Molecular Human Reproduction, Vol.15, No.4 pp. 205–213, 2009
Advanced Access publication on February 13, 2009 doi:10.1093/molehr/gap008

MHR Basic science of reproductive medicine

NEW RESEARCH HORIZON ***Invited Review***

Germ cell sex determination in mammals

Ayhan Kocer, Judith Reichmann, Diana Best, and Ian R. Adams[1]

MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK

[1]Correspondence address. Tel: +44-0131-332-2471; Fax: +44-0131-467-8456; E-mail: Ian.Adams@hgu.mrc.ac.uk

ABSTRACT: One of the major decisions that germ cells make during their development is whether to differentiate into oocytes or sperm. In mice, the germ cells' decision to develop as male or female depends on sex-determining signalling molecules in the embryonic gonadal environment rather than the sex chromosome constitution of the germ cells themselves. In response to these sex-determining cues, germ cells in female embryos initiate oogenesis and enter meiosis, whereas germ cells in male embryos initiate spermatogenesis and inhibit meiosis until after birth. However, it is not clear whether the signalling molecules that mediate germ cell sex determination act in the developing testis or the developing ovary, or what these signalling molecules might be. Here, we review the evidence for the existence of meiosis-inducing and meiosis-preventing substances in the developing gonad, and more recent studies aimed at identifying these molecules in mice. In addition, we discuss the possibility that some of the reported effects of these factors on germ cell development may be indirect consequences of impairing sexual differentiation of gonadal somatic cells or germ cell survival. Understanding the molecular mechanisms of germ cell sex determination may provide candidate genes for susceptibility to germ cell tumours and infertility in humans.

Key words: germ cell / oogenesis / spermatogenesis / sex determination

Introduction

Somatic sex determination

Sex determination in mice, as in most mammals, occurs through inheritance of the X and Y sex chromosomes. A number of genes have been implicated in translating the sex chromosome constitution of the embryo into sexual differentiation of the gonadal somatic cells (Fleming and Vilain, 2004; Wilhelm *et al*., 2007; Matzuk and Lamb, 2008), and only a simplified outline of somatic sex determination is presented here. The presence or absence of the Y-encoded male-determining *Sry* gene directs the developing gonad to differentiate into either a testis or an ovary, which in turn directs the sexual development of the rest of the embryo (Lovell-Badge and Robertson, 1990; Koopman *et al*., 1991). In mice, a transient burst of *Sry* expression in the gonadal supporting cell lineage at ~10.5 days post-coitum (dpc) directly leads to up-regulation of the transcription factor *Sox9* in XY gonads (Sekido *et al*., 2004; Sekido and Lovell-Badge, 2008). Both *Sry* and *Sox9* are necessary and sufficient to differentiate the supporting cell lineage into male Sertoli cells rather than female granulosa cells (Lovell-Badge and Robertson, 1990; Koopman *et al*., 1991; Vidal *et al*., 2001; Chaboissier *et al*., 2004). The Sertoli cells then signal to and masculinize other cell types in the gonad and adjacent mesonephros to induce testis differentiation. Thus, Sertoli cells directly or indirectly induce male differentiation of the germ cells, the testosterone-producing Leydig cells and the peritubular myoid cells that will surround the Sertoli cells and germ cells to form the testis cords (Palmer and Burgoyne, 1991; Ross and Capel, 2005). In addition, nascent Sertoli cells express the signalling molecule *Fgf9*, which signals back to the Sertoli cells to maintain up-regulated *Sox9* expression and male development in the supporting cell lineage (Colvin *et al*., 2001; Kim *et al*., 2006). However, it is not clear how Sertoli cells masculinize some of the other cell types, such as the germ cells, in the developing testis.

In XX embryos, the supporting cell lineage differentiates into female granulosa cells when *Sry* expression does not occur. It is not clear whether other gonadal cell types are directly or indirectly induced to differentiate along a female pathway by the developing granulosa cells, or differentiate as female by default. Wnt signalling molecules such as *Rspo1* and *Wnt4* are expressed by XX gonadal somatic cells and are required for female sex determination (Vainio *et al*., 1999; Chassot *et al*., 2008). *Rspo1* and *Wnt4* appear to induce female differentiation at least in part by down-regulating *Sox9* expression in the supporting cell lineage (Kim *et al*., 2006; Chassot *et al*., 2008). Thus, the antagonistic effects of Wnt signalling molecules and the *Sry*/*Sox9*-dependent Fgf signalling molecules

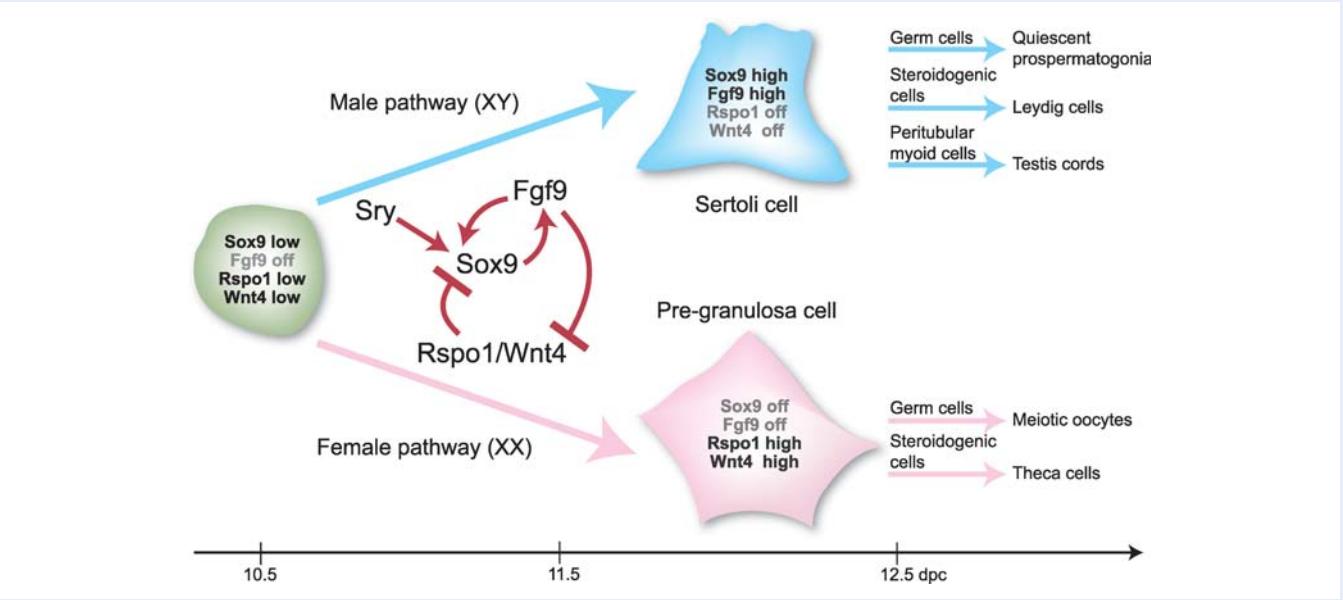

Figure 1 Somatic sex determination in mice. Schematic diagram showing differentiation of gonadal supporting cells (green) into male Sertoli cells (blue) or female pre-granulosa cells (pink). Changes in gene expression driving supporting cell differentiation and the downstream sexual differentiation of other gonadal cell types are indicated.

drive sexual differentiation of the supporting cell lineage and development of the gonad down either a male or a female pathway (Fig. 1).

