Evaluation and Management of Embryonic – Endometrial Synchrony

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The Endometrial Transcriptome

Excessive LH
Inadequate LH
Factors regulating implantation

- Embryo
- Endometrium
- Synchrony
Traditional View of Disorders of Embryonic-Endometrial Synchrony

- The Embryologists’ perspective
  - Temporal milestones of early development correlate with subsequent performance
    - Early cleavage
    - 6 or more cells on day 3
    - Time lapse
      - Time at 2 cell stage, 3 cell stage, etc
    - May be explained by failure to develop to the blastocyst stage
  - Timing of blastulation
    - Day 5 blastocysts
    - Day 6 blastocysts
    - Day 7 blastocysts
Day 5 versus Day 6
Fresh Blastocyst Transfer

Barrenetexea et al Fertil Steril 2005; 83:49-53
Day 5 versus Day 6 Blastocysts

Shapiro et al Fertil Steril 2008; 89:20-6
Traditional View of Disorders of Embryonic-Endometrial Synchrony

- The Endometrial Physiologists’ perspective
  - Luteal phase defects
    - Endometrial biopsies
    - Mid-luteal Progesterone assays
- Abnormal endometrial development (ultrasound)
  - Thin endometria (late follicular)
  - Hyperechoic endometria (late follicular)
  - Hyper-contractile endometria (luteal)
- Abnormal timing for the window of receptivity
  - Evidenced by the transcriptome (ERA)
Histologic Dating of the Endometrium is Imprecise

Out of Phase Endometrial Biopsies are Equivalent in Fertile and Infertile Women

Coutifaris et al Fertil Steril 2004; 82:1264-72
Serum Progesterone Levels..

- Natural cycle
- Different in stimulated cycle with hCG present
- Demonstrates tolerance for varying levels of P
- May explain limited predictive value of serum P monitoring

Filicori et al  
*J Clin Invest* 1984;73:1638-47
Varying Degrees of Hyperechoic Endometria and Clinical Outcomes
Impaired Receptivity

- **Histology**
  - Delayed or dysynchronous maturation
- **Endocrinology**
  - Progesterone
- **Proteomics**
  - Integrins
  - Selectins
  - Other candidates...
- **Transcriptomics**
  - *Altered transcriptome profile (ERA™)*
- Others.....

- Represents pathology – a deviation from a normal endometrial response
- Should be reproducible from cycle to cycle
Non-Traditional View of Embryonic-Endometrial Synchrony

• Can there be abnormalities in synchrony when:
  – embryonic blastulation and expansion are completely normal?
  – Endometrial receptivity is capable of being completely normal?
Window of Receptivity

- Day 15 = day 1 of P administration
- Day 2 embryos
- Documented a three day window of transfer

• Day 15 = day 1 of P administration
• Day 2 embryos
• Documented a three day window of transfer


• Pregnancies subsequently reported within a 5 day window
• Day 2 embryos.
Revisiting the Window of Receptivity

SUSTAINED IMPLANTATION RATES DECLINE OUTSIDE OF “OPTIMAL WINDOW”

Delayed Implantation versus Poor Embryo Quality

Wilcox et al NEJM 1999
What goes wrong?
Altered Rates of Endometrial Maturation

**Endometrial Receptivity Assay (ERA™)**

- Fixed P exposure
- Evaluation of the endometrial transcriptome
- Unique patterns for each day
- Results
  - In phase
  - Pre-receptive
  - Post-receptive

Ruiz-Alonso *et al* Fertil Steril 2013

Adapted from www.iviomics.com
Endometrial Receptivity Assay

• Diagnosis abnormalities in the endometrial response
  – Sufficient progesterone
  – Appropriate timing

• Reproducible
  – Intrinsic abnormality
  – Occurs in every cycle

• True deviation in normal physiology

• Most common in women with unexplained Recurrent Implantation Failure

Adapted from www.iviomics.com
Dysynchrony

- Loss of the temporal coordination *between* the embryo and the endometrium
- BOTH the embryo and the endometrium have normal reproductive potential
- Is not automatically reproducible from cycle to cycle
- About timing of the stimulus..
Dysynchrony versus Pathology

