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INVITED REVIEW

Germ Cell Biology

# The role of sex chromosomes in mammalian germ cell differentiation: can the germ cells carrying X and Y chromosomes differentiate into fertile oocytes?

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The sexual differentiation of germ cells into spermatozoa or oocytes is strictly regulated by their gonadal environment, testis or ovary, which is determined by the presence or absence of the Y chromosome, respectively. Hence, in normal mammalian development, male germ cells differentiate in the presence of X and Y chromosomes, and female germ cells do so in the presence of two X chromosomes. However, gonadal sex reversal occurs in humans as well as in other mammalian species, and the resultant XX males and XY females can lead healthy lives, except for a complete or partial loss of fertility. Germ cells carrying an abnormal set of sex chromosomes are efficiently eliminated by multilayered surveillance mechanisms in the testis, and also, though more variably, in the ovary. Studying the molecular basis for sex-specific responses to a set of sex chromosomes during gametogenesis will promote our understanding of meiotic processes contributing to the evolution of sex determining mechanisms. This review discusses the fate of germ cells carrying various sex chromosomal compositions in mouse models, the limitation of which may be overcome by recent successes in the differentiation of functional germ cells from embryonic stem cells under experimental conditions.

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## INTRODUCTION

In mammalian development, gonadal sex, either testis or ovary, is determined by the presence or absence of the *Sry* gene on the Y chromosome. Subsequently, germ cells undergo sexual differentiation dependent on the gonadal environment. Therefore, spermatogenesis takes place in the presence of X and Y chromosomes, and oogenesis in the presence of two X chromosomes. However, sex reversal occurs at the frequency of 1 in 20 000 newborn boys and is reported less frequently among girls.<sup>1,2</sup> In the XX testis, the XX germ cells enter a resting stage, the first phase of spermatogenesis, and become prospermatogonia; however, they are eliminated at the developmental stages during which the prospermatogonia normally resume mitotic activity and initiate differentiation into spermatogonia. By contrast, in the XY ovary, the XY germ cells enter meiosis and continue to differentiate as the primary oocytes, but their fertility depends on species, genetic background, and causes of sex reversal (reviewed by Amleh *et al.*<sup>3</sup>). It has recently been shown that mouse embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) can differentiate into primordial germ cell (PGC)-like cells in culture and then into functional sperm and oocytes after transfer into the testicular and the ovarian somatic environment, respectively.<sup>4,5</sup> Given that XX and XY ESCs are available, they can theoretically be differentiated into XX prospermatogonia and XY oocytes. This review summarizes what is known about the role of sex chromosomes in germ cell sex differentiation and functions and discusses the prospect of producing XY oocytes from the ESCs. Most

of the discussion is focused on mouse models, which have been best studied as to this aspect; however, human cases are also included where information is available.

## SEX DETERMINATION AND DIFFERENTIATION OF GONADS

In mammals, a single exon gene *SRY* on the Y chromosome is activated in the XY gonadal primordium and initiates a cascade of molecular and morphological events leading to testicular differentiation (See reviews<sup>6–9</sup>). *SRY*-encoded protein (SRY) is a transcription factor containing an HMG-box DNA-binding motif that directly targets *SOX9*, which encodes another transcription factor sharing the DNA binding motif with *SRY*. *SOX9* upregulates other genes, such as *FGF9* and *PGD2*, which are involved in the differentiation of the Sertoli cells that compose the testis cords. By contrast, in the absence of *SRY* or *SOX9*, ovarian differentiation is initiated in the XX gonad without distinct morphological changes by the activation of genes, such as *WNT4* and *FOXL2*, whose protein products antagonize testicular differentiation in gonadal somatic cells (see reviews<sup>10,11</sup>). Follicular structures are formed after germ cells have differentiated into primary oocytes and reached the diplotene stage of the meiotic prophase (see below). More details about the molecular mechanisms regulating testicular and ovarian differentiation are given in the above-mentioned reviews and not repeated here.

