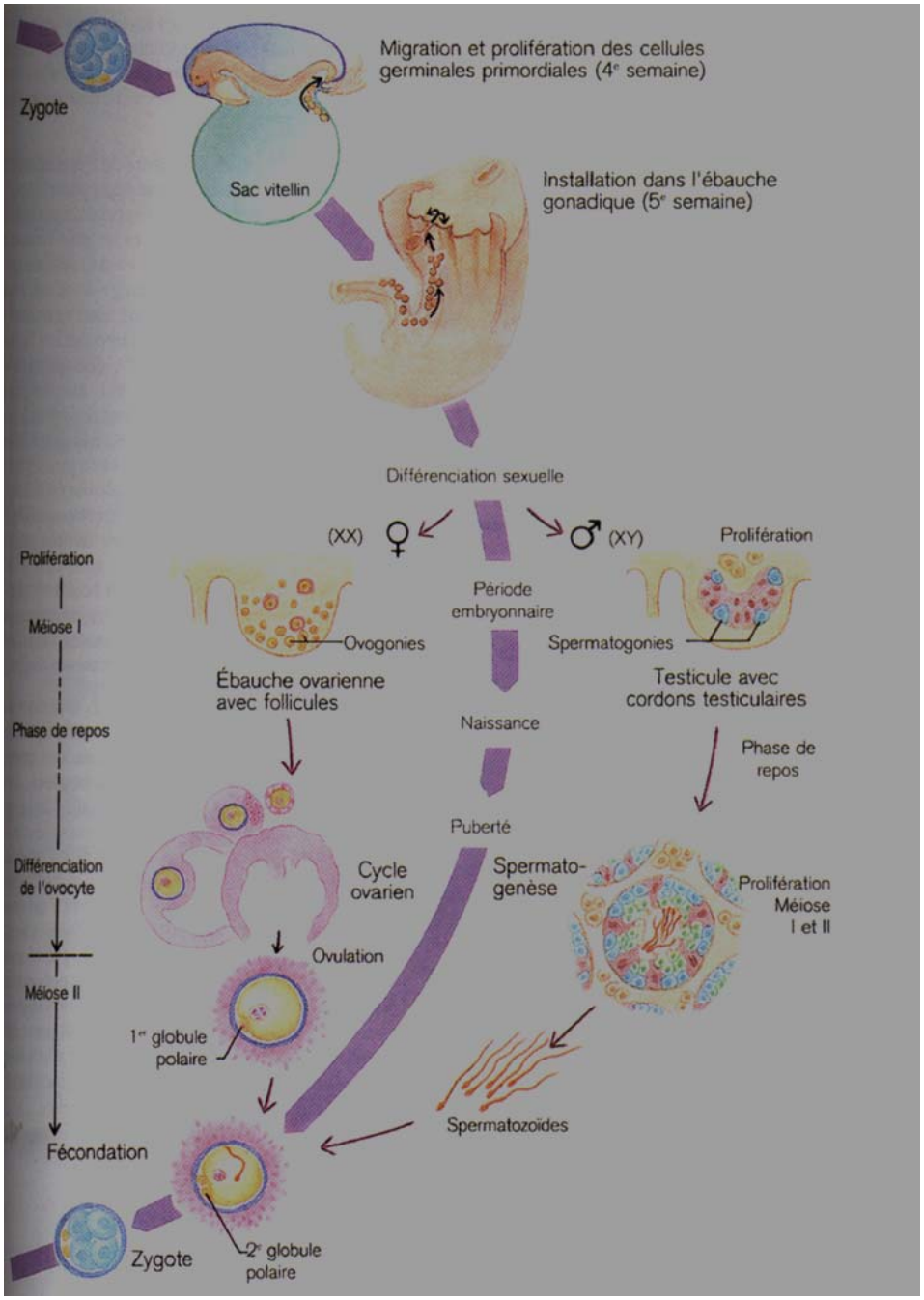
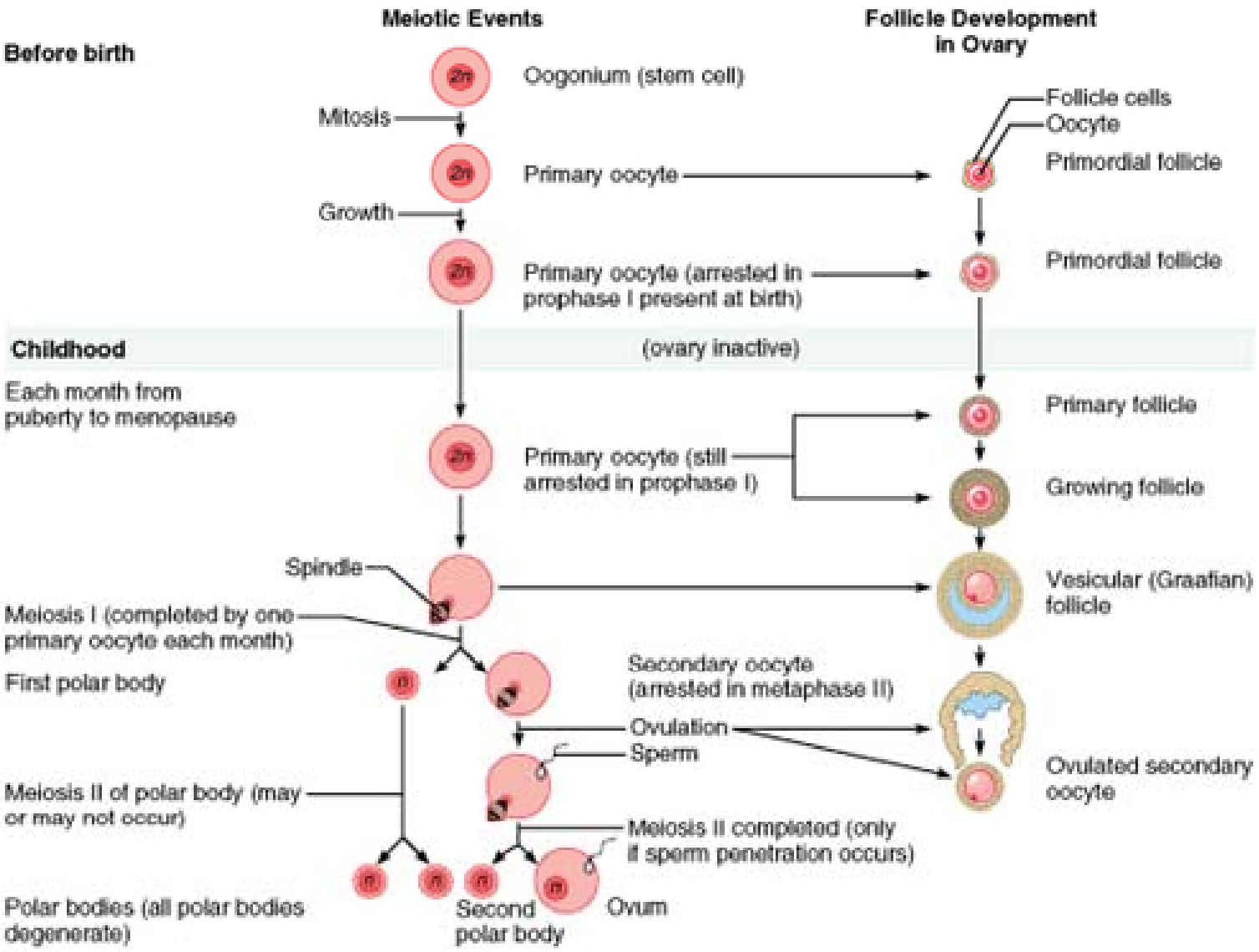
Do adult stem cells reside in the mammalian human ovary?

