Name: _____ Student ID:_____

Zoo 470 - Exam 1 - Spring 2009 – February 25, 2009

This exam has a total of **6** pages and a total of **50 points**. You will have **90 minutes** to complete it. Please put your name and student ID on all pages. Good luck!

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

a. spermatid: *Haploid product of male meiosis that, upon further differentiation, becomes a mature sperm cell..*

b. Smad: *Proteins activated by the TGF-b/BMP pathway. Smads transduce TGF-b signals to the nucleus by forming a complex that enters the nucleus to alter transcription.*

c. compaction: *Cadherin-dependent increase in adhesion of mammalian embryos at the 8-cell stage.*

d. transdifferentiation: *Process by which a differentiated cell can be converted to another differentiated cell. Recent work by the Melton lab showed this is possible in the mammalian pancreas.*

e. fate mapping: *Process by which cells in an early embryo are followed to see what progeny they make later in development. Fate mapping can involve applying bits of color to the exterior of the embryo, simply watching embryos carefully, or injection of a tracer into the cytoplasm of individual cells.*

f.: broadcast spawning: *Process of gamete release often exhibited by aquatic species, in which large numbers of gametes are released, and gametes rely on chance encounters for successful fertilization. Sea urchins are an example.*

2. MPF and cytostatic factor are known to regulate meiotic cell divisions. Answer the following questions, based on your knowledge of these factors' effects on cell division.

a. If purified CSF were injected into one cell of a two-cell embryo immediately after first cleavage, what would you predict would happen at the time of the next division? **Clearly state your reasoning.** (**2 points**)
At the next division, the injected cell would arrest at metaphase, since CSF would stabilize MPF, preventing its breakdown.

2 (cont)
b. Recall that frog eggs can be artificially activated by pricking them with a needle, initiating the same physiological changes as fertilization. What would you expect would happen to levels of MPF within a few minutes after pricking an egg? **Clearly state your reasoning**. (**2 points**)
Calcium elevation leads to CSF breakdown in such an oocyte, quickly leading to MPF inactivation (i.e., decrease in MPF activity)

3. You have isolated a gene that you believe encodes a novel sperm cell surface protein, *headline*, and you are making a presentation to a major pharmaceutical corporation for research support to work on this protein.
a. You come to the presentation armed with the following pieces of data. For each of the following facts, state the technique(s) you used to obtain your data. **If more than one technique is required, list all techniques necessary.** (**4 points**)

Discovery	**Technique(s) Used for Discovery**
When viewed under the microscope, human sperm extracted from the epididymis lack *headline* mRNA	*immunostaining*
The mouse *headline* genomic DNA region has a size of 2.6 kB	*Southern blot*
Excess *headline* protein in male zebrafish results in sperm that cannot fertilize eggs	*mRNA overexpression (would also accept transgenics, since it's fish!)*
You isolate a *single* primary spermatocyte from the testis of an infertile man, and you determine that the headline mRNA produced by this cell has a mutation at nucleotide 267.	*RT-PCR, sequencing*

b. *headline* mutant sperm are inefficient at undergoing the acrosome reaction. Suppose you find that *headline* encodes a calcium channel protein that allows calcium to enter sperm cells when they contact the zona pellucida. Describe **one** experiment that you could perform that would show that if the normal ionic events that trigger the acrosome reaction occur, the mutant sperm will undergo a normal acrosome reaction. (**2 points**)

Treat with calcium ionophore, which should trigger calcium elevation even in mutant sperm, triggering the acrosome reaction.

c. *Headline* has multiple roles in development. You learn that *C. elegans headline* is a **zygotic** gene required for embryonic survival. If a mother heterozygous for a recessive mutation in *headline* mates with a father heterozygous for the same mutation, what percentage of her offspring would you expect to survive? **(1 point)**

Percentage of dead offspring: *25%*

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4. Regulation of reproduction in humans involves a number of secreted factors.
a. For each of the following identify the source of the hormone or factor and the primary tissue(s) affected by the hormone or factor. (**2 points**)

