The Perspective of Ovum

Peter Sjoblom

Nottingham University Research and Treatment Unit In Reproduction

NURTURE

Overview

- General observations
- Differences between in vivo and in vitro reproduction from nthe eggs perspective

More questions than answers!

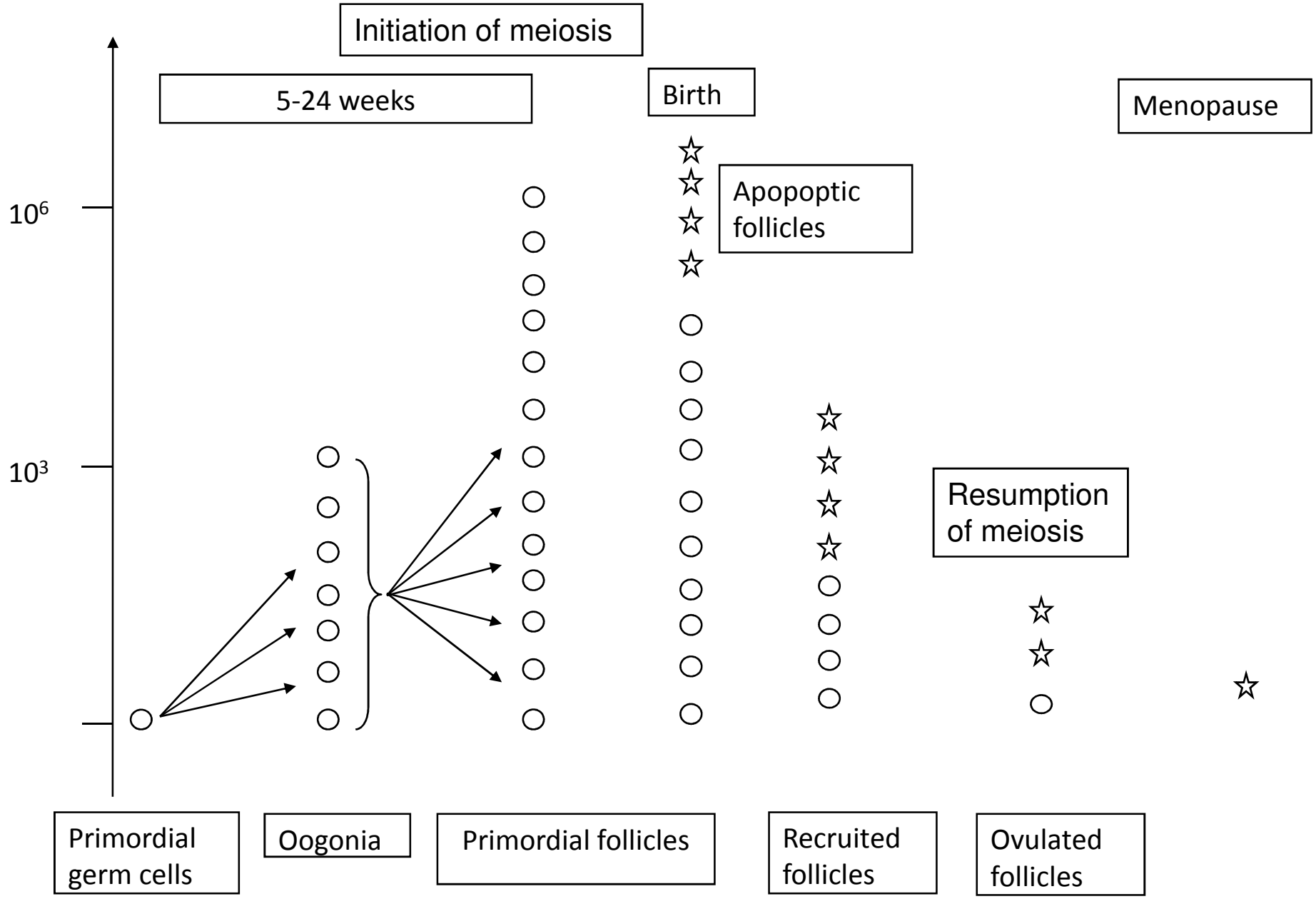
What's So Special About Gametes?

- Highly charged cells waiting to burst into activity after encountering each other
- Require close contact with somatic cells for normal differentiation
- Each cell is genetically unique
- Phenotype largely determined by somatic genome

=> No biological basis for natural selection of unique properties

What's So Special About Eggs?