## SEXUAL DIFFERENTIATION OF GERM CELLS

The PGCs originate in the proximal epiblast and migrate into a gonadal primordium that is undergoing early sexual differentiation.<sup>12–14</sup> Parental

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origin-specific DNA methylation is largely eliminated in the PGCs during this period.<sup>15–17</sup> The subsequent sexual differentiation of germ cells is regulated by their gonadal environment<sup>18,19</sup> (Figure 1). In the testis, the PGCs become arrested at the G<sub>0</sub>/G<sub>1</sub> stage of the mitotic cell cycle, undergo male-specific DNA methylation, and resume proliferation after birth.<sup>20</sup> In the entire male reproductive life, while the population of spermatogonial stem cells continually proliferates, a cohort of spermatogonia enter meiosis and differentiate into haploid spermatozoa in a cyclic manner. In the ovary, most, if not all, PGCs enter meiosis and reach the end of the first meiotic prophase, named the diplotene stage, at 5–7 months of pregnancy in humans or around the day of delivery in the mouse.<sup>21–23</sup> The diplotene oocytes become surrounded by granulosa cells and together form primordial follicles, which remain at this stage as an oocyte reserve until they are recruited into follicular growth.<sup>24–26</sup> This resting stage can last for decades in humans or months in mice. Female-specific DNA methylation takes place in oocytes during the growth phase.<sup>27–30</sup> Fully-grown oocytes resume the meiotic cell cycle and divide into haploid oocytes upon ovulation and fertilization. Thus, germ cells of both sexes enter meiosis to become haploid, but at very different stages of life and in different manners. It has been shown that the entry of germ cells into meiosis is regulated by local signaling from the surrounding somatic cells, and can be manipulated by pharmacological or genetic modifications<sup>31–37</sup> (Figure 1). Another distinct difference between the two sexes is the ability of germ cells to proliferate continuously; female germ cells cease proliferation when they enter meiosis in fetal ovaries. Hence, a female has a finite number of oocytes in reserve, and her reproductive life ends when her oocyte reserve decreases below the threshold that is required for sustaining ovarian functions.

## DIFFERENTIATION OF SPERM AND EGGS FROM EMBRYONIC STEM CELLS

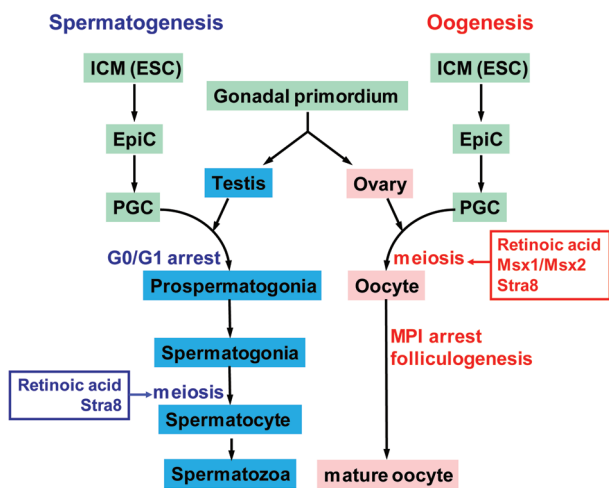
Since the first reports in 2003,<sup>38,39</sup> numerous attempts have been made to differentiate ESCs into germ cells, and eventually into mature haploid gametes that have the capacity to produce normal offspring (see reviews<sup>40,41</sup>). PGC-like cells appear spontaneously when murine and human ESCs are cultured under various conditions.<sup>38,39,42–45</sup> However, most attempts result in a failure to generate functional gametes; the major obstacle appears to be a lack of proper meiotic events in the

nucleus.<sup>46</sup> Although meiosis is central to normal gametogenesis, it has been reported that “oocyte-like” cells can differentiate in the absence of meiosis,<sup>47</sup> and ultimately, the production of offspring is the only reliable evidence for functional gametes. The first success in obtaining the birth of live offspring from ESC-derived male haploid cells was reported in 2006.<sup>42</sup> The key to this success may be the selection of ESCs expressing STRA8, which is essential for the initiation of meiosis.<sup>33,34</sup> However, the resultant pups showed growth abnormalities with incorrect DNA methylation profiles, and most died within the first month of life.

