

Video Article

A Seminiferous Tubule Squash Technique for the Cytological Analysis of Spermatogenesis Using the Mouse Model

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Abstract

Meiotic progression in males is a process that requires the concerted action of a number of highly regulated cellular events. Errors occurring during meiosis can lead to infertility, pregnancy loss or genetic defects. Commencing at the onset of puberty and continuing throughout adulthood, continuous semi-synchronous waves of spermatocytes undergo spermatogenesis and ultimately form haploid sperm. The first wave of mouse spermatocytes undergoing meiotic initiation appear at day 10 post-partum (10 dpp) and are released into the lumen of seminiferous tubules as mature sperm at 35 dpp. Therefore, it is advantageous to utilize mice within this developmental time-window in order to obtain highly enriched populations of interest. Analysis of rare cell stages is more difficult in older mice due to the contribution of successive spermatogenic waves, which increase the diversity of the cellular populations within the tubules. The method described here is an easily implemented technique for the cytological evaluation of the cells found within the seminiferous tubules of mice, including spermatogonia, spermatocytes, and spermatids. The tubule squash technique maintains the integrity of isolated male germ cells and allows examination of cellular structures that are not easily visualized with other techniques. To demonstrate the possible applications of this tubule squash technique, spindle assembly was monitored in spermatocytes progressing through the prophase to metaphase I transition (G2/M1 transition). In addition, centrosome duplication, meiotic sex chromosome inactivation (MSCI), and chromosome bouquet formation were assessed as examples of the cytological structures that can be observed using this tubule squash method. This technique can be used to pinpoint specific defects during spermatogenesis that are caused by mutation or exogenous perturbation, and thus, contributes to our molecular understanding of spermatogenesis.

Video Link

The video component of this article can be found at <https://www.jove.com/video/56453/>

Introduction

Meiosis is a complex cellular event in which a single round of DNA replication is followed by two successive rounds of cell division. Several meiosis-specific events must be coordinated during the initial stages of meiosis to ensure accurate chromosome segregation. These events include the completion of homologous recombination, co-orientation of sister kinetochores during the first meiotic division, and the stepwise loss of cohesin complexes to resolve chiasmata between homologs. Precise regulation of these processes is necessary to maintain fertility and to prevent chromosome missegregation events that can lead to genetic developmental disorders and spontaneous miscarriage¹.

While the key events of meiosis take place in both males and females, significant temporal and mechanistic differences exist between spermatogenesis and oogenesis². For example, during female meiosis, prophase I occurs during embryonic development and arrests at the dictyate stage until puberty. In contrast, spermatogenesis commences at puberty and progresses in waves throughout adult life without arrest. The differences between male and female meiosis emphasizes the need to develop methods that are specifically catered towards assessing these processes in both spermatocytes and oocytes. Currently, assessing meiotic progression largely relies on the use of chromatin spreads^{3,4,5}. While chromatin spreads are useful for studying meiotic chromosomes, they fail to preserve cellular integrity, preventing evaluation of cellular structures such as spindle microtubules, centrosomes, the nuclear envelope, and telomere attachments. Live imaging and long-term culturing techniques have greatly advanced our understanding of female meiosis; similar approaches to visualize the entire intact cell, however, are less frequently implemented for the study of spermatogenesis^{6,7}. In order to visualize dynamic events throughout male meiosis, we have adapted established tubule squash techniques to rapidly assess the cytological features of developing mouse spermatocytes^{8,9}. The method described here maintains the integrity of the cell, enabling the study of multiple cellular structures during different stages of spermatogenesis.

This tubule squash technique is a whole cell approach, which allows for the assessment of cellular structures via immunofluorescence microscopy. Common histological approaches to visualize meiotic progression in male mice such as haematoxylin and eosin staining of paraffin embedded testes, and immunofluorescent labeling of cryosections allow for a broad overview of meiotic progression. However, these techniques fail to resolve single cells to the extent necessary for detailed analysis of the events occurring throughout meiosis^{10,11}. Alternative techniques to visualize meiotic processes rely on significant chemiosmotic disruption to the spermatocyte to isolate and fix nuclear materials^{3,4,5}.

