YALE UNIVERSITY

SCHOOL OF MEDICINE

Update Fertility Preservation (Cancer Patients) and Fertility Postponement (Social Reasons)

Pasquale Patrizio, M.D., MBE

Department Obstetrics & Gynecology

Yale University Fertility Center

New Haven, CT-USA

Topics

- Epidemiology of Cancer
- Risk Factors
- Options to preserve Fertility for Women
- **Updates**:
  - In Vitro Folliculogenesis
  - Whole Ovary Perfusion and Directional Freezing
- Fertility Postponement

Young Women Exposed to Sterilizing Cancer Treatment/Year in USA

4 % of Cancers (~55,000/Year): diagnosed in women under the age of 35

- 3,000 Cervix ca
- **3,500 Leukemia and 3,000 Lymphomas**
- 15% of **Breast** cancer (~**40,000/year**)
- Bone Marrow –Stem Cell Transplantation
- SLE, Glomerulonephritis, Behcet, Sickle cells, etc.

Incidence & Survival

Lymphoma/ leukemia (female)	Total number women newly diagnosed with cancer in 2011	Number and percentage women under age 34 with newly diagnosed cancer in 2011	5 Year relative Survival
HL	4,000	1,760 (44%)	90-95%
NHL	30,300	1,650 (5.5%)	80-85%
ALL	2,410	1,750 (70.6%)	64%
CLL	600	20 (0.3%)	78%
AML	6,120	810 (12.7%)	23%
CML	2,150	220 (10.3%)	57%

http://seer.cancer.gov 2013

Chemo/Radiotherapy are **Gonadotoxic** and **Risk** of Early Menopause

- **Type** of chemotherapy drug
- Cumulative **dose of chemotherapy**
- Concomitant use and **dose of radiation**
- **Age** of patient (>35 high risk)

Goodwin PJ et al. *J Clin Oncol.* 1999;17:2365-2370.

Chemo Drugs Risks for Gonadotoxicity

➢ High Risk

- **Cyclophosphamide**
- Chlorambucil
- Melphalan
- Busulfan
- Nitrogen Mustard
- **Procarbazine**

➢ Intermediate Risk

- Cisplatin
- Adriamycin

➢ Low Risk

- Methotrexate
- 5-Fluorouracil
- Vincristine
- Bleomycin
- Actinomycin D

➢ Unknown Risk

- Oxaliplatin
- Irinotecan

Lee S et al. ASCO guidelines, JCO (2006 and 2013)

Effects of Breast Cancer Treatment on Ovarian Function

Factors responsible for gonadotoxicity are Age, Dose and Number of cycles of the Alkylating agent

- **Six** cycles of ***CMF*** (cyclophosphamide, methotrexate, fluorouracil): **33% of Amenorrhea**

- **Six** cycles of ***FEC*** (fluorouracil, epirubicin, CTX): **50-65% of Amenorrhea**

*After 6 cycles of CTX containing polychemotherapy, ovarian age can be advanced up to 10 years.

Kim et al., 2011, Fertil Steril

Overview Fertility preservation Strategies

- Hormonal suppression (evidence inconclusive)
- Surgery: Ovarian transposition/Trachelectomy (established)
- **Oocyte freezing** (established)
- **Embryo freezing** (established)
- **Ovarian freezing and Transplantation** (experimental)
  - Cortical strips
  - Whole Ovary
- **In vitro folliculogenesis** (experimental)
- **In vitro ovary perfusion** (experimental)

Oocyte Cryopreservation

- Single women
- Young (<40 years old)
- Ethical objections to embryo freezing
- Need time (about 2 weeks) before start of chemo or radiotherapy
- No contraindications to hormonal stimulation
- Should be offered prior to starting potentially sterilizing cancer treatment

Results-Oocyte Cryo Vitrification is the Winner!!

	Survival Rate/thawed oocyte	Fertilization Rate/thawed oocyte	Implantation Rate/thawed oocyte	Pregnancy Rate/thawed oocyte
Slow Freezing	**71.9%** [67.44, 75.89] 95% CI	**51.2%** [42.2,60.1] 95% CI	**7%** [4.3-11.2] 95% CI	**4.2%** [3.08, 5.56] 95% CI
Vitrification	**78.6%** [70, 85.18] 95% CI	**55.96%** [47.4, 67.1] 95% CI	**7.7%** [5.35, 11] 95% CI	**7.6%** [4.98, 11.4] 95% CI

Embryo Cryopreservation

- Need time (about 2 weeks) before start of chemo or radiotherapy
- Need partner
- No contraindication to hormonal stimulation
- Should be offered prior to starting sterilizing cancer treatment

Embryo Freezing Stages

- **Pronuclear (1, 2 Propanediol)**
  - Single cell-No Spindle
  - Easy to assess survival – most viable divide
- **Cleavage (1, 2 Propanediol)**
  - Can freeze at all cleavage stages
  - No time urgency
  - Survival considered if > 50% blastomeres viable
- **Blastocyst (Vit Protocols) ↑↑**
  - More than 100 cells
  - Loss of some cells does not compromise the entire embryo

Courtesy Noyes N MD, NYU

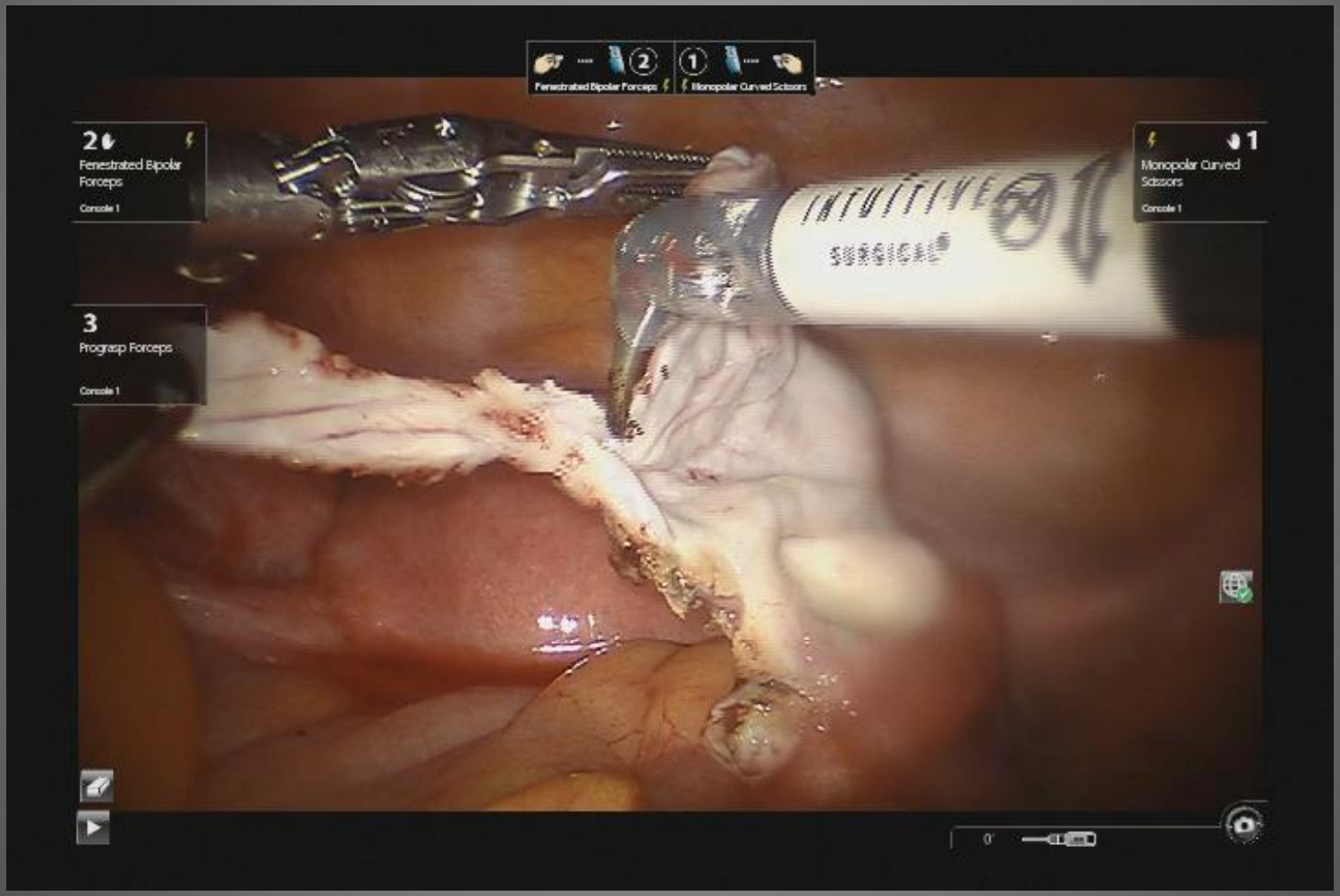
Breast Cancer:
Protocols for Egg/Embryo Freezing

- Natural cycle IVF
- Tamoxifen
- Tamoxifen + FSH
- ****Letrozole + FSH+ GnRH antagonist**
  (5 mg) (150/225IU)

**** The winner!!**

Ovarian Tissue Cryopreservation

- Cancer patients who do not have enough time for ovarian stimulation (2 weeks) or not safe
- Have no partner (and/or wants to freeze more than few oocytes)
- Pre-pubertal girls


2
Fenestrated Bipolar Forceps
Console 1
3
Prograsp Forceps
Console 1
1
Monopolar Curved Scissors
Console 1
Fenestrated Bipolar Forceps
Monopolar Curved Scissors
INTUITIVE
SURGICAL

Pre-Freezing Evaluation (**Safety**)

- Realistic Chance of long term survival
- Cancer work-up negative for metastasis
- Oncologist approves procedure
- Pelvic exam and ultrasound normal
- Negative histological biopsies
  - Light microscopy and Molecular Markers

Risk of Ovarian Involvement in Cancer patients-**Safety**

Low Risk (<1%)	**Mod. Risk (1%-11%)**	**High Risk (>11%)**
Wilm's Tumor	**Stage III-IV Breast Cancer**	**Leukemia**
Lymphomas	Adeno Cancer Cx	Neuroblastoma
Stage I-II Breast Cancer	**Colorectal Cancer**	
Nongenital-Rhabdonyosarcoma		
Osteogenic Sarcoma		
Squamous Cell Cx Cancer		
Ewing Sarcoma		

Oktay K. *Hum Reprod Update.* 2001;7:526-534.

