Int. J. Dev. Biol. 42: 855-860 (1998)

Germ cells and germ cell transplantation

ANNE MCLAREN*

Wellcome/CRC Institute of Cancer and Developmental Biology, Cambridge, United Kingdom

The germ cell lineage carries the potential for both totipotency and immortality. It forms the fragile link between one generation and the next, and so is of central importance for the survival and evolution of living organisms. Yet it is only recently that we have begun to gain some understanding of the establishment and early development of the germ cell lineage in mammals.

Establishment of the germ cell lineage

Unlike the situation in *Drosophila*, nematode worms and frogs, in mammals the germ cell lineage does not appear to be set aside early in embryonic life, nor have any cytoplasmic determinants for germ cell development (germ plasm, pole plasm) been identified.

At the onset of gastrulation the mouse embryo contains epiblast tissue that will give rise to the future fetus, and extraembryonic ectoderm that will contribute to the placenta and the various extraembryonic membranes. Experiments injecting a fluorescent lineage marker into single epiblast cells of embryos maintained *in vitro* (Lawson and Hage, 1994) have shown that the cells whose descendants will form the germ cell lineage are located in the proximal part of the epiblast, adjacent to the extraembryonic ectoderm, but that in this location they are not yet lineage-restricted. Clonal analysis established that lineage restriction does not occur until midway through gastrulation. By this time the proliferating cells have moved into an extra-embryonic position, posterior to the primitive streak where we were previously able to identify them as a cluster of cells expressing high levels of alkaline phosphatase activity (Ginsburg *et al.,* 1990). The size of the initial germ cell pool is estimated to be about 45 cells (Lawson and Hage, 1994).

When epiblast cells are transplanted from the distal to the proximal region, adjacent to the extraembryonic ectoderm, they too will give rise to germ cells, while no germ cells develop in the distal region when the reciprocal transplantation is carried out (Tam and Zhou, 1996). Clearly the proximal location is important for germ cell determination, although the cells are not yet lineage restricted. Given appropriate signals to provide positional information, the potential for germ cell development, and hence the capacity to retain totipotency, may reside in all epiblast cells.

Germ cell migration

About 24 h after initial germ-cell lineage restriction, mouse germ cells move from their extra-embryonic location, and are carried along with the invaginating hind gut towards the genital ridges, the site of the future gonads. Extracellular matrix molecules along the migration pathway are clearly important, and during the later stages of migration, up the gut mesentery and into the ridges, there is evidence that many of the germ cells are linked together by cell processes to form a network (Gomperts *et al.,* 1994). Little is known

***Address for reprints:** Wellcome/CRC Institute of Cancer and Developmental Biology, Tennis Court Road, Cambridge, CB2 1QR, United Kingdom. FAX: 1223 334089

0214-6282/97/$05.00

Printed in Spain

about how the germ cells find their way to the ridges, though contact guidance, differential adhesion and chemotaxis have all been postulated.

Throughout the three days of migration and a subsequent couple of days in the genital ridges, the primordial germ cells are dividing at a steady rate. The survival and proliferation of germ cells during this period depends on the presence of an intact c-kit/steel factor signal transduction pathway. If either the c-kit receptor on the germ cells is disrupted, as in mutations at the *W* locus (Dominant White Spotting), or its ligand (Steel factor, stem cell factor) is absent, as in *Steel* mutants, few germ cells reach the genital ridges and fertility is abolished or greatly reduced.

Using DNA genotyping to identify W^e/W^e embryos in segregating litters, we were able to establish that the number of primordial germ cells at the beginning of the migratory period did not differ significantly between homozygous mutant and wild-type embryos (Buehr *et al.,* 1993). Thus for the first 24 h after establishment of the lineage, survival and/or proliferation of germ cells is not dependent on the c-kit/Steel signal transduction pathway. Thereafter, while germ cells in wild-type and heterozygous embryos proliferate at a steady rate, germ cells in homozygous mutants fail to increase in number. Germ cells in mutant embryos tended to be clumped together, suggesting a defect in their adhesion properties; they were often found in ectopic sites and few reached the genital ridges.