These chemical treatments hinder the observation of cell types other than primary spermatocytes. A recently described method by Namekawa has enabled the research community to preserve the nuclear architecture of isolated spermatocytes, but requires the use of a cytospin and accessories that may not be readily available to some laboratories⁴. In contrast, the tubule squash technique only requires equipment that is generally standard in most cell biology laboratories.

The tubule squash method described here can be used to visualize the diverse cell types found within the seminiferous tubule, including sertoli cells, spermatogonia, primary and secondary spermatocytes, and spermatids. By coupling this technique with the near-synchronous first wave of spermatogenesis in juvenile mice, it is possible to obtain enriched populations of spermatogenic cells as they progress through meiosis¹². This process permits the detailed analysis of processes throughout spermatogenesis, such as early prophase events, the G2/M1 and metaphase to anaphase transitions, and spermiogenesis. Furthermore, tubule squash preparations can be used to visualize cytological features of the chromosomes (e.g. interchromatid domains (ICDs) and kinetochores) and centrosomes (centrioles and pericentriolar material/matrices). The squash method can be readily performed in parallel with other experimental approaches, such as chromatin spreads and protein extraction. In addition, this technique has been successfully modified to deposit living spermatogenic cells on slides for direct visualization¹³.

The method described here involves a whole cell seminiferous tubule squash technique to analyze the G2/M1 transition in wild-type C57BL/6J mice. The cytological features of primary spermatocytes entering the first meiotic division were visualized with immunofluorescence microscopy to observe the meiotic spindle. This versatile technique can be easily modified to visualize other meiotic stages and different cell types. The technique is also amenable to alternative visualization strategies, such as DNA and RNA FISH approaches.

Protocol

The use of mice was approved by the Institutional Animal Care and Use Committee of Johns Hopkins University. Experiments were performed on juvenile (20 - 26 days post-partum, dpp) C57BL/6J mice, taking advantage of the semi-synchronous first wave of spermatogenesis. However, this technique can also be performed using adult mice.

1. Dissection and isolation of mouse seminiferous tubules

1. Prepare the fix/lysis solution and antibody dilution buffer (ADB) described in **Table 1** and **Table 2**.
2. Prepare one 35-mm Petri dish with 3 mL of 1x phosphate buffered saline (1x PBS, pH 7.4), and another 35-mm dish with 2 mL of fix/lysis solution per mouse.
3. Sacrifice the mouse via cervical dislocation or CO₂ asphyxiation and spray the ventral abdomen with 70% ethanol. Open the abdominopelvic cavity using sterile scissors, making a V-shaped opening. Then remove the testes by pulling on the epididymal fat pad using forceps, and avoid disturbing the *tunica albuginea*. Place the testes in a 35-mm Petri dish containing 1x PBS.
NOTE: Utilize the first wave of spermatogenesis in order to observe an enriched cell population of interest¹⁴. Specific stages of spermatogenesis are enriched at different mouse ages (**Table 3**).
4. Remove the testicular *tunica albuginea* by puncturing the tissue with sharp tipped forceps and collect the loose seminiferous tubules. Transfer the tubules to a new 35-mm Petri dish containing 2 mL of fix/lysis solution.
5. Incubate the tubules in the fix/lysis solution for 5 min at room temperature.

2. Preparation of seminiferous tubules

1. Prepare a poly-L-lysine coated glass slide by outlining the edges of the slide with a liquid blocker pen.
2. Apply 100 μ L of fix/lysis solution to the center of the glass slide.
3. Using sterile forceps and scissors gently tease apart the seminiferous tubules. Cutting out long individual tubule segments approximately 20 mm in length.
NOTE: To visualize structures that are sensitive to prolonged fixative exposure, such as the centrosomes, Separate the tubules first in the 1x PBS, then directly mince the tubules on the surface of the poly-lysine slides coated in 100 μ L of fix-lysis solution.
4. Transfer five 20 mm long seminiferous tubule segments to the prepared poly-L-lysine coated glass slide containing fix/lysis solution.
5. Using sterile scissors mince seminiferous tubules into 1.5 to 3.0 mm segments.
6. Using sterile forceps arrange the tubule segments on the glass slide so that no tissue overlaps, and the tubules are distributed evenly.
NOTE: The optimal number of tubule segments on a glass slide is between 20 and 40.