- Mitosis
  - Finalised in fetal period
- Meiosis
  - Put on hold
- Genetic control of early development
  - Major transcription initiated long after fertilisation
- Needs sperm centrosome for post-fertilisation mitosis
- Mitochondria



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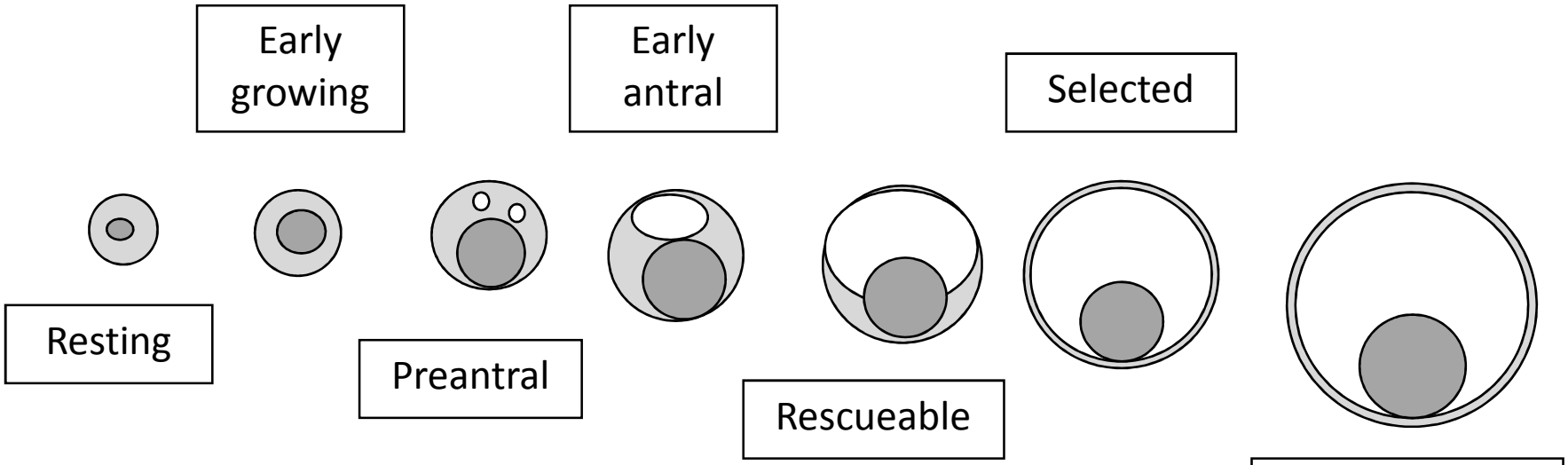
Timeline



About 1/10,000 of the primordial oocytes reach ovulation

≈180 from initiation to ovulation;
only last 14 days modifiable by exogenous FSH

After Gougeon , 1996

Structural and Functional Changes in Oocytes

- Primary follicles
  - Mitochondrial proliferation
  - Increase in endoplasmic reticulum
- Early secondary follicles
  - Secretion of zona pellucida proteins by the oocyte
  - Cortical granules scattered in the cytoplasm
  - Transcription, translation, accumulation of untranslated mRNA (truncation of polyA, possibly clustering in RNP)

Structural and Functional Changes in Oocytes

- Secondary follicles
  - Growth from 35 μm to 110 μm, almost finished at antrum formation
- Large antral follicles
  - Migration of cortical granules
  - Polarised distribution of proteins and RNA
- Ovulation
  - Progress of meiosis
  - Uncoupling of gap junctions

The Ovum Is A Charged Cell

- Finite life after ovulation
  - Degradation of RNA?
- Waiting to explode into action after contact with sperm
  - Prevention of polyspermia
  - Progression of meiosis

