The Prostaglandin Transporter (PGT): A novel indispensable mediator of ovulation

From Basic Research to Clinical Implications

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Disclosure

- Consultant to EarlySense®
Clinical Reports – First IVM Pregnancy

1: Cha KY, Koo JJ, Ko JJ, Choi DH, Han SY, Yoon


ED Program

270 Oocytes were aspirated from 23 ovaries

ET of 5 embryos to a woman with POF

Triplets pregnancy
IVM Protocols

First US-day 2-3 (AFC)

Second US- day 6
hCG administration when leading follicle is 12-14 mm and ES>6 mm

OPU day 10-14
Estrogen supplementation

AFC
Hormonal Profile

Leading Follicle: 12-14 mm
ES≥6mm
hCG

OPU

Day of Cycle

1 2 3 4 5 6 7 8 9

4 mm
10 mm
12-14 mm

6 mm
In vitro maturation (IVM) of oocytes

Advantages:
- Does not require gonadotropin stimulation
- Shorter treatment
- Less expensive
- Simpler
- Safer, avoiding OHSS

Unfortunately ...
- Maturation rate ~50-70%
- Reduced developmental potential
- Lower fertilization
- Lower pregnancy and live birth rates
IVM / IVF

Not only a treatment tool......
Hypothesis:

• Using the IVM / IVF model we generated a cDNA library of genes significantly up or down-regulated during the final stages of *in vivo* maturation and ovulation.

• We hypothesize that these regulated ovulatory genes might be critical determinants of ovulation and oocyte developmental potential.
Materials and Methods

Primordial     Primary     Secondary      Pre-Antral     Antral     Pre-Ovulatory

3-5 Months
FSH Independent

14 days
FSH Dependent

Ovulation

GV-IVM sample

IVM

IVF

M2-IVF sample
Flow chart of RNAseq

- Total RNA extraction
- cDNA library preparation
- WTA
- Raw read whole library
- Transcripts assembly
- Align reads to quantify expression
- Bioinformatics analysis
Different signature for GV-IVM and M2-IVF samples

- Cluster analysis of relative expression of RNAseq samples from GV-IVM and M2-IVF
- 1100 genes were significantly upregulated in the expanded CC cells and 646 were downregulated.
Major processes during cumulus maturation/expansion

Ingenuity IPA analysis

Yerushalmi et al., MHR, 2014
Prostaglandin transporter (PGT) regulation of PGE2 signaling is essential for ovulation
Why did we choose to explore Prostaglandin Transporter (PGT)?
1. PGT is among the highly up-regulated genes in Expanded Cumulus Cells Obtained from MII Oocytes (CCMII) Compared With Compact Cumulus Cells Obtained from GV Oocytes (CCGV)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Base Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCGV</td>
<td>83.6</td>
</tr>
<tr>
<td>CCMII</td>
<td>32825.2</td>
</tr>
</tbody>
</table>

Fold change: 392.3

**qRT-PCR**

- CCGV: 0.3
- CCMII: 26.9

* indicates significance.
Prostaglandins (PGs) have been recognized as key mediator of ovulation for more than 30 years.

Ovarian PGs production is increased in response to the LH surge.

PGs biosynthesis is under the control of the Cyclooxygenase (COX) enzyme.

$\text{PGE}_2$ is the major COX-2 product that mediates the ovulation process.

Disruption of $\text{PGE}_2$ production or $\text{PGE}_2$ receptor expression prevent ovulation.

$\text{PGE}_2$ is essential for follicle rupture, oocyte release, cumulus expansion.
3. There is no report on PGT expression, regulation and function in the ovary.

