NU-BIRD
Northwestern University Biology Investigations in Reproduction & Development

Goldberg and LaBonne Modules: Fertilization and Beyond

NU-BIRD is a collaborative effort between Northwestern University and Evanston Township High School
## Goldberg & LaBonne Modules:
### Schedule for Saturday March 1, 2014
### Pancoe Pavilion, Evanston Campus

<table>
<thead>
<tr>
<th>Time &amp; Location</th>
<th>Event</th>
<th>Facilitator</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:30 – 8:30 am</td>
<td>Arrive at the entrance to the Pancoe Building on the Northwestern Campus (see map, follow signs)</td>
<td>Ms Sarah Stein – call or text if you get lost 773-706-2292</td>
</tr>
<tr>
<td>8:30 – 8:45 am</td>
<td>Welcome and light breakfast service</td>
<td>Dr. Murdoch and Ms Stein</td>
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<tr>
<td>Pancoe 3103</td>
<td>Lecture: The male reproductive system and fertilization</td>
<td>Erwin Goldberg, PhD Carole LaBonne, PhD</td>
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<tr>
<td>8:45 – 9:45 am</td>
<td>Students will split into two groups and rotate between the Goldberg and LaBonne labs</td>
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<tr>
<td>Tech MG78</td>
<td>• Sea Urchin egg and sperm collection</td>
<td>Drs. Goldberg, Tang, Duan, and Monahan</td>
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<tr>
<td>Pancoe 3321</td>
<td>• Sea Urchin fertilization</td>
<td></td>
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<tr>
<td></td>
<td>• Sea Urchin development</td>
<td></td>
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<tr>
<td>9:45 –12:30 pm</td>
<td>Lunch</td>
<td></td>
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<tr>
<td>12:30 – 1:00 pm</td>
<td>Pancoe 3103</td>
<td>Lunch</td>
</tr>
<tr>
<td>1:00 – 3:00 pm</td>
<td>LaBonne Laboratory</td>
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<tr>
<td>Pancoe 3321</td>
<td>• Fluorescence Microscopy</td>
<td>Dr. LaBonne</td>
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<tr>
<td>Pancoe 3103</td>
<td>• Panel Discussion</td>
<td>NU undergraduate students</td>
</tr>
<tr>
<td>3:00 – 3:30 pm</td>
<td>Departure</td>
<td></td>
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<tr>
<td>Pancoe 3103</td>
<td>• Evaluations</td>
<td>Dr. Murdoch</td>
</tr>
<tr>
<td></td>
<td>• Pick-up personal items</td>
<td></td>
</tr>
</tbody>
</table>
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Northwestern University Biology Investigations in Reproduction and Development

Goldberg Group

Erwin Goldberg, PhD

Fern Murdoch, PhD
Huanghui Tang, PhD
Chongwen Duan, PhD
Pamela Monahan, PhD
Rexxi Prasasya

LaBonne Group

Carole LaBonne, PhD

Joe Nguyen
Elsy Buitrago Delgado
Kara Nordin
Maneeshi Prasad, PhD
Guiding Questions and Outcomes

Guiding Question: How do gametes produce a new individual?

<table>
<thead>
<tr>
<th>Laboratory Questions</th>
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<tbody>
<tr>
<td>• What are the structures of the male reproductive system?</td>
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<tr>
<td>• How does fertilization happen in sea urchins?</td>
</tr>
<tr>
<td>• How does fertilization happen in frogs?</td>
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<tr>
<td>• How can we track the development of embryonic cells?</td>
</tr>
<tr>
<td>• How can exposures during development cause birth defects?</td>
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</tbody>
</table>

Learning Outcomes:
Identify male reproductive anatomical structures.
Describe embryonic and early fetal development.
Describe how environmental factors can affect embryonic development.
Describe the usefulness of animal models.
Describe how fertilization occurs in sea urchins and in frogs.
Describe how embryo microinjection works and why it is used.
Male Reproductive Vocabulary

**Penis** – the male organ of copulation and, in mammals, of urinary excretion

**Testis** – the male gonad or reproductive gland; either of two oval glands located in the scrotum that produce spermatozoa

**Scrotum** – the pouch of skin that contains the testes

**Epididymis** – coiled tubule next to the testes where sperm mature and may be stored for a short time

**Vas Deferens** – the duct that transports the sperm from the epididymis to the penis

**Urethra** – part of both the urinary and reproductive system that passes urine and sperm outside of the body

**Prostate Gland** – a donut shaped gland that adds fluid to the semen

**Seminal Vesicles** – ducts that are about 5 cm long that add nutrients and fluid to semen

**Ejaculatory Duct** – canal that passes from the seminal vesicle and vas deferens, conveying semen to the urethra
Male Reproductive Vocabulary

