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Fertilization of Squirrel Monkey and Hamster Ova in the Rabbit Oviduct (Xenogenous Fertilization)

Abstract. *Homologous sperm and ova of either squirrel monkeys or hamsters were placed in the oviducts of pseudopregnant rabbits. Xenogenous fertilization rates of 36 and 60 percent were obtained for squirrel monkey and hamster gametes, respectively.*

The homologous mammalian oviduct provides the optimal environment for fertilization, but the oviduct of a pseudopregnant rabbit is able to support the development of embryos of foreign species (*1*). Additionally, the porcine oviductal environment can support fertilization of bovine ova by bovine sperm (*2*). In a preliminary study, we have reported the potential capacity of the pseudopregnant rabbit oviduct to support fertilization of squirrel monkey (*Saimiri sciureus*) ova (*3*). The present study was designed to test whether the oviduct of a pseudopregnant rabbit could support fertilization of hamster (*Mesocricetus auratus*) and squirrel monkey ova.

We initially transferred hamster oviductal ova (flushed from the excised oviduct with 0.2 ml of medium) in 5 μl of Medium 199 (Gibco) with added newborn calf serum (Agamma) in a ratio of 4:1 (*4*) to the fimbriated end of the rabbit oviduct (surgical transfer) 2 to 9 days after the rabbit received 100 international units (I.U.) of human chorionic gonadotropin (hCG) to induce ovulation and subsequent pseudopregnancy. Sperm from the minced cauda epididymis were added in 0.05 ml of medium to the oviduct. Fertilization was assessed only by the percentage of ova cleaved to the two-cell stage when recovered 30 hours later. Under these conditions, 9 of 66 (or .14) ova were fertilized. In subsequent trials with rabbits of 1, 2, 4, or 10 days of pseudopregnancy and mean inseminated sperm concentration of 2.83×10^8 sperm per milliliter (range, 4.51×10^6 to 8.96×10^8 sperm per milliliter), ova were recovered and examined for evidence of fertilization of either (i) the presence of two pronuclei and two polar bodies or (ii) cleavage of the fertilized ovum.

Squirrel monkey ova were collected by laparoscopically aspirating the developed follicles on the ovary (*5*). Follicular growth was induced with an ovulatory regimen of gonadotropins (*6*), and sperm were collected by electroejaculation (*7*), with mean inseminated sperm concentration of 4.60×10^7 sperm per milliliter (range, 5.00×10^6 to 2.00×10^8 sperm per milliliter). The gametes were then transferred to the rabbit oviduct. The monkey trials were all carried out with rabbit surgery scheduled 24 hours after intravenous injection of 100 I.U. of hCG to the rabbit (1 day of pseudopregnancy). Hamster ova were recovered 28 hours after being deposited by flushing the excised rabbit oviduct with saline through the tubal-uterine junction. Because we suspected slower development on the basis of preliminary trials, we recovered monkey ova by the same procedure 72 hours after they were deposited in the rabbit oviduct. The recovery and xenogenous fertilization rates are shown in Table 1. In all, 16.3 percent of the hamster ova reached the two-cell stage, a value comparable to the preliminary trials.

The overall recovery rates of hamster and squirrel monkey ova were 33 and 55 percent, respectively, and fertilization rates were 60.1 and 36.4 percent. Xenogenously fertilized hamster embryos did not develop beyond the two-cell stage in the pseudopregnant rabbit oviduct, but squirrel monkey embryos developed as far as the eight-cell stage. In one instance a squirrel monkey ovum from a Guyanan-type female (seven acrocentric pairs of chromosomes; point of

Table 1. Xenogenous fertilization of hamster and squirrel monkey ova in oviducts of the pseudopregnant rabbit. Proportions are given in parentheses.

Species	Day of rabbit pseudo-pregnancy	Ovum donor/ rabbit	Embryos recovered	Fertilization
Hamster	1	20/10	108/372 (.29)	59/108 (.55)
Hamster	2	2/1	50/75 (.67)	36/50 (.72)
Hamster	4	2/1	23/58 (.40)	12/23 (.52)
Hamster	10	2/1	7/65 (.11)	6/7 (.9)
Squirrel monkey	1	52/10	22/40 (.55)	8/22 (.36)

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export, Georgetown, Guyana) (*8*), was fertilized by sperm from a Bolivian-type male (six acrocentric pairs of chromosomes; point of export, Santa Cruz, Bolivia). Four similar types of "hybrid" embryos have previously been produced in our laboratory from in vitro fertilization. One live birth has occurred of a "hybrid" squirrel monkey as a result of a natural mating.

With this demonstrated success with primate and hamster ova, xenogenous fertilization may be an alternative method for in vitro fertilization of a variety of species, including humans, and may be more effective and represent less risk to the embryo. It also may be useful as a technique for assessing the fertilizability of sperm samples independent of the need for in vitro or homologous in vivo fertilization.