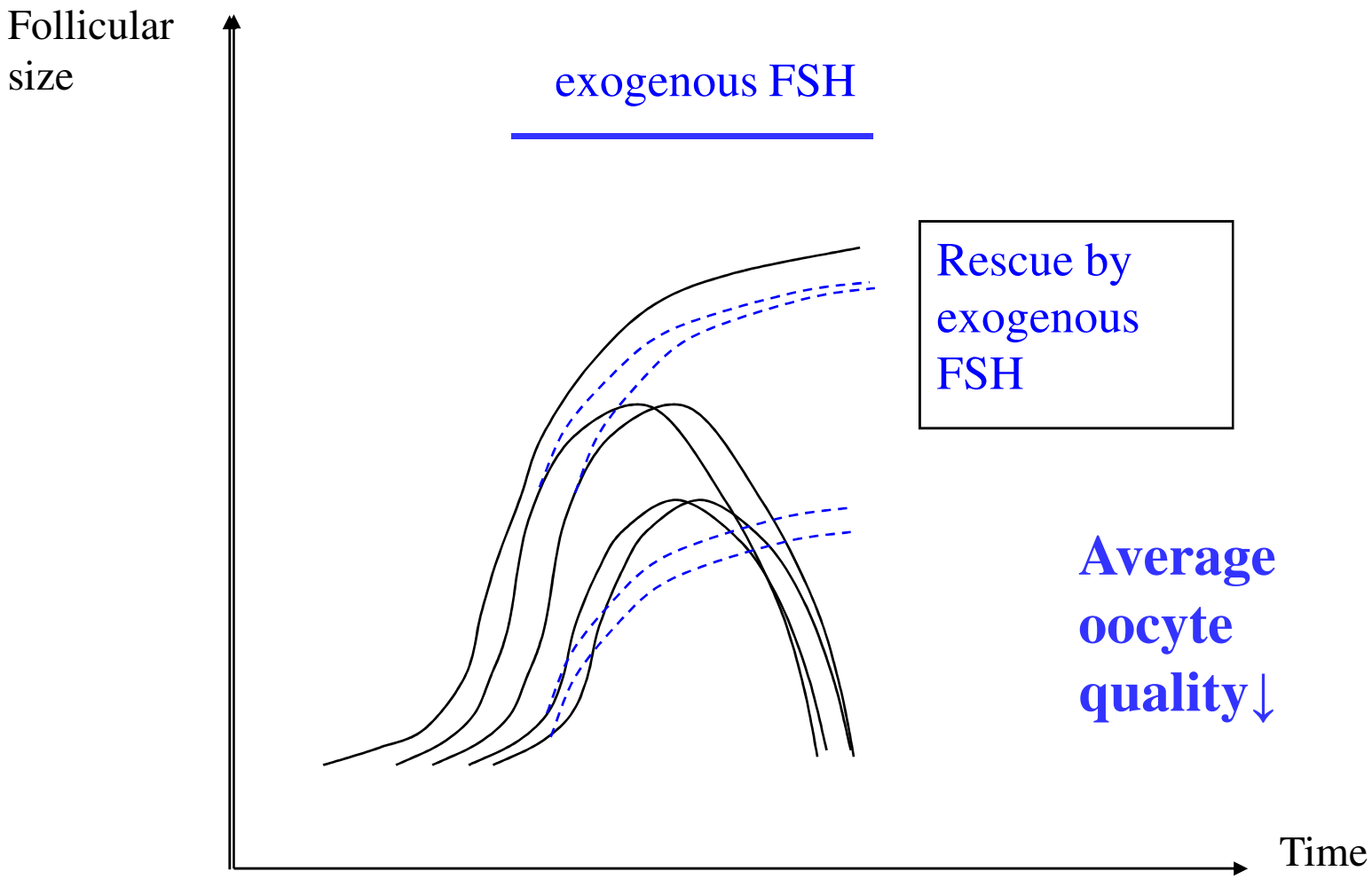
Fertilisation

- Sperm penetration through zona
- Sperm-egg fusion
- Polyspermia block
- 2nd meiotic division
- Decondensation of sperm nucleus
- Protamin-histone replacement
- Formation of pronuclei

What Does The Sperm Contribute?

- Genetic (DNA) inheritance
- Epigenetic inheritance
- Structural inheritance (centrosome)
- Activation signal

Development *in vitro vs in vivo*



Development *in vitro vs in vivo*

- On average <3 oocytes in each cycle has good developmental potential
- About 2-5% of oocytes retrieved in vitro become babies (What is the appropriate comparison in vivo?)
- Why do we give such large doses of hormones and why do we collect so many oocytes?

