

BIO380HF—HUMAN DEVELOPMENT
Third Midterm Test—October 26, 2011
Professor Danton H. O'Day
Test Length: 50min; 100 Marks Total

Answer all questions giving the best or most appropriate answer in each case. Answers should be organized and points clearly explained or marks will be deducted for lack of clarity. No aids allowed.

KEY

1. List three types of molecules that are digested by acrosomal enzymes and give at least one example of each, mentioning how that enzyme works. Then discuss the most studied of these enzymes in detail—do not use this enzyme as the example provided above. (15 marks)

Marking: 1 mark for each type of digestion (3 marks); 2 marks for single example of each with proper explanation of how it works (6 marks), then Acrosin detail (6 marks); note if acrosin used for both protein digestion and enzyme detail (-2 marks).

Protein Digestion

Acid Proteinase: a general protease that hydrolyzes protein at an acid pH

Collagenase: a protease that digests collagen

Acrosin: A special human acrosomal protease that digests zona pellucida proteins may also mediate sperm-zona pellucida binding. However, mouse null acrosin mutants can still penetrate the zona indicating that acrosin may not be critical in the mouse.

Novel Serine Proteases: Found in mice but not yet in humans.

Carbohydrate Digestion

Beta-glucuronidase: An enzyme that removes Beta-glucuronic acid residues from carbohydrates

Hyaluronidase: An enzyme that digests carbohydrates containing hyaluronic acid residues

Neuraminidase: An enzyme that cleaves neuraminic (sialic) acid residues from carbohydrates

Lipid Digestion

Phospholipase C: A lipase that hydrolyzes phosphoinositol phospholipids, in this case, in the egg membrane

Enzyme Detail

Human Acrosin

- Acrosomal serine protease
- Bound to the inner surface of acrosomal membrane
- Digests pathway through the zona pellucida which is primarily made up of three proteins: ZP1, ZP2, ZP3
- May act with other proteins to mediate the species-specific binding of sperm to the zona and hold the sperm cell in place as it digests its way through the zona

2. Using point form, explain the different ART procedures that are available and their frequency of use. (15 marks)

Marking: 4 marks for each example of each with proper explanation (12 marks), then 1 mark each for frequency of use (3 marks).

IVF (In Vitro Fertilization) involves extracting a woman's eggs, fertilizing the eggs in the laboratory, and then transferring the resulting embryo(s) into the woman's uterus through the cervix.

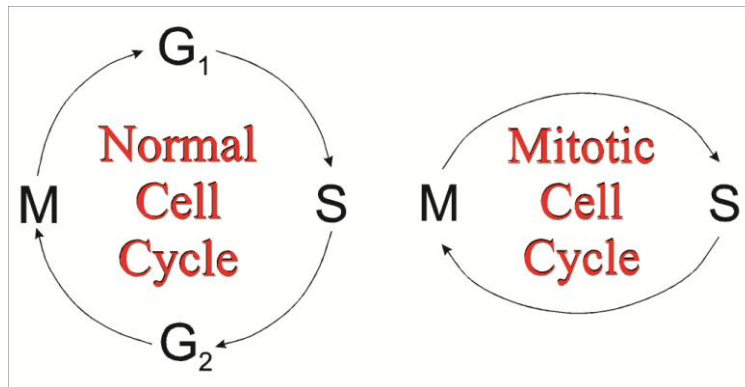
GIFT (Gamete IntraFallopian Transfer) involves using a fiber-optic instrument called a laparoscope to guide the transfer of unfertilized eggs and sperm (gametes) into the woman's fallopian tubes through small incisions in her abdomen.

ZIFT (Zygote IntraFallopian Transfer) involves fertilizing a woman's eggs in the laboratory and then using a laparoscope to guide the transfer of the fertilized eggs (zygotes) into her fallopian tubes. -Most IVF, GIFT, and ZIFT cycles used fresh, non-donor eggs or embryos.

- IVF = 73.3% of couples used standard IVF with their own (nondonor) eggs
- GIFT = 1.4% of couples used GIFT with their own (nondonor) eggs
- ZIFT = 1.8% of couples used ZIFT with their own (nondonor) eggs

3. Using drawings and point form, compare the cell cycle of cleavage versus somatic cells. Explain the significance of any differences and indicate if changes occur in the cell cycle during early embryonic development. (10marks)

Marking: 4 marks for correct drawings then 2 marks each for specific points (6 marks).



