Reproductive Steroid Hormones and Cardiovascular Function in Young Healthy Women

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Introduction

Hormone Replacement in Postmenopausal Women: Conflicting Data

Past Clinical Trials (HERS and WHI)

- **No benefit or ↑ CV risk with E+P HRT in PM women**
- **Many Studies: Estrogen benefits vascular health**
- **MPA antagonizes E benefits in PM women & animals**

Is Hormonal Information Being Translated to Younger Women?

- CVD is more prevalent in young women than in the past
- 72% of young women are on hormonal therapy
  - Gynecological or contraceptive purposes
- Progestin effects have been varied
  - Levonorgestrel (VLD) and MPA antagonize estrogen's effects
  - Desogestrel (LD), etonorgestrel (ring), and drospirenone do not antagonize estrogen's effects
- Unknown what *progesterone* does to the vasculature of young healthy women

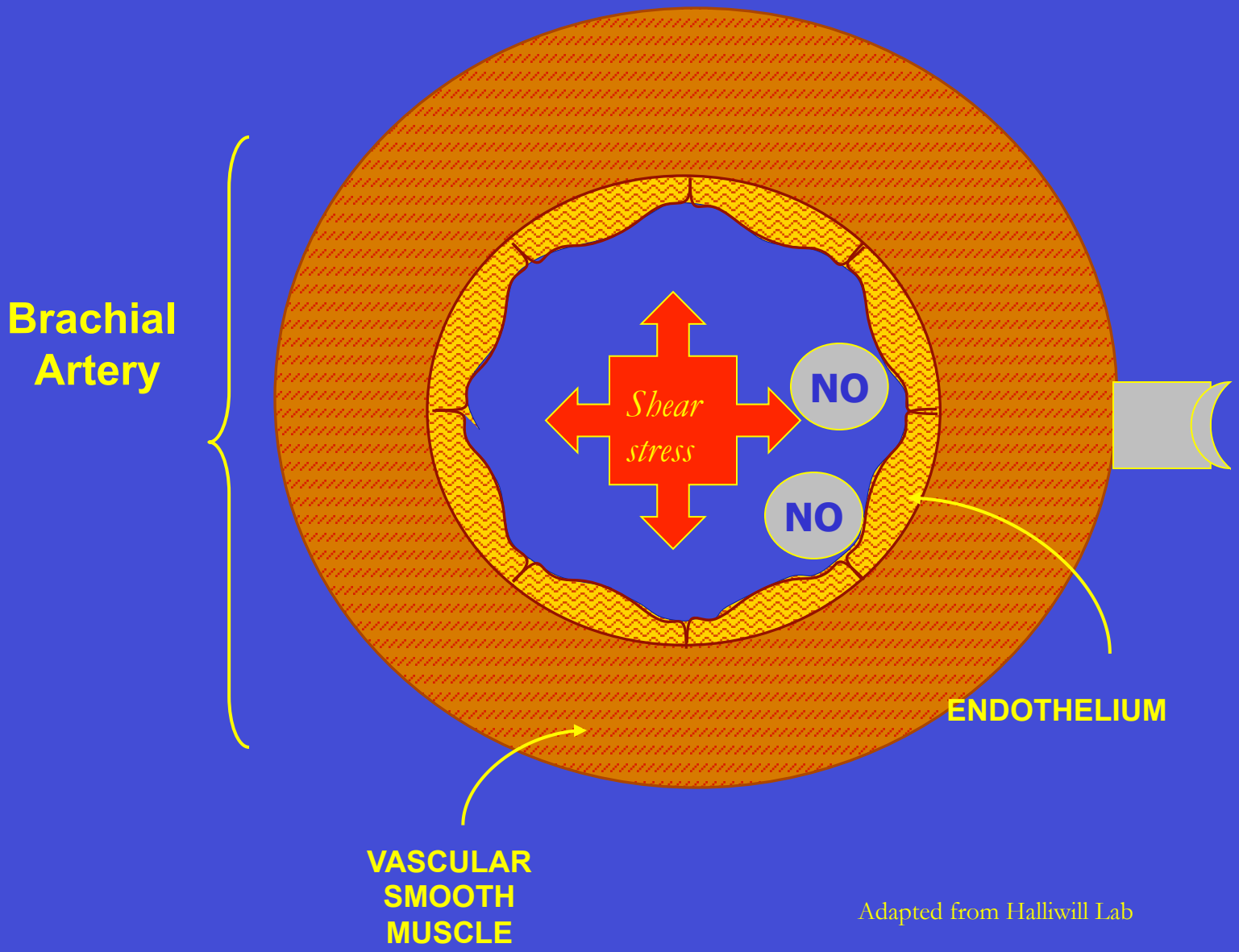
Why Study Endothelial Function?

- Brachial artery and coronary artery endothelial function parallel each other (*Anderson TJ, et al. J of Amer C of Card, 1995)*
- Endothelial dysfunction has been found in young symptom-free subjects with risk factors for CVD, before atherosclerosis (*Celermajer DS, et al. Lancet, 1992)*
- Previous research demonstrated traditional risk factors fail to explain up to 50% of CVD morbidity and mortality

Endothelium-Dependent Vasodilation (FMD)

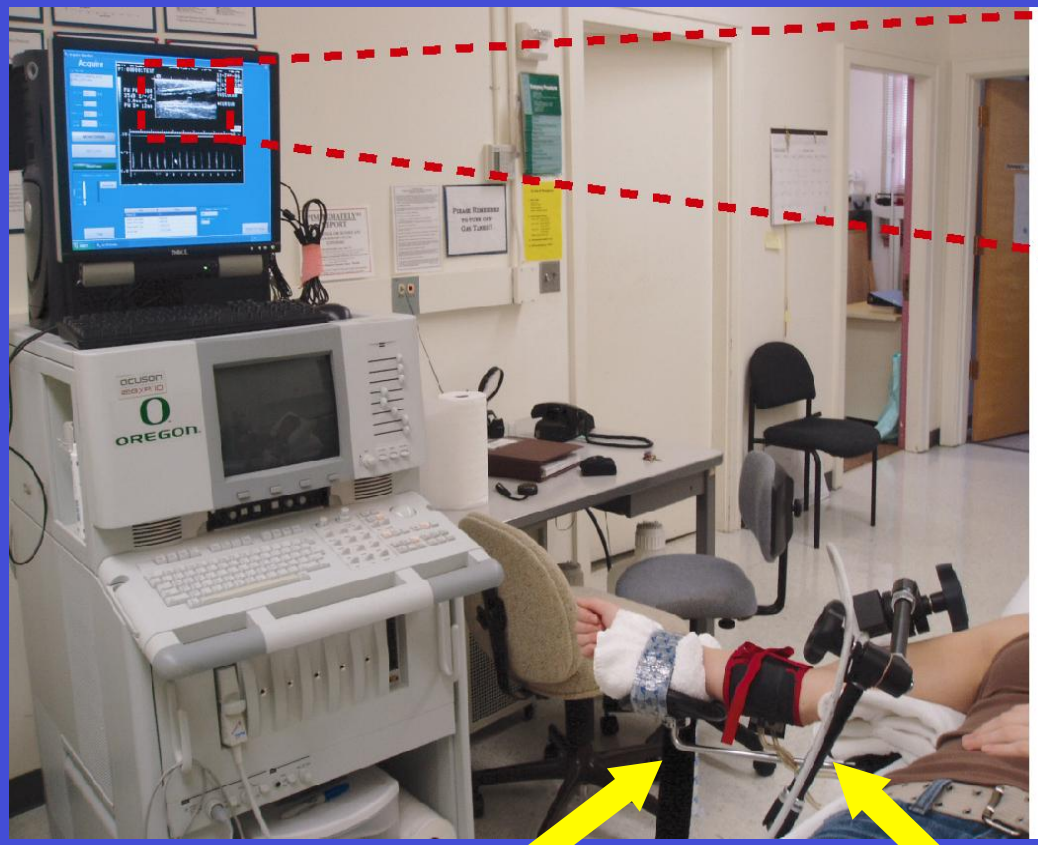
The rise in flow following a *distal* vascular occlusion creates a shear-stress across the brachial artery, causing the production and release of NO that is dependent on a healthy endothelium (Flow-Mediated Dilation or "FMD").

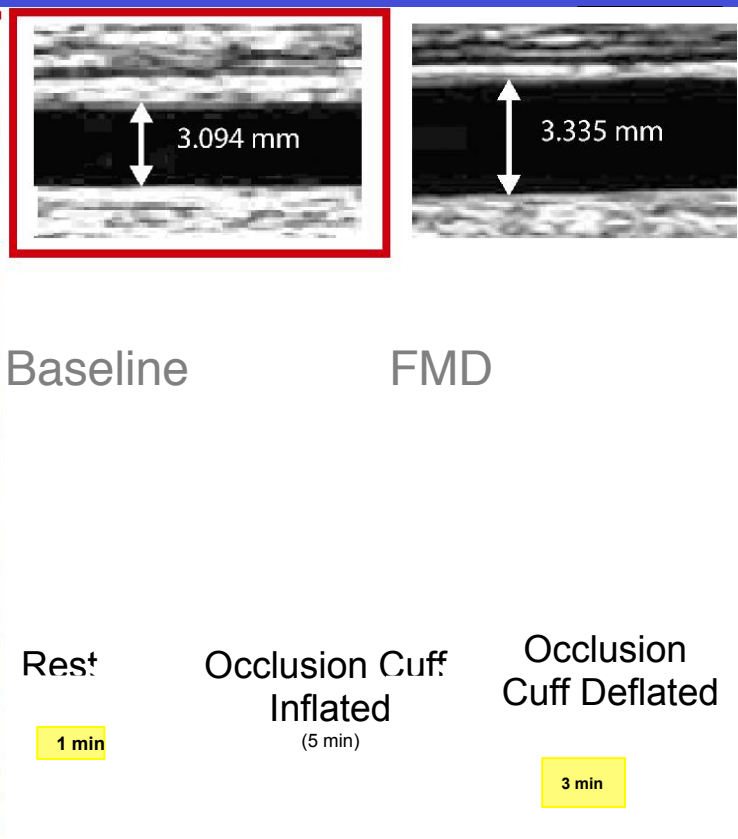
Role of the Vascular Endothelium



Adapted from Halliwill Lab

FMD Protocol





Blood pressure cuff for 5 minute forearm occlusion (distal to ultrasound transducer)

