Transplantation of the gonads of hypogonadal (*hpg*) mice*

S. Bamber, Carol A. Iddon, H. M. Charlton and B. J. Ward

Department of Human Anatomy, South Parks Road, Oxford OX1 3QX, U.K.

Summary. Testes from hypogonadal (*hpg*) mice transplanted under the tunica albuginea of the testes of normal mice displayed full spermatogenic activity after 84 days. When ovaries of *hpg* mice were transplanted to the periovarian capsule of ovariectomized normal females ripe follicles and corpora lutea developed. Although small remnants of normal ovary were found after this operation, the fact that 5 out of 11 normal females bearing transplanted ovaries and mating with heterozygous males raised litters containing *hpg* mice shows that the ovary of the mutant is capable of producing ova which can be fertilized.

Introduction

The failure of postnatal gonadal development in homozygous recessive hypogonadal (*hpg*) mice appears to be due to a deficiency in biologically active hypothalamic gonadotrophin hormone-releasing hormone (Cattanach, Iddon, Charlton, Chiappa & Fink, 1977; Iddon, Charlton & Fink, 1980). In the present study, testes and ovaries from hypogonadal mice have been transplanted into normal animals to test the functional capacity of the *hpg* gonads.

Materials and Methods

The animals used were hypogonadal and normal males and females reared in the Department of Anatomy as described by Iddon *et al.* (1980). Female mice used as recipients in Exp. I were F1 hybrids of the inbred strains C3H/Heh and 101/H in which the mutation first arose (Cattanach *et al.*, 1977). Animals were between 3 and 4 months of age and weighed 24–30 g.

Males

Hypogonadal males were killed by decapitation and their testes removed and placed on filter paper soaked with warm saline (9 g NaCl/l). At the same time normal adult males were anaesthetized with ether and the right testis was exposed. The tunica albuginea was incised with an iridectomy knife and a testis of an *hpg* mouse was inserted underneath the tunica. The testis plus transplant was replaced in the abdominal cavity and the animal was allowed to recover. Groups of animals treated in this way were killed by decapitation after 21, 42 and 84 days. The left (control) and right (+ transplant) testes were removed and fixed in Bouin's fluid before embedding in paraffin wax. Sections were cut at 7 µm and stained with haematoxylin and eosin.

Females

Experiment I. Eleven normal adult females not carrying the *hpg* gene were anaesthetized with ether and the ovary and oviduct on one side were exposed. The periovarian capsule was

* Reprint requests to Dr H. M. Charlton.

punctured with a pair of fine forceps and the normal ovary was 'pinched out' and removed. At the same time ovaries were taken from anaesthetized *hpg* females and kept on saline-moistened filter paper until insertion within the torn periovarian capsule. The tear was covered with a small fragment of absorbable gelatin sponge (Sterispon: Allen & Hanburys Ltd, England) and the capsule and transplant and the oviduct were replaced in the abdominal cavity. The process was repeated on the other side, each female therefore receiving both ovaries of an *hpg* female. After recovery daily vaginal smears were taken and, when cornified cells were seen 5–10 days later, the females were caged separately with a male known to be heterozygous for the *hpg* gene. Animals which failed to become pregnant after 4–6 months were killed by decapitation and their ovaries and uteri were removed, weighed, fixed in Bouin's fluid and processed for routine histology.

Experiment II. To check for the completeness of ovariectomy by the method described in Exp. I, 9 normal adult females in a control group (A) were subjected to the same operation but did not receive ovaries. Daily vaginal smears were taken and the animals were killed 30 days later. The uteri were removed, weighed and, together with the entire periovarian capsule and oviduct, fixed in Bouin's fluid for routine histology. A second group (B) of 7 control females without ovarian transplants was similarly prepared. Each was caged separately with a normal male.

Experiment III. Seven normal adult females were anaesthetized with ether and the oviducts and ovaries, with ovarian capsules intact, were removed. Vaginal smears were taken daily. After 30 days the mice were killed and the uteri were removed and weighed and fixed in Bouin's fluid for routine histology.

As a further control group for all of the above experiments 7 normal adult females were selected at random and killed by decapitation and their ovaries and uteri were removed, weighed and fixed in Bouin's fluid for routine histology.

Results

Males

Transplanted testes of *hpg* mice demonstrated variable degrees of development. All transplants showed some degree of central necrosis, with peripheral tubules showing greater development. In some transplants lymphocytic invasion had taken place. By its very nature the transplant operation causes damage to the host testicular tissue and those transplanted testes which appeared less well advanced tended to be found in association with testes showing signs of damage.