What to do with Patients with Leukemia?

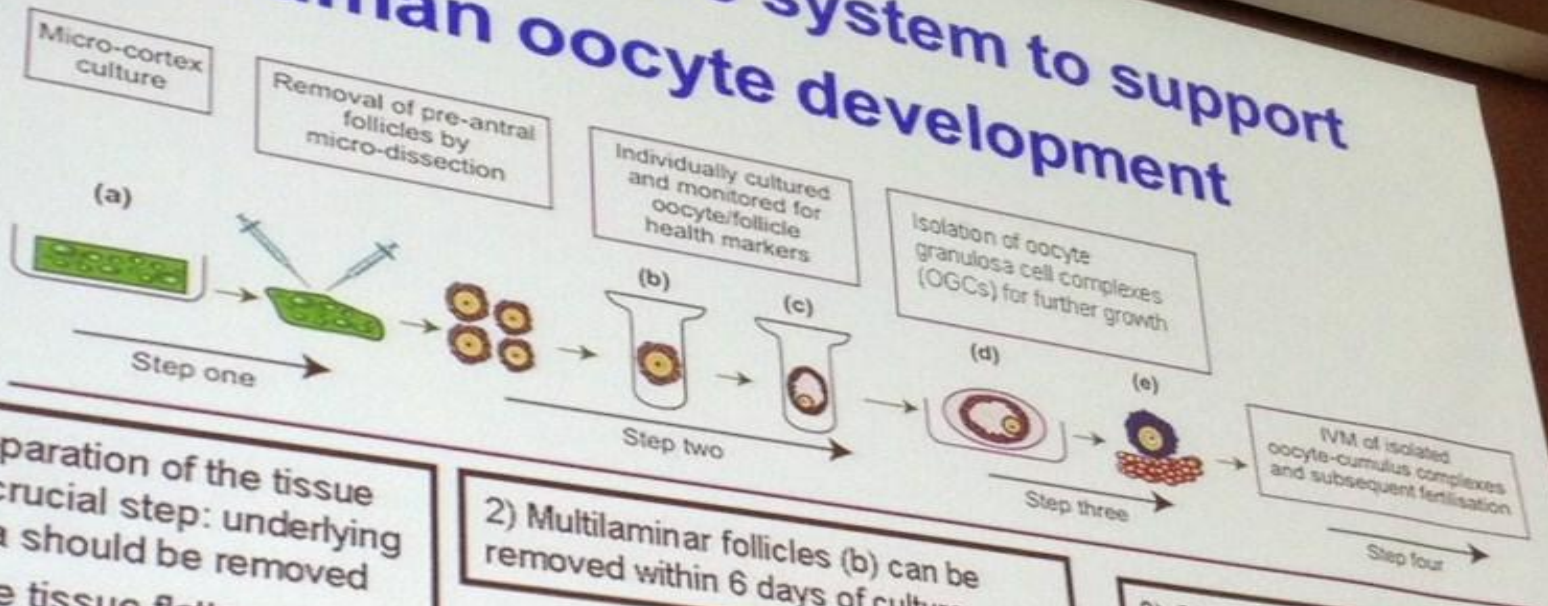
- No Time
- High risk of ovarian metastastic disease
- What is the best option?
- **In Vitro Folliculogenesis from cortical strips**
- **In vitro Whole Ovary perfusion and Freeze**
- **Artificial Follicles**

In vitro Folliculogenesis

Follicle culture performed using fresh cortical strips (IRB-approved protocol) [collaboration with E. Telfer, Edinburgh]

Hypothesis: Manipulation of the Target of Rapamycin (TOR) kinase allows control of follicle survival and growth (should improve likelihood of generating fertilizable mature eggs)

Multi-step Culture system to support human oocyte development



1) Preparation of the tissue (a) is crucial step: underlying stroma should be removed and the tissue flattened.

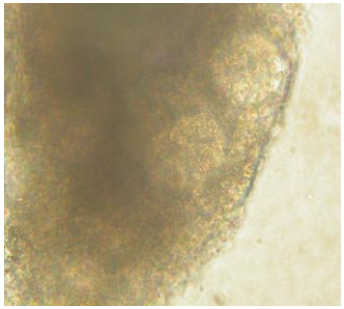
2) Multilaminar follicles (b) can be removed within 6 days of culture.

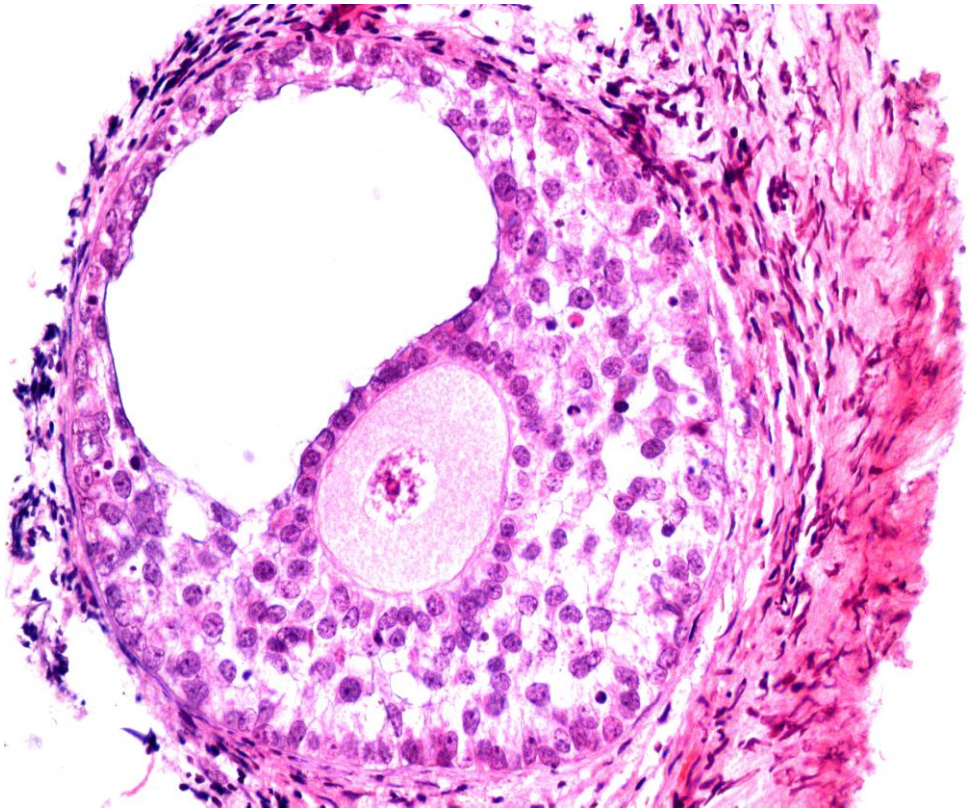
3) Once antral formation has been achieved (c), oocyte complexes can be removed and grown for a further period.

Approx 30% of oocytes that complete culture process can reach Metaphase II: Epigenetic Status and Fertilisation potential?

McLaughlin, Albertini, Wallace, Anderson & Telf

Antral development from *in vitro* grown human primordial follicles within 10 days



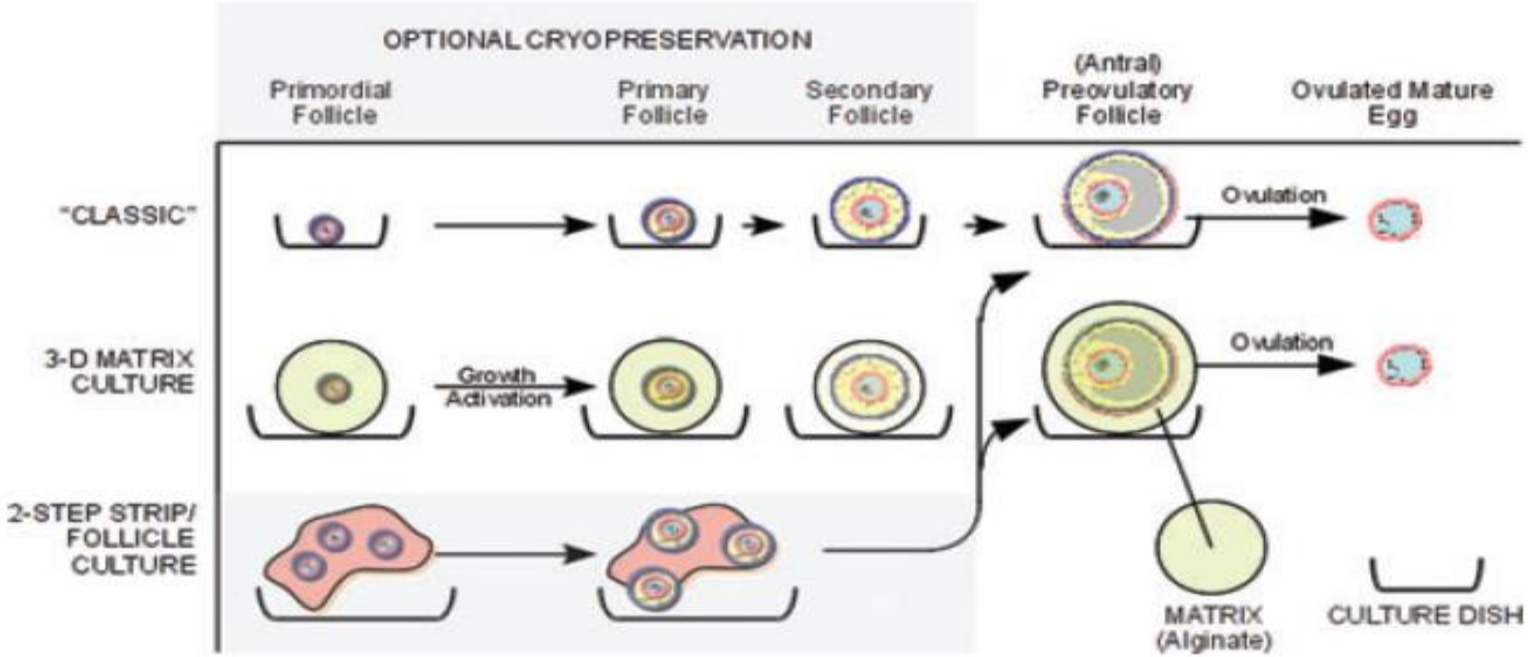


Telfer et al., 2008: A two step serum free culture system supports development of human oocytes from primordial follicles in the presence of activin. **Human Reproduction** 23: 1151-1158

