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Germ cell-specific disruption of the *Meig1* gene causes impaired spermiogenesis in mice

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Summary

Meiosis expressed gene 1 (*Meig1*) was originally identified in a search for mammalian genes potentially involved in meiosis. Seven mouse *Meig1* transcripts with the same coding region, but different 5′-UTRs, have been identified. These transcripts have different tissue distributions, two are only present in the testis. In the testis, *Meig1* is present in germ cells and Sertoli cells. A *Meig1* conditional knockout model has been generated. When *Meig1* was inactivated globally by crossing with *Cmv-Cre* transgenic mice, the *Meig1*-deficient males were sterile due to severe spermiogenic defects, and had no obvious defects in meiosis. To further study its role in individual cell types in the testis, the *Meig1*^{flox} mice were crossed with *Hsp2a-Cre*, *Prm-Cre*, and *Amh-Cre* mice, in which the Cre recombinase is driven by the heat shock protein 2 (*Hsp2a*) gene promoter (expressed in spermatocytes), the protamine 1 gene promoter (expressed in post-meiotic spermatids) and the anti-Mullerian hormone (*Amh*) gene promoter (expressed in Sertoli cells) respectively. Both *Meig1* mRNA and protein were undetectable in testis of the *Hsp2a-Cre; Meig1*$^{flox/flox}$ mice and all the mutant adult males tested were sterile. This phenotype mirrors that of the *Cmv-Cre; Meig1*$^{flox/flox}$ mice. Even though the total testicular *Meig1* mRNA and protein expression levels were dramatically reduced in testis of the *Prm-Cre; Meig1*$^{flox/flox}$ males, all the mice tested were fertile, and there was no significant difference in sperm count and sperm motility compared with age-matched *Meig1*$^{flox/flox}$ male mice. Disruption of *Meig1* in the Sertoli cells did not affect the MEIG1 protein expression. *Amh-Cre; Meig1*$^{flox/flox}$ males were fertile, and produced the same amount of spermatozoa as age-matched *Meig1*$^{flox/flox}$ mice. The testicular histology was also normal. Our results indicate that MEIG1 regulates spermiogenesis through effects in germ

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cells alone, and that the *Meig1* gene must be active during a discrete period in spermatogenesis after which it is dispensable.

Keywords

germ cells; -Meig1; mouse; spermiogenesis; testis

Introduction

Mouse meiosis expressed gene 1 (*Meig1*) was originally identified in a search for mammalian genes involved in meiotic processes (Don & Wolgemuth, 1992). Two murine *Meig1* transcripts, 11a2 and 2a2, were identified previously (11a2 and 2a2 are ID number of the clones identified, 11a2 also called *Meig1_v1*, and *Meig1*-002 in Ensembl database; 2a2 also called *Meig1_v2*, and *Meig1*-001 in Ensembl database); both contain three exons. The two isoforms share the same open reading frame and 3′-UTR, but differ in their 5′-UTRs. Each has a non-translated exon 1. *Meig1_v1* message was found to be expressed specifically in somatic cells in the testis. However, the predominant *Meig1_v2* isoform was found to be germ cell-specific. The *Meig1_v2* transcript begins to accumulate in testis at days 8–9 of post-natal (pn) development, coinciding with the entry of germ cells into meiosis, and is expressed most abundantly at pn day 14, and at subsequent stages, when spermatocytes enter the pachytene stage. In situ hybridization analysis showed that the *Meig1* expression level was low in leptotene cells, and increased as the cells progressed through zygotene and pachytene stages. In addition, *Meig1* message was also detected in embryonic ovary after day 15 of gestation when the cells entered the pachytene stage of meiosis 1, but not in adult ovary, suggesting that *Meig1* is a meiosis-associated gene (Don *et al.*, 1994; Chen-Moses *et al.*, 1997; Steiner *et al.*, 1999). Recent transcriptional profile studies revealed that *Meig1* message is also present in Sertoli cells in foetal gonads, and a Sertoli cell line TTE3 (Tabuchi *et al.*, 2006; Bouma *et al.*, 2007).

Although the molecular weight of MEIG1 is estimated to be 10 kDa based on its amino acid composition, the protein migrates as a 14 kDa band in Western blots, probably because of its basic isoelectric point. However, MEIG1 protein contains multiple consensus sequences for serine and threonine phosphorylation. Therefore, post-translational modifications might also affect MEIG1 mobility in the electrophoretic separation. There is evidence that MEIG1 protein is phosphorylated and can form a dimer in vivo. Furthermore, it has been suggested that the phosphorylated dimer enters the nucleus during the first meiotic prophase and binds to meiotic chromatin (Ever *et al.*, 1999).

Several studies suggested that MEIG1 is essential for spermatogenesis, and is related to ciliary function. *Meig1* message is dramatically reduced in heat shock transcription factor 2 (*Hsf2*) mutant mice, and this may result in impaired spermatogenesis and reduced fertility in *Hsf2* mutant mice (Wang *et al.*, 2003). Bioinformatic analysis revealed that *Meig1* is most abundantly expressed in tissues rich in highly ciliated cells, such as, testis, lung and olfactory sensory neurons, and is therefore predicted to be important for cilia (McClintock *et al.*, 2008). Our previous study showed that MEIG1 is associated with WD repeats of

SPAG16 protein (Zhang *et al.*, 2004), and *Spag16* mutant mice lacking the WD repeat region have a severe spermatogenesis defect due to haploinsufficiency, suggesting that MEIG1 might function in the same complex as SPAG16 protein.

To study the function of mouse MEIG1 protein, we further characterized this gene. Instead of two, seven mouse *Meig1* transcripts have been identified in our laboratory, all are translated into the same protein (Zhang *et al.*, 2009). These seven transcripts have different tissue distributions. As noted above, two are only present in the testis (Zhang *et al.*, 2009). Even though no known functional domains have been identified, MEIG1 protein sequences were found to be highly conserved among different species (Salzberg *et al.*, 2010).

To further study the role of the *Meig1* gene, a *Meig1* conditional knockout model has been generated in our laboratory. The Meig1 gene was inactivated globally by crossing the conditional knockout mice with *Cmv-Cre* transgenic mice. The global *Meig1* knockout mice revealed that homozygous mutant mice are viable, but the males are sterile, producing only a few spermatozoa that are morphologically abnormal. Unexpectedly, the *Meig1* mutant male mice had no obvious defect in meiosis, as was originally predicted from earlier work by Don and colleagues (Don & Wolgemuth, 1992; Don *et al.*, 1994; Chen-Moses *et al.*, 1997; Steiner *et al.*, 1999). The mice were sterile as a result of impaired spermatogenesis at the stage of elongation and condensation (Zhang *et al.*, 2009). The manchette was dramatically disrupted in the *Meig1*-deficient spermatids when viewed by transmission electron microscopy. The male infertility phenotype was later confirmed by a conventional *Meig1* knockout model generated recently by the Don laboratory (Salzberg *et al.*, 2010).

