

# Media for IVM



**MONZA - Italy**  
**2008**

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# General experience with IVM

- **IVM has a lower pregnancy rate per OPU - due to lower no. of oocytes/embryos resulting in lower transfer rate**
- **IVM has a lower pregnancy rate per ET - due to lower no. of embryos resulting in lack of selection possibilities, and possibly in a smaller no. of embryos transferred**
- **IVM has a lower implantation rate - due to lower no. of embryos resulting in lack of selection possibilities**
- **IVM has probably a higher rate of early pregnancy loss?**

# **We need to improve**

- **Culture media**
- **Patient selection**
- **General clinical techniques**
- **Synchronization of embryo and endometrial development ('put it back in the right place at the right time')**

# **What is the purpose of IVM Media?**

**Provide the in vitro environment for the oocyte-cumulus-complex (OCC) to mature:**

- **Oocyte nuclear maturation**
- **Oocyte cytoplasmatic maturation**
- **OCC maturation**

# What has been used as Human IVM Media?

- **Pure human donor follicular fluid**
- **Basal cell culture medium + fetal bovine serum or bovine follicular fluid**
- **Basal cell culture medium + patient serum or human donor follicular fluid (+ hormones)**
- **Basal cell culture medium + serum replacement + patient serum (+ hormones)**
- **Basal cell culture medium + serum replacement**
- **Basal cell culture medium + serum replacement + growth factors and hormones**

# Composition of an IVM Medium

- **A suitable basal medium**
- **A protein source?**
- **'Active molecules' / 'signal molecules'**

# Composition of a basal medium for IVM

- **water**
- **salts incl. bicarbonate as pH buffer**
- **glucose**
- **amino acids**
- **vitamins**
- **serum replacement?**

# **Basal media for IVM**

**Traditionally used: TCM199 or modifications**

**New formulation suitable for serum-free conditions?**

# Composition of an IVM Medium

- **A suitable basal medium**
- **A protein source?**
- **'Active molecules' / 'signal molecules'**

# Why do we need a protein source?

- **Because we need protein as such?** **Probably not**
- **Because we need factors found in this protein source?** **If not otherwise provided**
- **Because we need protein for detoxification of the media?** **Probably**
- **Because we need appropriate molecules to coat the surface of the plastic vessels?** **Probably**

# Protein sources

- **Follicular fluid**
- **Serum**
- **Fractions of serum**

# Follicular fluid

- **Oocytes mature in vivo in follicular fluid**
- **The composition of follicular fluid change with time during maturation**
- **Follicular fluid must be harvested from IVF patients**

# Serum

- **Provides nutrients, lipids, hormones and other signal molecules**
- **Serum is not identical to follicular fluid, and cannot be expected to have the correct concentrations of the correct components at any time**
  - E.g. many PCOS patients have a too high concentration of insulin in their serum
  - E.g. the concentration of LH and EGF is probably too low
- **Binds and neutralizes toxins and surplus nutrients, which may be inhibitory in too large concentrations**
- **May also release such substances**
- ***Serum is a complex, undefined substance***

# Fractions of serum

- Plasmanate
- SSS
- SPS
- Human serum albumin (HSA)

**May to various degrees reduce the unwanted effects of serum, but beneficial effects of serum or follicular fluid must be compensated for:**

- serum replacement (metal ion buffer, chelating system etc.)
- supplementation of nutrients - lipids, vitamins etc.
- supplement of appropriate signal molecules (hormones, growth factors etc.)

**This calls for a serum-free  
medium – or probably  
sequential media**

# Contents of a Synthetic Serum Replacement

- **Metal ion buffer (metals and excess of metal-binding chelators)**
- **Iron (Fe) and transferrin replacement to induce uptake of iron**
- **Trace elements: Zn, Cu, Mn, Cr, Co, Se, and possibly others**
- **Surfactant (weak detergent), to reduce surface tension**
- **The most important hormone: insulin may be included in a serum replacement**
- **Important lipids as cholesterol and fatty acids may be included**
- **Albumin or synthetic albumin-like substances to bind fatty acids and small organic toxic molecules (environmental buffer)**

# Composition of an IVM Medium

- **A suitable basal medium**
- **A protein source?**
- **'Active molecules' / 'signal molecules'**

# **'Active molecules' / 'signal molecules'**

**We want to promote maturation  
and secure  
synchronization of maturation**

**Nuclear maturation**

**Cytoplasmic maturation**

# Nuclear maturation

- **Oocytes resume meiosis once removed from follicular environment**
- **Measured by extrusion of 1<sup>st</sup> polar body**

