Review

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Regulation of meiotic entry and gonadal sex differentiation in the human: normal and disrupted signaling

Abstract: Meiosis is a unique type of cell division that is performed only by germ cells to form haploid gametes. The switch from mitosis to meiosis exhibits a distinct sex-specific difference in timing, with female germ cells entering meiosis during fetal development and male germ cells at puberty when spermatogenesis is initiated. During early fetal development, bipotential primordial germ cells migrate to the forming gonad where they remain sexually indifferent until the sex-specific differentiation of germ cells is initiated by cues from the somatic cells. This irreversible step in gonadal sex differentiation involves the initiation of meiosis in fetal ovaries and prevention of meiosis in the germ cells of fetal testes. During the last decade, major advances in the understanding of meiosis regulation have been accomplished, with the discovery of retinoic acid as an inducer of meiosis being the most prominent finding. Knowledge about the molecular mechanisms regulating meiosis signaling has mainly been established by studies in rodents, while this has not yet been extensively investigated in humans. In this review, the current knowledge about the regulation of meiosis signaling is summarized and placed in the context of fetal gonad development and germ cell differentiation, with emphasis on results obtained in humans. Furthermore, the consequences of dysregulated meiosis signaling in humans are briefly discussed in the context of selected pathologies, including testicular germ cell cancer and some forms of male infertility.

Keywords: carcinoma *in situ*; fetal gonad development; germ cell differentiation; meiosis; mitosis-meiosis switch; testicular germ cell cancer.

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Introduction

The mitotic-meiotic switch is a unique feature of germ cell development and is one of the first manifestations of sex differentiation in the developing gonad. The current understanding of the molecular mechanisms of germ cell differentiation and regulation of meiosis is primarily derived from studies in mice (1–4), with only few experimental studies conducted on human fetal gonads thus far (5, 6). In contrast, the physiological manifestations of sex differentiation that take place in humans during fetal gonad development and the expression pattern of key proteins have been described (7–13) and are briefly summarized here.

The bipotential gonad begins to develop from around gestational week (GW) 4 in humans, when the genital ridges appear as thickenings of the intermediate mesoderm. Initially, the bipotential gonads do not contain germ cells, but primordial germ cells (PGCs) start migrating from the posterior proximal epiblast toward the genital ridge and enter the gonad during GW 5 (14). Shortly after their arrival to the bipotential gonad, the PGCs become enclosed by somatic precursor cells that differentiate into Sertoli or granulosa cells based on the presence or absence of the Y chromosome and the sex-determining gene *SRY* (15, 16). Sex differentiation is initiated around GW 6, and the germ cells are directed toward the alternative pathways of oogenesis and spermatogenesis by the action of the somatic cells in the niche.

In fetal ovaries, germ cells are termed oogonia once they reside within the gonad (17), and they irreversibly commit to the female developmental pathway when initiating the first meiotic division (5, 6). During oogenesis, the mitotically dividing oogonia differentiate to primary oocytes by initiation of meiosis, coinciding with

a permanent downregulation of pluripotency genes, i.e., *OCT4*, *NANOG*, *C-KIT*, and *LIN28* (8, 11, 17–20). Because meiotic entry in humans is asynchronous, some oogonia initiate meiosis and differentiate to oocytes from GW 10–11, while another subpopulation of oogonia continues to express the pluripotency markers and remains proliferating until around GW 19–20 (6, 21). This proliferating subpopulation of oogonia is mainly found in the periphery of the ovaries, whereas the more differentiated oocytes are found deeper within the medulla (22). After GW 20, the majority of germ cells within the human fetal ovaries have initiated meiosis, and the oocytes arrest in diplotene stage of first meiotic division where they will be surrounded by primitive granulosa cells, resulting in the formation of single-layered primordial follicles (21, 23, 24) (Figure 1).

In human fetal testes, the first morphological sign of sex differentiation is the formation of seminiferous cords, which occurs gradually between GW 7–8 (13). Germ cells residing within the developing fetal testes are termed gonocytes at this stage, and they become enclosed by the immature Sertoli cells that are also located within the testis cords to ensure direct cell contact (13). The somatic cells in fetal testes initiate differentiation from GW 8–9 (Sertoli cells) and GW 9 (Leydig cells) (13) and continue to proliferate until around GW 14 (25). In contrast to fetal ovaries, germ cells in fetal testes do not enter meiosis until puberty, but the gonocytes differentiate asynchronously to pre-spermatogonia from around GW 17–18 (8–10, 12, 26). Fetal gonocytes are generally distinguished from pre-spermatogonia based on difference in protein expression profiles, with gonocytes characterized by expression of pluripotency factors, mainly determined by expression of OCT4, whereas pre-spermatogonia are OCT4-negative but express MAGE-A4 (10). This profound change in the protein expression profile coincides with migration of the human germ cells toward the basement membrane, thus completing the differentiation from gonocyte to pre-spermatogonia (27). Around GW 20, most germ cells present in fetal testes are OCT4-negative; however, a few gonocytes occasionally remain in the testes until 3–4 months of postnatal age (8). From around GW 20, the pre-spermatogonia enter a quiescent phase lasting for the remaining fetal life (28).

Initiation of meiosis in fetal ovaries

Meiosis is the germ cell-exclusive cell division, which produces haploid cells; it involves one round of DNA replication followed by two rounds of cell divisions, resulting in four haploid spermatids in males and one mature egg and three small cells, termed polar bodies, in females. Meiosis is generally divided into two stages, based on the two rounds of divisions: meiosis I and meiosis II. Each stage can be further subdivided into prophase, metaphase, anaphase, and telophase. Among these substages, prophase I entails the events unique for meiosis and is further divided into leptotene (chromosome condensation), zygotene (pairing of homologous chromosomes through the synaptonemal complex), pachytene (initiation of recombination between chromosome pairs and formation of chiasmata between homologues at sites crossing over), and diplotene (chromosomes begin to separate, but remain attached) (29). The details of the specific events in each stage and substage of meiosis are beyond the scope of this review, but several reviews focusing on this have recently been published (21, 29–31).

The generally accepted model for initiation of meiosis in mammalian species stipulates that meiotic entry is induced by the action of retinoic acid (RA). This has been demonstrated in several studies in mice (1, 2) and humans (5, 6). The sex-specific response to RA is regulated by the somatic cells within the gonad, which mediate the initiation of meiosis in fetal ovaries and inhibition of meiosis in fetal testes (Figure 1). In fetal ovaries, RA induces *Stra8* expression, both in mice (1, 2) and humans (5) and is considered a pre-meiosis marker because STRA8 regulates initiation of meiosis I and is required for pre-meiotic DNA replication (32, 33). In mice, DAZL is involved in rendering the germ cells competent to enter meiosis and could be the intrinsic signal necessary to initiate meiosis (34). In fetal mouse testes, RA-mediated initiation of meiosis is prevented by the action of the RA-degrading enzyme CYP26B1 (1, 2) and subsequently by NANOS2 (3), FGF9 (35, 36), DMRT1 (4), and NODAL (37, 38). In 2011, the model for RA-induced meiosis was challenged by Kumar et al. (39), who found *Stra8* expression in fetal ovaries from mice lacking RA synthesis (*Raldh*$^{-/-}$) in mesonephros and gonads, thereby suggesting that RA is not the only inducer of *Stra8* expression and meiosis. The authors suggest that an additional yet unidentified meiosis inducer is present and involved in the initiation of meiosis (39). These results were thoroughly discussed in a recent review by Griswold et al. (40).

