Vasorin: A Newly Identified Regulator of Ovarian Folliculogenesis and Ovulation

Nava Dekel

The Weizmann Institute of Science

Israel
Fertilization

Ruth Shalgi & David Phillips
Vasorin
Talk Outlines

• Introduction to Vasorin
• Mechanism of Vasorin action
• TGFβ superfamily members
• Research aims
• Experimental model & design
• Results
• Conclusions
Vasorin(Vasn)

• *A type I transmembrane glycoprotein*

• *Highly conserved at the DNA and protein levels (95% and 83% identity between murine and human)*

• *First identified in vascular smooth muscle cells*  
  *(Ikeda et al, 2004)*
Vasn Regulates the TGFβ Signaling Pathway

- ADAM17 cleaves Vasn releasing its soluble fraction
- sVasn competes with the TGFβ1R on TGFβ binding lowering its availability

Malapeira J et al, Oncogene, 2011
**TGFβ Superfamily Members in Folliculogenesis and Ovulation**

**Gonadotropin-Independent Preantral Follicle Growth**

- PGCs
- Germ Cells Clusters
- PMF
- Primary Follicle
- Secondary Follicle

**Gonadotropin-Dependent Antral Follicle Growth**

- Antral Follicle
- Preovulatory Follicle
- Corpus Luteum

**Activin** is involved in primordial follicle assembly

**GDF9 and BMP15** are required for early stages of folliculogenesis

**AMH** Inhibits primordial follicle growth

**TGFβ1** is involved in ovulation, CL function and angiogenesis
Life History of Ovarian Follicles

INITIAL RECRUITMENT

Primary

Primordial

Secondary

CYCLIC RECRUITMENT

Antral

human (2-5 mm)
rat (0.2-0.4 mm)

Selection & Dominance

Graafian Follicles

Atretic

Ovulation

Hsueh and McGee, 2000
The Big Question

The mechanism that controls the number of follicles recruited for growing and ovulation thus maintaining the ovarian reserve
Research Aim

To elucidate the role of Vasn in ovarian physiology and female fertility, focusing on its potential involvement in the protection of the ovarian reserve
Experimental Model

- Wild type mice
- Vasn systemic KO mice
- Vasn cKO mice, in which the deletion was directed at the granulosa cells
Experimental Design

• **Expression of Vasn in the ovary**
  
  Regulation of Vasn expression by LH

• **The role of Vasn in ovulation**
  
  Animal model: cKO and systemic KO mice

• **The role of Vasn in early folliculogenesis**
Vasn is Expressed in the Ovary

In-situ hybridization

mRNA

IHC

Protein
Vasn mRNA is Upregulated by LH
Upregulation of Vasn mRNA in Response to LH

\[ p < 0.05 \text{ Vs. } 0\text{hr} \]
Upregulation of Vasn Protein by LH

4hr after hCG

48hr after PMSG
LH-induced Upregulation of Vasn Expression is Mediated by EGFR and MAPK
Experimental Design

• Expression of Vasn in the ovary
  Regulation of Vasn expression by LH

• The role of Vasn in ovulation
  Animal model: cKO and systemic KO mice

• The role of Vasn in early folliculogenesis
Vasn cKO Mice are Fertile

The number of pups/litter and the average accumulated number of pups are similar in the cKO and WT mice

N=6
Vasn Regulates Ovulation Size

\[ N=5-\text{cKO, 7-wt} \]
\[ \ast p<0.05 \]
Systemic Knock Out Ovary Transplantation Model

**Donor**
Vasn\(^{-/-}\) or Vasn\(^{+/+}\) mouse

**Recipient**
Ovariectomized WT mouse

one week

Induction of ovulation

Transplantation Assay

![Graph showing follicle type and number of follicles](image)
There are Less Apoptotic Antral Follicles in cKO Mice

A larger pool of antral follicles may account for the higher size of ovulation in the cKO mice
TGFβ Signaling Pathway is Activated in cKO mice

![Diagram showing TGFβ pathway activation in cKO mice.](image)

- WT
- cKO

Fluorescence mean intensity for pSmad2:

- WT: 20
- cKO: 60

* indicates significant difference between WT and cKO groups.
Experimental Design

• Expression of Vasn in the ovary
  Regulation of Vasn expression by LH

• The role of Vasn in ovulation
  Animal model: cKO and systemic KO mice

• The role of Vasn in early folliculogenesis
Ovaries of Vasn cKO Mice Contain Less Primordial and Primary Follicles

15-day-old cKO mouse ovaries
Some details about our cKO Mouse Model:

The deletion of Vasn is coupled to the expression of Cyp19, which takes place only in growing follicles. Thus, the lower number of small follicles is due to the lack of Vasn in growing follicles.
In the WT animals, Vasn, which is expressed by growing follicles inhibits the death or the activation of PMFs, thus protecting the ovarian reserve.
Summary

• Vasn is expressed in the ovary
• Vasn expression is regulated by LH
• The effect of LH is mediated by MAPK and EGFR
• Vasn controls the size of ovulation
• Vasn regulates the TGFβ signaling pathway
• Vasn maintains the ovarian reserve in early folliculogenesis
Conclusions

- **Vasn is a new central player in folliculogenesis**
- **As Vasn is evolutionary conserved and as it has been recently identified in human follicular fluid it may be targeted for possible clinical implications, such as fertility preservation**
Vasorin Conditional Knock Out (cKO) Mouse Model

P  \( \text{Vasn}^{f/f} \times \text{Cyp19-Cre}^{+/-} \)

F1  \( \text{Vasn}^{f/-} \times \text{Cyp19}^{+/-} \times \text{Vasn}^{f/f} \)

F2  \( \text{Vasn}^{f/f} \times \text{Cyp19-Cre}^{+/-} \)  cKO

Ai9 Cyp19-Cre

cKO Verification - RT-PCR on GC 3hr after hCG

Fold change

<table>
<thead>
<tr>
<th>Genotype</th>
<th>WT-3hr</th>
<th>cKO-3hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*
Vasorin- Systemic Knock Out Mouse Model

- **Vasn-KO was confirmed in mRNA level.**
- **Vasn-KO mice die by the age of 30 days before reaching sexual maturation.**
The Ovarian Reserve in Vasn Systemic KO mice is more than Doubled

15-day-old mouse ovaries

Vasn may be involved in the establishment of the ovarian reserve.