Ultrasound transducer held in place with a clamp

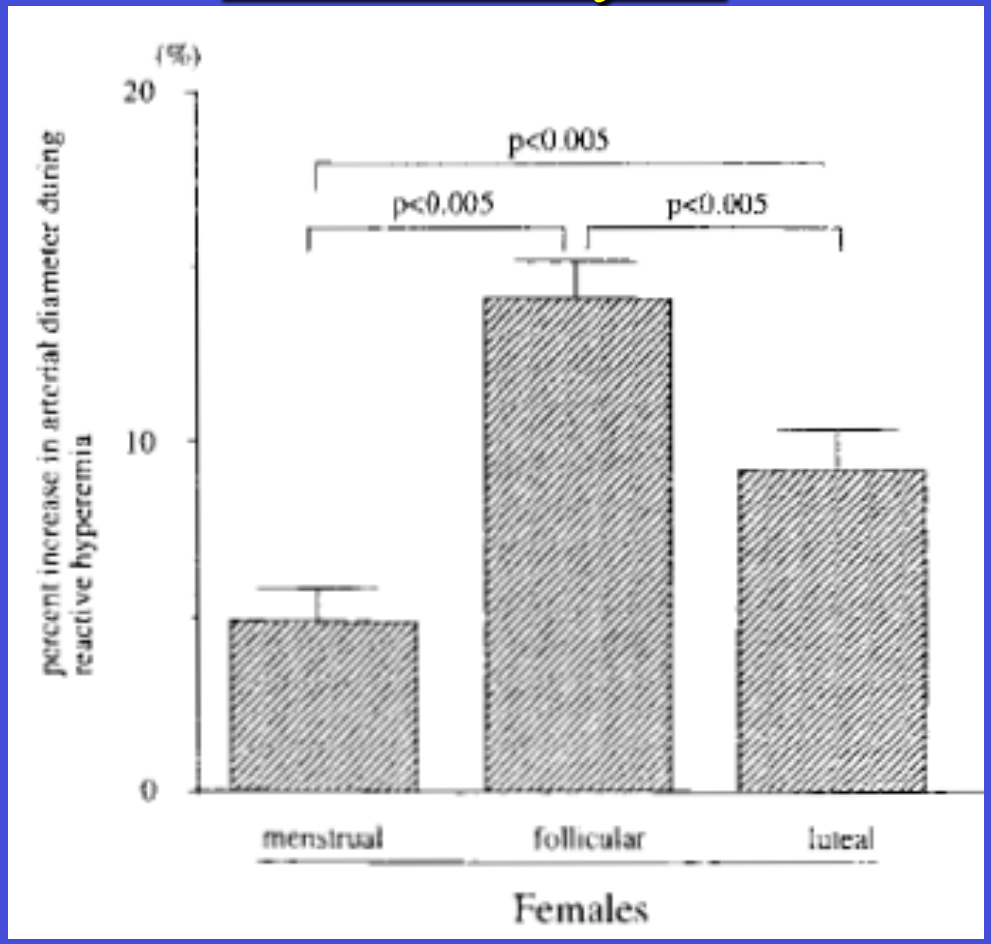
FMD (% change) = ((FMD diameter – Baseline diameter) / Baseline diameter) * 100

Prognostic Role of FMD

- Prospective study of 2,264 PM Women (age 54 ± 6 years)
- Follow-up for 45 months
- Controlled for other risk factors
- 90 confirmed CV events
  - ↓ FMD = ↑ Cardiac Events: Cardiac-related death, myocardial infarction, revascularization procedure, TIA, Stroke
- "FMD was an independent risk determinant, and adds prognostic information above and beyond traditional risk factors"

Rossi R, et al. *J of Amer Col of Card*, 51;10:2008

Hormones change endothelial function across the menstrual cycle



FMD increases when estrogen is high during the follicular phase & decreases during the luteal phase.

EFFECTS OF ESTRADIOL AND MPA ON VASCULAR FUNCTION IN YOUNG WOMEN

Am J Physiol Heart Circ Physiol 294: H1630–H1637, 2008.
First published February 15, 2008; doi:10.1152/ajpheart.01314.2007.

Estrogen, medroxyprogesterone acetate, endothelial function, and biomarkers of cardiovascular risk in young women

Jessica R. Meendering,[1] Britta N. Torgrimson,[1] Nicole P. Miller,[1] Paul F. Kaplan,[1,2] and Christopher T. Minson[1]

[1]*Department of Human Physiology, University of Oregon, Eugene, and* [2]*Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Oregon Health and Sciences University, Portland, Oregon*

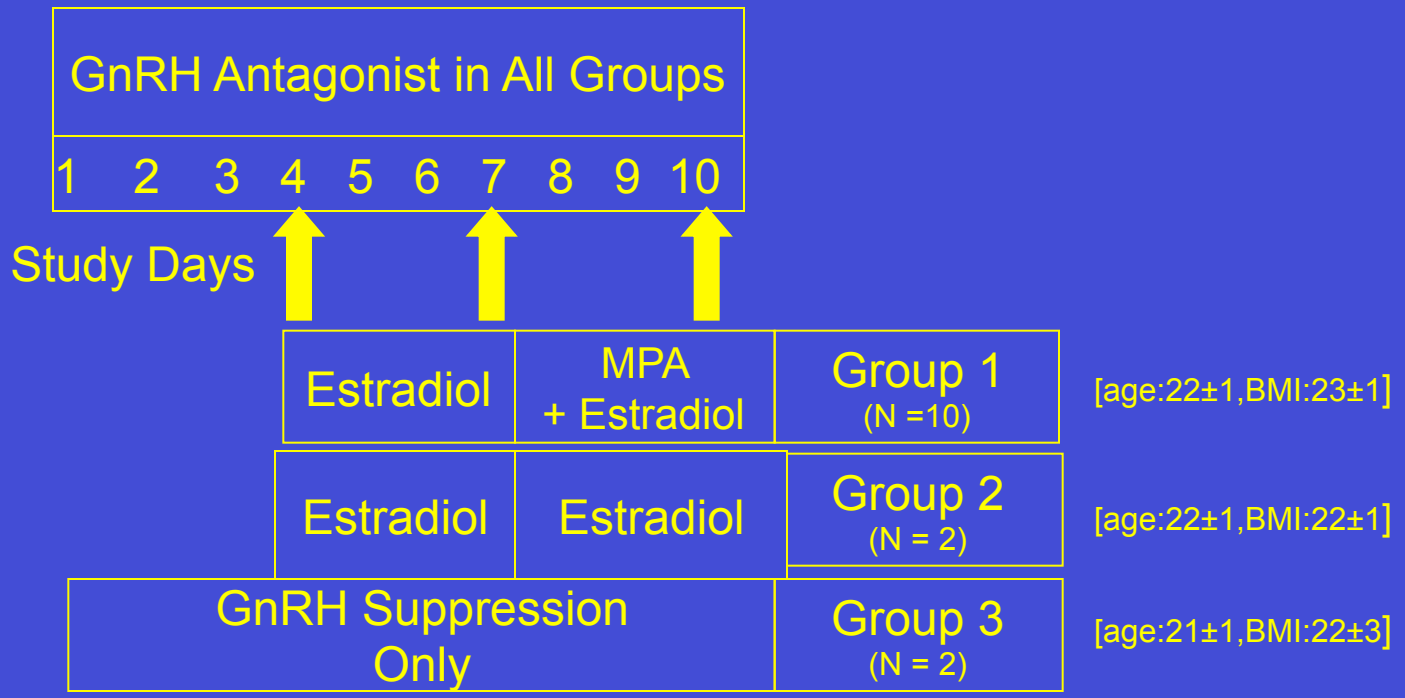
Submitted 9 November 2007; accepted in final form 11 February 2008

Meendering JR, Torgrimson BN, Miller NP, Kaplan PF, Minson CT. Estrogen, medroxyprogesterone acetate, endothelial function, and markers of cardiovascular risk in young women. *Am J Physiol Heart Circ Physiol* 294: H1630–H1637, 2008. First published February 15, 2008; doi:10.1152/ajpheart.01314.2007.—Medroxyprogesterone acetate (MPA) is widely known for its use in combination hormone therapy for postmenopausal women. However, MPA is also commonly used in young women for contraception and treatment of a number of gynecological conditions. Despite its widespread use, the cardiovascular effects of MPA in young women are unclear. Therefore, the purpose of this study was to determine the acute effects of MPA when used in combination with estradiol on

raise questions about the use of progestins, and specifically MPA, in hormone treatments.

In addition to postmenopausal women, premenopausal women are also commonly prescribed MPA. MPA is used in the injectable progestin-only contraceptive Depo-Provera, which is a popular contraceptive choice, particularly for younger premenopausal women because of the ease of use and high compliance. Oral MPA hormone treatments are also used to treat a number of gynecological conditions in young women, such as endometriosis, polycystic ovarian syndrome, and irregular uterine bleeding (7). Despite numerous reports that MPA may impair