Germ cell sex determination

The germ cells are one of the main cell types that respond to sex-determining signalling molecules in the embryonic gonad. Migrating primordial germ cells reach the genital ridge at ~10.5 dpc in mice and differentiate into either meiotic oocytes or quiescent prospermatogonia depending on the sex of the embryo. Germ cells proliferate during migration and for a few days after colonizing the gonad in both male and female embryos. The first morphological sign of sex-specific germ cell development is seen at ~13.5 dpc when female germ cells initiate meiosis. These meiotic oocytes proceed through the leptotene, zygotene and pachytene stages of meiotic prophase before birth, then arrest in diplotene until adulthood. In contrast, male germ cells do not initiate meiosis in the embryo, but rather enter a period of quiescence during late embryogenesis. After birth, some of the male germ cells will resume mitotic proliferation and differentiate into spermatogonial stem cells. The first wave of male germ cells to initiate meiosis will not do so until around a week after birth (McLaren, 1984; McLaren, 2003).

The germ cells' decision to develop as male or female depends on external signals in their surrounding environment rather than the chromosomal sex of the germ cells themselves: XY germ cells can develop as oocytes in female chimaeric embryos and XX germ cells can develop as prospermatogonia in male chimaeric embryos (Ford *et al.*, 1975; Palmer and Burgoyne, 1991). These observations are consistent with the sex-determining activity of the Y chromosome acting only in the supporting cell lineage and the resulting Sertoli cells influencing sexual differentiation of other gonadal cell types through extracellular signals (Palmer and Burgoyne, 1991). The developmental timing of the germ cells' response to sex-determining signals in the gonad differs between male and female embryos (Fig. 2). Germ cells in XY gonads commit to spermatogenesis between 11.5 and 12.5 dpc (McLaren and Southee, 1997; Chuma and Nakatsuji, 2001; Adams and McLaren, 2002). Thus, germ cells in 11.5 dpc XY gonads can be induced to sex-reverse and initiate meiosis by co-culturing on feeder cells, in embryonic lung tissue, and in female embryonic urogenital ridge tissue (McLaren and Southee, 1997; Chuma and Nakatsuji, 2001; Adams and McLaren, 2002). However, by 12.5 dpc germ cells in XY gonads have responded to the XY gonadal environment, are committed to differentiate along a male pathway and will not sex-reverse or initiate meiosis even when co-cultured in female embryonic urogenital ridge tissue (Adams and McLaren, 2002). Germ cells in XX gonads appear to commit to oogenesis a day later than germ cells in XY gonads commit to spermatogenesis. Thus, germ cells in 12.5 dpc XX gonads can be induced to sex-reverse and differentiate into prospermatogonia by co-culture in male embryonic urogenital ridge tissue, but germ cells in 13.5 dpc XX gonads continue to differentiate as meiotic oocytes in these conditions (Adams and McLaren, 2002).

The germ cells' commitment to male development occurs at around the same time that germ cells and Sertoli cells become enclosed in the developing testis cords in XY gonads. This has led to some suggestions that the formation of testis cords may help to determine germ cell sex by providing a physical barrier that prevents signalling molecules from reaching the germ cells. However, germ cells can differentiate into prospermatogonia in the testis interstitium between testis cords, as well as when testis cord formation is prevented in culture, suggesting that the testis cords are not required for male germ cell sex determination (McLaren, 1984; Buehr *et al.*, 1993; Yao and Capel, 2002). Thus, germ cell masculinization and testis cord formation do not appear to be causally linked and their

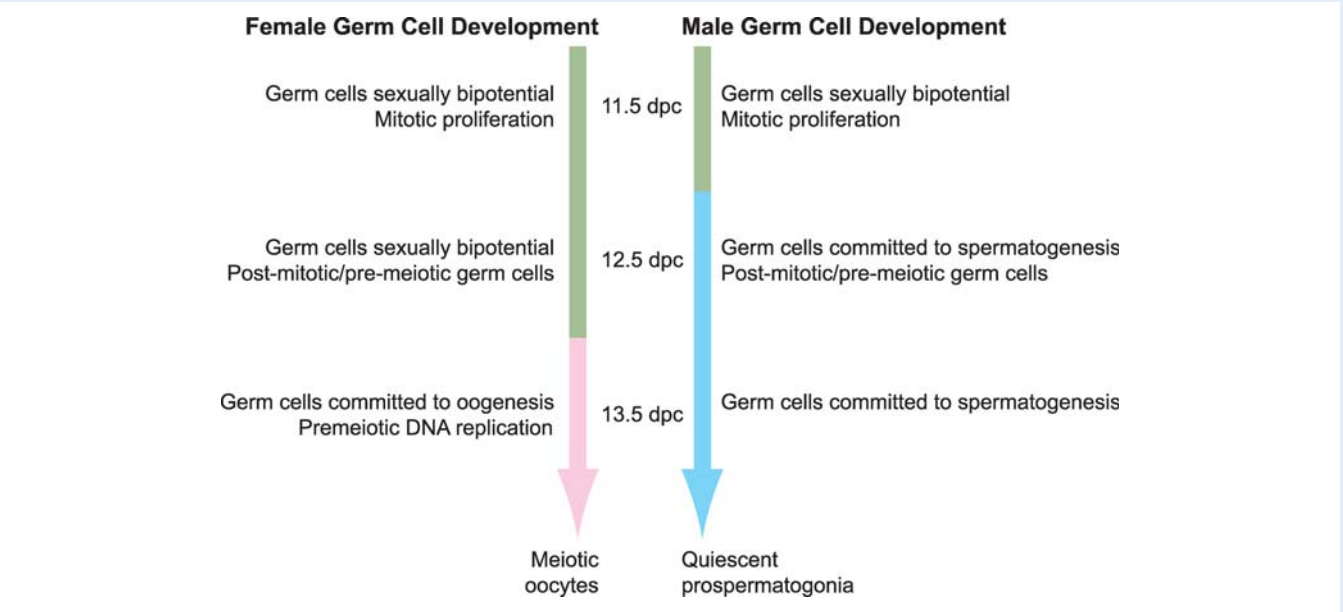

Figure 2 Developmental timing of germ cell sex determination. Schematic diagram of when germ cells commit to spermatogenesis and oogenesis during mouse embryogenesis. Germ cells are sexually bipotential (green) at 11.5 dpc, and commit to spermatogenesis (blue) between 11.5 and 12.5 dpc in male gonads or to oogenesis (pink) ~1 day later in female gonads. dpc, days post-coitum.

coincidence may simply reflect these events being parallel downstream consequences of Sertoli cell differentiation.

