The oocyte
From GV to MII

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Overview

- Ovarian reserve, oocyte maturation
- Follicle growth from the oocytes point of view
- Nuclear vs. cytoplasmic maturation
- Optimisation of methods
- IVM - *In Vitro* Maturation

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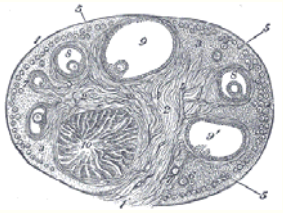
Ovary

- Cortex, central medulla
- Produces germ cells and hormones



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Ovary

- Primordial follicles in a dense stroma
- Grow inwards towards the medulla when follicle maturation commences

Primordial

Primary

Secondary

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Follicle growth

- Long process, >100 days (estimated 220 days), the initial stages are independent of gonadotrophins
- After antrum formation the follicles become dependent on FSH for continued development
- The oocyte communicates with surrounding granulosa cells and partially controls its own development
- The ”health” of the follicle is reflected in the developmental potential of the oocyte
  - Blood flow, growth factors

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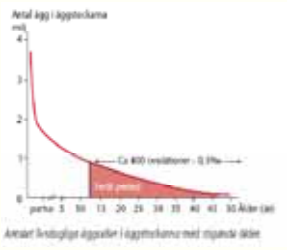
Ovarian reserve

- Oocyte numbers decrease with age
- Independent of IVF treatment
- Approximately 1000 follicles disappear every months, 999 become atretic, 1 matures



Infertilitet 2005, Landgren & Hamberger

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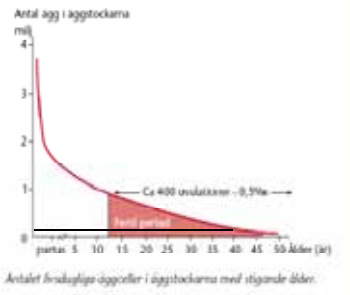
When does the fertile period start?

- 1.st attempt at pregnancy at the age of 38 years

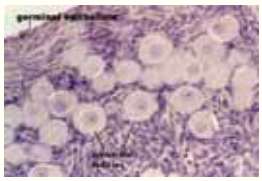


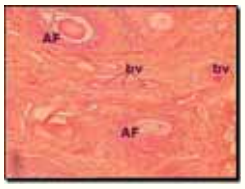
Antalet livsdugliga äggceller i äggstockarna med stigande ålder.

Infertilitet 2005, Landgren & Hamberger

Ovarian reserve

- Primordial follicles, changes with age



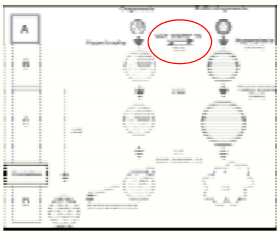


Follicle growth

- A continuous trickle of primordial follicles are released from dormancy throughout reproductive life
- The oocyte plays an active and dominant role in directing follicle growth – bidirectional communication
- Captain of the follicle instead of a passenger

Follicle growth

- Pre-antral follicle growth occurs independent of endocrine stimuli
- Early stages show oocyte growth, limited granulosa cell proliferation
- GDF-9
- BMP-15
- Kit-L



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Hutt, Albertini, RBM Online, 2007

Growth factors in follicle growth

- GDF-9: Stimulates granulosa cell growth and cumulus cell mucification, reduces apoptosis
- BMP-15: Stimulates granulosa cell growth
  - Both are very important at early and later stages of oocyte and follicle growth
- Kit-Ligand: Produced by pre-antral granulosa cells, promotes oocyte growth
- Granulosa cells transfer important metabolites to the oocyte

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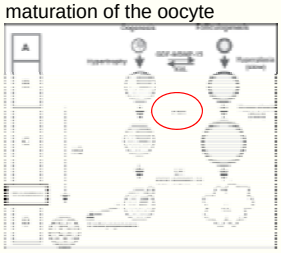
Follicle growth

- Later stages show granulosa cell proliferation and cytoplasmic maturation of the oocyte
- FSH



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Hutt, Albertini, RBM Online, 2007

Follicle growth

- Signals regulating growth initiation of primordial follicles are still unknown - not conclusively confirmed
  - Probably involve AMH (anti-Müllerian hormone) which inhibits initiation
  - c-kit and kit-ligand which stimulate growth initiation
- At the antral stage, the follicles become dependent on gonadotrophins for growth and development
- That is why neither FSH stimulation nor birth control pills affect ovarian reserve of follicles
- Granulosa cells inhibit meiotic progression through transfer of meiosis-arresting signals via gap junctions
- High levels of c-AMP in the oocyte maintain meiotic arrest

