

Chapter 9

Preparation of Mammalian Meiotic Chromosomes and Spermatozoa/ Obtaining Early Mammalian Embryos and Preovulation Oocytes

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Introduction

Decades of teaching have demonstrated that although many interests come and go young adults' interests in reproduction endures. This is hardly surprising. Nevertheless, the lack of sophistication among students about the biology of reproduction is often substantial. I have found this to be the case among students in one of the courses I regularly teach, Human Life Before Birth, even among women who have given birth to several children.

The procedures described here may be used at several levels including general biology and upper division courses in embryology or developmental biology. The gametes span the generations, they are the only physical link between parents and offspring. What parents physically transmit to their offspring is funneled through two cells, sperm and egg.

Conceptually, we will first look at cells that result in the formation of the male gametes; that is the germ cells that participate in spermatogenesis. We will particularly focus on chromosomes of these cells; spermatogonia, primary spermatocytes, and secondary spermatocytes. Incidentally, we will also make note of spermatids, those cells that undergo a remarkable type of differentiation, spermiogenesis. This part of the work will cause us to look at cells of the testis. For a preparation of living spermatazoa we will go to the tail (cauda) of the epididymis.

The other part of the laboratory work will have us isolate the living ovaries and oviducts of the mouse. We will macerate the ovaries to release preovulation oocytes, which we may then examine, note their structure and that of their envelopes, and compare them to follicle cells, which are not germ cells but somatic cells. We will observe the oviducts and try to see fertilized eggs or 2-cell embryos through their translucent walls. Subsequently, we will macerate the oviducts and isolate the fertilized eggs or embryos for wet-mount preparations.

All or parts of these procedures may be introduced into a variety of courses where they may be truncated or expanded upon to suit the needs of the instructor.

Materials

Mammalian Meiotic Chromosomes (numbers in parentheses are procedure steps in the section *Student Outlines*)

- (1) male mice (1/pr. of students); preinjected with colchicine solution
disposal bags for mice
- (2) fine forceps (stainless steel), 2/student
fine scissors (stainless steel), 1/student
sodium citrate solution (2.2% in water), 100 ml/student desk
glass petri dishes, small, 3/pair
kimwipes, 1 box/student desk
- (3) pasteur pipettes & bulbs/student desk
centrifuge tubes, 15-ml, sterile, graduated, disposable, plastic, 2 /pair
- (4) clinical centrifuge, table-top, that will spin 15-ml tubes at 500 rpm, centrifuge should accommodate as many tubes as there are pairs of students
timer
sodium citrate solution (1% in water), 100 ml/student desk
- (5) methanol, 75 ml/student desk
glacial acetic acid, 25 ml/student desk
- (7) microscope slides, 1 box/student desk
- (8) giemsa stain- see Appendix
- (9) buffer- see Appendix
microscopes (incl., 10x, 40x, 100x objectives), 1/student
immersion oil, 1 bottle/student desk

Mammalian Spermatozoa

- (4) Hanks balanced salt solution, 100 ml/student desk
- (5) dissecting needles, 4/pair
- (6) depression slides & coverslips, 1/student

Obtaining Early Mammalian Embryos and Preovulation Oocytes

- (1) mice, female, day after vaginal plug, 1/pair of students
- (3) glass petri dishes, small, 3/pair
- (5) dissecting microscope, stereoscopic, 1-60x mag., with both reflected and transmitted light (diffuse & direct), 1/student
- (6) tuberculin syringes, 1cc, with needles, 2/student

Mice

If your experience with mice is minimal, see Dye (1993).

Notes for the Instructor

Part A: Preparation of Mammalian Meiotic Chromosomes

Objectives

The objectives of this laboratory work are to:

- (1) introduce the student to a method for obtaining spreads of chromosomes from mammalian testes (which will include both mitotic and meiotic chromosomes) and
- (2) reinforce the student's knowledge of meiosis and spermatogenesis by critical interpretation of what is seen through the microscope.

Part B: Mammalian Spermatozoa

Objectives

The objectives of this laboratory work are to:

- (1) obtain living spermatozoa from the cauda epididymis of the mouse,
- (2) observe the morphology and behavior of normal and abnormal sperm,
- (3) and compare characteristics of sperm to those of eggs.