- PGs are organic anions and diffuse poorly through plasma membranes.
- Their diffusion rate is too low to initiate biological response.
- Carrier-mediated transport mechanism is needed for PGs to cross the biological membranes.
In vivo Expression Pattern of PGT mRNA in Human Granulosa Cells
Expression of PGT mRNA in Cumulus (CGCs) and Mural Granulosa Cells (MGCs)

PGT mRNA Expression Pattern During the Late Antral Follicle Development in mGCS

![Graph showing PGT/β-actin mRNA expression pattern](image)

- Small (<10 mm)
- Large (10–14 mm)
- Preovulatory (>17 mm)
PGT mRNA Expression in CGCs According to Maturation Stage of the Corresponding Oocyte.
In vitro PGT Regulation in Human Granulosa Cells
hCG Effect on PGT Expression in Cultured MGCs Cells

PGT expression in the human ovary during folliculogenesis

Early Antral

Post ovulatory

Corpus Luteum

A-Antrum  GC-granulosa cells  T-Theca cells  V-Luteal blood Vessels
Intracellular Signaling Pathways Regulating PGT Gene Expression: cAMP, PKC Pathways

![Diagram showing intracellular signaling pathways and gene expression levels](image)

- **PGT mRNA (fold change)**

  - Control
  - hCG: b
  - FSK: b
  - PMA: b
  - FSK + PMA: ab

Legend:
- a
- b
Intracellular Signaling Pathways Regulating PGT Gene Expression: cAMP-PKA pathway

![Graph showing the effects of hCG, FSK, and H89 on PGT mRNA expression](graph.png)
Intracellular Signaling Pathways Regulating PGT Gene Expression: PI3K/Akt, MAPK pathways

![Graph showing fold change in PGT mRNA levels with treatments.]

- Control
- hCG
- LY294002 + hCG
- U0126 + hCG

- LY294002 inhibits PI3K/Akt pathway.
- U0126 inhibits MEK/ERK1/2 pathway.

Legend:
- P: Phosphorylation
- LHR: Luteinizing Hormone Receptor
- PI3K: Phosphatidylinositol 3-Kinase
- Akt
- MEK: Mitogen-Activated Protein Kinase Kinase
- ERK1/2: Extracellular Signal-Regulated Kinase 1/2
- hCG: Human Chorionic Gonadotropin
Intracellular Signaling Pathways Regulating PGT Gene Expression: PKA, PKC and MAPK pathways.
Summary & Conclusions

- In the preovulatory follicles PGT is induced in response to LH surge.
- LH-induced expression of PGT is mediated by PKA and PKC pathways.
- Both pathways signal through the activation of the MEK-ERK pathway.

In vitro PGT Function in Human Granulosa Cells
Prostaglandin Transporter (PGT)

- PGT has been identified as major PGE\textsubscript{2} transporter.
- Three possible roles were postulated for PGT:
  1. PGT mediate the **efflux** of newly-synthesized PGs from cells.
  2. PGT mediate the **influx** of PGs for their inactivation.
  3. PGT mediate vectorial PG transport.
PGT Mediated Uptake of PGE$_2$

Time-Course of PGT Mediated PGE$_2$ Uptake

In Vivo Mouse Model
The Role of PGT in the Ovulatory Process
Superovulation Protocol

- **10U PMSG**
- After 48h:
  - **10U hCG**
- After 16h:
  - Collection of oocytes from oviducts

**23-25 day old**

- **10U PMSG**
- After 48h:
  - **10U hCG + DIDS**
PGT mRNA Expression Pattern in the Mouse Ovary Throughout the Pre-ovulatory Period

![Bar graph showing PGT/β-actin mRNA expression levels across different time points following hCG administration.](Image)

- **Saline**: a
- **0h**: b
- **3h**: b
- **6h**: c
- **9h**: c
- **12h**: b

**Legend**:
- a
- b
- c

**Note**:
- Values are expressed as PGT/β-actin mRNA (×10^3)
Dose-dependent Inhibition of hCG-induced Ovulation by DIDS in PMSG-primed mice

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. ovulating mice/no. treated (%)</th>
<th>Mean no. ova/ovulating mice</th>
<th>Range of oocytes in ovulating mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>hCG</td>
<td>16/16 (100%)</td>
<td>56.2±6.6</td>
<td>10-111</td>
</tr>
<tr>
<td>hCG+Vehicle</td>
<td>5/5(100%)</td>
<td>60.6±3.5</td>
<td>55-74</td>
</tr>
<tr>
<td>hCG+DIDS 50 mg/kg</td>
<td>1/25 (4%)</td>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td>hCG+DIDS 20 mg/kg</td>
<td>7/12 (58.3%)</td>
<td>22±6.8</td>
<td>1-51</td>
</tr>
<tr>
<td>hCG+DIDS 10 mg/kg</td>
<td>9/13 (69.2%)</td>
<td>27.4±6.8</td>
<td>10-60</td>
</tr>
<tr>
<td>hCG+DIDS 5 mg/kg</td>
<td>12/12 (100%)</td>
<td>53.5 ±5.1</td>
<td>7-77</td>
</tr>
</tbody>
</table>