Entry into meiosis

Throughout the migratory period, germ cells in female and male embryos behave in a similar manner, but after entering the genital ridges their fates diverge (McLaren, 1995). Following two or three days of continued proliferation, germ cells in female embryos enter prophase of the first meiotic division, passing through leptotene, zygotene and pachytene stages to arrest after birth as diplotene oocytes in primordial follicles. At the same time, about 13 days *post coitum*, germ cells in male embryos cease dividing, and remain in G0/G1 (McLaren, 1984) until after birth, when proliferation resumes. The first meiotic spermatocytes are not seen in the testis until at least a week after birth.

Germ cells in female and male embryos of course differ in their sex chromosome constitution, but this is not the basis for their different fates, since XY germ cells in a female genital ridge enter meiosis before birth, while XX germ cells in a testis enter mitotic arrest (for references, see McLaren, 1995). Further, germ cells in a male embryo that migrate into the adrenal primordium rather than into the adjacent genital ridge all enter meiosis at the same time as do germ cells in a female embryo; so also do germ cells in a male genital ridge if the somatic tissue has been disrupted, for example by disaggregation before 12 days *post coitum*, followed by reaggregation and culture in an organ culture system (McLaren and Southee, 1997). This result suggests that entry of germ cells into meiosis is prevented in the male genital ridge by the presence of some inhibitory factor, the production of which is blocked by prior disaggregation.

To investigate whether the mouse urogenital ridge (which gives rise to the adrenal as well as to the gonad) produces a substance that induces germ cells to enter meiosis before birth, McLaren and Southee (1997) recovered germ cells from female and male genital ridges at various stages of development, and cultured them in a reaggregate of embryonic lung. The germ cells from female embryos entered meiosis at the expected time, whatever the stage from which they were recovered. If the organ cultures were maintained for 3 weeks, the germ cells developed into growing oocytes. The germ cells from male embryos also entered meiosis if they were recovered from embryos younger than 12 days *post coitum*, but after 12 days they went into mitotic arrest and developed as prospermatogonia in the lung aggregates as they would have done *in vivo*.

It therefore seems possible that mouse germ cells have a cell-autonomous tendency to enter meiosis at a certain stage of development, perhaps after a certain number of mitotic cycles (8-9) from germline lineage restriction. (In the adult mouse testis, germ cells are believed to enter meiosis 12 mitotic divisions after budding off from the spermatogonial stem cells). Entry into meiosis before birth is inhibited in the testis by the production of some as yet unidentified factor.

Culture of primordial germ cells

If mouse germ cells do indeed have a cell-autonomous tendency to enter meiosis, this must require the permissive environment of surrounding somatic tissue, whether genital ridge, adrenal or lung, in order to be realized. There have been no published reports of primordial germ cells entering meiosis *in vitro*, when maintained as isolated cells.

When germ cells were harvested from mouse genital ridges with minimal somatic cell contamination (De Felici and McLaren, 1982) and cultured in plastic dishes (De Felici and McLaren, 1983), those taken from female ridges after 13 days, that had already entered meiosis, survived for several days and progressed through the various stages of first meiotic prophase *in vitro* as *in vivo*. Male germ cells and premeiotic female germ cells, on the other hand, survived for only a short time and did not proliferate. Subsequent experiments using substrates such as fibronectin, laminin and collagen and culture on feeder layers, also addition of cytokines to the culture medium, gave improved viability and some proliferation of germ cells from 8.5 days *post coitum* onwards (see Buehr, 1997 for references), but increase in cell numbers ceased after no more than a week and the cultures could not be maintained for longer periods.