Study Design

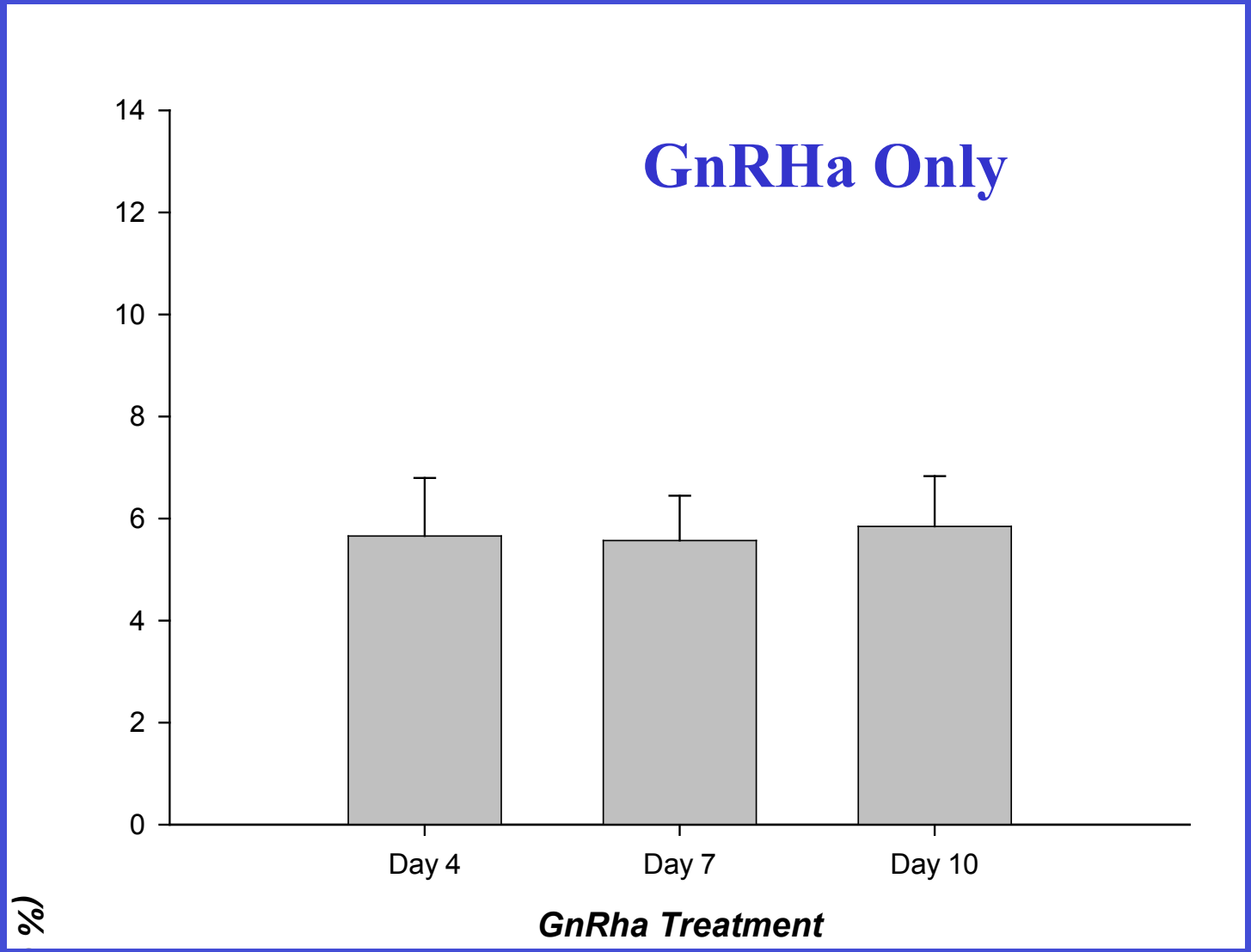


GnRH antagonist = ganarelix 250µg/0.5 ml per day
Transdermal Estradiol = 0.1 mg/day
MPA = 5 mg per day

Endothelial-Dependent FMD in Group 3 (n=2)

FMD Response

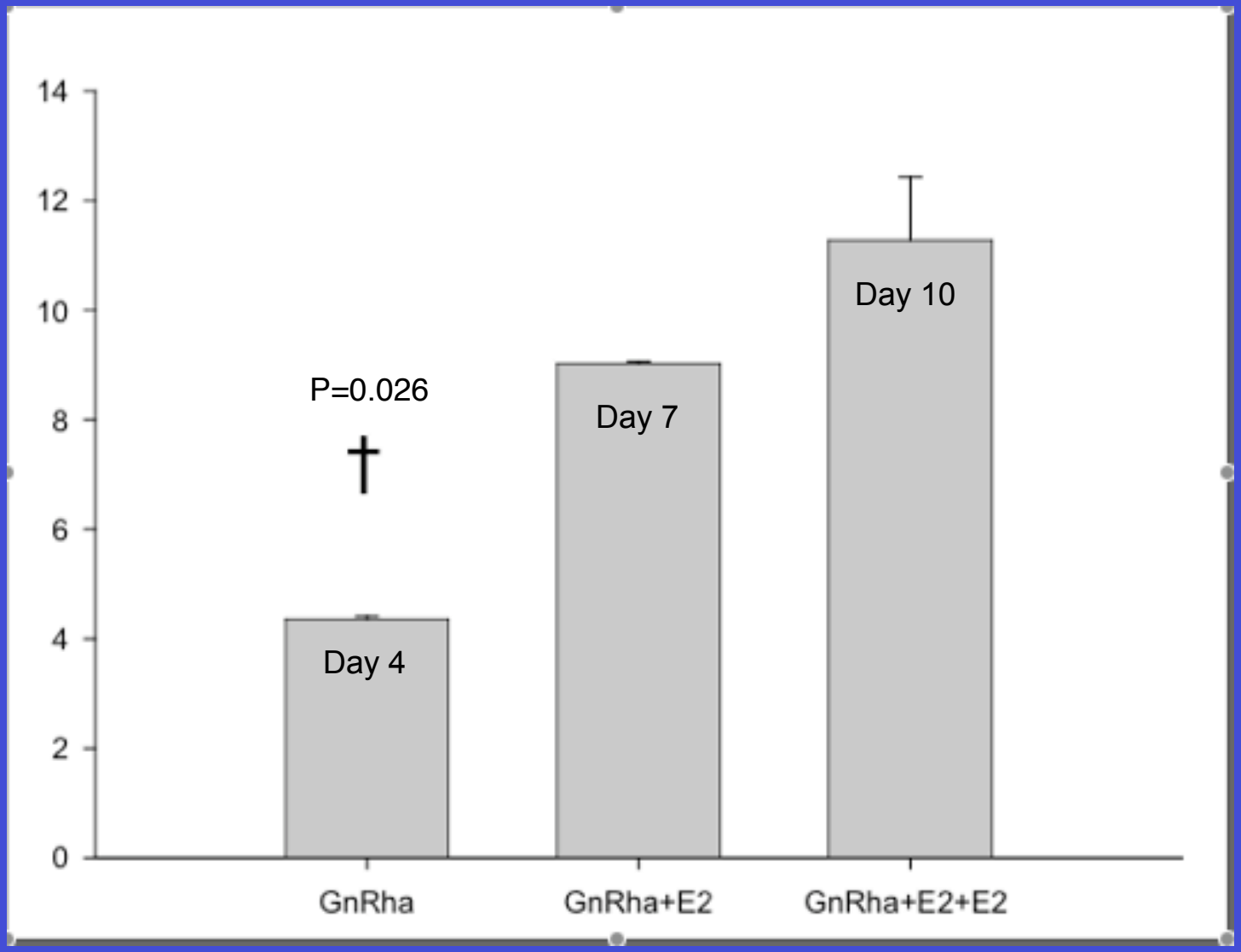
(% Change in Brachial Artery Diameter from Baseline)



Endothelial-Dependent FMD in Group 2 (n=2)

FMD Response

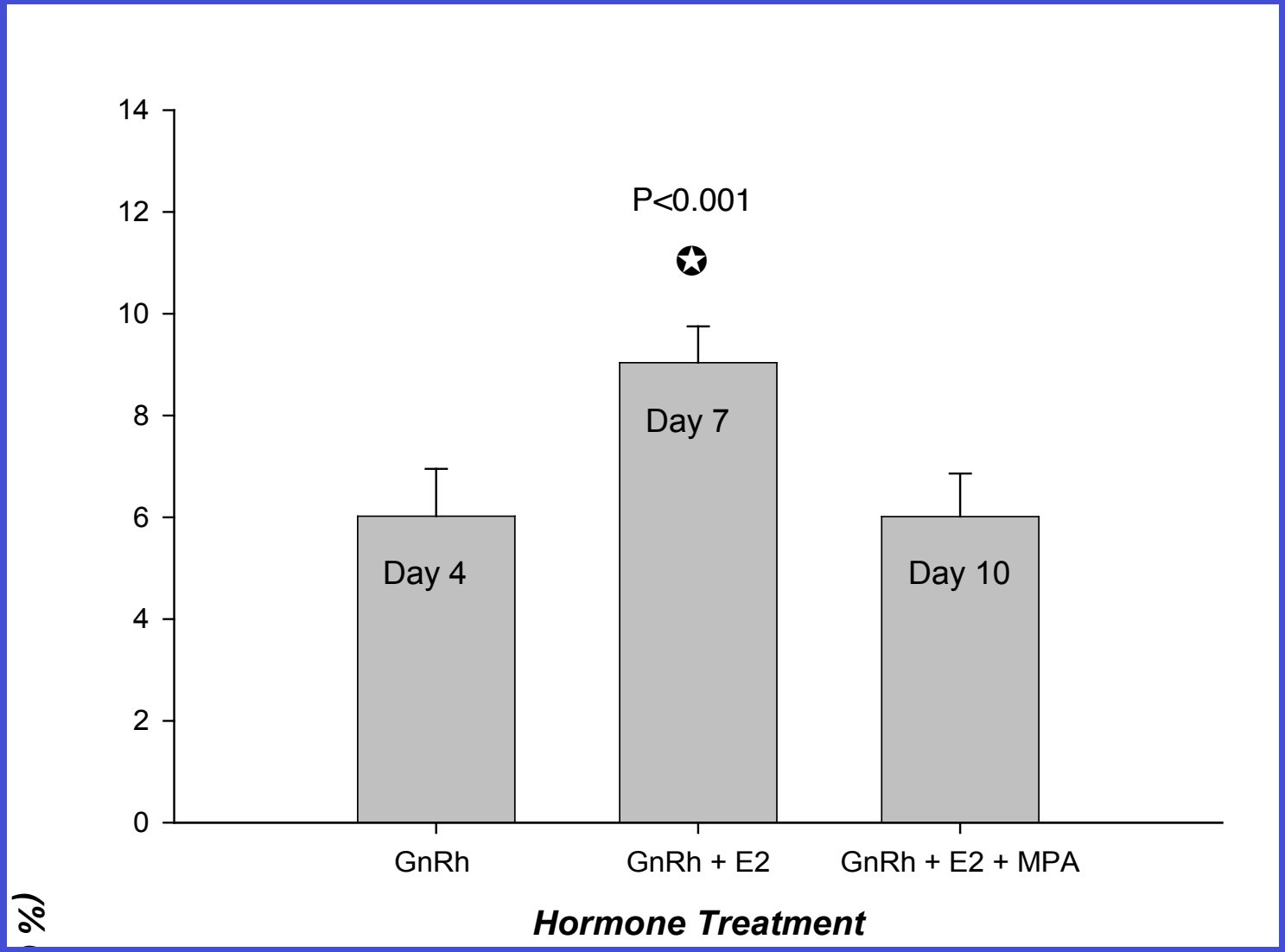
(% Change in Brachial Artery Diameter from Baseline)



Endothelial-Dependent FMD in Group 1 (n=10)

FMD Response

(% Change in Brachial Artery Diameter from Baseline)



Estradiol/MPA FMD Results

- **Administration of E2 improved endothelium-dependent vasodilation (ED-FMD) in all groups.**
- **MPA antagonized the benefits of E2.**
- **There were no observed changes in endothelium-independent vasodilation in any group, consistent with a specific role for endothelial NO production.**

EFFECTS OF PROGESTERONE AND ESTRADIOL ON VASCULAR FUNCTION IN YOUNG WOMEN

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Am J Physiol Heart Circ Physiol 301: H1716–H1722, 2011.
First published August 19, 2011; doi:10.1152/ajpheart.00405.2011.