Germ cell development requires timely transition from a pluripotent germ cell to meiotic differentiation. This is associated with widespread changes in protein expression, including downregulation of pluripotency factors, OCT4, NANOG, C-KIT, and LIN28 (8, 11, 17–20), and upregulation of markers of germ cell differentiation and meiosis, such as VASA, DAZL, STRA8, and SYCP3 (5, 6, 20). In human fetal ovaries, meiotic entry is initiated asynchronously from around GW 10–11 and constitutes the transition from oogonia to oocytes (17, 20, 22, 41). This was recently confirmed by the presence of γH2AX-positive (marks double-strand

breaks) oogonia at GW 10 (5) and SCP3-positive oogonia from GW 12–14 (42, 43). The pre-meiosis marker *STRA8* was expressed before and around this developmental time point (5, 6, 37, 44), with low transcript levels of *STRA8* detectable from GW 8–9 (6, 44). The expression of *STRA8* in fetal ovaries peaks around GW 12–14 but remains until GW 18–20 (5, 6, 44). In contrast to fetal ovaries, *STRA8* expression was not detected in human fetal testes (6, 42, 44). The expression of *STRA8* is followed by upregulation of *SCP3*, *SPO11*, and *DMC1*, which are markers of meiotic progression. In accordance with this general notion, expression of *SCP3* was detected in ovaries from GW 11–12 (42, 44) and *DMC1* from GW 13 (5, 42). In addition, *SPO11* and *SCP1* expression was detected from GW 13 in human fetal ovaries (5, 44).

The expression pattern of key meiosis regulators investigated so far in human fetal gonads indicates that several aspects of meiosis initiation are conserved between mice and humans. An interesting difference is that in mice, RA is produced in the mesonephros and diffuses to the gonads (45), whereas several recent studies indicate that the human gonads also possess an intrinsic capacity to synthesize RA, based on the expression pattern of the RA-synthesizing enzyme *ALDH1A* (5, 6, 42). *ALDH1A1* was expressed at similar levels in fetal testes and ovaries and in samples with and without mesonephros (5, 6, 42). Based on increased expression of *ALDH1A* coinciding with the initiation of meiosis, Le Bouffant et al. (5) suggested that *ALDH1A1* might play a role in the initiation of meiosis in fetal ovaries. However, Childs et al. (6) did not find an increase in *ALDH1A1* expression at the time of meiosis initiation in fetal ovaries. Instead, the authors implicated *NANOS3* in meiosis, based on expression level and timing in the fetal gonads (6). At the protein level, however, no clear sex-specific difference in NANOS3 expression level or pattern was observed between fetal testes and ovaries at the time of meiosis initiation in the fetal ovaries (42).

Another important intrinsic factor in the regulation of meiosis is Dazl, which is expressed in pre-meiotic germ cells in mouse gonads and enables germ cells to initiate meiosis in response to RA within a restricted developmental window (34). The Dazl-mediated meiosis-competent cell state exists in both male and female embryonic germ cells since the addition of exogenous RA induced *Stra8* expression in fetal testes and hence overcame the physiological inhibition of meiosis, but this effect was abolished in *Dazl*-deficient mice (34). In human fetal gonads, a marked increase in the expression of DAZL and VASA (both at the transcript and protein levels) was reported between GW 9–14, coinciding with meiotic entry in fetal ovaries (22, 41), which could indicate a conserved role for DAZL as an intrinsic factor involved in meiotic competence. A similar increase in DAZL and VASA was observed in male germ cells, suggesting that the upregulation of DAZL may be required to enable meiosis signaling or germ cell differentiation in human fetal gonads.

Recently, an additional regulator of meiosis was found in mice based on the finding that Dmrt1 promotes oogenesis through transcriptional activation of *Stra8* in fetal mice and is involved in initiation of meiotic prophase to ensure formation of a normal number of ovarian follicles postnatally (46). Dmrt1 is only transiently expressed in fetal ovaries of mice, with expression disappearing from germ cells by E15.5 (46). A similar expression pattern is found in human fetal ovaries where DMRT1 is expressed in the oogonia and a subset of early oocytes until around GW 20, coinciding with meiotic entry (42). This relatively conserved expression pattern indicates that DMRT1 might also be required for initiation of meiosis in humans.

Only one study to date has investigated the initiation of meiosis in human fetal ovaries by treatment experiments. Here, organ cultures of human fetal ovaries were treated with RA (1 μM) for 14 days, resulting in an increased number of meiotic germ cells (5). Also, increased transcriptional levels of *STRA8*, *REC8*, and *SPO11* were found after the addition of both RA and fetal calf serum to culture media, whereas no difference in the expression of *DMC1* and *SYCP3* was observed (5). Interestingly, oogonia present in human fetal ovaries before GW 7 did not initiate meiosis in response to RA treatment, indicating that the lack of expression of a meiotic facilitator renders the oogonia unresponsive to RA or that a currently unidentified meiotic inhibitor may exist in the early fetal ovaries. These results emphasize that several aspects of meiosis initiation in human cannot be explained based on current knowledge and that further studies are needed.

Inhibition of meiosis in the fetal and perinatal testes

In the developing mice testes, initiation of meiosis is inhibited by the action of the RA-degrading enzyme *Cyp26b1*, which is expressed in the gonads from 11.5 days post coitum (dpc) (1, 2). The expression pattern suggests that CYP26B1 prevents the action of RA between 12.5 and 14.5 dpc (45), and this function was confirmed by several different experimental approaches. Addition of exogenous RA to gonad-mesonephros cultures induced expression of meiosis markers in XY germ cells (1, 2), and in male *Cyp26b1*-null mice, upregulation of *Stra8* and *Scp3* was found at 13.5 dpc, indicating that the germ cells are entering meiotic prophase I like XX germ cells at

this developmental time point (1, 47). Also, conditional knockout of CYP26B1 in the Sertoli cells after germ cells have entered mitotic arrest resulted in XY germ cells initiating meiosis (48). A recent study found that CYP26B1 promotes male germ cell differentiation both by removal of RA, and thereby suppression of STRA8-dependent meiosis, and by a STRA8-independent pathway (49). Another important inhibitor of meiosis in fetal mouse testes is FGF9, which suppresses meiosis by maintaining expression of pluripotency factors, thereby actively promoting male fate (35, 36). *In vivo*, FGF9 prevents germ cells from upregulating *Stra8* indirectly by reducing germ cell responsiveness to RA and by promoting the male-specific pathway *via* upregulation of *Nanos2* and *Dnmt3l* expression (35). Addition of FGF9 to mice XX gonads results in the downregulation of *Stra8* expression, whereas expression of *Cyp26b1* is unaltered (35), indicating that FGF9 and CYP26B1 inhibit meiosis by different mechanisms. Following the action of FGF9 and CYP26B1, inhibition of meiosis in fetal testes is enforced by NANOS2, which ensures continued inhibition of *Stra8* expression (3). NANOS2 is also suggested to directly promote the male differentiation program (50) by inducing expression of *Tdrd1* and *Dnmt3l* (3). A recent study found that the NANOS2-mediated promotion of male germ cell development was independent of its role in meiosis suppression (51). The male germ cells in the fetal mice have entered mitotic quiescence at 14.5 dpc (52, 53), which has been suggested to also be partly explained by the *Cyp26b1*-mediated removal of RA (2).