Hormone/factor	**Source**	**Target Tissue**
gonadotropin releasing hormones	*Hypothalamus*	*Ant. pituitary*
follicle stimulating hormone (FSH)	*Ant. Pituitary*	*Ovary (follicle)*

b. The standard birth control pill suppresses ovulation in human females. In addition to estrogen, the standard birth control pill contains one other steroid hormone. Based on what you know about the inhibitory effects of hormones during the ovulatory cycle, what is this hormone? (**1 point**)

Hormone: *progesterone*

5. Sperm-egg binding is crucial for normal development, and can be affected by many factors. Please complete the following table, which asks whether or not a sperm will bind to beads or eggs, based on your knowledge of the acrosome reaction and sperm/egg interactions in various species. (**6 points**):

Sperm type	**Egg type**	**Fertiliza-tion (yes/no)**	**Reasoning**
Sea urchin sperm not exposed to egg jelly	Sea urchin egg stripped of its egg jelly	*no*	*Sperm did not undergo acrosome reaction, so no bindin on the surface [half credit for saying something about RESACT only]*
Untreated sea urchin sperm	Normal eggs from a different species	*no*	*Fertilization is species-specific. In this case, bindin from one species won't interact with eggs from another.*
Human sperm extracted directly from the seminiferous tubule	Human oocyte/ cumulus complex	*no*	*The sperm hasn't undergone full maturation/capacitation*
Sea urchin sperm	Sea urchin egg held at -70 mV by a "voltage clamp"	*yes*	*Fast block can't occur, but fertilization will still take place*

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6. One cell, called EMS, gives rise to some cells that make endoderm in the *C. elegans* (nematode worm) embryo. You suspect that EMS receives a Wnt signal from a neighboring cell (called P2) that ultimately results in endoderm formation.

a. You wish to perturb the expression of the receptor on the surface of EMS that is required for this event. What kind of molecule will you perturb? (**1 point**)

Molecule: ___*Frizzled*_____

b. You suspect that the receptor clusters near the site of contact between EMS and P2. Name one technique you could use to show this. (**1 point**)

Technique: ____*immunostaining*__

7. You have identified a new species of ascidian, *Wisconsinus buckii,* that is closely related to other well characterized species, but with a difference: the one-celled zygote has a crescent-shaped region of red cytoplasm that is inherited by cells that form most of the tail muscles in the tadpole.

a. You believe that the red crescent contains localized determinants for muscle formation. **Without knowing what molecules are involved in the red crescent's activity,** describe **one** experiment that you could perform to show that the red crescent is **sufficient** for muscle formation **(3 points)**
Accepted several answers. The textbook answer is the following: Use a needle to press on the egg, redistributing the red crescent, so that some cells inherit some of the crescent material that would not normally. Then see if these cells make muscle. If they do, the red crescent material is sufficient for muscle formation.

b. The National Science Foundation has decided to sequence the genome of *W. bucki*. You cloned the *W. buckii* equivalent of *macho-1*, a gene whose mRNA, but not protein, is expressed in the yellow crescent (myoplasm) of other ascidians. What technique would you use to show that mRNA for the *W. bucklii* version of *macho-1* is in the red crescent? **(1 point)**.

Technique: *in situ hybridization*

8. Proteins associated with the cytoskeleton are important for many developmental processes.

a. Cytokinesis (physical separation of dividing cells) in sea urchin zygotes requires the actin cytoskeleton. How could you show that actin is **necessary** for this process? (**1 point**):

Treat with cytochalasin or latrunculin; this should block cytokinesis. Also accepted several possibilities relating to morpholinos. "Knockouts" got partial credit (these aren't; mice!).

b. Vanadate was used classically to show that a "motor" protein is involved in flagellar bending of sperm tails. What motor protein is disrupted? (**1 point**)

Motor protein: *flagellar dynein*

c. What molecule is hydrolyzed to generate the shape changes in motor proteins that are required for their activity? (**1 point**)

Molecule: *ATP*

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9. Circle the appropriate response (T = true, F = false) for each of the following statements. (**6 points**):

F Before Dolly, it was assumed that cloning from embryonic mammalian cells was impossible *[Cloning from embryonic nuclei had been achieved long before. It was somatic nuclei wouldn't work]*