## **Aim:**

- **Increase maturation rate**
- **Decrease "GV-to-MII" time**

## **Ways:**

- **Adding Growth Hormone (GH)** (Iga et al 1998)
- **Priming with hCG**
- **Other**

# Maturation enhancers

## Some suggestions:

- FF-MAS** (Follicular Fluid Activating Sterol)
- LIF / IL6** (Leukemia Inhibiting Factor/Interleukin 6)
- VEGF** (Vascular Endothelial Growth Factor)
- EGF** (Epidermal Growth Factor)
- EGF-like Superfamily**
  - TGF $\alpha$**  (Transforming Growth Factor)
  - HB-EGF** (Heparin-binding EGF)
  - Amphiregulin**
  - $\beta$ -cellulin**
  - Epiregulin**

# Maturation enhancers

## More suggestions:

**FSH** (Follicle Stimulating Hormone)

**LH** (Luteinizing Hormone)

**IGF-1** (Insulin-like Growth factor)

**BMP** (Bone morphogenic protein)

**GDF9** (Growth differentiation factor)

**GH** (Growth Hormone)

**FGF** (Fibroblast Growth Factor)

**Statins**

**Neurotrophin**

# Cytoplasmic maturation

- **Developing competence to regulate fertilization and development during early stages of embryogenesis, by:**
- **Growth and relocation of cytoplasmic organelles and accumulation of RNA and proteins for regulation of**
- **Changes in membrane transport systems**
- **Measured by ???**

# Cytoplasmic maturation

## **Aim:**

- **Synchronize cytoplasmic and nuclear maturation**

## **Ways:**

- **Prolonged culture system (4-5 days)**
- **Oocyte is kept meiotically arrested**
- **Provide appropriate growth factors and hormonal supplements**

Smitz et al 2001

# **Maturation “synchronizers”**

## **(Nuclear maturation inhibitors)**

**e.g.**

**Cilostamide**

**PDE3-I (phosphodiesterase 3-inhibitor)**

**(L. Vanhoutte et al., Ghent)**

# Cytoplasmic maturation

## **Aim:**

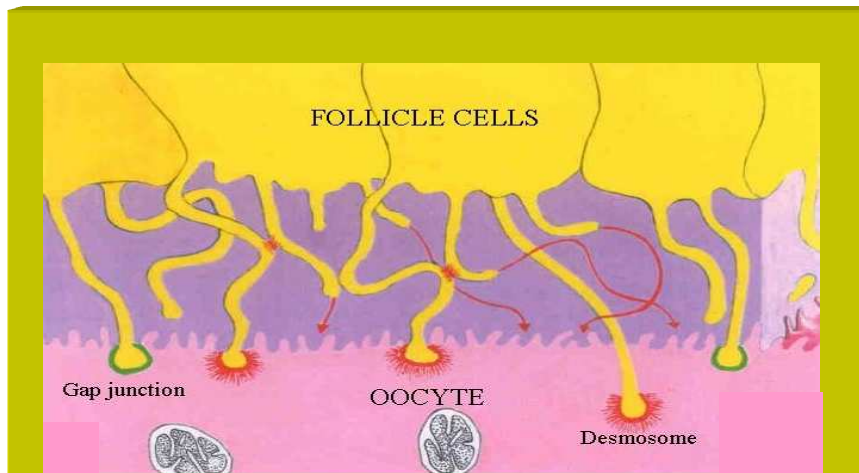
- **Synchronize cytoplasmic and nuclear maturation**

## **Ways:**

- **Prolonged culture system (4-5 days)**
- **Oocyte is kept meiotically arrested**
- **Provide appropriate growth factors and hormonal supplements**
- **Maintain transzonal connection between granulosa cells and oocyte**

Smitz et al 2001

# OCC interaction and maturation



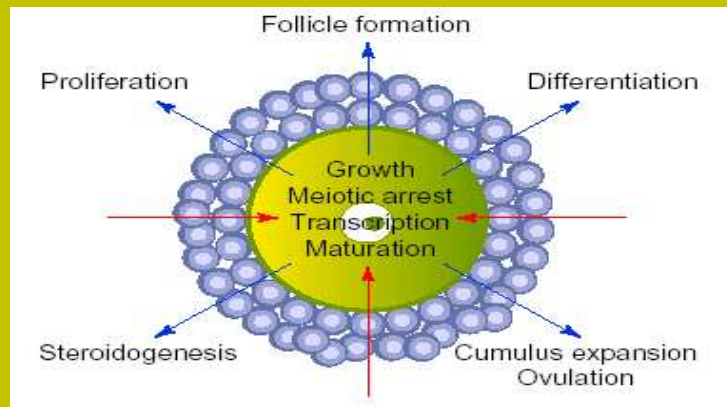
**Granulosa cells activate gap junctions with the oocytes.**

