

Zoology 470 - Exam #1 – 2008

This exam has **5** pages and a total of **50 points**. You will have **90 minutes** to complete it. Answer all short answer questions as briefly as possible. **Make sure your name and ID number are on all pages.** Good luck!

1. Provide concise definitions for each of the following (**6 points**)

a. RESACT: *A peptide derived from egg jelly in sea urchins. RESACT acts as a chemoattractant for sperm.*

b. axoneme: *Microtubule-based structure that forms the core of flagella and cilia. The axoneme has a characteristic "9 + 2" microtubule structure in cross-section, in which the outer doublet MTs are connected to the central pairs by spoke proteins, and the outer doublets are connected to one another by linker proteins and flagellar/ciliary dynein.*

c. "Organizer": *Originally identified by Hans Spemann and Hilde Mangold, this group of cells that originates at the dorsal lip of the blastopore in amphibian gastrulae is responsible for inducing nearby cells to become the main embryonic axis. Implanting this tissue elsewhere will induce a secondary axis.*

2. Reproduction in humans is regulated by hormones. A major pharmaceutical company has used this fact to develop commercial products.

a. One way to produce "superovulation" (i.e., production of more than one oocyte at a time) for in vitro fertilization uses an estrogen-blocking drug called Clomiphene. Based on your knowledge of hormonal signaling during ovulation, explain why this would promote ovulation (**2 points**):

At a particular point in the ovulatory cycle, estrogen exerts negative feedback on the hypothalamus (and hence, indirectly, on the anterior pituitary). By blocking estrogen, this inhibition is removed, cause multiple follicles to mature simultaneously.

b. A second product produced by this company is a home pregnancy test that tests for successful conception (i.e., fertilization) within 7 days of the event. What hormone does this product test for? (**1 point**)

Hormone: _____ *hCG* _____

c. RU486 interferes with the normal function of progesterone during early pregnancy. What tissue is the most important target of progesterone that is affected by the drug? (**1 point**)

Tissue: _____ *uterus* _____

d. What tissue(s) or cells normally produce progesterone? (**1 point**)

Tissue/cells: _____ *corpus luteum in the ovary* _____

3. Circle true (T) or false (F) for each of the following (7 points)

F	Targeted gene manipulation by homologous recombination is a technique that is specific to mouse ES cells, and not generally applicable to other mammals such as humans
T	Unlike some European Union countries, the United States has not passed legislation outlawing human cloning
F	Smoothed receptors are important in embryos because they allow attachment of migrating cells to the extracellular matrix
T	Unlike mammalian sperm, sea urchin sperm produce a long acrosomal process
F	Hans Driesch's experiments involving separation of sea urchin blastomeres indicate that the right and left halves of early sea urchin embryos are autonomously specified in the 2- or 4-cell embryo
T	The slow block to polyspermy in mammals involves enzymatic remodeling of the zona pellucida, but not production of a fertilization envelope
T	Wnt signaling sometimes leads to accumulation of β -catenin in the nucleus of the responding cell, leading to changes in its state of differentiation
F	If a female zebrafish is homozygous for a maternal effect mutation that is lethal and she is mated with a wild-type male, one quarter of her offspring will die
F	Cloning by somatic cell nuclear transfer is considered to be too difficult and expensive to use it to clone pets
F	Once sperm are produced following meiosis, their final maturation occurs in the seminiferous tubule
F	In mammalian cloning experiments, Dolly was a surprise, because cloning from embryonic cells had previously been shown to be impossible in mammals
F	Growth factors typically act on cells far from their site of production
F	Cloning by nuclear transfer in frogs indicates that it is easier to obtain normal embryos when the donor nucleus comes from highly differentiated cells than from embryonic cells
F	Altered nuclear transfer (ANT) has been proposed as an ethically acceptable way to generate human ES cells, because ANT results in trophoblast cells without functional inner cell mass cells

4. Isolation of cell with extensive developmental plasticity is a major goal of regenerative medicine.

a. Provide **two** pieces of evidence that pre-compacted mammalian embryos are **totipotent**. (3 points)

Evidence #1: *Allophenic mice – combining three embryos to form a single giant, yet normally differentiating, blastocyst*

Evidence #2: *Embryo splitting/blastomere isolation: separated blastomeres will develop into complete embryos*

Others: blastomere removal -> normal embryo

4 (cont) c. Are inner cell mass cells, from which embryonic stem cells are produced, *totipotent*? **Explain your answer. (2 points)**

Are ICM cells totipotent? **Circle one:** Yes No

Explanation: *ICM cells are pluripotent; they cannot make extraembryonic cells under normal circumstances.*

d. You are in Doug Melton's lab at Harvard, and you are competing with Jamie Thomson's group to find new ways to perform "induced pluripotency". You have induced cells that you believe are pluripotent. Describe **one** experiment that would show that your induced cells are pluripotent, and not merely multipotent **(2 points)**:

Several possibilities: (1) chimeric mouse; the cells engage in the full range of fates; (2) teratoma formation in a nude mouse; (3) could also accept testing pluripotency in vitro using a variety of growth factor/exogenous factor treatments, and showing that they can make lots of things beyond standard definitions of multipotency