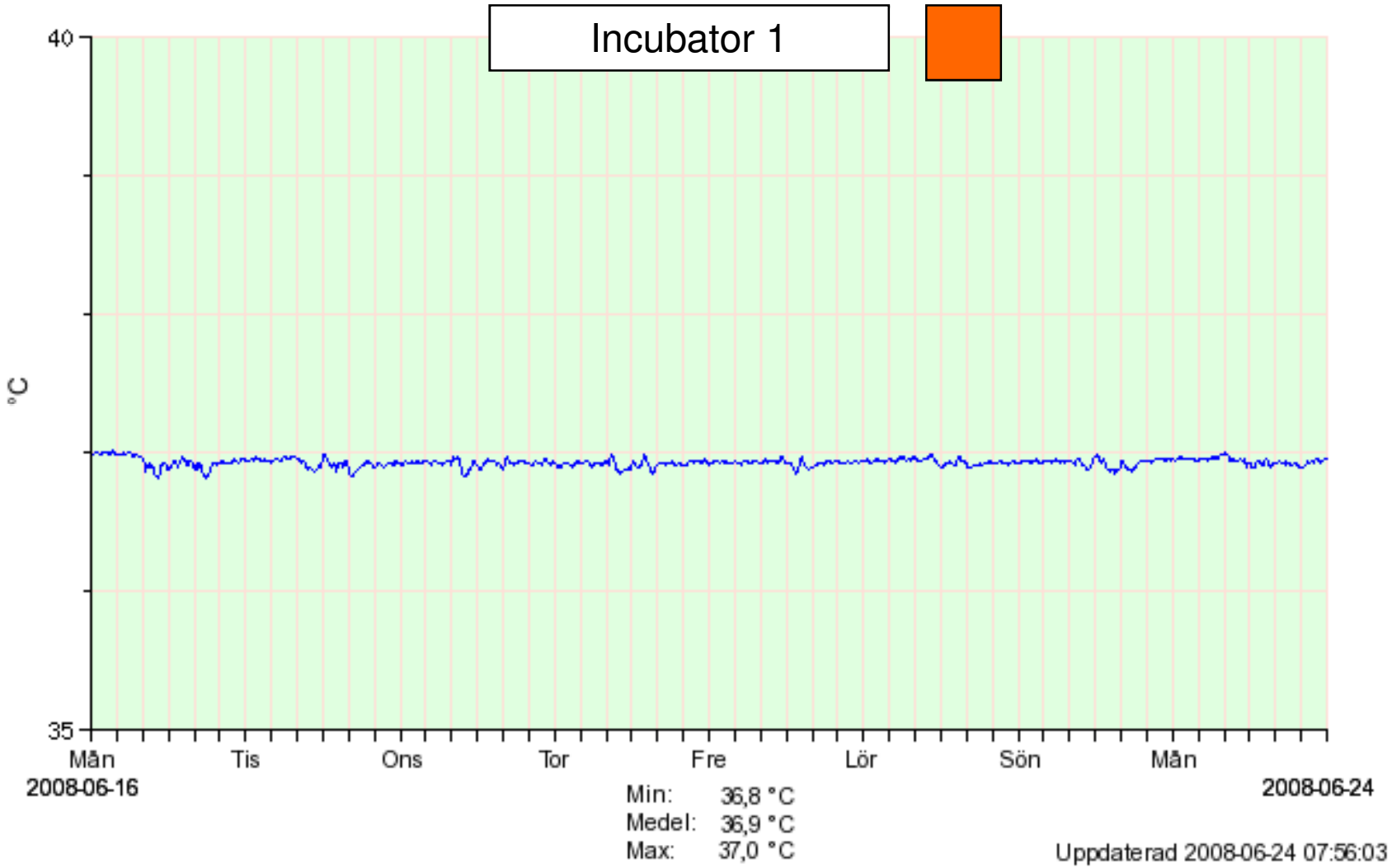
Development *in vitro vs in vivo*

- Environmental factors
  - Physical-chemical factors (T, pH, osm, oxygen, light)
  - Nutritional factors (media, metabolites)
  - Cellular factors (epithelial cells, sperm concentration)

Development *in vitro vs in vivo*
Temperature

- Chemical reaction rates
- Solubility
- Macromolecular structure
  - Proteins and nucleic acids
  - Lipid bilayers
  - Tubulin