Continued proliferation of premeiotic germ cells was eventually achieved when germ cells from 8.5 day *post coitum* embryos were cultured on feeder layers expressing the membrane-bound form of Steel Factor, with soluble Steel Factor, LIF and bFGF all present in the culture medium (Matsui *et al.,* 1992; Resnick *et al.,* 1992). Under these conditions, the germ cells continued to survive and proliferate, they could be subcultured and cell lines established. These “immortalized” germ cells have been termed embryonic germ (EG) cells: injected back into mouse blastocysts, they are able to colonize all lineages of the developing embryo, including the germline (Stewart *et al.,* 1994). In this they resemble embryonic stem (ES) cells, which they also resemble in appearance.

EG cell lines have also been derived from both male and female germ cells 11.5 and 12.5 days *post coitum*. These resemble normal ES cells less closely, since on injection back into blastocysts they cause fetal overgrowth and skeletal abnormalities (Tada *et al.,* 1998), resembling those seen in chimeras made with androgenetic ES cells. The methylation status of alleles at several imprintable

loci suggested that by 11.5 days *post coitum* germ cells had undergone initial epigenetic changes, similar in both males and females (Tada *et al.,* 1998). Lymphocytes fused with these EG cells undergo loss of allele-specific methylation, perhaps because EG cells (and by implication germ cells also) lack functional demethylation protection factors (Tada *et al.,* 1997).

Preliminary results (Garcia-Castro *et al.,* 1997) suggest that immortalized cell lines can also be derived from germ cells taken from the genital ridges of mice from a strain transgenic for the gene coding for the temperature-sensitive SV40 large T antigen. These germ cell lines differ in some respects from both EG and ES cell lines.

Gametogenesis

Although primordial germ cells can be cultured throughout the migratory phase, and immortalized by the influence of various growth factors, and although EG cells retain their totipotency and can contribute to every cell lineage in the developing embryo if introduced into a blastocyst, it is not yet possible in mammals to support the development *in vitro* of diploid, premeiotic germ cells through to mature gametes, in the absence of supporting tissue.

In fish, Nagahama and his colleagues (e.g. Miura *et al.,* 1991) have devised an organ culture system in which fragments of immature Japanese eel testis, containing only spermatogonia and inactive testicular somatic cells, can be maintained in chemically defined medium. Spermatogenesis can be induced hormonally: gonadotrophin stimulation leads to the production by Leydig cells of 11-ketotestosterone, which in turn activates Sertoli cells to stimulate premeiotic spermatogonia to complete spermatogenesis.

In mice, premeiotic oogonia will enter meiotic prophase and give rise to growing oocytes in fragments of embryonic ovary maintained in an organ culture system (McLaren and Buehr, 1990); this transition does not require stimulation from the somatic tissue of the ovary, since oogonia released from the embryonic ovary and transferred to embryonic lung reaggregates will also enter meiosis and give rise to growing oocytes after a period of culture (McLaren and Southee, 1997). Primordial follicles, in which the oocytes are arrested in the diplotene stage of first meiotic prophase, have been recovered from the neonatal mouse ovary and matured *in vitro* by appropriate hormone treatment. After removal of the surrounding follicle cells, the mature oocyte can be fertilized and the resulting embryo transferred to the uterus, where it will develop to term, though the success rate is still very low (Eppig and O'Brien, 1996).

Spermatogenesis in the testes of male mammals continues throughout life. A population of spermatogonial stem cells (Huckins, 1971; Clermont, 1972), which divide slowly and are relatively resistant to irradiation and other environmental hazards, gives rise to waves of proliferating and differentiating spermatogonia that enter meiosis and develop through the stages of primary and secondary spermatocytes, round and elongated spermatids, eventually to form mature spermatozoa. The timing of spermatogenesis and the structure and ultrastructure of the testis have been exhaustively researched in many mammalian species; the various spermatogenic stages can be isolated and analyzed biochemically; the hormonal requirements for spermatogenesis have been established, and many stage-specific genes have been isolated and cloned. The interactions between spermatogenic cells and the Sertoli cells that nurture them are being intensively studied, both *in vivo* and *in vitro* (e.g., Grandjean *et al.*, 1997), yet it has proved extremely difficult to devise a culture system that will support development all the way from spermatogonia to spermatozoa. So far the only report that has been published of diploid spermatogonia progressing *in vitro* to haploid spermatids involves coculture with an immortalized Sertoli cell line (Rassoulzadegan *et al.,* 1993).

