Targeting mTOR signaling to promote reproductive longevity

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• American Society for Reproductive Medicine
• Foundation for Women’s Wellness

Study approved by the New York University Institutional Animal Care and Use Committee (IACUC)
Aging

- Stem cell exhaustion
- Cellular senescence
- Mitochondrial dysfunction
- Deregulated nutrient sensing
- Genomic instability
- Telomere attrition
- Epigenetic alterations
- Loss of proteostasis
Dietary restriction: IIS and mTOR inhibition AMPK and sirtuin activation

Clearance of senescent cells

Mitohormetics, mitophagy

Stem-cell-based therapies

Anti-inflammatory drugs
Blood-borne rejuvenation factors

Elimination of damaged cells

Telomerase reactivation

Activation of chaperones and proteolytic systems

Epigenetic drugs

Lopez-Otin et al, Cell 2017
mTOR and aging
Rapamycin extends lifespan in mice
Reproductive aging in the context of somatic aging
Fixed primordial follicle pool declines progressively with age

Adapted from De Vos et al. Lancet 2010

Total primordial follicles

Age (y)

Mean age of first birth

Menopause

Birth

Female life expectancy

Adapted from De Vos et al. Lancet 2010
Delayed childbearing

Mean age by birth order: United States, 2000-2014

## Delayed childbearing in the U.S

<table>
<thead>
<tr>
<th>First child between 35-39y</th>
<th>First child between 40-44y</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Massachusetts</td>
<td>1) Washington, D.C.</td>
</tr>
<tr>
<td>3) New York</td>
<td></td>
</tr>
</tbody>
</table>

**New York 2012:**
Births in women >35y up 57% from 2000-2012

Adapted from CDC.gov
## Culture of competing interests

### For Most Highly Educated Women, Motherhood Begins in the Thirties

*Age at birth of first child, by educational attainment*

<table>
<thead>
<tr>
<th>Education Level</th>
<th>UNDER 30</th>
<th>30 AND OLDER</th>
<th>MEDIAN AGE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>under 25 yrs</td>
<td>25-29</td>
<td>30-34</td>
</tr>
<tr>
<td>Master’s degree+</td>
<td>18%</td>
<td>28%</td>
<td>34%</td>
</tr>
<tr>
<td>Bachelor’s degree</td>
<td>26%</td>
<td>34%</td>
<td>27%</td>
</tr>
<tr>
<td>Two-year degree/some college</td>
<td>49%</td>
<td>29%</td>
<td>15%</td>
</tr>
<tr>
<td>High school or less</td>
<td>62%</td>
<td>22%</td>
<td>11%</td>
</tr>
</tbody>
</table>

Note: Based on women ages 40-50 who have ever given birth.

PEW RESEARCH CENTER
Culture of competing interests

Majority believe delaying or avoiding motherhood is the best way to achieve career success.

A Woman Who Wants to Reach a Top Position in Business Is Better Off...

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Having children early on in her career</td>
<td>36</td>
<td>36</td>
<td>37</td>
</tr>
<tr>
<td>Having children later in her career</td>
<td>40</td>
<td>38</td>
<td>41</td>
</tr>
<tr>
<td>Not having children at all</td>
<td>22</td>
<td>24</td>
<td>20</td>
</tr>
</tbody>
</table>

Note: “No answer” not shown.
Source: Pew Research Center survey, Nov. 12-21, 2014 (N=1,835)
Autologous IVF over 40

SART 2005

SART 2014

Age (years) vs Percent

- Pregnancies
- Live births
- Singleton live births

Curves show the percentage of pregnancies, live births, and singleton live births for different age groups.
Physiologic ovarian folliculogenesis

- Primordial
- Primary
- Secondary
- Antral
Promoting and inhibitor factors

**Promoting**
- mTOR
- AKT/PIK3C
- Nobox
- Kit/KitLG
- GDF9

**Inhibiting**
- PTEN
- S6K1
- FOX03
- AMH
- FOXL2

**Primordial**
- GDF9

**Primary**
- IGF1
- GDF9

**Secondary**
- LH/LHR
- FSH/FSHR
- ESR1/2
- CYP19A1
- FMR1
- GDF9

**Antral**
- TP53
- BAX

Adapted from Wood-Trageser M, Rajkovic A. Sem Reprod Med 2013
Promoting and inhibitor factors

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Adapted from Wood-Trageser M, Rajkovic A. Sem Reprod Med 2013
mTOR is central to ovarian folliculogenesis

Nutrients
Growth factors
Cancer

PI3K → AKT → mTORC1, mTORC2

mRNA translation
Cell growth
Survival
Proliferation

Primordial → Primary → Secondary → Antral

Reddy et al. Science 2008
Adhikari D et al. Mol Hum Reprod 2009
mTOR pathway critical to primordial follicle activation

mTOR pathway critical to primordial follicle activation

- Nutrients
- Growth factors
- Chemotherapy
- Cancer

- PI3K
- AKT
- mTORC1
- mTORC2
- S6K1
- 4EBP1

mRNA translation
Cell growth
Survival
Proliferation

Primordial
Primary
Secondary
Antral

Chronically activated PI3K/AKT activity results in follicular depletion

PI3K \[\rightarrow\] PTEN

AKT \[\downarrow\] TSC1/2

mTORC1 \[\downarrow\] mTORC2

S6K1 \[\downarrow\] 4EBP1

Primordial \[\rightarrow\] Primary \[\rightarrow\] Secondary \[\rightarrow\] Antral

