Suppressive Effect of Fetal Testes on Development of Fetal Ovaries Transplanted into Adult Males in the Rat

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(Received 17 May 1994/Accepted 27 August 1994)

ABSTRACT. The age-related testicular effect on the ovarian primordia was studied by combined transplantation of fetal testes and ovaries in adult male hosts. First, ovarian primordia of 14-day fetal rats were transplanted into a renal subcapsular position of castrated or intact adult male rats. In both the castrated and the intact hosts, most of the ovarian transplants developed normally with only 3 of them having in part seminiferous tubule-like structures in addition to normal ovarian structure. Second, a 14-day ovary was combined with a fetal testis the age of which varied from 13- to 18-day, and the combination was transplanted. In the combination of a 14-day ovary and a 13-day testis, the results varied in such a way that the ovary or the testis alone developed or otherwise, both gonads developed well. In union with 15- to 18-day testes, the ovaries did not develop, although the testes developed well. These results suggest that the 14-day ovarian primordia have a slight reactivity to androgens of host rats and that the 13-day fetal testes begin to inhibit the development of the 14-day ovaries co-transplanted with them.—**KEY WORDS:** differentiation, fetus, ovary, rat, testis.

J. Vet. Med. Sci. 56(6): 1113–1118, 1994

The development of ovaries of genetic females is inhibited under some conditions. For example, in the combined transplantation of 15-day fetal ovaries and 15-day fetal testes into a renal subcapsular position of gonadectomized adults of both sexes in the rat, the ovaries are severely inhibited in development in spite of the fact that the testes develop normally [7]. However, control experiments, in which 15-day fetal ovaries are transplanted alone, result in normal ovarian development [7]. Similarly, in organ culture of 14.5-day fetal ovaries combined with 17.5-day fetal testes, the ovaries show testicular structures [2]. For another example, most of the fetal rat ovaries (days 12.5 to 15.5 of gestation) transplanted within the scrotal testes of adults consist entirely of seminiferous tubules [15]. On the other hand, in the mouse, following transplantation into a renal subcapsular position of adult males, 12- or 13-day fetal ovarian primordia frequently show ovotestes, testicular in part and ovarian in part [12].

Following transplantation into a renal subcapsular position of adult rats, various combinations of fetal ovaries and testes varying in age with each other bring about various cases in such a way that ovarian development is inhibited, that testicular development is suppressed and that both developments are not interfered with [8].

The present work was conducted with the transplantation of fetal ovarian primordia alone or combined with fetal testes of different gestational ages into a renal subcapsular position of castrated or intact adult male rats. The objectives of this work were, first, to know whether transplanted fetal ovarian primordia can develop normally and, second, to test how fetal testes can suppress age-relatedly the development of fetal ovarian primordia as heterosexual co-transplants.

MATERIALS AND METHODS

Rats of Wistar strain (CLEA, Tokyo, Japan) were given a commercial diet (Labo MR Breeder) and water both *ad libitum*. Females were placed with males overnight and were examined the next morning for the presence of sperm in the vaginal smear. The day on which sperm was detected was designated as day 0 of gestation.

Experiment 1: The sexes of 14-day fetuses were easily determined by observation of gonads under a dissecting microscope. Ovarian primordia were removed *en bloc* with the adjacent mesonephrotic tissues. Each primordium was transplanted into a renal subcapsular position of a castrated or intact adult male rat, 12–13 weeks old, under ether anesthesia. Castration had been carried out at least 3 weeks prior to transplantation.

Experiment 2: A 14-day ovarian primordium was combined with a testis the age of which varied from 13- to 18-day. The combination was transplanted into a renal subcapsular position of an adult male rat. The 13-day testicular primordia were not easily determined, so that the contralateral gonad was transplanted alone to determine its sex by later histologic examination. Testes of 13- and 14-day fetuses were removed *en bloc* with the adjacent mesonephrotic tissues.

After 2-week transplantation, the transplants were removed from the hosts, fixed in Bouin's fluid, dehydrated in a graded series of ethanol and embedded in Paraplast (Sherwood Medical, St. Louis, U.S.A.). They were sectioned serially at 5 μ m and stained with hematoxylin and eosin.

