7.013 Problem Set 5 FRIDAY April 2nd, 2004

Problem sets will NOT be accepted late.

Problem 1

A great medical revolution has occurred with the ability to assist the large number of couples that previously would be incapable of having children. Generally, about half of infertility cases are due to paternal issues.

Congratulations, after inspiration from 7.013 you decided to forsake a lucrative career in computer science and enter the world of medicine. After an additional four years of medical school, and numerous years of training you are fiscally broke, but are now a medical professional treating infertility.

A young and otherwise healthy couple approaches you after experiencing difficulty in conceiving despite over one year of attempts. The woman has had a child from a previous husband. You suspect that the man has infertility issues and begin a work-up to determine the problem.

A. A sample of freshly collected semen is analyzed microscopically for sperm number and activity. Unlike the videos shown in class of vigorous sperm ascending towards the egg, the sperm sample indicates a normal number of sperm swimming rather lazily in a dish.

a. How many sperm are typically present in a human ejaculate? Why are there so many?

There are approximately $5x10^7$ sperm in a normal ejaculate. That many sperm are necessary because very few sperm actually reach the egg, and the more sperm are included the higher the chance for fertilization.

b. What (other than an actual defect) might explain the languorous behavior of the freshly ejaculated sperm *in vitro*?

Sperm require capacitation by the vaginal environment in order to achieve full activity.

c. Human ejaculate is a complex mixture of many substances. One of the primary ingredients is fructose (a simple disaccharide). What two properties of sperm may explain the presence of fructose in ejaculate?

Sperm are very small and highly motile. They have little storage for energy sources but have high energy demands. The presence of fructose can supply the sperm with fuel for their journey.

B. After determining that the number, morphology, and activity of the sperm are normal, you next seek to test whether the sperm are capable of fertilizing an intact egg. However, with human eggs hard to come by you are forced to use hamster eggs. Hamster eggs are easily obtained by hormonal treatment of female hamsters inducing super-ovulation. A large number of eggs can be recovered by removal and gentle tapping of the uterine horn with eggs dropping out into a dish.

a) What hormone was likely used to induce superovulation of the hamster? What is the normal fate of these extra eggs (without the introduction of hormone)?

FSH stimulates the development of more immature follicles to produce mature ovum. Normally, in humans, only a single egg is released and the rest of the follicles recede and the immature ova die.

b) Have the collected eggs completed meiosis? If not, at what meiotic stage are the eggs frozen? What prompts the completion of meiosis?

No, at the time of ovulation eggs are frozen at Metaphase II. Only fertilization of the egg is able to induce the completion of meiosis.

c) Would you expect a human sperm to fertilize an intact hamster egg? What component of the hamster egg prevents fertilization from occurring?

No, human sperm would not be able to bind to the outer coating of the egg called the zona pelucida.

C. A simple enzymatic treatment of the hamster eggs can effectively remove the component that you named above. After removal of this component, human sperm can now fertilize the treated hamster egg to form a zygote. Worry not; the resulting zygote cannot actually develop into a human/hamster hybrid.

a) Assuming that the man's sperm can fertilize the treated hamster egg, at what stage of fertilization are the man's sperm possibly failing.

Because the sperm are active, and capable of fertilizing an egg lacking a zona, it is likely that the sperm are either failing to bind to the zona or there is a defect in the acrosomal reaction.

b) Are the couple's plans for children doomed? What mode of ART would you suggest to overcome the man's difficulties?

No, the sperm can be injected directly into mature eggs harvested from his wife. This is called Intracytoplasmic Sperm Injection (ICSI)

Problem 2

Teratocarcinoma is a rare form of cancer that typically forms on the testes or ovary. As opposed to most cancers that are composed of accumulations of cells that are similar in appearance, teratocarcinomas are quite heterogenous. Both gross and histologic examination of a teratocarcinoma reveal a variety of differentiated tissues present within the cancerous mass including: hair, teeth, intestinal epithelium, skin, etc..

a) Was the cell that gave rise to the teratocarcinoma differentiated?

No, the presence of the many different differentiated cell types within the tumor suggests that the primary tumor was made up of non-determined non-differentiated cells.

b) Assuming that the teratocarcinoma cells can give rise to all the tissues present in an adult how would you describe their potency?