To investigate MEIG1's role in germ cells and Sertoli cells, the $Meig1^{flox/flox}$ mice were crossed with transgenic mice that express Cre recombinase specifically in these individual cells, and the phenotypes were analysed. Our results demonstrate that MEIG1 plays an essential role in germ cells, but not in Sertoli cells.

Materials and Methods

Generation of cell type-specific *Meig1* mutant mice

Amh-Cre (Stock Number: 007915), *Hsp2a-Cre* (Stock Number: 008870), and *Prm-Cre* (Stock Number: 003328) mice were purchased from Jackson laboratory (Bar Harbor, ME, USA). These transgenic mice were crossed with $Meig1^{flox/flox}$ mice, and the resulting *Cre;* $Meig1^{flox/+}$mice were backcrossed with $Meig1^{flox/flox}$ mice to create the *Cre;* $Meig1^{flox/flox}$ mice. The detailed breeding strategy used to create conditional *Meig1* mutant mice is shown in Supplemental Figures 1, 3 and 5.

Genotyping and determination of tissue/cell type-specific disruption of the *Meig1* gene

Genotypes of floxed *Meig1* mice were determined by PCR using DNA isolated from tail slips following the same strategy as described previously (Zhang *et al.*, 2009). PCR was also conducted to identify *Cre*-positive mice using a primer set that can amplify the Cre recombinase gene with the following primers: forward primer: 5′-CAGTTCGATTACGATCAG-3′, and reverse primer: 5′-GACTTAGGCTATCGTAC-3′.

To determine tissue-specific deletion of the floxed *Meig1* region, DNA was isolated from the indicated tissues of the *Cre-Meig1*$^{flox/flox}$ mice, and PCR was conducted using the primer set that flanks the floxed region. Testicular germ cells and somatic cells were also collected from adult *Cre-Meig1*$^{flox/flox}$ mice, and DNA was isolated for PCR to determine cell type-specific deletion of floxed *Meig1* region. Briefly, mixed testicular cells were prepared from adult *Cre-Meig1*$^{flox/flox}$ mice by enzymatic dissociation with collagenase (1 mg/mL) at 34 °C for 12 min, followed by digestion with trypsin (0.5 mg/mL) for 10 min at 34 °C. The cells were washed with DMEM, and suspended with DMEM (10% FBS), and cultured at 34 °C. Six hours after plating, somatic cells stick to the culture plates, and germ cells are suspended in the medium, thus the two populations were separated (Chang *et al.*, 2011). Beside Sertoli cells, the somatic cell preparation also contains a small number of Leydig cells and peritubular cells.

Northern blot analysis

Total RNA was isolated from mouse testes with Trizol (Life Technologies, Inc., Grand Island, NY, USA). A quantity of 30 μg of these RNAs was separated on a 1% denaturing agarose gel. The RNA was subsequently transferred to a Hybond N+ nylon membrane. The blots were hybridized with the ^{32}P-α-dCTP labelled *Meig1* probe generated previously (Zhang *et al.*, 2009). After hybridization, the membranes were exposed to X-ray films.

Western blot analysis

Equal amounts of protein (50 μg/lane) were heated to 95 °C for 10 min in 4 × sample buffer, loaded onto 10% sodium dodecyl sulphate-polyacrylamide gels, electrophoretically separated and transferred to polyvinylidene difluoride membranes. The membranes were blocked, and then incubated with indicated antibodies overnight at 4 °C. After being washed, the blots were incubated with an anti-rabbit, or anti-mouse immunoglobulin conjugated to horseradish peroxidase for 1 h at room temperature. The proteins were detected with Super Signal Pico Chemiluminescent system (Pierce, Rockford, IL, USA).

Assessment of fertility and fecundity

To assess fertility and fecundity, littermate males (3–4 months old) of different genotypes were each placed in cages with two mature wild-type females for at least 2 months. The number of mice achieving a pregnancy and the number of offspring from each mating set or pregnancy were recorded.

Sperm motility assays

Spermatozoa were collected after swimming out from the caudae epididymides into modified Whitten's medium (15 mM Hepes-sodium salt, 1.2 mM $MgCl_2$, 100 mM NaCl, 4.7 mM KCl, 1 mM pyruvic acid and 4.8 mM lactic acid) for 10 min at 37 °C. The solution had a final pH of 7.35. Spermatozoa were counted and diluted to 2×10^6 spermatozoa/mL, and motility was analysed in a 100-μm chamber (Cell Vision-USA, Delvavan, WI) using the IVOS Sperm Analyzer (Hamilton-Thorne Research, Beverly, MA, USA). The parameters that were used for the analysis setup are described in the technical guide for the IVOS system, version 12.2 (mouse setup 1). Sperm populations were analysed as soon as possible

after their release from the epididymides. For each sperm sample, a total of 10 fields were analysed (Zhang *et al.*, 2006).

Histology, immunochemistry and transmission electron microscopy

Testes, epididymides and cauda epididymal spermatozoa were prepared for light and transmission electron microscopy using standard methods, as previously described. The same mice that were used to assess fertility were used for histological analysis of the testes (Zhang *et al.*, 2002).

Ethics

Procedures involving animals were conducted under the approval of the Institutional Animal Care and Use Committee of the Virginia Commonwealth University in accordance with the Guide for Care and Use of Laboratory Animals (Protocol #AM10297).

Results

Disruption of the *Meig1* gene at the stage of meiosis in germ cells results in a severe spermatogenesis defect

In the testis, MEIG1 is predominantly expressed in the germ cells. To investigate the role of MEIG1 in germ cells, *Meig1*$^{flox/flox}$ males were crossed with *Hsp2a-Cre* females so that *Meig1* is disrupted in germ cells at the stage of meiosis (Supplemental Figure 1).