Temperature



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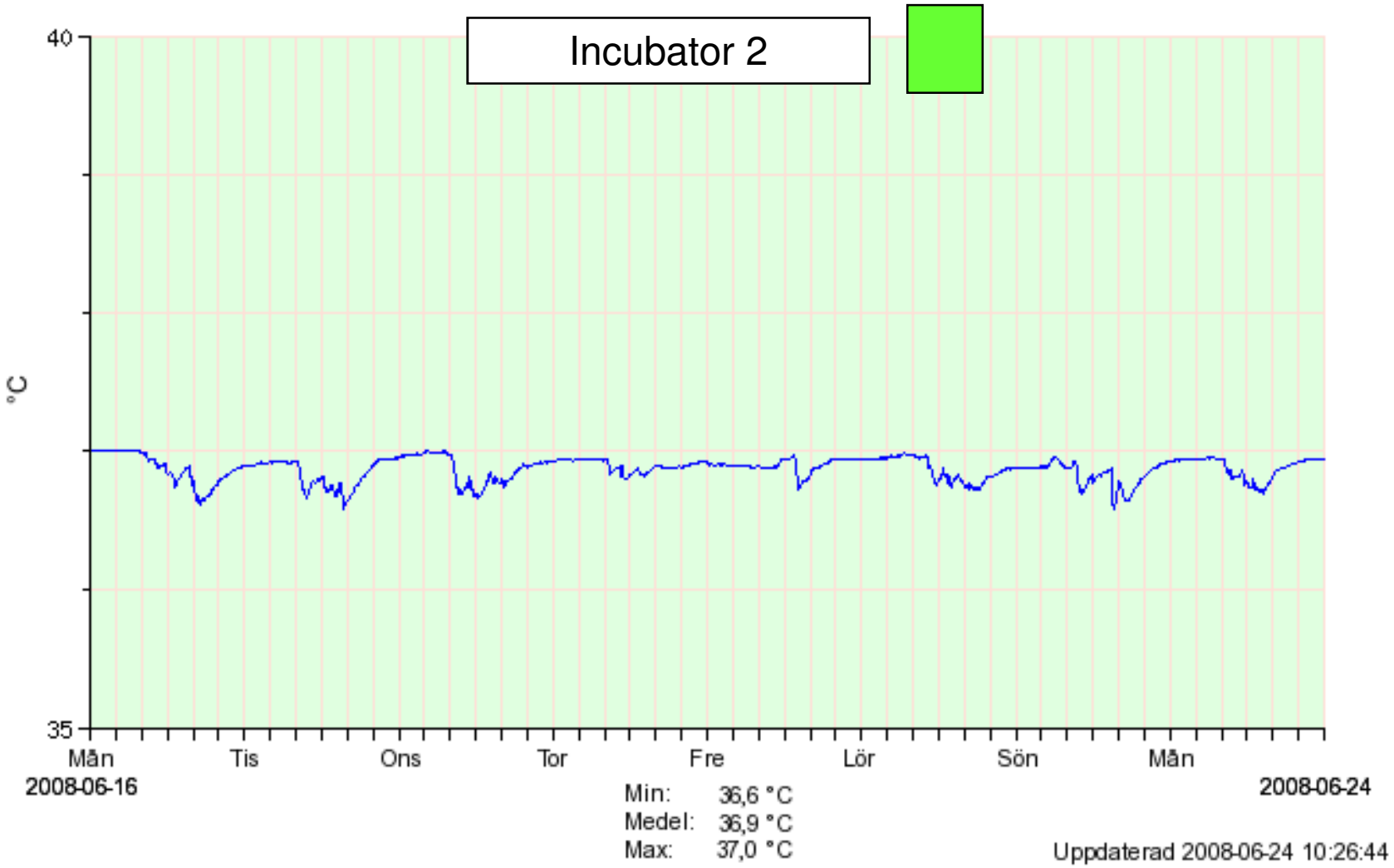
Temperature