Germ cell transplantation

It was therefore of particular interest when Brinster and Zimmermann (1994) reported that spermatogonial stem cells recovered from the testes of baby mice could repopulate the seminiferous tubules of sterile male mice, and produce mature spermatozoa. The recipient males were rendered sterile either genetically (owing to absence of functional c-kit receptors, few if any germ cells populate the gonads of mice homozygous for mutations at the *W* locus, though the Sertoli cells remain normal) or by treatment with the chemotherapeutic drug busulfan, which kills all spermatogenic stages with the exception of a small number of the highly resistant stem cells (Bucci and Meistrich, 1987). Active spermatogenesis was established in about one third of the injected testes; in the Busulfan-treated males, the donor origin of the spermatogenic cells was confirmed by using a donor strain carrying a *LacZ* transgene expressed in spermatids and spermatozoa. The number of spermatozoa present in the ejaculate was too low to restore fertility. In a subsequent series (Brinster and Avarbock, 1994), the proportion of recipients in which the donor cells successfully established spermatogenesis was increased to over 70% by using somewhat older but still immature donor males; while decreasing the dose of busulfan to allow some endogenous spermatogenesis led to successful pregnancies following mating of the treated mice, with up to 80% of the progeny being sired by the transplanted cells. Since donor-derived progeny were still being produced 8 months following transplantation, it is clear that donor-derived spermatogenesis was initiated by spermatogonial stem cells rather than by later spermatogenic stages.

As the authors point out, spermatogonial transplantation potentially provides a simpler and cheaper route to germline modification by homologous recombination than the existing use of embryonic stem cells injected into the blastocyst. However, this would depend on being able to culture spermatogonial stem cells for long enough to carry out the required genetic modification and subsequent selection. This objective has not yet been fully realized, though preliminary results look promising (Nagano and Brinster, 1998). In an attempt to combine the two approaches, Brinster and Avarbock (1994) injected mouse embryonic stem cells into seminiferous tubules of sterile recipients, but rather than giving rise to spermatogenic cells, the injected cells formed tumors.

In farm animals, where the economic rewards for genetic modification could be very great, embryonic stem cell lines have not yet been produced. The birth of lambs derived from the transfer of nuclei of cultured cells to enucleated oocytes (Wilmut *et al.,* 1997) offers an alternative route to genetic manipulation, but the technique at present is very inefficient. If an adequate culture system for spermatogonia could be devised, transplantation of genetically modified cells to a host testis would ensure germline transmission.

Interspecific transplantation

In the spermatogonial transplantation experiments described above, donor and host strains were selected to be immunologically compatible. Male germ cell transplantation between the testes of two immunologically diverse strains of rat has also been successful in supporting spermatogenesis but in minitubules formed within the lumen of the host seminiferous epithelium, i.e. protected from immunological attack by the blood-testis barrier (Jiang and Short, 1995). The stage of spermatogenesis in donor and adjacent host epithelium was closely synchronized. Not surprisingly, immature rat spermatogonia failed to colonize the seminiferous tubules of immunologically competent mice (Clouthier *et al.,* 1996); however, when immunodeficient nude (lacking T cells) or SCID (lacking both B and T cells) mice were used as hosts, rat spermatogenesis occurred and rat spermatozoa with normal morphology were produced, over a period of several months following transplantation. The host mice were treated with a level of busulfan that eliminated most but not all of the endogenous mouse spermatogenesis; rat spermatogenic stages (other than spermatozoa, which retain very little cytoplasm) could be distinguished by staining for β-galactosidase, since the donor rat strain carried a *LacZ* transgene. Rat and mouse spermatozoa could readily be distinguished by the shape of the sperm head, as well as by their tail length.