Mice were genotyped by PCR and tested to determine if they carried the *Hsp2a-Cre* transgene (Supplemental Figure 2A). To evaluate tissue-specific deletion of the *Meig1* gene, genomic DNA was isolated from the testis, heart and liver from *Hsp2a-Cre; Meig1*$^{flox/flox}$ mice. PCR was conducted using the primer set flanking the floxed region. The deletion band was observed only in the testis, but not in the heart and liver (Supplemental Figure 2). Testis somatic cells and germ cells were separated, and DNA was isolated from them. PCR results indicated that the deletion band was present only in the germ cells, not in the somatic cells (Supplemental Figure 2).

Northern blot and Western blot results revealed that testicular *Meig1* mRNA and protein were undetectable in the *Hsp2a-Cre; Meig1*$^{flox/flox}$ mice (Fig. 1). Even though all the *Hsp2a-Cre; Meig1*$^{flox/flox}$ mice were viable and showed no gross abnormalities, and the testis weight was also comparable to age-matched *Meig1*$^{flox/flox}$ mice, all of the males tested were infertile, and the epididymidal sperm number was significantly lower than that in the *Meig1*$^{flox/flox}$ mice (Table 1).

Microscopy revealed that spermatogenesis is arrested in the *Hsp2a-Cre; Meig1*$^{flox/flox}$ mice. Most spermatids failed to develop to the elongating stage [Fig. 2(A)]. Epididymides from *Hsp2a-Cre; Meig1*$^{flox/flox}$ mice only contained debris and degenerating spermatozoa [Fig. 2(B)]. The remaining epididymidal spermatozoa showed no motility as examined by visual inspection with a light microscope, and most spermatozoa had round or detached heads [Fig. 2(C)].

Transmission electron microscopy demonstrated that spermatids in wild-type mice undergo normal developmental processes, including flagellum formation with '9 + 2' axonemes and chromatin condensation associated with normal manchette structure [Fig. 3(A)]. However, in the *Hsp2a-Cre; Meig1*$^{flox/flox}$ mice, very few flagella were formed, and the sperm axonemes and accessory structures were disorganized [Fig. 3(B)]. In particular, manchette structures in spermatids were either disrupted or absent [Fig. 3(B)a–b], spermatid tails were not formed normally [Fig. 3(B)c] and the redundant nuclear envelope appeared to be rather enlarged [Fig. 3(B)d].

Disruption of the *Meig1* gene post-meiotically does not affect spermatogenesis and sperm motility

Germ cells undergo dramatic morphological changes after meiosis. To determine if disruption of the *Meig1* gene post-meiotically affects spermatogenesis, the *Meig1*$^{flox/flox}$ mice were crossed with *Prm-Cre* mice to create *Prm-Cre; Meig1*$^{flox/flox}$ mice (Supplemental Figure 3).

The mice were genotyped following the same strategy as for the *Hsp2a-Cre; Meig1*$^{flox/flox}$ mice (Supplemental Figure 4A), and similar to the *Hsp2a-Cre; Meig1*$^{flox/flox}$ mice, the deletion band was observed only in the testis germ cells, but not in other somatic tissues, including the heart, liver, lung and testis somatic cells (Supplemental Figure 4B).

Testicular *Meig1* mRNA and protein expression levels were significantly reduced in the *Prm-Cre; Meig1*$^{flox/flox}$ mice (Fig. 4). However, all the males tested were fertile and sired normal numbers of offspring. Epididymidal sperm counts were normal (Table 2). Testis and epididymides were indistinguishable under light microscope observation compared with the *Prm-Cre; Meig1*$^{flox/flox}$ and *Meig1*$^{flox/flox}$ mice. Both showed normal histology (Fig. 5A,B). Sperm morphology was normal [Fig. 5(C)], and there was no reduction in sperm motility in the *Prm-Cre; Meig1*$^{flox/flox}$ mice [Fig. 5(D)].

Disruption of the *Meig1* gene in Sertoli cells does not affect spermatogenesis

Besides germ cells, *Meig1* is expressed in the Sertoli cells in the testis. To investigate whether MEIG1 also regulates spermatogenesis through Sertoli cells, *Amh-Cre* mice were crossed with the *Meig1*$^{flox/flox}$ mice so that *Meig1* was disrupted only in Sertoli cells (Supplemental Figure 5).

Amh-Cre; Meig1$^{flox/flox}$ mice were identified using the same strategy as described in identifying the *Hsp2a-Cre; Meig1*$^{flox/flox}$ and *Prm-Cre; Meig1*$^{flox/flox}$ mice (Supplemental Figure 6A). The deletion band was observed only in the total testis and testis somatic cells, but not in testis germ cells and other somatic tissue, including the heart (Supplemental Figure 6).

Northern blot and Western blot analysis showed that there were no differences in *Meig1* mRNA and protein expression between the *Amh-Cre; Meig1*$^{flox/flox}$ mice and the *Meig1*$^{flox/flox}$ mice (Fig. 6).

Like the *Hsp2a-Cre; Meig1*$^{flox/flox}$ mice, all the *Amh-Cre; Meig1*$^{flox/flox}$ mice were viable and showed no gross abnormalities. The testis weight was also comparable to age-matched *Meig1*$^{flox/flox}$ mice. All the males tested were fertile, and sired normal number of offspring, and these mice had normal number of spermatozoa collected from epididymides (Table 3).

Amh-Cre; Meig1$^{flox/flox}$ males showed normal testis architecture. All stages of spermatogenesis were present [Fig. 7(A)]. Epididymidal morphology from *Amh-Cre*, *Meig1*$^{flox/flox}$ and *Meig1*$^{flox/flox}$ mice was also comparable. Mature spermatozoa filled the whole cavity [Fig. 7(B)]. The epididymidal spermatozoa showed normal morphology under light microscopy [Fig. 7(C)], and normal motility when estimated by visual inspection with a light microscope.

Discussion

Our previous studies and others demonstrated that MEIG1 plays an essential role in spermiogenesis. The mouse *Meig1* gene encodes at least seven isoforms that are present in multiple tissues (Zhang *et al.*, 2009). Some isoforms are expressed only in the testis (Zhang *et al.*, 2009). The testis contains both germ cells and somatic cells, and the somatic cells also play a role in regulating spermatogenesis (Ge *et al.*, 2008; Johnson *et al.*, 2008; Cool & Capel, 2009). To determine the roles of MEIG1 in germ cells and testis somatic cells, *Meig1*$^{flox/flox}$ mice were crossed with the *Hsp2a-Cre* mice to disrupt the *Meig1* gene only in male germ cells. The *Hsp2a*-Cre directs expression of Cre recombinase under the control of the mouse *Hsp2a* (heat shock protein 2 [Hsp70-2]) promoter (Dix *et al.*, 1996). *Hsp2a-Cre* sequences of the transgene are flanked by *Acrv1* (acrosomal vesicle protein 1 [SP-10]) insulator elements that are believed to tether the *Acrv1* gene to the nuclear matrix in somatic cells to prevent transcription (Reddi *et al.*, 2003; Abhyankar *et al.*, 2007). Our initial immunochemistry experiments also confirmed that the Cre recombinase was expressed in the spermatocytes. When these transgenic mice are bred with mice containing a *loxP*-flanked sequence of interest, Cre-mediated recombination is expected to result in deletion of the floxed sequences in leptotene/zygotene spermatocytes of the male offspring. Thus, the *Hsp2a-Cre* mice are useful for generating conditional mutations for studying mammalian spermatocyte development, gametogenesis and meiosis (Inselman *et al.*, 2010).

