PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 176, 148–153 (1984)

# Induction of Testicular Differentiation in the Fetal Mouse Ovary by Transplantation into Adult Male Mice (41855)

TERUKO TAKETO, HORACIO MERCHANT-LARIOS,* AND SAMUEL S. KOIDE

*The Population Council, Center for Biomedical Research, 1230 York Avenue, New York, New York 10021, and *Instituto de Investigaciones Biomedicas, Universidad Nacional Autonoma de Mexico, Apartado Postal 70228, Mexico 20 D.F., Mexico*

*Abstract.* Mouse ovarian primordia on the 12th or 13th day of gestation frequently developed testicular structures in addition to ovarian structures by the 14th day after transplantation into a site beneath the kidney capsules of adult male mice. Electron microscopic examination of the testicular portion of ovotestes revealed all types of testicular somatic cells, i.e., Sertoli, myoid, and Leydig cells, but not spermatogenic cells. On the other hand, after transplantation into adult female mice ovarian grafts developed only ovarian structures. We conclude that female gonadal somatic cells differentiate into testicular components in a male host environment.

Sexual differentiation in mammals involves a sequence of events. Genetic sex is determined at fertilization by the combination of sex chromosomes, which are in turn responsible for gonadal development. A gene(s) located on the Y chromosome, interacting with autosomal genes, induces testicular differentiation, and in its absence, gonadal primordia differentiate into ovaries (1). However, the mechanism by which the genes control gonadal differentiation is not clear. One way to elucidate the mechanism is to study the condition(s) under which gonadal sex can be reversed experimentally.

Gonadal sex reversal in mammals that is caused by mutation or translocation of genes involved in testis determination has been reported in the human, mouse, and goat. For example, sex-reversed (Sxr) female mice develop as phenotypic males with testes because a segment of the Y chromosome is translocated to the X chromosome (2). However, sex reversal of mammalian gonads with normal chromosomes is very rare. One example is the freemartin condition in cattle, sheep, and goats, where testicular components are formed in the ovary when the circulation of a female fetus is shared with that of a male (3, 4). The mechanism of this phenomenon remains unclear, primarily because of the difficulty in reproducing the freemartin condition experimentally. For studying the regulation of gonadal sex differentiation, it is important to establish an experimental system in which gonadal sex can be reversed frequently. It has been reported that fetal rat ovaries develop testis-cord-like structures when transplanted into a site beneath kidney capsules (5), into the omenta (6), or into a subcutaneous (7), intraocular (8), or scrotal (9) position of adult rats. However, the tubular structures developed in the transplanted ovaries were not considered "testicular" by some investigators (10).

We report here that when transplanted into adult male mice, mouse fetal ovaries frequently developed testicular structures which contained all types of testicular somatic cells. On the other hand, fetal ovaries transplanted into adult female hosts developed only ovarian components. The results show that gonadal somatic cells undergo testicular differentiation in a male host environment, regardless of the genetic sex of the gonads.

**Materials and Methods.** Ovarian primordia with the mesonephroi attached were isolated from mouse fetuses between the 12th and 16th day of gestation (dg). The day when copulation plugs were found was defined as Day 0. SJL/J strain and NCS strain (Rockefeller University) mice were used. On the 12th dg or later, fetal testes were distinguished from fetal ovaries by observing for striation of testis cords under a dissecting microscope. One of each pair of fetal gonads was transplanted into a site beneath the kidney capsules of an adult male mouse, and the other into the same site of an adult female mouse. On the 14th day after transplantation, the gonadal grafts were dissected out and processed for histological examinations. For light microscopic study,



0037-9727/84 $1.50

tissues were fixed in Bouin's solution, sectioned with paraffin, and stained with hematoxylin and eosin. For electron microscopic study, tissues were fixed in Karnovsky's solution (11) and embedded in Epon after $OsO_4$ fixation. Semithin sections (100-nm thickness) were stained with silver–methenamine solution and examined with a light microscope.

**Results.** Twenty-five fetal ovaries of NCS strain on the 12th or 13th dg were transplanted into male mice. As summarized in Table I, 20 ovarian primordia developed testicular structures. Of these masculinized ovaries, 17 also developed follicular structures (ovotestes) while 3 developed only testicular structures. The ratio of testicular to ovarian structures varied with each ovotestis. The other 5 ovarian primordia developed only follicular structures. In female hosts, on the other hand, all 12 ovarian primordia developed typical follicular structures. Since the NCS mouse is outbred, we repeated this experiment in SJL/J inbred strain mice; 6 of 13 ovarian primordia developed testicular structures when transplanted into male hosts, whereas all 14 ovarian primordia developed only ovarian structures when transplanted into female hosts. In contrast to ovarian primordia transplanted on the 12th or 13th dg, fetal ovaries transplanted on the 14th dg or later into male hosts did not develop testicular structures (except for one transplant in SJL/J), and formed well-advanced follicles containing oocytes of normal morphology.

