

1  **ANAT2300 - Vertebrate Development A
Lecture 2**

School of Medical Sciences
The University of New South Wales
Dr Mark Hill
Cell Biology Laboratory
Room G20 Wallace Wurth Building
Email: m.hill@unsw.edu.au

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3  **Fertilization and Early Development**

- Gamete formation
 - Oogenesis (egg)
 - Spermatogenesis (sperm)
- Fertilization
 - migration sperm and egg
 - egg 2nd meiotic division, cortical reaction
 - sperm activation, binding, acrosome reaction
- Formation of the Zygote
 - Pronuclei male and female
 - first mitotic division
 - sex determination
- Early blastocyst formation

4  **Mitosis and Meiosis**

- Following DNA replication
 - 2 nuclear (and cell) divisions are required to produce haploid gametes
 - Each diploid cell in meiosis produces 4 haploid cells
 - Each diploid cell mitosis produces 2 diploid cells
 - only one pair of homologous chromosomes is shown
 - Text modified from MBoC

5  **Meiosis Recombination/Segregation**

6  **Female Reproductive Tract**

7  **Gamete Formation- Oogenesis**

- Process of oogonia mature into oocytes
 - ova, ovum, egg
 - all oogonia form primary oocytes before birth
- Maturation of pre-existing cells in the ovary
- Humans release usually only 1 ovum
 - IVF super-ovulation
- Follicle
 - oocyte and surrounding cells
 - primary -> secondary -> ovulation releases

8  **Egg Development**

- Egg
 - Meiotic Division by stages
- Specialized cortex/ecm
 - Cortical granules

- Zona pellucida
- Supporting cells
- Release
 - ovulation

9  Ovary Histology

10  Menstrual Cycle

11  Oogenesis- Preantral Follicle

12  Primary Oocyte

- arrested at early Meiosis 1
- diploid: 22 chromosome pairs + 1 pair X
- chromosomes (46, XX)
 - autosomes and sex chromosome

13  Oogenesis- Antral Follicle

14  Movie: Follicle Development

15  Secondary Oocyte

- 1 Day before ovulation completes (stim by LH)
- Meiosis 1
 - haploid: 22 chromosomes + 1 X chromosome (23, X)
 - Nondisjunction
 - abnormal chromosome segregation
- begins Meiosis 2 and arrests at metaphase
 - no interphase replication of DNA
- only fertilization will complete Meiosis 2

16  Second Meiotic Division

17  Ovulation

- Endocrine Axis
 - Hypothalamus (GRH) -> Pituitary FSH and LH -> ovary
 - GRH gonadotropin releasing hormone
 - FSH follicle stimulating hormone
 - LH lutenizing hormone
- release of the secondary oocyte
- formation of corpus luteum
- secondary oocyte encased by
 - zona pellucida
 - corona radiata

18  Ampulla Movement

19  Ovulation

20  Zona Pellucida

- glycoprotein shell ZP1, ZP2, ZP3
- involved in the fertilization process
- sperm binding
 - adhesion of sperm to egg

- acrosome reaction
 - releases enzymes to locally breakdown
- block of polyspermy
 - altered to prevent more than 1 sperm penetrating
- may also have a role in development of the
- blastocyst

21 Corona Radiata

- granulosa cells and extracellular matrix
 - protective cells for egg during transport
 - cells are also lost during transport along oviduct
 - nutrition

22 Testis Anatomy

23 Spermatogenesis

- process of spermatogonia mature into spermatozoa
 - sperm
- continuously throughout life occurs in the
- seminiferous tubules in the male gonad- testis
 - plural testes
- at puberty spermatogonia activate and proliferate
 - mitosis
- primary spermatocyte -> secondary spermatocyte-> spermatid->sperm
- Maturation involves meiosis and spermeogenesis

24 Human Germ Cell Formation

- Meiosis
 - reductive cell division
 - 1 spermatogonia (diploid) = 4 sperm (haploid)
- 46, XY diploid
 - also written 44+XY
 - 23, X
 - 23, X
 - 23, Y
 - 23, Y

25 Seminiferous Tubule

26 Spermiogenesis

27 Spermatogenesis- Spermeogenesis

- Cell shape change
 - round spermatids -> elongated sperm
- Loose cytoplasm
- Transform golgi apparatus
 - acrosome (in head)
- Organize microtubules for motility
 - flagellum (tail)
- Segregate mitochondria for energy
 - (in tail)

28 📺 Spermogenesis

29 📺 Ejaculate

- 200-600 million sperm, 3.5 ml
 - Oligospermia (Low Sperm Count)
 - less than 20 million sperm after 72 hour abstinence from sex
 - Azoospermia (Absent Sperm)
 - blockage of duct network
 - Immotile Cilia Syndrome
 - lack of sperm motility
- By volume <10 % sperm
- Accessory Glands
 - 60 % seminal vesicle
 - 10 % bulbourethral
 - 30 % prostate