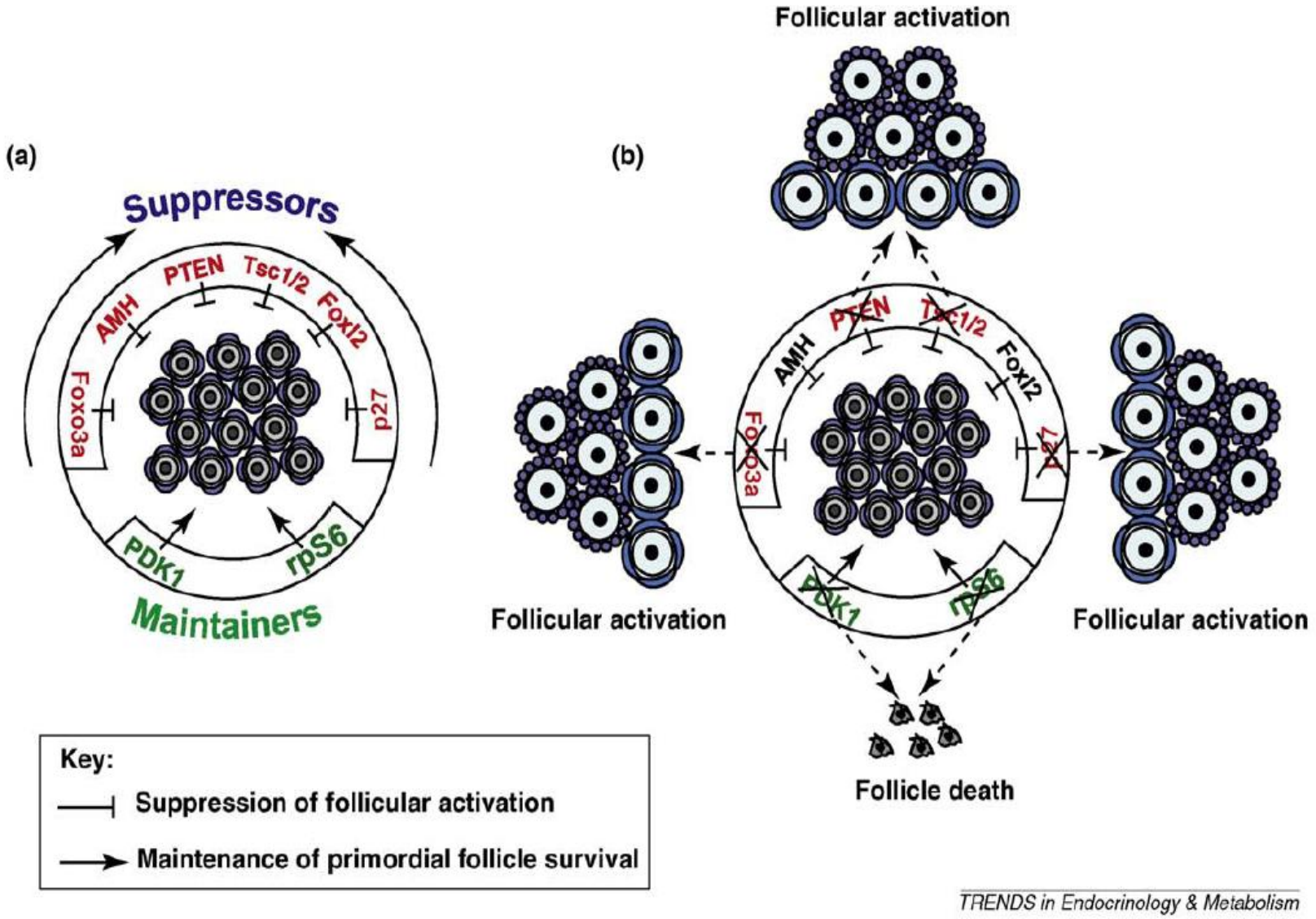
ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Issue: *Reproductive Science*

Ovarian cryopreservation strategies and the fine control of ovarian follicle development *in vitro*

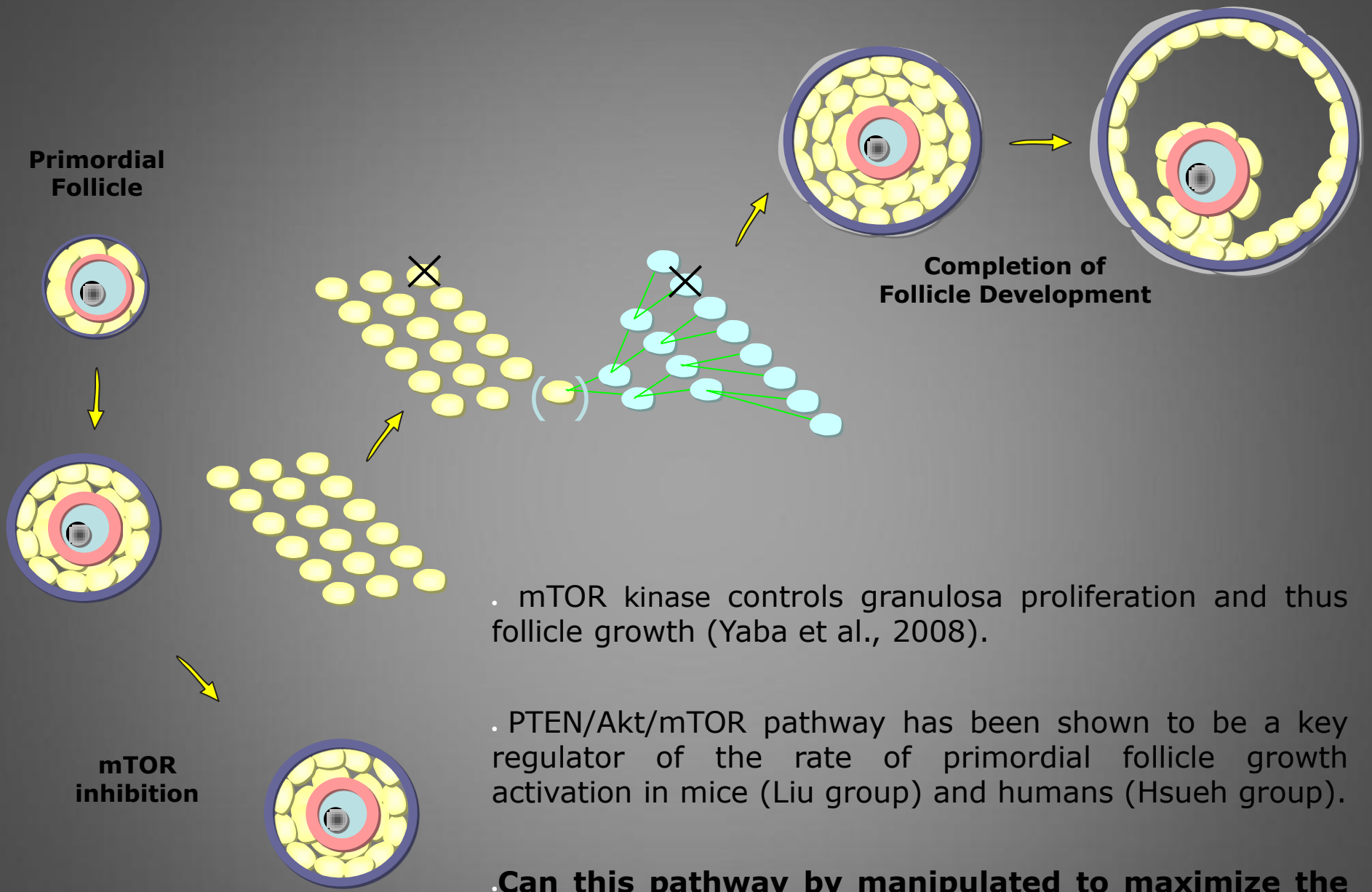
Joshua Johnson and Pasquale Patrizio



Akt/mTOR signaling and Growth Activation



Reddy, Zheng, & Liu, 2009



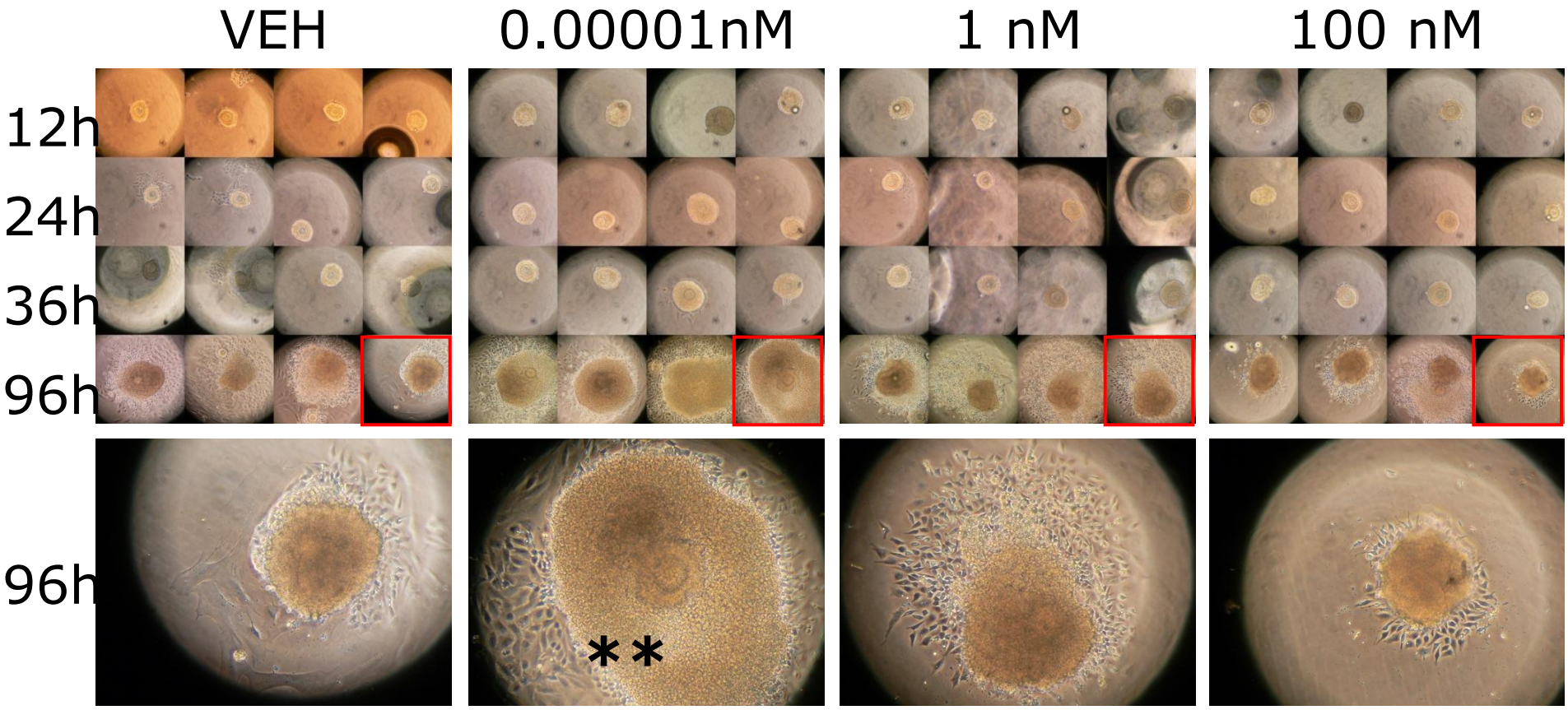
. mTOR kinase controls granulosa proliferation and thus follicle growth (Yaba et al., 2008).