5. You are studying ZP3 in the laboratory of its discoverer, Paul Wassarman.

a. You and others have used a variety of techniques to study ZP3's function. Match the statements about ZP3 on the left with the appropriate response from the right-hand column that could be used to obtain that information. **Note: Choices from the right may be used more than once, but each discovery should be matched with the single best answer from the right-hand column (3 points)**

Discovery	Possible technique(s) used
ZP3 mRNA is present at high levels in the granulosa (cumulus) cells of the ovary ___g___	a. Immunostaining
The glycosylated form of ZP3 protein is 130 kilodaltons in size ___d___	b. Northern blot
The absence of ZP3 surrounding the oocyte results in defects in follicle formation ___e___	d. Western blot (immunoblotting)
The rat ZP3 mRNA has an approximate size of 1.3 kilobases ___b___	e. Targeted mutation ("knockout")
After fertilization, individual zona pellucidat were examined microscopically and found to lack ZP3 protein ___a___	f. DNA sequencing
When mice overexpress ZP3, their oocytes are more easily fertilized than wild-type ___h___	g. in situ hybridization
	h. None of the above

b. You are a conservation biologist testing whether or not proteins found in the zona pellucida around the oocytes of a rare rainforest monkey confer sperm binding. Assume you can biochemically purify all of the major proteins from the zona, and that standard in vitro fertilization techniques are possible. Describe **one** way that you could identify which protein(s) confer sperm/egg binding **(3 points)**:

Use a competition experiment like that used to show that glycosylated ZP3 is the protein to which bind in the zona. The protein in the monkeys that is the corresponding protein should effectively compete away binding to sperm when sperm are preincubated with the protein. Other answers that are possible: radiolabeling the proteins, and then see which binds to sperm, or similar answers.

6. Cytostatic factor (CSF) is known to regulate meiotic cell divisions. Complete the following table, which predicts the level of CSF in oocytes or zygotes under a variety of conditions (3 points)

Type of oocyte/zygote	CSF activity (high/low)
Frog primary oocyte	Low
A fertilized frog zygote immediately after completion of all meiotic divisions	Low
Mature frog oocyte 1 hr after treatment with a calcium ionophore	Low

7. Successful fertilization requires that sperm interact with the egg or oocyte in specific ways. Complete the following table regarding sperm/egg interactions under a variety of circumstances (6 points):

Type of sperm	Type of egg	Fertilization successful (yes/no)?
Sea urchin sperm treated with calcium ionophore	Normal sea urchin egg from same species with its egg jelly removed	Yes
Sea urchin sperm exposed to egg jelly	Sea urchin egg from same species pretreated with purified bindin	No
Mouse sperm extracted directly from the epididymis	Mouse oocyte with normal cumulus complex	No
Normal sea urchin sperm	Sea urchin egg held at a constant +20 mV by using an electrophysiological "voltage clamp"	No

8. Egg activation is known to involve a number of ionic and signal transduction events.

a. You inject a sea urchin egg with a calcium indicator, treat it with the Src inhibitor, BMS-354825, and then fertilize it. What effects do you expect on calcium dynamics? **Explain your reasoning (2 points)**

Src is thought to lead to activation of PLC γ , which in turns leads to calcium release from the ER as part of the calcium wave. Blocking Src would block PLC activation, leading to lack of calcium release/wave and reduced egg activation and cortical granules release as part of the slow block to polyspermy.

b. Your lab has run into funding trouble, so you cannot use calcium indicators to monitor early events in fertilized eggs, but you still want to pursue experiments involving a new drug that mimics the effects of inositol trisphosphate (IP3) on sea urchin eggs. What feature under the microscope could you look for to assess whether eggs treated with your drug behave as expected? (1 point)

Feature: _____ fertilization envelope _____

9. Cloning by somatic cell nuclear transfer in mammals is under intensive investigation.

a. Most scientists oppose using somatic cell nuclear transfer to produce human babies (reproductive cloning). What common molecular defect in the vast majority of cloned mammals leads them to adopt this position? (1 point)

Defect: *defects in epigenetic reprogramming of DNA (methylation) that normally occurs in the embryo*

b. Many scientists are in favor of therapeutic cloning for production of stem cells. Has there ever been a **documented** case of **successful** therapeutic cloning in **primates**? **Circle the correct answer.** (1 point)

Yes No

c. Dolly, the first mammal cloned by somatic cell nuclear transfer, had several known abnormalities. Name **one** abnormality (1 point)

Abnormality: _____ Possibilities: *arthritis, short telomeres* _____

10. Match the following events with the major protein system that it requires. **Note: Choices from the right may be used more than once, but each event should be matched with the single best answer from the right-hand column (4 points)**

Event	Protein required
Acrosomal process formation in sea urchins _____ <i>a</i> _____	a. actin microfilaments
Sorting out in dissociated cells _____ <i>b</i> _____	b. cadherins
Establishment of planar polarity in neural plate cells _____ <i>g</i> _____	c. microtubules
Flagellar bending in human sperm _____ <i>c</i> _____	d. integrins
Attachment of a mesenchymal cells to fibronectin _____ <i>d</i> _____	e. gonadotropin-releasing hormone
Stimulation of follicular maturation during the ovulatory cycle _____ <i>i</i> _____ (accept e) _____	f. proteoglycans
Lifting of the vitelline envelope away from the sea urchin zygote _____ <i>f</i> _____	g. Frizzled
Sliding of microtubules in the sperm flagellum _____ <i>i</i> _____	h. kinesin
	i. none of the above