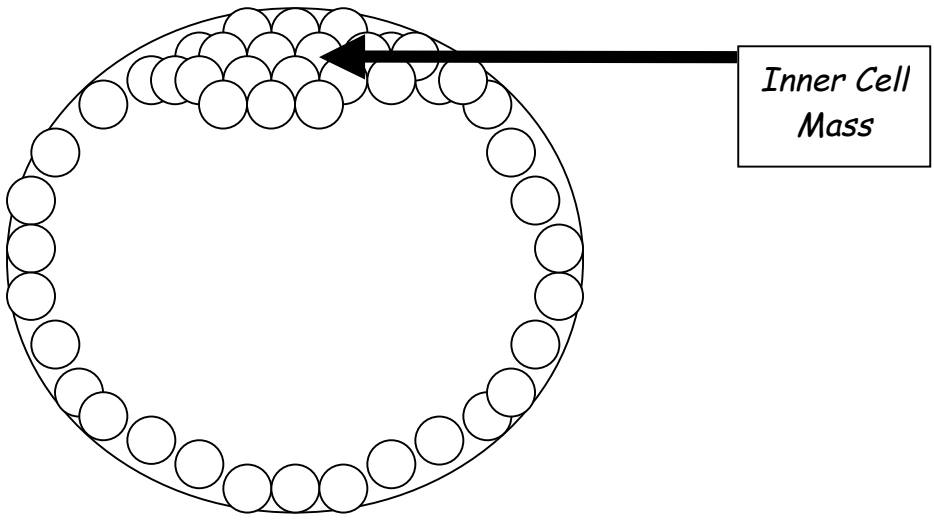
The teratocarcinoma cells are totipotent.

Teratocarcinoma cells have proven quite useful *in vitro* (grown in culture) to define both inducers and determinants of cell differentiation. Exposure of undifferentiated teratocarcinoma cells to a given extracellular signal results in their specific differentiation into a given cell fate.

c). Despite their similar potency as embryonic stem cells, teratocarcinoma cells are not used in vivo and have no therapeutic potential. Why not?

As opposed to embryonic stem cells, teratocarcinoma cells are cancerous. In general one does not want to inject cancer as a therapy.

Below is an illustration showing the cross-section of a blastocyst…



d) Label the section of the blastocyst that gives rise to embryonic stem cells.

Grown in culture, embryonic stem cells form colonies of cells. You would like to use the cells to identify novel determinants of differentiation.

You expose a plate of ES cells to retinoic acid (Vitamin A). Immediately after exposure the ES cells begin to express Gene N. One week after exposure you observe that the cells in the dish have the appearance of mature neurons.

e) Please match the items below with all of the terms that apply.

i. ES cell	1. Inducer
ii. Retinoic acid	2. Undifferentiated Cell
iii. Cells after exposure to retinoic acid	3. Terminally Differentiated Cell
iv. Neurons	4. Determined Cell

i.- 2 ii.-1 iii.-4 iv. 3,4

You wish to explore whether Gene N is acting as a determinant for neuron formation. Transfection is the process by which a DNA plasmid can be put into a eukaryotic cell (similar to transformation in bacteria).

f) What experiment could you perform to test the hypothesis that Gene N acts as a determinant in neuron formation?

Transfect a plasmid that expresses Gene N into the ES cells and see if this can induce neuron formation in the absence of retinoic acid, the inducer.

g) Can the nucleus of a differentiated cell, such as a neuron, be used to clone an animal? If so, why?

Yes, with few exceptions (immune cells), none of the changes that take place during differentiation involve changes to the sequence of the DNA. The nucleus can be transferred into an enucleated egg which is then activated. This "resets" the differentiation program of the nucleus and results in a totipotent zygote.