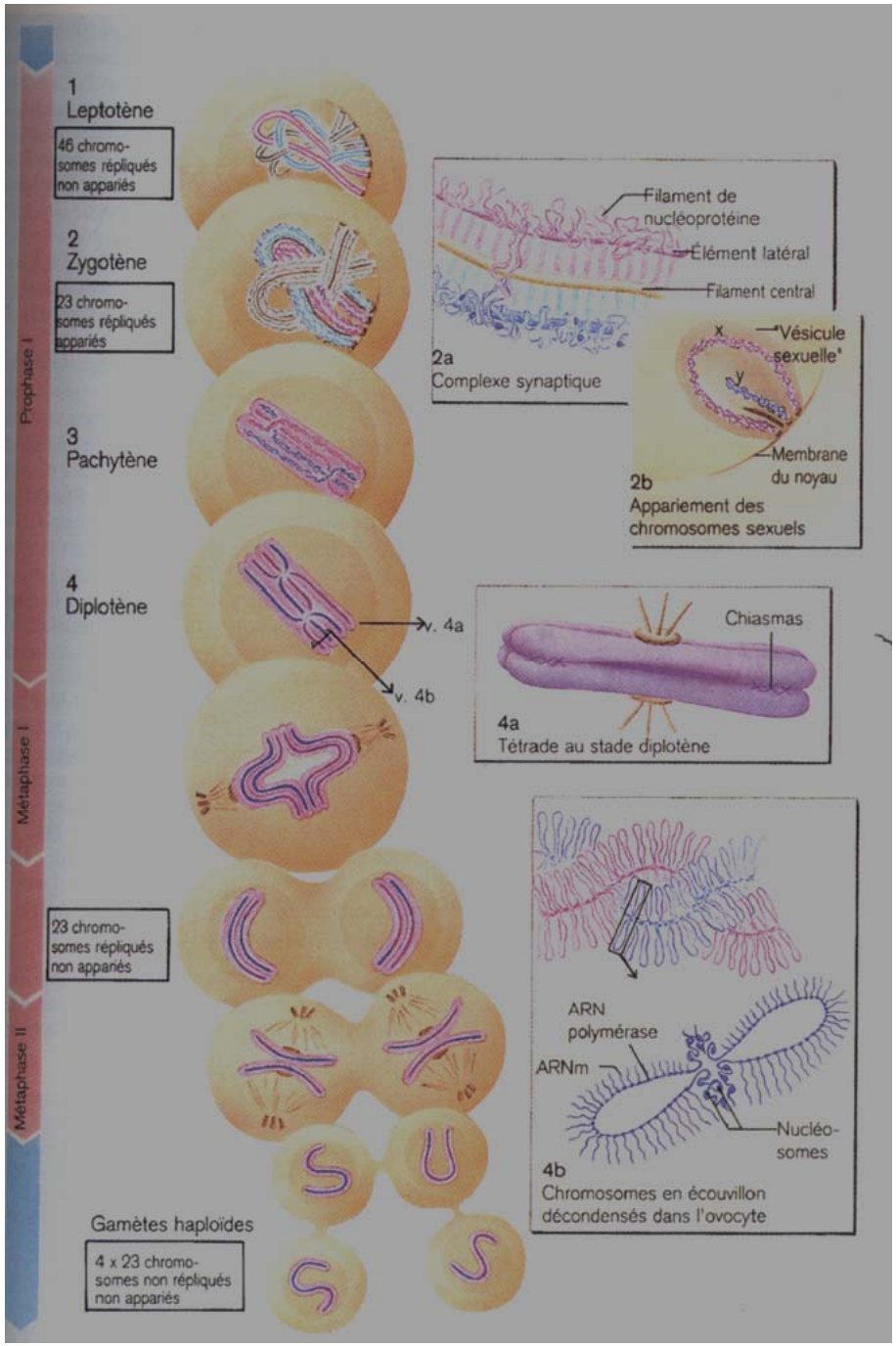
Anna Veiga

Centre de Medicina Regenerativa de Barcelona

Institut Universitari Dexeus. Barcelona


Zygote
Migration et prolifération des cellules germinales primordiales (4e semaine)
Sac vitellin
Installation dans l'ébauche gonadique (5e semaine)
Différenciation sexuelle
(XX) ♀
♂ (XY)
Prolifération
Période embryonnaire
Ovogonies
Spermatogonies
Ébauche ovarienne avec follicules
Testicule avec cordons testiculaires
Naissance
Phase de repos
Puberté
Prolifération
Méiose I
Phase de repos
Différenciation de l'ovocyte
Cycle ovarien
Spermato-genèse
Prolifération Méiose I et II
Ovulation
Méiose II
1er globule polaire
Spermatozoïdes
Fécondation
Zygote
2e globule polaire


Meiotic Events
Follicle Development in Ovary
Before birth
2n
Oogonium (stem cell)
Mitosis
Follicle cells
Oocyte
Primordial follicle
2n
Primary oocyte
Growth
2n
Primary oocyte (arrested in prophase I present at birth)
Primordial follicle
Childhood
(ovary inactive)
Each month from puberty to menopause
Primary follicle
2n
Primary oocyte (still arrested in prophase I)
Growing follicle
Spindle
Vesicular (Graafian) follicle
Meiosis I (completed by one primary oocyte each month)
First polar body
n
Secondary oocyte (arrested in metaphase II)
Ovulation
Sperm
Ovulated secondary oocyte
Meiosis II of polar body (may or may not occur)
Meiosis II completed (only if sperm penetration occurs)
n
n
n
n
Polar bodies (all polar bodies degenerate)
Second polar body
Ovum


Prophase I
Métaphase I
Métaphase II
1
Leptotène
46 chromosomes répliqués non appariés
2
Zygotène
23 chromosomes répliqués appariés
3
Pachytène
4
Diplotène
v. 4a
v. 4b
23 chromosomes répliqués non appariés
Gamètes haploïdes
4 x 23 chromosomes non répliqués non appariés
Filament de nucléoprotéine
Élément latéral
Filament central
2a
Complexe synaptique
x
y
Vésicule sexuelle*
Membrane du noyau
2b
Appariement des chromosomes sexuels
Chiasmas
4a
Tétrade au stade diplotène
ARN polymérase
ARNm
Nucléosomes
4b
Chromosomes en écouvillon décondensés dans l'ovocyte

- Before birth, the cortex of the female ovary contains its peak number of follicles (about 7×10^6).
- The supply of follicles decreases to 2×10^6 by birth and 300,000 by puberty. By virtue of the "inefficient" nature of folliculogenesis only 400 of these follicles will ever reach the preovulatory stage.
- It is commonly said that when oocytogenesis is completed, no additional primary oocytes are created.

THE RELATION OF AGE TO NUMBERS OF OOCYTES

BY ANITA M. MANDL AND S. ZUCKERMAN

From the Department of Anatomy, University of Birmingham

(*Received* 30 *October* 1950)

