Video Article

Isolation and *In vitro* Activation of *Caenorhabditis elegans* Sperm

Gunasekaran Singaravelu[1], Indrani Chatterjee[1], Matthew R. Marcello[1], Andrew Singson[1]

[1]Waksman Institute of Microbiology, Rutgers University

URL: https://www.jove.com/video/2336
DOI: doi:10.3791/2336

Keywords: Developmental Biology, Issue 47, spermatid, spermatozoa, spermiogenesis, protease, pseudopod, nematode

Date Published: 1/31/2011

Citation: Singaravelu, G., Chatterjee, I., Marcello, M.R., Singson, A. Isolation and *In vitro* Activation of *Caenorhabditis elegans* Sperm. *J. Vis. Exp.* (47), e2336, doi:10.3791/2336 (2011).

Abstract

Males and hermaphrodites are the two naturally found sexual forms in the nematode C. *elegans*. The amoeboid sperm are produced by both males and hermaphrodites. In the earlier phase of gametogenesis, the germ cells of hermaphrodites differentiate into limited number of sperm - around 300 - and are stored in a small 'bag' called the spermatheca. Later on, hermaphrodites continually produce *oocytes*[1]. In contrast, males produce exclusively sperm throughout their adulthood. The males produce so much sperm that it accounts for >50% of the total cells in a typical adult worm[2]. Therefore, isolating sperm from males is easier than from that of hermaphrodites.

Only a small proportion of males are naturally generated due to spontaneous non-disjunction of X chromosome[3]. Crossing hermaphrodites with males or more conveniently, the introduction of mutations to give rise to Him (High Incidence of Males) phenotype are some of strategies through which one can enrich the male population[3].

Males can be easily distinguished from hermaphrodites by observing the tail morphology[4]. Hermaphrodite's tail is pointed, whereas male tail is rounded with mating structures.

Cutting the tail releases vast number of spermatids stored inside the male reproductive tract. Dissection is performed under a stereo microscope using 27 gauge needles. Since spermatids are not physically connected with any other cells, hydraulic pressure expels internal contents of male body, including spermatids[2].

Males are directly dissected on a small drop of 'Sperm Medium'. Spermatids are sensitive to alteration in the pH. Hence, HEPES, a compound with good buffering capacity is used in sperm media. Glucose and other salts present in sperm media help maintain osmotic pressure to maintain the integrity of sperm.

Post-meiotic differentiation of spermatids into spermatozoa is termed spermiogenesis or sperm activation. Shakes[5], and Nelson[6] previously showed that round spermatids can be induced to differentiate into spermatozoa by adding various activating compounds including Pronase E. Here we demonstrate *in vitro* spermiogenesis of *C. elegans* spermatids using Pronase E.

Successful spermiogenesis is pre-requisite for fertility and hence the mutants defective in spermiogenesis are sterile. Hitherto several mutants have been shown to be defective specifically in spermiogenesis process[7]. Abnormality found during *in vitro* activation of novel Spe (Spermatogenesis defective) mutants would help us discover additional players participating in this event.

Video Link

The video component of this article can be found at https://www.jove.com/video/2336/

Protocol

1) Enrichment of male population

1. Depending on the experimental need, large numbers of males can be obtained by employing one of the following strategies:
    1. large population of wild type males can be obtained by crossing 5 wild type males and 1 hermaphrodite on a small lawn of OP50 seeded at the center of NGM plate. Roughly 50% of the succeeding generation will be wild type males.
    2. *him-5*(*e1490*) or *him-8*(*e1489*) hermaphrodites throw large number of males. *him-5* and *him-8* males are fertile and there is no obvious defect in sperm morphology and function. So, *him-5* or *him-8* males can be used *in lieu* of wild type males for many experiments.

2) Identification and isolation of males

1. Examine the tail morphology of worms; the male worm's tail is rounded.

2. Pick L4 stage males and transfer them to a NGM plate seeded with *E. coli* OP50 and let them grow for a day or two. Growing celibate males in the absence of hermaphrodites prevent the loss of sperm and thus large number of spermatids would be available during experimental procedure.
3. On the day of dissection, transfer the celibate males on to a NGM plate without bacteria and let them crawl for few minutes. This step helps to remove small layer of *E. coli* adhered on the surface of worms. Alternatively, pipette small volume of M9 buffer (6g Na_2HPO_4, 3g KH_2PO_4, 5g NaCl, 0.25g $MgSO_4.7H_2O$ per liter) in a watch glass and transfer males into the buffer.