Similar to the *Cmv-Cre; Meig1*$^{flox/flox}$ mice, all *Hsp2a-Cre; Meig1*$^{flox/flox}$ males tested were infertile, and severe spermiogenesis defects occurred in these mice. Sperm number was significantly reduced, and the epididymides from these mice only contained debris and degenerating spermatozoa. The manchette structure was disrupted or absent, the '9 + 2' axonemes were not present in the remaining flagella, and the redundant nuclear envelope was enlarged. It has been reported that expression of *Hsp2a-Cre* may be 'leaky' and recombination of *loxP*-flanked sequences can occur in some cells in somatic tissues (Inselman *et al.*, 2010). Our PCR using DNA isolated from tail slips and multiple somatic tissues indicates that the *loxP*-flanked sequence remained intact in these tissues, distinguishing them from the *Cmv-Cre; Meig1*$^{flox/flox}$ mice, in which the *loxP*-flanked sequence was deleted globally (Schwenk *et al.*, 1995). PCR using DNA isolated from testicular somatic cells and germ cells demonstrated that the floxed region was deleted in germ cells. A trace amount of intact floxed region was also amplified in the germ cells of the

Hsp2a-Cre; Meig1$^{flox/flox}$ mice. This may be due to early-stage germ cells when the Cre recombinase is not active. These early-stage germ cells may not transcribe sufficient *Meig1* mRNA and translate enough MEIG1 protein, which explains why *Meig1* mRNA and protein were not detected in the *Hsp2a-Cre; Meig1*$^{flox/flox}$ mice.

The *Prm-Cre* mice have a transgene comprising the mouse protamine 1 promoter and Cre recombinase coding sequence, which mediates the efficient recombination of a *cre* target transgene in the male germ line, but not in other tissues (O'Gorman *et al.*, 1997). Compared with the *Hsp2a-Cre* mice, the Cre recombinase is expressed at a later stage in the *Prm-Cre* mice, which was confirmed in initial immunochemistry experiment using specific antibody against Cre. As the *Meig1*-deficient mice showed a phenotype post-meiotically, we wished to know if disruption of *Meig1* after meiosis also gives rise to spermatogenesis defects by crossing the *Meig1*$^{flox/flox}$ mice with the *Prm-Cre* mice. Even though expression of both *Meig1* mRNA and protein was significantly reduced, the *Prm-Cre; Meig1*$^{flox/flox}$ males were fertile, and testis histology, sperm number, morphology and motility were also normal. This suggests that MEIG1 protein translated during the meiosis stage is sufficient for the spermatids to support normal spermatogenesis.

A major challenge in conditional gene targeting is that Cre expression can sometimes be ‘leaky’, even though its expression is driven by tissue/cell-specific promoters. The reduction in *Meig1* mRNA and protein that we observed in the testis of *Meig1*$^{flox/flox}$*; Prm1-Cre* males might be explained by the fact that some Cre protein was expressed at an earlier stage than expected from the known pattern of *Prm1* promoter activity. This unscheduled Cre expression could have partially disrupted the *Meig1* gene. If that is the case, the residual *Meig1* that was expressed was clearly sufficient to support normal spermiogenesis. An alternative explanation is that the reduction in *Meig1* mRNA and protein is the result of turnover of these molecules, and the failure to replace them in the later stages of spermiogenesis due to disruption of *Meig1* expression in the *Meig1*$^{flox/flox}$*; Prm1-Cre* testis.

Besides the germs cells, somatic Sertoli cells are another major population in the seminiferous tubules (Griswold, 1998). These cells have been proposed to maintain the integrity and compartmentalization of the seminiferous epithelium, secrete fluid to form a tubular lumen, participate in spermiation, and phagocytosis (Fisher, 2002; Kawasaki *et al.*, 2002; Vernet *et al.*, 2008; Lie *et al.*, 2009), and these functions are essential for normal spermatogenesis. Even though *Meig1* mRNA and protein were underdetectable in the testis of *Hsp2a-Cre; Meig1*$^{flox/flox}$ by Northern blot and Western blot analyses, PCR revealed that intact *Meig1* is present in the somatic cells of *Hsp2a-Cre; Meig1*$^{flox/flox}$ (Supplemental Figure 2). As *Meig1* is present in the Sertoli cells, we wished to know if MEIG1 also regulates spermatogenesis through a role on this cell type. *Amh-Cre* mice were crossed with the *Meig1*$^{flox/flox}$ mice so that *Meig1* was disrupted only in the Sertoli cells. The *Amh-Cre* transgenic mice express Cre recombinase directed by the Sertoli cell-specific promoter elements of the anti-Mullerian hormone (*Amh*) gene (Holdcraft & Braun, 2004). When *Amh-Cre* transgenic males are bred with female mice containing a *loxP*-flanked sequence of interest, Cre-mediated recombination will result in deletion of the flanked sequence specifically in Sertoli cells. These *Amh-Cre* transgenic mice have been useful in generating conditional knockouts in Sertoli cells for studying several genes that play roles in male

embryonic sexual differentiation and the regulation of spermatogenesis (Elliott *et al.*, 2010; Meng *et al.*, 2011). PCR results indicated that the floxed region of *Meig1* gene was deleted in somatic cells in the testis, but not in the germ cells. Given that the testicular somatic cell preparation also contains small numbers of Leydig cells and peritubular cells, and the *Meig1* gene in these cells remains intact in the *Amh-Cre; Meig1*$^{flox/flox}$ mice, a trace amount of *Meig1* expression can be expected (Supplemental Figure 6). Amh-Cre; Meig1$^{flox/flox}$ males showed normal fertility, produced normal number of spermatozoa and had normal testicular histology. These findings suggest that MEIG1 does not play a fundamental role in spermatogenesis in the Sertoli cells.