3. Squashing of the seminiferous tubules

1. Transfer the glass slide containing dispersed seminiferous tubule segments onto a benchtop. Remove excess liquid using a laboratory wipe to avoid losing tissue during the squash step.
2. Apply a coverslip (22 x 60 - 1.5 mm) on top of the glass slide and apply pressure with the heel of the palm for 10 - 20 seconds. It is important to apply enough force to disperse spermatocytes from the seminiferous tubules.
NOTE: Observe the tubules using a dissection microscope in order to optimize the squashing step so that all tubule segments are disrupted.
3. Using slide forceps, immediately flash freeze the glass slide in a small dewar of liquid N₂ for 15 seconds, or until the liquid ceases to bubble. If immunolabeling immediately, remove the coverslip with a straight edge razor blade, fine tip forceps or 21-gauge needle.
NOTE: For optimal results, immunolabel the slides immediately (see step 4). However, at this point slides can be preserved at -80 °C for up to two weeks. To maximize the lifetime of proteins and cellular structures, immediately store the slides on dry ice or transfer to a -80 °C freezer, and keep the coverslip on the slide. When immunolabeling stored slides, remove the coverslip by immersing the slides in liquid N₂ for 15 seconds, as described in step 3.3.

4. Immunolabeling of mounted seminiferous tubules

1. Immerse the slide in 1x PBS, and wash three times for 5 min in a 50-mL Coplin jar containing 1x PBS.
NOTE: Never allow the slides to completely dry during immunolabeling.
2. Apply 1 mL of antibody dilution buffer onto the glass slide to block for 1 - 2 h in a humidified chamber.
3. Remove the antibody dilution buffer and apply 100 μ L of primary antibody diluted in antibody dilution buffer in a humidified chamber.
NOTE: For best results, incubate primary antibodies overnight at 4 °C. Use smaller volumes of antibody (e.g. 50 μ L) by covering the slide with a coverslip or parafilm. Validate and optimize primary antibodies¹⁵.
4. Rinse slides three times for 5 min in a 50 mL Coplin jar containing 1x PBS.
NOTE: To enhance washes, agitate Coplin jars by using a small magnetic stir bar and place on a magnetic stir plate at low speed, or place the Coplin jar on a tabletop mixer at low speed.
5. Apply 100 μ L of secondary antibody diluted in antibody dilution buffer for 1 to 1.5 h in a humidified chamber at room temperature. Incubate the slides in a dark box to avoid photo bleaching of fluorophore conjugated antibodies.
NOTE: Use smaller volumes of antibody (e.g. 50 μ L) by covering the slide with a coverslip or parafilm.
6. Rinse slides two times for 5 min in a 50-mL Coplin jar containing 1x PBS.
7. Mount the slides in mounting medium containing 4',6-diamidino-2-phenylindole (DAPI, 1.5 μ g/mL) and apply coverslips (22 x 60 - 1.5 mm).
8. Seal the coverslips to the slides with clear nail polish to preserve the slides and prevent the coverslips from moving.

5. Analysis and imaging tubule squash preparations

1. Use an epifluorescence microscope with automated stage that enables precise Z-axis movement and a high-resolution camera for image capturing. Alternatively, use a laser confocal microscope.
NOTE: Use any available hardware and software for this step. For example, images displayed in **Figure 1** and **Figure 2** were captured using a Zeiss Cell Observer Z1 linked to an ORCA-Flash 4.0 CMOS camera and analyzed with the Zeiss ZEN 2012 blue edition image software.
2. Assess the slides using a 20X objective to determine the quality of the squash preparation. Good quality slides have a monolayer of nuclei that are evenly distributed.
NOTE: Some sections of the slide may be better than others, it is useful to note the coordinates of where the best regions of the slide can be found for further analysis using higher magnification.
3. Using higher magnification objective (e.g. 63X or 100X), capture regions of the slide that have a consistent monolayer of nuclei. Once a region is selected, set the upper and lower Z-stacks to ensure that all in-focus light is captured.
NOTE: The optimal number of Z-stacks captured between the upper and lower limit is generally designated by the image acquisition software and is different for each objective.
4. Use the image processing software to compile an extended depth focus image. The software combines the in-focus light from each Z-stack, and is essential for optimal imaging of tubule squash preparations.