The timing of the germ cells' decision to differentiate as male or female also coincides with the appearance of germ cells with a distinctive histological appearance that have been described as both post-mitotic and pre-meiotic (Hilscher *et al*., 1974; McLaren, 1984, 2003). These post-mitotic/pre-meiotic germ cells are present in both male and female embryonic gonads around 12.5–13.5 dpc (McLaren, 1984, 2003) and represent cells in transition between the end of a mitotic cell cycle and the start of a meiotic cell cycle (Hilscher *et al*., 1974; Wartenberg *et al*., 1998). Interestingly, both male and female germ cells initiate expression of some meiotic genes at ~12.5 dpc, but while female germ cells then undergo pre-meiotic DNA replication and proceed through meiotic prophase, male germ cells do not initiate meiosis and meiotic gene expression gradually diminishes (Di Carlo *et al*., 2000; Chuma and Nakatsuji, 2001; McLaren, 2003). The induction of at least some aspects of meiotic gene expression in male and female germ cells at ~12.5 dpc suggests that the initial transcriptional activation of these meiotic genes occurs independently of the sex-determining cues present in the XY gonadal environment that allow germ cells to commit to spermatogenesis at this stage.

Evidence for masculinizing and feminizing factors regulating germ cell sex determination

The somatic environment of the embryonic gonad could potentially influence germ cell sex determination through masculinizing factors in the testis-promoting germ cells to initiate spermatogenesis rather than a default female pathway; or through feminizing factors in the ovary-promoting germ cells to initiate oogenesis rather than a default male pathway; or through masculinizing and feminizing factors in testes and ovaries, respectively (Fig. 3).

Conceptually, these masculinizing and feminizing factors may each comprise one or multiple signalling molecules that induce different aspects of sex-specific germ cell behaviour in male and female embryos. However, as the initiation or prevention of meiosis has often been used to monitor sex-specific differentiation of germ cells, studies have focused on whether a feminizing meiosis-inducing substance (MIS) and/or a masculinizing meiosis-preventing substance (MPS) direct sex-specific germ cell behaviour in the embryonic gonads (Fig. 3). Unfortunately, many of these organ co-culture studies are contradictory and open to alternative interpretations. Evidence for an MIS mainly implicates the mesonephros as its source: in cultured hamster ovaries, or grafted mouse embryonic ovaries, pre-meiotic germ cells only initiate meiosis if the mesonephros is present, although the germ cells do not sex-reverse in the absence of the mesonephros (Byskov, 1974; O and Baker, 1976). Organ co-culture experiments suggest that the mesonephros-derived MIS can induce germ cell sex-reversal in embryonic testes (Byskov and Saxén, 1976; O and Baker, 1976; Byskov, 1978a), but this is typically accompanied by poor development of testis cords, which may indicate impaired Sertoli cell differentiation. If an MPS exists, its expression would depend on Sertoli cell function; therefore, the meiosis seen in these co-culture experiments may be an indirect consequence of a mesonephros-derived factor impairing Sertoli cell gene expression, differentiation or survival.

Some of the early studies reporting a male MPS to counteract the putative mesonephros-derived MIS present in both male and female embryos describe a factor that causes meiotic germ cells to arrest during meiotic prophase in organ co-culture experiments (Byskov and Saxén, 1976), an activity that may be related to the degenerative effects that the male gonadal environment has on germ cells that are

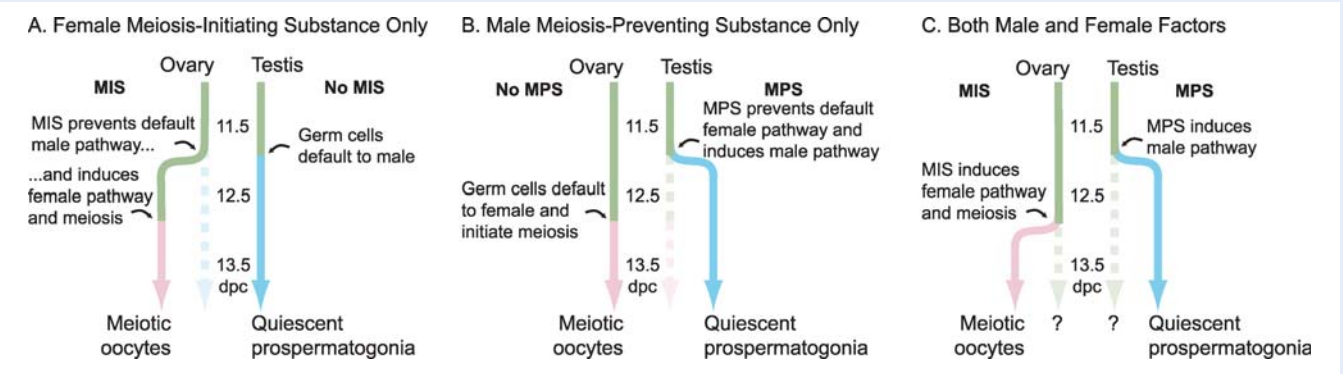

Figure 3 Potential mechanisms for germ cell sex determination. Schematic diagram outlining models for how the embryonic gonadal environment could determine germ cell sex. (**A**) Using a female MIS only. (**B**) Using a male MPS only. (**C**) Using both a female MIS and a male MPS. Green indicates sexually bipotential germ cells, blue indicates commitment to spermatogenesis and pink indicates commitment to oogenesis. dpc, days post-coitum.

already in meiosis rather than a putative MPS activity that prevents germ cells from initiating meiosis (Evans *et al*., 1982; Dolci and De Felici, 1990). However, although some organ co-culture experiments do suggest that a diffusible male MPS can prevent pre-meiotic ovarian germ cells from initiating meiosis (Evans *et al*., 1982), it is not clear whether this is a direct effect on the germ cells or an indirect consequence of testicular factors disrupting somatic cell gene expression, differentiation or survival in the developing ovary. In contrast to the putative MIS, any putative MPS would appear to originate from the gonad itself rather than the mesonephros as removing the mesonephros from 11.5 dpc XY gonads does not prevent the germ cells from developing into male prospermatogonia in culture (Buehr *et al*., 1993).

Thus, it is not clear whether many of the often contradictory organ co-culture studies assaying MIS and MPS activity are analysing direct effects on germ cell development or indirect consequences of changing somatic cell gene expression, differentiation or survival in the gonad. In addition, it can also be difficult to distinguish whether changes in the number of meiotic germ cells reflect changes in pre-meiotic or meiotic germ cell survival rather than changes in the number of germ cells initiating meiosis (McLaren, 1984). Moreover, studies performed in culture systems that do not recapitulate the normal development of both male and female germ cells can add to the confusion over how germ cell sex is determined. As a consequence there is, at present, little conclusive *in vitro* evidence that either a putative MIS or a putative MPS can directly influence germ cell sex determination and further work is needed to demonstrate whether one or both of these factors exists.