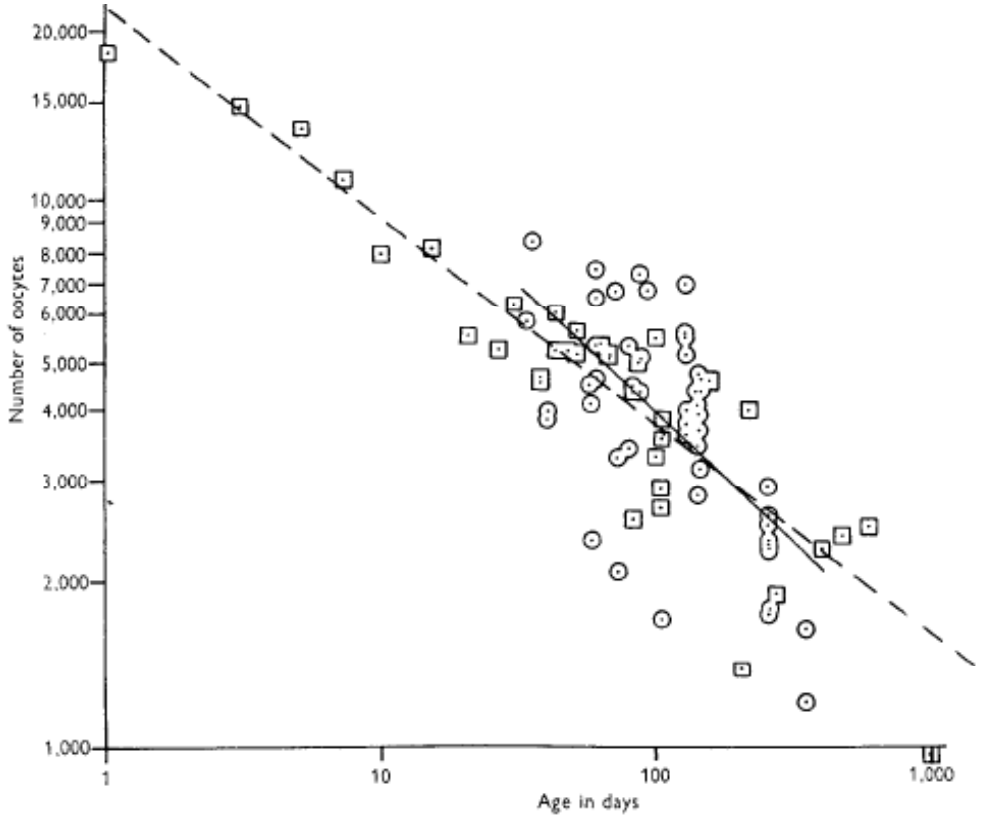


Fig. 1. The interrelation of oocyte numbers and age in the rat (in logs). Regression line ——— and items ⊙ apply to Birmingham series. Regression line – – – – and items ⊡ apply to Arai's data [1920].

Journal of Endocrinology

(based on data

from Arai, 1920

and Mandl, 1950

Human Reproduction vol.11 no 7 pp 1484–1486, 1996

A model conforming the decline in follicle numbers to the age of menopause in women

Faddy and Gosden,

1996

110 pairs of ovaries
(0-51 years of age)

Step change to
gradual change

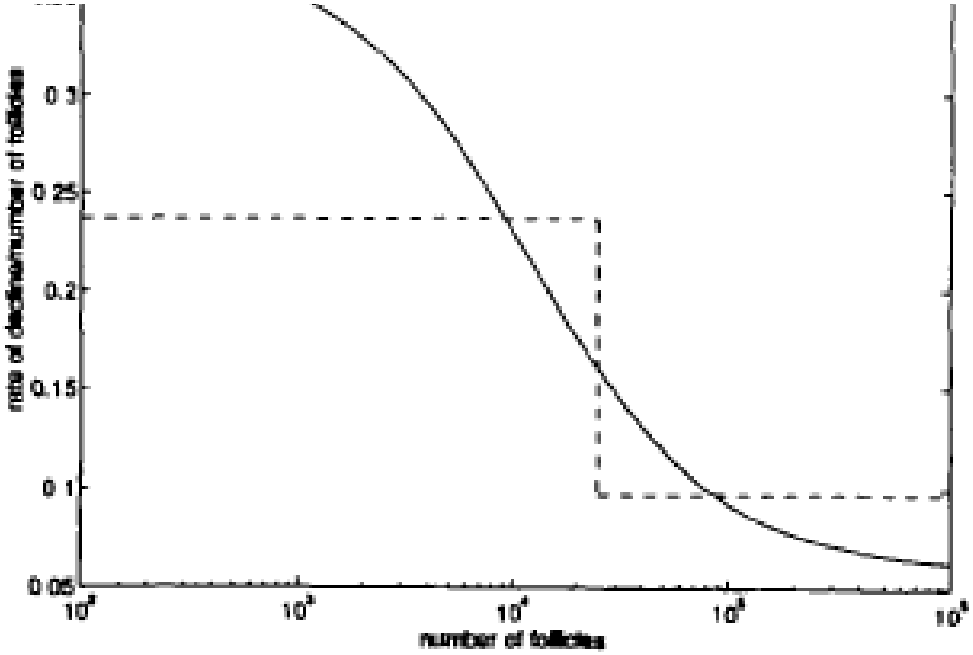


Figure 1 A comparison between two models accounting for the accelerated rate of disappearance of follicles in older ovaries: 'bi-exponential' model showing a step change in the rate when 25 000 follicles remain (– – –), and after fitting a model with a more gradual change (———). Note that the *x*-axis representing numbers

Human Reproduction vol.11 no 7 pp 1484–1486, 1996

A model conforming the decline in follicle numbers to the age of menopause in women

Faddy and Gosden,

1996

Distribution of predicted menopausal ages (number of fertile years remaining)