. PTEN/Akt/mTOR pathway has been shown to be a key regulator of the rate of primordial follicle growth activation in mice (Liu group) and humans (Hsueh group).

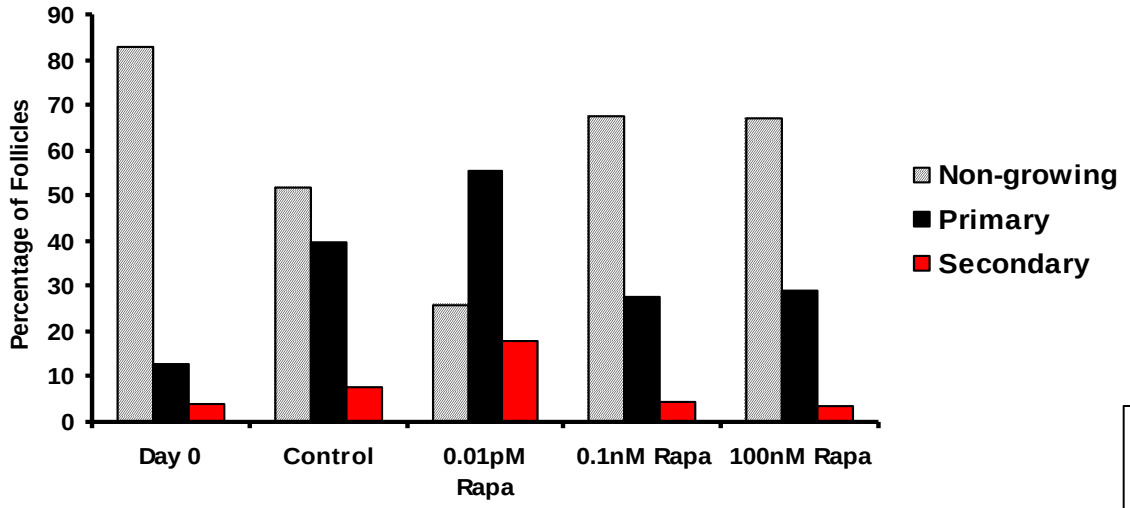
.**Can this pathway by manipulated to maximize the growth activation, survival, and oocyte maturation in human cortical strip cultures?**

Rapamycin treatment of **mouse** follicles in vitro: dose-dependent reduction in follicle growth