Part C: Obtaining Early Mammalian Embryos and Preovulation Oocytes

Objectives

The objectives of this laboratory work are to:

- (1) obtain living embryos from oviducts of the mouse,
- (2) obtain oocytes from mouse ovaries, and
- (3) note the dynamics of oviducts, including smooth muscle contraction of oviducts and movement of embryos within the lumen of the living oviduct.

Student Outlines

Part A: Preparation of Mammalian Meiotic Chromosomes

Laboratory Work

- 1) Inject a male mouse with 0.1 ml of colchicine solution (0.1%, aqueous), IP, 2 hours before sacrificing it. The animal is sacrificed by cervical dislocation.
- 2) Remove a testis and place it in sodium citrate solution (2.2%) at room temperature. Pierce the tunica, grasp the tunica with 2 sharp forceps and strip it off the testicular tubules, and swirl the tubules in the solution to remove adherent fat.
- 3) Transfer the testis into fresh 2.2% citrate solution contained in a small petri dish and gently pull out the tubules. Hold the mass of tubules with fine, straight forceps and thoroughly tease out (by constantly squeezing the tubules with the forceps - avoid shredding the tubules) their contents with an additional

fine forceps. When the tubules appear “flat” and opaque allow them to settle; then transfer the supernatant fluid, with a pasteur pipette, into a 15 ml centrifuge tube.

- 4) Centrifuge the cell suspension obtained at 500 rpm for 5 minutes (accelerate slowly, so actually use a 7 minute total run). Discard the supernatant fluid, with a pasteur pipette, and resuspend the sedimented cells in approximately 3 ml of 1% sodium citrate (hypotonic) solution.
- 5) Leave the cells in hypotonic solution for 12 minutes at room temperature. Centrifuge at 500 rpm for 5 minutes with slow acceleration.
- 6) Remove as much of the supernatant fluid as possible. Resuspend the cells in the remainder by flicking the tube with thumb or forefinger so that a thin film of suspension adheres to the wall of the tube. Add about 0.25 ml of fixative (3:1; methanol:glacial acetic acid) rapidly, directly into the suspended cells. Add more fixative down the side of the tube, flicking meanwhile, until 1 ml is added. After 5 minutes, sediment the cells again by centrifuging and then resuspend in fresh fixative. Repeat the change of fixative after a further 10 minutes.
- 7) Take up some of the suspension into a pasteur pipette. Allow a drop to fall, from an altitude of 5 cm, onto a grease-free slide at room temperature. Blow gently on the slide to hasten the final evaporation.
- 8) When dry, stain for 5 minutes in giemsa.
- 9) Rinse in buffer, wipe the bottom of the slide, and observe with the microscope.

Interpretation

Carefully examine the slides for chromosome spreads. Remember the mouse has a diploid number of 40 chromosomes.

1. How many chromosomes would you expect to find in a mitotic metaphase spread?
2. How many chromosomes would you expect to find in a meiosis I metaphase spread? How would you expect these chromosomes to be arranged?
3. How many chromosomes would you expect to find in a meiosis II metaphase spread?

Make sketches of two of each of the above kinds of metaphase spreads. In addition to including the normal sketch specifications, beneath each sketch record the number of chromosomes in the metaphase spread.

Part B: Mammalian Spermatozoa

Laboratory Work

- 1) The animal is sacrificed by cervical dislocation.
- 2) Open the abdominal cavity in the usual fashion (the testes will be retracted into the abdominal cavity).
- 3) With forceps, gently grasp the fat mass at the anterior pole of a testis and pull the testis into plain view. Note at the posterior pole of the testis the sac-like cauda of the epididymis.
- 4) With fine scissors, cut the cauda epididymis away from its testis and immerse it in physiological saline solution.
- 5) With two dissecting needles, macerate the cauda in the saline solution. This will release the active sperm and create a suspension of these highly differentiated cells in saline solution.
- 6) With a depression slide and coverslip, make a wet-mount preparation, of sperm suspension, in the usual fashion.

- 7) Observe the wet-mount preparation with your microscope. Note the morphology of the sperm, including normal, two-headed, and headless sperm. Note the behavior of the sperm, including vigor and directionality of movement.