Representative Results

Here, we have used the tubule squash method to visualize transitory cell populations undergoing the prophase to metaphase I (G2/MI) transition, which were enriched by harvesting testes from juvenile wild-type mice undergoing the first wave of spermatogenesis (24 dpp). **Figure 1** depicts representative images of the various cell stages that can be visualized using the tubule squash method. Enriched populations of metaphase I spermatocytes were visualized using antibodies against alpha-tubulin (**Figure 1A**). We utilized markers for early and late meiosis I to stage meiotic progression. Tubule squash preparations were immunolabeled with an antibody to the synaptonemal complex protein, SYCP3, to visualize stages of prophase I. Leptotene/zygotene and pachytene/diplotene spermatocytes are shown in **Figure 1B**. Spermatocytes undergoing the first meiotic division can be clearly identified using antibodies against alpha-tubulin (**Figure 1A** and **1C**). To study late meiotic events, tubule squashes were immunolabeled using antibodies against the cell cycle kinase Aurora B (AURKB), which localizes to the inner centromere during metaphase, then relocalizes to the spindle midzone and cleavage furrow during anaphase and cytokinesis, respectively (**Figure 1C**). Cytological features of chromosomes were visualized during the first meiotic division. To observe ICDs, tubule squash preparations were immunolabeled using antibodies against the meiosis-specific alpha-kleisin subunit of cohesin, REC8 (**Figure 1D**). Chromosome dynamics during anaphase were assessed using alpha-tubulin immunolabelling and the DNA stain, DAPI. A spermatocyte successfully completing the first meiotic division is shown in **Figure 1E**, while a spermatocyte containing an anaphase bridge is shown in **Figure 1F**. Using the tubule squash technique, meiotic spindle length, chromosome alignment and segregation can be measured and compared between mutant and control mice.

The utility of this method for visualization of additional cell-cycle transitions and subcellular structures associated with prophase I is demonstrated in **Figure 2**. During the leptotene substage of prophase I, initiation of homologous chromosome pairing is mediated by dynamic changes in telomere attachments to induce formation of chromosome clusters facing a single pole of the nuclear envelope (NE)^{16,17,18,19,20}. This conformation, known as the chromosome "bouquet" is thought to increase the likelihood and frequency of inter-homolog interactions (**Figure 2A**). Initiation and completion of synapsis between homologous chromosomes occurs during the zygotene (**Figure 2B**) and pachytene substages (**Figure 2C**), respectively, corresponding with progressive dispersal of the chromosome bouquet. To simultaneously assess synapsis and chromosome bouquet dynamics in juvenile mice, we immunolabeled tubule squash preparations with antibodies to SYCP3 and the centromeres, and stained for DNA using DAPI. Mouse chromosomes are telocentric, therefore centromere immunolabeling was used to detect changes in telomere NE attachments.

Meiotic sex chromosome inactivation (MSCI) is a hallmark unique to male meiosis, whereby the non-homologous X and Y chromosomes circumvent checkpoint activation by undergoing chromosome-wide phosphorylation of histone variant H2AFX (yH2AX; pSer139)^{21,22,23}. The X-Y chromosome pair is transcriptionally silenced and compartmentalized into a dense nuclear subdomain called the "sex body." Spermatocytes undergoing MSCI can be readily identified during the pachytene and diplotene substages by immunolabeling tubule squash preparations with antibodies to yH2AX (Ser139). **Figure 2D** depicts a spermatocyte at the pachytene substage immunolabelled with antibodies against yH2AX (Ser139), the centromeres and stained with DAPI to visualize the DNA.

Centrosome duplication is semi-conservative and occurs during prophase I of male meiosis upon completion of DNA repair to ensure that each resulting daughter cell inherits two centrioles (one centrosome) following cell division^{24,25}. Centrosomes consist of two orthogonally arranged centrioles connected by a flexible linker and are surrounded by an amorphous matrix of proteins known as the pericentriolar material/matrix (PCM)²⁶. After duplication in the pachytene substage, newly formed centriole pairs disengage from one another and undergo centrosome maturation and migration to opposite poles during the diplotene stage to facilitate formation of bipolar spindles. An early diplotene spermatocyte undergoing disjunction of newly formed centriole pairs was visualized by immunolabeling tubule squash preparations with antibodies against pericentrin (PCNT), a protein component of the PCM and Centrin-3 (CETN3) to mark centrioles (**Figure 2E**). Staging was determined using antibodies against SYCP3, and DAPI was used to stain DNA.

