Ovarian Biological Clock Rhythm and Its Effects on Follicle Reserve

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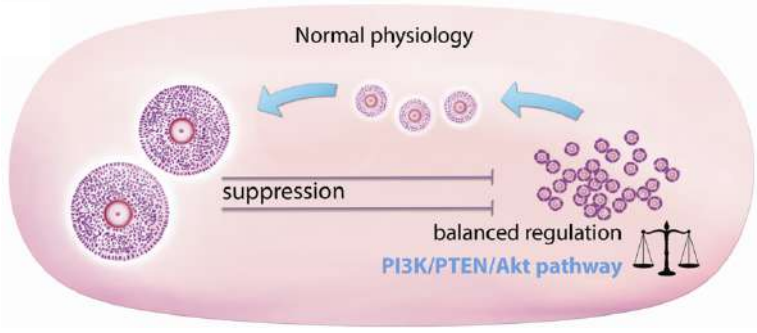
Pathways involved in follicle dormancy and activation

- PI3K/AKT pathway
- Ant Müllerian Hormone
- Hippo signaling
- Genetic
- Other pathways



Genetic etiologies leading to POI

Accelerated loss of primordial follicles - **Turner Syndrome**

Inadequate follicle pool - **Galactosemia**

- Normal development of primordial follicles during fetal development.
- Accelerated follicle loss.
- Individual genes responsible for the ovarian syndrome have not been identified.
- Etiology leading to follicle loss - unknown.

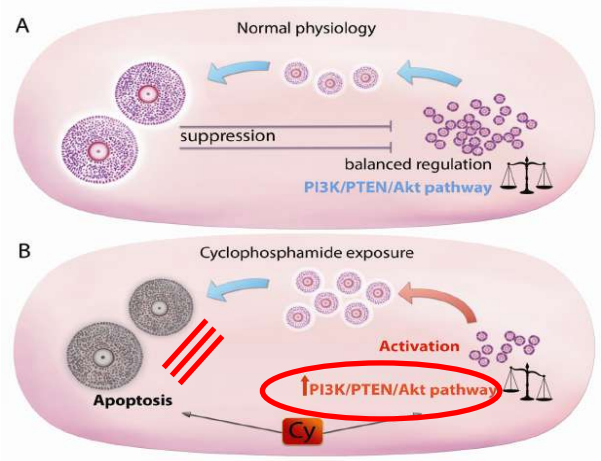
Acute Follicle loss

Massive follicle loss occurs immediately post chemotherapy – Whole ovary

Immediate follicle loss is evident post ovarian tissue transplantation – Fragment of Ovarian Cortex

What is the mechanism?

Follicle Activation: Potential Target for Drug Intervention



- Death of growing follicles which are responsible for producing AMH, a negative regulator of follicle activation.
- Upregulation of the PI3K-Akt signalling pathway.

Kalich-Philosoph et al,
Science Translational Medicine, 2013

Manuscripts supporting Follicle Activation

PLoS One. 2015 Dec 14;10(12):e0144245. doi: 10.1371/journal.pone.0144245. eCollection 2015.

Cisplatin Induces Overactivation of the Dormant Primordial Follicle through PTEN/AKT/FOXO3a Pathway which Leads to Loss of Ovarian Reserve in Mice.

Chang EM[1], Lim E[2], Yoon S[1,2], Jeong K[3], Bae S[2], Lee DR[1,2], Yoon TK[1], Choi Y[2], Lee WS[1].

Int J Mol Sci. 2016 May 30;17(6). pii: E836. doi: 10.3390/ijms17060836.

Follicle Loss and Apoptosis in Cyclophosphamide-Treated Mice: What's the Matter?

Chen XY[1,2,3], Xia HX[4], Guan HY[5,6,7], Li B[8], Zhang W[9].

Reprod Biomed Online. 2017 Jan;34(1):104-114. doi: 10.1016/j.rbmo.2016.10.005. Epub 2016 Oct 17.

Short-term exposure of human ovarian follicles to cyclophosphamide metabolites seems to promote follicular activation in vitro.

Lande Y[1], Fisch B[1], Tsur A[2], Farhi J[3], Prag-Rosenberg R[1], Ben-Haroush A[1], Kessler-Icekson G[4], Zahalka MA[4], Ludeman SM[5], Abir R[6].