Hayashi *et al.*<sup>4</sup> have achieved a step-by-step induction of XY ESCs into epiblast-like cells and then into PGC-like cells with high efficiency (Figure 1). After these PGC-like cells had been dissociated into single cells and transplanted into germ cell-deficient mouse testes, they colonized the host seminiferous tubules and differentiated into spermatozoa-like cells, which produced healthy and fertile offspring of both sexes. Enrichment of donor cells based on the genes that they expressed not only increased the efficiency of gamete derivation but also prevented the development of teratomas in the host testis. When the PGC-like cells derived from XX ESCs by the same protocol had been aggregated with fetal ovarian somatic cells to form reconstituted ovaries *in vitro* and transplanted into the ovarian bursa or kidney subcapsular site of recipient mice, growing follicles containing oocyte-like cells were obtained.<sup>5</sup> When these oocyte-like cells were allowed to mature and were fertilized *in vitro*, they developed into blastocyst-stage embryos. After fertilized eggs had been transferred into foster females, healthy and fertile offspring of both sexes was generated. These findings indicate that ESCs can be induced to differentiate into PGCs under defined *in vitro* conditions, but also that their sexual differentiation into spermatozoa and oocytes depends on their testicular and ovarian somatic environment, respectively. However, the efficiency of healthy offspring production from ESCs is still limited, particularly through the female germ-line and similar attempts starting with iPSCs of embryonic fibroblast origin yielded much less success.<sup>4,5</sup> We still poorly understand whether certain oocytes are eliminated during the meiotic prophase (see below), excluded from follicular recruitment or eliminated by follicular atresia under physiological conditions. Circumventing the normal surveillance mechanism due to artificial conditions may decrease the proportion of oocytes that are competent for embryonic development.

## SEX CHROMOSOMES IN EMBRYONIC STEM CELLS

In early studies, oocyte-like cells were generated from ESC lines that had been established from 129SY male mouse embryos, which are inevitably of the XY karyotype.<sup>38,43</sup> None of these attempts succeeded in producing functional oocytes. It has been reported that when germ-cell-like cells were derived from human ESCs of either XX or XY origin, their gene expression profiles indicated that both male and female programs, namely the expression of *TEKT1* and *GDF9* respectively, were activated regardless of karyotype.<sup>44</sup> Using a very different protocol, differentiation of haploid cells with male germ-line gene expression patterns was obtained from human iPSCs (but not ESCs) of either XX or XY origin.<sup>48</sup> These observations suggest the bidirectional potential of ESCs regardless of their chromosomal sex. However, the only successful derivation of functional oocytes so far used mouse XX ESCs.<sup>5</sup> It remains to be evaluated whether the PGC-like cells derived from XY ESCs can differentiate into functional oocytes in the ovarian environment. A caveat is the stability of the sex chromosomes in ESCs and iPSCs. It has been reported that both XX and XY human ESCs tend to lose the second sex chromosome to become XO at high frequencies (3.0% and 1.5%, respectively) after multiple



**Figure 1:** Sexual differentiation of germ cells. ICM: innercell mass; ESC: embryonic stem cells; EpiC: epiblast cells; PGC: primordial germ cells; MPI: meiotic prophase I.

passages in culture.<sup>49</sup> Therefore, the karyotype in the initial cell line does not warrant its maintenance during gametogenesis.