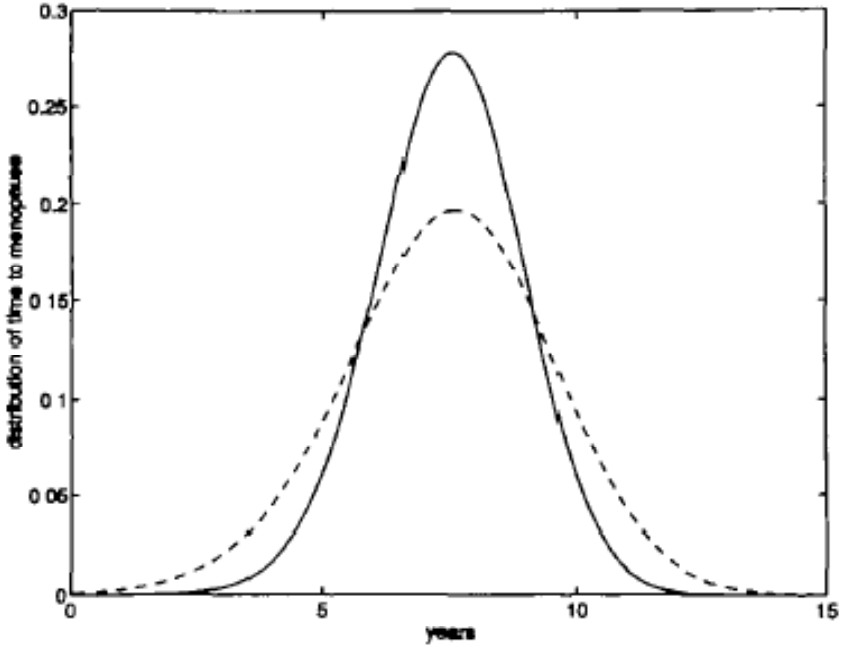


Figure 3. Years to menopause predicted from a stochastic threshold model, when 10 000 follicles remain. The probability distributions represented are when this number of follicles is known exactly (———) and when estimated with a 50% standard error (– – –)

Continuous loss of oocytes throughout meiotic prophase in the normal mouse ovary

Kelly A. McClellan,[a] Roger Gosden,[b] and Teruko Taketo[a,c,*]

2003

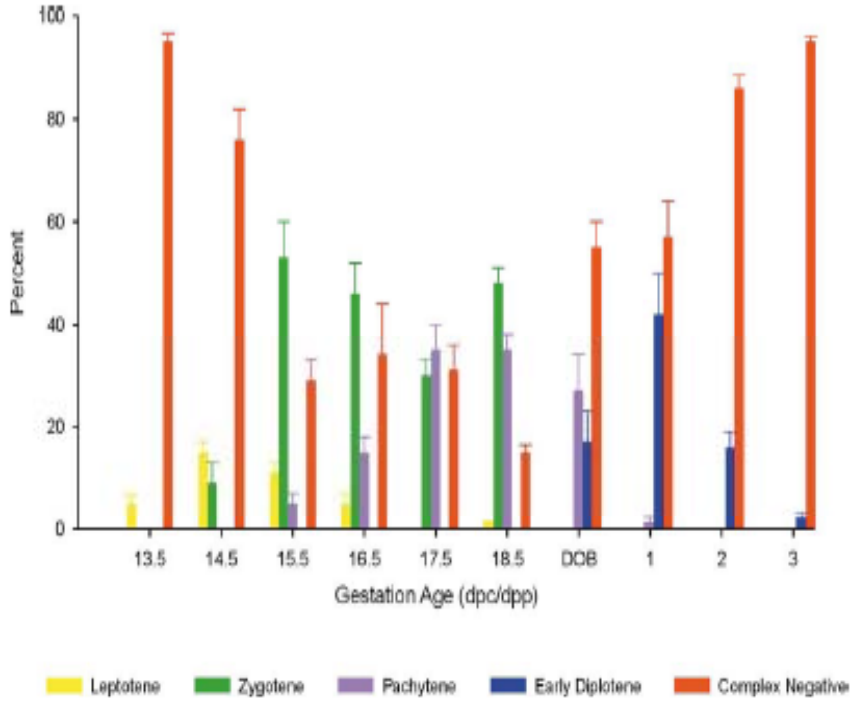


Fig. 3. Progress of meiotic prophase in GCNA-1-labeled cells. Each meiotic prophase stage represents the proportion of total cells (mean ± SEM) obtained from six pairs of ovaries from two litters at each gestation age.

Quantificaction of murine GC (markers GCNA)

- Most GCNA + cells entered and progressed though meiotic prophase during fetal development
- Continuous decline in GCNA + cells during fetal development

Establishment of ovarian reserve: a quantitative morphometric study of the developing human ovary

Antonino Forabosco, M.D., Ph.D.,[a] *and Chiarella Sforza, M.D., Ph.D.*[b]

Fertil. Steril 2007

TABLE 2

Total number of primordial and primary follicles per ovary and ovarian reserve.

Case no.	Age (wk)	N_V PF mm^{-3}	Total no. PF	N_V F1 mm^{-3}	Total no. F1	Total no. of follicles per ovary	% of F1 of total follicles per ovary	Ovarian reserve
1	15	3,885	51,884			51,884		103,768
2	17	3,116	59,469			59,469		118,937
3r	19	2,410	124,538			124,538		—
3l	19	2,179	88,028			88,028		212,565
4r	20	7,205	186,563			186,563		—
4l	20	7,227	176,120			176,120		362,682
5	20	6,704	197,361			197,361		394,722
6	25	8,007	251,763			251,763		503,526
7	34	6,001	341,354			341,354		682,708
8	38	4,023	462,748	162	18,634	481,382	3.81	925,496
9	38	4,390	297,095	146	9,881	306,975	4.77	594,190
10	38	2,828	325,730	171	19,696	345,426	5.70	651,460
11	38	2,257	176,764	106	8,302	185,065	4.48	353,538
12	38	2,289	368,120	116	18,655	386,775	4.82	736,240
13	66	1,398	342,095	34	8,313	350,408	2.30	684,190

Note: Age is in weeks of development; r = right specimen; l = left specimen; N_V = number per unit volume; PF = primordial follicles; F1 = primary follicles.