Short-term oral progesterone administration antagonizes the effect of transdermal estradiol on endothelium-dependent vasodilation in young healthy women

Jennifer A. Miner,[1] Emily R. Martini,[1] Michael M. Smith,[1] Vienna E. Brunt,[1] Paul F. Kaplan,[1,2] John R. Halliwill,[1] and Christopher T. Minson[1]

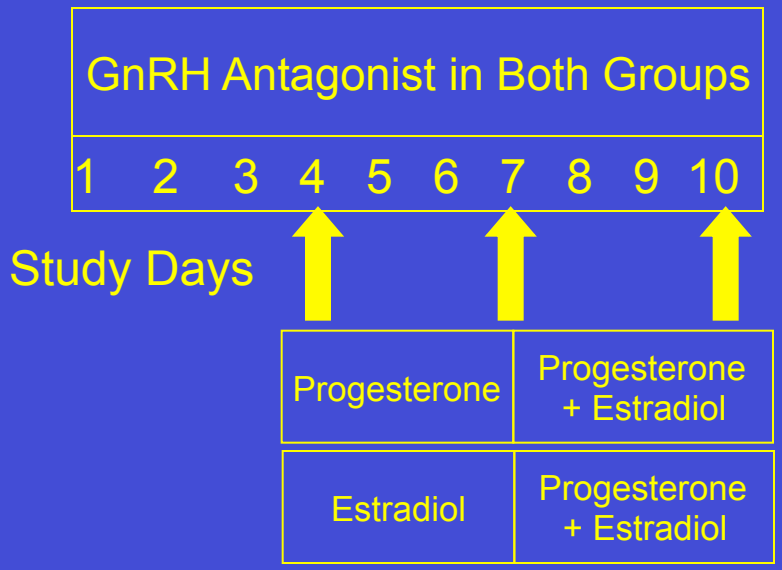
[1]*Department of Human Physiology, University of Oregon, Eugene, and* [2]*Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Oregon Health Sciences University, Portland, Oregon*

Submitted 22 April 2011; accepted in final form 12 August 2011

Miner JA, Martini ER, Smith MM, Brunt VE, Kaplan PF, Halliwill JR, Minson CT. Short-term oral progesterone administration antagonizes the effect of transdermal estradiol on endothelium-dependent vasodilation in young healthy women. *Am J Physiol Heart Circ Physiol* 301: H1716–H1722, 2011. First published August 19, 2011; doi:10.1152/ajpheart.00405.2011.—Very few studies have explored the cardiovascular effects of progesterone in premenopausal women, despite the fact that progesterone is one of the most frequently prescribed progestogens, the need to understand the influence of progesterone on cardiovascular health is great.

One of the primary methods used to investigate the effect of sex hormones on vascular health is via flow-mediated dilation (FMD). FMD, measured as the percent change in brachial artery diameter in response to an increase in shear stress, has

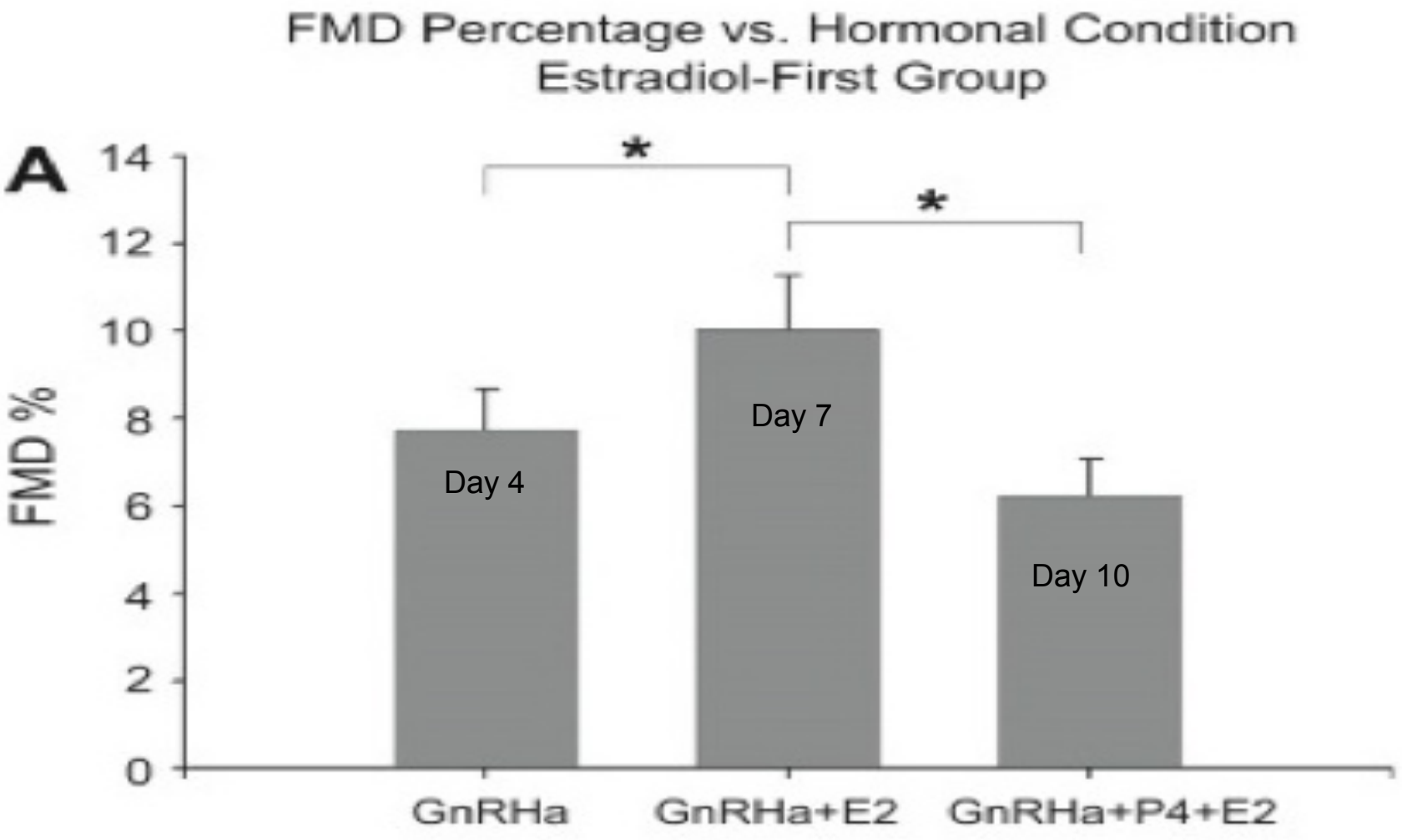
Study Design



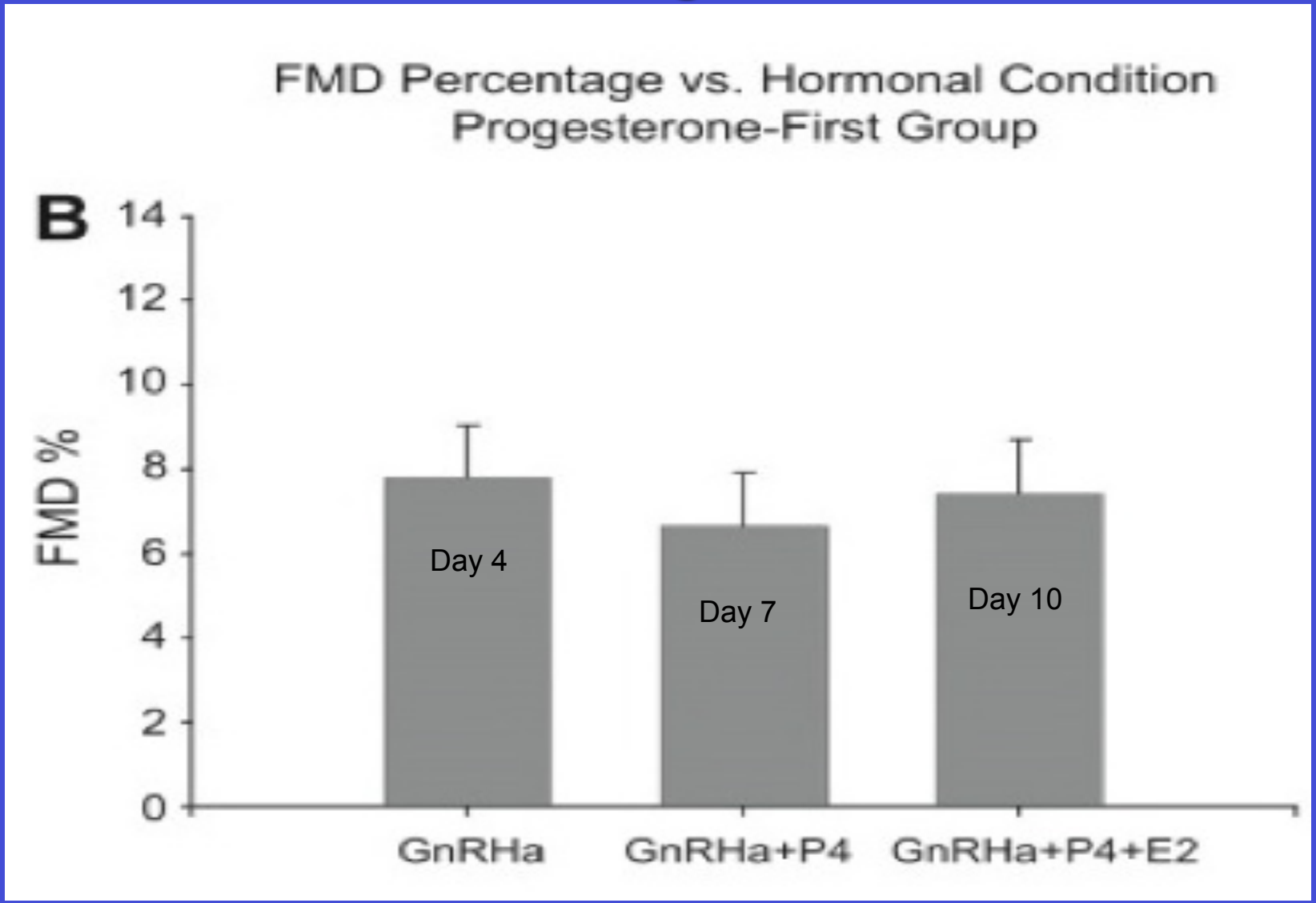
Baroreflex	EDVD
N = 16	N = 8
N = 15	N = 9