### ROLE OF SEX CHROMOSOMES IN SPERMATOGENESIS

In XX sex-reversed males, the XX germ cells enter the early phase of spermatogenesis and become arrested, but do not survive to resume the mitotic cell cycle. It has been established that the presence of the second X chromosome is incompatible with spermatogenesis since XO germ cells lacking the second X chromosome survive to initiate spermatogonial proliferation (reviewed by Burgoyne<sup>50</sup>). However, these cells do not complete spermatogonial proliferation and differentiation, and thus fail to enter meiosis. In mice and men, deletions of specific regions of the Y chromosome have been linked to an early failure of spermatogenesis and consequent sterility.<sup>51–55</sup> In the mouse, deletion mapping of the Y chromosome located a gene necessary for spermatogonial proliferation to a 1.3 Mb deletion on the short arm (Yp) that removed six single copy genes and created a *Zfy2/1* fusion gene; subsequent Y transgene additions identified *Eif2s3y* as the spermatogonial proliferation gene.<sup>54</sup> Some genes on the mouse long arm (Yq) such as *Sly* are required for postmeiotic gene expression and morphological differentiation of spermatozoa.<sup>56</sup> However, the problems associated with late spermatogenesis can be circumvented either by intracytoplasmic sperm injection or by round spermatid injection (ROSI) into the oocytes, to produce live offspring.<sup>57,58</sup> Using the ROSI technique, the *Eif2s3y* gene alone was shown to be sufficient to allow for the production of live offspring when added to an XOSry male mouse.<sup>54,58</sup> The encoded EIF2S3Y protein is the third subunit of the eukaryotic translation initiation factor 2, which is ubiquitously expressed, but plays a specific role in spermatogonial proliferation in the testis. Its X homolog *Eif2s3x* is also ubiquitously expressed, and its protein shares biological activity with EIF2S3Y. Accordingly, it has been proposed that the maintenance of a double EIF2S3 dose affords a selective advantage in both sexes since *Eif2s3x* is one of only a few mouse X-linked genes known to escape X inactivation.<sup>59</sup> Although *Eif2s3y* is not conserved in any of the simian primates, including humans, the human Y chromosome harbors EIF1AY, a Y-encoded version of the elongation and initiation factor EIF1A. The caveat is that the spermatids generated by *Eif2s3* expression without any other Y-linked genes but *Sry* often contain diploid, instead of haploid, DNA contents, and their success in offspring production is limited.<sup>58</sup> The lack of a pairing partner for the X chromosome was initially suspected to be the cause of low fertility,<sup>58</sup> but overcoming X chromosome univalence by providing its pairing partner did not improve meiotic arrest or increase ROSI success,<sup>58</sup> suggesting that other Y-linked genes might also be required for obtaining spermatids that are fully functional in ROSI. It has recently been established that mouse *Zfy1* and *Zfy2* (encoding zinc finger transcription factors) are required for promoting the second meiotic division in spermatocytes, thus generating haploid spermatids.<sup>60</sup>

### ROLE OF SEX CHROMOSOMES IN OOGENESIS

In the normal ovary, the germ cells carry two X chromosomes. The second X chromosome is initially inactivated as in any somatic cells, but gradually reactivated prior to the onset of meiosis.<sup>61–63</sup> Hence, the oocyte develops in the presence of two transcriptionally active X chromosomes for the rest of its reproductive life. Consequently, the absence of the second X chromosome from the XO oocyte may be disadvantageous when compared to its presence in the XX oocyte. Anomalies of XO females in humans are known as Turners syndrome (TS). Ninety-nine percentage of XO fetuses die in the womb, and TS girls who have been

born develop short stature, congenital cardiovascular defects, and metabolic abnormalities.<sup>64,65</sup> In addition, most TS women are infertile due to the early loss of oocytes.<sup>66–68</sup> By contrast, XO female mice are viable, healthy, and fertile.<sup>69</sup> Since the X chromosome is subjected to genomic imprinting during gametogenesis, XO female mice carrying paternal X chromosomes show more severe phenotypes than are expected from the mere half-dosage of X chromosome contents.<sup>70–73</sup> Nonetheless, they can be fertile, and their counterparts, XO females carrying maternal X chromosomes, are quite normal. The striking difference between humans and mice with the XO karyotype has been explained by the fact that many fewer X-linked genes escape X inactivation in the mouse as compared to humans; 15% of X-linked genes consistently escape X inactivation, and a further 10% escape in certain tissues or individuals in humans, whereas only 3.3% of X-linked genes escape X inactivation in the mouse.<sup>74–76</sup> Therefore, it has been assumed that the absence of the second X chromosome can be detrimental in humans, but less so in mice. However, it was recently proposed that human XO embryos may die due to a haploinsufficiency of placental gene expression.<sup>49</sup> If this hypothesis is correct, the early loss of XO oocytes may be a consequence of placental defects, and they may survive and become functional if they have been placed in a healthier environment.