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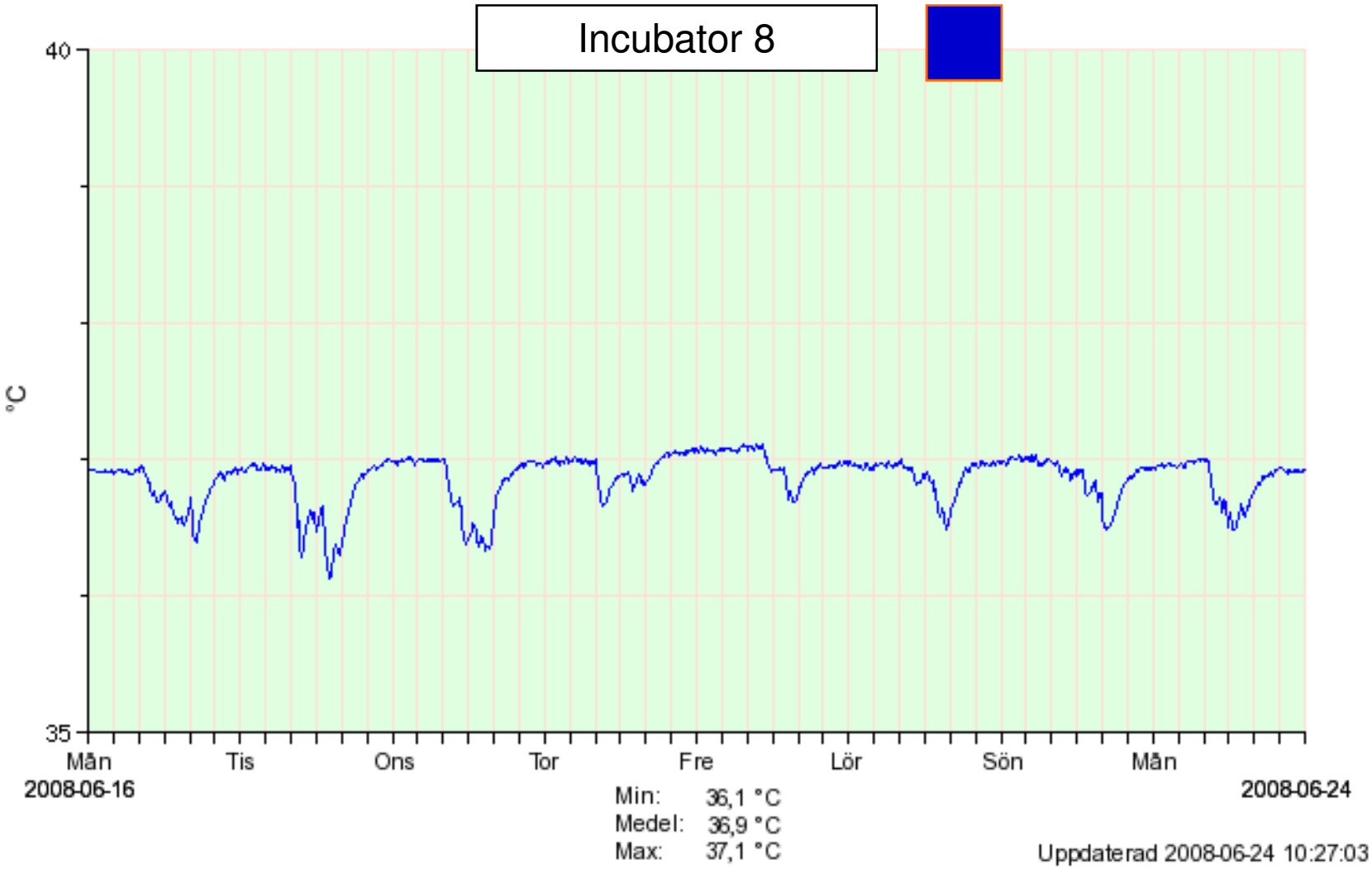
Temperature