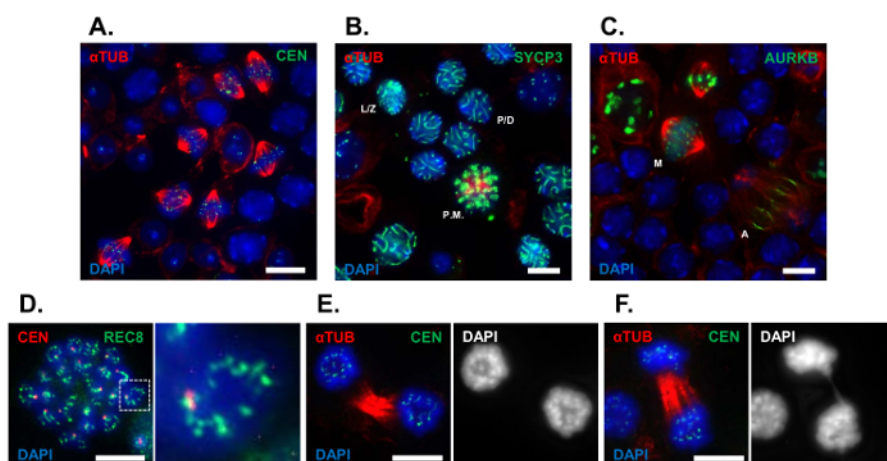


Figure 1: Representative analysis of the meiotic G2/M transition using tubule squash preparations. Tubule squash preparations were performed on seminiferous tubule segments of C57BL/6J mice aged 24 dpp. **(A)** Representative field of developing spermatocytes immunolabeled with antibodies against alpha-tubulin (red), centromeres (green) and the DNA stain DAPI (4', 6-diamidino-2-phenylindole, blue). **(B)** Wild-type tubule squashes were stained with DAPI (blue) as well as immunolabeled with antibodies against alpha-tubulin (red), and the lateral element of the SC, SYCP3 (green). **(C)** Wild-type tubule squashes were stained with DAPI (blue) as well as immunolabeled with antibodies against alpha-tubulin (red), and the cell cycle kinase AURKB (green). Cytological features of chromosomes such as ICDs and anaphase bridges can be visualized using tubule squash preparations. **(D)** To observe ICDs, prometaphase spermatocytes were immunolabeled with antibodies against centromeres (red) as well as a meiotic specific cohesin component, REC8 (green) and stained with DAPI (blue). **(E, F)** Chromosome morphology was visualized during anaphase using antibodies against alpha-tubulin (red), centromeres (green), and the DAPI stain (blue). A full complement of developing spermatocytes can be identified using the tubule squash technique (L/Z, leptotene/zygotene stage spermatocytes; P/D, pachytene/diplotene stage spermatocytes; P.M., prometaphase spermatocytes; M, metaphase spermatocytes; A, anaphase spermatocytes). Scale bar = 10 μ m. [Please click here to view a larger version of this figure.](#)