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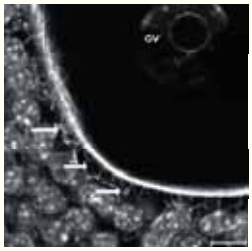
Follicle growth

- Trans-zonal projections between cumulus cells and oolemma
- Bi-directional communication



Hutt, Albertini, RBM Online, 2007

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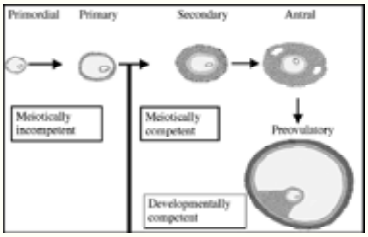
Follicle and oocyte growth



Telfer& McLaughlin, RBM Online 2007

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Oocyte growth

- Nuclear maturation – ability to resume meiosis. Can be initiated by removal from the antral follicle
- Cytoplasmic maturation - relocation of organelles, synthesis of proteins, modification of mRNAs
- Imprinting processes
- Of these complex processes, only GV-breakdown/polar body extrusion are visible in the light microscope. Complex processes occur on the molecular level – microscopic evaluation gives limited information on oocyte status
- High oocyte quality is a prerequisite for good embryo quality and foetal viability

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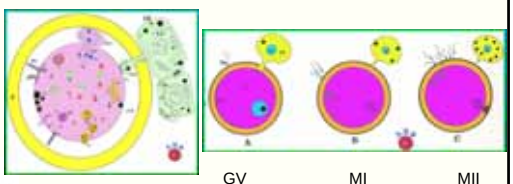
Final oocyte maturation

- Germinal vesicle (GV), the visible nucleus disappears, 1.st polar body is formed, granulosa cells withdraw from the cell membrane of the oocyte
- Flow of cAMP and nutrients to the oocyte stops, inhibition of maturation ceases



GV MI MII

sathembryoart.com

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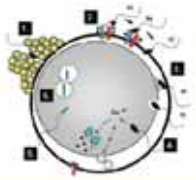
Oocyte capacity and fertilisation



Important stages in fertilization

Swain & Pool HR Update 2008

Oocyte secreted factors facilitate cumulus expansion: GDF 9, BMP 15

Synthesis of ZP proteins

Sperm binding, gamete fusion

Oocyte activation

Sperm processing

Formation and migration of pronuclei

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How to optimize the maturation period?

- Optimal time period from aspiration to ICSI
- Selection of sperm and injection with physiological substances
- Should we use MI oocytes for ICSI?
- What happens if vital processes are disrupted? IVM

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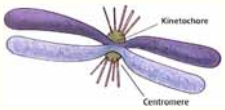
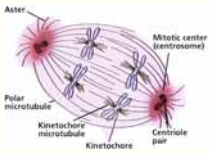
Temperature sensitivity

- Microtubules are temperature sensitive – start to decompose at temperatures under 35°C
- Reformation after re-heating is not effective
- Aneuploidy
- At temperatures under 30°C the oocyte is destroyed
- Work on a heating plate, continuous temperature control





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Aneuploidy in oocytes and embryos

- Control mechanisms for chromosome arrangement on the metaphase plate are ineffective for the first three days of embryo development
- Separation of chromosomes in meiosis or mitosis may be defective
- Anomalies occur both before and after fertilization
- Mosaicism
- The oocyte is extremely sensitive to environmental fluctuations

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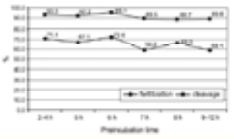
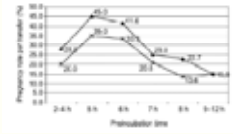
Optimal time from aspiration to ICSI

- Pre-incubation of oocytes before ICSI is beneficial (Rienzi *et al.*, 1998; Isiklar *et al.*, 2004)
- Most appropriate incubation time for mature oocytes before ICSI is 5-6 hrs (Falcone *et al.*, 2008)





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Selection of sperm and injection

- Polyvinylpyrrolidone (pvp) is a plastic substance
- Hyaluronic acid (HA) is a naturally occurring alternative which is biodegradable
- HA seems to play an important role in physiological sperm selection - present in ECM in cumulus
- Sperm which are able to bind to HA in vitro
  - Plasma membrane remodelling and maturity
  - Lower DNA fragmentation (5% vs 11% in pvp), less nuclear anomalies 14,5% normal in HA vs 11% normal in pvp), better embryo quality
  - Parmegiani *et al.*, 2009