PNAS 20170.1073/pnas.1620729114

AMH/MIS as a contraceptive that protects the ovarian reserve during chemotherapy

Kano M, Sosulski AE, Zhang L, Saatcioglu HD, Wang D, Nagykery N, Sabatini ME, Gao G, **Donahoe**PK, Pépin D.
Proc Natl Acad Sci U S A. 2017 Feb 28;114(9):E1688-E1697. doi: 10.1073/pnas.1620729114. Epub 2017 Jan 30.

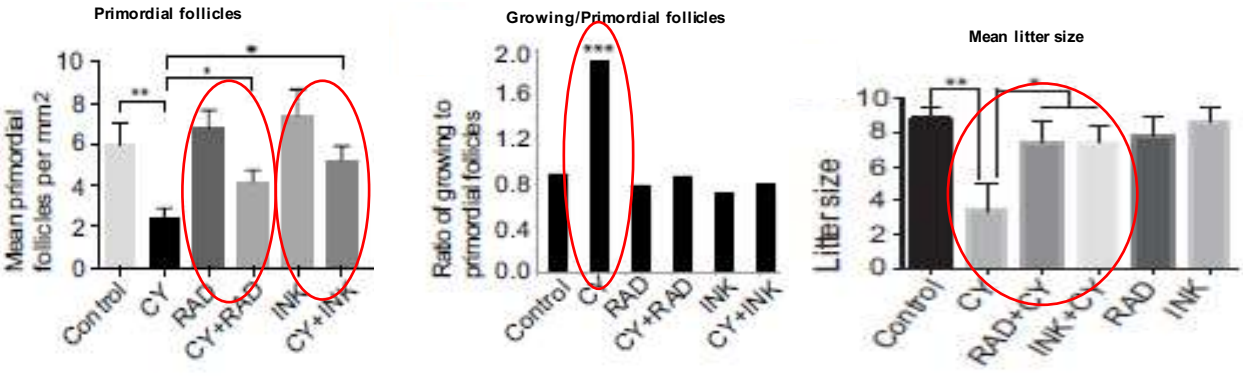
Chemotherapy-induced follicle activation and loss in human ovaries. First documentation of the "Burn-Out" effect in clinical cases

Investigating ovaries of patients recently treated with alkylating agents compared with normal ovaries.

This study demonstrate, for the first time, that alkylating agents activate the growth of the quiescent PMF pool in human ovaries exposed in vivo to chemotherapy.

Dr Shai Daniel et.al. ISFP 2017 Vienna

mTORC1/2 inhibition preserves ovarian function and fertility during genotoxic chemotherapy



RAD – MTORC1 inhibitor

INK – MTORC1/2 inhibitor

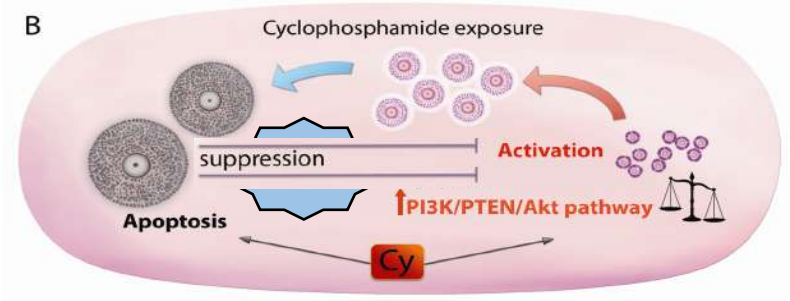
Goldman et al, PNAS, 2017

Co-treatment of temsirolimus with Cy reduced the depletion of primordial follicles *in vivo,*

providing a proof of principle that

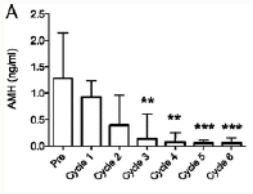
reduction of activation via the PI3K pathway increases survival of primordial follicle pool.