Recently, additional players involved in the regulation of meiosis signaling in fetal testes were identified. Matson et al. (4) demonstrated that loss of *Dmrt1* expression in fetal male mice causes spermatogonia to precociously enter meiosis. Specifically, Dmrt1 expressed in spermatogonia restricts RA responsiveness, directly represses *Stra8* transcription, and activates transcription of the spermatogonial differentiation factor *Sohlh1* (4). Also, the TGFβ pathway ligand Nodal was recently shown to be involved in the inhibition of premature initiation of meiosis in murine fetal male germ cells as well as in the suppression of female reprogramming of somatic cells (37, 38). However, Spiller et al. (54) did not find increased *Stra8* expression in their mouse model with suppressed Nodal signaling as expected if Nodal inhibits premature meiosis entry, indicating that future studies are needed to clarify the exact role of Nodal in meiosis regulation.

The mechanisms of meiosis regulation in human fetal testes have not yet been thoroughly investigated. However, meiosis is apparently actively prevented in fetal testes because several studies have detected expression of proteins involved in synthesis of RA (ALDH1A) in analogy to fetal ovaries (5, 6, 42), but without initiation of meiosis in the testes at this developmental time point (Figure 1). In contrast to mice, the expression pattern and level of *CYP26B1* appears to be similar in human fetal testes and ovaries, suggesting that CYP26B1-mediated degradation of RA is most likely not the primary mechanism of meiosis inhibition (5, 6, 42). In contrast, the role of *NANOS2* appears to be more conserved with an expression pattern that is developmentally regulated in a sex-dimorphic manner also in human gonads (6). Furthermore, an abundant expression of RA receptors, RARα, RARβ, RXRα (protein level) as well as *RARα*, *RARβ*, *RARγ*, *RXRα*, *RXRβ*, and *RXRγ* (transcriptional level), was found in human fetal testes (6), which is in contrast to mice where RARα and RARγ were undetectable (55). Together, this indicates that retinoid signaling takes place in human fetal testes, but its meiosis-inducing function is blocked by a yet unidentified mechanism. In a recent study by Childs et al. (6), human fetal testis (second trimester) were treated with RA (1 μM) for 24 h, which resulted in a higher expression of *STRA8*. In contrast, no difference in expression levels of *SCP3* and *DMC1* were found (6), but this could be due to the fact that the testes were disaggregated to yield a single-cell suspension, which may not provide the optimal conditions for meiotic progression of germ cells. Alternatively, the simultaneous presence of meiosis inhibitors in fetal testes could have prevented further meiotic progression.

Meiotic entry in mature testes

In the postpubertal human testes, the two rounds of meiotic cell division take place immediately after each other during spermatogenesis. Spermatogenesis is a tightly regulated differentiation process in which spermatogonial stem cells differentiate to ultimately give rise to spermatozoa. Overall, spermatogenesis can be separated into three types of cellular events: the proliferative phase (spermatogonia), the meiotic phase (spermatocytes), and the differentiation phase or spermiogenesis (spermatids), which is followed by a series of post-testicular maturation processes required for the formation of fully functional spermatozoa (that are capable of motility and fertilization). In contrast to mice, mature human testes contain only two types of A-spermatogonia: the stem spermatogonial cells A_{dark} and the self-renewing spermatogonial cells A_{pale} that mature to B-spermatogonia, of which there is only one type in humans (56). Primary spermatocytes that are derived from the B-spermatogonia enter the first meiotic division, starting with a long prophase that can be