### Inhibition of Ovulation with BCG

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<td>10-111</td>
</tr>
<tr>
<td>hCG+BCG 500mg/kg</td>
<td>0/4 (0%)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>hCG+BCG 350mg/ml</td>
<td>0/4 (0%)</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>hCG+BCG 300mg/ml</td>
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<td>1</td>
<td>-</td>
</tr>
<tr>
<td>hCG+BCG 250mg/ml</td>
<td>15/16(93.8%)</td>
<td>58.4±7.4</td>
<td>12-61</td>
</tr>
</tbody>
</table>
Ovulation cascade

- Oocyte maturation
- Luteinization
- Cumulus expansion
- Follicular rupture
Induction of ovulation in mice - Ovarian Histology

48hr PMSG

Intact granulosa and Theca Layers

Thick wall

Compact cumulus
Induction of ovulation in mice-Ovarian Histology

- **Cumulus Expansion**
- Post hCG (12-16h)
- GC invasion and membrane thinning
- Follicle rupture

**Ovarian Histology post hCG (12-16h)**
PGT inhibition by DIDS block hCG effects (12hr)

**hCG**
- GC invasion and membrane thinning
- Cumulus expansion

**hCG+DIDS**
- Intact GC and theca layers
- Compact CC + GV oocyte

PGT inhibition by DIDS block hCG effects (16hr)

- **hCG**
  - Early corpus luteum

- **hCG+DIDS**
  - Compact CC + GV oocyte
PGT inhibition by DIDS block hCG effects (40hr)

Progesterone levels

Molecular effect of inhibition of PGT by DIDS on Ovulation

- LH targets
Molecular effect of inhibition of PGT by DIDS on Ovulation

- LH targets
- Cumulus expansion and ovulation
Molecular effect of inhibition of PGT by DIDS on Ovulation

- LH targets
- Cumulus expansion and ovulation
- Steroidogenesis
Summary

• PGT blockage results in
  – Complete blocking of all ovulatory processes
  – Inhibition in the molecular level

• PGT is responsible for PGE2 influx in granulosa cells

• PGT blocking leads to accumulation of extracellular PGE2.

Mechanism?
• Elevated extracellular PGE\(_2\) levels leads to EP receptor desensitization.
• This effect may be mitigated by PGT expression.
• PGT is a modulator of EP receptor signaling
PGE2 desensitization in Granulosa cells culture

- Primary granulosa cells were treated with PGE2.
- cAMP levels were measured after 10 min to estimate EP receptor activity (short term desensitization).
- EP4 receptor levels following incubation with PGE2 for 24h (long term desensitization).
Modeling the role of PGT in LH/hCG-induced ovulation

PG receptor desensitization

hCG

↑ COX-2

↑ PGT

↑ PGE2

Normal PGE2 Signaling

↓ PGE2

Ovulation

hCG + PGT inhibitor

↑ COX-2

↑ PGT

↑ PGE2

PGE2 receptor desensitization

→ Blocked PGE2 signaling

↓ PGE2

No ovulation
LH-PG signaling in ovulation

- LH
- EGF like (AREG, EREG)
  - COX-2
    - PGE2↑
    - EP2 signaling
    - EGF like (AREG, EREG)
      - ERK 1/2
        - FSH action
          - Oocyte maturation
          - COC expansion
          - TNFAIP6
          - Follicle rupture
            - PR
            - ADAMTS1
            - CTSL
          - Luteinization
            - StAR
  - PGE2↓
    - Sensitized cell

Ben-ami et al. MHR 2006
Shrestha K et al. Reproduction 2015
Translational therapeutic significance

Non hormonal contraception – the PG cascade

The combined approach
Collaborators:

Rubens Fadini
Giovanni Cotticio
Mariabeatrice Dal Canto
Fausta Brambillasca
Biogenesi Reproductive Medicine Centre, Istituti Clinici Zucchi
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Ettie Maman

Svetlana Merkman