****Ultra-low dose @ 0.00001nM increases follicle size and improves morphology**

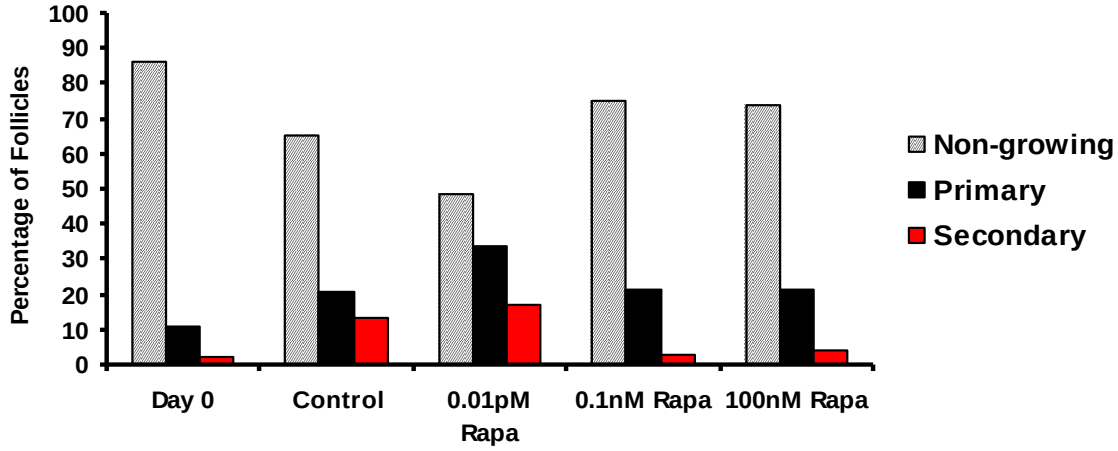


Distribution of Bovine Ovarian Follicles: Effect of Rapamycin

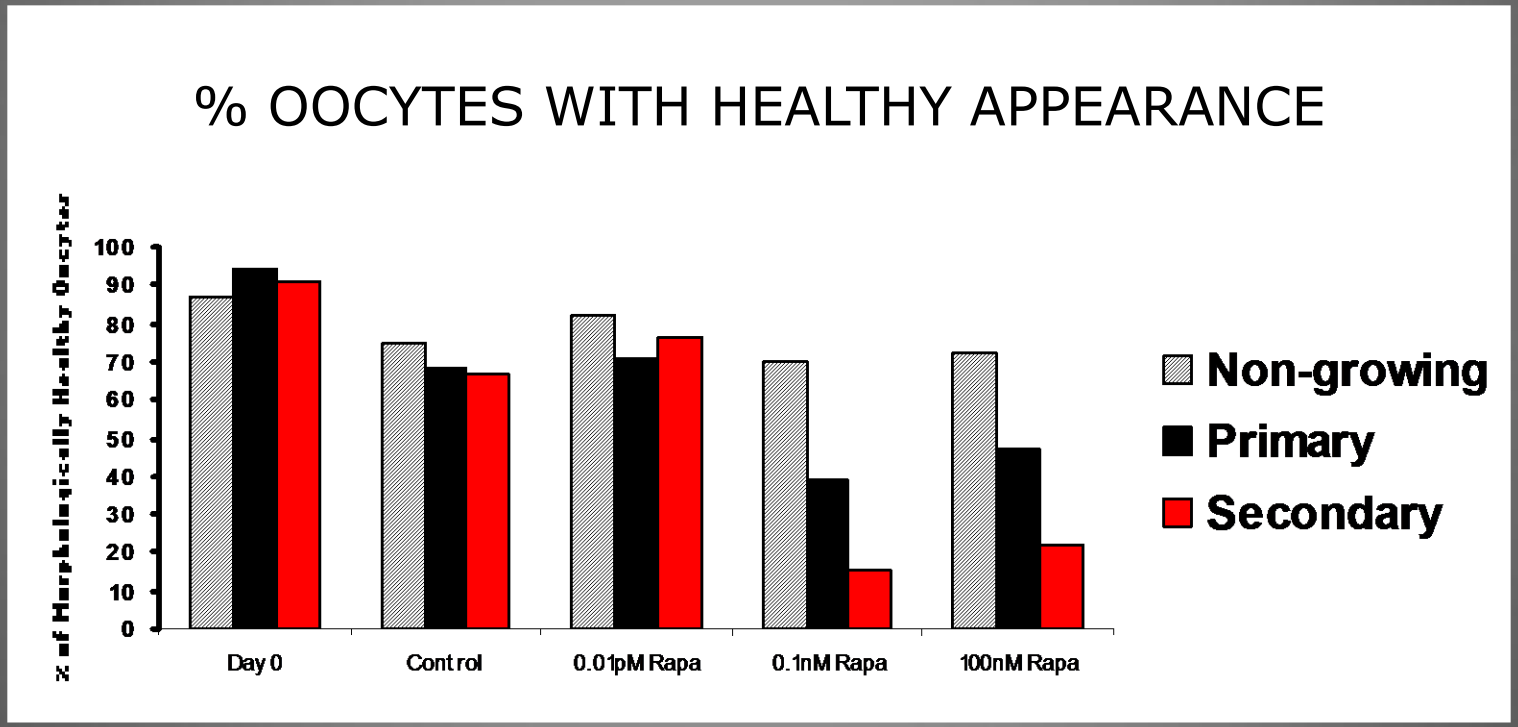


120-360 Follicles Assessed per TREATMENT

Distribution of HUMAN Ovarian Follicles: Effect of Rapamycin



Oocyte "Quality" in **Human** Ovarian Strip Cultures

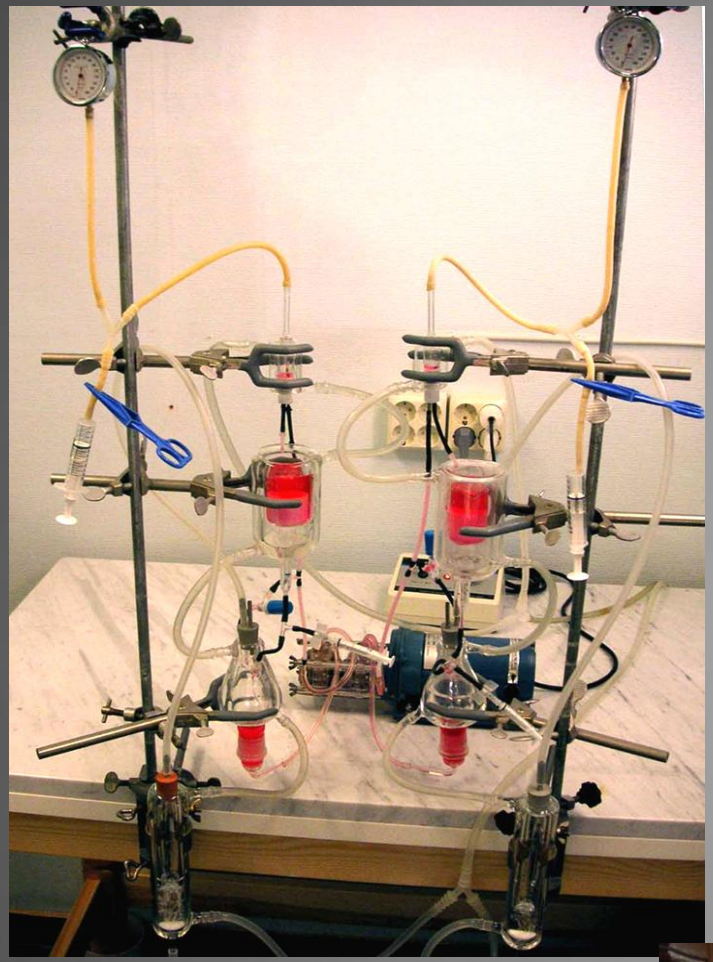


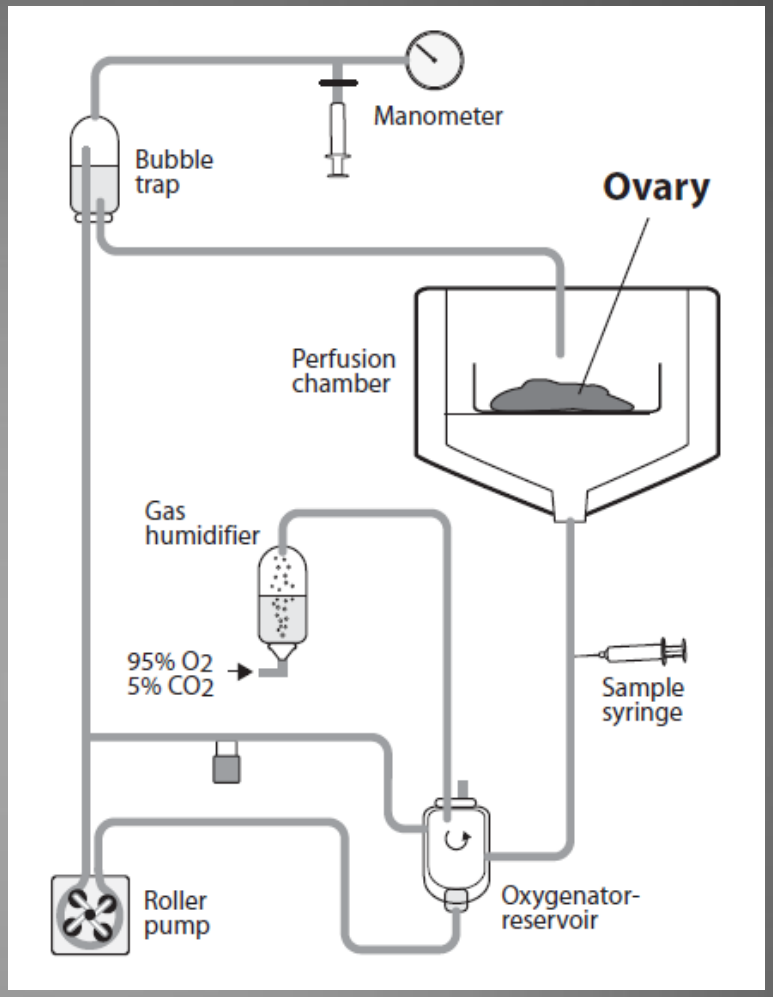
Ultra-low dose Rapamycin and Ovarian Follicles in Vitro

a) Enhances primordial follicle growth activation and oocyte 'viability' (mouse, cow, and **human**)

b) Picomolar dose significantly alters granulosa cell gene expression at the level of transcription

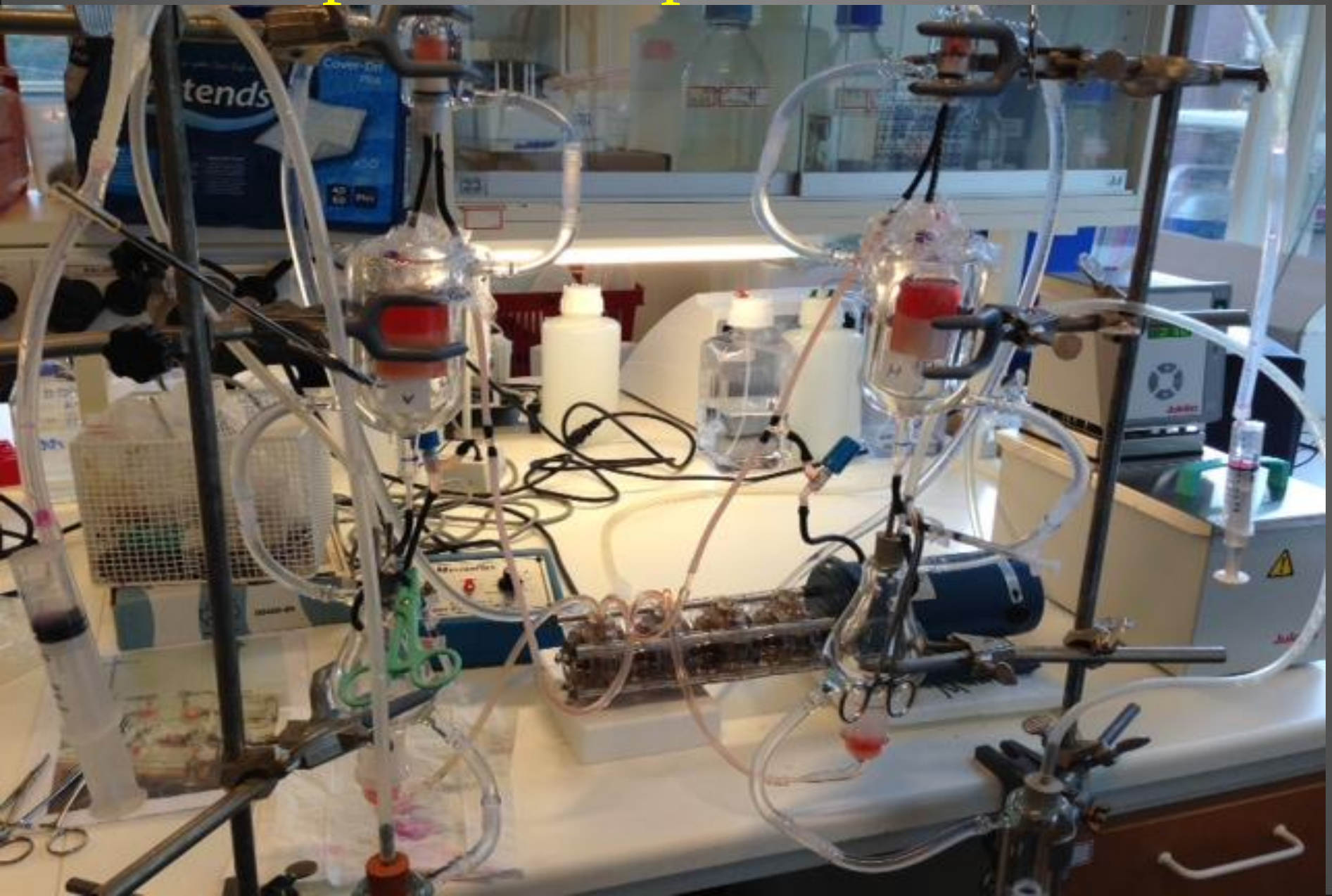
c) Clinically attractive (? in vitro oocyte production)





In vitro perfusion apparatus

Sheep Ovaries perfused 36 hrs.



Sheep ovaries after 36 hrs: after hMG and hCG (retrieval)