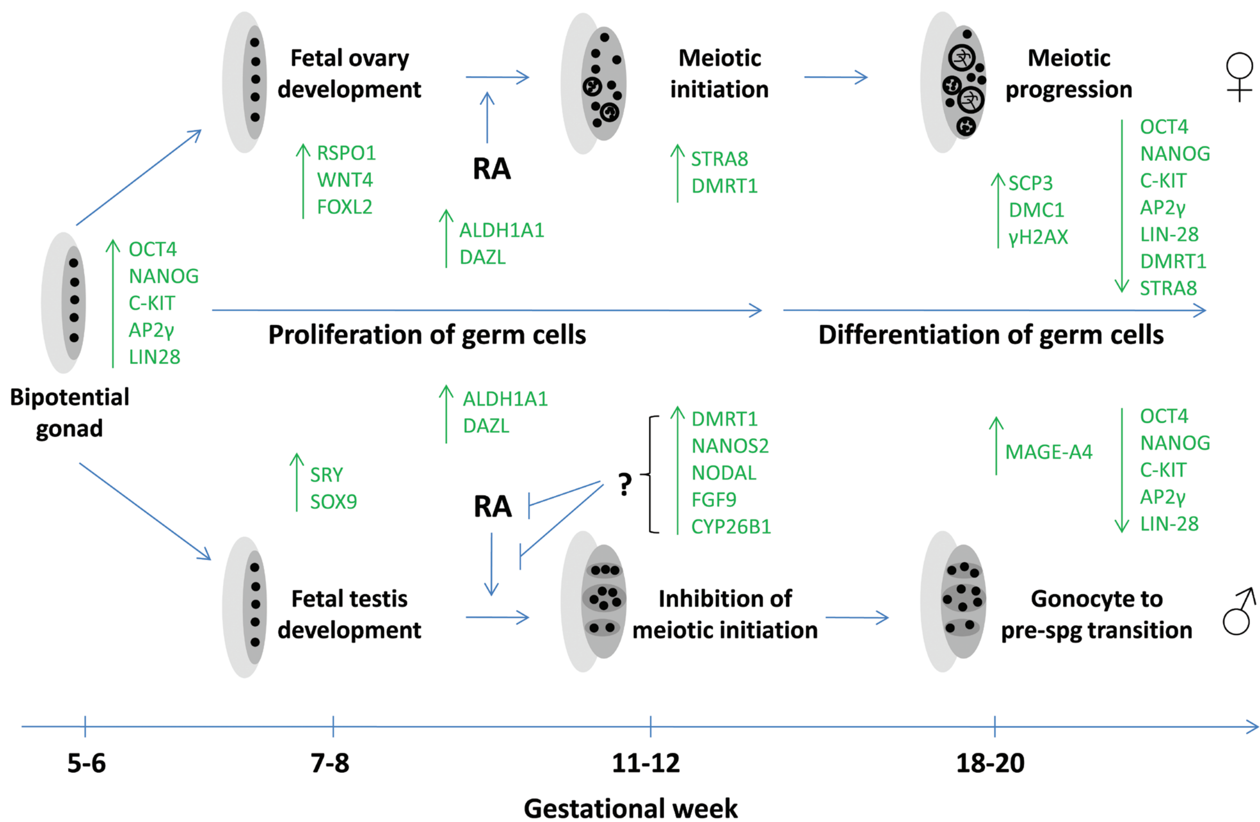

Figure 1 Meiosis regulation in human fetal gonads.
PGCs, which express embryonic pluripotency factors (*OCT4*, *NANOG*, *C-KIT*, *AP2γ*, *LIN28*), migrate to the forming gonads from GW 5. From GW 6–7, the somatic cells within the bipotential gonad direct the germ cells toward the alternative pathways of testis and ovary development. In the developing testes, this differentiation is directed by SRY and SOX9, and in the ovary, by FOXL2, WNT4, and RSPO1. Around GW 11–12, meiosis is initiated asynchronously in the ovaries by the action of RA, which leads to the upregulation of the pre-meiosis marker STRA8. The action of RA is prevented in the testes at this developmental time point by a mechanism that is not yet resolved but may involve DMRT1, NANOS2, NODAL, FGF9, and CYP26B1. In humans, synthesis of RA appears to take place in both fetal testes and ovaries based on expression of the RA-synthesizing enzyme ALDH1A1. Furthermore, the expression pattern of DAZL suggest a conserved role in mediating meiotic competence in germ cells of human fetal fetal ovaries and testes. In the ovaries, meiosis then progresses, resulting in the upregulation of meiosis markers SCP3, DMC1, γH2AX. Around GW 18–20, the majority of germ cells have initiated meiosis, and the oocytes arrest in the diplotene stage of the first meiotic division. During the initiation of meiosis, a permanent downregulation of pluripotency factors and the meiosis regulators STRA8 and DMRT1 is observed. In the testes, gonocytes differentiate asynchronously to pre-spermatogonia and migrate toward the basement membrane. During this transition, which takes place from around GW 18–20, a marked change in the expression profile is observed with the upregulation of MAGE-A4, coinciding with permanent downregulation of pluripotency factors.

subdivided into five different stages: leptotene, zygotene, pachytene, diplotene, and diakinesis. The second meiotic division occurs in secondary spermatocytes and results in the formation of round haploid spermatids. The molecular mechanisms of chromosome pairing, meiotic recombination, chromatin modifications, and cell cycle control during meiotic division in spermatogenesis are outside the scope of this review but have been recently reviewed by others (57–59).

In mice, the switch from mitosis to meiosis in postnatal testes requires the action of RA [reviewed in detail in (40, 60)]. In brief, RA signaling is required for the transition from undifferentiated to differentiating spermatogonia and promotes meiosis by activating the transcription of *Stra8* in preleptotene spermatocytes (61–63). In a recent study, treatment of neonatal mice with RA resulted in increased proliferation of germ cells, thus promoting transition of fetal spermatogonia into mature spermatogonia. However, meiosis was not triggered probably due to a quick removal of RA by CYP26A1 and B1 enzymes (64). In vitamin A-depleted adult mice, significantly lower *Stra8* expression was found as well as impaired meiotic progression in germ cells during the first wave of spermatogenesis (65). This resulted in the presence of only undifferentiated

type A-spermatogonia and Sertoli cells in testis tubules; however, upon vitamin A replacement, spermatogonial differentiation and meiosis was triggered (66), thereby indicating that removal of RA leads to a reversible block of spermatogenesis. Additional evidence was provided by a transgenic mouse model that expresses β-galactosidase in response to RA, where β-galactosidase activity was primarily detected in STRA8-positive spermatogonia (67). This was confirmed in Stra8 mutant mice, where the majority of preleptotene spermatocytes fail to enter meiosis (33, 68). Together, these results indicate that RA and STRA8 control the switch from spermatogonial differentiation to meiosis.

It remains poorly understood how the switch from mitosis to meiosis is controlled in spermatogonia, but it was shown that DMRT1 likely plays a central role in this transition (4). DMRT1 is expressed in both undifferentiated and differentiating spermatogonia, but not in meiotic or postmeiotic germ cells (4). Deletion of *Dmrt1* in undifferentiated spermatogonia results in upregulation of *Stra8* and precocious meiosis entry (4). Based on these results, DMRT1 was suggested to function as a gatekeeper that controls the mitosis vs. meiosis decision in male germ cells, both by suppressing RA signaling and by directly inhibiting transcription of *Stra8*.

Regulators of meiotic entry in human adolescent males remain largely unidentified. However, a recent study described the expression pattern of meiosis markers, including STRA8, SCP3, and DMC1, in human postnatal testis samples (42). All three meiosis markers were predominantly found in spermatocytes, as expected, given their role in the initiation and progression of meiosis. Interestingly, a strong expression of STRA8 was found in a subpopulation of B-spermatogonia (42), which was in accordance with a previous study in mice (61). This indicates that STRA8 is also a pre-meiosis marker in adult human testes. Interestingly, DMRT1 was expressed in a distinct pattern in adult human testes, with DMRT1 completely absent in A-dark spermatogonia and with a transient strong expression in A-pale and B-spermatogonia followed by a complete downregulation in the zygotene and pachytene spermatocytes (42). This finding was in accordance with previous reports of DMRT1 expression in human spermatogonia (69, 70). The expression pattern in human testes is slightly different compared with mice, where DMRT1 is highly expressed in all A-type spermatogonia, and expression decreases in B-spermatogonia and disappears with initiation of meiosis (4). Interestingly, an overlap in the expression of DMRT1 and STRA8 was observed in a small subset of B-spermatogonia in adult human testes (42), which is in contrast with results from mice, where DMRT1 and STRA8 are mutually exclusive (4). Despite these small differences, the overall similarity in the expression pattern between mice and humans indicates a conserved function of DMRT1 as regulator of the mitosis-meiosis switch presumably through regulation of the STRA8 expression.

As previously described, both CYP26B1 and NANOS2 are involved in the regulation of meiosis in mice by preventing premature initiation of meiosis during fetal development (1–3, 71). However, it is not known whether CYP26B1 and/or NANOS2 play a role in regulating initiation of meiosis in adult testes. In human postpubertal testis samples, CYP26B1 was primarily expressed in Leydig cells, whereas NANOS2 was expressed in a subpopulation of spermatogonia and spermatocytes (42), in accordance with a previous study that found NANOS2 expressed in spermatogonia, spermatocytes, and round spermatids (72). Another plausible candidate to be involved in meiosis regulation is the *NANOS2*-related gene, *NANOS3*, as suggested by Childs et al. (6). In adult human testes, NANOS3 was expressed in germ cells throughout spermatogenesis (42, 73), with the highest expression detected in germ cell nuclei in which the protein co-localized with chromosomal DNA during mitosis/meiosis (73). Reduction of NANOS3 expression levels results in a lower germ cell number and a decreased expression of germ cell-intrinsic genes required for the maintenance of pluripotency and meiotic initiation and progression (73). This could also indicate a role for NANOS3 in the regulation of meiosis in the adult testes, but this needs to be investigated further.