Our Approach: AMH replacement



HYPOTHESIS: That replacement of AMH during the initial period of loss following chemotherapy treatment would prevent the burst of follicle activation and prevent follicle loss

Effects of chemo on AMH levels

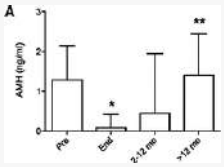


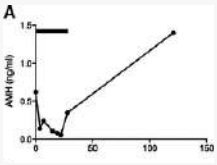
Clinical measurements of serum AMH show that chemotherapy induces a **reduction in AMH during treatment** – *"reflects increased follicular atresia"*

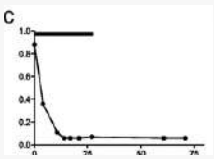
(Brougham, 2012)

AMH levels recover (partially) following cessation of treatment depending on toxicity of chemotherapy

AMH recovery after non-sterilizing treatment and sterilizing treatment

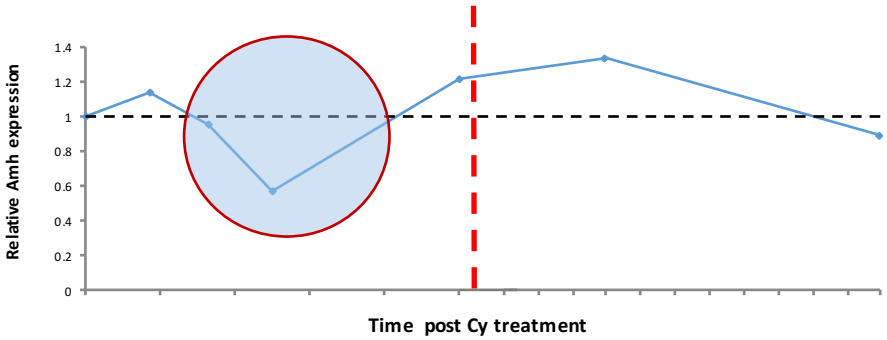






Short Term Loss of AMH Opportunity for Replacement

Real time qtPCR analysis of AMH levels in mice ovaries after in vivo administration of Cyclophosphamide: AMH levels in the ovary drop triggering a burst of follicle activation and growth, which in turn leads to an increase.



rAMH: Novel Treatment for Fertility Preservation

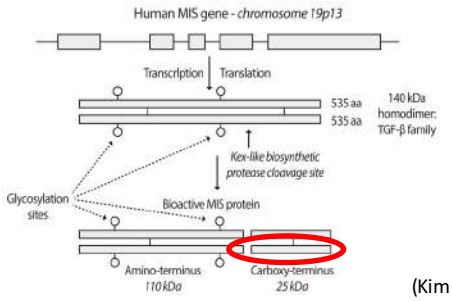
AMH: Anti Mullerian hormone. A dimeric glycoprotein hormone with a molar mass of 140 kDa

The Composition tested: Active C-terminus homodimer of 11.7 kDa

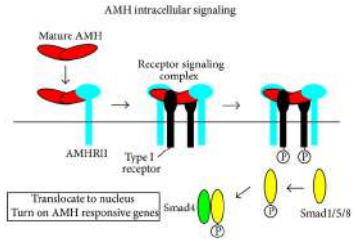
According to P. Donahoe and D. Pépina

Only the whole molecule works (PNAS 2017)

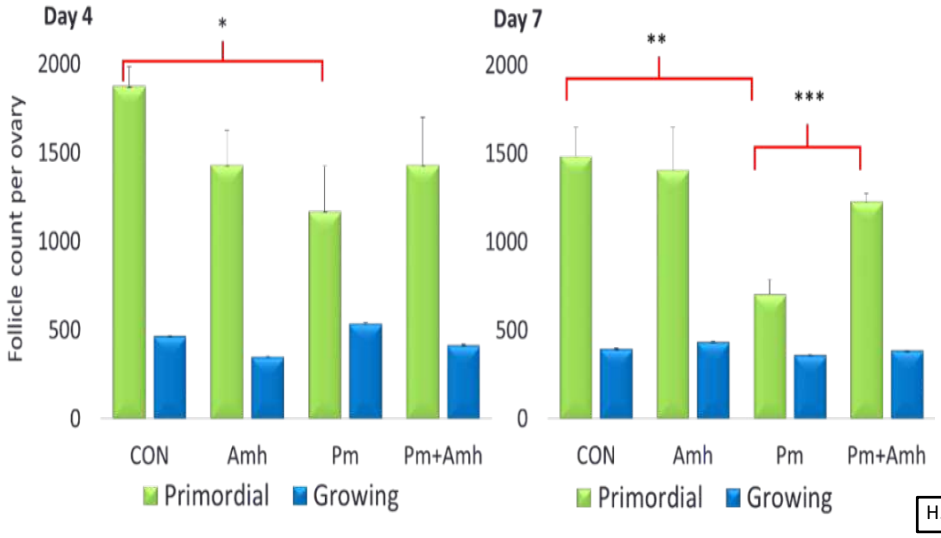
Our results are different



(Kim et al., 2014)