3) Dissection of males and *in vitro* activation

1. Take a glass slide and mark a small circle using a pap pen. Pipette 30-50 µl of 1X Sperm Medium (50mM HEPES, 25mM KCl, 45mM NaCl, 1mM $MgSO_4$, 5mM $CaCl_2$, 10mM Dextrose; pH 7.8) within the circle. The hydrophobic circle helps to retain the sperm medium within its boundary.
2. Transfer the 5-10 males on to the sperm medium.
3. Viewing the slide under microscope, hold two syringes attached with 27 gauge needles on both of your hands.
4. Use one needle to 'hold' the worm and use the other needle to cut the posterior end of males to release spermatids.
5. Cutting the worm usually expels vast number of spermatids instantaneously. The spermatids are visible as minute 'granules' under the stereo microscope. However, sometimes part or most of the spermatids may be retained within the carcass. In that case, gently 'dragging' the carcass over the glass slide may facilitate the release of spermatids from the carcass.
6. To activate the spermatids, dissect the worms in 1X Sperm Medium containing Pronase E(20µg/ml) and let them sit for 5 minutes.
7. Do not allow the slides to become dry at any time point of the experiment. Add more 1X Sperm Medium if needed.

4) Visualization of spermtids and spermatozoa

1. Gently place a coverslip on the surface of dissected sample.
2. Find the field of spermatids under low magnification (10X)
3. Fine details of spermatids or spermatozoa can be observed by moving to higher magnification - usually at 100X oil immersion.

5) Representative Results

An example of DIC image of spermatids isolated from male C. *elegans* is shown in Figure 1. Spermatids are spherical in shape and the nuclei are prominent. *In vitro* activated sperm are shown in Figure 2.

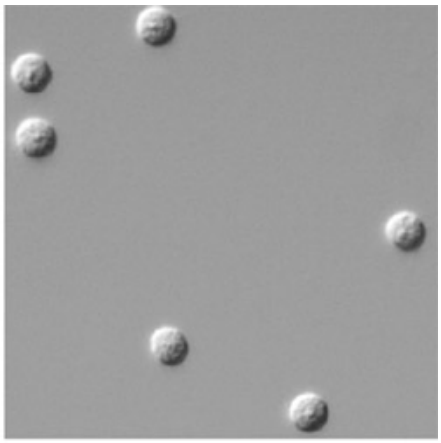

Figure 1. DIC image of *C. elegans* spermatids.

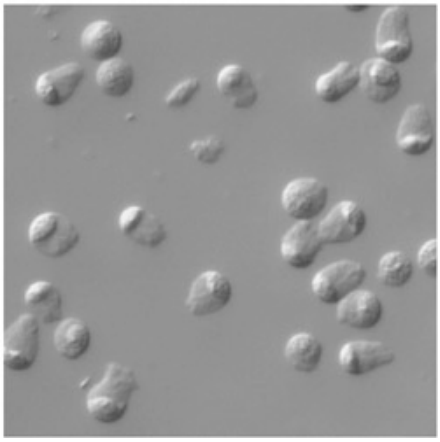

Figure 2. DIC image of *in vitro* activated *C. elegans* spermatozoa.

Discussion

In addition to analyzing Spe mutants, this protocol has other important applications, such as analyzing sperm morphology with aging. Spermatids and spermatozoa isolated using this protocol can be used in other downstream experiments such as, immunostaining of wild type and mutant sperm[8, 9], motility of sperm on slides[10], physiological measurements[9, 11], or even artificial insemination[12].

Disclosures

No conflicts of interest declared.

Acknowledgements

We thank Samuel Ward, Diane C. Shakes and Gregory Nelson for pioneering this technique. We thank Pavan Kadandale and Brian Geldziler for videos showing activated spermatozoa; members of the Singson lab for helpful discussions; CGC for strains and NIH for supporting us through grant (R01HD054681).

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