In vivo evidence for the activity of an MIS or an MPS in germ cell sex determination is also somewhat inconclusive. The observation that meiotic gene expression occurs in an anterior–posterior wave in developing ovaries (Menke *et al*., 2003; Yao *et al*., 2003) provides some *in vivo* evidence consistent with a mesonephros-derived MIS diffusing into the anterior embryonic ovary. Alternatively, this wave of gene expression may reflect preferential colonization of the anterior gonad by the most advanced migrating germ cells. Interestingly, the spatial distribution of meiotic oocytes within the developing human ovary is not consistent with a mesonephros-derived MIS diffusing into the anterior ovary in this species (Childs *et al*., 2008).

If an MIS is required for mouse germ cells to initiate meiosis, its activity cannot be restricted to embryonic ovaries as ectopic germ cells in the adrenal gland initiate meiosis (Zamboni and Upadhyay, 1983), and germ cells cultured with lung tissue, with feeder cells or even in a feeder-free system also initiate meiosis (McLaren and Southee, 1997; Chuma and Nakatsuji, 2001; Farini *et al*., 2005). Indeed, groups of meiotic oocytes are present inside and outside the testis cords in the anterior region of the testis in normal mouse embryos (Byskov, 1978b; Yao *et al*., 2003), suggesting that any MIS must also be present in the anterior embryonic testis. Interestingly, ectopic germ cells and cultured germ cells initiate meiosis at around the same developmental stage as ovarian germ cells (Zamboni and Upadhyay, 1983; McLaren and Southee, 1997; Chuma and Nakatsuji, 2001). Thus if a widely expressed MIS exists, a cell-autonomous timing mechanism must regulate when germ cells respond to that external signal. Alternatively, this timing mechanism may simply trigger germ cells to initiate meiosis and develop as female by default without any requirement for an external MIS.

In vivo evidence for an MPS mainly comes from the observation that ectopic germ cells in the mesonephros of male embryos usually differentiate into prospermatogonia and are prevented from initiating meiosis (McLaren, 1984). In contrast, ectopic germ cells in a female mesonephros initiate meiosis (McLaren, 1984). These data suggest that a diffusible MPS in the embryonic testis can influence germ cell development in the adjacent mesonephros and is difficult to reconcile with germ cell sex determination being regulated solely by a mesonephros-derived MIS. Thus the MIS-only model for germ cell sex determination (Fig. 3A), and variants of the MIS-only model where the local testicular environment protects the germ cells from the action of the diffusible mesonephros-derived MIS, may not be operating in mice.

New developments

Molecular candidates for factors involved in germ cell sex determination

Recent studies have attempted to identify molecular candidates for the putative MIS and MPS. The finding that the retinoic acid (RA)-inducible

Stra8 gene is expressed in female germ cells from 12.5 dpc, and that *Cyp26b1*, which encodes an enzyme that metabolizes RA, is expressed in male gonads from 12.5 dpc has led to the role of RA in germ cell sex determination being investigated (Bowles *et al*., 2006; Koubova *et al*., 2006). In male and female embryos, RA appears to be synthesized in the mesonephros and diffuses into the adjacent gonad, where metabolism by Cyp26b1 in the male gonad generates a difference in RA levels between the sexes (Bowles *et al*., 2006). The behaviour of RA in the developing urogenital system has clear parallels with the putative mesonephros-derived MIS, and the widespread distribution of RA in the embryo and in serum-containing culture systems, would be consistent with the MIS acting in these diverse embryonic locations and culture systems (Bowles and Koopman, 2007).

Several lines of experimental evidence support RA being an MIS. Culturing female gonads with RA receptor antagonists is reported to prevent *Stra8* expression (Bowles *et al*., 2006; Koubova *et al*., 2006) and germ cell meiosis (Bowles *et al*., 2006). Culturing male gonads with exogenous RA, or Cyp26 inhibitors, induces ectopic *Stra8* expression (Bowles *et al*., 2006; Koubova *et al*., 2006) and germ cell meiosis (Bowles *et al*., 2006). Also, meiotic germ cells have been reported in *Cyp26b1*$^{-/-}$ embryonic testes (MacLean *et al*., 2007). The difference in RA levels between male and female embryonic gonads has therefore been proposed to regulate germ cell sex determination (Bowles and Koopman, 2007).

However, although culturing female gonads with RA receptor antagonists in a serum-free culture system abolishes *Stra8* expression (Bowles *et al*., 2006), the histology suggests that any reduction in the number of meiotic germ cells is caused primarily by the loss of germ cells, rather than germ cell sex-reversal. Culturing 11.5 dpc female gonads with RA receptor antagonists in a serum-containing culture system inhibits *Stra8* expression without the widespread loss of germ cells (Koubova *et al*., 2006). However, the role of *Stra8* in the initiation of meiosis appears to be modified by genetic background (Baltus *et al*., 2006; Anderson *et al*., 2008; Mark *et al*., 2008), and it is not clear if meiosis is prevented or delayed in these cultures, or if the germ cells sex-reverse and develop as prospermatogonia. Interestingly, a region of the *Stra8* promoter containing the RA-response elements can drive transgene expression in male germ cells in the adult testis, but not in meiotic oocytes, suggesting that currently undefined elements of the *Stra8* promoter located outside the RA-response elements may play a role in expressing *Stra8* in meiotic oocytes (Sadate-Ngatchou *et al*., 2008).

Furthermore, although increasing RA levels in 12.5 dpc male gonads cultured in a serum-free system induces *Stra8* expression (Bowles *et al*., 2006), the morphology of the germ cells in 11.5 dpc male gonads cultured in these conditions more closely resembles apoptosis than meiosis. Similar experiments performed in a serum-containing system found that only ~1% of the germ cells in the 11.5 dpc male gonads cultured with RA or Cyp26 inhibitor were in meiosis, whereas the remaining ~99% were male prospermatogonia (Best *et al*., 2008). Increasing RA levels in 12.5 dpc male gonads also induces *Stra8* expression, but meiotic chromosome condensation is not observed (Koubova *et al*., 2006). The failure of RA to induce meiosis in 12.5 dpc embryonic testes is not surprising as germ cells have become committed to spermatogenesis by this stage (Adams and McLaren, 2002). Thus, RA appears to be able to alter gene expression, but may not be able to induce meiosis in the developing testis.