Forabosco. Formation of ovarian reserve in humans. Fertil Steril 2007.

15 ovaries from fetuses, neonates and one 8 month baby

PF pool shows an exponential increase till 8th month of prenatal life to, at least, the 8th month of postnatal life

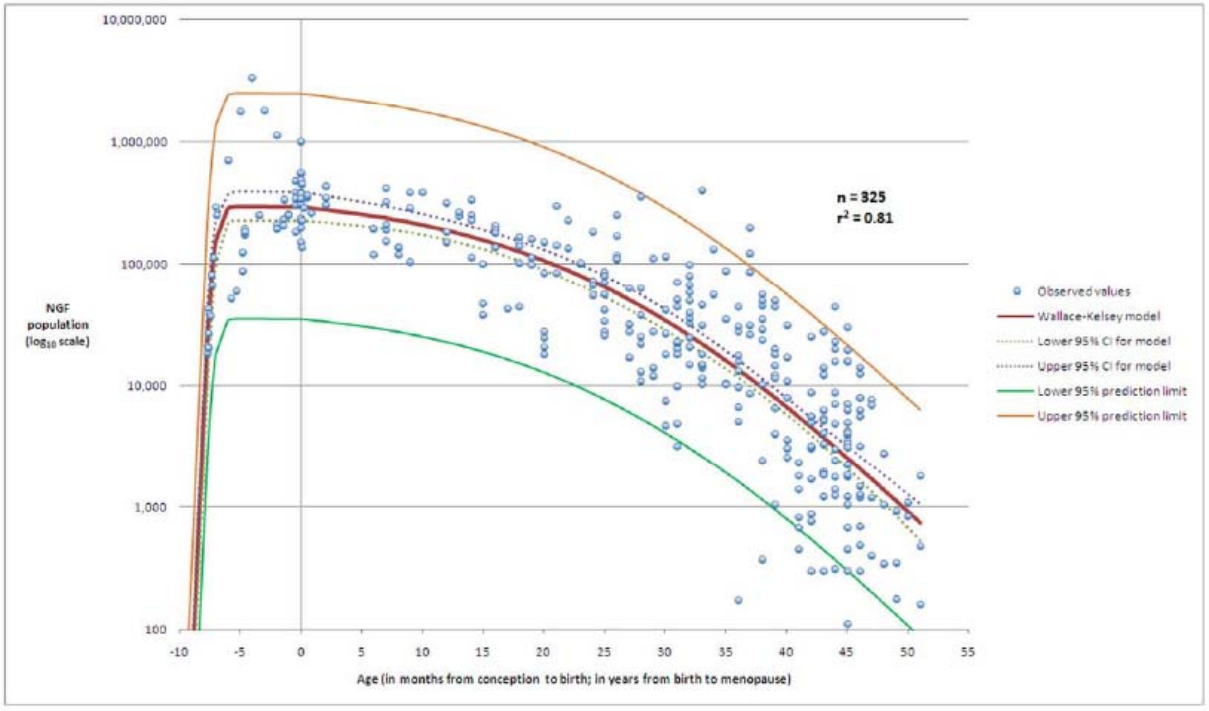
OPEN ACCESS Freely available online

PLoS one

Human Ovarian Reserve from Conception to the Menopause

W. Hamish B. Wallace[1]*, Thomas W. Kelsey[2]

2010



Mathematical model of age related population of NGF in the human ovary from conception to menopause

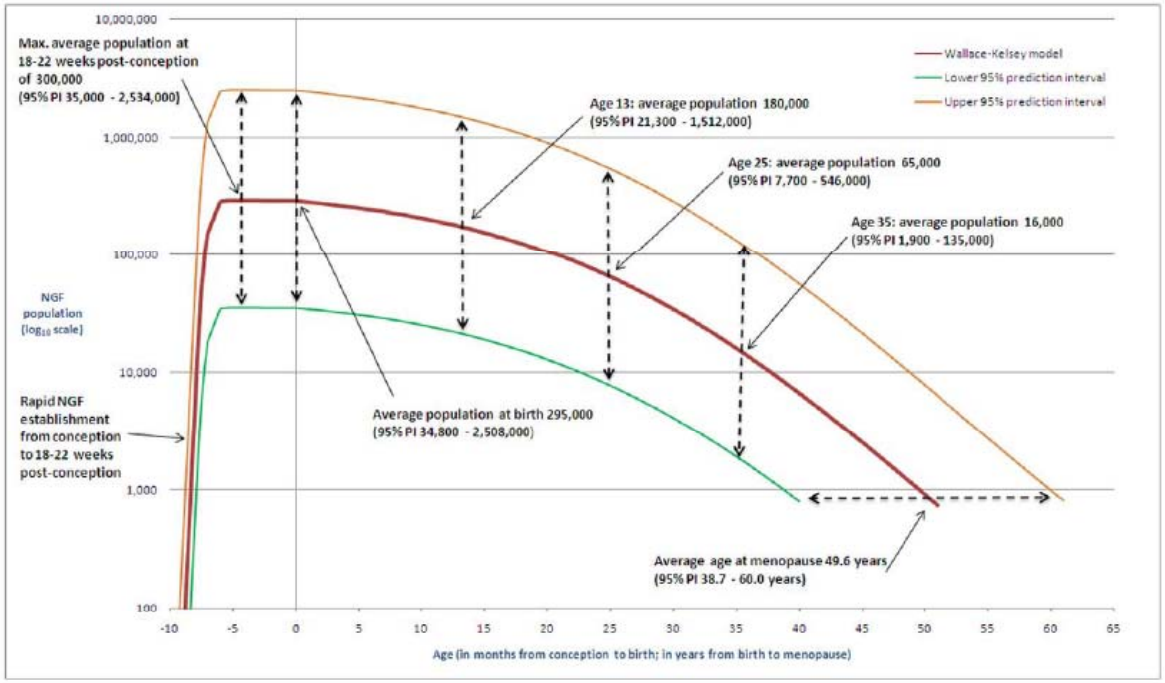
8 separate quantitative histological studies (n=325)