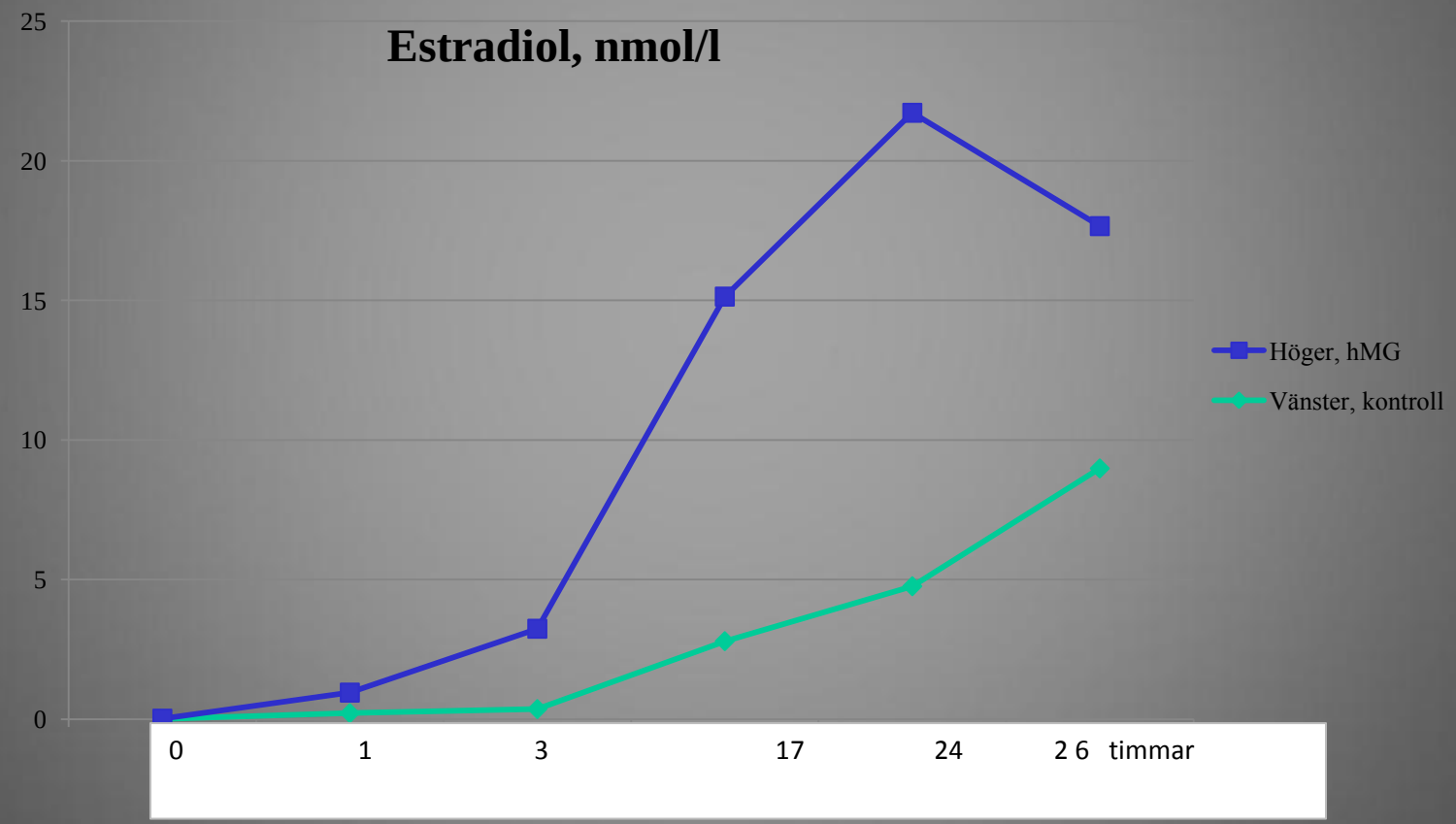
Whole Sheep Ovaries Perfused in Vitro

6 Follicles between 7-8 mm

4 Oocytes retrieved

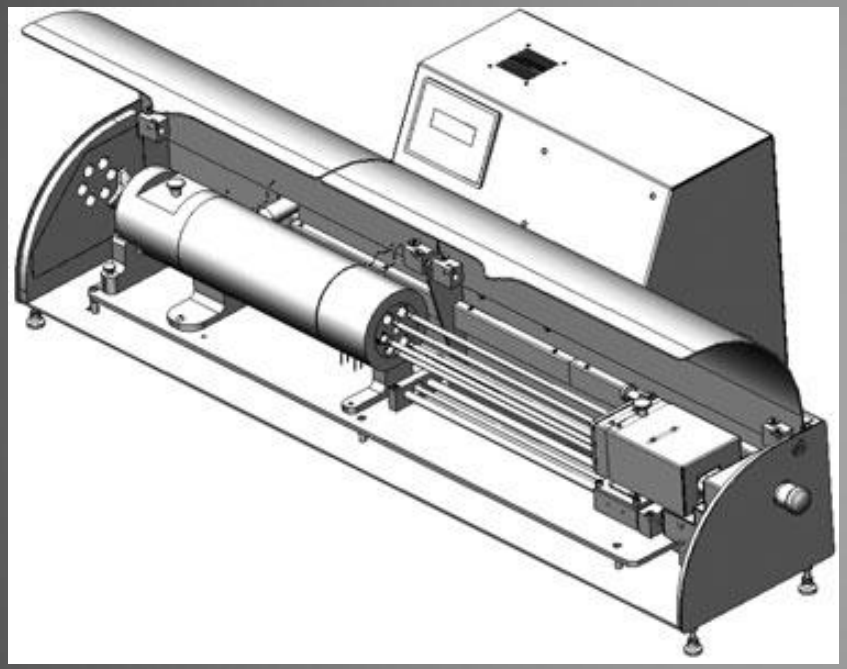
1MII and 1 MI and 2 GV

Estradiol secretion during in Vitro Perfusion



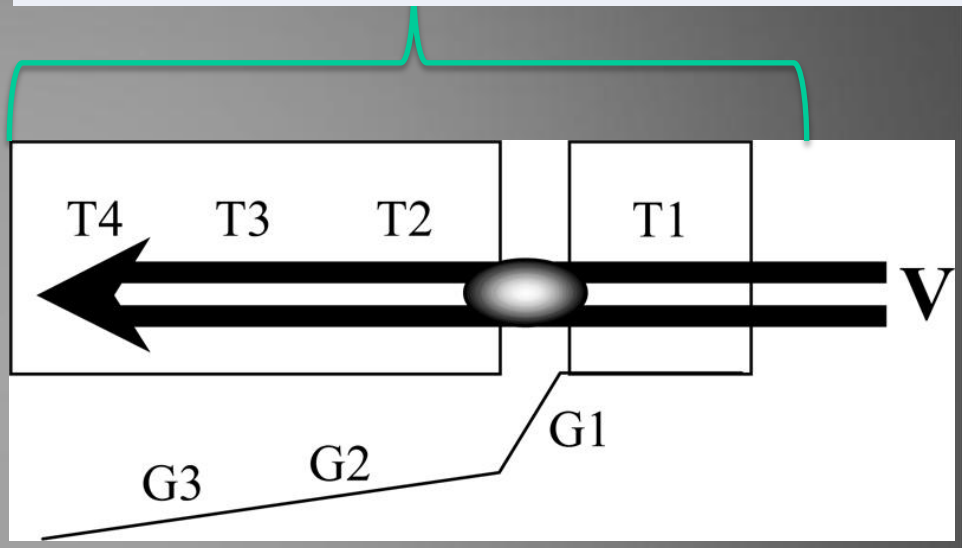
Whole Ovary Cryo new data

Beneficial effect of Directional Freezing
[Maffei S et al. Hum Reprod Oct. 2013]



MTG directional freezing device

Gradient T along the track



Sketch of directional freezing apparatus

Arav & Natan, Semin Reprod Med, 2009; Reprod Dom Anim, 2012
Arav et al RBMO (2010); Patrizio and Bromer Semin Reprod Med 2009

Study design

[Maffei S et al, Hum Reprod 2013]

**experimental groups*
(n=10 each)**

DIRECTIONAL FREEZING
- whole ovary (DFwo)
- Cortical tissue (Dfof)

CONVENTIONAL FREEZING
- whole ovary (CFwo)
- Cortical tissue (Cfof)

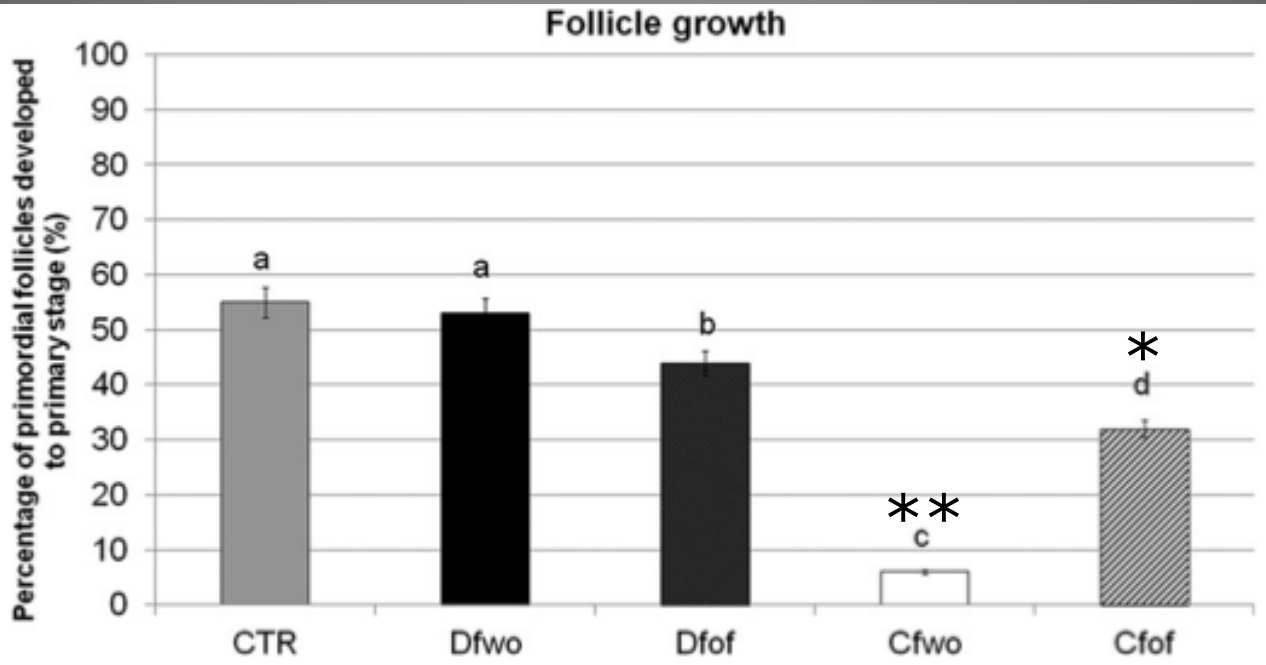
controls (CTR)
(n=8)