Much of the data supporting the hypothesis that RA is an MIS is open to similar caveats described for the data supporting the existence of a mesonephros-derived MIS (McLaren, 1984). Specifically, does changing RA levels influence the survival of pre-meiotic or meiotic germ cells under the experimental conditions tested, rather than the initiation of meiosis; and does manipulating RA levels affect germ cell behaviour directly or indirectly through altering the gonadal somatic cells?

The possibility that RA primarily influences germ cell survival is supported by studies on cultured primordial germ cells, on cultured embryonic ovaries and in the embryo (Koshimizu *et al*., 1995; Morita and Tilly, 1999). Both these studies found that RA stimulates germ cell proliferation and promotes germ cell survival. Germ cells in male and female gonads appear to use different molecular pathways to regulate both proliferation and survival (Kasai *et al*., 2003; Molyneaux *et al*., 2003; DiNapoli *et al*., 2006; Tanaka *et al*., 2000). Perhaps, some of the reported effects of adding RA receptor antagonists to cultured embryonic ovaries reflect endogenous RA having a role in germ cell proliferation or survival in female embryos.

The possibility that RA primarily influences gonadal somatic cells rather than germ cells is particularly relevant for developing testes as any treatment that inhibits Sertoli cell differentiation would indirectly cause XY germ cells to sex-reverse and initiate meiosis. Even in the absence of experimental treatments, Sertoli cell differentiation may be somewhat impaired in cultured gonads: although 11.5 dpc XX and XY urogenital ridges develop as ovaries or testes, respectively, in a serum-containing culture system, cultured 10.5 dpc XY urogenital ridges contain meiotic oocytes and do not develop testis cords (Buehr *et al*., 1993). 11.5 dpc urogenital ridges cultured in a serum-free system may also have some problems with Sertoli cell differentiation: *Stra8*, which is female-specific in embryonic gonads (Menke *et al*., 2003), is expressed at similar levels in XX and XY control gonads cultured in this serum-free system (Bowles *et al*., 2006), possibly reflecting impaired Sertoli cell differentiation and/or impaired *Cyp26b1* expression. Any treatment that further impairs the differentiation, proliferation or survival of Sertoli cells in culture is therefore likely to indirectly trigger germ cell sex-reversal.

RA can induce homeotic transformations and alter cell identity in various developmental systems (Kessel and Gruss, 1991; Duester, 2008) and can severely impair Sertoli cell survival or differentiation in cultured rat embryonic testes (Li and Kim, 2004). It is not clear if the number of Sertoli cells is reduced in studies suggesting that increasing RA in cultured embryonic testes induces germ cell meiosis. Thus, reports suggesting that increasing RA levels in cultured embryonic testes induce germ cell meiosis may be detecting the indirect consequences of impaired Sertoli cell differentiation, proliferation or survival.

Although RA has been proposed to act as an MIS, the experimental evidence for this comes mainly from organ culture experiments where it can be difficult to separate the direct effects of RA on germ cells from its effects on gonadal somatic cells. Exogenous RA is not required for germ cells to initiate meiosis in a feeder-free culture system (Farini *et al*., 2005), although it remains to be determined whether the serum in this system contributes physiologically relevant levels of RA (Bowles and Koopman, 2007). Further development of this type of sophisticated culture system should allow the role of RA in inducing germ cell meiosis to be tested directly.

The finding that meiotic germ cells are present in *Cyp26b1*$^{-/-}$ knockout embryonic testes provides some support to RA being an MIS (MacLean *et al*., 2007). However, the apparent abundance of aberrant mitotic germ cells in *Cyp26b1*$^{-/-}$ embryonic testes (MacLean *et al*., 2007) may indicate that reducing RA levels primarily allows prospermatogonia to become quiescent after germ cell sex has been determined, a possibility that is supported by organ culture experiments (Trautmann *et al*., 2008). As some meiotic germ cells can be present in wild-type embryonic testes (Byskov, 1978b; Yao *et al*., 2003) and RA promotes survival of meiotic oocytes *in vivo* (Morita and Tilly, 1999), it would be informative to quantify the proportion of meiotic and non-meiotic germ cells in *Cyp26b1*$^{-/-}$ embryonic testes to assess the magnitude of any germ cell sex reversal caused by this mutation.

One piece of data that is difficult to reconcile with mesonephros-derived RA acting as an MIS is that ectopic germ cells found in the mesonephros of male embryos usually develop as prospermatogonia and are prevented from initiating meiosis (McLaren, 1984). Recent evidence that secretion of an MPS occurs in the developing testis has come from pharmacological inhibition of membrane trafficking in embryonic testis cultures. Transient treatment of 11.5 dpc embryonic testes with a reversible inhibitor of secretion was sufficient to induce XY germ cells to sex-reverse and develop as meiotic oocytes enclosed in testis cords (Best *et al*., 2008). Sertoli cell differentiation and cord formation were not overtly disrupted by this treatment. Furthermore, this germ cell sex-reversal does not appear to result from altering RA levels as directly increasing RA did not induce germ cell sex-reversal in embryonic testes cultured in this system (Best *et al*., 2008). Thus, the action of an unidentified male MPS, which is probably secreted by the Sertoli cells, appears to be important for germ cell sex determination. Secretion of this MPS by embryonic Sertoli cells may be facilitated by changes that occur in the membrane trafficking pathway during Sertoli cell differentiation (Best *et al*., 2008).

One potential candidate for an MPS in the developing testis is leukaemia inhibitory factor (LIF), a cytokine that signals through the gp130 receptor family. The addition of LIF has been shown to prevent germ cell meiosis, even in feeder-free culture, and LIF is more highly expressed in embryonic testes than the embryonic ovaries (Chuma and Nakatsuji, 2001; Farini *et al*., 2005). However, it is not clear whether female germ cells exposed to exogenous LIF sex-reverse and differentiate into male prospermatogonia or follow some other developmental fate. Male embryos carrying mutations in the gp130 receptor have fewer germ cells at 13.5 dpc than wild-type embryos, but at present there is no evidence of germ cell sex-reversal in gp130-deficient embryos (Molyneaux *et al*., 2003). Another potential candidate for an MPS in the developing testis is Fgf9. Mutations in *Fgf9* cause male-to-female germ cell sex reversal in XY embryos, but as Fgf9 plays a role in supporting cell sex determination it is not clear whether *Fgf9* mutations influence germ cell sex determination directly or indirectly (Colvin *et al*., 2001). Similarly mutations in *Rspo1*, which antagonises Fgf signalling in supporting cell sex determination, induce female-to-male germ cell sex reversal in XX embryos (Chassot *et al*., 2008). Elegant genetic experiments will be required to dissect out any direct effects that mutations in *Fgf9* and *Rspo1* have on germ cell sex determination from their effects on supporting cell sex determination.