Consequences of dysregulated meiosis signaling in the testes

Sex-dimorphic meiotic entry regulation is a fundamental aspect of sex differentiation, and alterations in the timing or expression level of players in this pathway can have consequences later in life, including disorders of sex development (DSD), testicular cancer, and infertility. Individuals with DSD, in which the phenotypic gender is uncertain and morphological differences between the testis and the ovary are blurred, have an increased prevalence of gonadal pathologies, including the testicular cancer precursor carcinoma *in situ* (CIS), gonadoblastoma, testicular germ cell tumors (TGCT) as well as low serum testosterone levels and infertility (74–76).

In severe forms of DSD, for example, in rare cases of ovotestes, sex-specific expression of Sertoli and granulosa cell markers is clearly observed in specific parts of the gonad, for example, FOXL2 and SOX9 are never

co-expressed within the same cell (77). In fetal mice, both male and female sex determination can be overruled by changes in the expression pattern of key genes/proteins. In adult mice, loss of FOXL2 expression can lead to reprogramming of granulosa cells into Sertoli cells, and the opposite is observed when DMRT1 expression is lost in Sertoli cells, resulting in the initiation of FOXL2 expression and trans-differentiation into granulosa cells (78). This recently suggested concept of sex maintenance in adult life led us to speculate that an even slightly lower expression of DMRT1 in Sertoli cells in the postnatal human testes could result in a more blurred phenotype of the Sertoli cells, which could have consequences related to meiosis signaling and germ cell differentiation.

In milder forms of DSD, under-masculinization of the testes is most likely an effect of imbalance between the male- and the female-promoting factors, which leads to a slightly impaired or delayed differentiation of Sertoli cells. It is now well documented that CIS cells most likely originate from developmentally arrested gonocytes that failed to differentiate due to a disturbed or reduced signaling from the somatic niche during early fetal development (79–81), but the precise pathogenesis of CIS cells is not well understood. The arrested fetal gonocytes (also sometimes termed pre-CIS cells) persist in a dormant state within the testes until after puberty where it is believed that alterations in hormone levels induce proliferation and gain of invasive capacity of CIS cells and, eventually, further malignant transformation to tumors [reviewed in (82)]. We recently found some evidence of apparent dysregulation of the mitosis-meiosis switch in premalignant CIS cells, which are relatively frequently found in dysgenetic testes. In CIS cells, we detected simultaneous expression of meiosis-inducing (STRA8, SCP3, DMC1) and meiosis-inhibiting (DMRT1, NANOS2) factors (83). We believe that CIS cells may be rendered ‘sexually confused’ by the inadequately virilized somatic niche during testis development (82). Accordingly, we hypothesize that a conflict between meiosis-inhibiting (male pathway) and meiosis-inducing signals (female pathway) in the fetal gonads could be a contributing factor in the development of gonadal dysgenesis and milder forms of DSD. Furthermore, the dysregulation of the meiosis signaling in CIS cells may putatively lead to polyploidization, genomic amplification of some chromosomal regions including 12p, and genetic instability that is frequently observed in CIS cells and TGCT (82, 84, 85). Despite the observed expression of some pre-meiosis and meiosis markers in CIS cells, the neoplastic germ cells do not complete meiosis and appear unable to progress through meiotic prophase I. This is most likely due to simultaneous expression of meiosis inhibitors, resulting in an insufficient response to the postpubertal somatic environment that normally promotes meiosis. By contrast, CIS cells continue mitotic proliferation, which thereby may lead to a gain of invasive capacity (83, 86).

Disruption of meiosis regulation also has severe consequences when occurring in postpubertal testes, leading to spermatogenic arrest, cessation of spermiogenesis, and infertility. In humans, neither the frequency of meiotic mutations nor the rate of infertility due to such mutations is known, but bilateral spermatogenic arrest was observed in 12.5% of patients who underwent testicular biopsy due to infertility (87). In recent years, the identification of genes involved in infertility spanning all aspects of spermatogenesis, including meiotic defects, has been accelerated mainly by studies of mouse models (88, 89). Despite the numerous knockout mouse models presenting with spermatogenic arrest, studies in humans have largely failed to identify specific gene mutations. The main problem is the heterogeneity of human infertility phenotypes and the large number of genes involved in the regulation of spermatogenesis. We can mention only few studies specifically investigating patients with failure of meiosis manifested as spermatogenic failure. Mutational analysis of genes known from murine studies, including *SYCP3*, *MSH4*, and *DNMT3L* found mainly polymorphic sequence alterations, which were also detected in normospermic controls (90, 91). In a more unrestricted approach, Stouffs et al. (92) analyzed genome-wide gene copy number variation (CNV) by array comparative genomic hybridization in patients with spermatogenic arrest at the primary spermatocyte level. They found a few promising regions harboring several genes that potentially may be involved in the regulation of meiosis, but their biological function needs to be elucidated.

Among the most common large CNVs that may cause failure of meiotic entry in humans are microdeletions in the *AZF* (azoospermia factor) region of the Y chromosome. The *AZF* region is highly repetitive and consists of numerous palindromic regions thought to have arisen out of repeated rounds of gene duplication and inversion (93, 94). The repetitive nature of the *AZF* region makes it particularly prone to homologous recombination between direct repeats, resulting in deletions. There is now a fairly comprehensive chromosomal map of the *AZF* regions with three specific regions currently identified, *AZFa*, *AZFb*, and *AZFc*, which represent multigene segments on the Y chromosome (93). The most commonly deleted of these three is the *AZFc* (b2–b4) region, resulting in phenotypes that range from complete absence of the germ cell lineage (Sertoli cell-only syndrome) to severe oligozoospermia or cryptozoospermia (95, 96). Patients with complete

deletions of either *AZFa* or *AZFb* region (or both) are azoospermic and often display a histological pattern or spermatogenic arrest at the stage of spermatogonia or primary spermatocytes, consistent with a failure of the meiotic entry. Despite their deletion frequency, the functions of genes that map to the Y chromosome are still poorly understood and more studies are needed to understand the genetic aspects of disrupted meiosis regulation.

Conclusion

The switch between mitosis and meiosis is a unique feature of germ cells and constitute a key step in the sex-dimorphic gonadal differentiation during early fetal development as well as in spermatogenesis in the adult testes. Great progress in the understanding of the molecular mechanisms regulating meiotic entry has been made in rodent models within the last decade, and recent results from human studies suggest some conservation of the meiosis regulation machinery. However, there are also differences between rodent and human meiosis signaling, and there is growing evidence for much greater complexity and redundancies in the control of meiotic entry in both rodents and humans than initially thought. It is of importance to increase the understanding by filling the gaps of knowledge because dysregulation of meiosis signaling may lead to serious health problems, including DSD, germ cell cancer, and infertility.