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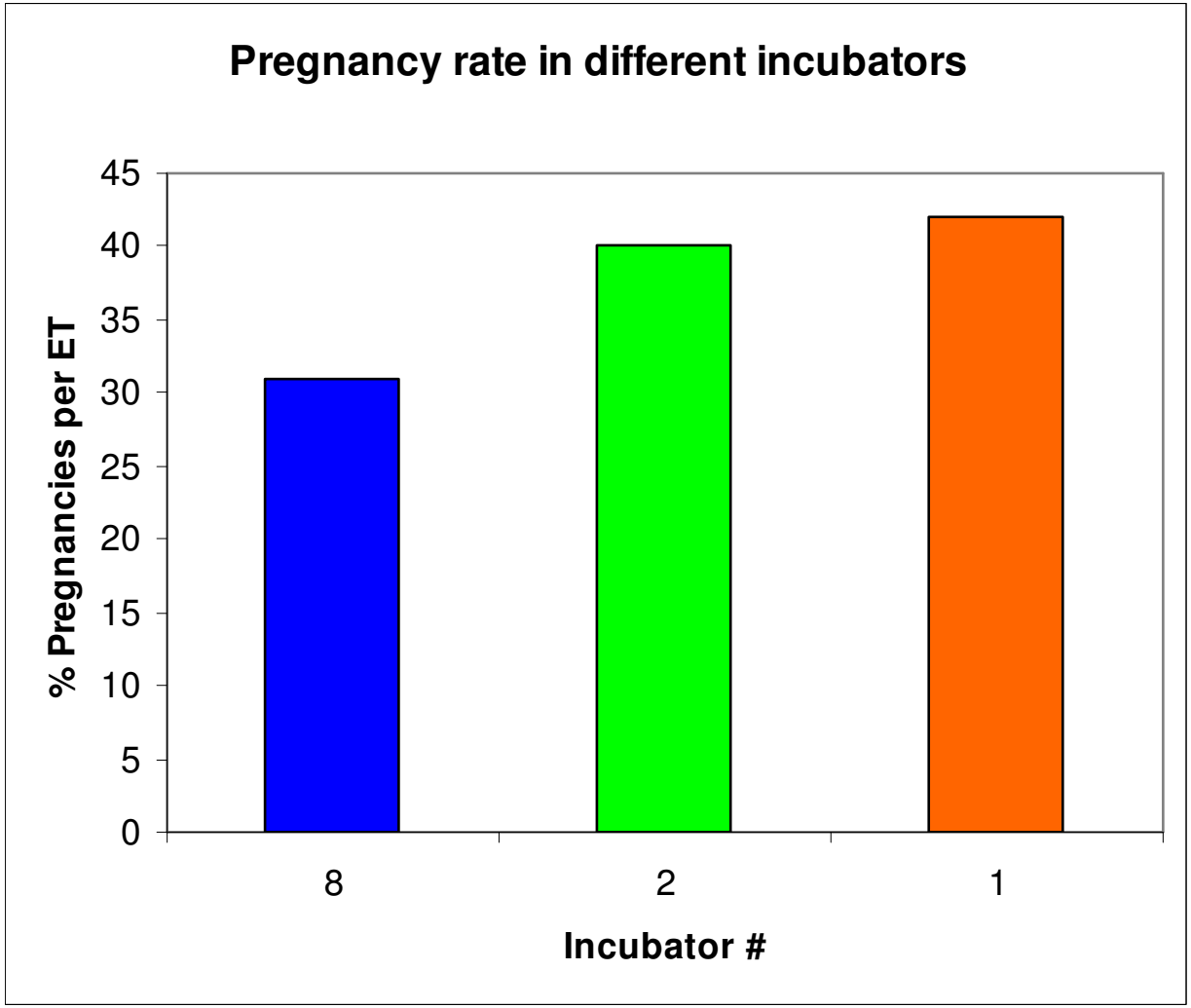
Development *in vitro vs in vivo*



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Development *in vitro vs in vivo*
pH

- Macromolecular conformation
  - Charge of $-NH_2$ and COOH groups; hydrogen bonds
- Reaction rates
- Protein function
- Energy storage

Development in vitro vs in vivo
pH

- Oocytes incapable of regulating internal pH
- Internal pH regulation requires presence of bicarbonate in surrounding medium

Fertilisation in vitro vs in vivo
Nutritional factors

- Energy requirements differ between developmental stages
  - Abrupt vs gradual change
- Composition of media
  - Simple versus complex
  - Degradation
- Removal of metabolites
  - NH_4^+