Pharmacological control of folliculogenesis via the PI3K/AKT/mTOR pathway

**ACTIVATION**
- PTEN inhibitors
- PI3K activators
- Actin-polymerization enhancing drugs
- mTOR stimulators

**INHIBITION**
- AMH
- SIRT1 activators
- Resveratrol
- AS101
- mTOR inhibitors
mTOR inhibitors

RAD001
Everolimus

mTORC1 mTORC2

PI3K

AKT

S6K1 4EBP1

Protein translation

Cell growth, survival, proliferation

INK128
MLN0128
mTOR inhibitors preserve ovarian reserve and fertility during gonadotoxic chemotherapy

Baseline (8 weeks)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AMH (ng/mL)</th>
<th>Litter size (pups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Cy75</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>RAD + Cy75</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td>INK + Cy75</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>RAD</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>INK</td>
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</tbody>
</table>

Control (75 mg/kg CY) + RAD + INK + Cy75

* Indicates significance.
mTOR inhibitors preserve primordial follicles compared to control

>30% more PMFs in mice treated with a short course of mTOR inhibitors

Goldman et al., unpublished
mTOR and longevity

- mTOR de-regulation implicated in aging-related disease
- mTOR inhibitors prolong lifespan across multiple species

mTOR and reproductive longevity

- Calorie restriction – prolongs reproductive lifespan (McShane and Wise, 1996)
  - Mediated through insulin signaling and mTOR
- Resveratrol prevents age-related fertility decline in mice (Liu et al, 2013)
  - Induces autophagy by directly inhibiting mTOR (Park et al, 2016)
Hypothesis: mTOR inhibitors promote reproductive longevity

mTOR inhibitor (RAD, INK)

Birth
Reproductive maturity 8 weeks
Peak fertility 2-6 months
Declining fertility
Reproductive senescence 13-14 months
Methods

C57BL/6
8 weeks (n=7/group)

Control (PVP)
RAD001 (mTORC1 inhibitor) 2.5mg/kg
INK128 (mTORC1/2 inhibitor) 0.3mg/kg

Daily RAD
4 weeks

Daily INK
8 weeks

Consecutive breeding
11 months old
Study timeline and C57BL/6 reproductive function

- **Birth**: 8 weeks of age
- **Reproductive maturity**: 2-6 months
- **Peak fertility**: 6-9 months
- **Declining fertility**: 13-14 months
- **Reproductive senescence**: 13-14 months

**Study timeline**

8 weeks of age: treatment initiated (4 week duration)

8 week interval

5 months to 11 months of age

Sacrifice

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Franks LM, Payne J. J Reprod Fertil 1970
Outcomes analyzed

Breeding data
- Time to delivery
- Total litters
- Total pup number

Birth outcomes
- Birth weight
- Litter size (pups per litter)
- Gross anomalies

Tissue
- Weights
- Serum AMH
- Bilateral ovaries
  - Histological follicle counts
  - Immunoblots
Outcomes analyzed

**Breeding data**
- Time to delivery
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- Litter size (pups per litter)
- Gross anomalies

**Tissue**
- Weights
- Serum AMH
- Bilateral ovaries
  - Histological follicle counts
  - Immunoblots
No systemic toxicity between groups
Weight and surface area

**Ovarian weight**

- **Control**
- **RAD**
- **INK**

**Uterine weight**

- **Control**
- **RAD**
- **INK**

**Ovarian surface area (mm²)**

- **Control**
- **RAD**
- **INK**

*Note: The comparison between Control and INK is statistically significant.*

**control**

**INK**
No difference in follicle counts or serum AMH
Outcomes analyzed

Breeding data
- Time to delivery
- Total litters
- Total pup number

Birth outcomes
- Birth weight
- Litter size (pups per litter)
- Gross anomalies

Tissue
- Weights
- Serum AMH
- Bilateral ovaries
  - Histological follicle counts
  - Immunoblots
Twice as many litters and pups among INK-treated mice

Total litters throughout breeding period

- Control: 2.6 ± 1.1
- RAD: 4.2 ± 1.3
- INK: 5.2 ± 0.8

Pups throughout breeding period

- Control: 19 ± 4
- RAD: 29 ± 4
- INK: 36 ± 5
No difference in pups per litter or pup survival

No evidence of gross anomalies among pups
Pup weight greater among RAD and INK-treated mice

Pup birth weight

Weight (g)

Control RAD INK

*** ns *

Pup weight

Litter 1 Litter 2 Litter 3 Litter 4 Litter 5 Litter 6
INK-treated mice fertile for 1.5 months longer than control

Age (days) last litter

Age (months) last litter
Treated mice produced litters at advanced reproductive ages.
Litter size over time remained higher in RAD and INK-treated
Conclusion: Greater reproductive potential achieved amongst mTOR-inhibitor-treated mice

- Longer reproductive lifespan
- More litters and pups
- No change in litter size over time
Limitations

- Mouse model
- Vaginal smears
- Study timing

Strengths

- Proof of principle
- Findings supported by growing body of literature
  - Rapamycin and ovarian lifespan (Dou et al, Aging Cell 2017)

Future directions

- Offspring health
- Further investigate mechanism and more precise targets
References

Thank You

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NYU Fertility Center Staff

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