RESULTS

Experiment 1: Of the 21 14-day ovarian grafts in intact hosts, 19 developed normally, containing primary and secondary follicles and the rete ovarii (Table 1, Figs. 1a,

Fig. 1. Photomicrographs of 14-day ovaries transplanted alone into intact (a, b) or castrated male rats (c-e) (a: $\times 175$, b, d, f: $\times 350$, c: $\times 228$). a: A 14-day ovary transplanted into an intact adult male rat. This ovary has developed normally. b: Higher magnification of a. There are follicles with single or several layers of granulosa cells (arrow). c: An ovary showing tubular structures (T) near ovarian structures (O). d: A part of the ovarian graft in c. The seminiferous cord-like structures are devoid of germ cells. e: A part of the ovarian graft in c. Oocytes have developed with one or several layers of granulosa cells.

Table 1. Development of ovarian primordia transplanted into intact or castrated adult male rats

Host	No. of Samples	Development into	
		Ovaries	Ovotestes
Intact male	21	19	2
Castrated male	20	19	1

b). These follicles were surrounded by 1 to 3 layers of follicular cells (Fig. 1b). However, the remaining 2 grafts showed tubular structures resembling seminiferous tubules in addition to the follicles. These tubules did not contain primordial germ cells in their lumina. These 2 grafts were regarded as ovotestes. The growth and development of the follicles of these ovotestes were inhibited compared with normally developed grafts.

Of the 20 14-day ovarian grafts in castrated hosts, 19 developed normally just as the foregoing 19 grafts in intact hosts (Table 1). However, the remaining one had seminiferous tubule-like structures (Figs. 1c-e), similar to the ovotestes observed in the grafts in intact hosts.

Experiment 2: The results in *Experiment 1* showed that the 14-day ovarian primordial grafts developed normally regardless of castrated or non-castrated hosts. Therefore, in *Experiment 2*, all grafts were transplanted into intact adult male rats.

The 13-day testicular primordia that had been transplanted alone developed testicular structures with seminiferous tubules and proliferated germ cells (Figs. 2a, b). When the 14-day ovarian primordia were transplanted with the 13-day testes, both gonads developed in 3 manners as follows: The testis only (8 cases, Fig. 3a), the ovary only (7 cases, Fig. 3b) or both gonads (5 cases, Figs.

Table 2. Results of combined transplants of ovaries and testes of various ages

Ages of Transplants		No. of Samples	Testis*			Ovary*		
Ovaries	Testes		+	+	-	-	+	+
14	13	20	8	5	7			
14	14	22	21	1	0			
14	15	19	19	0	0			
14	16	22	22	0	0			
14	17	22	22	0	0			
14	18	19	19	0	0			

Ages: Gestational days.

*: (+): Testis or ovary well developed, (-): Testis or ovary inhibited.

3c, d, e) developed well (Table 2). When either the testis or the ovary showed good structures, such as primary and secondary follicles in the ovarian primordia or seminiferous tubules and proliferated germ cells in the testicular primordia, the degree of these developments was similar to that of the gonads transplanted alone. On the other hand, the combined and inhibited gonads did not show their particular structures.

The 14-day testicular primordia that had been transplanted alone developed well. When the 14-day ovaries were transplanted with the 14-day testes, only one ovary developed well, but the other ovaries were inhibited, although all testes developed well.

All the 14-day ovaries that had been transplanted with the 15- to 18-day testes did not develop, but all testes of every age showed good structures in development.

Fig. 2. Photomicrographs of a 13-day testis transplanted alone (a: $\times 175$, b: $\times 350$). a: This graft has developed normally. b: Higher magnification of a showing seminiferous cords and proliferated germ cells (arrow).

Fig. 3. Photomicrographs of combined transplants of ovaries and testes (a: $\times 47$, b, d, e: $\times 350$, c: $\times 228$). When 14-day ovaries and 13-day testes were co-transplanted, gonads have developed in different ways. a: Only the testis has developed (T), but there are no follicles in the ovarian portion (O). b: Only the ovary has developed, with well-developed follicles, but there are no tubular structures (figures not shown). c: Both gonads have developed normally (T: testicular portion, O: ovarian portion). d: Enlarged view of ovarian portion in c. There are follicles with single or several layers of granulosa cells. e: Enlarged view of testicular portion in c. The testes show normal structures, seminiferous cords and proliferated germ cells.