**Spermatozoan** – the male reproductive cell, usually consisting of a round or cylindrical nucleated cell (head), a short neck (midpiece), and a thin motile tail (flagellum)

**Spermatocyte** – a diploid cell that undergoes meiosis to form four spermatids, haploid cells which then develop into sperm

**Spermatid** – one of the cells that result from the meiotic divisions of a spermatocyte and mature into spermatozoa

**Spermatogonium** – a cell in the male gonads that undergoes mitosis to form spermatocytes

**Seminiferous Tubules** – one of two or three twisted, curved tubules in each lobule of the testis in which spermatozoa develop

**Sertoli Cell** – elongated cells found in the seminiferous tubules of the testis where spermatids attach during spermiogenesis for nourishment

**Leydig Cell** – a cell in the testes that secretes the hormone testosterone
Male Reproductive Vocabulary

**Acrosome** – an organelle covering the head of animal sperm and containing enzymes that digest the egg cell coating, thus permitting the sperm to enter the egg

**Tail** – allows the sperm to swim toward the egg

**Middle Piece** – contains energy-producing mitochondria
Fertilization and Development Vocabulary

**Polar Body** – a small cell containing little cytoplasm that is produced along with the oocyte and later discarded

**Vitelline Membrane** – the membrane enveloping the egg

**Zona Pellucida** – a thick, solid, transparent outer membrane of a developed mammalian ovum; can be penetrated by one sperm in the fertilization process; usually remains around the fertilized egg until it is implanted in the wall of the uterus

**Fertilization** – the process by which a male and female gamete fuse to form a zygote

**Fertilization membrane** – specialized membrane that form around the fertilized egg to prevent entry by additional sperm

**Polyspermy** – the fusion of more than one sperm with an egg

**Pronucleus** – the haploid nucleus of a sperm or egg before fusion of the nuclei in fertilization

**Cleavage** – a series of synchronized mitotic cell divisions immediately following fertilization that results in the formation of the blastomeres and changes the single-celled zygote into a multicellular embryo

**Other Vocabulary**

**Fluorescence microscopy** – a type of light microscopy in which the specimen is irradiated at wavelengths that excite a fluorophore, causing it to emit light through fluorescence; can be used to track which cells a particular blastomere gives rise to
What are the structures of the male reproductive system?

How is the male reproductive tract adapted to produce millions of sperm a day?

In what ways might the sperm in other species resemble the sperm that human males produce?

Questions to Think About:

Students Notes or Questions:

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Diagram of male reproductive system
How does fertilization happen in sea urchins?

Female Urchin shedding eggs after Potassium Chloride (KCI) injection

The spawn of males is creamy white and should be left undiluted until used.
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Goldberg Lab Station 1: Sea Urchin Gamete Collection
Goldberg Lab Station 1: Sea Urchin Gamete Collection

Spawning

Sea urchin and human

Sperm comparison

NOTE THE 5 MICRON SIZE.
Remember –
1000 microns = 1 mm
25 mm = 1 inch

Egg comparison

NOTE THE 100 MICRON SIZE
Fill a 1 ml syringe fitted with a 0.5 inch 25 gauge needle with 0.55 M KCl solution. You lab leader will assist with this.

Fill a beaker to the brim with sea water: this will be needed if your specimen is a female. Also, open a small plastic Petri dish: this will be needed if your specimen is male.

Inject 0.2 ml portions of KCl in 2-3 places around the mouth

Return the injected urchin to a pad of paper toweling, oral side down, and monitor the genital pores on the aboral surface for the emergence of gametes.

If the exuding material is a milky-white fluid, your specimen is **male** and should be inverted immediately over the plastic Petri dish.

If the exuding material is pigmented (pinkish-red, orange, or creamy-yellow) and granular, your specimen is a **female** and should be inverted immediately over the beaker so that the genital pores are beneath the surface of the water.

Questions to Think About:

- How does working with sea urchins help researchers understand how fertilization works in humans?
- Why do researchers use sea urchins in particular to study fertilization?
**Viewing Gametes**

**Eggs (Ovum).** To view eggs, remove a sample of eggs and seawater from the beaker with an transfer pipette. A good egg: seawater mixture is \(\frac{1}{4}\) eggs and \(\frac{3}{4}\) seawater. Place a drop of egg solution on a flat slide, add cover slip. Begin with the 4X objective on your microscope and find an egg to view. Increase magnification until you can see the pronucleus of the ovum.

**Sperm.** To observe active sperm combine one drop of “dry” sperm with sea water in a small beaker. Place a drop of solution on a flat slide, add cover slip. Begin with the 4X objective on your microscope and increase magnification carefully. Sperm are much smaller than eggs.