We examined the seminiferous tubules that developed in ovarian transplants and identified well-differentiated testicular somatic cells. Light micrographs of a representative ovotestis are shown in Figs. 1 and 2. For comparison, a light micrograph of an ovary transplanted into a female host is shown in Fig. 3; the morphology, with follicular structures at various stages of development (Types 2–5b according to Peters' classification) (12), was comparable to that of 1-week-old mouse ovaries (13, 14). The ovarian portions of the ovotestes contained oocytes surrounded by follicular cells whose developmental stages (mainly Types 2–3b) were considerably retarded in comparison with those of ovaries transplanted into female hosts. Similar developmentally retarded follicles were seen in the ovaries that were transplanted into male hosts and did not develop any testicular structures, although these ovaries also contained more advanced follicles (Types 2–5b). The testicular portions of the ovotestes contained Sertoli cells and myoid cells (or interlamellar cells) (15) composing seminiferous tubules, and Leydig cells in the interstitial region. In the seminiferous tubules, oocytes were occasionally present, but spermatogenic cells were never observed. Electron micrographs of the Sertoli cells (Fig. 4) showed slightly advanced morphology, typical of that observed in testes of the 2-week-old mouse (16). Electron micrographs of myoid cells and Leydig cells in ovotestes are shown in Figs. 5 and 6. As in the normal testis, numerous microfilaments were seen in the myoid cells of the testicular portion of ovotestes, but not in the theca cells enclosing the follicles in the ovarian portion (and in the ovaries transplanted into female hosts). Furthermore, ultrastructures characteristic of steroidogenic (Leydig) cells were found only in the interstitial region of testicular portions, whereas in the ovarian portions, such steroidogenic cells were found only inside the follicles.

TABLE I. DEVELOPMENT OF OVARIAN PRIMORDIA TRANSPLANTED INTO ADULT MICE

| Mouse strain | Developmental age of transplants[a] | Sex of hosts | Number of experiments | Development into: | | |
|---|---|---|---|---|---|---|
| | | | | Testes | Ovotestes | Ovaries |
| NCS | 12–13 | Male | 25 | 3 | 17 | 5 |
| | 12–13 | Female | 12 | 0 | 0 | 12 |
| | 14 | Male | 9 | 0 | 0 | 9 |
| SJL/J | 12 | Male | 13 | 2 | 4 | 7 |
| | 12 | Female | 14 | 0 | 0 | 14 |
| | 14–16 | Male | 9 | 0 | 1 | 8 |

[a] Day of gestation.