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Immature oocytes for ICSI

- MI oocytes at ICSI, exposed to gonadotrophins, still immature
- Strassburger *et al.*, 2009: Matured for 2, 4-8 and 24 hrs
- Nuclear maturation achieved in up to 72% of MI oocytes at 24 hrs (30%, 62%, 72%) - tempting to use
- Lower fertilisation rates, 51% vs 71%, poorer embryo quality
- High aneuploidy rates (40-100%), especially after prolonged culture
- Reduced cytoplasmic competence - avoid use

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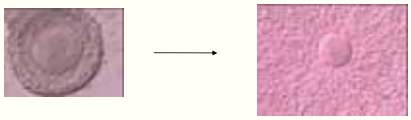
In-vitro Maturation IVM

- IVM-culture for 32-36 hours, then ICSI (IVF)
- Maturation medium with 10% patient serum, collected on the day of OPU, FSH and hCG added to the culture medium
- Patients at risk of OHSS, for example PCOS - OHSS is avoided; Younger women with good ovarian reserve; Male factor infertility



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In-vitro Maturation IVM

- Clinical pregnancy rates 20-30% per transfer (Suikkari 2007)
- In most publications somewhat lower results than after conventional IVF / ICSI
- Varying results may be explained partly by different patient groups and numbers of embryos for ET
- Better results in IVM cycles with in-vivo matured oocytes (Son et al., 2008)
- Are IVM-oocytes competent to support embryo and foetal development?

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In-vitro Maturation IVM

- Reported rates of miscarriage are between 22-57%
  - Lin et al. 2003 22%
  - Chian et al. 2000 25%
  - Buckett et al. 2007 25%
  - Le Du et al. 2005 33%
  - Söderström Anttila et al. 2005 36%
  - Cha et al. 2005 37%
  - Child et al. 2001 40%
  - Mikkelsen, Lindenberg 2001 57%
- Not possible to calculate the mean, but clearly elevated

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In-vitro Maturation IVM

- Miscarriage rates have been proposed to be PCO-related (Buckett et al. 2007), however other groups with even higher rates of miscarriage have not had a majority of PCO patients
- Rates of caesarean section are also elevated
  - Söderström Anttila et al. 2005 35%
  - Buckett et al. 2007 39%
- Obstetric complications such as bleeding are usually not reported

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In-vitro Maturation IVM

- Higher birth weight after IVM compared to IVF or national average - IVF is usually under the national average except for cryopreserved embryos
  - Cha et al. 2005 3252g vs. 3165g (Korean average)
  - Mikkelsen et al. 2005 3720g vs. 3532g (Danish average) vs. 3457g (IVF/ICSI, Pinborg)
  - Buckett et al. 2007 3482g vs. 3260g (controls) vs. 3189g (IVF/ICSI)
  - Söderström Anttila 2006 3550g vs. 3541g (controls) vs. 3364g (IVF/ICSI)

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In-vitro Maturation IVM

- The differences are not great, but the pattern is clear and the number of children born is relatively high
- Same pattern is seen after IVM in farm animals
  - "Large offspring syndrome", also associated with other complications
- How can we explain these observations?
- What mechanism might cause higher miscarriage rates, higher rates of pregnancy interventions and higher birth weight

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In-vitro Maturation IVM

- Genomic imprinting is achieved at different stages in germ cell development
- During embryonic development, germ cells erase imprinting marks and establish new imprinting depending on their sex (Reik, Walter 2001; Tada et al 1998)
  - Male germ cells re-establish their imprinting marks at the round spermatid stage (Shamanski et al 1999)
  - Oocytes complete their imprinting **just before ovulation** in each ovulatory cycle (Schaefer et al 2007; Obata et al 1998)
  - It is logical that imprinting defects are more likely to affect oocytes then sperm
  - Imprinting effects may be most frequently seen in placenta

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In-vitro Maturation IVM

- Endometrial effects
- Growth factor deficiency - GDF 9
- IVF culture media are moving from rich to focused/specified
- Some indications that this decreases risk for epigenetic effects (Marees *et al.*, 2009)
- IVM uses serum for culture in the human
- c-AMP modulators
- IVF has not needed to prove it's safety, only to show it is not unsafe. This situation may turn around – and it has – for IVM

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Conclusions

- Knowledge of detailed mechanisms of oocyte maturation, development and morphology allows us to improve results of ART treatments
- Optimising oocyte competence is one of the key issues in optimising results in ART

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Thank you for your attention!

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