**Viewing Fertilization**

1. Place one drop of your egg-seawater mixture (\(\frac{1}{4}\) eggs and \(\frac{3}{4}\) seawater) on a flat slide, cover with a cover slip being careful not to “squash” your eggs. Bring the eggs into focus and move to higher magnification.

2. Prepare sperm by combining one drop of “dry” sperm with 10-20 ml of sea water in a small beaker. **NOTE:** Sperm lose their ability to fertilize eggs approximately 10 minutes after they have been mixed with seawater. Prepare your sperm mixture and act quickly!

3. Add sperm to your slide by placing one drop next to (touching) the coverslip. The surface tension will pull the sperm under the coverslip to the eggs.

Fertilization may occur in as little as 30 seconds, but may take up to two minutes. You should be able to observe swimming sperm as they gather around the eggs. When the sperm penetrates the egg, the fertilization coat will begin to rise. The fertilization coat may first appear as a bubble but will eventually completely surround the egg.

**Questions to Think About:**

How does working with sea urchins help researchers understand how fertilization works in humans?

Why do researchers use sea urchins in particular to study fertilization?
**Observation of Cleavage**

1. Slides with live urchin embryos have been prepared by your lab instructors. The time of fertilization is noted.

2. Permanent slides of preserved urchin embryos are also available.

3. Examine slides at various times after fertilization. Look for the features in the figures at right. Determine if the stage of development matches the development timeline seen under ideal conditions in the table below.

<table>
<thead>
<tr>
<th>Developmental Stage</th>
<th>Time after fertilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formation of fertilization coat</td>
<td>2-5 min</td>
</tr>
<tr>
<td>First cleavage</td>
<td>60-70 min</td>
</tr>
<tr>
<td>4 cell embryo</td>
<td>2 hours</td>
</tr>
<tr>
<td>8 cell embryo</td>
<td>3 hours</td>
</tr>
<tr>
<td>16 cell embryo</td>
<td>4 hours</td>
</tr>
<tr>
<td>32 cell embryo</td>
<td>5 hours</td>
</tr>
<tr>
<td>Early blastula</td>
<td>9 hours</td>
</tr>
<tr>
<td>Blastula</td>
<td>11 hours</td>
</tr>
<tr>
<td>Gastrula</td>
<td>32 hours</td>
</tr>
<tr>
<td>Pluteus (larval form)</td>
<td>48-68 hrs</td>
</tr>
<tr>
<td>Metamorphosis to adult</td>
<td>&gt; 5 weeks upon environmental cue</td>
</tr>
</tbody>
</table>

**Questions to Think About:**

How does working with sea urchins help researchers understand how fertilization works in humans?

Why do researchers use sea urchins in particular to study fertilization?
How does fertilization happen in frogs?
Labonne Lab Station 1: Frog Fertilization

Adult female and male Xenopus Laevis

Unfertilized Xenopus eggs inside their jelly coat

A white spot at the animal pole indicates GVBD (Germinule Vesicle Break Down) within the oocytes
**How does fertilization happen in frogs?**
**LaBonne Lab Station 1: Frog Fertilization**

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>The night before the frogs were “primed”</td>
<td>Let them incubate for ten minutes to allow sperm entry.</td>
</tr>
<tr>
<td>to lay eggs by injection with human chorionic gonadotropin</td>
<td>The first sign of successful fertilization is a contraction of the pigmented animal hemisphere as a result of the block to polyspermy.</td>
</tr>
<tr>
<td>Primed frogs are”squeezed” to release matured eggs into a petri dish</td>
<td>Flood the dish with water for an additional thirty minutes to allow cortical rotation.</td>
</tr>
<tr>
<td>Cut a thin slice of pre-removed testis and place into a quarter-sized droplet of water</td>
<td>Remove the jelly coat from the embryos by incubation in cysteine hydrochloride.</td>
</tr>
<tr>
<td>Macerate the testis with forceps to release sperm</td>
<td>Rinse well with a low salt solution and incubate until cleavage.</td>
</tr>
<tr>
<td>Mix the eggs and sperm together with a glass pipette</td>
<td></td>
</tr>
</tbody>
</table>

**Questions to Think About:**

How does working with frogs help researchers understand how fertilization works in humans?

Why do researchers use these types of frogs in particular to study fertilization?

**Students Notes or Questions:**

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____________________________________________________________________
How can we track the development of embryonic cells?
LaBonne Lab Station 2: Embryo Microinjection

Microinjection of green fluorescent protein into 2-cell embryo visualized one day later

Microinjection station

Cleavage stage *Xenopus* embryos

Questions to Think About:

- Does it damage the embryo to have the fluorescent protein injected into it?
- Why is it useful to research embryonic development?