The Sertoli cells that line seminiferous tubules nourish and regulate spermatogenic cells at all stages of their development, from spermatogonia to spermatids. Signals of many different types must pass between Sertoli cells and germ cells in both directions, though as yet only a few have been identified (McGuinness and Griswold, 1994; Fritz, 1994). When donor spermatogonia are injected into host seminiferous tubules, some donor Sertoli cells are transferred along with the germ cells. In the experiments using mouse donor cells, it was not possible to ascertain whether any of the Sertoli cells lining the seminiferous tubules after transplantation were of donor origin, since the *LacZ* transgene carried by the donor mouse strain was not expressed in Sertoli cells. In the rat into mouse transplantation experiments, on the other hand, the donor rat strain carried a *LacZ* transgene that was expressed in Sertoli cells, and it was evident that all the Sertoli cells lining the tubules were of host origin (Clouthier *et al.,* 1996). This result was confirmed by ultrastructural observations (Russell and Brinster, 1996), since mouse and rat Sertoli cells can be distinguished at the ultrastructural level, for example by the appearance of the mitochondria. Whether the spermatogonia and later spermatogenic stages in a given tubule were of mouse or rat origin, the Sertoli cells supporting them were always of host (i.e. mouse) type.

The finding that mouse Sertoli cells can fully support rat spermatogenesis, in spite of the ten million years or so of evolution that separate the two species, is remarkable. The differences in the shape of the sperm head between different inbred mouse strains has been shown to be a cell-autonomous character, not affected by the strain of origin of the associated Sertoli cell (Burgoyne, 1975). Evidently the same is true of the more striking species-specific differences in head shape that characterize rat and mouse spermatozoa. There is also a marked difference in the length of the spermatogenic cycle between mice (35 days) and rats (52 days). For rat spermatogenesis taking place in mouse seminiferous tubules, it will be important to know whether the timing is determined by the germ cells or the Sertoli cells or both.

Future applications

The ability to grow spermatozoa of one species in the testis of another opens up intriguing possibilities – especially now that *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) have removed the need for large numbers of spermatozoa in an ejaculate to achieve pregnancies. There seems no reason why species other than the mouse could not be used as hosts: Ogawa *et al.* (1997) discuss the possible advantages in larger animals of spermatogonial injection into the rete testis [the route used by Jiang and Short (1995) in rats] or into the efferent ducts rather than directly into the seminiferous tubules. Although Sertoli cells are believed to secrete immunosuppressive substances (Bellgrau *et al.,* 1995), xenotransplantation would require the use of either immunodeficient hosts, or hosts rendered immunologically compatible with the donor species by genetic manipulation. We do not know how big a species gap between donor and host would still permit spermatogenesis to proceed: it may be that the mouse with its convenient immunodeficient strains could be used as a "universal host".

Avarbock *et al.* (1996) report that both mouse and rat spermatogonial stem cells can be successfully cryopreserved at -196°C, using the simple freezing techniques that are adequate for somatic cells rather than the more elaborate protocols designed for storing sperm samples, which vary from one species to another. After thawing, both mouse and rat stem cells were able to generate spermatogenesis in mouse testes. By storing testicular tissue, it should be possible to preserve indefinitely the germ lines of unique experimental animals, or valuable farm animals that die before puberty, or that are too old to breed. Testicular tissue from endangered species could be preserved for future xenotransplantation.