GnRH antagonist = ganarelix 250µg/0.5 ml per day
Oral Progesterone = 200 mg per day
Transdermal Estradiol = 0.1 mg/day

Results
EDVD/FMD: Estradiol First



Results
EDVD/FMD: Progesterone First



Hormones as Predictor of FMD

- **Multi-level prediction model**
  - Comparing estrogen and progesterone levels in blood with percent FMD
  - Hormone levels across all subjects, regardless of condition
  - Nests observations within subjects
- **Progesterone & estrogen both predict FMD:**
  - **Progesterone is associated with lower FMD (p=0.022)**
  - **Estrogen is associated with higher FMD (p=0.006)**

Conclusions

- **Acute administration of oral progesterone antagonizes the effect of estradiol on endothelial function at these study doses**

- **Progesterone is associated with decreased endothelial function, and estradiol with increased endothelial function**

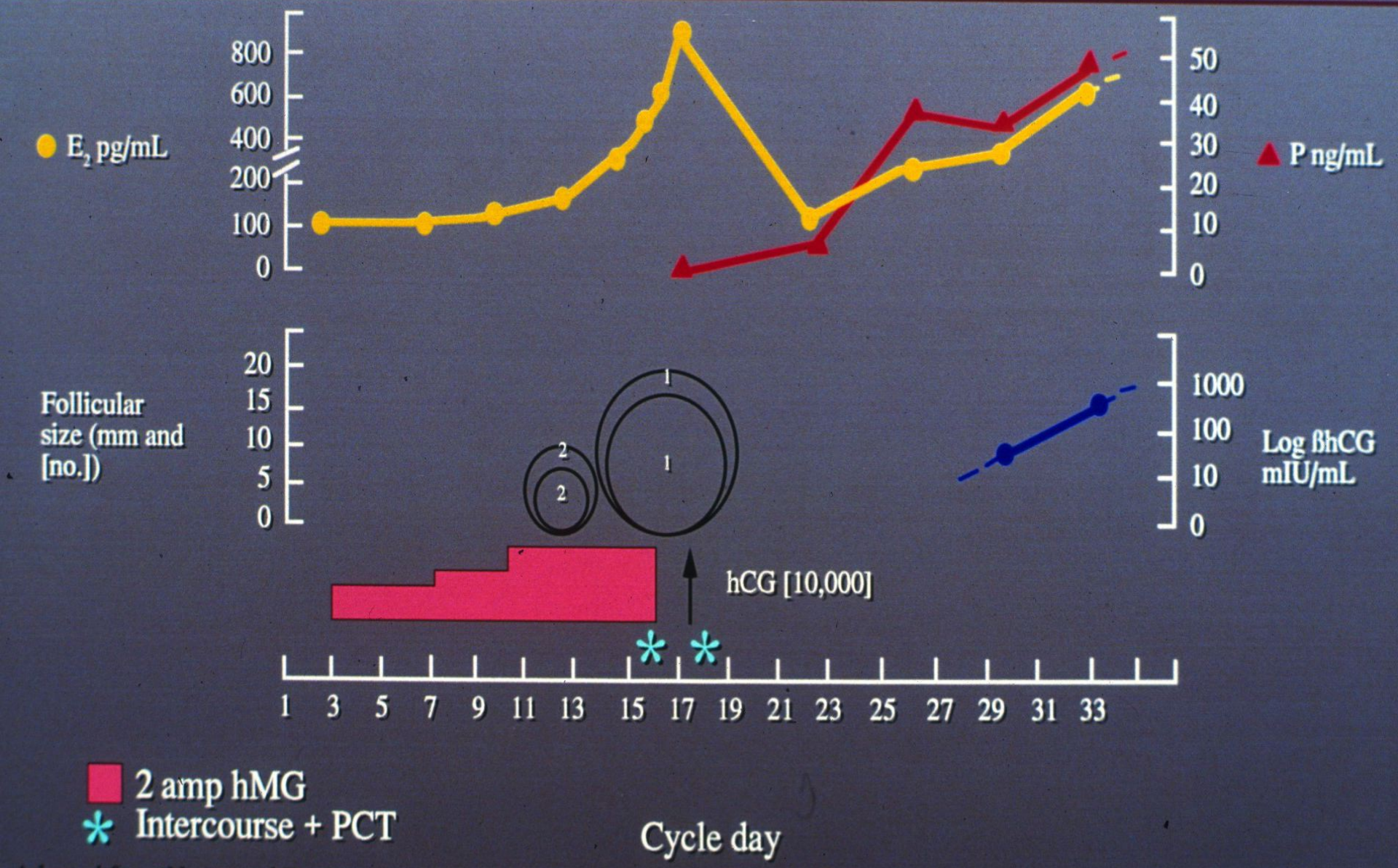
- **Next Phase: Role of Testosterone ??**

Assisted Reproductive Technologies: Present and Future

The Assisted Reproductive Technologies (ART)

- In Vitro Fertilization (IVF)
- Intracytoplasmic Sperm Injection (IVF/ICSI)
- Donor Oocyte IVF
- Frozen Embryo Thaw and Transfer
- In Vitro Maturation/Freezing of Oocytes

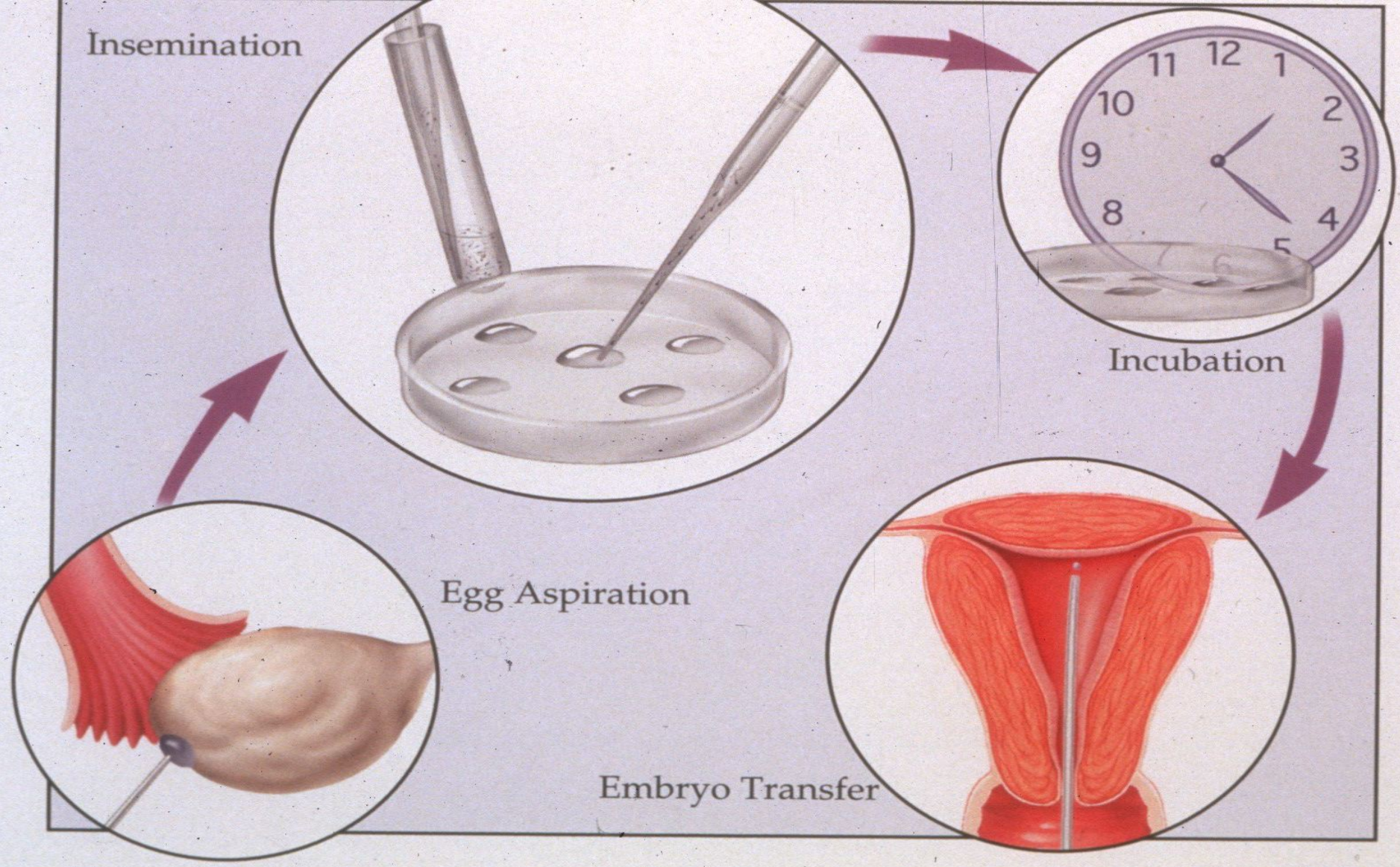
An hMG–hCG Cycle



Adapted from Navot and Rosenwaks, 1987.