Students Notes or Questions:
Experiment 4: *Xenopus laevis* Injection

1) Calibrate injection needle

2) Draw solution for injection slide into needle from a droplet on a glass slide

3) Set 2-cell-staged embryos in 3% Ficoll solution to prepare embryos for injection

4) Transfer approximately 30-50 embryos onto injection slide in a large enough volume of 3% Ficoll to completely submerge the embryos

5) Hold one embryo stable with a forceps in your left hand

6) Using your right hand, insert the injection needle into the embryo targeting the desired tissue

7) Using floor pedal inject desired volume of solution into the embryo

8) Carefully withdraw needle from embryo and move to next embryo

You will be injecting a fluorescent dye called Rhodamine Dextran.

It is used as lineage tracer to follow the fate of injected cells.

We will view your injections after lunch.
Fluorescent dye injected into early blastomeres marks the cell and its progeny so that the tissues they give rise to can be identified.

Questions to Think About:

Why is it useful to identify the origin of different tissues?

Does this procedure differ when it used with the embryos of different animals?
Alcohol (ethanol) is a known teratogen. Teratogens are chemicals that can interfere with normal development leading to birth defects. Prenatal exposure to alcohol can result in several birth defects including:

- Brain damage
- Cognitive defects
- Epilepsy and seizures
- Microcephaly (small head size)
- Craniofacial abnormalities

The extent of the defects of fetal alcohol syndrome is determined by three factors:

- The amount of alcohol consumed
- The time of exposure - when during pregnancy the alcohol is consumed
- Frequency of consumption during pregnancy

There is no recognized “safe” amount of alcohol that can be consumed during pregnancy.

In this module, you will examine the effects of ethanol on the zebrafish.

Zebrafish have clear embryos which will allow us to look at various embryonic structures after ethanol exposure.

You will be testing two factors that contribute to the effects of ethanol on birth defects using zebrafish embryos.

1. The amount, or concentration, of ethanol
2. The time when ethanol is present

You will compare ethanol treated animals to untreated controls to see if ethanol causes brain or jaw deformities in the fish embryos.
**How does alcohol affect the physical development of zebrafish?**

LaBonne Lab Station 3: Zebrafish Alcohol Experiment

<table>
<thead>
<tr>
<th>Part 1: Testing ethanol effects on zebrafish</th>
<th>Part 2: Examining embryos 3 days after Ethanol treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1.</strong> Collect zebrafish embryos and determine what stage of development they are at.</td>
<td>The experiment you conducted today was also run 3 days ago. These embryos were exposed to ethanol at 2 different stages.</td>
</tr>
<tr>
<td><strong>2.</strong> Divide the embryos up into 4 petri dishes.</td>
<td>Examine these embryos and determine if any developmental abnormalities are apparent.</td>
</tr>
</tbody>
</table>
| **3.** Immerse the embryos in different amounts of ethanol:  
  1. 0.0% Ethanol (Control)  
  2. 0.5% Ethanol  
  3. 1.0% Ethanol  
  4. 1.5% Ethanol | What do you see? |
| **4.** After 10 minute incubation in ethanol, remove the ethanol solutions with a pipette and rinse the embryos with embryo water. | __________________________________________ |
| **5.** Observe your embryos under the microscope to check for any immediate changes in appearance. | __________________________________________ |
| **6.** Set embryos in 28C incubator in hallway. | Did the time of treatment matter? |

**Questions:**

What stage were your embryos in when you added Ethanol?

__________________________________________

Did your embryos change appearance immediately after the ethanol treatment?

__________________________________________

Was the effect greater at higher amounts of ethanol?

__________________________________________
How can we track the development of embryonic cells?

LaBonne Lab Station 4: Fluorescence Microscopy

** Viewing your injected embryos

Observe the fluorescent microscope, its working parts, and how images are captured digitally by the camera.

Observe your injected embryos using the fiber optic light and then with the fluorescent light.

Observe older embryos injected with Rhodamine dextran.

Take a picture of your injected embryos.

Three blastomeres injected with three different dyes, and the progeny of those cells one day later.

Injections such as these tell scientists which tissues (for example, brain or heart) that a specific cell in the early embryo will contribute to.

This allows experiments to be designed that can tell us how a particular tissue is formed by that cell.

** Questions to Think About:

How do researchers develop techniques such as fluorescence microscopy? Are the techniques useful in other fields?

How long do the dyes last? Would researchers find longer or shorter-lasting dyes useful?

** Students Notes or Questions:___________________

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