**Gap junctions are involved in the maturation processes:**

- **to complete cytoplasmic maturation**
- **to complete meiosis**

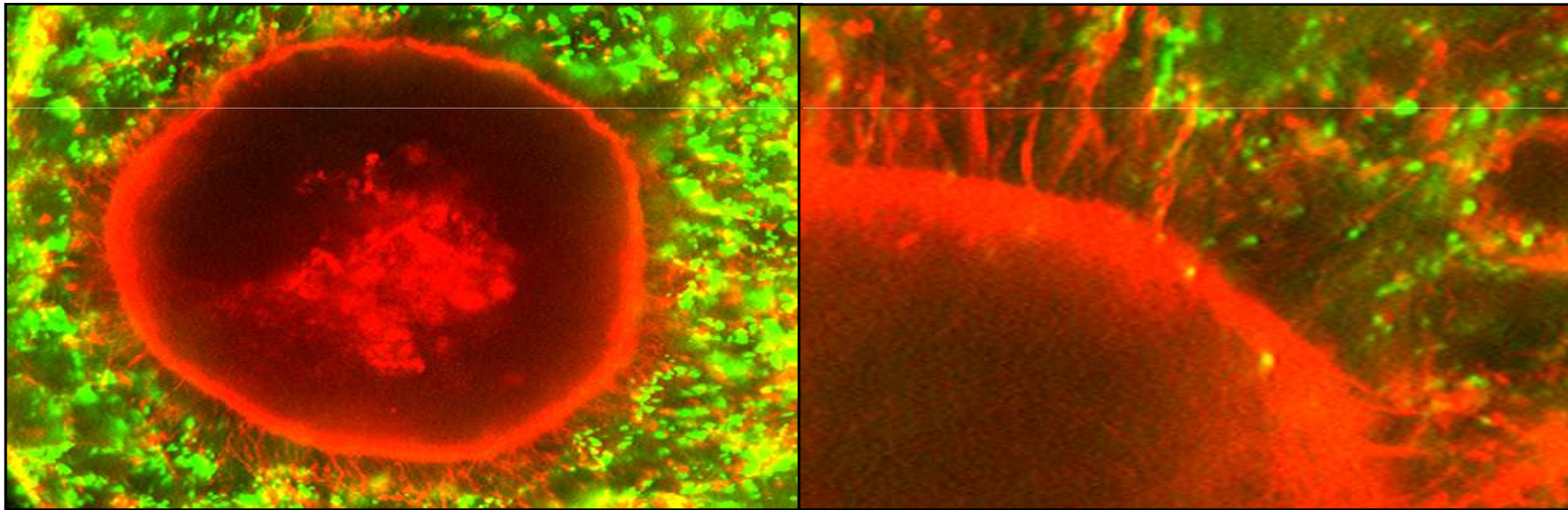
*Simon et al., 1997*

**OCC communication is regulated by FSH**



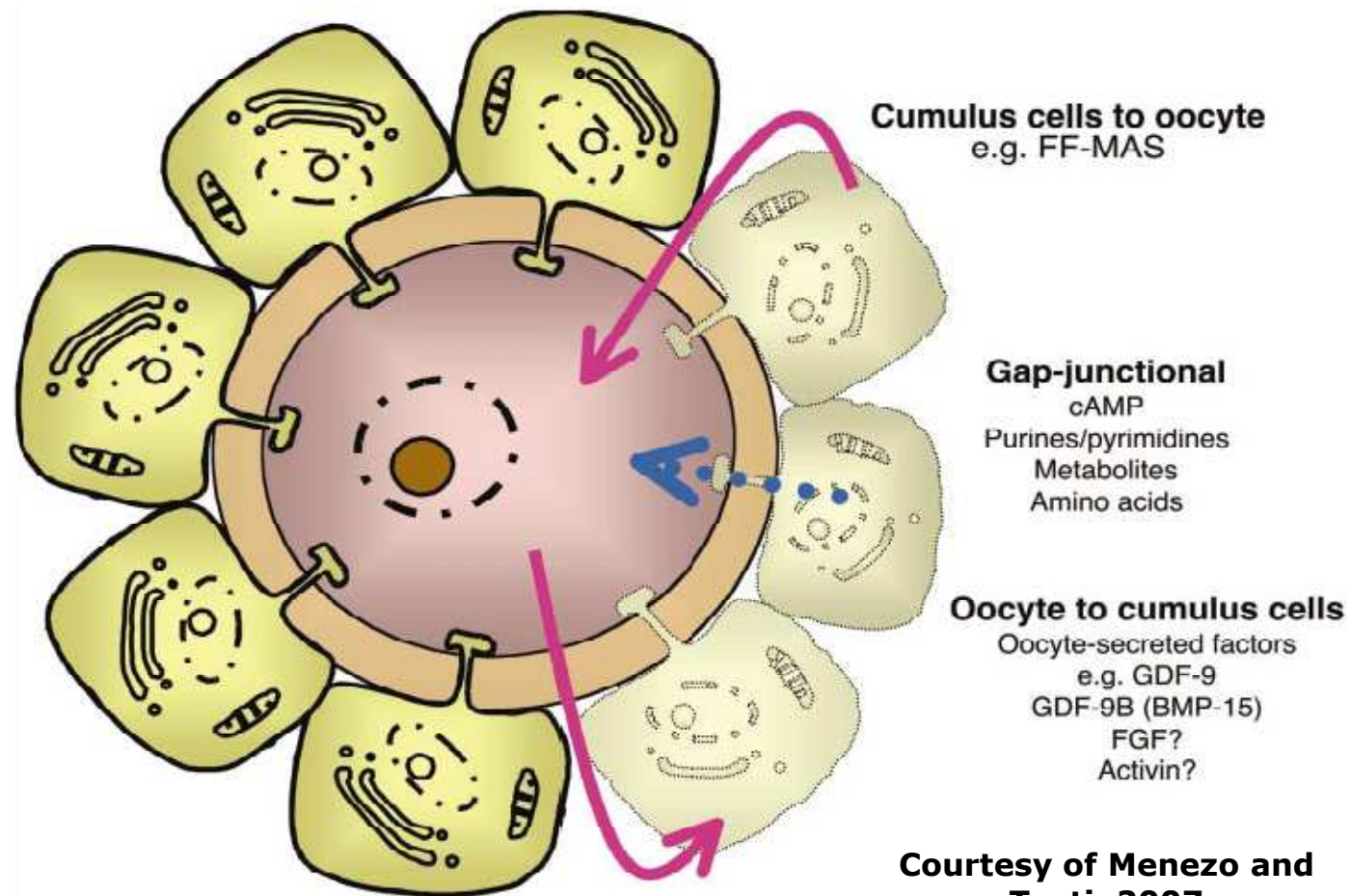
# OCC interaction and maturation

**Transzonal projections (TZPs) as dynamic mediators of information exchange at the Oocyte-Granulosa Interface**



Courtesy of D. Albertini

# Paracrine/juxtacrine interactions during oocyte maturation



Courtesy of Menezo and  
Tosti, 2007

# **Maintain transzonal connection between granulosa cells and oocyte**

## **Three-dimensional culture (collagen)**

**in the presence of PDE3-I (phosphodiesterase  
3-inhibitor)**

**(L. Vanhoutte et al., Ghent)**

# What is the problem?

## Considerations in connection with adding of *one* of these factors

- **Concentration**
- **Shelf life**
- **Expenses**
- **Safety issues**
  - **Production staff**
  - **Laboratory staff**
  - **Embryos / fetus**
- **Proprietary issues (patents)**

# **What is the problem?**

**Addition of just one of these factors cannot realistically be expected to do the trick**

# What is the problem?

## Considerations in connection with adding of combinations of factors

- Which combination
- Concentrations of individual components
- Shelf life of individual components
- Increased development time and costs
- Costs of components
- Increased safety issues
  - Production staff
  - Laboratory staff
  - Embryos / fetus
- **Proprietary issues (patents) for components *and combinations***

# **The biggest problem**

**for the development of media for a better treatment of IVM patients are:**

**Proprietary rights / patents**

# Example 2003

**Additions used: FSH, hCG + patient serum**

**Suggested serum-free medium:**

**FSH, LH, Estradiol, Progesterone, Corticosterone, EGF,  
FGF, IGF-1, SSR Serum Replacement, HSA**

**A pilot study in Japan showed promise, *but ...***

**Combining these factors in a medium caused  
proprietary problems (patents). Project stopped.**

## **A few more examples**

- **Neurotrophin: Suggested as addition to IVM media in 2004. Turned out to conflict with an existing patent. Project stopped.**
- **FF-MAS: Lengthy negotiations about patent and costs have delayed and eventually probably stopped the development of media.**
- **BMP: Proprietary issues stopped development of media.**
- **EGF in combination with certain hormones is patented.**



Rubens  
**Thank you**  
**for your attention!**