Anomalies of XY females in humans are known as Swyer syndrome.<sup>2,77,78</sup> Fifteen to twenty percentages of XY sex reversal can be attributed to *SRY* mutations, including point mutations, frame-shifts and deletions.<sup>79–81</sup> In addition, XY females may develop as a consequence of gonadal dysgenesis, a failure in gonadal differentiation, because female phenotype is a default pathway. XY women are infertile due to the absence of oocytes although exceptional fertile cases have been reported.<sup>82</sup> Around 30% of all XY females and 60% of familial cases of XY sex-reversed individuals are reported to develop gonadoblastom.<sup>83–87</sup> Therefore, gonads are surgically removed in most cases of XY sex reversal when identified. Donor oocytes are the only option for these women in order to conceive babies.<sup>82,88</sup>

In the mouse, in addition to *Sry*, over ten genes have been identified to play critical roles in testicular differentiation; their functional deletion results in sex reversal in the XY gonad (see reviews<sup>7–9,89</sup>). However, complete sex reversal by autosomal gene mutations is rare since the XY ovary develops only when testicular components are absent or limited to a small proportion.<sup>90,91</sup> In other words, sex reversal must be nearly complete in order to develop XY ovaries. Therefore, XY female mouse models for studying the influence of the Y chromosome on female fertility are limited to those with impaired *SRY* functions. One exception is the XY female mouse, whose sex has been reversed by a conditional deletion of *SOX9* from gonadal somatic cells while the *Sry* gene remains intact.<sup>92</sup> The fertility of XY female mice depends on the cause of sex reversal as well as the genetic background (**Table 1**). When sex reversal is caused by the deletion of the Y chromosome region harbouring *Sry*, the resultant XY<sup>Tdym1</sup> female on a mixed genetic background has severely reduced fertility; nonetheless, it occasionally produces offspring.<sup>93</sup> Better fertility was reported for the XY<sup>Tdym1</sup> female on the outbred MF1 background after being delivered by XXY<sup>Tdym1</sup> females.<sup>94</sup> By contrast, when most copies of *Rbmy* repeats on the Y chromosome are deleted but *Sry* remains intact, *Sry* is repressed during gonadal differentiation and sex reversal ensues. On an MF1 background, these XY<sup>dl</sup> females are nearly as fertile as XO females.<sup>95</sup> This difference in female fertility has recently been attributed to the expression versus repression of the Y-linked *Zfy2* gene.<sup>96</sup> In XY females of the B6.Y<sup>TIR</sup> or B6.Y<sup>POS</sup> strain, the *Sry* gene is intact and expressed during gonadal differentiation and yet fails to initiate testicular differentiation.<sup>97–99</sup> These XY females are

**Table 1: Fertility of XY female mice**

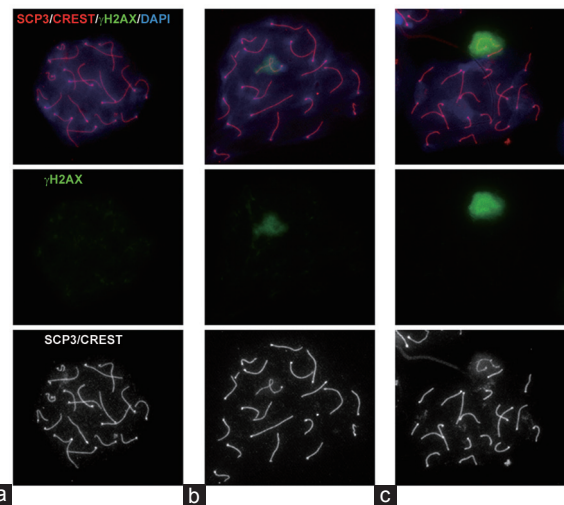
Genotype	X chromosome	Y chromosome	Sry	Fertility	Comments
XX	2	Absent	Absent	++	
XO	1	Absent	Absent	+	Shorter reproductive life
XO.Zfy	1	Zfy+	Absent	-	Block at the two-cell stage
XY <sup>Tdym1</sup>	1	Sry-	Absent	±	Occasional offspring
XY <sup>d</sup>	1	Rbmy-	Suppressed	+	Similar to XO females
XY <sup>Sox9</sup>	1	Intact	Expressed	±	Occasional offspring
B6.Y <sup>TIR</sup>	1	Intact	Expressed	-	Early oocyte loss Failure in the second meiotic division