Interpretation:

Review your background in mammalian spermatogenesis, including spermiogenesis.

1. How would you explain the origin of two-headed or headless sperm?
2. Which organelle(s) in sperm may be defective when sperm swim with little vigor?
3. Which organelle(s) in sperm may be defective when the sperm swim with apparent lack of direction? Might lack of direction instead be due to lack of environmental signals? Explain.

Part C: Obtaining Early Mammalian Embryos and Preovulation Oocytes

Laboratory Work

- 1) The animal is sacrificed by cervical dislocation.
- 2) Open the abdominal cavity in the usual fashion (the ovaries, oviducts and uterine horns are found in the posterior portion of the abdominal cavity). The uterine horns and the short body of the uterus have an inverted-wishbone configuration.
- 3) With forceps, grasp one of the uterine horns and pull it away from its ovary- this will stretch out the oviduct and ovary. With fine scissors, make one cut between the ovary and oviduct and a second cut through the uterine horn behind (opposite the oviduct side) the forceps. With forceps transfer the oviduct and short length of uterine horn into a dish of physiological saline.
- 4) Work the tips of fine forceps between an ovary and its surrounding fat mass, squeezing the ovary away from the fat. With fine scissors cut the ovary away from the forceps and place it into a dish of physiological saline.
- 5) Observe the living oviduct in its dish of saline solution with a dissecting microscope, using transmitted light. It should be possible to observe the oviduct undergoing peristaltic contractions. By carefully focusing up and down through the translucent walls of the oviduct it is possible to observe moving embryos in the lumen of the oviduct.
- 6) With two needles of 1-cc hypodermic syringes, cut the oviduct into several lengths and press on each one. Then, carefully observe the floor of the dish for 2-cell, living, mouse embryos.
- 7) Observe the other dish containing the ovary. Note that, unlike the translucent oviduct, the ovary is opaque. Look for evidence of developing ovarian follicles on the surface of the ovary. With two dissecting needles, thoroughly macerate the ovary. Then, carefully observe the floor of the dish for living, preovulatory, mouse oocytes.

A good marker for the oocytes, as well as for the embryos referred to above, is the shiny, zona pellucida around each oocyte.

Interpretation

It is instructive to make sketches of oocytes and embryos. The 2-cell, mouse embryos are found in the fluid-filled perivitelline space within the clear, noncellular, zona pellucida. In addition to two blastomeres, and their nuclei, polar bodies should also be visible. Preovulatory oocytes may be found within an intact ovarian follicle. In any case, the oocyte will be within a zona pellucida not separated from the oocyte by a fluid-filled, perivitelline space.

1. What is the origin of the blastomeres and polar bodies of the 2-cell mouse embryo?
2. What is the origin, chemical composition, and function of the zona pellucida?
3. If you find ovarian follicles, what are the cells called adhering to the outside of the zona pellucida? What is the human equivalent of the cumulus clot of the mouse?
4. The oocyte, in the ovarian follicle, just about to be ovulated is in what stage of maturation? It is in what stage of meiosis? An oocyte in a younger follicle is in what stage of maturation? It is in what stage of meiosis?

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APPENDIX
Giemsa Stain and Colchicine

Giemsa Stain

Buffer Stock Solution

Monobasic ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$); FW = 137.99

0.1 M = 13.8 g/liter = 1.38 g/100 ml

Dibasic ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$); FW = 268.07

0.1 M = 26.8 g/liter = 2.68 g/100 ml

Phosphate Buffer (pH 7; 0.1 M)

45 ml monobasic + 55 ml dibasic

Giemsa Stock Solution

1) 1 g of Giemsa (Fisher G-146) ground up in 66 ml of glycerol with a mortar and pestle; place in oven, 55-60 °C for 2 hours.

2) Add 66 ml of methanol; stir = ready to use.

Giemsa Working Solution

1 ml of Giemsa Stock Solution in 50 ml of diluted buffer (i.e., 5 ml of phosphate buffer brought up to 50 ml with distilled water = 0.01 M = 10 mM phosphate buffer).

Colchicine

Sigma C-3915