30 📺 Sperm and Egg Migration

- Sperm migration
 - deposited in vagina
 - have approximately 24-48h to fertilize
 - migrate through cervix into uterus
 - from uterus into oviduct (=uterine horn, fallopian tube)
- Egg migration
 - following ovulation from ovary into fimbria
 - then into oviduct

31 📺 EM Egg and Sperm

32 📺 Fertilization Site

- Fertilization usually occurs in first 1/3 of oviduct
- Fertilization can occur outside oviduct
 - associated with ectopic pregnancy
- In vitro Fertilization- IVF, GIFT, ZIFT....
- the majority of fertilized eggs do not go on to form an embryo

33 📺 Fertilization- Sperm

- capacitation
 - removal of glycoprotein coat and seminal proteins
 - alteration of sperm mitochondria
- binding
 - ZP3 acts as receptor for sperm
- acrosome reaction
 - exocytosis of acrosome contents (Ca mediated)
 - enzymes to digest the zona pellucida
 - exposes sperm surface proteins to bind ZP2
- membrane fusion
 - between sperm and egg
 - allows sperm nuclei passage into egg cytoplasm

34 📺 Movie: Human Sperm Motility

35  **Fertilization**

36  **Fertilization**

37  **Fertilization- Egg**

- sperm membrane fusion
 - causes depolarization of egg membrane
 - primary block to polyspermy
- cortical reaction
 - IP3 pathway elevates intracellular Calcium
 - exocytosis of cortical granules
 - enzyme alters ZP3 so it will no longer bind sperm plasma
 - membrane
- 2nd meiotic division
 - completion of 2nd meiotic division
 - forms second polar body

38  **Cortical Reaction 1**

39  **Cortical Reaction 2**

40  **Cortical Reaction 3**

41  **Comparison of Fertilization**

42  **Formation of the Zygote**

- male and female pronuclei
 - 2 nuclei approach each other
 - nuclear membranes break down
 - DNA replicates
- first mitotic division
 - sperm contributes centriole which organizes mitotic spindle

43  **Sex Determination**

- based upon whether an X or Y carrying sperm has fertilized the egg
- should be 1.0 sex ratio
- is actually 1.05, 105 males for every 100 females.
 - some studies show more males 2+ days after ovulation
 - cell totipotential
- Men- Y Chromosome
 - carries sry gene
 - gene activates pathway for male gonad
- Women- X Chromosome
 - one X chromosome in each cell has to be inactivated

44  **Cleavage of the Zygote**

- cleavage of zygote forms 2 blastomeres
- cleavage with no cytoplasm synthesis
 - therefore individual cell volume decreases
 - initially synchronously, then asynchronously

- slow- centre cells, larger
- fast- peripheral cells, flattened against zona pellucida

45 Early cell division

46 Formation of the Morula

- there are now 16-20 cells
- at the end of the oviduct
- ball of cells

47 Formation of the Blastocyst

- Day 5
- zona pellucida lost
 - broken down by uterine secretions
- compaction
 - central cells now compacte
 - located on a region of the wall
- blastocele formation
 - cavity formed by compaction
 - fluid filled blastocele

48 Blastocyst

49 Blastocyst Development

50 Online References

- UNSW Embryology
 - ANAT2300 Lectures
 - <http://anatomy.med.unsw.edu.au/cbl/embryo/class.htm>
 - Week 1
 - <http://anatomy.med.unsw.edu.au/cbl/embryo/Notes/week1.htm>
 - Menstrual Cycle
 - <http://anatomy.med.unsw.edu.au/cbl/embryo/wwwhuman/MCycle/MCycle.htm>
- Online Books
 - Developmental Biology
 - <http://www.ncbi.nlm.nih.gov/80/books/bv.fcgi?db=books&rid=dbio.chapter.1334>
 - Molecular Biology of the Cell
 - <http://www.ncbi.nlm.nih.gov/80/books/bv.fcgi?db=books&rid=cell.section.5435#5442>

51 Differences in Mammalian Meioses

1

- Female oogenesis
 - Meiosis initiated once in a finite population of cells
 - 1 gamete produced / meiosis
 - Completion of meiosis delayed for months or years
 - Meiosis arrested at 1st meiotic prophase and reinitiated in a smaller population of cells
 - Differentiation of gamete occurs while diploid
 - in first meiotic prophase
 - All chromosomes exhibit equivalent transcription and recombination during meiotic prophase

2

- Male spermatogenesis
 - Meiosis initiated continuously in a mitotically dividing stem cell population
 - 4 gametes produced / meiosis
 - Meiosis completed in days or weeks
 - Meiosis and differentiation proceed continuously without cell cycle arrest

- Differentiation of gamete occurs while haploid
 - after meiosis ends
- Sex chromosomes excluded from recombination and transcription during first meiotic prophase

52  Meiosis- Chromosome reduction

53  Gonads (rabbit)