sterile except for one litter at an early stage of backcross generation.<sup>97,100</sup> Similarly to XY<sup>Tdym1</sup> females, XY females with a conditional deletion of SOX9 are subfertile, occasionally producing offspring.<sup>92</sup> Thus, the physical presence of the Y chromosome does not prevent female fertility, but the configuration or expression of genes on the Y chromosome appears to critically influence female fertility.

### INFERTILITY OF THE B6.Y<sup>TIR</sup> FEMALE MOUSE

Our laboratory has been particularly interested in the infertility of XY oocytes in the B6.Y<sup>TIR</sup> female mouse since they carry intact X and Y chromosomes. Their male siblings that have developed testes are fertile. Therefore, this mouse model provides a unique opportunity for comparing spermatogenesis and oogenesis under identical chromosomal compositions. We have reported that more than half of B6.Y<sup>TIR</sup> gonads develop into ovaries, in which germ cells enter meiosis and go through the meiotic prophase.<sup>100–102</sup> The X and Y chromosomes do not pair in most XY oocytes during the first meiotic prophase, unlike in XY spermatocytes (Figure 2). Subsequently, a greater number of XY oocytes are eliminated by the end of meiotic prophase, compared to XX oocytes, which can be attributed to a surveillance mechanism of chromosome asynapsis (described later). Nonetheless, a considerable number of XY oocytes survive to reach the diplotene stage and form follicles. The XY ovary contains follicles at all stages at young ages, but rapidly loses its oocytes with age, and retains no, or very few, oocytes/follicles at 2 months after birth.<sup>100</sup> When XY fully-grown oocytes are subjected to meiotic resumption and maturation by *in vitro* culture, the unpaired X and Y chromosomes are segregated independently.<sup>103,104</sup> However, their distribution is not random; 70% of MII-oocytes retain single sex chromosomes, equally X or Y, 24% retain both X and Y, and the rest retain none. Regardless, very few MII-oocytes reach the two-cell stage or beyond after fertilization, thus diminishing the chance for reproduction.

The developmental incompetence of the oocytes from XY females can be attributed to their cytoplasmic defects; when the nuclei of XY oocytes have been transferred into enucleated XX oocytes, either at the GV- or MII-stage, the reconstructed oocytes generate healthy offspring after *in vitro* fertilisation and embryo transfer.<sup>105</sup> The offspring faithfully inherit the sex chromosomes identified in the MII-oocytes from XY females except for the death of YY and YO embryos.<sup>104,105</sup> It appears that sex chromosome aneuploidy *per se* does not block oogenesis or embryonic development. We compared gene expression profiles in fully-grown oocytes collected from XX, XO, and XY ovaries.<sup>106</sup> We