In our own species, female infertility arising from ovulatory problems responds well to hormonal treatment. Male infertility, on the other hand, which is involved in nearly half of all the cases of infertility for which couples seek medical advice, has proved much more intractable to therapy. For men whose ejaculates contain even a few sperm, ICSI, in which a single sperm is injected into the cytoplasm of the egg, has proved unexpectedly successful, giving a pregnancy rate equalling that of normal IVF. Fertilization has been achieved even when sperm motility and morphology is poor. Sperm recovered from the epididymis or from the testis can also be used in ICSI, and a few live births have been reported following the injection of spermatids. Many infertile men have been enabled to father their own children with the aid of these procedures.

If however the infertile patient's testis supports no active spermatogenesis but contains only spermatogonial stem cells, it might be possible to transplant these into a host testis of a different species, and obtain sufficient sperm of donor origin to achieve a pregnancy using ICSI. Since testicular tissue can still be used after cryopreservation, tissue from prepubertal boys undergoing cancer treatment could be stored for their future use, and if their own testes could not support spermatogenesis, xenotransplantation could be considered.

Two potential problems in any clinical application can be foreseen: acceptability and immunological compatibility. Immunodeficient strains of mice can support rat spermatogenesis; we do not know if they

would support human spermatogenesis. An alternative might be the transgenically humanized lines of pigs produced as potential xenograft organ donors. Infertile couples are prepared to endure a great deal to achieve a pregnancy; however, women might not find it acceptable to have their eggs fertilized by sperm matured in a pig or a mouse, while men might prefer a different species, perhaps a bull or a stag, or even a hamster, since hamsters have associations with infertility treatment and are widely regarded as attractive animals.

Whatever the future holds, the demonstration that spermatogenesis can be successfully carried out in a testis of a different species not only raises fascinating problems for basic studies on gametogenesis, but is likely also to have important practical applications.

Summary

The germ cell lineage in mice is established about a week after fertilization, in a group of cells that have left the epiblast and moved to an extraembryonic site. They migrate back into the embryo, along the hind gut and into the gonads. Germ cells in male and female embryos then pursue different pathways: in the testis the germ cells cease proliferating and enter mitotic arrest, while germ cells in the ovary, like those in male embryos that remain outside the gonads, enter meiotic prophase. Studies on explanted germ cells suggest that all germ cells may enter meiosis at a certain stage of their development, unless prevented from doing so by some inhibitory influence of the testis.

Germ cells during the migratory stage can be cultured, but do not enter meiosis unless embedded in somatic tissue. Addition of certain growth factors and cytokines to the culture medium allows germ cells to proliferate indefinitely *in vitro*: Like embryonic stem cells, these immortalized EG (embryonic germ) cells will colonize all cell lineages if introduced into a blastocyst.

After birth, germ cells undergo gametogenesis; oogenesis in the female, spermatogenesis in the male. Brinster and his colleagues have shown that spermatogonial stem cells injected into a germ-cell depleted testis will repopulate the seminiferous tubules and undergo spermatogenesis, giving rise to functional spermatozoa. Stem cells from frozen testicular tissue are still capable of giving rise to spermatogenesis in a host testis. Rat testicular tissue can undergo spermatogenesis in a mouse testis, to form morphologically normal rat spermatozoa, even though the Sertoli cells that support them are of endogenous mouse origin. These findings are of fundamental importance for our understanding of spermatogenesis and the interactions between germ cells and Sertoli cells; but they also have significant practical implications, in relation to both agricultural practice and clinical treatment of infertility.

KEY WORDS: *primordial germ cells, germ cell lineage, spermatogenesis, spermatogonial transplantation, spermatogenic xenografting*

Acknowledgments

I am grateful to the Wellcome Trust for financial support.

References

AVARBOCK, M.R., BRINSTER, C.J. and BRINSTER, R.L. (1996). Reconstitution of spermatogenesis from frozen spermatogonial stem cells. *Nature Med. 2* : 693-696.

BELLGRAU, D., GOLD, D., SELAWRY, H., MOORE, J., FRANZUSOFF, A. and DUKE, R.C. (1995). A role for CD95 ligand in preventing graft rejection. *Nature 377*: 630-632.