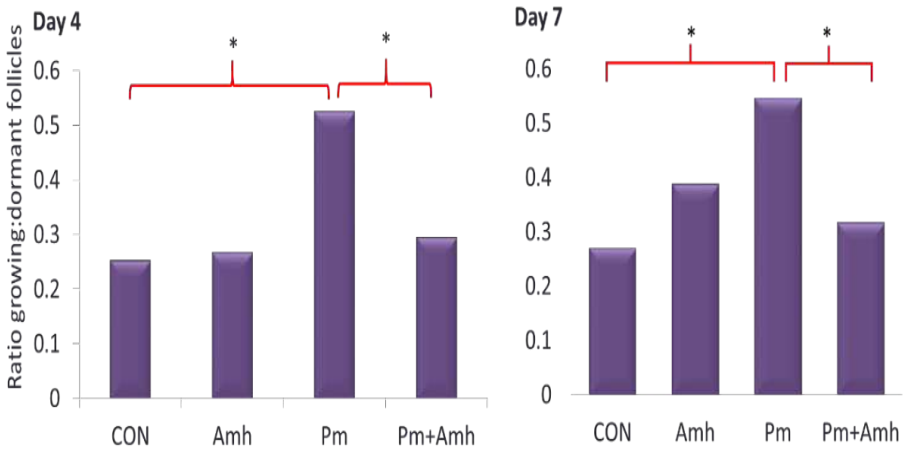


rAMH Reduces Chemo-Induced Follicle Loss In *ex-vivo*



H. Ronnes et al., 2015

AMH Replacement *ex vivo* Reduces Follicle Activation



H. Ronnes et al., 2015

AMH Replacement *In Vivo*

- **Efficacy of IP injection** – will rAMH injected IP reach the ovary?
- **Bioavailability** – how long will rAMH persist in the ovary after administration?
- **Dose** – what is the minimum effective dose that achieves the maximal results?
- **Safety** – does rAMH administration have any side effects?

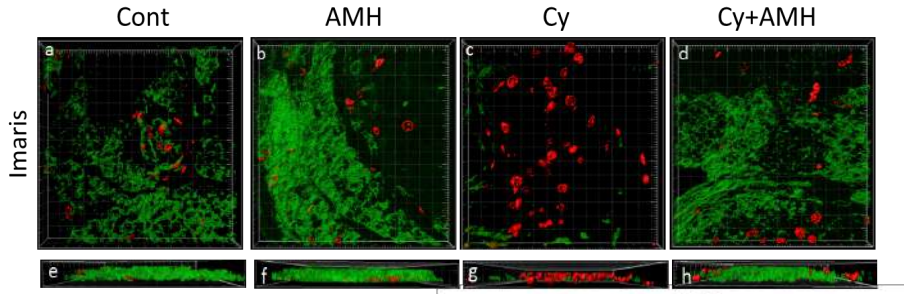
rAMH prevents Cy induced follicle activation in-vivo

Short term after treatment, mice who received rAMH along with Cy:

- Significantly higher number of primordial follicles as well as fewer growing follicles ($p<0.001$).
- Ratio of growing: dormant follicles was significantly increased in mice treated with Cy, but those which received rAMH had a ratio similar to controls.

AMH prevents the follicle activation caused by Cy

rAMH: reduced proliferation



AMH

Ki67

Conclusions

biological clock- chemotherapy / AMH

- The active fragment rAMH can be delivered to the ovary via IP injection
- rAMH has physiological activity
- **Co-administration of rAMH protects the ovarian reserve from chemotherapy-induced follicle activation and loss *in-vivo.***

Hadassa Ronnes et al submitted

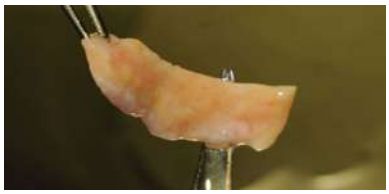
Transplantation of ovarian tissue and Follicle activation