Although the molecular identity of the MPS is not yet clear, one of the downstream targets of this factor is likely to be *Nanos2*. *Nanos2* is expressed in male but not female germ cells from 13.5 dpc, and although *Nanos2*$^{-/-}$ germ cells normally undergo apoptosis in embryonic testes, *Nanos2*$^{-/-}$ Bax$^{-/-}$ germ cells that cannot initiate apoptosis were found to be in meiosis in 17.0 dpc XY embryos (Tsuda *et al*., 2003; Suzuki and Saga, 2008). *Nanos2*$^{-/-}$ Bax$^{-/-}$ germ cells express *Stra8* in an embryonic testis environment, suggesting that *Nanos2* represses *Stra8* during male germ cell development (Suzuki and Saga, 2008). It is not clear whether RA, or some other developmental signal, induces *Stra8* expression in the *Nanos2*$^{-/-}$ Bax$^{-/-}$ testes. Furthermore, ectopic expression of *Nanos2* in female germ cells down-regulates *Stra8* expression, prevents meiosis and appears to direct ovarian germ cells down a male developmental pathway (Suzuki and Saga, 2008). As *Nanos2* appears to be an important regulator of germ cell sex determination, any signalling molecules that induce *Nanos2* expression in germ cells in the developing testis would presumably act as an MPS and determine male germ cell sex. It is therefore clearly of significant interest to elucidate how *Nanos2* expression is induced in germ cells developing in embryonic testes.

Implications

Germ cell sex determination, infertility and cancer

The main clinical consequence of defects in germ cell sex determination during human fetal development is likely to be infertility. Mutations in genes involved in the primary sex determination decision in the supporting cell lineage such as *SRY*, *SOX9* and *WNT4* may lead to XX germ cells differentiating as male or XY germ cells differentiating as female as a consequence of somatic sex-reversal in the gonad (Fleming and Vilain, 2004). Such sex-reversed patients are typically infertile and have azoospermic testes or streak ovaries that contain few germ cells. The atypical sex chromosome constitution of sex-reversed germ cells is likely to cause problems during gametogenesis that lead to germ cell death (Speed, 1986; Burgoyne *et al*., 1992; Alton *et al*., 2008), and hence the significant reduction in the number of germ cells seen in human sex-reversed patients.

One might predict that defects in the germ cell sex determination machinery could cause germ cell sex-reversal in the absence of somatic sex-reversal resulting in the appearance of XX prospermatogonia in developing ovaries or XY meiotic oocytes in developing testes. However, the sex-reversed germ cells would presumably encounter problems caused by their atypical sex chromosome constitution, in addition to the survival problems associated with being in an inappropriate gonadal environment. Therefore, mutations in the germ cell sex determination machinery are likely to resolve into agametic gonads and cause infertility in human patients. It will be of interest to discover whether any cases of human infertility are caused by mutations in some of the genes and pathways recently implicated in germ cell sex determination.

Another consequence of germ cell sex reversal in humans is increased susceptibility to germ cell tumours. XY female sex-reversed patients exhibit a high incidence of gonadoblastomas, a mixed germ cell-somatic cell tumour that appears to originate from sex-reversed XY oogonia/oocytes (Cools *et al*., 2006). Furthermore, carcinoma

in situ, the non-malignant precursor of seminomatous and non-seminomatous testicular germ cell tumours, has also been proposed to originate from impaired or delayed germ cell differentiation during fetal testis development (Skakkebak *et al*., 2001; Oosterhuis and Looijenga, 2005). In particular, environmental or genetic factors that disrupt the communication between Sertoli cells and germ cells during fetal development may be causative factors for the development of testicular germ cell tumours in adult life. Carcinoma *in situ* cells express molecular markers and phenotypic characteristics associated with post-migratory germ cells, but these cells do not appear to have differentiated correctly down the male developmental pathway to spermatogonia (Skakkebak *et al*., 2001; Oosterhuis and Looijenga, 2005). It will be interesting to determine whether mutations in genes involved in communication between Sertoli cells and germ cells in mouse embryogenesis turn out to be risk factors for testicular germ cell tumours in humans.

Germ cell sex and aneuploidy in humans

Sexual differentiation of the germ cells has additional repercussions for human genetic disease; in that some types of *de novo* chromosomal abnormalities are transmitted through male and female germlines with significantly different frequencies. For example, aneuploidy, which can cause miscarriage, infertility and conditions such as Down's syndrome in humans, is present in human oocytes at a ~10-fold higher frequency than in human sperm (Hassold and Hunt, 2001). The incidence of aneuploidy in human gametes is also influenced by age in addition to sex. In men, ~2% of sperm are aneuploid and this modestly increases around 2-fold with age mainly due to sex chromosome aneuploidies (Sloter *et al*., 2004). The effect of maternal age on aneuploidy is far more dramatic: among women in their early 20s ~2% of all clinically recognized pregnancies are trisomic, but this increases to >35% towards the end of a woman's reproductive life span. As many aneuploid embryos do not survive long enough to become clinically recognized pregnancies, the incidence of trisomic pregnancies is likely to be significantly lower than the frequency of aneuploidy in mature oocytes (Hassold and Hunt, 2001).

Aneuploidies arise from errors in chromosome segregation during meiosis, and there are two significant differences in the timing and regulation of meiosis between the sexes that might contribute to the high rate of aneuploidy in older human oocytes. First, male germ cells proceed through meiosis without interruption in adult men, whereas female germ cells initiate meiosis in the embryo and remain arrested in meiotic prophase for decades until hormonal stimulation prior to ovulation. During the oocytes' meiotic arrest, homologous chromosomes are physically held together as bivalents by crossover events and cohesion between the DNA molecules. The gradual loss of these physical connections between homologous chromosomes during the prolonged meiotic arrest, and/or age-dependent defects in the machinery involved in aligning and segregating the homologous chromosomes on the meiotic spindle upon resumption of meiosis, could contribute to the high rates of aneuploidy in older females by causing mis-segregation of meiotic chromosomes (Hassold and Hunt, 2001; Jones, 2008). Second, at least in mouse models, female germ cells appear to respond less stringently than male germ cells to abnormalities that can arise during meiotic chromosome synapsis and segregation (Morelli and Cohen, 2005; Jones, 2008). These differences in the timing and regulation of meiosis between male and female germ cells may contribute to the sex-bias in aneuploidy rates in humans.

As sex-specific differences in meiosis are likely to influence the incidence of aneuploidy in human gametes, it is of interest to understand the differences between male and female meiosis at a molecular level. These differences may well be consequences of gene expression cascades initiated when germ cells respond to the embryonic gonadal environment to make their sex-determining decision. Therefore, understanding how germ cells in the embryonic gonad change their gene expression profiles and cell biology in response to sex-determining cues to embark down a male or a female developmental pathway should provide insight into how this fundamental decision in germline development impacts on the aetiology of some types of human genetic disease.

Acknowledgements

We thank Howard Cooke (MRC-HGU) for critical reading of the manuscript.

Funding

This work was supported by the Medical Research Council.

References

Adams IR, McLaren A. Sexually dimorphic development of mouse primordial germ cells: switching from oogenesis to spermatogenesis. *Development* 2002;**129**:1155–1164.