- Cleavage is characterized by a shortened cell cycle: growth G₁ and G₂ stages are by-passed so cells simply progress from S (DNA synthesis) to M (mitosis) without the intervening growth phases
- As a result cleavage cells continue to decrease in size until they approximate the size of somatic cells
- At the blastocyst stage the transition occurs to a normal cell cycle: G₁, S, G₂, M.

4. Clearly define the following abbreviations covered so far in this course. Explain their importance/significance/success and provide a specific example for each. (20 marks)

ICSI:

Intra-Cytoplasmic Sperm Injection (ICSI) involves the injection of a sperm cell into an egg via a fine needle
Success rate 50-70% of eggs injected begin development
Allow for fertilization with defective spermatozoa that would otherwise not be able to penetrate the egg naturally
Typically used when low sperm motility, abnormal shape, etc.
No congenital abnormalities result; only small increase in minor congenital anomalies except for hypospadias which may be due to hypofertility of male donors
Specific example: globospermia case report: sperm injected followed by calcium ionophore activation of egg; result: successful, normal birth

Kit:

- a receptor tyrosine kinase
- a subpopulation of cells on the most dorsomedial aspect of the neural tube express the receptor tyrosine kinase Kit (previously known as c-kit)
- These cells emigrate exclusively into the developing dermis where they develop into melanocytes—
- Cells expressing Kit are thus melanocyte progenitor cells (or, Kit+ cells were then demonstrated to represent a sub-population of neural crest cells that formed melanocytes).
- Kit expression provided direct in vivo evidence for NC lineage segregation within the mouse neural tube.

5. Explain the sequence of steps in moving sperm from their site of formation to the outside world during ejaculation. Where possible, indicate the significance of each stage in this process. (10 marks)

Sperm Transport in Males

- Sperm are produced in seminiferous tubules but are not motile (i.e., they can't move) and can't fertilize an egg (i.e., they are functionally immature although they appear mature morphologically)
- The sperm which are surrounded by testicular fluid passively move into the epididymus where they are stored and undergo biochemical maturation
- 4-12 day maturation phase in epididymus
- For ejaculation, sperm rapidly move to vas deferens
- Fluid is added increasing volume of semen and altering its characteristics: seminal vesicles--fructose for energy; prostate gland--ions, citric acid, acid phosphatase

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6. Explain why we say the “human blastocyst hatches”. Also indicate what can cause delayed and premature hatching and their significance. (10 marks)

- Hatching occurs just prior to implantation
- Embryo breaks through ZP due to proteases secreted by blastocyst
- Inability to hatch is a reason for infertility; could be due to altered zona pellucida or to the absence of essential protease for zona digestion
- Often assisted hatching is done *in vitro* during ART procedures
- In mutant mouse embryos lacking ZP1, the zona pellucida is weak and the embryos hatch prematurely which can lead to the embryo implanting in oviducts or elsewhere.

7. As clearly as possible, using point form list and explain the sequence of events in embryo implantation. Then detail what is known about the proteins involved in these events. (20 marks)

Implantation: Summary of Events (Lecture 11)

1. Apposition: of blastocyst to endometrial epithelium
2. Adherence: via cell adhesion molecules; the proteins involved are L-selectin; trophinin
3. Formation of Syncytiotrophoblast: Fusion of cytotrophoblast cells results in giant multinucleate cell that will surround complete embryo
4. Penetration: Syncytiotrophoblast is invasive and works way into uterine tissue ultimately making contact with maternal blood vessels
5. Decidual Reaction: Uterine tissue responds to embryonic invasion by setting up an immunological barrier, the decidua; thus the decidua is derived from maternal tissues

Two cell adhesion molecules have been shown to function during embryo implantation: L-selectin and trophinin.

L-selectin (Lymphocyte selectin)

- a lectin (binds carbohydrate groups) cell adhesion molecule
- present in cells of blastocyst
- binds to sugar groups on extracellular matrix of endometrial cells
- weak adhesion; involved in initial adherence
- initiates events leading to trophinin expression by maternal cells

Trophinin

- homophilic cell adhesion molecule
- once trophinin molecules appear on endometrial cells, trophinin-trophinin binding occurs
- strong adhesion event