OPEN ACCESS Freely available online PLoS one

Human Ovarian Reserve from Conception to the Menopause

2010

W. Hamish B. Wallace[1]*, Thomas W. Kelsey[2]



- 95% of women by 30 years of age have 12% of their max. prebirth number of NGF, 3% by 40.
- 81% of the variance in NGF population is due to age alone

- Recently, a number of publications have challenged the ovarian biology dogma that a finite number of follicles are set around the time of birth.
- Renewal of ovarian follicles from germline stem cells (originating from bone marrow and peripheral blood as well as ovarian stem cells) have been reported in the mouse and human ovary

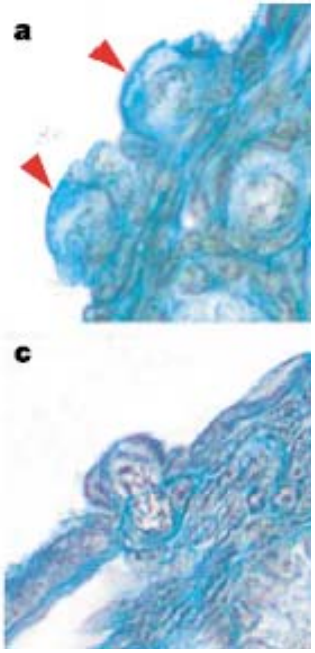
Nature 2004

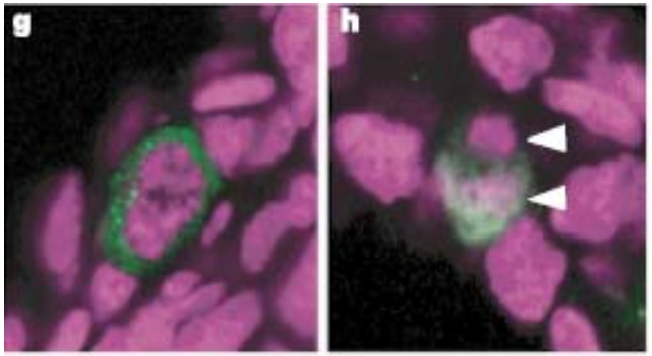
articles

Germline stem cells and follicular renewal in the postnatal mammalian ovary

Joshua Johnson*, Jacqueline Canning*, Tomoko Kaneko, James K. Pru & Jonathan L. Tilly

- Juvenile and adult mouse ovaries possess mitotically active germ cells that, based on rates of oocyte degenetration are needed to replenish the oocyte pool.





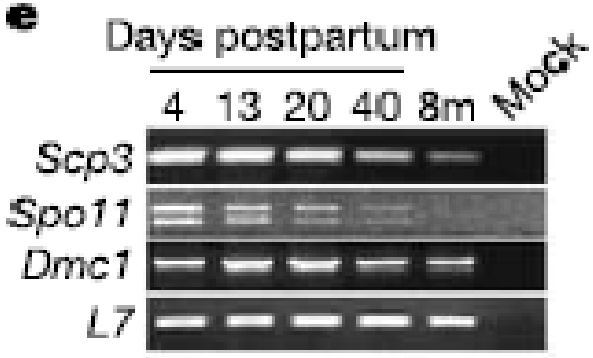
Nature 2004

articles

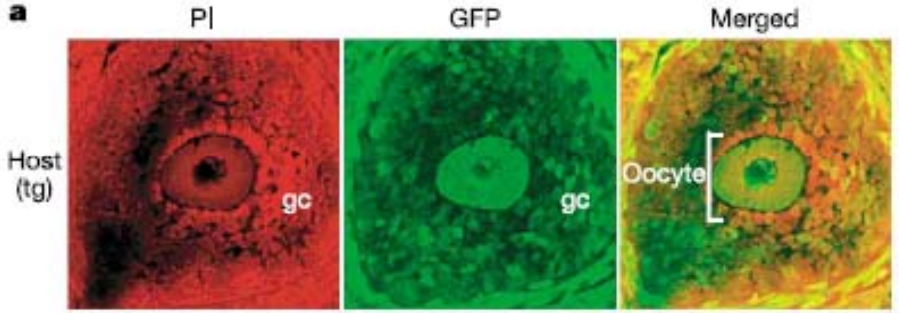
Germline stem cells and follicular renewal in the postnatal mammalian ovary

Joshua Johnson*, Jacqueline Canning*, Tomoko Kaneko, James K. Pru & Jonathan L. Tilly

- Presence of cells expressing meiotic markers in juvenile and adult mouse ovaries



- Wild type ovaries grafted into transgenic mice (GFP) become infiltrated with GFP positive cells that form follicles