In Vitro Fertilization (IVF)

- Daily S/C or IM FSH/hMG injection
- Follicular monitoring with serum estradiol and transvaginal ultrasound
- HCG given to trigger ovulation (LH surge)
- Transvaginal oocyte retrieval and insemination
- Embryo culture and transcervical embryo transfer
- Embryo cryopreservation for future F.E.T.
- Pregnancy rate of 40-50 % per cycle



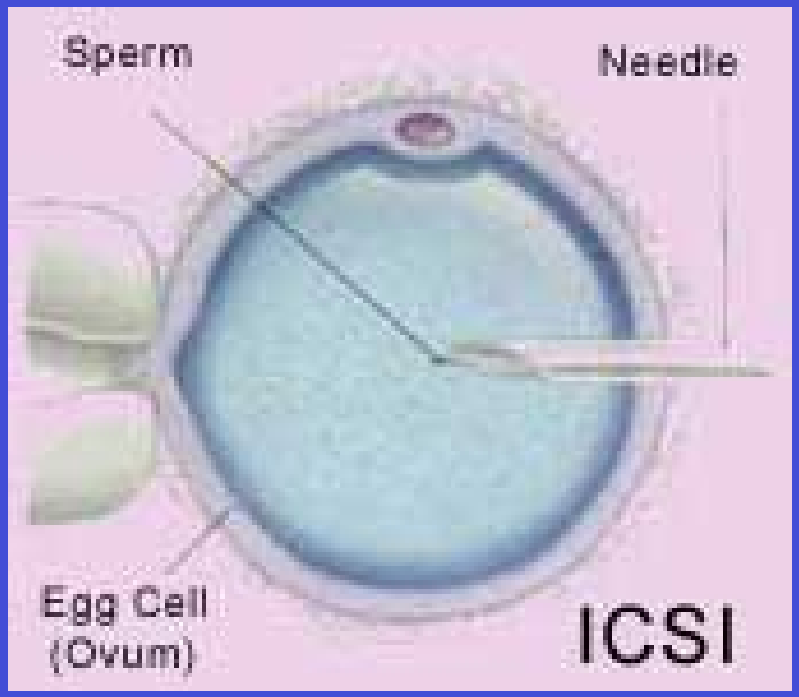
In IVF, eggs are harvested from the woman's ovary and fertilized in the laboratory with sperm. The embryos are then transferred into the uterus.

IVF Embryo Culture and Transfer

Intracytoplasmic Sperm Injection (ICSI)

- Standard IVF Stimulation and oocyte retrieval
- Injection of a single sperm into each oocyte
- Embryo culture and transcervical embryo transfer
- Currently used in almost 50% of IVF cycles for treatment of male factor and unexplained causes
- Pregnancy rate of 40-50 % per cycle

Intracytoplasmic Sperm Injection (ICSI)



Future Directions in Infertility Treatment

- The " *–omics* " Revolution
- Preimplantation genetic diagnosis (PGD)

  - with transgenic therapy ?

- Nuclear and/or cytoplasmic oocyte transfer
- Oocyte Cryopreservation
- Embryonic Stem Cell Line Development

The "-omics" Revolution in Infertility

- **Genomics: The branch of molecular biology concerned with the structure, function, evolution, and mapping of genomes.**
- **Proteomics: The set of proteins expressed by the genetic material of an organism under a given set of environmental conditions.**

The "-omics" Revolution in Infertility

- **Metabolomics:** The systematic study of the unique chemical fingerprints that specific cellular processes leave behind.
- **Embryomics:** The identification, characterization and study of the diverse cell types which arise during embryogenesis.

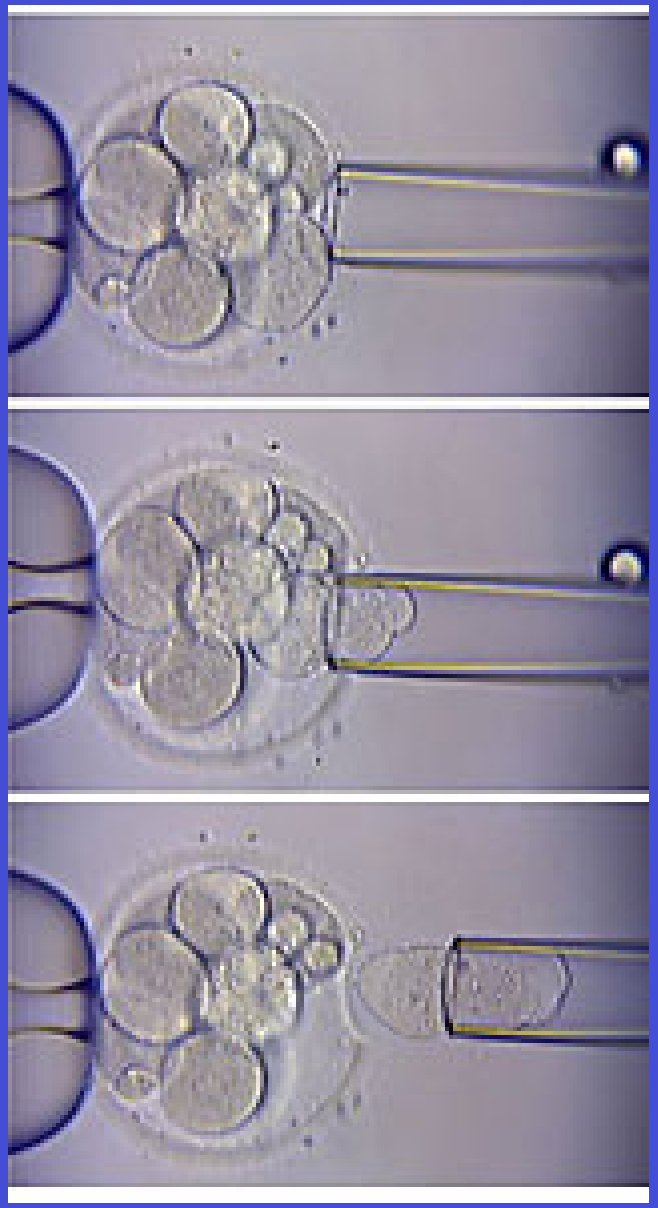
Future Directions in Infertility Treatment(con't)

- Embryo Cloning - Reproductive/Therapeutic
- Embryonic Stem Cell Gamete Development
- Fertility Preservation Techniques (cancer)
- Adult Cell Gamete Cloning - sperm/oocyte
- Adult Somatic Cell Cloning

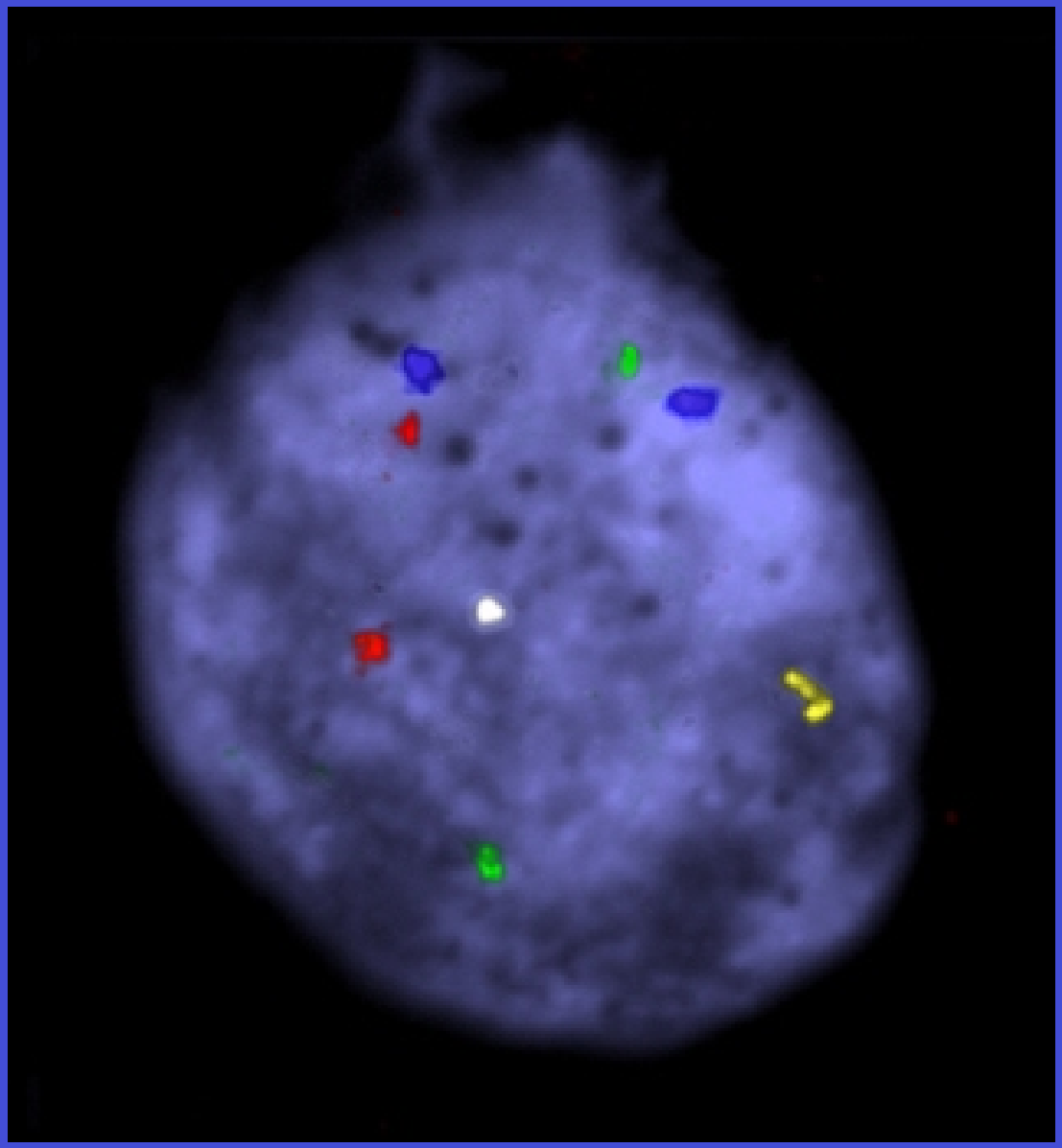
Preimplantation Genetic Diagnosis (PGD)

- Goal: Identify Genetically Abnormal Embryos
- IVF/ICSI + Embryo Culture
- Blastomere Biopsy of 8-cell Embryo
- FISH/PCR Genetic Studies (X,21,single gene,etc)
- Transfer of Normal Blastocysts/Frozen Embryos