Transplantation induced follicle activation-

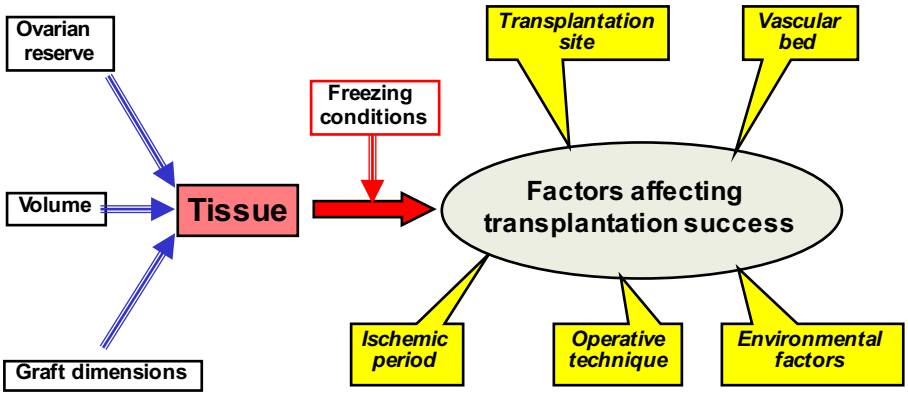
Universality,

AMH- pharmacological administration,

Mechanisms



Factors affecting transplantation success

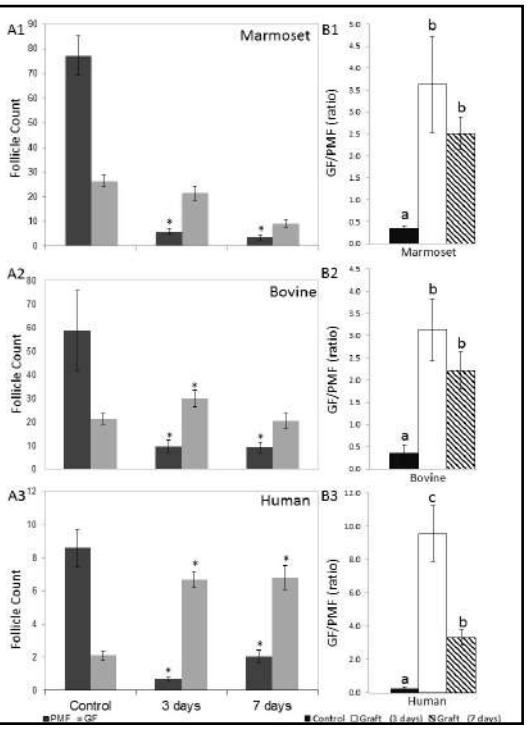


Transplantation induced follicle activation:

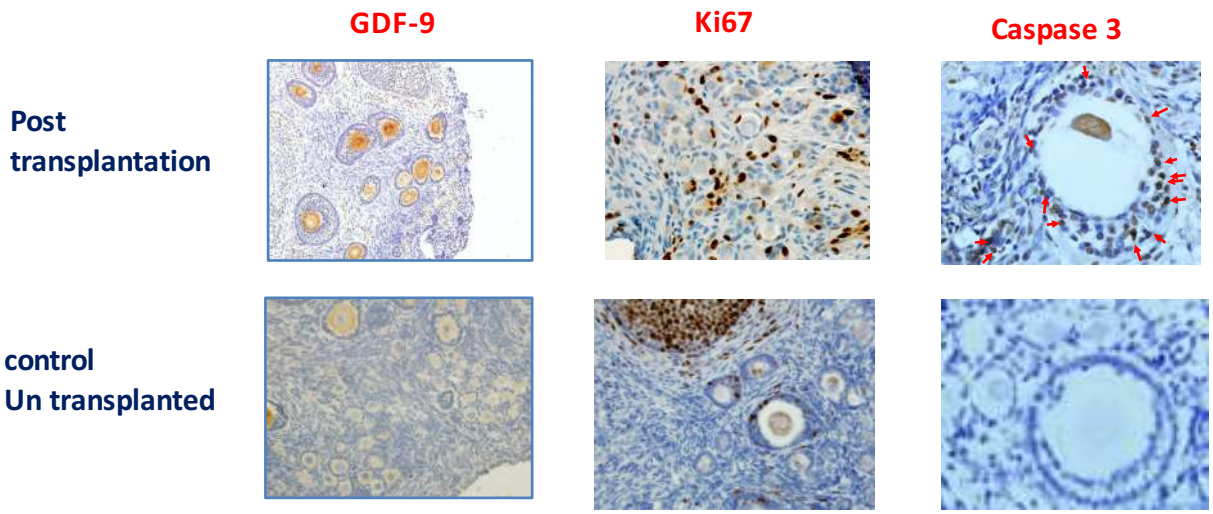
- Extensive PMF activation and loss was observed already 3 days post transplantation ($p<0.001$).
- In contrary, **growing follicles numbers increased** significantly 3 days post transplantation ($p<0.05$).