In conclusion, our results demonstrate that MEIG1 regulates spermiogenesis through an action in germ cells, not in Sertoli cells, and that the Meig1 gene must be active during a discrete period in spermatogenesis after which it is dispensable.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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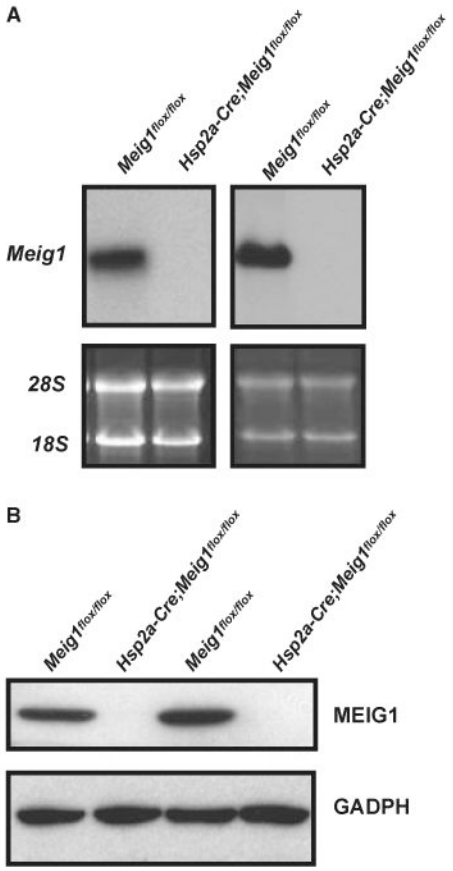

Figure 1.

Expression of *Meig1* mRNA and protein is underdetectable in the testis of the *Hsp2-Cre; Meig1*$^{flox/flox}$ mice. Total testicular RNA and protein were extracted from adult *Meig1*$^{flox/flox}$ and *Hsp2a-Cre; Meig1*$^{flox/flox}$ mice. Northern blot (A) was conducted using a full-length *Meig1* cDNA as the probe; Western blot (B) was performed using an anti-MEIG1 antibody. Notice that there were no detectable *Meig1* mRNA and protein in the testis of *Hsp2a-Cre; Meig1*$^{flox/flox}$ mice.

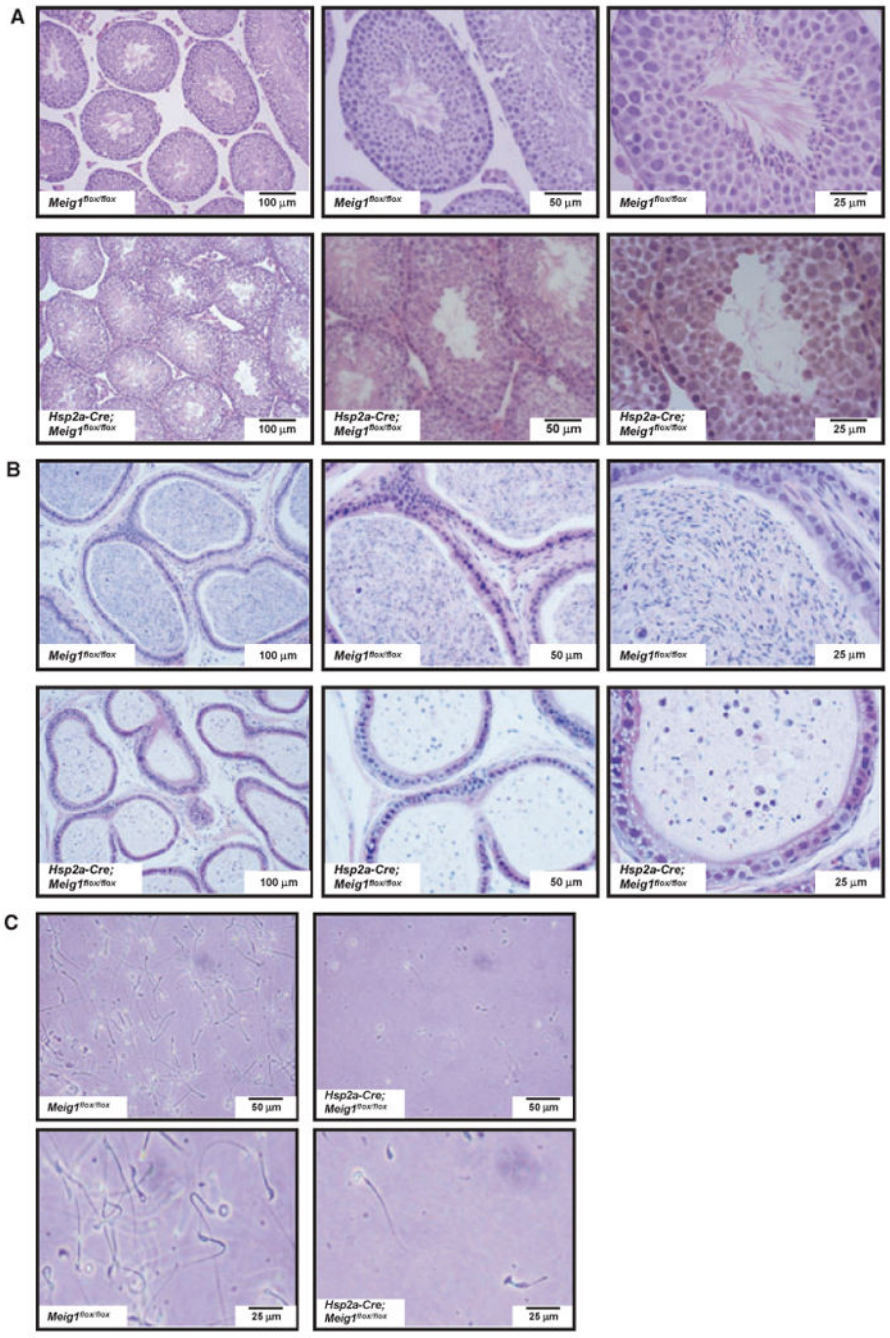

Figure 2.