**Figure 2:** Pairing of sex chromosomes at the pachytene stage of meiotic prophase I. Microspread ovarian or testicular cells were immunostained with human autoantibody CREST (red), with specific antibodies against SCP3 (red) and  $\gamma$ H2AX (green), and counterstained with DAPI (blue). The merged image (top) is followed by  $\gamma$ H2AX signals alone (middle) and red signals of SCP3 and CREST alone (bottom). (a) A pachytene oocyte nucleus collected from an XX fetal ovary at 18.5 dpc. 20 sets of homologous chromosomes including the XX pair are fully synapsed. No  $\gamma$ H2AX signals are seen. (b) A pachytene oocyte nucleus collected from a B6.Y<sup>TIR</sup> fetal ovary at 18.5 dpc. The short Y chromosome is apart from the single X chromosome, the latter of which is covered by a  $\gamma$ H2AX domain, a characteristic of unsynapsed chromosome. Nineteen autosomal pairs are fully synapsed without  $\gamma$ H2AX signals. (c) A pachytene spermatocyte nucleus collected from a B6.Y<sup>TIR</sup> adult testis. The X and Y chromosomes are partially synapsed at their pseudoautosomal regions and covered by a  $\gamma$ H2AX domain, named XY or sex body. Nineteen autosomal pairs are fully synapsed.

found that all the genes tested showed comparable transcript levels between XX and XO oocytes, indicating that the storage of mRNAs is well-adjusted in these oocytes despite the difference in X chromosome numbers. In contrast, many X-linked and autosomal genes showed higher or lower transcript levels in XY oocytes, suggesting that mRNA storage is altered. Many of these differentially expressed genes are also included in the gene expression profile of those XO oocytes which carry a Y-linked Zfy2 transgene, despite their different genetic backgrounds.<sup>96</sup> mRNA-profile differences may contribute to the fertility of XO oocytes and the infertility of XY and XOZfy2 oocytes.

### SURVEILLANCE MECHANISM OF GERM CELLS

In attempts to produce functional gametes *ex vivo*, it is important to consider the presence of a surveillance mechanism by which a large population of gametes is eliminated under normal physiological conditions. A target of the surveillance mechanism can be a failure in chromosome synapsis, which would otherwise lead to aneuploidy, the major cause of embryonic loss. Chromosome asynapsis, irrespective of the underlying cause, is almost always associated with spermatogenic arrest at the mid-pachytene stage of the meiotic prophase or at the metaphase stage of the first meiotic division, resulting in subfertility or sterility in males (see reviews<sup>107,108</sup>). A “pachytene checkpoint” has been proposed as a mechanism to eliminate the meiocytes with synaptic defects in *Saccharomyces cerevisiae*,<sup>109</sup> and this hypothesis has been adapted to mammals.<sup>110,111</sup> However, whether an analogous checkpoint operates in mammalian gametogenesis remains to be verified. One major issue is the sex difference; various mutations that affect meiotic synapsis block spermatogenesis but not oogenesis during the first meiotic prophase (reviewed by Morelli and Cohen<sup>108</sup>). If the pachytene

checkpoint mechanism has been conserved from yeast to mammals, it is difficult to explain its inefficiency in the mammalian female germ-line.

An alternative hypothesis accommodates the sex difference; a surveillance mechanism may have evolved to cooperate with the presence of X and Y chromosomes in the spermatocyte, deviating from that in the oocyte. The mammalian X and Y chromosomes have poor homology except for the small pseudoautosomal region (PAR). Yet, their pairing and recombination is obligatory for successful spermatogenesis.<sup>112</sup> This is achieved by the formation of the XY or sex body to accommodate X and Y chromosomes and facilitate their pairing at their PARs. However, the remaining parts of the X and Y chromosomes become separated, showing all the characteristics of unsynapsed chromosomes, such as accumulations of ART, BRCA1, and phosphorylated histone variant H2AX ( $\gamma$ H2AX)<sup>113–117</sup> (Figure 2). A pachytene checkpoint, if it exists, would recognize the X-Y asynapsis and block spermatogenesis. Instead, both the X and Y chromosomes are subjected to transcriptional silencing, named meiotic sex chromosome inactivation (MSCI), and processed in the XY body differentially from the autosomal pairs.<sup>117–119</sup> Overall transcriptional activity is suppressed at the premeiotic S-phase and resumes in the autosomes prior to the pachytene stage in the normal gametogenesis. Therefore, the transcriptional repression of the X and Y chromosomes throughout the pachytene stage is distinct. Evidence has accumulated to prove that MSCI is obligatory for spermatogenesis, and also that interference with MSCI results in unwanted expression of X and Y-linked genes and subsequent loss of mid-pachytene spermatocytes.<sup>107,116</sup> Asynapsis of the autosomes, which occurs naturally or is caused by gene mutations, often interferes with X-Y pairing and MSCI, leading to spermatogenesis arrest.<sup>120–122</sup> It has recently been reported that misexpression of Y-linked *Zfy1/Zfy2* is sufficient to arrest pachytene spermatocytes.<sup>116</sup>