Z. Gavish et al Hum Reprod 2014

Z Gavish, I Spector et al.
JARG 2017



Activation and apoptosis markers in Marmosets ovaries post transplantation



Conclusion transplantation

These results indicate that at least part of the massive follicle loss observed post transplantation is caused by follicle activation and loss in “Burn Out” mechanism, that occurs up to 3 days post transplantation.

Post transplantation follicle activation is universal and occurs in all species evaluated.

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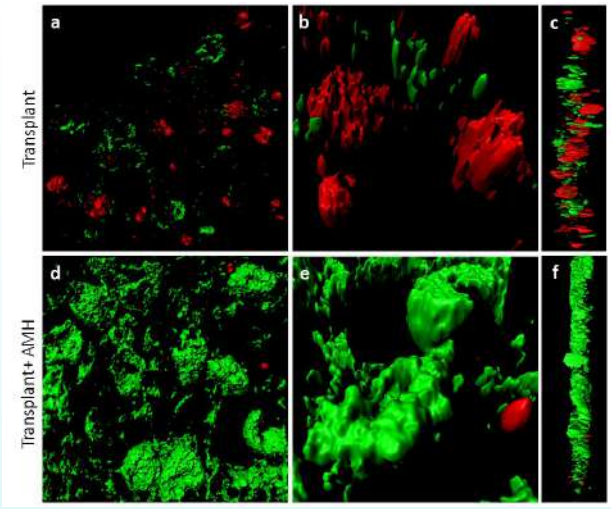
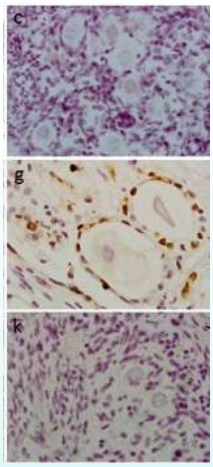
Can AMH administration
prevent post transplantation follicle activation and loss?

AMH- inhibits proliferation

AMH
Ki67



KI67

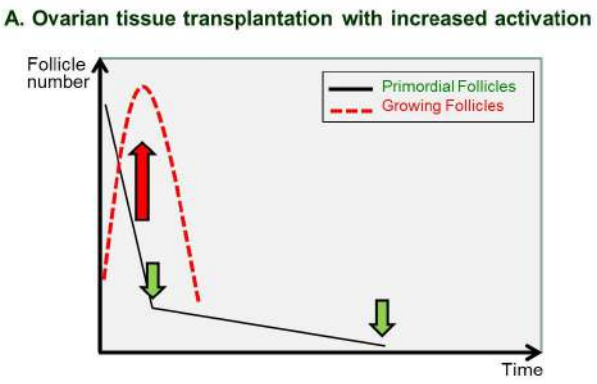


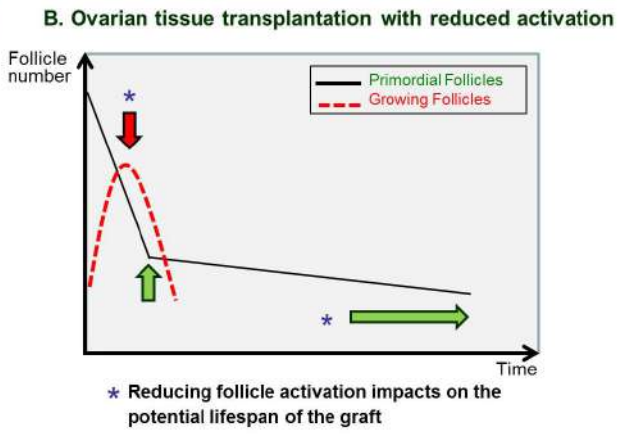
Un-transplant

transplant

Transplant +AMH

Gavish and spector 2017





Gavis Z. Spector I. et al JARG 2018

Conclusions

- rAMH prevents follicle activation and loss post tissue transplantation.
- Therefore, rAMH presents as a potentially useful clinical option for extending the life of ovarian tissue grafts and maximizing fertility for women undergoing OTCP-TP.

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