Disruption of *Meig1* gene in the germ cells during meiosis stage results in severe spermatogenesis defect. (A) Representative testicular histology from a *Meig1*$^{flox/flox}$ mouse and a *Hsp2a-Cre; Meig1*$^{flox/flox}$ mouse. The *Meig1*$^{flox/flox}$ animal reveals normal architecture of the seminiferous tubules and interstitial tissue, but spermatogenesis is arrested in the *Hsp2a-Cre; Meig1*$^{flox/flox}$ mouse. (B) Representative epididymides histology from a *Meig1*$^{flox/flox}$ mouse and a *Hsp2a-Cre; Meig1*$^{flox/flox}$ mouse. The lumen is filled with mature spermatozoa in the *Meig1*$^{flox/flox}$ animal, but only cell debris can be seen in the *Hsp2a-Cre;*

Meig1$^{flox/flox}$ animal. (C) Morphology of epididymal spermatozoa collected from a *Meig1*$^{flox/flox}$ mouse and a *Hsp2a-Cre; Meig1*$^{flox/flox}$ mouse. All spermatozoa in the *Meig1*$^{flox/flox}$ animal show normal morphology. However, very few spermatozoa can be observed in the *Hsp2a-Cre; Meig1*$^{flox/flox}$ animal, and most collected spermatozoa had round or detached heads.

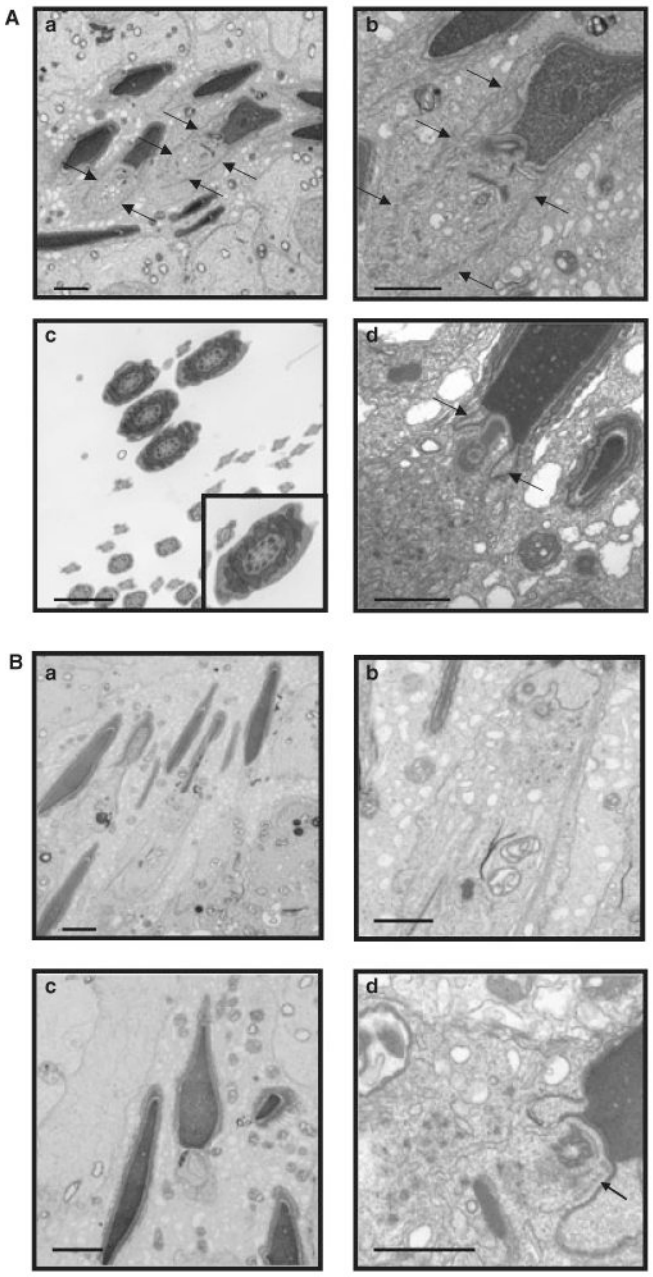

Figure 3.

Testicular ultrastructure in adult *Meig1*$^{flox/flox}$ and *Hsp2a-Cre; Meig1*$^{flox/flox}$ mice. (A) Representative transmission electronic microscopy images from adult *Meig1*$^{flox/flox}$ mice. (a) Condensing spermatids with normal manchette structure (arrows) in *Meig1*$^{flox/flox}$ testes. (b) Higher magnification of a condensing spermatid in (a) with normal manchette structure (arrows). (c) Tail cross-sections with normal '9 + 2' axonemes. (d) Normal condensing spermatid head and redundant nuclear envelope (arrows). Bars = 2 microns. (B) Representative transmission electronic microscopy images from adult *Hsp2a-Cre; Meig1*$^{flox/flox}$ mice. (a) Condensing spermatids with missing or disorganized manchettes. (b) Higher magnification of a condensing spermatid in (a) with abnormal manchette structure.

(c) Spermatid with abnormal tail formation (centre). (d) Spermatid with enlarged redundant nuclear envelope (arrow). Bars = 2 microns.

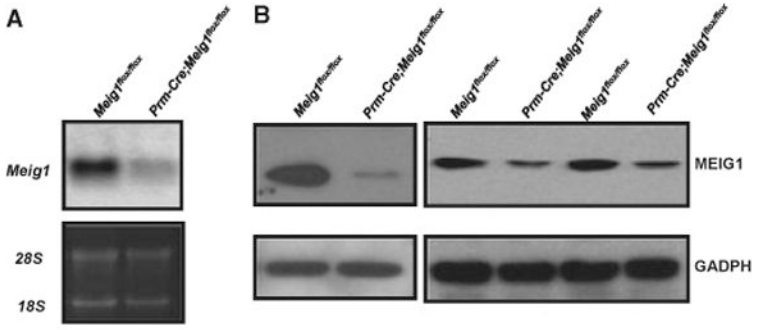

Figure 4.

Expression of *Meig1* mRNA and protein is significantly reduced in the testis of the *Prm-Cre; Meig1*$^{flox/flox}$ mice. Total testicular RNA and protein were extracted from adult *Meig1*$^{flox/flox}$ and *Prm-Cre; Meig1*$^{flox/flox}$ mice. Northern blot (A) was conducted using a full-length *Meig1* cDNA as the probe; Western blot (B) was performed using an anti-MEIG1 antibody. Notice that both *Meig1* mRNA and protein expression levels in the *Prm-Cre; Meig1*$^{flox/flox}$ mice are significantly lower than that in the control *Meig1*$^{flox/flox}$ mice.