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References

1. Bowles J, Knight D, Smith C, Wilhelm D, Richman J, Mamiya S, Yashiro K, Chawengsaksophak K, Wilson MJ, Rossant J, Hamada H, Koopman P. Retinoid signaling determines germ cell fate in mice. Science 2006; 312: 596–600.
2. 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: 2474–9.
3. Suzuki A, Saga Y. Nanos2 suppresses meiosis and promotes male germ cell differentiation. Genes Dev 2008; 22: 430–5.
4. Matson CK, Murphy MW, Griswold MD, Yoshida S, Bardwell VJ, Zarkower D. The mammalian doublesex homolog DMRT1 is a transcriptional gatekeeper that controls the mitosis versus meiosis decision in male germ cells. Dev Cell 2010; 19: 612–24.
5. Le Bouffant R, Guerquin MJ, Duquenne C, Frydman N, Coffigny H, Rouiller-Fabre V, Frydman R, Habert R, Livera G. Meiosis initiation in the human ovary requires intrinsic retinoic acid synthesis. Hum Reprod 2010; 25: 2579–90.
6. Childs AJ, Cowan G, Kinnell HL, Anderson RA, Saunders PT. Retinoic acid signalling and the control of meiotic entry in the human fetal gonad. PLoS One 2011; 6: e20249.
7. Rajpert-De Meyts E, Jørgensen N, Graem N, Müller J, Cate RL, Skakkebaek NE. Expression of anti-Müllerian hormone during normal and pathological gonadal development: association with differentiation of Sertoli and granulosa cells. J Clin Endocrinol Metab 1999; 84: 3836–44.
8. Rajpert-De Meyts E, Hanstein R, Jørgensen N, Graem N, Vogt PH, Skakkebaek NE. Developmental expression of POU5F1 (OCT-3/4) in normal and dysgenetic human gonads. Hum Reprod 2004; 19: 1338–44.
9. Hoei-Hansen CE, Nielsen JE, Almstrup K, Sonne SB, Graem N, Skakkebaek NE, Leffers H, Rajpert-De Meyts E. Transcription factor AP-2gamma is a developmentally regulated marker of testicular carcinoma in situ and germ cell tumors. Clin Cancer Res 2004; 10: 8521–30.
10. Gaskell TL, Esnal A, Robinson LL, Anderson RA, Saunders PT. Immunohistochemical profiling of germ cells within the human fetal testis: identification of three subpopulations. Biol Reprod 2004; 71: 2012–21.
11. Stoop H, Honecker F, Cools M, de Krijger R, Bokemeyer C, Looijenga LH. Differentiation and development of human female germ cells during prenatal gonadogenesis: an immunohistochemical study. Hum Reprod 2005; 20: 1466–76.
12. Hoei-Hansen CE, Almstrup K, Nielsen JE, Brask Sonne S, Graem N, Skakkebaek NE, Leffers H, Rajpert-De Meyts E. Stem cell pluripotency factor NANOG is expressed in human fetal gonocytes, testicular carcinoma in situ and germ cell tumours. Histopathology 2005; 47: 48–56.
13. Ostrer H, Huang HY, Masch RJ, Shapiro E. A cellular study of human testis development. Sex Dev 2007; 1: 286–92.
14. Culty M. Gonocytes, the forgotten cells of the germ cell lineage. Birth Defects Res C Embryo Today 2009; 87: 1–26.
15. Sinclair AH, Berta P, Palmer MS, Hawkins JR, Griffiths BL, Smith MJ, Foster JW, Frischauf AM, Lovell-Badge R, Goodfellow PN. A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. Nature 1990; 346: 240–4.
16. Koopman P, Gubbay J, Vivian N, Goodfellow P, Lovell-Badge R. Male development of chromosomally female mice transgenic for Sry. Nature 1991; 351: 117–21.
17. Byskov AG, Høyer PE, Yding Andersen C, Kristensen SG, Jespersen A, Møllgård K. No evidence for the presence of oogonia in the human ovary after their final clearance during the first two years of life. Hum Reprod 2011; 26: 2129–239.
18. Robinson LL, Gaskell TL, Saunders PT, Anderson RA. Germ cell specific expression of c-kit in the human fetal gonad. Mol Hum Reprod 2001; 7: 845–52.