DISCUSSION

Androgens administered to pregnant rats and mice, while Wolffian and Müllerian systems coexist in the embryos, cause the females of the litter to be permanently intersexed; the males are unaffected [15]. In the rat, testosterone propionate administered to the mother on days 13 and 14 of gestation induced intersexuality in the female offspring, but did not masculinize the ovaries [15]. In the present *Experiment 1*, transplanted ovarian primordia of 14-day fetuses developed normally except for 3 cases. Therefore, these results show that the development of ovaries of this stage is not largely affected by the testes of host rats, probably androgens. In the exceptional cases, 2 ovaries in normal male hosts and one ovary in a castrated host showed small-sized tubular structures. This suggests that the 14-day ovary may have a slight sensitivity to some unknown factors, other than testicular androgens, to induce tubular structures.

In the mouse, it has been reported that 13-day fetal undifferentiated ovaries transplanted into a renal subcapsular position of adult males frequently developed tubular structures which contained all types of testicular somatic cells and which were devoid of germ cells except for occasional oocytes [12, 13]. In the present study, among the 14-day ovaries transplanted into male rats, castrated or intact, only 3 were inhibited in their normal development and had both the immature follicles and the seminiferous tubular-like structures without germ cells. The lack of male germ cells in this rat case as well as in the foregoing mouse case can be explained by a notion that XX germ cells do not transform into spermatogonia and cannot survive in the testicular environment [11]. Therefore, sex determination of somatic elements and that of germ cells seem to be under the control of different factors.

Experiment 2 in the present study shows that ovarian primordia cannot develop normally when transplanted with testicular primordia 13–18 days of age. Since the testes from 13-day fetuses partly, those from 14-day almost and those from 15–18 day completely inhibit the development of the ovary, the degree of this inhibitory ability of the fetal testis increases according to the gestational age. Such an inhibitory effect of fetal testes has been shown by many studies in the rat [7–9, 15].

Anti-Müllerian hormone (AMH) is also known as a Müllerian-inhibiting substance (MIS) secreted by Sertoli cells, causing the regression of Müllerian ducts which would otherwise give rise to female reproductive tracts [1, 4, 14]. In male rats, MIS mRNA is first detected on the medial aspect of the urogenital ridge early in the morning on day 13 of gestation before testicular differentiation, localized to the more obvious Sertoli cells later on day 13. This mRNA remains at maximal levels between day 14.5 and day 17.5 of gestation, and then disappears by 3 weeks after birth [5].

Incidentally, *Sry*, maps to the highly conserved sex-determining region on Y-chromosome, has been recently

detected in the mouse [6, 10]. In the rat fetus, the developmental timing and tissue proximity of *Sry* and MIS gene expression have prompted speculation that the MIS gene might be induced by *Sry* and that MIS may then act slightly later to support continued testicular differentiation [3, 5]. Accordingly, it may be possible that the foregoing suppressive effect of fetal testes on the normal development of ovaries transplanted together with them is due to an action of their AMH (MIS). AMH is not present over 3 weeks after birth, and if, by transplantation with testes from males over 3 weeks of age, the fetal ovaries could develop normally, it would be further likely that the suppressive effect of fetal testes is resulted mainly from AMH action. Moreover, the reason why 15- to 18-day testes completely suppress the ovarian development seems to be related with a high level of this AMH.

According to Vigier *et al.* [16], when 13-day ovaries are exposed to purified bovine AMH for 3 to 10 days *in vitro*, AMH consistently induces seminiferous cord-like structures with a continuous basal membrane and fibronectin. Furthermore, according to Charpentier and Magre [2], the 14.5-day ovaries when cultured with 17.5-day testes for 4 days show a functional as well as a morphological masculinization as a result of acquisition of an ability to produce AMH by ovaries themselves. These findings by Vigier *et al.* [16] and Charpentier and Magre [2] indicate that the *in vitro* effect of AMH on fetal ovaries is not only to suppress their developments but also to masculinize them. However, the *in vivo* effect of AMH as shown in the present transplantation study is only to suppress the development of fetal ovaries, but not to induce testicular structures in them.

It is of interest that, in the present study, some 14-day ovaries inhibit the development of co-transplanted 13-day testes, although the 13-day testicular primordia develop normally when transplanted alone. MacIntyre *et al.* [8] observed a suppression of testicular differentiation in some combined transplants in which the ovary was older than the testis. For example, the greatest percentage (36%) of the cases showing testicular suppression was achieved in the combinations of 13-day testes with 16-day ovaries.

As to the failure of some 13-day testes to suppress the development of the co-transplanted 14-day ovaries, premature production of AMH may be concerned, since testicular AMH mRNA first appears on day 13 of gestation as stated in the foregoing. Inversely, the reason why some 14-day ovaries suppressed the development of 13-day testes remains to be solved in future.

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