DEVELOPMENT

Generation of Live Young from Xenografted Mouse Ovaries

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Assisted reproductive technologies (ARTs) are being used to aid in overcoming reproductive problems and to preserve endangered wildlife. However, high numbers of mature, fertilizable oocytes are often difficult to obtain. The culture of primordial follicles as a method of oocyte maturation has had limited success. A recent study (*1*) has shown that ovarian germ cells can be matured in vitro and give rise to live young, but only if the donor oocyte nucleus is transferred to a mature enucleated oocyte. Xenografting (transfering tissue from one species to a different species) provides an alternative method for the in vivo maturation of oocytes, and in this process, both nuclear and cytoplasmic maturation can be completed.

Ovarian xenografting has provided a valuable experimental tool to study follicular growth in vivo for a range of species including the human (*2–7*) and allows follicular development to the antral stage (*2–4*, *6*, *7*). Whereas female rodents are typically used as recipients of ovarian tissue, the use of male mice as recipients for human ovarian tissue appeared to result in more antral follicles (*7*). However, to date, the viability of the oocytes produced by ovarian tissues xenografted to male and female rodents has never been tested.

This study aimed to determine whether mouse ovarian tissue xenografted into rats would produce fully viable oocytes that are capable of fertilization and development into viable young. Ovaries from 3-week-old FVB mice (white coat color) were halved and xenografted under the kidney capsule of intact adult male (n = 2) or ovariectomized female (n = 7) nude rat recipients (2 to 6 grafts per recipient). Three of the female and both the male rats were given 10IU pregnant mare serum gonadotrophin (+PMSG group) 19 days later. The remaining female rats received no hormone treatment (−PMSG group). Twenty-one days after grafting, germinal vesicle stage (GV) oocytes and expanding cumulus oocyte complexes were collected from the xenografts, matured in vitro for 18 hours, and then inseminated with sperm from FVB male mice. Control groups were included for each of the xenograft groups and, after 24 hours in embryo culture media, two-cell mouse embryos were transferred to suitably synchronized foster mice. The fertility of the resulting pups was assessed when they reached 2 months of age (*8*).

Significantly more ovarian xenografts ($P < 0.05$) were recovered from female (78.9% −PMSG; 83.3% +PMSG) than from male recipients (9.1%). The average number of oocytes recovered per xenograft was 5.7, 3.8, and 10.0 for the female −PMSG, female +PMSG, and male +PMSG groups, respectively. Of these, 40.0, 57.9, and 20.0%, respectively, formed two-cell embryos. The rate of development to two-cell embryos in the female +PMSG xenograft group was comparable to that obtained for control −PMSG and control +PMSG groups (Table 1) and was significantly higher than the rate obtained for the other xenograft groups ($P < 0.05$).

After embryo transfer, five FVB pups in total were born to females receiving embryos derived from the xenografts grown in female −PMSG and female +PMSG rats (Table 1; fig. S1). All pups developed normally and produced healthy live young when mated at 2 months of age (*8*). Pups were also produced in each of the control groups (Table 1).

In all experimental groups, the number of implantation scars exceeded the number of pups born (Table 1), indicating some fetal wastage, but there was no difference in fetal losses after implantation between the xenograft and control groups ($P > 0.05$). It remains unclear whether the embryos obtained from the male +PMSG xenograft group were viable. These embryos were placed (together with six control PMSG + hCG embryos) in the only surrogate mother that was subsequently shown to have no implantation scars and may thus not have been receptive at the time of embryo transfer.

We demonstrate that mouse ovarian tissue, when xenografted into a rat recipient, can produce mature oocytes that can subsequently be fertilized and develop into fertile adult mice. This suggests that oocytes grown in xenografted ovarian tissue from other species, including domestic animals or wildlife (*2*, *3*, *5*, *6*), may also be viable. In addition, it may be possible to collect ovarian tissue from other living animals or salvage it from recently deceased animals to subsequently aid in the propagation of rare and endangered species.

Although this strategy could potentially be applied to humans, its use in human-assisted reproduction should be considered with caution.

Table 1. Number of xenografts and ovaries, oocytes collected, two-cell embryos formed, embryos transferred, implantation scars, and pups weaned for xenograft and control groups. *n*, number. The same superscript letters within columns indicate groups that are statistically the same ($P > 0.05$). *Expressed as *n* and as a percentage of two-cells transferred. †1 pup in each of these groups died within 24 hours of birth.

Xenograft and control groups	*n* ovaries	*n* oocytes (% to two-cell)	*n* two-cells transferred (*n* fosters)	Total implantation scars*	Pups weaned*
Female −PMSG	15	85 (40.0^{a})	32 (5)	12 (37.5)^{a,b}	3 (9.4)^{a}
Female +PMSG	15	57 (57.9^{b})	33 (5)	8 (24.2)^{a}	2 (6.1)^{a}
Male +PMSG	1	10 (20.0^{a})	2 (1)	0 (0)^{a,b}	0 (0)^{a,b}
Control −PMSG	4	75 (65.3^{b})	12 (2)	7 (58.3)^{b,c}	1 (8.3)^{a}
Control +PMSG	4	53 (69.8^{b})	18 (3)	14 (77.8)^{c,d}	6† (33.3)^{b}
Control PMSG + hCG	4	53 (94.3^{c})	30 (5)	26 (86.7)^{d}	15† (50.0)^{b}

References and Notes

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8. Materials and methods are available as supporting material on *Science* Online

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Supporting Online Material

www.sciencemag.org/cgi/content/full/297/5590/2227/DC1

Materials and Methods

Fig. S1

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