Follicle Culture 7 days

Morphological Analysis
Tunel Assay
Immunohistochemistry
Western blot

*Same freezing/thawing solutions applied for both protocols

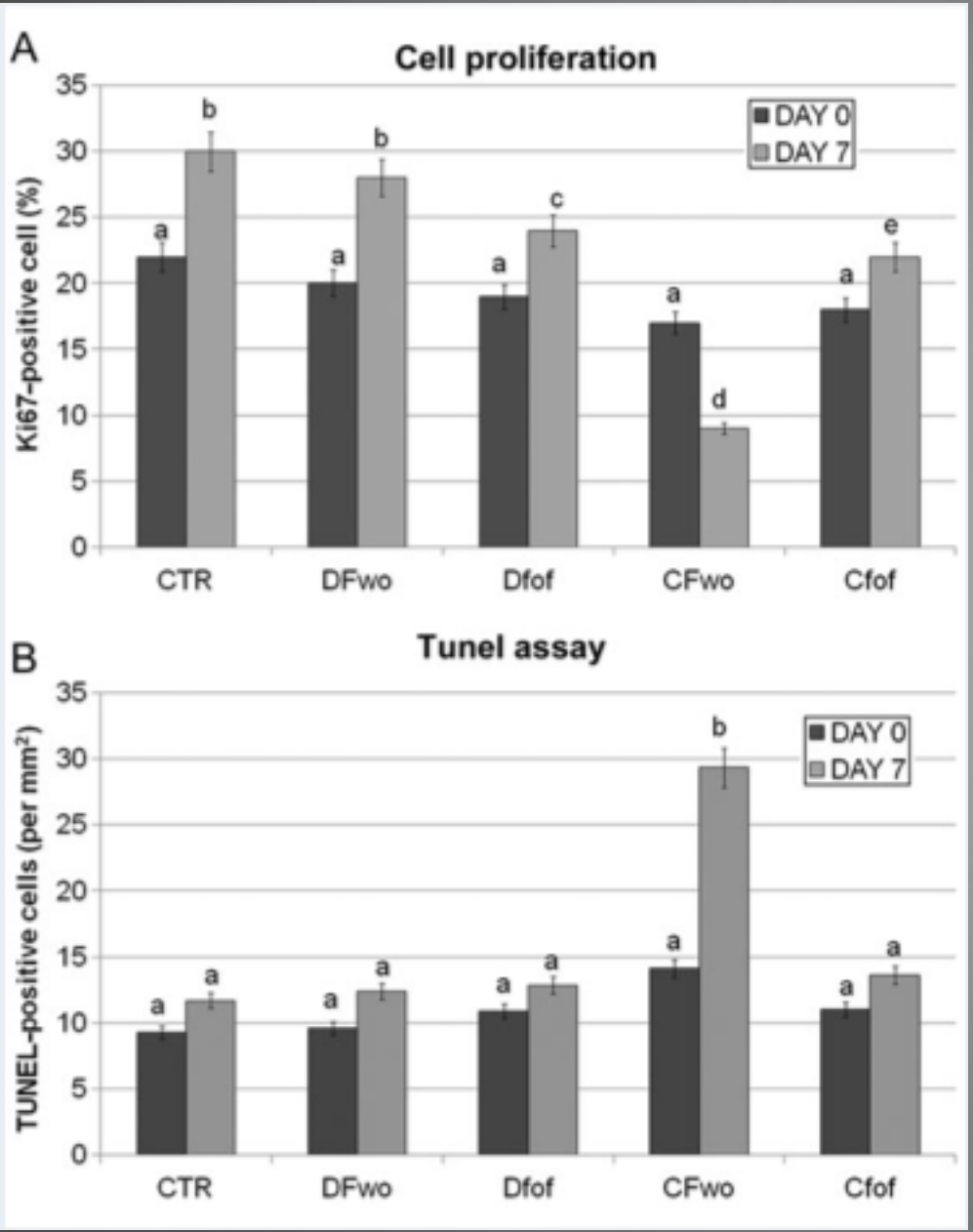
Result (i) – follicle growth after 1 week



Development of PMF into primary follicles *p<0.05

- Whole ovarian cryo provided higher yields of primary follicles development
- Directional freezing leads to higher rates of follicle growth

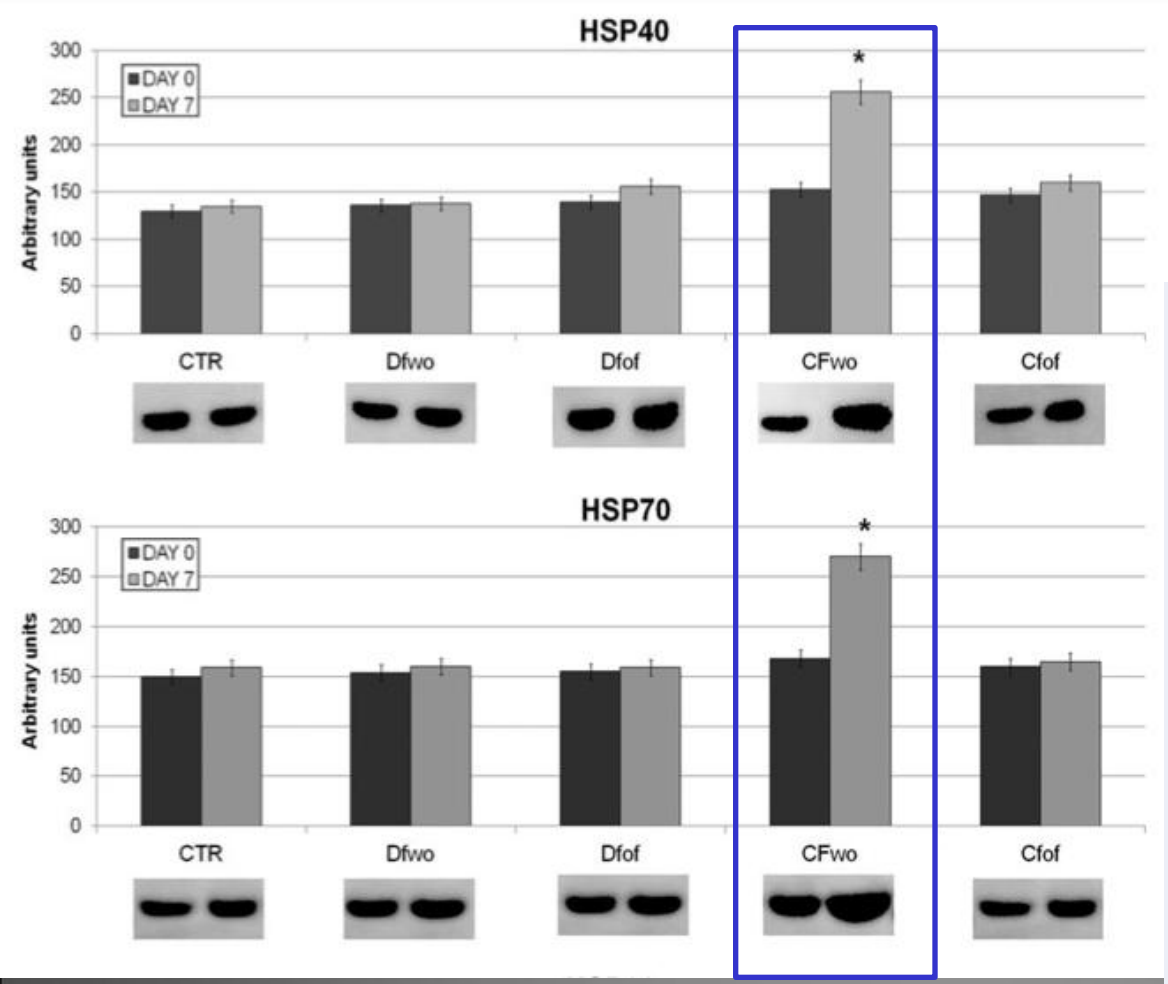
Result (ii)-cell Proliferation and Apoptosis



DFwo shows comparable proliferation rate (Ki67) to CTR
More Ki67-cells in Dfof compared to Cfwo and Cfof ($p<0.05$)

At day 0 apoptotic rate comparable between groups
After 7 days of follicle culture **Cfwo** shows a **significant increase** of **apoptotic cells** ($p<0.05$)

Result (iii)-expression of HS proteins



HSP70 = most abundant heat shock protein in cells

Conventional freezing whole ovary induces the **activation of proteins involved in stress-response** pathways

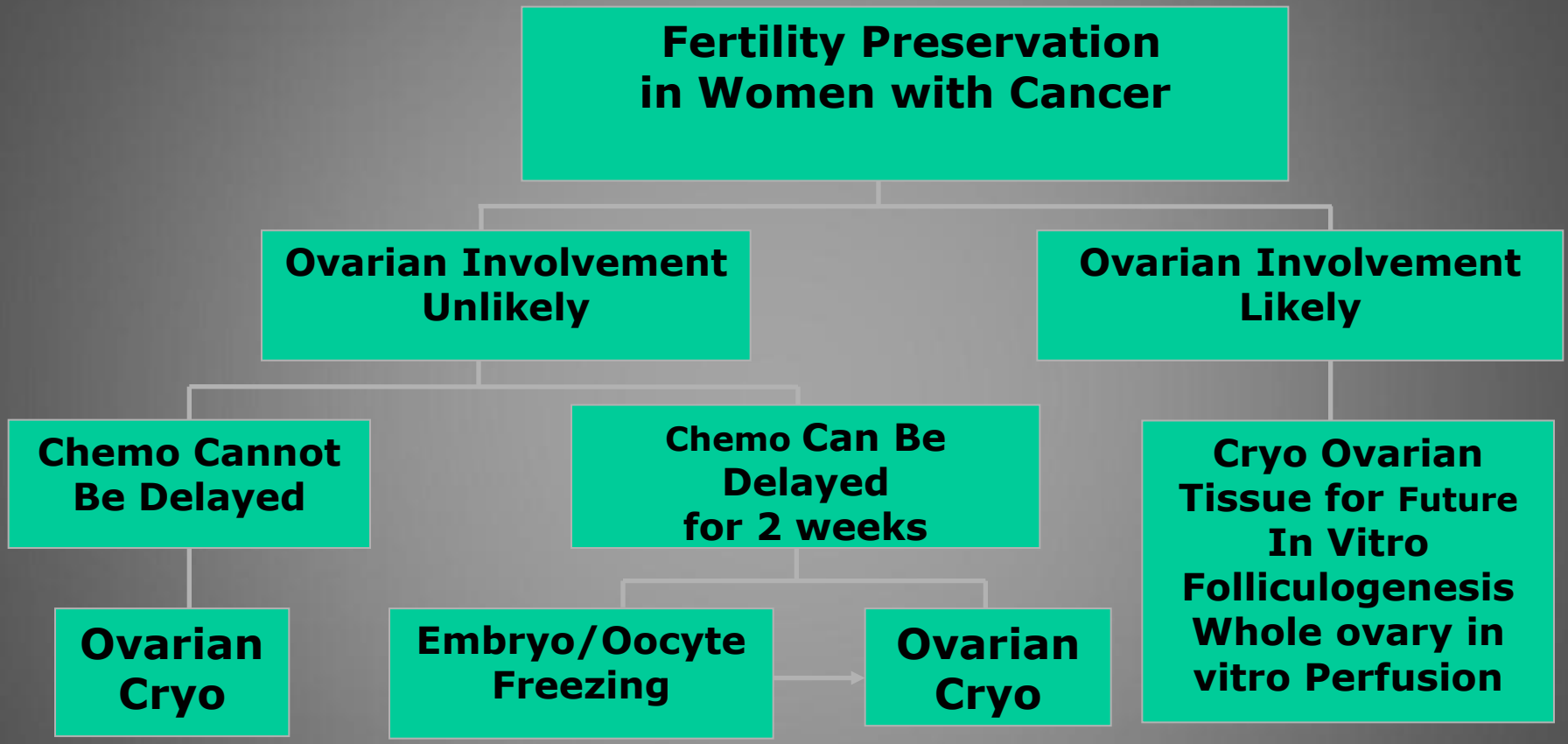
No differences in other groups

Summary Directional Freezing

- DF significantly improve the integrity of follicular structure from primordial to secondary transition; is able to remove the latent heat produced by ice crystal formation (most likely the cause of tissue cryoinjury), and decreases rate of intracellular ice formation