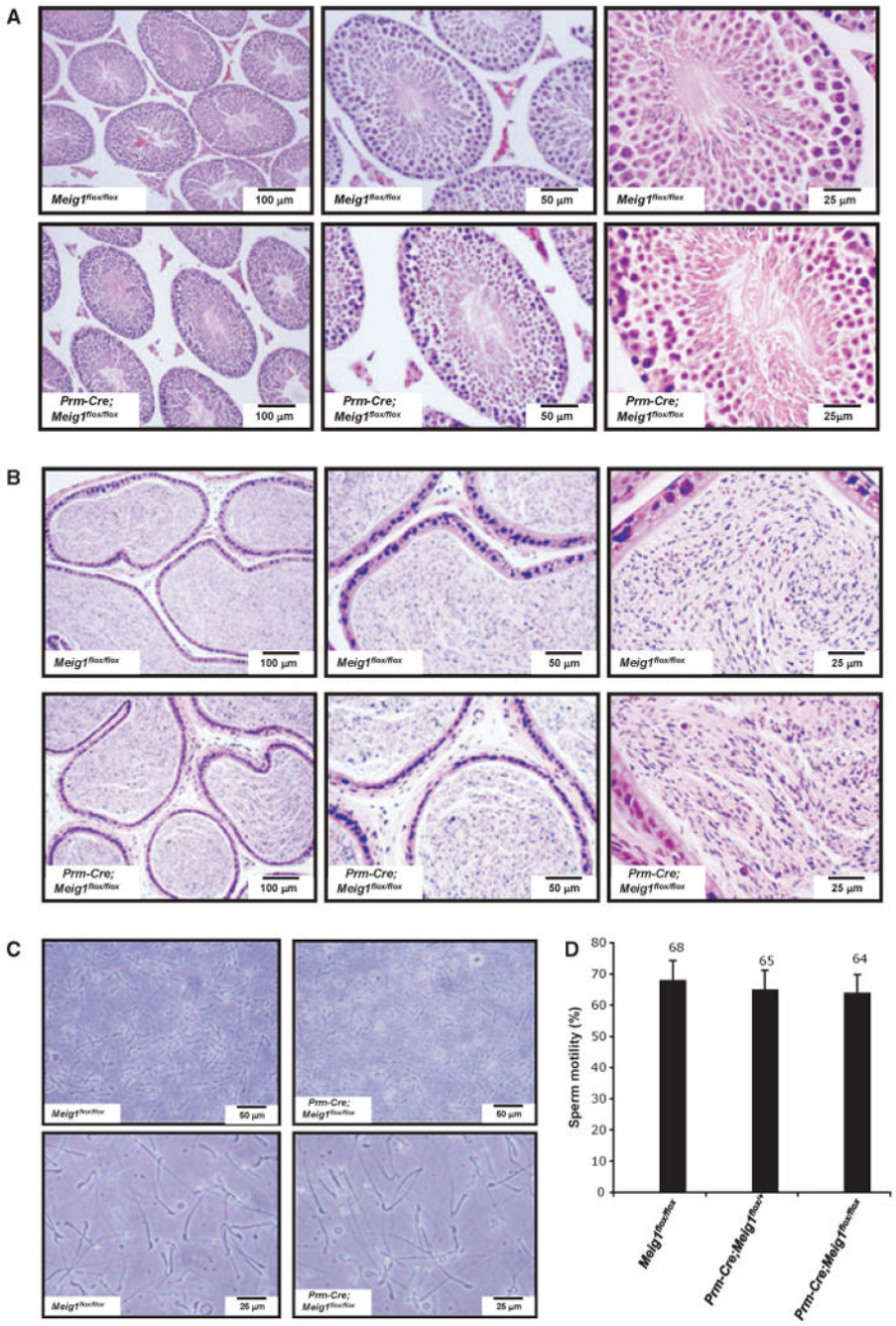

Figure 5.

Disruption of *Meig1* gene in the germ cells post-meiotically does not result in spermatogenesis defect. (A) Representative testicular histology from a *Meig1*$^{flox/flox}$ mouse and a *Prm-Cre; Meig1*$^{flox/flox}$ mouse. Both animals show normal architecture of the seminiferous tubules and interstitial tissue. (B) Representative epididymides histology from a *Meig1*$^{flox/flox}$ mouse and a *Prm-Cre; Meig1*$^{flox/flox}$ mouse. The lumen is filled with mature spermatozoa in both animals. (C) Morphology of epididymal spermatozoa collected from a

Meig1$^{flox/flox}$ mouse and a *Prm-Cre; Meig1*$^{flox/flox}$ mouse. (D) All spermatozoa in both groups appear to be normal.

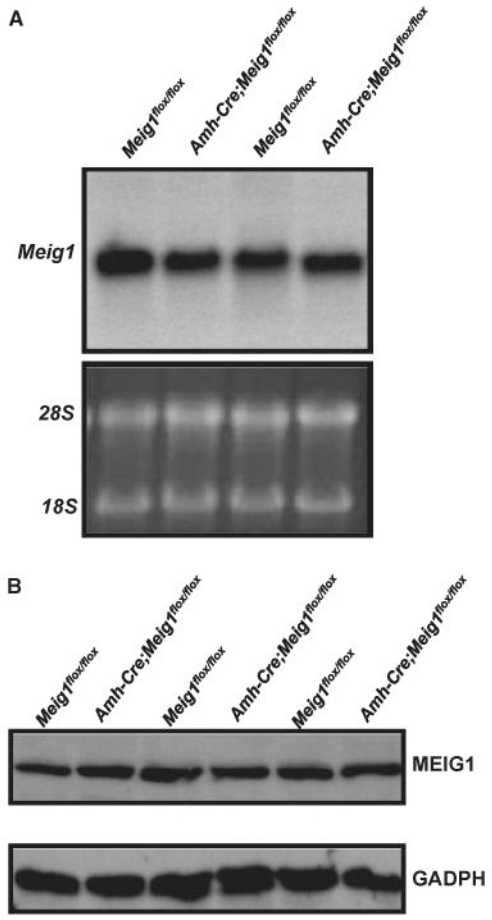

Figure 6.

Expression levels of *Meig1* mRNA and protein are not changed in the testis of the *Amh-Cre; Meig1*$^{flox/flox}$ mice. Total testicular RNA and protein were extracted from adult *Meig1*$^{flox/flox}$ and *Amh-Cre; Meig1*$^{flox/flox}$ mice. Northern blot (A) was conducted using a full-length *Meig1* cDNA as the probe; Western blot (B) was performed using an anti-MEIG1 antibody. Notice that there was no difference in *Meig1* mRNA and protein expression between the two groups.