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Therapy of Spontaneous Metastases by Intravenous Injection of Liposomes Containing Lymphokines

Abstract. *Mice of two different strains were injected subcutaneously with spontaneously metastasizing syngeneic melanomas. After 4 to 6 weeks, the local tumors were removed and, 3 days after surgery, treatment of the metastases was initiated. The treatment consisted of intravenous injections of liposomes containing lymphokines or control supernatant fluids. Liposomes were injected twice weekly for 3 weeks, and the mice were killed 2 weeks later. Seventy-three percent of the mice injected with liposomes containing lymphokines were free of metastases, whereas only 10 percent of the mice treated with control liposomes were tumor-free. These experiments suggest that this form of therapy may provide a valuable addition to the more conventional approaches to the eradication of cancer metastases.*

Metastasis, the formation of secondary tumors at sites distant from the primary tumor, is responsible for most failures in cancer treatment. Recent studies suggest that neoplasms are heterogeneous with regard to many phenotypic characteristics, including metastatic potential (*1*), and that metastases may result from the proliferation of a minor subpopulation of cells preexisting within the primary tumor. These studies imply that a successful therapy of metastases will be one that circumvents this problem of cellular diversity between primary cancers and their metastases and among various metastases.

Tumoricidal macrophages distinguish between normal and cancerous cells by some as yet unknown mechanism. Further, the destruction of cancer cells, at least in vitro, occurs independently of such phenotypic characteristics as antigenicity, invasiveness, metastatic potential, and drug sensitivity. Although tumor cells resistant to most other toxic regimens have been described, attempts to select tumor cells that are resistant to macrophage cytotoxicity in vitro have been unsuccessful (*2*).

Normal macrophages can be rendered tumoricidal in vitro by activation with a variety of agents, including macrophage-activating factor (MAF), a lymphokine released by sensitized lymphocytes during their interaction with antigens or mitogens in culture (*3*). Evidence of the effectiveness of tumoricidal macrophages in controlling cancer metastasis in vivo was obtained from studies in which macrophages activated in vitro were injected intravenously into syngeneic mice bearing pulmonary tumors (*4*). This approach, however, has serious clinical limitations, such as the need to transfuse large numbers of autologous or histocompatible macrophages. Although macrophages from tumor-bearing animals can respond to MAF to become tumoricidal (*5*), it would be preferable to activate these cells by delivering the activating agent to them in vivo. Advances in liposome technology (*6*) have provided a mechanism for accomplishing this task. Recent studies demonstrated that MAF encapsulated within liposomes is much more efficient at activating macrophages in vitro than free MAF (*7*). This raises the possibility that liposome-encapsulated MAF might also be highly efficient in activating macrophages in vivo (*8*), thereby providing a new approach for the treatment of metastases.

To test this possibility, we used a variant line of the C57BL/6 B16 melanoma, B16BL-6 (*9*), and a clone of the recently derived C3H K-1735 melanoma (*10*). In a preliminary study, both metastasized to pulmonary and other sites in over 85 percent of mice in which the tumors were grown subcutaneously. Syngeneic mice (C57BL/6 and C3H) were injected subcutaneously in the footpad or external ear with 2.5×10^4 living tumor cells grown in vitro in a volume of 0.1 ml of Hanks balanced salt solution (HBSS). Four or 6 weeks later, the mice injected with B16 or K-1735, respectively, were anesthetized by methoxyflurane inhalation, and the foot or ear, bearing tumors ranging in diameter from 10 to 15 mm, was amputated. Liposome treatment began 3 days later. Each treatment consisted of an intravenous injection of 5 μmole of multilamellar large vesicle (MLV) liposomes suspended in 0.2 ml of HBSS. The liposomes consisted of chromatographically pure egg phosphatidylcholine and bovine brain phosphatidylserine at a 1 : 1 mole ratio and contained either MAF, normal lymphocyte supernatant (NLS), or supplemented Eagle's minimum essential medium (SMEM) (*11*). The liposomes were injected twice weekly for 3 weeks, and the mice were killed 2 weeks after the final injection. The number of pulmonary tumor colonies in each animal was counted in double-blind fashion under a dissecting microscope by two independent observers. All suspected pulmonary and extrapulmonary metastases were confirmed by microscopic examination of fixed histological sections.

Spontaneous pulmonary metastases were well established in the animals at the time liposome treatment was initiated. Many consisted of hundreds of tumor cells. Some superficial metastases could even be detected under a dissecting microscope (Fig. 1a). Without therapy, these metastases developed into macroscopic tumor nodules by the end of the experiment (Fig. 1b). How-

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