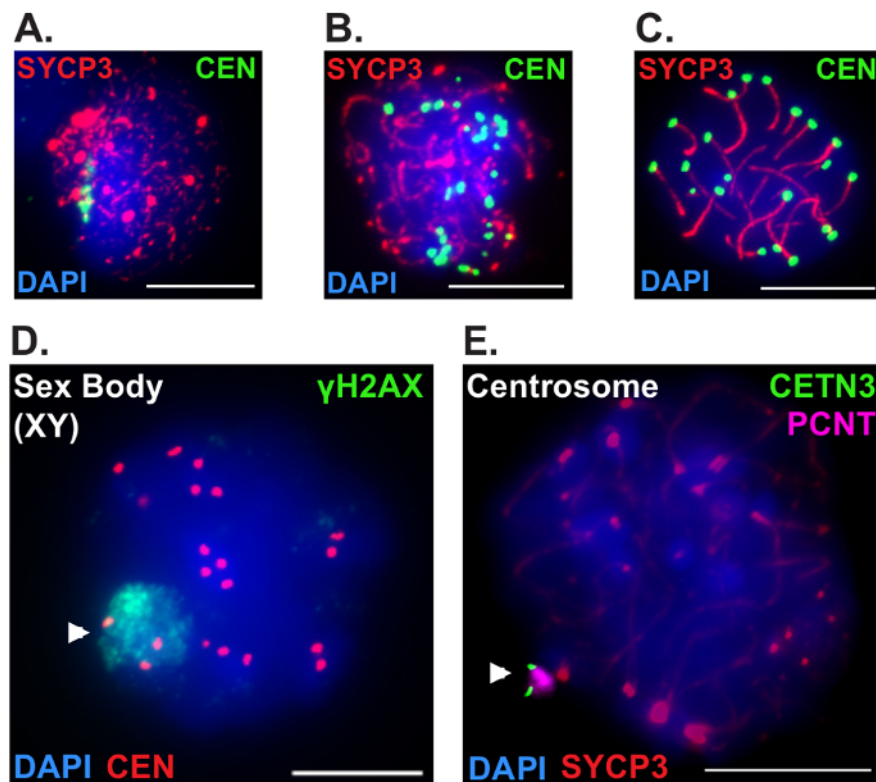


Figure 2: Examples of additional morphological changes and structures associated with meiotic prophase progression in males using tubule squash preparations. Tubule squash preparations were performed on seminiferous tubule segments of C57BL/6J mice aged 12-18 dpp and were stained with DAPI (4', 6-diamidino-2-phenylindole, blue) to label DNA. **(A-C)** Homolog synapsis is coupled to the formation and progressive dispersal of the chromosome "bouquet" during prophase progression. Leptotene **(A)**, zygotene **(B)**, and pachytene **(C)** stage spermatocytes were immunolabeled with antibodies against SYCP3 (red) and the centromeres (green). **(D-E)** Tubule squash preparations can be used to detect unique cytological changes and structures during male prophase I progression including meiotic sex chromosome inactivation (MSCI) and centrosome duplication. **(D)** Pachytene stage spermatocytes undergoing MSCI were detected using antibodies to γ H2AX (Ser139) to label the sex-body (green, arrowhead), and centromeres are shown in red. **(E)** Diplotene stage spermatocytes were identified using antibodies against SYCP3 (red), and centrosomes (arrowhead) were detected using antibodies against Centrin-3 (green) and Pericentrin (magenta) to label the centrioles and the pericentriolar material/matrix, respectively. Scale bar = 10 µm. [Please click here to view a larger version of this figure.](#)

Item	Amount	Final Concentration
1x PBS	10 mL	1x
16% PFA	500 µL	0.8% (v/v)
10% TritonX-100 in PBS	100 µL	0.1% (v/v)

Table 1: Fix/lysis solution. Description: Adjust pH to 9 with NaOH [50mM]. Wrap the container of solution in aluminum foil to protect from light and store at 4 °C. Do not use the fix/lysis solution if more than a week old. Solid PFA can also be used to make the solution. Use of a fumehood is recommended to avoid PFA exposure.

Item	Amount	Final Concentration
1x PBS	50 mL	1x
BSA	1.5 g	3% (w/v)
Horse Serum	5 mL	10% (v/v)
10% TritonX-100 in PBS	250 µL	0.05% (v/v)

Table 2: Antibody dilution buffer (ADB) recipe. Description: Store ADB at 4 °C or freeze stocks at -20 °C if making larger quantities. ADB can become contaminated, make sure good aseptic techniques are used and assess the solution for contamination prior to each use. Prepare smaller aliquots of ADB to minimize contamination.

Cell type	Cellular Process	Mouse Age (dpp)
Leptotene	DNA DSB formation	10 - 12
	Assembly of axial elements	
Zygotene	Initiation of homologous DSB repair and synapsis	12 - 16
Pachytene	Completion of autosomal DSB repair	16 - 20
	Maturation of crossover recombination events	
	Complete synapsis between homologs	
	Meiotic Sex Chromosome Inactivation (MSCI)	
	Centriole Duplication/Centrosome Maturation	
Diplotene and Diakinesis	SC Desynapsis	18 - 22
	Centrosome separation	
Metaphase I	Spindle Checkpoint	22 - 26
Round Spermatid	Protamine replacement	>24
	Acrosome formation	

Table 3: Near-synchronous first wave of spermatogenesis in juvenile mice. Description: Specific stages of spermatogenesis are enriched at different stages of juvenile mouse development.