- **Endometrial Pathology**
  - Accelerated or retarded rate of development
  - Does NOT vary from cycle to cycle

- **Dysynchrony**
  - Normal endometrial responsiveness
  - Reflects an abnormality in the timing of the stimuli which induce endometrial receptivity
Altered Window of Endometrial Receptivity

Onset of P Exposure

Pathologic

Normal

Dysynchronous

Onset of P Exposure

Blastocyst Expansion

Window of Receptivity

Onset of P Exposure

Pre-Receptive

Post-Receptive

Dysynchronous
Progesterone and clinical outcomes

- Serum Progesterone on day of HCG administration
- 2 critical breakpoints
- Could exogenous LH be the cause?

Late follicular rise in progesterone

- Retrospective study
- 4032 patients
- $P_4 \geq 1.5$ ng/mL associated with lower ongoing pregnancy rates

Progesterone and the Endometrial Transcriptome

Adapted from S. Young, MD, PhD
Progesterone Pharmacokinetics

Usadi RS, et al. JCEM 2008 & Young Lab, Unpublished

Adapted from S. Young, MD, PhD
### Progesterone and the Endometrial Transcriptome

<table>
<thead>
<tr>
<th>Number of genes differentially expressed vs. 40 mg P</th>
<th>Natural Cycle</th>
<th>10 mg P</th>
<th>5 mg P</th>
<th>2.5 mg P</th>
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</thead>
<tbody>
<tr>
<td>≥ 2-fold change</td>
<td>0</td>
<td>0</td>
<td>70</td>
<td>236</td>
</tr>
<tr>
<td>≥ 1.5-fold change</td>
<td>0</td>
<td>0</td>
<td>605</td>
<td>1186</td>
</tr>
</tbody>
</table>

Young Lab, Unpublished

Adapted from S. Young, MD, PhD
Rise in $P_4$ with time after hCG administration

Hours Since hCG Administration

Progesterone (ng/mL)

N=12,463
Controlling for the number of mature follicles increasing luteinization prognosticates more oocytes recovered.
Late follicular rise in progesterone

- Prior to hCG: suboptimal

- After hCG:
  - Accompanies optimal mature oocyte yield
  - ? Impact on endometrial receptivity

Natural Cycle

Onset of LH Surge

Ovulation

Progesterone Rise

Embryonic Window of Implantation

Endometrial Window of Implantation

time
Stimulated Cycle

Synchronous versus Dysynchronous

- hCG administration
- Progesterone Rise
- Embryonic Window of Implantation
- Endometrial Window of Implantation
- Retrieval

24h
embryo and endometrium synchrony - revisited

Franasiak et al ASRM 2013
How do you isolate the components impacting synchrony?

- **Endometrium**: difficult to measure and control for
- **Embryo**: Can assess embryonic development in the laboratory

**Sustained Implantation Rate by D5 Expansion**

- MOR: 36.1%
- B1: 35.3%
- B2: 58.7%
- B3: 59.1%
- B4: 60.0%
- B5-6: 60.0%
Fresh day 6 embryo transfer

Removes those embryos which do not progress to B2+

Franasiak et al ASRM 2013
Frozen synchronous cycle

Progesterone Start

Endometrial Window of Implantation

time
Frozen day 6 embryo transfer

Fransasiak et al ASRM 2013
Embryonic Endometrial Synchrony

*It take two.....*
Progesterone and Impaired Implantation: A Pilot Study of *Euploid* Blastocysts

All patients had P levels < 1.5 ng/mL prior to the administration of hCG
Older patients are more likely to have “slow” embryos

Proportion of "Slow" Blastocysts

- <35 years old: 31%
- ≥35 years old: 46%

P < 0.0001

Forman et al ASRM 2013
Embryonic-Endometrial Asynchrony Increases with Maternal Age