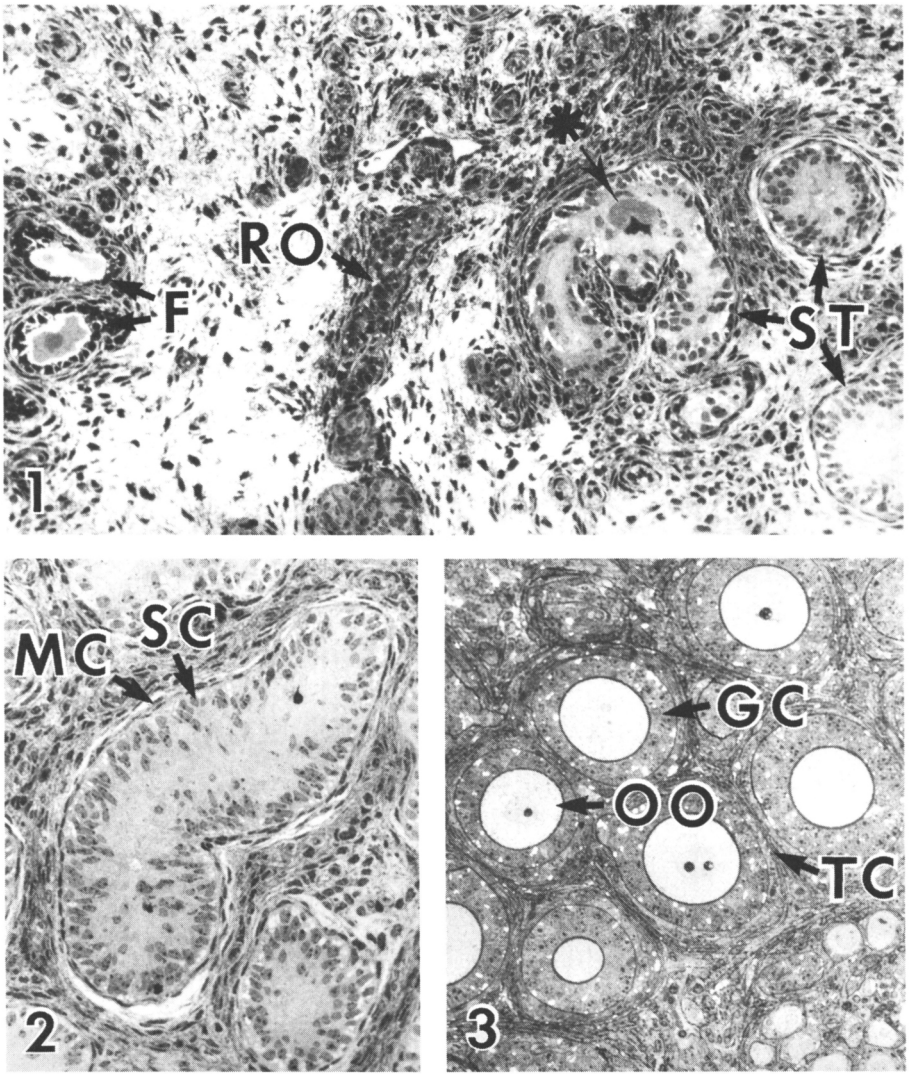



FIG. 1. An ovotestis developed in a male host. Note three types of epithelial structures surrounded by basal lamina and dense connective tissue; ovarian follicles (F), rete ovarii (RO), and seminiferous tubules (ST). A remnant of degenerating oocyte (*) is seen in a seminiferous tubule. ×60.

FIG. 2. Testicular part of the ovotestis shown in Fig. 1. The seminiferous tubule is surrounded by a layer of myoid cells (MC), whereas Sertoli cells (SC) are arranged around the inside of the basement membrane of the seminiferous cord. Note absence of germ cells. ×90.

FIG. 3. An ovary developed in a female host. Well-developed follicles contain multilayers of granulosa cells (GC) and large oocytes (OO). TC, thecal cells. ×90.

**Discussion.** We showed that testicular structures that developed in ovarian transplants did not merely represent the different arrangement of ovarian components, but involved the differentiation of ovarian precursor cells into testicular components. The capacity of ovarian primordia to form testicular structures was restricted to those transplanted at early stages of development (until the 13th dg) and it appeared to be lost during the course of ovarian differentiation. In NCS mice, testicular differentiation in male fetuses begins on the 12th dg, whereas fetal ovaries remain undifferentiated until the 13th dg, when pri-