Discussion

Mice have proven to be a useful model organism for studying the cellular events that govern meiotic progression during spermatogenesis. Further, it is necessary to develop tools catered to the study of spermatogenesis because many events, such as exit from meiotic prophase I, are sexually dimorphic. This protocol describes a seminiferous tubule squash method for visualization and study of different stages of mouse spermatogenesis. This method preserves cellular integrity and thereby allows detailed analyses of nuclear and cytoplasmic structures. The representative results depicted in this study demonstrate the utility of this protocol in assessing primary spermatocytes undergoing meiotic progression. Moreover, this protocol can also be used to study other cellular processes during spermatogenesis, including proliferation and differentiation of spermatogonia and stages of spermiogenesis^{27,28}. The methods described are within are adapted from previously described tubule squash techniques^{8,9}. However, this protocol is the first detailed guide, covering all reagents and steps required, from obtaining the tubules to visualizing single cells via fluorescence microscopy. Furthermore, we have presented images on additional cellular features, including the centriole and the telomere bouquet, which can be visualized with the technique.

There are a few technical elements that are key for optimizing tubule squash preparations. Firstly, one must apply the proper amount of force to the glass coverslip. Failure to apply sufficient force will produce slides with few adherent cells, as most cells will remain within the seminiferous tubules. Secondly, one must incubate the testis material in the fix/lysis solution for the appropriate length of time. Generally, fixation should be limited to 5 min, but a range between 5 and 15 min is acceptable. Tubules that have been fixed for a prolonged period will be difficult to manipulate and yield poor results. It is possible, however, to extend the incubation time by reducing the concentration of PFA. For example lowering the percent PFA to 0.5% would allow for increased incubation times in the fix/lysis solution. In the method we have presented, the permeabilization and fixation steps occur simultaneously, however these steps can be performed one after another, respectively. Separating the permeabilization and fixation steps can help to reduce levels of cytoplasmic background signal. Variation of fixation and/or permeabilization strategies can also be tested to optimize conditions for assessment of a specific subcellular structure or antibody. For example, visualization of centrosomal and spindle proteins can be enhanced by using methanol instead of PFA^{29,30,31}. Additionally, it is beneficial to use juvenile mice that are progressing through the first wave of spermatogenesis (approximately 10 - 26 dpp) in order to specifically and robustly visualize infrequent meiotic cellular events¹². Alternatively, it is possible to isolate stage-specific meiotic cells from adult mice by observing the light absorption pattern within the seminiferous tubule³². Given the asynchronous cycle of spermatogenesis in adult mice, however, analyzing rare meiotic events in detail can be challenging. This limitation can be overcome by injecting mice with bisdichloroacetyldiamine, WIN 18,446, which blocks vitamin A metabolism and halts spermatogonial differentiation³³. Following treatment with WIN 18,466, resumption of spermatogenesis will occur synchronously, yielding large quantities of enriched germ cell populations from a mature animal³⁴. This synchronization strategy can be used prior to the tubule squash technique to investigate the differences between the first semi-synchronous waves of spermatogenesis to those that occur in the adult^{35,36,37}. Furthermore, correlation between paternal aging and aneuploidy could also be assessed using WIN 18,466 synchronization and assessing chromosome segregation with the tubule squash method.

In summary, this method can be used in conjunction with immunofluorescence microscopy to observe cells in different stages of spermatogenesis. Applied more broadly, this technique can also be used to assess sub-populations of spermatogonia, spermatocytes and spermatids, and can be tailored to study a wide variety of processes during spermatogenesis. This approach has been used for live cell imaging of spermatocytes, and modified to conduct a variety of cellular and biochemical studies^{38,39,40}. The tubule squash technique has been used for mouse and rat seminiferous tubules, suggesting that it should be readily applied to other mammalian systems, including human⁴⁰. This technique can help define the cellular perturbations that give rise to infertility and sperm aneuploidy.

Disclosures

The authors have nothing to disclose.

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