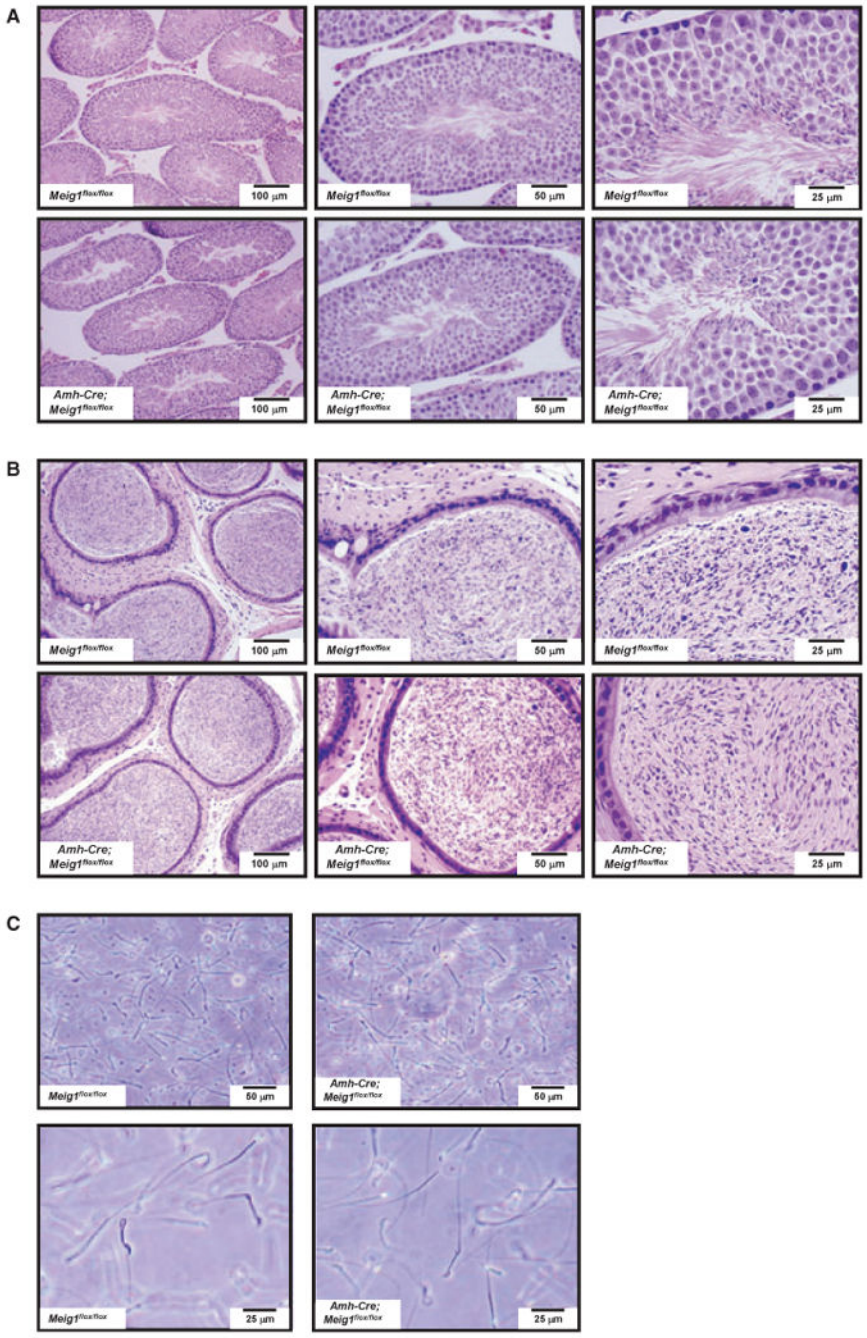

Figure 7.

Disruption of *Meig1* gene in the Sertoli cells does not affect normal spermatogenesis. (A) Representative testicular histology from a *Meig1*$^{flox/flox}$ mouse and a *Amh-Cre; Meig1*$^{flox/flox}$ mouse. Both reveal normal architecture of the seminiferous tubules and interstitial tissue. (B) Representative epididymides histology from a *Meig1*$^{flox/flox}$ mouse and a *Amh-Cre; Meig1*$^{flox/flox}$ mouse. The lumen is filled with mature spermatozoa in both mice. (C)

Morphology of epididymal spermatozoa collected from a *Meig1*$^{flox/flox}$ mouse and a *Amh-Cre; Meig1*$^{flox/flox}$ mouse. All spermatozoa in both groups appear to be normal.

Table 1

***Hsp2a/Cre*; *Meig1*$^{flox/flox}$ males are infertile**

Mice genotype	Male fertility	Litter size (n = 8)[a]	Sperm count (10^6, n = 8)[ab]	Testis/body weight (mg/g)[a]	Seminal vesicle/body weight (mg/g)[a]
Meig1$^{flox/flox}$	8/8	9.2 ± 0.7	25.68 ± 1.32	8.06 ± 0.66	6.34 ± 0.56
Hsp2a-Cre; Meig1$^{flox/flox}$	8/0	0	0.051 ± 0.004	8.02 ± 0.46	6.18 ± 0.39

[a] Values are Means ± SEM.

[b] $p < 0.01$.

Table 2

***Prm-Cre; Meig1$^{flox/flox}$* males demonstrate normal fertility**

Mice genotype	Male fertility	Litter size (n = 8)[a]	Sperm count (10^6, n = 8)[a]	Testis/body weight (mg/g)[a]	Seminal vesicle/body weight (mg/g)[a]
Meig1$^{flox/flox}$	10/10	9.1 ± 0.6	24.52 ± 1.32	8.14 ± 0.76	6.27 ± 0.35
Prm-Cre; Meig1$^{flox/flox}$	10/10	8.9 ± 0.7	21.86 ± 1.48	8.22 ± 0.46	6.38 ± 0.42

[a]Values are Means ± SEM.

Table 3

***Amh-Cre; Meig1$^{flox/flox}$* males have normal fertility**

Mice genotype	Male fertility	Litter size (n = 9)[a]	Sperm count (10^6, n = 8)[a]	Testis/body weight (mg/g)[a]	Seminal vesicle/body weight (mg/g)[a]
Meig1$^{flox/flox}$	9/9	8.9 ± 0.7	24.71 ± 2.68	7.95 ± 0.52	6.20 ± 0.42
Amh-Cre; Meig1$^{flox/flox}$	9/9	8.6 ± 0.6	23.15 ± 2.13	8.05 ± 0.68	6.08 ± 0.36

[a]Values are Means ± SEM.