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Fertilizability of Ova Ovulated and Recovered from Rabbit Ovaries Perfused in vitro

Abstract. *Ovaries removed from New Zealand White rabbits were perfused and exposed to gonadotropin in vitro. The ova ovulated in vitro (N = 56) were recovered and cultured and then transferred to the oviducts of six previously mated Dutch Belted hosts. Twelve of the resulting 36 offspring (33.3 percent) were white. In control matings between 12 Dutch Belted females (six randomly selected and the six hosts) and New Zealand White males, only one of 80 (1.2 percent) offspring was white. These data indicate that ova ovulated in vitro can be transferred to the oviduct of a host rabbit where they may be fertilized and after implantation may develop into viable embryos.*

Perfusion of the rabbit ovary in vitro has served as a valid model for studying mammalian ovulation. Serial observation of the final stages of follicle development and disruption facilitates assessment of the effects of various agents added to the perfusate which may have a direct ovarian action (*1–6*). The effluent can be serially sampled for determination of substances produced by the ovary during the ovulatory process (*7*). The stage of maturity of ova recovered immediately after ovulation in vitro can be correlated with the time interval from gonadotropin stimulation to the occurrence of ovulation (*8*). Ovulation in the rabbit occurs within 12 hours after the administration of human chorionic gonadotropin (hCG) or mating (*9*). In the study described here we determined that ova extruded from ovaries in vitro achieve the same degree of maturation after exposure to gonadotropins in vitro as they do in vivo and that such ova are capable of being fertilized in vivo.

Rabbits were isolated for a minimum of 3 weeks with controlled temperature and light and were given free access to a diet of Purina Rabbit Chow and water. Ovaries from sexually mature virgin New Zealand White (NZW) rabbits served as a source of ova. Sexually mature virgin Dutch Belted (DB) female rabbits were used as hosts for ova ovulated in vitro. The characteristic black fur of the DB rabbit serves as a marker to distinguish DB offspring of the host rabbit from those of the NZW rabbits which were used as ovum donors. Male NZW rabbits of proved fertility served as inseminators.

Both ovaries of untreated NZW female rabbits were removed after arterial cannulation at laparotomy and perfused individually according to the operative procedure and perfusion technique described previously (*1, 6, 8*). At the onset of perfusion 100 IU of hCG was added to the perfusion fluid of all ovaries. This dose promotes follicular development, follicular rupture, and ovum maturation consistently in this system (*7, 8*). Perfusion was carried out in a constant temperature room (37°C) maintained at 100 percent humidity. Ovaries were observed continually for follicle development and ovulation. Follicles that continued to grow throughout perfusion and achieved a diameter of greater than 1.5 mm were considered mature. Ovulation in vitro was characterized by disruption of the follicle wall and extrusion of the ovum surrounded by cumulus cells.

The ovulatory efficiency, defined as the percentage of mature follicles which rupture by 12 hours after hCG administration, of the 14 perfused ovaries used in this experiment was 75.5 ± 2.2 percent (mean ± standard error); the mean time of ovulation was 6.03 ± 0.29 hours after the addition of hCG. These data are consistent with previous observations (*8*).

Each ovum was aspirated by Pasteur pipette from the surface of the ovary immediately after ovulation. Ova were washed three times in Brackett's defined medium and cultured according to the method of Brackett *et al.* (*10*) until 12 hours had elapsed from the initial time of exposure to hCG in vitro. At 12 hours after hCG addition, ova were removed from the culture dish together with a small amount of culture medium for transfer to the host rabbit.

The time period selected for ovum culture after hCG treatment was based on an experiment in which 20 NZW ovaries were perfused as described above. Seventy-eight ova were cultured after ovulation in vitro until a total of 12 hours had elapsed from the time of hCG administration. All ovulations in vitro occur by 12 hours after exposure to hCG (*8*). Ova were microscopically examined for stage of maturity. Ova demonstrating cytolysis, necrosis, vacuolation, or loss of spherical shape were classified as degenerated. Of the ova treated in this fashion, 1.3 percent contained an intact germinal vesicle with no degeneration, 15.4 percent achieved metaphase 1 (germinal vesicle breakdown) with no degeneration and 51.3 percent were at metaphase 2 (first polar body extruded) and had not degenerated. The 12-hour interval from hCG administration to transfer was therefore selected to yield a high percentage of mature ova (metaphase-2 ova) to be transferred to the host rabbits for fertilization in vivo.

A DB female host was mated with an NZW buck at the same time as each ovarian perfusion was begun (Fig. 1). The vagina of the DB host was examined 8 hours after mating to confirm the presence of sperm. Twelve hours after mating, each host underwent a laparotomy in preparation for transfer of ova from the donor rabbit. Ova were transferred from the culture dish to both oviducts of the host by means of a glass pipette in which the flow was controlled by a screw mechanism to prevent leakage of fluid and loss of ova. The pipette was inserted into the ampulla of the oviduct and the ova, in approximately 20 μl of Brackett's medium, were gently deposited into each oviduct. The number of ova transferred in each experiment corresponded to the number of ova recovered from the donor

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ovary after ovulation in vitro (range two to eight). The number of ovulation points observed on each ovary of the host was also recorded. After laparotomy, each rabbit received intramuscular penicillin G benzathine (Bicillin 300,000 units, Wyeth).