Alton M, Lau MP, Villemure M, Taketo T. The behavior of the X- and Y-chromosomes in the oocyte during meiotic prophase in the B6.YTIR sex-reversed mouse ovary. *Reproduction* 2008;**135**:241–252.

Anderson EL, Baltus AE, Roepers-Gajadien HL, Hassold TJ, de Rooij DG, van Pelt AMM, Page DC. Stra8 and its inducer, retinoic acid, regulate meiotic initiation in both spermatogenesis and oogenesis in mice. *Proc Natl Acad Sci USA* 2008;**105**:14976–14980.

Baltus AE, Menke DB, Hu Y-C, Goodheart ML, Carpenter AE, de Rooij DG, Page DC. In germ cells of mouse embryonic ovaries, the decision to enter meiosis precedes premeiotic DNA replication. *Nat Genet* 2006;**38**:1430–1434.

Best D, Sahlender DA, Walther N, Peden AA, Adams IR. Sdmg1 is a conserved transmembrane protein associated with germ cell sex determination and germline-soma interactions in mice. *Development* 2008;**135**:1415–1425.

Bowles J, Koopman P. Retinoic acid, meiosis and germ cell fate in mammals. *Development* 2007;**134**:3401–3411.

Bowles J, Knight D, Smith C, Wilhelm D, Richman J, Mamiya S, Yashiro K, Chawengsaksophak K, Wilson MJ, Rossant J *et al.* Retinoid signaling determines germ cell fate in mice. *Science* 2006;**312**:596–600.

Buehr M, Gu S, McLaren A. Mesonephric contribution to testis differentiation in the fetal mouse. *Development* 1993;**117**:273–281.

Burgoyne PS, Mahadevaiah SK, Sutcliffe MJ, Palmer SJ. Fertility in mice requires X-Y pairing and a Y-chromosomal 'spermiogenesis' gene mapping to the long arm. *Cell* 1992;**71**:391–398.

Byskov AG. Does the rete ovarii act as a trigger for the onset of meiosis? *Nature* 1974;**252**:396–397.

Byskov AG. Regulation of initiation of meiosis in fetal gonads. *Int J Androl* 1978a;**2**:29–38.

Byskov AG. The meiosis inducing interaction between germ cells and rete cells in the fetal mouse gonad. *Ann Biol Anim Biochim Biophys* 1978b; **18**:327–334.

Byskov AG, Saxén L. Induction of meiosis in fetal mouse testis *in vitro*. *Dev Biol* 1976;**52**:193–200.

Chaboissier MC, Kobayashi A, Vidal VIP, Lutzkendorf S, van de Kant HJG, Wegner M, de Rooij DG, Behringer RR, Schedl A. Functional analysis of Sox8 and Sox9 during sex determination in the mouse. *Development* 2004;**131**:1891–1901.

Chassot AA, Ranc F, Gregoire EP, Roepers-Gajadien HL, Taketo MM, Camerino G, de Rooij DG, Schedl A, Chaboissier MC. Activation of β-catenin signaling by Rspo1 controls differentiation of the mammalian ovary. *Hum Mol Genet* 2008;**17**:1264–1277.

Childs AJ, Saunders PTK, Anderson RA. Modelling germ cell development *in vitro*. *Mol Hum Reprod* 2008;**14**:501–511.

Chuma S, Nakatsuji N. Autonomous transition into meiosis of mouse fetal germ cells *in vitro* and its inhibition by gp130-mediated signaling. *Dev Biol* 2001;**229**:468–479.

Colvin JS, Green RP, Schmahl J, Capel B, Ornitz DM. Male-to-female sex reversal in mice lacking fibroblast growth factor 9. *Cell* 2001; **104**:875–889.

Cools M, Drop SLS, Wolffenbuttel KP, Oosterhuis JW, Looijenga LHJ. Germ cell tumors in the intersex gonad: old paths, new directions, moving frontiers. *Endocr Rev* 2006;**27**:468–484.

Di Carlo AD, Travia G, De Felici M. The meiotic specific synaptonemal complex protein SCP3 is expressed by female and male primordial germ cells of the mouse embryo. *Int J Dev Biol* 2000;**44**:241–244.

DiNapoli L, Batchvarov J, Capel B. FGF9 promotes survival of germ cells in the fetal testis. *Development* 2006;**133**:1519–1527.

Dolci S, De Felici M. A study of meiosis in chimeric mouse fetal gonads. *Development* 1990;**109**:37–40.

Duester G. Retinoic acid synthesis and signaling during early organogenesis. *Cell* 2008;**134**:921–931.

Evans CW, Robb DI, Tuckett F, Susan C. Regulation of meiosis in the foetal mouse gonad. *J Embryol Exp Morphol* 1982;**68**:59–67.

Farini D, Scaldaferri ML, Iona S, La Sala G, De Felici M. Growth factors sustain primordial germ cell survival, proliferation and entering into meiosis in the absence of somatic cells. *Dev Biol* 2005;**285**:49–56.

Fleming A, Vilain E. The endless quest for sex determination genes. *Clin Genet* 2004;**67**:15–25.

Ford CE, Evans EP, Burtenshaw MD, Clegg HM, Tuffrey M, Barnes RD. A functional 'sex-reversed' oocyte in the mouse. *Proc R Soc Lond B Biol Sci* 1975;**190**:187–197.

Hassold T, Hunt P. To err (meiotically) is human: the genesis of human aneuploidy. *Nat Rev Genet* 2001;**2**:280–291.

Hilscher B, Hilscher W, Bülthoff-Ohnolz B, Krämer U, Birke A, Pelzer H, Gauss G. Kinetics of gametogenesis. *Cell Tissue Res* 1974;**154**:443–470.

Jones KT. Meiosis in oocytes: predisposition to aneuploidy and its increased incidence with age. *Hum Reprod Update* 2008;**14**:143–158.

Kasai S, Chuma S, Motoyama N, Nakatsuji N. Haploinsufficiency of Bcl-x leads to male-specific defects in fetal germ cells: differential regulation of germ cell apoptosis between the sexes. *Dev Biol* 2003;**264**: 202–216.

Kessel M, Gruss P. Homeotic transformations of murine vertebrae and concomitant alteration of Hox codes induced by retinoic acid. *Cell* 1991;**67**:89–104.

Kim Y, Kobayashi A, Sekido R, DiNapoli L, Brennan J, Chaboissier MC, Poulat F, Behringer RR, Lovell-Badge R, Capel B. Fgf9 and Wnt4 act as antagonistic signals to regulate mammalian sex determination. *PLoS Biol* 2006;**4**:e187.

Koopman P, Gubbay J, Vivian N, Goodfellow P, Lovell-Badge R. Male development of chromosomally female mice transgenic for *Sry*. *Nature* 1991;**351**:117–121.