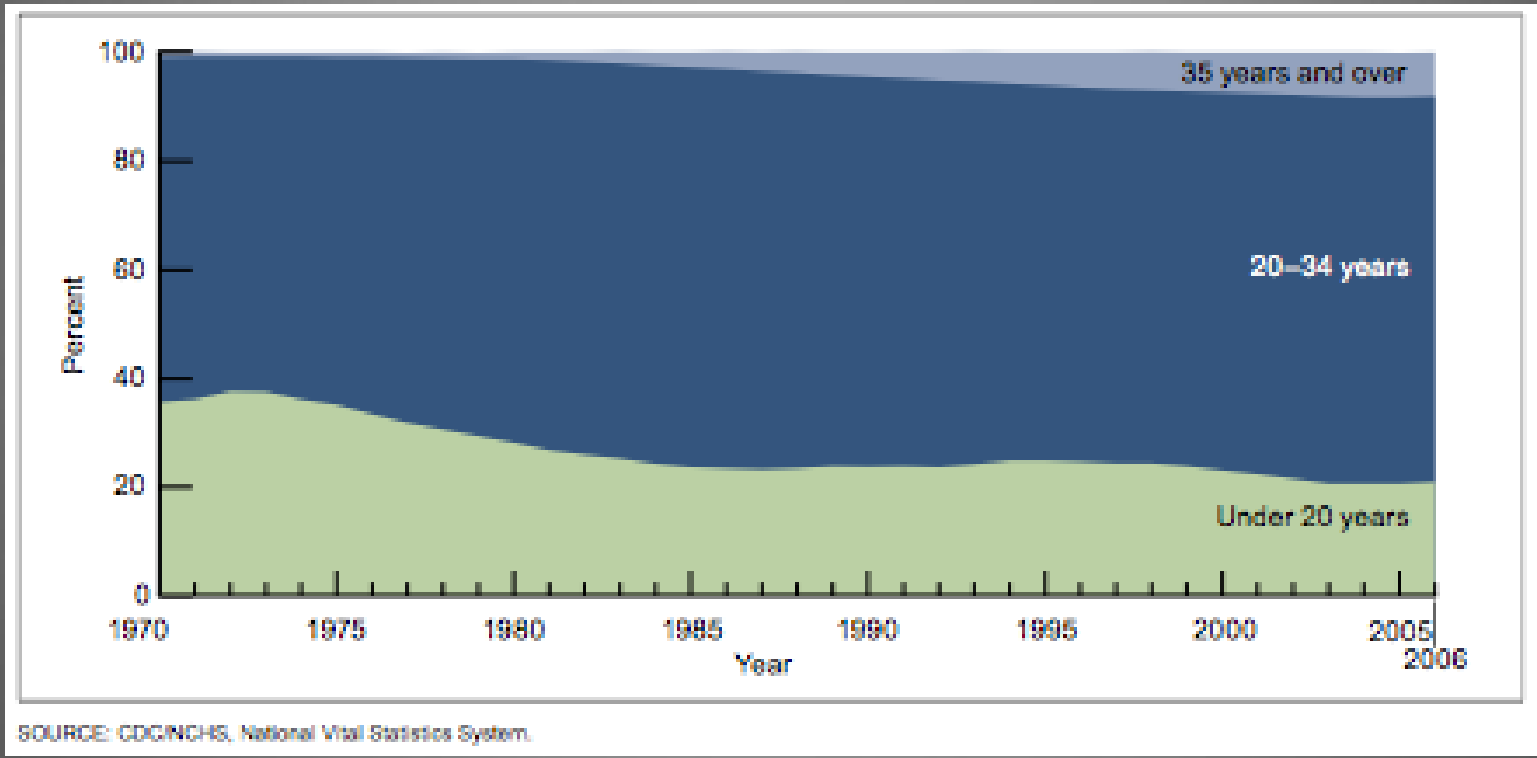
- Functional analysis showed that ovarian viability is well preserved in DF of Whole Ovaries:
  A) higher follicular proliferation rate;
  B) lower expression of HSP and
  C) capacity to activate DNA repairing system

Summary


Fertility Preservation in Women with Cancer
Ovarian Involvement Unlikely
Ovarian Involvement Likely
Chemo Cannot Be Delayed
Chemo Can Be Delayed for 2 weeks
Cryo Ovarian Tissue for Future In Vitro Folliculogenesis Whole ovary in vitro Perfusion
Ovarian Cryo
Embryo/Oocyte Freezing
Ovarian Cryo

Egg Freezing for “Social” Reasons Postponement of Fertility

Average Age of First Time Mothers in USA
Now age **26.3 first pregnancy** (2013 data)



Martin JA et al. Births:vol. 60(1) National Center for Health Statistics. **2011**

Birth rates	**20-24 y**	**25-29 y**	**30-34 y**	**35-39 y**	**40-44 y**	**45-49**
2009	96/1000	110/1000	97.7/1000	46.5/1000	**10.1/1000**	0.7/1000
2008	103/1000	115/1000	99.3/1000	46.9/1000	9.8/1000	0.7/1000
Variation	**-7%** declining last 20yrs	**-4%**	**-2%**	-----	**+1%** increasing last 20 yrs	------ **0.3 in 1992**

Fresh (non-donor) IVF Cycles 1999-2008 (CDC)

Year	Number of IVF Cycles/Ages					Total Cycles
	<35	35-37	38-40	41-42	>42	
1999	29,682	15,291	12,848	5,302	2,628	65,751
2000	33,453	17,284	14,701	6,118	3,401	74,957
2001	35,984	17,791	16,283	7,044	3,762	80,864
2002	37,591	19,110	17,454	7,733	3,938	91,032
2003	39,852	20,056	18,660	8,185	4,279	91,032
2008	43,296	23,326	21,793	9,783	4,907	103,105
2004	40,853	21.019	19,174	8,487	4,709	94,242
% Change	+45.8	+52.5	**+69.6**	+**83.0**	**+86.2**	+56.6

Reasons for Postponement

- Most common reason women give for their decision to postpone pregnancy is **uncertainty** about the stability **of their relationships**
- Another common reason for delaying pregnancy are **future goals and aspirations**
  - Women wait until reaching certain academic and career achievements
  - Women do not want to fall behind in the workplace
  - Desire to be financially secure when having a child

Need to Educate Women

Fertility and Sterility® Vol. 97, No. 5, May 2012

A persistent misperception: assisted reproductive technology can reverse the "aged biological clock"

Nichole Wyndham, B.A.,[a] Paula Gabriela Marin Figueira, M.D.,[b] and Pasquale Patrizio, M.D., M.B.E.[a,b]

[a] Center for Bioethics, and [b] Yale University Fertility Center, Yale University, New Haven, Connecticut

- Most women unsure what age infertility begins to take effect and how quickly it advances
  - Estimates suggest that as few as 75% of women understand that fertility decreases between ages 30-40
- Believe that ART can overcome infertility

Risks of Postponing Fertility

- Older women have more trouble naturally becoming pregnant
  - Fertility start decline after age 30 with rapid decrease after age 35
- Even with reproductive technologies older women have a low chance of pregnancy
  - **Only 8.8% of women over the age of 42 who use IVF will become pregnant**
  - **Only 4.1% of them will actually give birth to a child**

Moving Forward

- General practitioners and gynecologists who see women at an early age should have a discussion with their patients about:
  - **The risks of fertility postponement**
  - **Options Oocyte and Embryo cryopreservation**
- Societal practices that encourage women to postpone fertility need to be addressed
- We must not think of age-related infertility as a disease but rather a social harm

SOLUTION to the PROBLEM

OOCYTE FREEZING
(by Vitrification)

One more consideration……

Cost of Fresh EGG donation: $32,000

Cost of Frozen EGG donation: $18,000

Cost of storing own EGGS: $6,500 (plus storage $600/year)

Fertility Postponement

- **51 cycles** (42 patients) cryopreserved for social reasons**: Total oocytes= 487**
- **Mean age**: 38 (range 31-42)
- **Job classification**: 13 Businesswomen-5 MD-5 Teachers-2 Psychologists-2 Lawyers-1 Minister-1 Chemist- 3 Students.

Fertility Postponement

- **Total of 487 oocytes** cryopreserved
- 134 by slow freezing
- 353 by vitrification
- So far only two patients utilized oocytes [**41 years old,** minister- now 43, had 13 oocytes by slow freezing-9 (69%) survived-2 fertilized (22%)-NP] and [**39 years old,** teacher-now 42, 12 oocytes SF-9 survived, 4 fertilized, NP]

NYU cycle data stratified by age

(mean age:38; range: 23-42 y).

n = 499 (**2005-2010**)

Age (y)	≤34 (n = 41)	35 - 37 (n = 129)	≥38 (n = 329)	P (anova)
E2 day of OT (pg/ml±SD)	2612±1285	2416±1424	2248±1291	.07
LH morning after LA OT (IU/L; range) pt n=22	126 (70-170)	109 (45-201)	90 (19-211)	NS
Number oocytes retrieved n (range)	**21 (4-59)**	**17 (3-47)**	**14 (2-74)**	**.0001**
Number MII oocytes retrieved and frozen n (range)	**15** (2-35)	**12** (1-36)	**10** (1-55)	.0001
Number MII per total number of oocytes	73%	74%	71%	NS
Peak E2 per retrieved oocyte (pg/ml±SD)	153±81	162±83	196±118	.001

All values are means.

Werner, Knopman, Arslan, Noyes, ISFP, 2011

Conclusions Fertility Postponement

- The majority of patients are older than 35 yrs
- So far **low utilization rates** (eggs still frozen)
- The number of women that are using oocyte cryopreservation for fertility postponement is still **low**
- Although ASRM has **removed the label experimental, it is not encouraging Oocyte freezing for fertility postponement**

The TEAM

- J Johnson (Cell Biology, Yale)
- A Arav (Tel Aviv, Israel)
- M Brannstrom, M Milenkovich (Goteburg, Sweden)
- E Telfer (Edinburgh, Scotland)

References

Bromer J et al Sem Repr Med (2009)
Patrizio P (2010) e-Book,Fertility Preservation www.GFA.com
Donnez J (2011) Ann. Med.
Johnson & Patrizio Ann NY Acad Sci (2011)