19. Kerr CL, Hill CM, Blumenthal PD, Gearhart JD. Expression of pluripotent stem cell markers in the human fetal ovary. Hum Reprod 2008; 23: 589–99.
20. Childs AJ, Kinnell HL, He J, Anderson RA. LIN28 is selectively expressed by primordial and pre-meiotic germ cells in the human fetal ovary. Stem Cells Dev 2012; 21: 2343–9.
21. Hartshorne GM, Lyrakou S, Hamoda H, Oloto E, Ghafari F. Oogenesis and cell death in human prenatal ovaries: what are the criteria for oocyte selection? Mol Hum Reprod 2009; 15: 805–19.
22. Anderson RA, Fulton N, Cowan G, Coutts S, Saunders PT. Conserved and divergent patterns of expression of DAZL, VASA and OCT4 in the germ cells of the human fetal ovary and testis. BMC Dev Biol 2007; 7: 136.
23. Hilscher W. The genetic control and germ cell kinetics of the female and male germ line in mammals including man. Hum Reprod 1991; 6: 1416–25.
24. Fowler PA, Flannigan S, Mathers A, Gillanders K, Lea RG, Wood MJ, Maheshwari A, Bhattacharya S, Collie-Duguid ES, Baker PJ, Monteiro A, O'Shaughnessy PJ. Gene expression analysis of human fetal ovarian primordial follicle formation. J Clin Endocrinol Metab 2009; 94: 1427–35.
25. Svechnikov K, Söder O. Ontogeny of gonadal sex steroids. Best Pract Res Clin Endocrinol Metab 2008; 22: 95–106.
26. Honecker F, Stoop H, de Krijger RR, Chris Lau YF, Bokemeyer C, Looijenga LH. Pathobiological implications of the expression of markers of testicular carcinoma in situ by fetal germ cells. J Pathol 2004; 203: 849–57.
27. Pauls K, Schorle H, Jeske W, Brehm R, Steger K, Wernert N, Büttner R, Zhou H. Spatial expression of germ cell markers during maturation of human fetal male gonads: an immunohistochemical study. Hum Reprod 2006; 21: 397–404.
28. Culty M. Gonocytes, from the fifties to the present: is there a reason to change the name? Biol Reprod 2013; 89: 46.
29. Baillet A, Mandon-Pepin B. Mammalian ovary differentiation – a focus on female meiosis. Mol Cell Endocrinol 2012; 356: 13–23.
30. Skinner MK. Regulation of primordial follicle assembly and development. Hum Reprod Update 2005; 11: 461–71.
31. Maheshwari A, Fowler PA. Primordial follicular assembly in humans – revisited. Zygote 2008; 16: 285–96.
32. Baltus AE, Menke DB, Hu YC, 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: 1430–4.
33. Anderson EL, Baltus AE, Roepers-Gajadien HL, Hassold TJ, de Rooij DG, van Pelt AM, 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: 14976–80.
34. Lin Y, Gill ME, Koubova J, Page DC. Germ cell-intrinsic and -extrinsic factors govern meiotic initiation in mouse embryos. Science 2008; 322: 1685–7.
35. Bowles J, Feng CW, Spiller C, Davidson TL, Jackson A, Koopman P. FGF9 suppresses meiosis and promotes male germ cell fate in mice. Dev Cell 2010; 19: 440–9.
36. McClelland K, Bowles J, Koopman P. Male sex determination: insights into molecular mechanisms. Asian J Androl 2012; 14: 164–71.
37. Souquet B, Tourpin S, Messiaen S, Moison D, Habert R, Livera G. Nodal signaling regulates the entry into meiosis in fetal germ cells. Endocrinology 2012; 153: 2466–673.
38. Wu Q, Kanata K, Saba R, Deng CX, Hamada H, Saga Y. Nodal/activin signaling promotes male germ cell fate and suppresses female programming in somatic cells. Development 2013; 140: 291–300.
39. Kumar S, Chatzi C, Brade T, Cunningham TJ, Zhao X, Duester G. Sex-specific timing of meiotic initiation is regulated by Cyp26b1 independent of retinoic acid signalling. Nat Commun 2011; 2: 151.
40. Griswold MD, Hogarth CA, Bowles J, Koopman P. Initiating meiosis: the case for retinoic acid. Biol Reprod 2012; 86: 35.
41. He J, Stewart K, Kinnell HL, Anderson RA, Childs AJ. A developmental stage-specific switch from DAZL to BOLL occurs during fetal oogenesis in humans, but not mice. PLoS One 2013; 8: e73996.
42. Jørgensen A, Nielsen JE, Jensen MB, Græm N, Rajpert-De Meyts E. Analysis of meiosis regulators in human gonads: a sexually dimorphic spatio-temporal expression pattern suggests involvement of DMRT1 in meiotic entry. Mol Hum Reprod 2012; 18: 523–34.
43. Gkountela S, Li Z, Vincent JJ, Zhang KX, Chen A, Pellegrini M, Clark AT. The ontogeny of cKIT+ human primordial germ cells proves to be a resource for human germ line reprogramming, imprint erasure and in vitro differentiation. Nat Cell Biol 2013; 15: 113–22.
44. Houmard B, Small C, Yang L, Naluai-Cecchini T, Cheng E, Hassold T, Griswold M. Global gene expression in the human fetal testis and ovary. Biol Reprod 2009; 81: 438–43.
45. Bowles J, Koopman P. Retinoic acid, meiosis and germ cell fate in mammals. Development 2007; 134: 3401–11.
46. Krentz AD, Murphy MW, Sarver AL, Griswold MD, Bardwell VJ, Zarkower D. DMRT1 promotes oogenesis by transcriptional activation of Stra8 in the mammalian fetal ovary. Dev Biol 2011; 356: 63–70.
47. MacLean G, Li H, Metzger D, Chambon P, Petkovich M. Apoptotic extinction of germ cells in testes of Cyp26b1 knockout mice. Endocrinology 2007; 148: 4560–7.
48. 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: 656–64.
49. Saba R, Wu Q, Saga Y. CYP26B1 promotes male germ cell differentiation by suppressing STRA8-dependent meiotic and STRA8-independent mitotic pathways. Dev Biol 2014; 389: 173–81.
50. 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: 1239–41.
51. Saba R, Kato Y, Saga Y. NANOS2 promotes male germ cell development independent of meiosis suppression. Dev Biol 2014; 385: 32–40.
52. Guerquin MJ, Duquenne C, Lahaye JB, Tourpin S, Habert R, Livera G. New testicular mechanisms involved in the prevention of fetal meiotic initiation in mice. Dev Biol 2010; 346: 320–30.
53. Western PS, Miles DC, van den Bergen JA, Burton M, Sinclair AH. Dynamic regulation of mitotic arrest in fetal male germ cells. Stem Cells 2008; 26: 339–47.
54. Spiller CM, Bowles J, Koopman P. Nodal/Cripto signaling in fetal male germ cell development: implications for testicular germ cell tumors. Int J Dev Biol 2013; 57: 211–9.
55. Cupp AS, Dufour JM, Kim G, Skinner MK, Kim KH. Action of retinoids on embryonic and early postnatal testis development. Endocrinology 1999; 140: 2343–52.