Fourteen days after ovum transfer, each host rabbit underwent a second laparotomy for affirmation of pregnancy. At this time notation was made of the number, size, and location of implantations in each uterine horn. At 32 days after mating, cages were observed frequently for presence of offspring. Color of fur and number of offspring were recorded.

Seven DB hosts were used in the transfer experiments. Only six of these are considered in the results. One animal failed to deliver its litter despite pregnancies observed in both uterine horns at the time of laparotomy on day 14. Upon reexploration, signs of pregnancy had regressed, suggesting fetal resorption. Fifty-six ovulated ova from the NZW rabbit ovaries perfused in vitro were transferred to six DB female hosts. Of 36 offspring born to these rabbits, 12 (33.3 percent) were completely white. If the assumption is made that all of the white offspring were derived from NZW rabbit ova ovulated in vitro, 12 of the 56 (21.4 percent) transferred ova resulted in offspring.

Purebred DB females (1.4 to 1.8 kg) are unable to mate with NZW males (3.7 to 4.0 kg) by virtue of the size difference. Because of the possibility that the large DB females (2.1 to 2.8 kg) required for mating in this experiment may not have been genetically pure, two additional experiments were carried out. These experiments were designed to confirm that when white offspring resulted from the transfer of NZW ova ovulated in vitro to DB hosts mated to a NZW inseminator, these white offspring were derived from the NZW ova ovulated in vitro rather than from DB ova ovulated in vivo. Therefore, in the first control group, randomly selected DB females were mated with NZW males used in the original experiment. Each DB female underwent a laparotomy 12 hours after mating for the purpose of examining the ovaries for ovulation points. An additional laparotomy was performed 14 days after mating to examine the uteri for implantation sites. At the time of delivery, color of fur and number of offspring were recorded. In six matings, one of 33 (3.0 percent) offspring was white. In the second control experiment, the same DB hosts as used in the original experiment were remated with the NZW inseminators to which they had been mated in the original experiment. These animals were not subjected to exploratory laparotomies to confirm pregnancy. All 47 offspring resulting from these six matings were black.

The percentage of white offspring in the transfer experiments was significantly greater than the percentage of white offspring from the randomly mated group ($P < .005$) and the remated group ($P < .0005$). After ova transfer to the host rabbits, 37 implantations were observed in association with 37 ovulation points (100 percent). This ratio is significantly greater than the ratio of implantations to ovulations (85 percent) in the random matings ($P < .05$). Furthermore, in three of six host rabbits the number of implantations exceeded the number of ovulation points observed on the ovaries of the host rabbit at the time of transfer, indicating that some of the pregnancies in these rabbits resulted from the transfer of ova ovulated in vitro.

Only one white live-born offspring (male) from the transfer experiments survived; all others were rejected by the mother after birth as frequently happens under laboratory conditions.

Our experiments suggest that the ovulatory process of rabbit ovaries in vitro is similar to that in vivo. The observation that ova ovulated in vitro can be fertilized and develop after implantation in a host further demonstrates that the rabbit ovary perfused in vitro serves as a valid model for studying ovulatory physiology.

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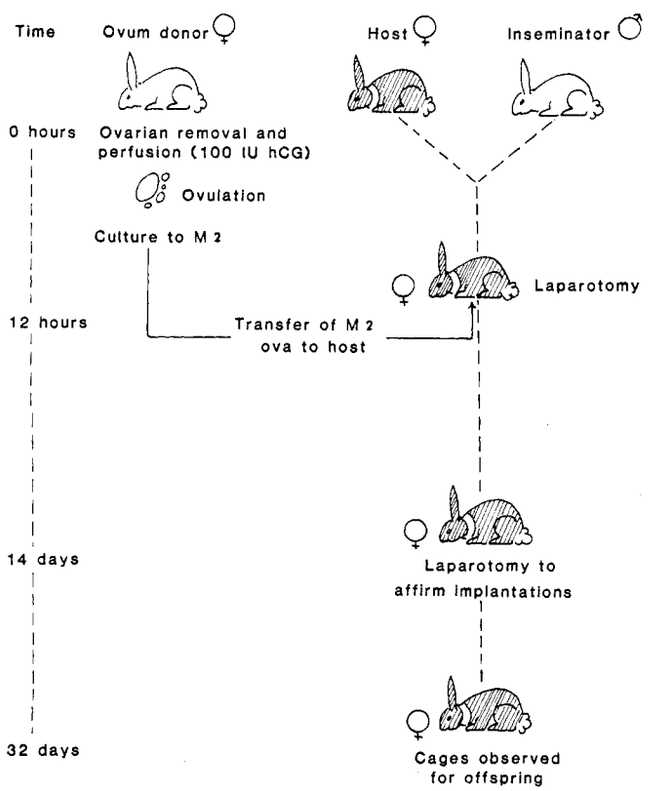

Fig. 1. Experimental design and time sequence for fertilization experiments in vivo, including the transfer of NZW ova ovulated in vitro to DB hosts (*M 2*, metaphase 2).

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