The normal oocyte carries two X chromosomes, which pair efficiently like autosomal pairs and do not need MSCI. Nonetheless, the majority (70%) of oocytes is eliminated during the first meiotic prophase in normal ovarian development.<sup>21,73,123</sup> The cause or mechanism of this major oocyte loss is not well understood. However, evidence is accumulating to support the presence of a surveillance mechanism during this period. First, apoptosis, a common form of “programmed cell death,” plays a major role in oocyte elimination; a deficiency in caspase 9, a key player in the mitochondrial apoptotic pathway, prevents oocyte loss during the first meiotic prophase.<sup>124</sup> Remarkably, caspase 9 and its upstream apoptotic pathway are constitutively activated in all oocytes; therefore, oocytes must be protected from apoptotic execution until they are destined for such a fate<sup>124</sup> (Chung and Taketo, unpublished results). Second, when the pairing partner for the single X chromosome is absent, such as in XO and XY oocytes, greater numbers of oocytes are eliminated by the end of the first meiotic prophase as compared to XX oocytes.<sup>73,101,125</sup> The pachytene checkpoint hypothesis cannot explain this oocyte loss since sufficient numbers of XO and XY oocytes survive through the first meiotic prophase to produce mature oocytes and make XO females fertile, although XY females encounter other infertility problems.

An alternative hypothesis, meiotic silencing of unsynapsed chromatin (MSUC), shares many aspects of molecular mechanisms with MSCI in the spermatocyte but acts less stringently.<sup>113,117,119</sup> It has been proposed that the single X chromosome is subjected to MSUC in XO oocytes, resulting in their elimination, but it occasionally synapses within self, or with the autosomes, and escapes from the MSUC response, resulting in oocyte survival.<sup>114,119,126</sup> However, the low frequency of X chromosome self-synapsis does not explain the number of XO oocytes that survive. Moreover, we found that the consequence of MSUC,

based on the localization of X-encoded ATRX, is heterogeneous in XY oocytes.<sup>127</sup> We hypothesize that the MSUC response in oocytes is variable because the first meiotic prophase, particularly at the pachytene stage, is very short in oocytes compared to spermatocytes (2 vs 7 days), leaving the interval between transcriptional activation and the MSUC response too short to give a consistent result. Consequently, oocyte survival may depend on the levels and repertoire of essential gene products. These studies are limited to sex chromosomes while normal oocytes may have a synaptic failure in any chromosomes.<sup>127</sup> Whether the MSUC hypothesis is applicable to autosomal asynapsis in oocytes remains to be evaluated.

## CONCLUDING REMARKS

It is possible to obtain XY oocytes carrying intact X and Y chromosomes as a consequence of sex reversal in the mouse. However, such a mouse is available only with a Y chromosome of a specific origin on a certain genetic background. Hence, functionality of XY oocytes has not yet been vigorously examined due to the limited availability of sex-reversed mouse models. It must be noted that sex reversal results in functional gametes in some rodent and nonmammalian invertebrate species.<sup>128–130</sup> It is important to know whether or not mammals have evolved mechanisms to prevent the reproduction of sex-reversed individuals. Furthermore, the XY oocyte becomes infertile or subfertile largely due to the expression of Y-linked gene (s) leading to ooplasmic defects in a mouse model. Such defects can be prevented by genetic or nongenetic manipulation of oocytes during culture. An understanding of the role of sex chromosomes in germ cell differentiation and functions will be promoted by deriving XY oocytes from XY ESCs on more diverse genetic backgrounds.

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## COMPETING INTERESTS

The authors declare that they have no competing interests.

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