After 21 days little advance in development was seen in the cellular populations of transplanted testes, although the diameter of peripheral seminiferous tubules appeared to be increased. Early signs of spermatogenesis were seen after 42 days, in that stage 8–9 spermatids were present in peripheral tubules in the more successful transplants. After 84 days (Pl. 1, Figs 1 and 2) the most advanced transplants had peripheral tubules demonstrating all stages of germ cell development.

Females

Experiment I. Out of the original 11 normal females given transplant ovaries (transplant females; see Pl. 2, Fig. 5) 5 have produced and still are producing litters. Out of 117 young born to these females 78 have been normal and 39 hypogonadal. The ovaries removed from transplant females that failed to produce litters were indistinguishable from normal ovaries (see Pl. 2, Figs 4 and 5); they contained corpora lutea and follicles in all stages of development. Adhesion of the transplant to the abdominal wall appeared to be the major cause of the

Fig. 1. In the testis of an *hpg* mouse, spermatogenesis is not fully developed; the tubules contain few premeiotic spermatogenic or Sertoli cells and the interstitial tissue is undeveloped. $\times 150$.

Fig. 2. In the transplanted testis of an *hpg* mouse under the tunica albuginea of a normal testis after 84 days, seminiferous tubules are present in various states of development and active interstitial cells can be seen. The tunica albuginea of the transplanted testis is arrowed. $\times 150$.

(Facing p. 250)

Fig. 3. In the ovary of the hypogonadal mouse there is very little evidence of antrum formation and very few follicles possess more than 6–8 layers of granulosa cells. $\times 50$.

Fig. 4. In the ovary of a normal mouse corpora lutea and follicles in various stages of development can be seen. $\times 50$.

Fig. 5. Corpora lutea and follicles in various stages of development are present in the ovary of an *hpg* mouse after transplantation to the ovarian capsule of a normal female. $\times 125$.

Fig. 6. A fragment of ovary can be seen in the periovarian tissue dissected from a female in Group A of Exp. II. $\times 125$.

infertility. The uteri of these transplant females were as large as those of untreated normal mice (see Table 1).

Table 1. Mean $\pm$ s.e.m. ovarian and uterine weights of female mice with or without ovarian transplants

	No. of mice	Paired ovarian wt (mg)	Uterine wt (mg)
Normal adult ♀	7	10.7 $\pm$ 1.5	136 $\pm$ 12.6
Exp. I (ovarian transplants)	6	13.6 $\pm$ 1.5	146 $\pm$ 13.0
Exp. II (Group A) (ovariectomy but no transplants)	9	—	158.5 $\pm$ 12.6
Exp. III (ovariectomy)	7	—	12.8 $\pm$ 1.7

Experiment II. The vaginae of the females in Group A remained perforate and the vaginal smear patterns exhibited irregular cycles of cornified cells. At autopsy the uteri were no different from those of transplant females or normal adult controls (see Table 1). In all females, a small fragment of ovarian tissue was identified histologically in the periovarian capsule (see Pl. 2, Fig. 6), but was never more than 10% of a normal ovary as assessed by a study of serial sections and was usually considerably less. The fragments remaining contained follicles, oocytes, and interstitial tissue. In Group B females caged with active males the presence of copulatory plugs indicated that the females were mating, but after 2 months these 7 females had produced only 1 young (born dead).

Experiment III. In this group, uterine weight was dramatically reduced (Table 1) and the uteri were atrophic. The vaginal smears never contained cornified cells.

Discussion

These results show that the gonads of *hpg* mice are capable of full gametogenic activity when exposed to a suitable hormonal environment. In the male, all stages of spermatogenesis can be found in the 84-day transplant testes (see Pl. 1, Fig. 2).

The production of *hpg* young from normal females with transplanted ovaries demonstrates that the mutant ovary is capable of performing its gametogenic function. The expected result of mating a male heterozygous for the *hpg* gene with a transplant female producing homozygous recessive oocytes would be 50% heterozygous phenotypically normal offspring and 50% homozygous mutants. In fact the ratio of normal to *hpg* offspring has been 2:1 from the matings analysed so far. The reasons for this deviation from the expected result are not immediately evident. They could be the result of pre- or post-implantation losses. The method used to remove the normal ovaries in the transplant animals quite obviously leaves hormonally active fragments of tissue behind (see Exp. II) and it is possible that ova from such fragments have been fertilized as in the single female in Group B in Exp. II, thereby increasing the numbers of normal offspring. Nevertheless, the fact that *hpg* offspring have been born to transplant females not themselves carrying the *hpg* gene indicates that the transplanted ovary from an *hpg* mouse is producing viable oocytes. The failure of full sexual development in the *hpg* mouse does not therefore result from a gonadal abnormality.

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