BRINSTER, R.L. and AVARBOCK, R.L. (1994). Germline transmission of donor haplotype following spermatogonial transplantation. *Proc. Natl. Acad. Sci. USA 91*: 11303-11307.

BRINSTER, R.L. and ZIMMERMANN, J.W. (1994). Spermatogenesis following male germ-cell transplantation. *Proc. Natl. Acad. Sci. USA 91*: 11298-11302.

BUCCI, L.R. and MEISTRICH, M.L. (1987). Effects of busalfan on murine spermatogenesis: Cytotoxicity, sterility, sperm abnormalities, and dominant lethal mutations. *Mutat. Res. 176*: 259-268.

BUEHR, M. (1997). The primordial germ cells of mammals: some current perspectives. *Exp. Cell Res. 232:* 194-207.

BUEHR, M., McLAREN, A., BARTLEY, A. and DARLING, S. (1993). Proliferation and migration of primordial germ cells in W^e/W^e mouse embryos. *Dev. Dynamics 198:* 182-189.

BURGOYNE, P.S. (1975). Sperm phenotype and its relationship to somatic and germline genotype. A study using mouse aggregation chimaeras. *Dev. Biol. 44*: 63-76.

CLERMONT, Y. (1972). Kinetics of spermatogenesis in mammals: seminiferous epithelium cycle and spermatogonial renewal. *Physiol. Rev. 52*: 198-236.

CLOUTHIER, D.E., AVARBOCK, M.R., MAIKA, S.D., HAMMER, R.E. and BRINSTER, R.L. (1996). Rat spermatogenesis in mouse testis. *Nature 381*: 418-421.

De FELICI, M. and McLAREN, A. (1982). Isolation of mouse primordial germ cells. *Exp. Cell Res. 142*: 477-482.

De FELICI, M. and McLAREN, A. (1983). *In vitro* culture of mouse primordial germ cells. *Exp. Cell Res.* 144, 417-427.

EPPIG, J.J. and O'BRIEN, M.J. (1996). Development *in vitro* of mouse oocytes from primordial follicles. *Biol. Reprod. 54*: 197-207.

FRITZ, I.B. (1994). Somatic cell-germ cell relationships in mammalian testes during development and spermatogenesis. In *Germline Development* (eds J. Marsh & J Goode)., Ciba Found. Symp. 182. pp271-281.

GARCIA-CASTRO, M., BRONNER-FRASER, M., SURANI, A.M and McLAREN, A. (1997). Immortalization of primordial germ cells. *13th Int. Cong. Dev. Biol.* S59 (Abstr.)

GINSBURG, M., SNOW, M.H.L. and McLAREN, A. (1990). Primordial germ cells in the mouse embryo during gastrulation. *Development 110*: 521-528.

GOMPERTS, M., GARCIA-CASTRO, M., WYLIE, C., and HEASMAN., J. (1994). Interactions between primordial germ cells play a role in their migration in mouse embryos. *Development 120*: 135-141.

GRANDJEAN, V., SAGE, J., RANC, F., CUZIN, F., and RASSOULZADEGAN, M. (1997). Stage-specific signals in germ line differentiation: control of Sertoli cell phagocytic activity by spermatogenic cells. *Dev. Biol. 184*: 165-174.

HUCKINS, C. (1971). The spermatogonial stem cell population in adult rats. Their morphology, proliferation, and maturation. *Anat. Res 169*: 533-558.

JIANG, F-X. and SHORT, R.V. (1995). Male germ cell transplantation in rats: Apparent synchronization of spermatogenesis between host and donor seminiferous epithelia. *Int. J. Androl. 18*: 326-330.

LAWSON, K.A. and HAGE, W.J. (1994). Clonal analysis of the origin of primordial germ cells in the mouse. In *Germline Development*, Ciba Found. Symp. 182. pp 68-91. Chichester: Wiley.