56. Waheeb R, Hofmann MC. Human spermatogonial stem cells: a possible origin for spermatocytic seminoma. Int J Androl 2011; 34: e296–305.
57. Baudat F, Imai Y, de Massy B. Meiotic recombination in mammals: localization and regulation. Nat Rev Genet 2013; 14: 794–806.
58. Wolgemuth DJ, Manterola M, Vasileva A. Role of cyclins in controlling progression of mammalian spermatogenesis. Int J Dev Biol 2013; 57: 159–68.
59. McNicoll F, Stevense M, Jessberger R. Cohesin in gametogenesis. Curr Top Dev Biol 2013; 102: 1–34.
60. Hogarth CA, Griswold MD. The key role of vitamin A in spermatogenesis. J Clin Invest 2010; 120: 956–62.
61. Oulad-Abdelghani M, Bouillet P, Décimo D, Gansmuller A, Heyberger S, Dollé P, Bronner S, Lutz Y, Chambon P. Characterization of a premeiotic germ cell-specific cytoplasmic protein encoded by Stra8, a novel retinoic acid-responsive gene. J Cell Biol 1996; 135: 469–77.
62. Vernet N, Dennefeld C, Rochette-Egly C, Oulad-Abdelghani M, Chambon P, Ghyselinck NB, Mark M. Retinoic acid metabolism and signaling pathways in the adult and developing mouse testis. Endocrinology 2006; 147: 96–110.
63. Zhou Q, Nie R, Li Y, Friel P, Mitchell D, Hess RA, Small C, Griswold MD. Expression of stimulated by retinoic acid gene 8 (Stra8) in spermatogenic cells induced by retinoic acid: an in vivo study in vitamin A-sufficient postnatal murine testes. Biol Reprod 2008; 79: 35–42.
64. Busada JT, Kaye EP, Renegar RH, Geyer CB. Retinoic acid induces multiple hallmarks of the prospermatogonia-to-spermatogonia transition in the neonatal mouse. Biol Reprod 2014; 90: 1–11.
65. Li H, Palczewski K, Baehr W, Clagett-Dame M. Vitamin A deficiency results in meiotic failure and accumulation of undifferentiated spermatogonia in prepubertal mouse testis. Biol Reprod 2011; 84: 336–41.
66. van Pelt AM, de Rooij DG. Synchronization of the seminiferous epithelium after vitamin A replacement in vitamin A-deficient mice. Biol Reprod 1990; 43: 363–7.
67. Snyder EM, Small C, Griswold MD. Retinoic acid availability drives the asynchronous initiation of spermatogonial differentiation in the mouse. Biol Reprod 2010; 83: 783–90.
68. Mark M, Jacobs H, Oulad-Abdelghani M, Dennefeld C, Féret 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: 3233–42.
69. Looijenga LH, Hersmus R, Gillis AJ, Pfundt R, Stoop HJ, van Gurp RJ, Veltman J, Beverloo HB, van Drunen E, van Kessel AG, Pera RR, Schneider DT, Summersgill B, Shipley J, McIntyre A, van der Spek P, Schoenmakers E, Oosterhuis JW. Genomic and expression profiling of human spermatocytic seminomas: primary spermatocyte as tumorigenic precursor and DMRT1 as candidate chromosome 9 gene. Cancer Res 2006; 66: 290–302.
70. von Kopylow K, Staege H, Spiess AN, Schulze W, Will H, Primig M, Kirchhoff C. Differential marker protein expression specifies rarefaction zone-containing human Adark spermatogonia. Reproduction 2012; 143: 45–57.
71. Kashimada K, Svingen T, Feng CW, Pelosi E, Bagheri-Fam S, Harley VR, Schlessinger D, Bowles J, Koopman P. Antagonistic regulation of Cyp26b1 by transcription factors SOX9/SF1 and FOXL2 during gonadal development in mice. FASEB J 2011; 25: 3561–9.
72. Kusz KM, Tomczyk L, Sajek M, Spik A, Latos-Bielenska A, Jedrzejczak P, Pawelczyk L, Jaruzelska J. The highly conserved NANOS2 protein: testis-specific expression and significance for the human male reproduction. Mol Hum Reprod 2009; 15: 165–71.
73. Julaton VT, Reijo Pera RA. NANOS3 function in human germ cell development. Hum Mol Genet 2011; 20: 2238–50.
74. Müller J, Skakkebaek NE, Ritzén M, Plöen L, Petersen KE. Carcinoma in situ of the testis in children with 45,X/46,XY gonadal dysgenesis. J Pediatr 1985; 106: 431–6.
75. Cools M, Looijenga LH. Tumor risk and clinical follow-up in patients with disorders of sex development. Pediatr Endocrinol Rev 2011; 9: 519–24.
76. Lindhardt Johansen M, Hagen CP, Rajpert-De Meyts E, Kjærgaard S, Petersen BL, Skakkebæk NE, Main KM, Juul A. 45,X/46,XY mosaicism: phenotypic characteristics, growth and reproductive function – a retrospective longitudinal study. J Clin Endocrinol Metab 2012; 97: E1540–9.
77. Hersmus R, Kalfa N, de Leeuw B, Stoop H, Oosterhuis JW, de Krijger R, Wolffenbuttel KP, Drop SL, Veitia RA, Fellous M, Jaubert F, Looijenga LH. FOXL2 and SOX9 as parameters of female and male gonadal differentiation in patients with various forms of disorders of sex development (DSD). J Pathol 2008; 215: 31–8.
78. Matson CK, Murphy MW, Sarver AL, Griswold MD, Bardwell VJ, Zarkower D. DMRT1 prevents female reprogramming in the postnatal mammalian testis. Nature 2011; 476: 101–4.
79. Skakkebaek NE, Berthelsen JG, Giwercman A, Müller J. Carcinoma-in-situ of the testis: possible origin from gonocytes and precursor of all types of germ cell tumours except spermatocytoma. Int J Androl 1987; 10: 19–28.
80. Almstrup K, Hoei-Hansen CE, Wirkner U, Blake J, Schwager C, Ansorge W, Nielsen JE, Skakkebaek NE, Rajpert-De Meyts E, Leffers H. Embryonic stem cell-like features of testicular carcinoma in situ revealed by genome-wide gene expression profiling. Cancer Res 2004; 64: 4736–43.
81. Sonne SB, Almstrup K, Dalgaard M, Juncker AS, Edsgard D, Ruban L, Harrison NJ, Schwager C, Abdollahi A, Huber PE, Brunak S, Gjerdrum LM, Moore HD, Andrews PW, Skakkebaek NE, Rajpert-De Meyts E, Leffers H. Analysis of gene expression profiles of microdissected cell populations indicates that testicular carcinoma in situ is an arrested gonocyte. Cancer Res 2009; 69: 5241–50.
82. Rajpert-De Meyts E. Developmental model for the pathogenesis of testicular carcinoma in situ: genetic and environmental aspects. Hum Reprod Update 2006; 12: 303–23.
83. Jørgensen A, Nielsen JE, Almstrup K, Toft BG, Petersen BL, Rajpert-De Meyts E. Dysregulation of the mitosis-meiosis switch in testicular carcinoma in situ. J Pathol 2013; 229: 588–98.
84. Adamah DJ, Gokhale PJ, Eastwood DJ, Rajpert De-Meyts E, Goepel J, Walsh JR, Moore HD, Andrews PW. Dysfunction of the mitotic:meiotic switch as a potential cause of neoplastic conversion of primordial germ cells. Int J Androl 2006; 29: 219–27.
85. Rajpert-de Meyts E, Hoei-Hansen CE. From gonocytes to testicular cancer: the role of impaired gonadal development. Ann NY Acad Sci 2007; 1120: 168–80.
86. Sharpe RM, Mitchell RT. The downside of ‘inappropriate messaging’: new insight into the development of testicular germ cell tumours in young men? J Pathol 2013; 229: 497–501.

87. McLachlan RI, Rajpert-De Meyts E, Hoei-Hansen CE, de Kretser DM, Skakkebaek NE. Histological evaluation of the human testis – approaches to optimizing the clinical value of the assessment: mini review. Hum Reprod 2007; 22: 2–16.
88. Matzuk MM, Lamb DJ. The biology of infertility: research advances and clinical challenges. Nat Med 2008; 14: 1197–213.
89. Jamsai D, O'Bryan MK. Mouse models in male fertility research. Asian J Androl 2011; 13: 139–51.
90. Martínez J, Bonache S, Carvajal A, Bassas L, Larriba S. Mutations of SYCP3 are rare in infertile Spanish men with meiotic arrest. Fertil Steril 2007; 88: 988–89.
91. Stouffs K, Vandermaelen D, Tournaye H, Liebaers I, Lissens W. Mutation analysis of three genes in patients with maturation arrest of spermatogenesis and couples with recurrent miscarriages. Reprod Biomed Online 2011; 22: 65–71.
92. Stouffs K, Vandermaelen D, Massart A, Menten B, Vergult S, Tournaye H, Lissens W. Array comparative genomic hybridization in male infertility. Hum Reprod 2012; 27: 921–9.
93. Kuroda-Kawaguchi, T, Skaletsky H, Brown LG, Minx PJ, Cordum HS, Waterston RH, Wilson RK, Silber S, Oates R, Rozen S, Page DC. The AZFc region of the Y chromosome features massive palindromes and uniform recurrent deletions in infertile men. Nat Genet 2001; 29: 279–86.
94. Skaletsky H, Kuroda-Kawaguchi T, Minx PJ, Cordum HS, Hillier L, Brown LG, Repping S, Pyntikova T, Ali J, Bieri T, Chinwalla A, Delehaunty A, Delehaunty K, Du H, Fewell G, Fulton L, Fulton R, Graves T, Hou SF, Latrielle P, Leonard S, Mardis E, Maupin R, McPherson J, Miner T, Nash W, Nguyen C, Ozersky P, Pepin K, Rock S, Rohlfing T, Scott K, Schultz B, Strong C, Tin-Wollam A, Yang SP, Waterston RH, Wilson RK, Rozen S, Page DC. The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. Nature 2003; 423: 825–37.
95. Reijo R, Lee TY, Salo P, Alagappan R, Brown LG, Rosenberg M, Rozen S, Jaffe T, Straus D, Hovatta O, de la Chapelle A, Silber S, Page DC. Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. Nat Genet 1995; 10: 383–93.
96. Reijo R, Alagappan RK, Patrizio P, Page DC. Severe oligozoospermia resulting from deletions of azoospermia factor gene on Y chromosome. Lancet 1996; 347: 1290–3.

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