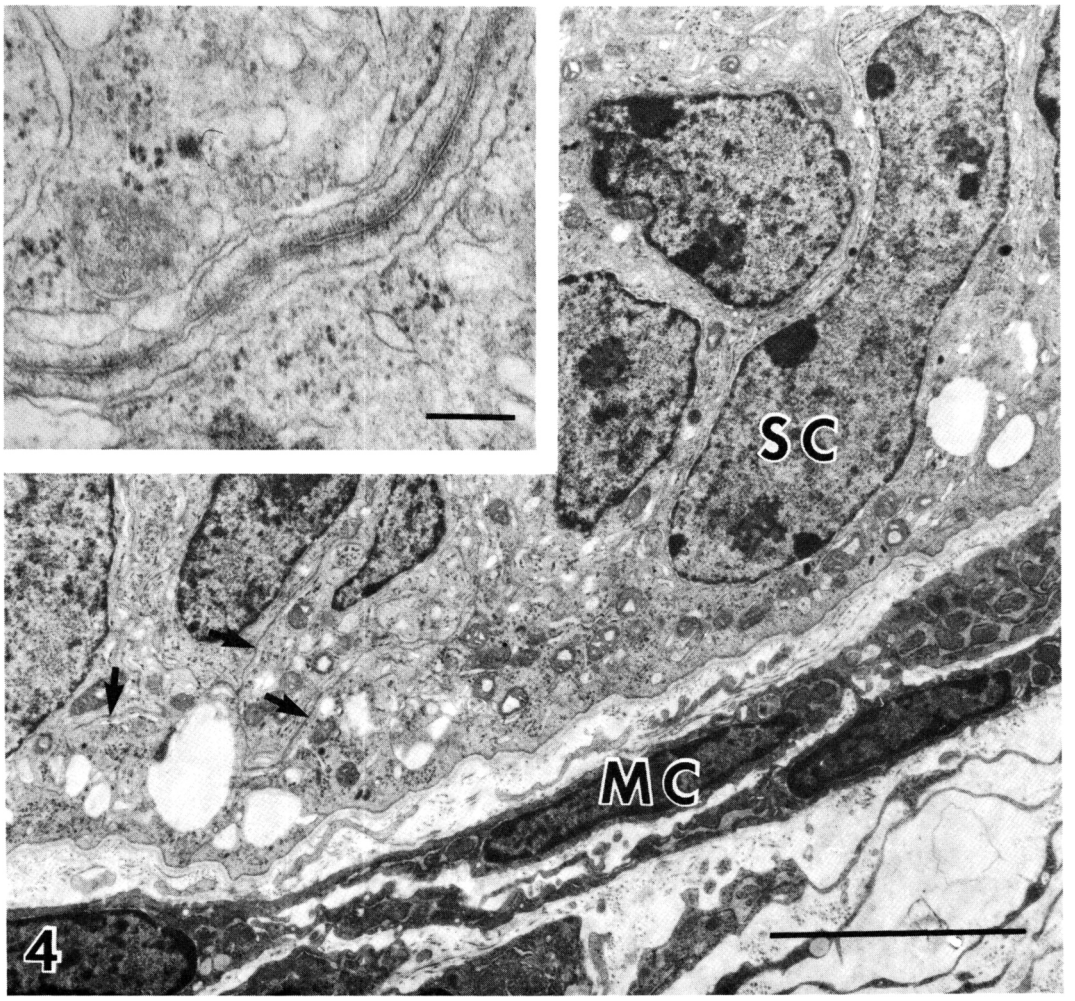
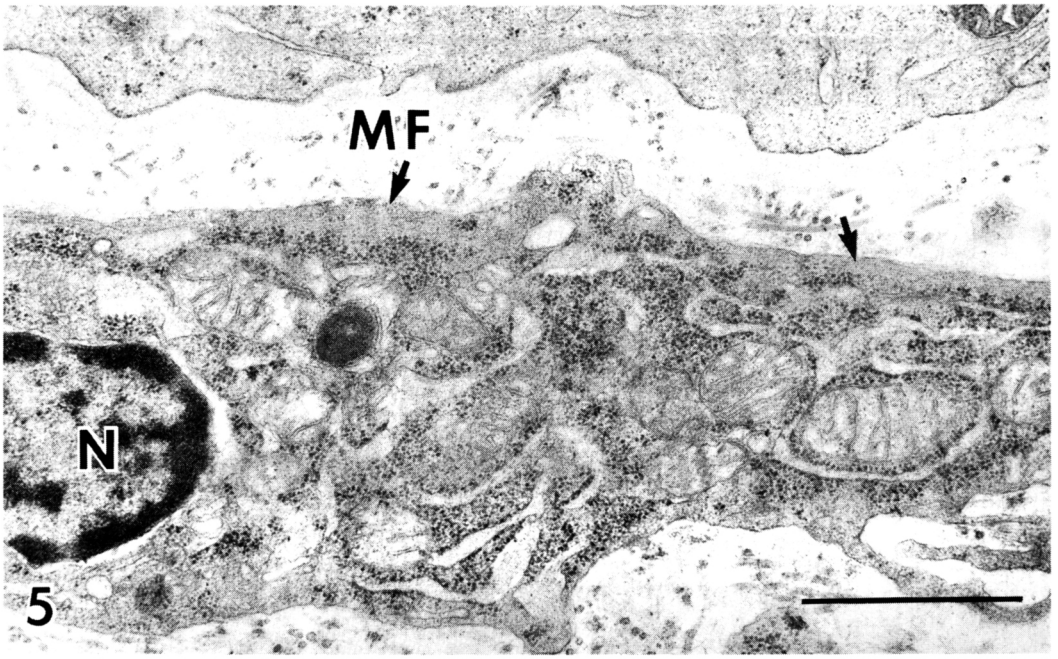