- Retrospective
- 1,341 IVF cycles
- Thresholds for Asynchrony (either)
  - $P > 1.5$ mg/mL on day of hCG
  - No blastulation prior to day 6
- Risk for asynchrony increases with maternal age
- Live birth predicted
  - Day 5 blastulation ($P < 0.0001$)
  - $P < 1.5$ ng/mL ($P = 0.0002$)

Shapiro BS et al Fertil Steril 2013 100:S287

Is it asynchrony or an intrinsic diminution in quality?
Follicular Progesterone Elevations

The Two Cell Theory

DHEA
Progesterone

C21 → C19

Theca Cell

C27

LH-R

Androgen

C19 → C18

Granulosa Cell

C21 ← C27

Estradiol

C21

Cholesterol

Circulation

FSH-R

Cholesterol
Optimal Ratio of exLH/exFSH

Colors separate statistically definable groups

% Progesterone Elevation (>1.5 ng/mL)

Werner et al – Fertil Steril 2014
Differences between response groups

% Cycles with Progesterone Elevations >1.5ng/mL

- Low Responder
- Normal Responder
- High Responder

Werner et al – Fertil Steril 2014
Synchrony in Low Responders

Experimental Design

Follow Up Clinical Outcomes

1999-2009 Cleavage Stage Transfer

- Transfer order based on what was available
- Day 3 Embryo Transfer

2009-2012 Extended Culture – Blastocyst Transfer

- Expanded blastocyst by 6 AM day 6?
  - No
    - Vitrification: Max of 2 blast transfer next cycle
  - Yes
    - Fresh transfer max of 2 blasts

-or-

-or-

-or-

-or-
Number of Embryos Present on day 3 of \textit{in vitro} development

![Bar chart showing the proportion of cases for different numbers of embryos on day 3. The chart compares Cleavage Transfer and Blast Transfer.}

Proportion of Cases (%) vs. Number of Embryos Present on Day 3

- **1 Embryo Present on Day 3:**
  - Cleavage Transfer: 20%
  - Blast Transfer: 15%

- **2 Embryos Present on Day 3:**
  - Cleavage Transfer: 30%
  - Blast Transfer: 30%

- **3 Embryos Present on Day 3:**
  - Cleavage Transfer: 40%
  - Blast Transfer: 50%

Statistical significance: $P<0.0007$
Sustained Implantation Rate

Proportion of Embryos which Delivered

% of embryos transferred which delivered

Cleavage: 11025
Blastocyst: 1251

P<0.0001
Delivery Rate

Proportion of Transfers

% Transfers

Cleavage

5001

Blastocyst

538

P<0.00001
Fresh versus Cryopreserved Blastocyst Transfer

Sustained Implantation Rates

P = 0.75

122 / 280

80 / 174
No Transfer Rate

Proportion of Embryos which Delivered

Cleavage

Blastocyst

P<0.00001

0 / 5001

84 / 538
Intent to Treat Delivery Rate

Delivery rate based relative to decision to transfer day 3 or go to blast

P<0.05

<table>
<thead>
<tr>
<th>Cleavage</th>
<th>Blastocyst</th>
</tr>
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<tbody>
<tr>
<td>1670 / 5001</td>
<td>202 / 538</td>
</tr>
</tbody>
</table>

Bar graph showing delivery rates for Cleavage and Blastocyst.
Beware of Interference in your P Assay

- Patients receiving DHEA have elevated DHEA-SO\textsubscript{4} levels
- These levels may falsely elevate P levels
- Assay dependent

Forman - RMANJ
Summary

• Understanding of Embryonic-Endometrial synchrony is still evolving

• Active management of synchrony improves clinical pregnancy rates

• Each program will need to refine its own limits for synchronous transfer

• It may lead programs to consider cryo all cycles in the future
Thoughts or Questions?