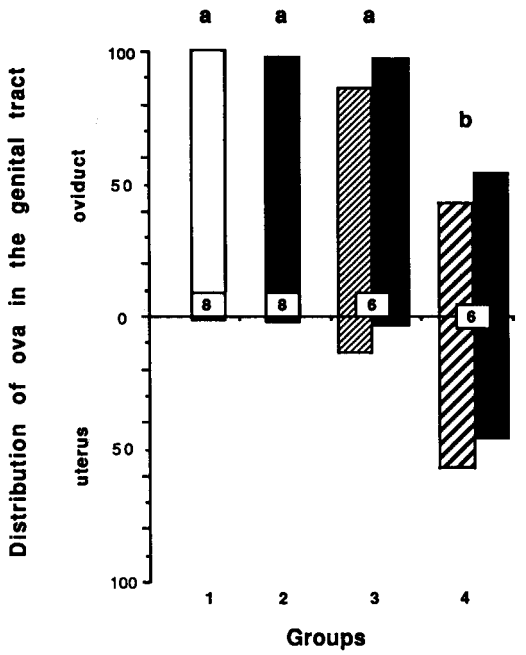

Fig. 6: Percent distribution of ova in the genital tract at 15.00 h on Day 4 of pregnancy, in rats. Open bar: undisturbed rats (Group 1). Shadowed bar: sham transferred rats (Group 2). Light hatched and solid bars: transferred hamster oocytes and native rat embryos respectively (Group 3). Hatched and solid bars: transferred hamster embryos and native rat embryos respectively (Group 4). Hamster ova were transferred to the rat oviducts on day 1 of pregnancy. The number of animals in each group are indicated at the foot of the bar. The percentage of uterine ova in animals transporting hamster embryos (Group 4) is different from the percentage of the other groups ($a \neq b$ $p < 0.001$).

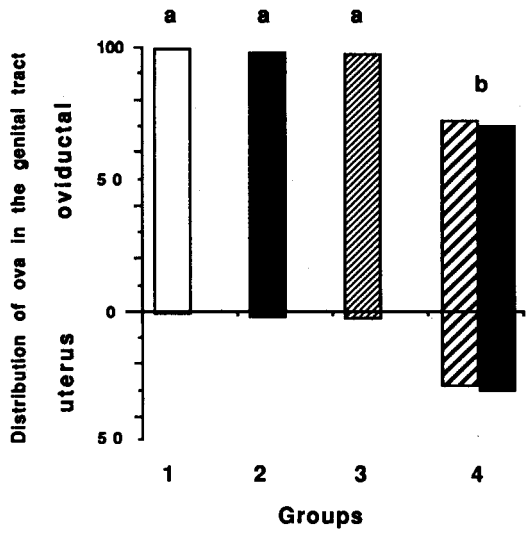

Fig. 7: Percent distribution of ova in the rat genital tract at 15.00 h on Day 4 of pregnancy. Open bar: undisturbed rats (Group 1). Shadowed bar: sham transferred rats (Group 2). Light hatched bar: native embryos and transferred rat 1-cell embryos (Group 3). Hatched and solid bar: transferred rat 4-cells embryos and native embryos respectively (Group 4). The percentage of uterine embryos in animals transporting more advanced rat embryos (Group 4) is different from the percentage of the other groups ($a \neq b$ $p < 0.001$).

junction in a way that advances their passage to the uterus.

Because in the presence of fertilized hamster eggs or more developed rat embryos the arrival of native and foster ova into the uterus was advanced, we postulate that: 1) the rat oviduct has built-in mechanisms that allow differential transport of embryos of different biological properties. 2) The egg properties that are relevant to this differential transport appear earlier in hamster embryos as compared to rat embryos. 3) Some foster ova may provide local signals that drive the oviduct or the utero-tubal junction in a way that advances the passage of other ova to the uterus. 4) Further experiments are needed to disclose the nature of the eggs signals as well as the mechanisms that allow the genital tract to recognize them.

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