MATSUI, Y., ZSEBO, K. and HOGAN, B.L.M. (1992). Derivation of pluripotential stem cells from murine primordial germ cells in culture. *Cell 70:* 841-847.

MCGUINNESS, M.P. and GRISWOLD, M.D. (1994). Interactions between Sertoli cells and germ cells in the testis. *Semin. Dev. Biol. 5*: 61-66.

McLAREN, A. (1984). Meiosis and differentiation of mouse germ cells. In *Controlling Events in Meiosis* (C.W. Evans and H.G. Dickinson, eds). 38th Symp. Soc., Exp. Biol., Company of Biologists, Cambridge, pp 7-23.

McLAREN, A. (1995). Germ cells and germ cell sex. *Philos. Trans. R. Soc. Lond. [Biol.] 350*: 229-233.

McLAREN, A. and BUEHR, M. (1990). Development of mouse germ cells in cultures of fetal gonads. *Cell. Differ. Dev. 31*: 185-195.

McLAREN, A. and SOUTHEE, D. (1997). Entry of mouse embryonic germ cells into meiosis. *Dev. Biol. 187*: 107-113.

MIURA, T., YAMAUCHI, K., TAKAHASHI, H., and NAGAHAMA, Y. (1991). Hormonal induction of all stages of spermatogenesis *in vitro* in the male Japanese eel (Anguilla japonica). *Proc. Natl. Acad. Sci. USA 88*: 5774-5778.

NAGANO, M. and BRINSTER, R.L (1998). Spermatogonial transplantation and reconstitution of donor cell spermatogenesis in recipient mice. *Acta Pathol. Microscop. Immunol. Scand. 106:* 47-57.

OGAWA, T., ARECHAGA, J.M. AVARBOCK, M.R. and BRINSTER, R.L. (1997). Transplantation of testis germinal cells into mouse seminiferous tubules. *Int. J. Dev. Biol. 41*: 111-122.

RASSOULZADEGAN, M., PAQUIS-FLUCKLINGER, V., BERTINO, B., SAGE, J., JASIN, M., MIYAGAWA, K., VAN HEYNINGEN, V., BESMER, P. and CUZIN, F. (1993). Transmeiotic differentiation of male germ cells in culture. *Cell 75*: 997-1006.

RESNICK, J.L., BIXLER, L.S., CHENG, L. and DONOVAN, P.J. (1992). Long-term proliferation of mouse primordial germ cells in culture. *Nature 359:* 550-551.

RUSSELL, L.D. and BRINSTER, R.L. (1996). Ultrastructural observations of spermatogenesis following transplantation of rat testis cells into mouse seminiferous tubules. *J. Androl. 17*: 615-627.

STEWART, C.L., GADI, I. and BHATT, H. (1994). Stem cells from primordial germ cells can re-enter the germ line. *Dev. Biol. 161*: 626-628.

TADA, M., TADA. T., LEFEBVRE, L., BARTON, S.C. and SURANI, M.A. (1997). Embryonic germ cells induce epigenetic reprogramming of somatic nucleus in hybrid cells. *EMBO J. 16*: 6510-6520.

TADA, T., TADA, M., HILTON, L., BARTON, S.C., SADO, T., TAKAGI, N. and SURANI, M.A. (1998). Epigenotype switching of imprintable loci in embryonic germ cells. *Dev.Genes Evol. 207:* 551-561.

TAM, P.P.L. and ZHOU, S.X. (1996). The allocation of epiblast cells to ectodermal and germ-line lineages is influenced by the position of the cells in the gastrulating mouse embryo. *Dev. Biol. 178*: 124-132.

WILMUT, I., SCHNIEKE, A.E., McWHIR, J., KIND, A.M. and CAMPBELL, K.H.S. (1997). Viable offspring derived from fetal and adult mammalian cells. *Nature 385*: 810-813.