FIG. 4. Electron micrographs of a part of the seminiferous tubule of an ovotestis developed in a male host. Note typical "intersertoli contact specializations" (arrows) between Sertoli cells (SC). Myoid cells (MC) are seen outside the basal lamina. Scale bar indicates 5 $\mu$m. Inset: A part of an "intersertoli contact specialization," which is characterized by the presence of an intercellular space (about 7–9 nm) and a flat cisternum of endoplasmic reticulum running parallel to juxtaposed Sertoli cell plasma membranes with some ribosomes on the cytoplasmic side. Note the accumulation of a layer of electron-dense filamentous material between the plasma membrane and the associated cisterna of the endoplasmic reticulum. Scale bar indicates 0.2 $\mu$m.

mordial germ cells start premeiotic DNA synthesis without accompanying ovary-specific organization (17). On the 14th and 15th dg, germ cells enter meiotic prophase and are frequently surrounded by epithelial cells, which are probably precursors of granulosa cells. These aggregates of somatic cells and germ cells are enclosed by basement membranes and designated as "sex cords" (14). It is our contention that, when placed in the male environment, these sex cords develop into seminiferous tubules, and the pregranulosa cells differentiate into Sertoli cells; this hypothesis is consistent with the findings of occasional oocytes within the seminiferous tubules in ovotestes (Fig. 1) and of epithelial cords containing both Sertoli cells and pregranulosa cells (data not shown). It is noteworthy that the development of the Sertoli cells always coincided with the differentiation of surrounding



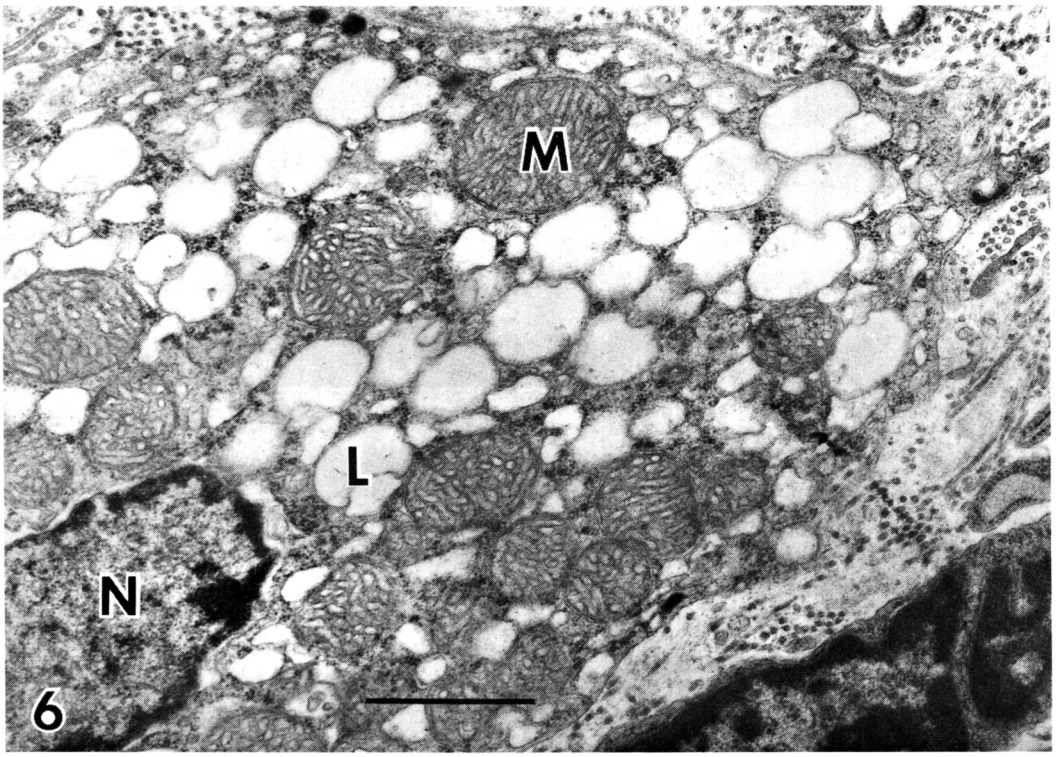




FIG. 5. A part of a myoid cell of an ovotestis with numerous microfilaments (MF) occupying the cytoplasmic matrix. N, nucleus. Scale bar indicates 1.0 $\mu$m.

FIG. 6. A part of a Leydig cell of an ovotestis with abundant lipid droplets (L), nucleus (N), and large mitochondria (M) with tubular cristae. Scale bar indicates 1.0 $\mu$m.

stromal tissue into testis-specific cells, i.e., myoid cells and Leydig cells. The same mechanism may be involved in the induction of these three types of testicular components, or, alternatively, the differentiation of one cell type may induce that of the other.