PGD 8-cell Blastomere Biopsy



PGD FISH - Normal Embryo

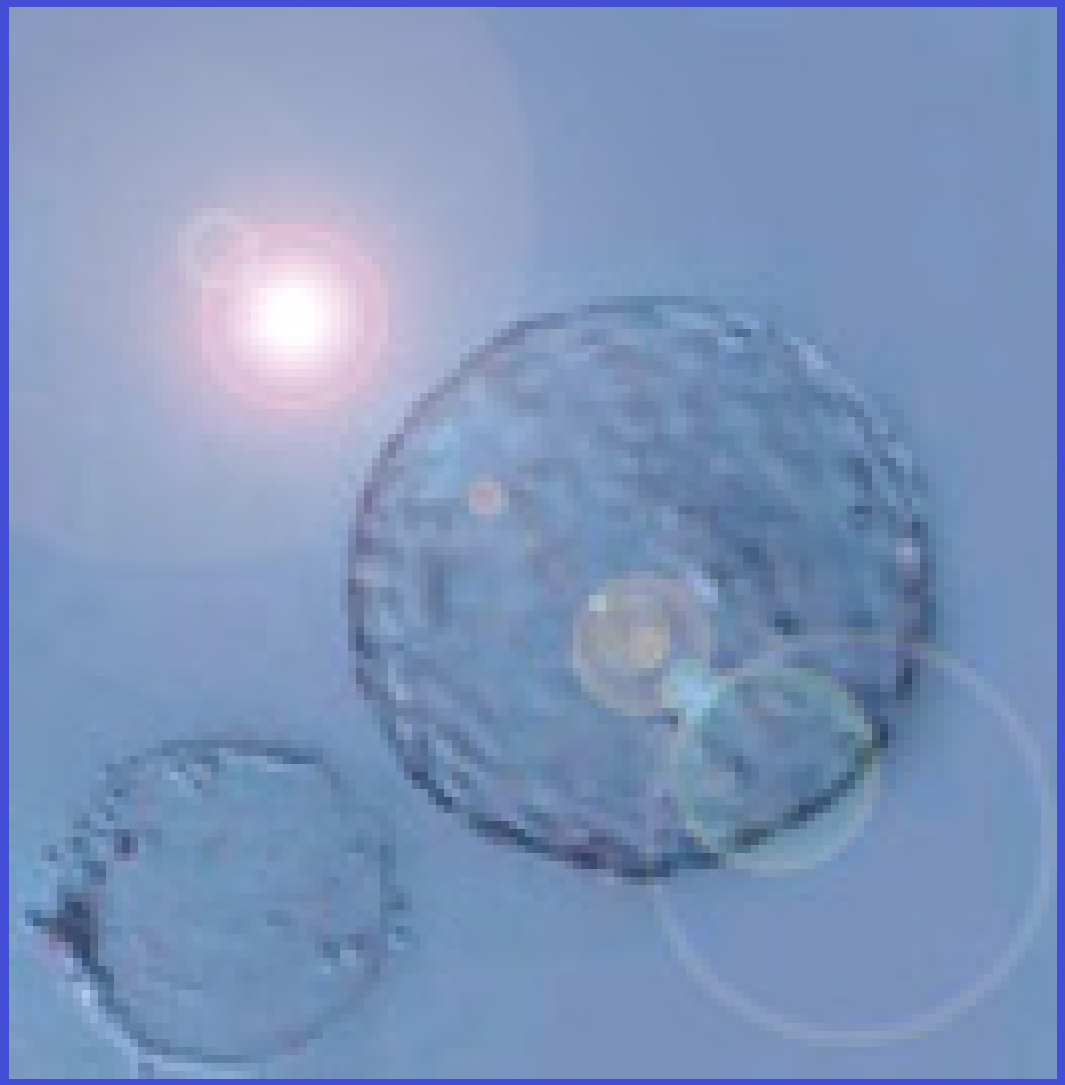


White= Y
Yellow= X
Blue= 18
Red= 21
Green= 13

Oocyte Cryopreservation

- Preservation of Oocytes Prior to Fertilization
- TV Retrieval of Stimulated Oocytes
  - Future: Unstimulated Oocytes with IVM
- Desiccation and Cryopreservation
- Delayed Thaw and IVF/ICSI Embryo Culture
- Transfer of Healthy Embryos

Oocyte Desiccation for Cryopreservation



Is Oocyte Cryopreservation a Successful Option?

A. 69 women had all IVF oocytes frozen (12/04-12/06)

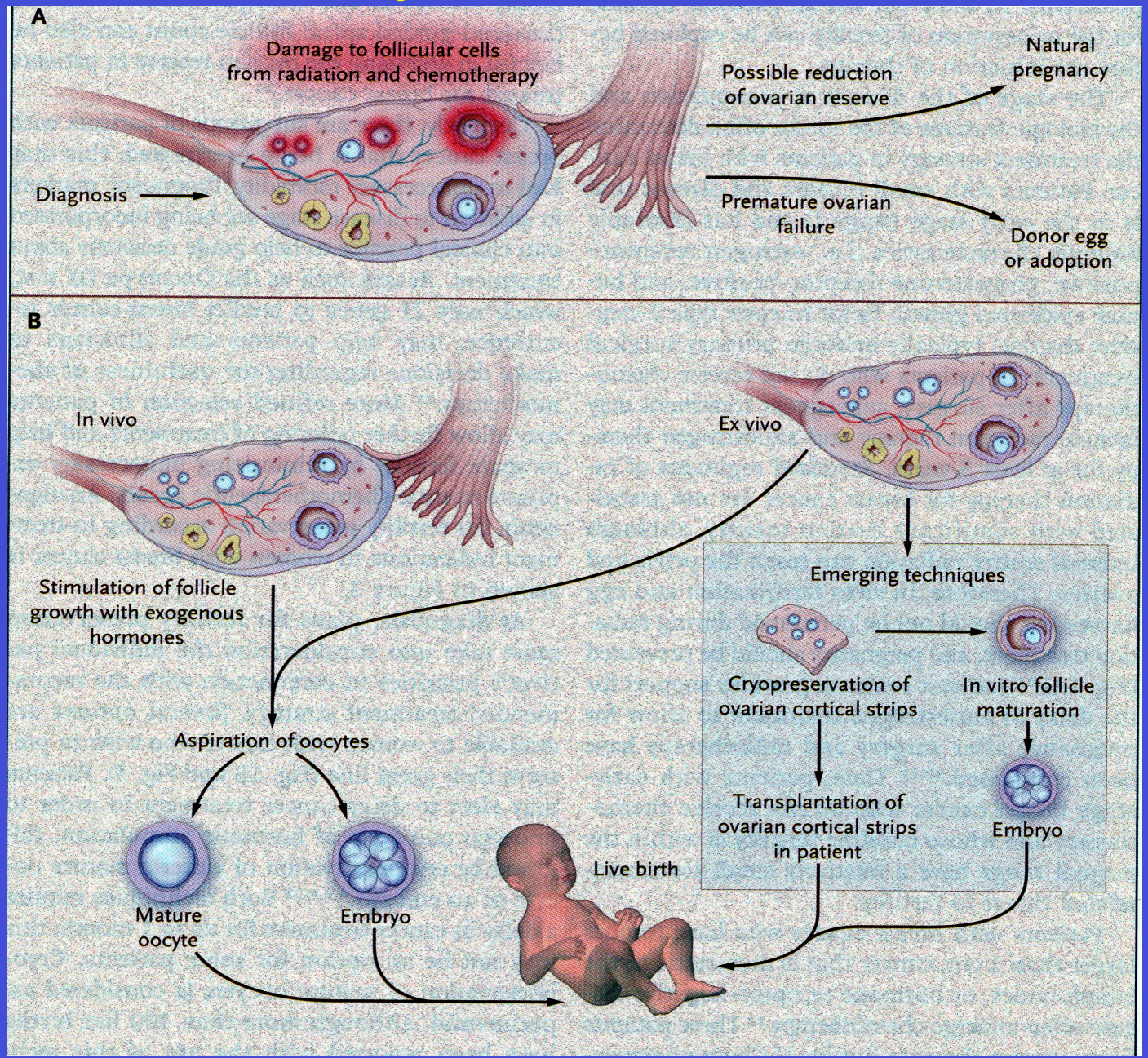
B. 254 oocytes thawed in 18 women for 24 transfers

C. 130 of 254 fertilized (52%)

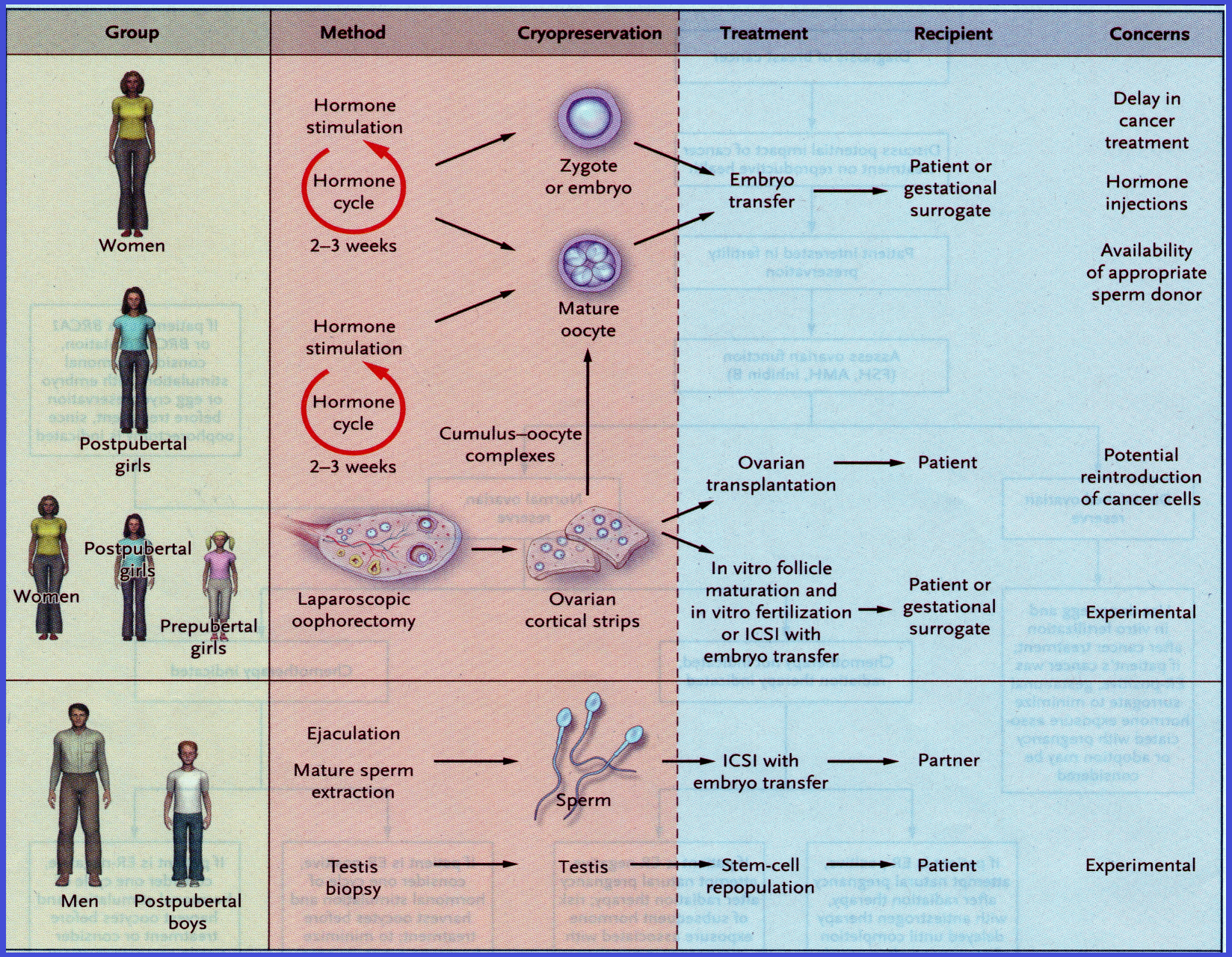
D. 84 embryos transferred. Clinical pregnancy rate 11/24 (45.8%) per embryo transfer and 10/18 (55.6%) per patient.