F In the organizer experiment of Spemann, an entire second axis is formed entirely from the implanted material *[Some is formed by induced tissue from the host]*

F Hans Driesch's experiments involving separation of sea urchin blastomeres indicate that the right and left halves of early embryos are irreversibly committed in the 2- or 4-cell embryo

F Like several European Union countries, the United States recently passed legislation outlawing human cloning *[Nope; US has no such laws]*

T The ion that is largely responsible for the fast block to polyspermy is sodium

T The inner cells of 32 cell mammalian embryo would be expected to be pluripotent, since they generate the cells of the inner cell mass of the blastocyst

T In contrast to "knockouts" produced by homologous recombination, transgenic mice are useful because they can be used to assess the effects of overexpressing a gene of interest

F Growth factors act in an "all-or-none" fashion, i.e., their effects are not concentration–dependent *[They are concentration-dependent in their action]*

F RU486 prevents implantation because it binds to progesterone, preventing it from interacting with its receptor *[It binds to the receptor, not the hormone]*

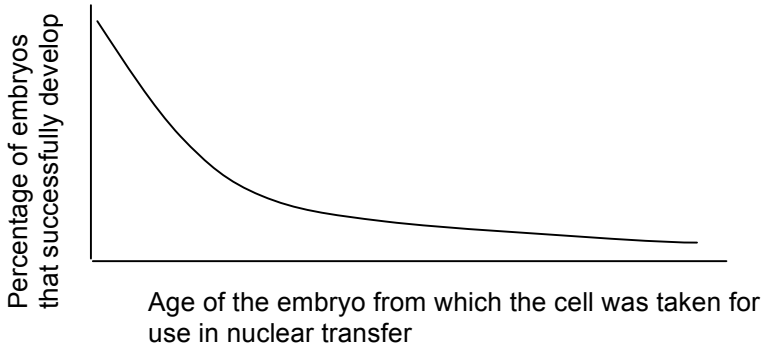
F Fertilization in humans typically occurs in the uterus *[Fallopian tube]*

T The formation of the fertilization envelope in sea urchins involves release of proteoglycans from cortical granules

T Integrins require firm attachment to the actin-based cytoskeleton

9. Cloning by nuclear transfer has been investigated in many vertebrates.

a. Early experiments on nuclear transfer involved amphibian oocytes and nuclei. Draw a graph that illustrates the relationship between the likelihood of successful development of an oocyte with a donor nucleus and the age of the embryo or animal from which the donor nucleus was obtained. (**2 points**):



9 (cont)
b. The U.S. National Academy of Science has declared that human reproductive cloning is a bad idea due to the potential for "large offspring syndrome". What major molecular defect is thought to result in this common problem in cloned mammals? (**1 point**)?

Major molecular defect: *Imprinting of growth regulatory genes, leading to defects in the expression of these genes, and hence growth defects in embryo/placenta.*

c. Although cloning by somatic cell nuclear transfer was thought to be impossible in primates, it has recently been accomplished in non-human primates. Circle the correct answer (**1 point**).

True

10. Induced pluripotent cells (iPS cells) have generated tremendous excitement as a new therapy in regenerative medicine.

a. Initial reports describing the production of iPS cells involved the introduction of four genes. What general type of proteins did these genes encode? (**1 point**)

Type of protein: _____*transcription factors*_____

b. The techniques used to test of pluripotency in mouse embryos are problematic for several reasons with human iPS cells. Describe **one** piece of evidence that would show iPS cells are pluripotent. (**2 points**).

Put into a nude mouse, they'd form teratomas. Partial credit: treating them with various things shows they can make many different cell types (this is not as stringent a test of pluripotency). Also for partial credit: putting into a mouse blastocyst. However, these are human cells, and so this is not an appropriate experiment.

c. iPS cells raise fewer ethical issue than human embryonic stem (ES) cells. Why is this true?(**2 points**)

ES cells are procured by removing the trophoblast, thereby destroying a human embryo. iPS start with cultured adult cells and transform them into pluripotent cells.