Fertilisation in vitro vs in vivo
Cellular factors

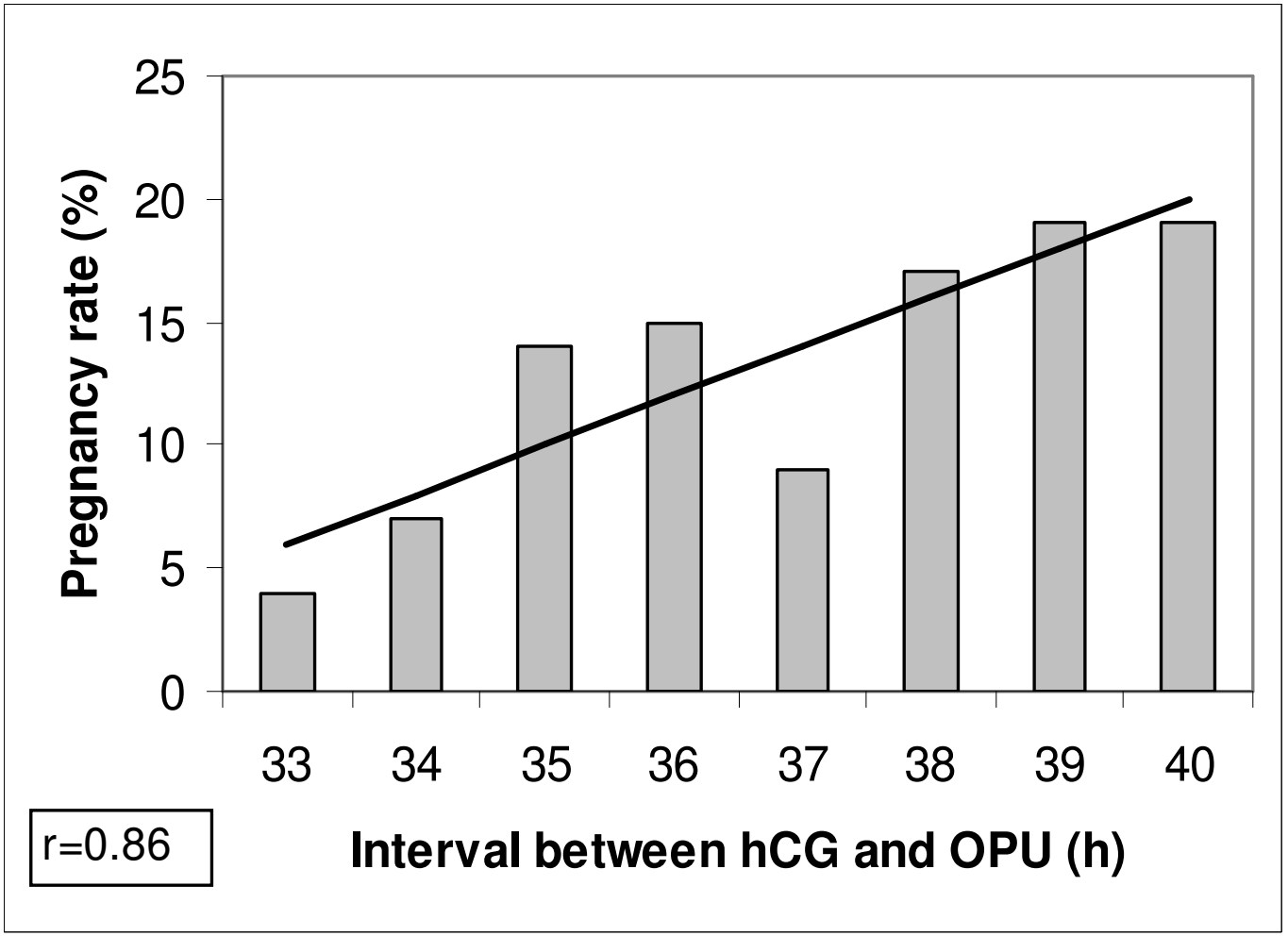
- Tubal epithelium
- Sperm concentration
  - 100,000 vs 100
  - Consumption of nutrients
  - Accumulation of metabolites

Development in vitro vs in vivo

- Escape from the intrafollicular environment
- Functional life of oocytes probably 6-10 h post ovulation
- Optimal time of insemination probably close to natural time of ovulation

Fertilisation *in vitro vs in vivo*

Interval hCG oocyte retrieval



(Nargund et al, 2001)

Oocyte Freezing

- Risk of aneuploidy due to degradation of meiotic spindle
- Efficacy of reproduction only marginally lower than with fresh oocytes

Ovarian Tissue Freezing

- Still very small numbers thawed and transplanted, but huge numbers frozen
- “Results are promising”
- Great need for fertility preservation

Future Challenges

- Understanding and manipulation of oogenesis and folliculogenesis
- Understanding ovarian senescence
- Identifying characteristics of oocytes with good developmental potential
- Improvement of culture conditions
- Understanding the developmental impact of in vitro manipulations