Paulson R et al. PCOGS 10-12-07

Fertility Preservation



Fertility Preservation



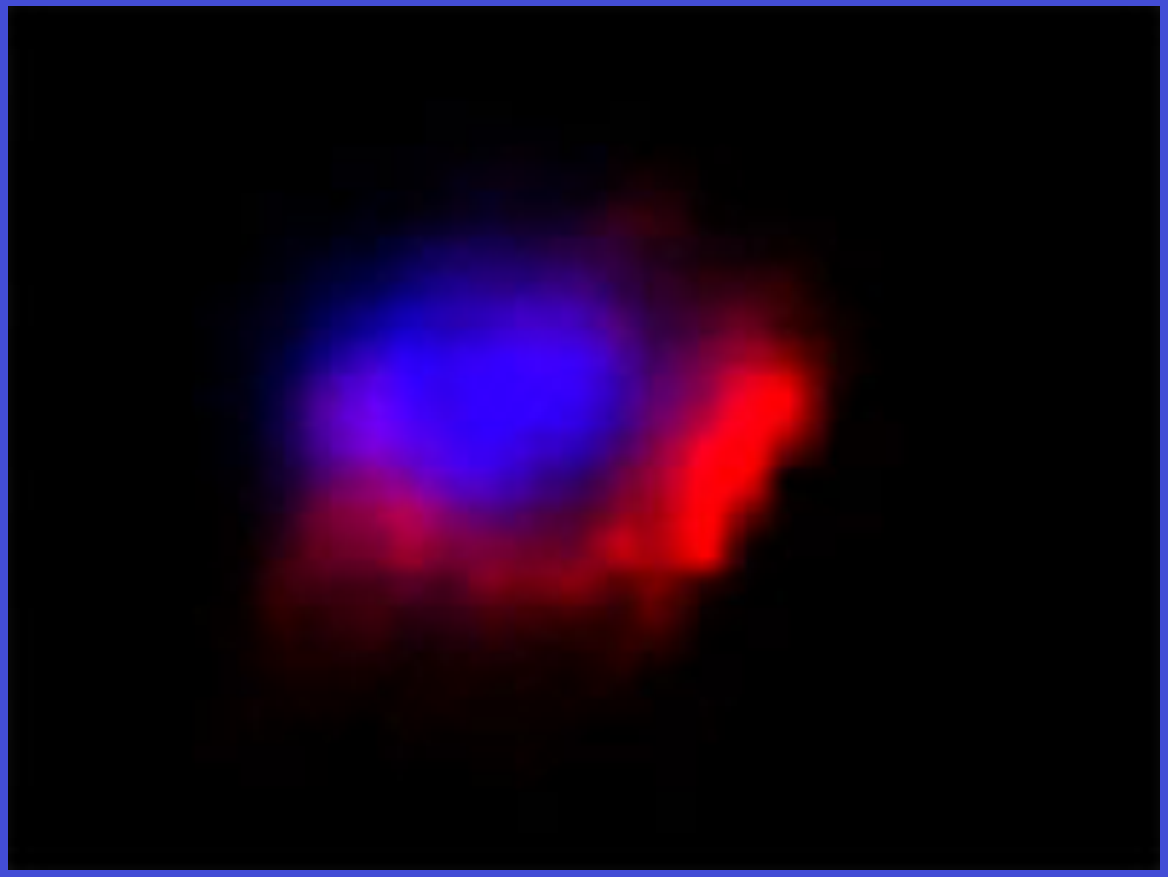
Stem Cell Gamete Production

A. Goal is cost-effective, ethically-acceptable source of sperm and oocytes
   - Reduction of risks with donor gametes
   - Alternative to somatic cell cloning

B. Potential treatment for cancer patients, age-related infertility, and severe male factor

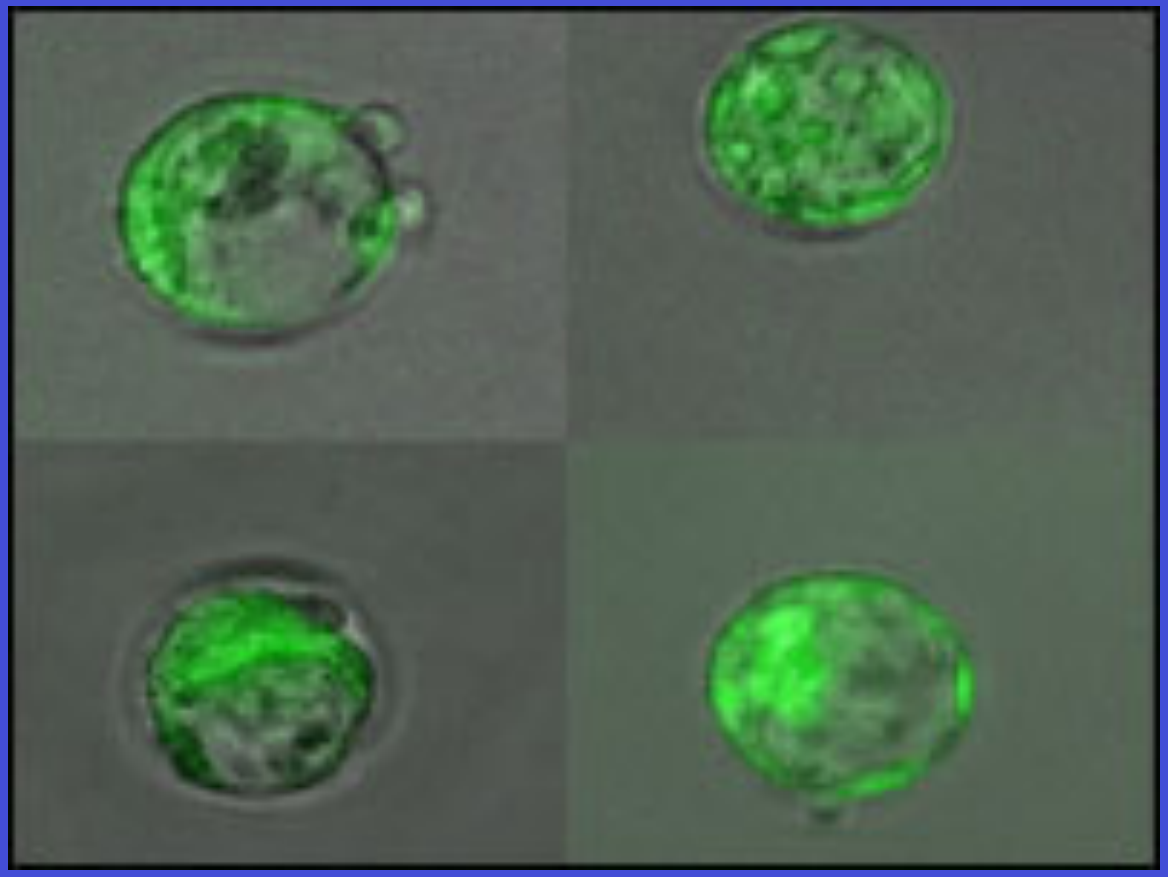
C. Reduction of multiple gestation by SET

Mouse Embryonic Stem Cell Spermatid



NPR Dec. 2004

Mouse Blastocysts Fertilized by Embryonic Stem Cell Spermatids



NPR Dec. 2004

Milestones of infertility medicine

1967
Clomid comes on the market

1969
Pergonal and human chorionic gonadotropin marketed

1978
Louise Brown, first "test-tube baby," born

1981
First IVF baby in America

1984
GIFT technique developed by Ricardo Asch of San Antonio

First "donor baby" (eggs and sperm) born to surrogate mother in Australia

1985
Maryland passes legislation requiring insurance coverage for IVF

First ultrasound-guided, nonsurgical IVF

1986
Richard Marrs delivers first U.S. baby developed from a frozen embryo

1987
ZIFT technique introduced

Lupron comes on the market

1990
Mark Sauer reports pregnancies in postmenopausal women

1991
First preimplantation genetic screening (for cystic fibrosis)

1992
Fertility Clinic Success Rate and Certification Act calls for uniform definition of success; to take effect October 1994

1993
Supreme Court decides frozen embryos cannot be implanted against the father's will

In Vitro Fertilization (IVF) - 2014

- SART Data: 61,740 IVF babies born in 2012 in U.S.
- IVF babies now constitute almost 2% of U.S. births
- Estimated 400,000 IVF babies born in 2012 in world
- IVF births now almost 4% of births in Europe
- Estimated 5,000,000 IVF births by Oct. 2013

- Who Knew ?????

The world's first IVF baby Louise Brown (2nd right) posing with her son Cameron, her mother Lesley Brown and IVF pioneer Professor Robert Edwards in 2008