Koshimizu U, Watanabe M, Nakatsuji N. Retinoic Acid is a potent growth activator of mouse primordial germ cells *in vitro*. *Dev Biol* 1995; **168**:683–685.

Koubova J, Menke DB, Zhou Q, Capel B, Griswold MD, Page DC. Retinoic acid regulates sex-specific timing of meiotic initiation in mice. *Proc Natl Acad Sci USA* 2006;**103**:2474–2479.

Li H, Kim KH. Retinoic acid inhibits rat XY gonad development by blocking mesonephric cell migration and decreasing the number of gonocytes. *Biol Reprod* 2004;**70**:687–693.

Lovell-Badge R, Robertson E. XY female mice resulting from a heritable mutation in the murine primary testis determining gene, Tdy. *Development* 1990;**109**:635–646.

MacLean G, Li H, Metzger D, Chambon P, Petkovich M. Apoptotic extinction of germ cells in testes of Cyp26b1 knockout mice. *Endocrinology* 2007;**148**:4560–4567.

Mark M, Jacobs H, Oulad-Abdelghani M, Dennefeld C, Feret B, Vernet N, Codreanu CA, Chambon P, Ghyselinck NB. STRA8-deficient spermatocytes initiate, but fail to complete, meiosis and undergo premature chromosome condensation. *J Cell Sci* 2008;**121**:3233–3242.

Matzuk MM, Lamb DJ. The biology of infertility: research advances and clinical challenges. *Nat Med* 2008;**14**:1197–1213.

McLaren A. Meiosis and differentiation of mouse germ cells. In Evans CW, Dickinson HG (ed). *Controlling Events in Meiosis. 38th Symposium of the Society for Experimental Biology*. Cambridge, UK: Company of Biologists, 1984. 7–23.

McLaren A. Primordial germ cells in the mouse. *Dev Biol* 2003;**262**:1–15.

McLaren A, Southee D. Entry of mouse embryonic germ cells into meiosis. *Dev Biol* 1997;**187**:107–113.

Menke DB, Koubova J, Page DC. Sexual differentiation of germ cells in XX mouse gonads occurs in an anterior-to-posterior wave. *Dev Biol* 2003; **262**:303–312.

Molyneaux KA, Schaible K, Wylie C. GP130, the shared receptor for the LIF/IL6 cytokine family in the mouse, is not required for early germ cell differentiation, but is required cell-autonomously in oocytes for ovulation. *Development* 2003;**130**:4287–4294.

Morelli MA, Cohen PE. Not all germ cells are created equal: aspects of sexual dimorphism in mammalian meiosis. *Reproduction* 2005;**130**:761–781.

Morita Y, Tilly JL. Segregation of retinoic acid effects on fetal ovarian germ cell mitosis versus apoptosis by requirement for new macromolecular synthesis. *Endocrinology* 1999;**140**:2696–2703.

O W, Baker TG. Initiation and control of meiosis in hamster gonads *in vitro*. *J Reprod Fertil* 1976;**48**:399–401.

Oosterhuis JW, Looijenga LHJ. Testicular germ-cell tumours in a broader perspective. *Nat Rev Cancer* 2005;**5**:210–222.

Palmer SJ, Burgoyne PS. *In situ* analysis of fetal, prepubertal and adult XX↔XY chimaeric mouse testes: Sertoli cells are predominantly, but not exclusively, XY. *Development* 1991;**112**:265–268.

Ross AJ, Capel B. Signaling at the crossroads of gonad development. *Trends Endocrinol Metab* 2005;**16**:19–25.

Sadate-Ngatchou PI, Payne CJ, Dearth AT, Braun RE. Cre recombinase activity specific to postnatal, premeiotic male germ cells in transgenic mice. *Genesis*. 2008;**46**:738–742.

Sekido R, Lovell-Badge R. Sex determination involves synergistic action of SRY and SF1 on a specific Sox9 enhancer. *Nature* 2008;**453**:930–934.

Sekido R, Bar I, Narvaez V, Penny G, Lovell-Badge R. SOX9 is up-regulated by the transient expression of SRY specifically in Sertoli cell precursors. *Dev Biol* 2004;**274**:271–279.

Skakkebak NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects: opinion. *Hum Reprod* 2001;**16**:972–978.

Sloter E, Nath J, Eskenazi B, Wyrobek AJ. Effects of male age on the frequencies of germinal and heritable chromosomal abnormalities in humans and rodents. *Fertil Steril* 2004;**81**:925–943.

Speed RM. Oocyte development in XO foetuses of man and mouse: the possible role of heterologous X-chromosome pairing in germ cell survival. *Chromosoma* 1986;**94**:115–124.

Suzuki A, Saga Y. Nanos2 suppresses meiosis and promotes male germ cell differentiation. *Genes Dev* 2008;**22**:430–435.

Trautmann E, Guerquin MJ, Duquenne C, Lahaye JB, Habert R, Livera G. Retinoic acid prevents germ cell mitotic arrest in mouse fetal testes. *Cell Cycle* 2008;**7**:656–664.

Tanaka SS, Toyooka Y, Akasu R, Katoh-Fukui Y, Nakahara Y, Suzuki R, Yokoyama M, Noce T. The mouse homolog of Drosophila Vasa is required for the development of male germ cells. *Genes Dev* 2000; **14**:841–853.

Tsuda M, Sasaoka Y, Kiso M, Abe K, Haraguchi S, Kobayashi S, Saga Y. Conserved role of nanos proteins in germ cell development. *Science* 2003;**301**:1239–1241.

Vainio S, Heikkila M, Kispert A, Chin N, McMahon AP. Female development in mammals is regulated by Wnt-4 signalling. *Nature* 1999;**397**:405–409.

Vidal VPI, Chaboissier MC, de Rooij DG, Schedl A. *Sox9* induces testis development in XX transgenic mice. *Nat Genet* 2001;**28**:216–217.

Wartenberg H, Hilscher B, Hilscher W. Germ cell kinetics during early ovarian differentiation: an analysis of the oogonial cell cycle and the subsequent changes in oocyte development during the onset of meiosis in the rat. *Microsc Res Tech* 1998;**40**:377–397.

Wilhelm D, Palmer S, Koopman P. Sex determination and gonadal development in mammals. *Physiol Rev* 2007;**87**:1–28.

Yao HH, Capel B. Disruption of testis cords by cyclopamine or forskolin reveals independent cellular pathways in testis organogenesis. *Dev Biol* 2002;**246**:356.

Yao HH, DiNapoli L, Capel B. Meiotic germ cells antagonize mesonephric cell migration and testis cord formation in mouse gonads. *Development* 2003;**130**:5895–5902.

Zamboni L, Upadhyay S. Germ cell differentiation in mouse adrenal glands. *J Exp Zool* 1983;**228**:173–193.

Submitted on December 22, 2008; resubmitted on January 20, 2009; accepted on January 26, 2009