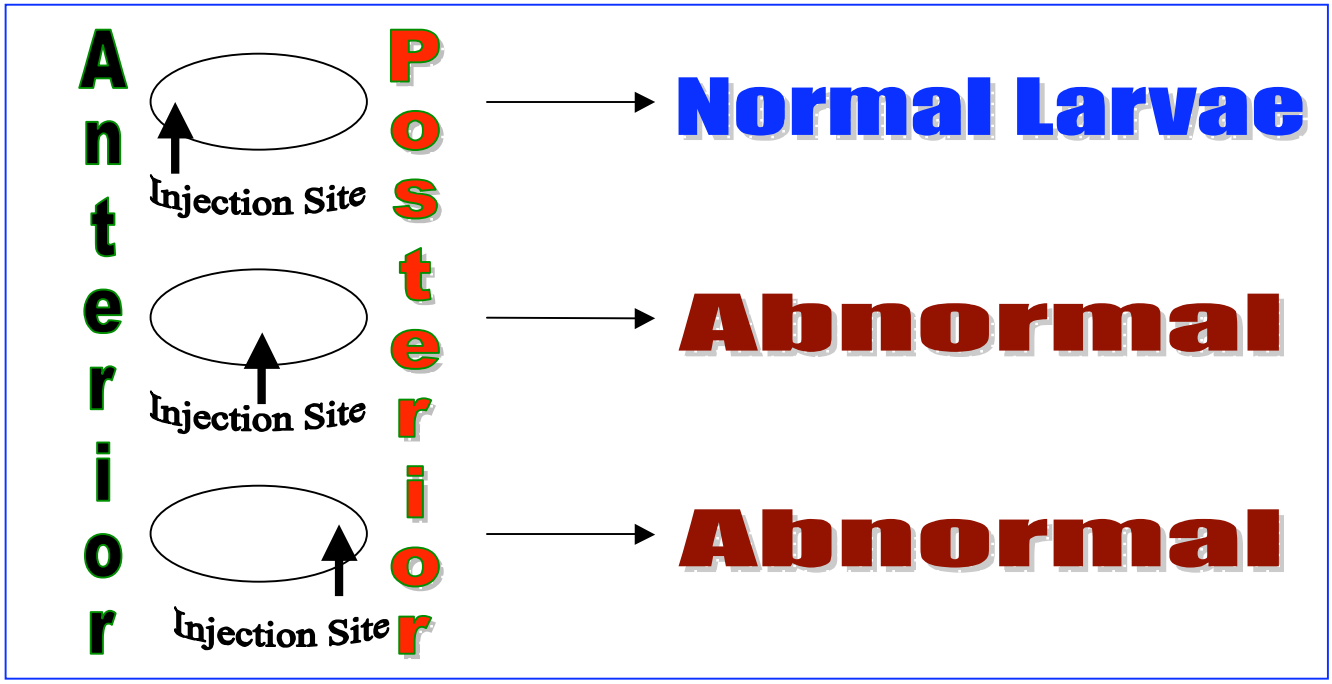
Problem 3

A large class of mutants in *Drosophila melanogaster* (fruit flies) result in female sterility. The defects in this class of mutants do not result from failure to produce eggs, but due to defects in offspring development. By comparison there are a paucity of mutants that cause male sterility due to similar defects.

a) Why may there be this discrepancy between the male and female flies? Why can you get a generation of homozygous mutants?

Female flies supply many gene products to the egg that are necessary for development. The homozygous mutants can exist, because their mother was a heterozygote and thus could supply the necessary genes to its eggs. This existence of maternal mRNAs in the egg are referred to as the MATERNAL EFFECT.

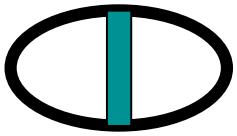
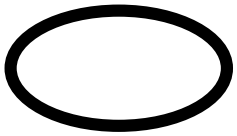
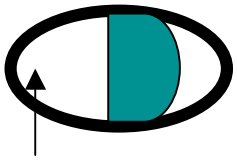
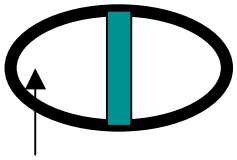
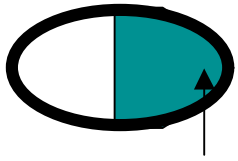
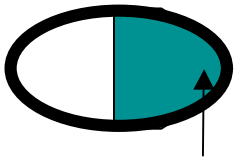
One such mutation is in the gene *biocoid.* Mutation of bicoid results in the formation of larvae lacking the anterior segments that normally develop into the head and thorax. To determine the mechanism of bicoid function your peform the following mRNA injections into Drosophila eggs prior to fertilization:



b) What may explain the sensitivity of development to the site of injection? What normally establishes this polarity?

Bicoid is normally present as mRNA in the egg with an anterior to posterior gradient that is established by the maternal nurse cells that border the egg.

You next look at the expression of a second gene (Gene B) that you believe may be regulated by bicoid. You perform a similar injection experiment, injecting bicoid mRNA into either bicoid mutant eggs or wildtype eggs and look for the pattern of expression of **Gene B**.

Genotype	Injection Site	Gene B Expression
Wildtype	None	
Bicoid Mutant	None	
Wildtype	Anterior	
Bicoid Mutant	Anterior	
Wildtype	Posterior	
Bicoid Mutant	Posterior	

c) Explain the likely function of Bicoid in the expression of Gene B?

Bicoid appears to activate Gene B expression.

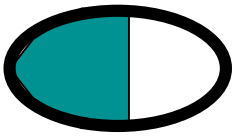
d) Is bicoid expression on its own sufficient for expression of Gene B?

Hypothesis 1: No, there appears to be a posterior factor that is also required for Gene B expression. This factor is present in a gradient from posterior to anterior (P→A).

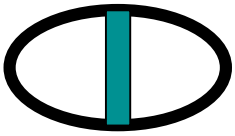
*Hypothesis 2: Yes, Bicoid activates gene B expression, but there is also an inhibitor with a **steeper** high to low gradient from the anterior to posterior end (A→P). This inhibitor limits gene B expression to the middle of the embryo.*

e) For a wild-type egg, draw the likely pattern of Gene B expression if some of the cytoplasm at the posterior of the egg were transferred to the anterior part.

Hypothesis 1



Hypothesis 2



f) For a bicoid mutant egg, draw the likely pattern of Gene B expression if some of the cytoplasm at the posterior of the egg were transferred to the anterior.