

## **Ontogeny of gametes in relation to susceptibility to gene insertion**

- **Primordial germ cells (PGC's) arise in yolk sac @ 3 weeks.**
- **Migrate by ameboid movement through dorsal mesentary to genital ridge. Multiply by mitosis.**
- **Arrive to genital ridge at 4-5 wks.**
- **During this period cells are unprotected and mitotically active, allowing infection by agents that require mitotic activity.**
- **Fetal gene therapy must take this risk into account**

# **Female Gametes**

- **PGC's become oogonia that divide by mitosis until about 5 months.**
- **Many begin to die, while others become primary oocytes.**
- **Primary oocytes enter meiosis and complete crossing over, then arrest, surrounded by follicle cells in the primordial follicle.**
- **Once in primordial follicle, oocytes become relatively inaccessible.**

# **Female Gametes, cont.**

- **At puberty, follicle develops in response to FSH from pituitary. Numerous follicle cells surround oocyte within the follicle wall, and begin to produce glycoprotein “egg shell” the zona pellucida. As follicle matures MI resumes. First polar body is released and chromosomes move to metaphase of MII.**
- **To enter egg, genes must pass follicle wall, traverse follicle cells around egg, and get through zona.**

# **Female Gametes, cont.**

- **At ovulation, egg is in metaphase of MII and is surrounded by zona and granulosa cell layer.**
- **Although immunoglobulin molecules will pass through zona, there is no evidence that naked DNA or viruses will do so. MII is completed after fertilization with release of second polar body and formation of female pronucleus.**
- **Micromanipulation to assist reproduction can assist genetic material in bypassing zona.**
- **Retroviruses and lentiviruses will infect MII oocytes to produce transgenic cattle, monkeys and mice.**

# Male Gametes

- **PGC's become dormant after arrival to genital ridge, where they are contained within "sex cords". They remain this way until puberty.**
- **Cells are mitotically inactive but relative unprotected.**

# **Male Gametes, cont.**

- **At puberty, PGC's become spermatogonia and begin dividing. Type A spermatogonium is renewable stem cell. Type B is committed to meiosis and spermatogenesis. Spermatogonia can be transduced with retroviruses and lentiviruses.**
- **Testis becomes organized into seminiferous tubules. All pre-meiotic cells are at tubule periphery, where agents able to penetrate tubule wall can access them.**

## **Male Gametes, cont.**

- **Sertoli cells, situated within seminiferous tubules, form tight junctions that sequester meiotic cells behind the “blood testis barrier”. Sperm move toward lumen of tubule as they complete meiosis and morphological transformation.**
- **Meiotic cells are difficult to access except retrograde through sex ducts.**

# **Male gametes, cont.**

- **Sperm maturation, or spermiogenesis, is characterized by loss of most cytoplasm, replacement of histones by much tighter binding protamines, and near complete cessation of gene expression.**
- **Highly condensed nucleus becomes surrounded by giant lysosome, the acrosome.**
- **It is difficult to access DNA in sperm head.**

# **Male Gametes, cont.**

- **When sperm bind to zona pellucida, they undergo acrosome reaction (AR). AR is fusion of outer acrosome membrane with plasma membrane to release contents of acrosome. Much of plasma membrane vesiculates and is lost.**
- **Genetic material on plasma membrane can be lost during zona penetration.**
- **Entire sperm often incorporated into egg.**

## **Male Gametes, cont.**

- **Shortly after fertilization, sperm head decondenses to form male pronucleus, DNA replication begins.**
- **Genetic material that enters egg with sperm can be included in pronucleus, where integration takes place with relatively high frequency.**

# **Early embryo**

- **Early embryo cleaves within protective zona until implantation, when hatching from zona occurs. Hatching and implantation occur concomitantly, and embryo is difficult to access.**
- **Micromanipulation can open zona and expose embryo to gene transfer agents for extended periods.**