The testicular structures that developed in ovarian transplants were devoid of germ cells except for occasional oocytes, which often degenerated. This observation supports the finding by other investigators that XX germ cells do not transform into spermatogonia and cannot survive in the testicular environment (18). In contrast, it has been reported that XY germ cells survive and enter meiosis precociously in an ovarian environment (18) or even in a male environment outside the testis cord (19). Therefore, sex determination of somatic elements and that of germ cells appear to be under the control of different factors. The present study shows that gonadal sex reversal resulting from transplantation is restricted to the somatic elements.

The experimental model reported here has provided a useful system for studying the mechanism regulating sex differentiation of gonadal somatic components. The present results indicate that mouse ovarian primordia are bipotential for the direction of sex differentiation, and the direction can be controlled by factor(s) present in adult, and probably fetal, male mice. This male-specific factor induces differentiation of testicular cells and thereby formation of seminiferous tubules. However, the induction of testicular structures in ovarian primordia might be indirect. It is possible that a male host environment caused the disappearance of oocytes, which in turn prevented follicular development, and permitted the precursor cells to differentiate into testicular components; these three phenomena were coincident in the development of ovotestes.

We thank Braulio Centeno for technical assistance. This work was partially supported by Rockefeller Foundation Grant GA PS 8208 and NICHD Grant HD-13184 and HD-18669.

1. Simpson E. Sex reversal and sex determination. Nature (London) **300**:404–406, 1982.
2. Singh L, Jones KW. Sex reversal in the mouse (*Mus musculus*) is caused by a recurrent nonreciprocal crossover involving the X and an aberrant Y chromosome. Cell **28**:205–216, 1982.
3. Lillie FR. The free-martin: A study of the action of sex hormones in the foetal life of cattle. J Exp Zool **23**:371–451, 1917.
4. Jost A, Vigier B, Prepin J, Perchellet JP. Studies on sex differentiation in mammals. Recent Progr Horm Res **29**:1–35, 1973.
5. Buyse A. The differentiation of transplanted mammalian gonad primordia. J Exp Zool **70**:1–41, 1935.
6. Holyoke EA. The differentiation of embryonic gonads transplanted to the adult omentum in the albino rat. Anat Rec **103**:675–699, 1949.
7. Moore CR, Price D. Differentiation of embryonic reproductive tissues of the rat after transplantation into post-natal hosts. J Exp Zool **90**:229–265, 1942.
8. Torrey TW. Intraocular grafts of embryonic gonads of the rat. J Exp Zool **115**:37–38, 1950.
9. Mangoushi MA. Scrotal allografts of fetal ovaries. J Anat **120**:595–599, 1975.
10. Ozdzenski W, Rogulska T, Batakier H, Brzozowska M, Bembiszewska A, Stepinska V. Influence of embryonic and adult testis on the embryonic ovary in the mouse. Arch Anat Microsc **65**:285–294, 1976.
11. Karnovsky MJ. A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J Cell Biol **27**:137A, abstr, 1965.
12. Peters H. The development of the mouse ovary from birth to maturity. Acta Endocrinol **62**:98–116, 1969.
13. Merchant H, Zamboni L. Presence of connections between follicles in juvenile mouse ovaries. Amer J Anat **134**:127–132, 1972.
14. Merchant-Larios H. Germ and somatic cell interactions during gonadal morphogenesis. In: Van Blerkom J, Motta PM, eds. Ultrastructure of Reproduction. Boston/The Hague/Dordrecht/Lancaster, Nijhoff, pp19–30, 1984.
15. Fawcett DW, Heidger MP, Leak VL. Lymph vascular system of the interstitial tissue of the testis as revealed by electron microscopy. J Reprod Fertil **19**:109–119, 1969.
16. Flickinger JC. The postnatal development of the Sertoli cells of the mouse. Z Zellforsch **78**:92–113, 1967.
17. Taketo T, Koide SS. *In vitro* development of testis and ovary from indifferent fetal mouse gonads. Dev Biol **84**:61–66, 1981.
18. Short RV. Sex determination and differentiation. Brit Med Bull **35**:121–127, 1979.
19. Upadhyay S, Zamboni L. Ectopic germ cells: Natural model for the study of germ cell sexual differentiation. Proc Natl Acad Sci USA **79**:6584–6588, 1982.

Received December 8, 1983. P.S.E.B.M. 1984, Vol. 176. Accepted March 6, 1984.