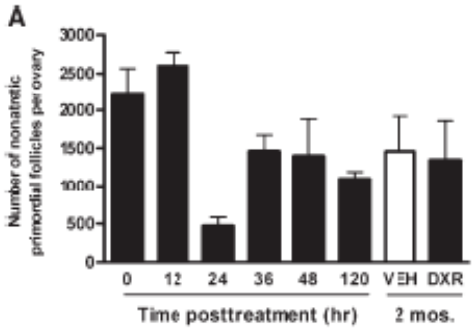


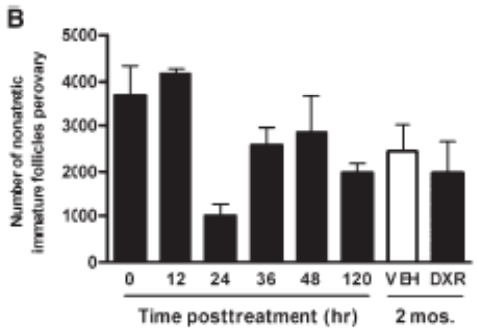
Cell, Vol. 122, 303–315, July 29, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.cell.2005.06.031

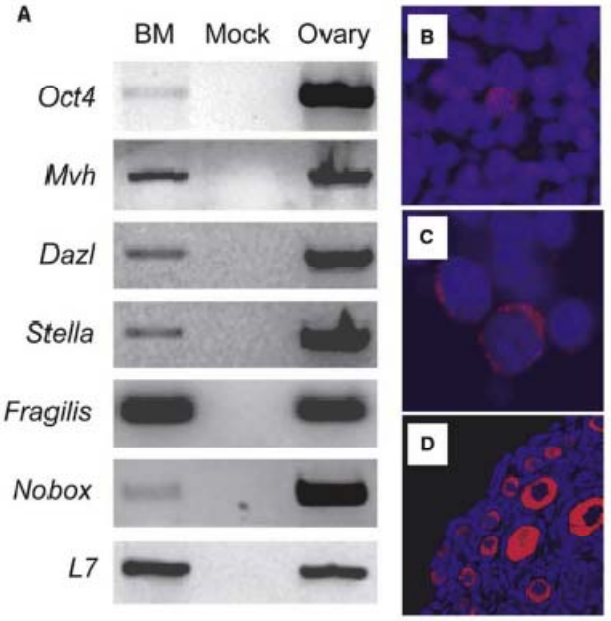
Oocyte Generation in Adult Mammalian Ovaries by Putative Germ Cells in Bone Marrow and Peripheral Blood

2005

- Rapid generation of 100s of oocytes
- Extragonadal source of germ cells?
- Germline markers in BM





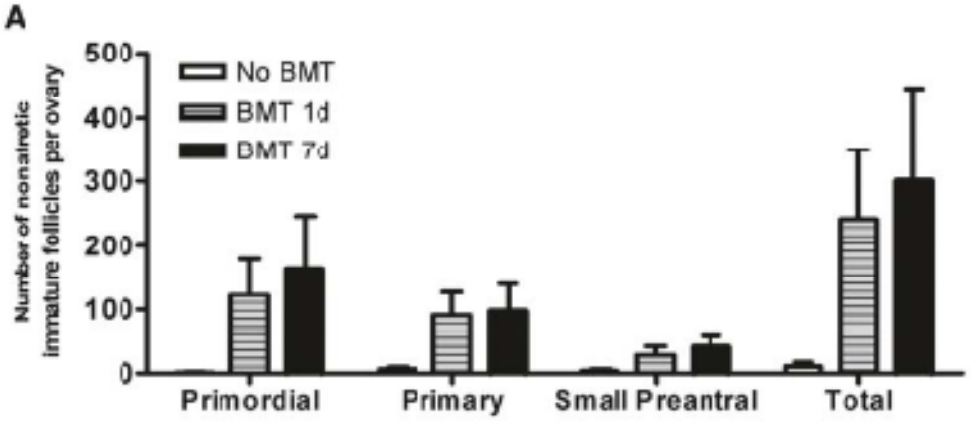


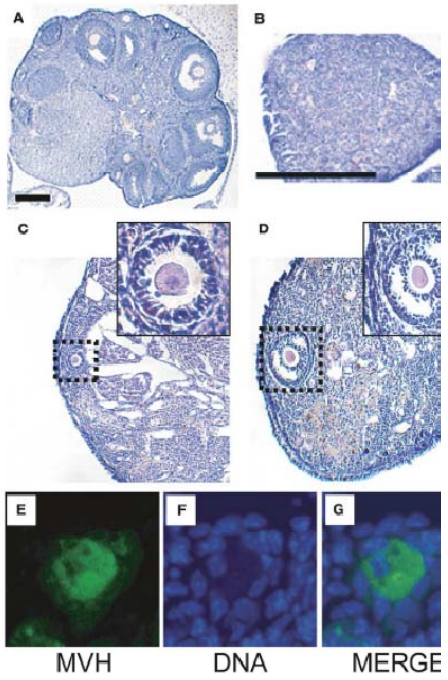
Cell, Vol. 122, 303–315, July 29, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.cell.2005.06.031

Oocyte Generation in Adult Mammalian Ovaries by Putative Germ Cells in Bone Marrow and Peripheral Blood

2005

- BM transplantation restores oocyte production in wild type mice sterilized by chemotherapy
- Donor derived oocytes observed in females following peripheral blood transplantation





BM is a potential source of GC that sustain oocyte production in adulthood

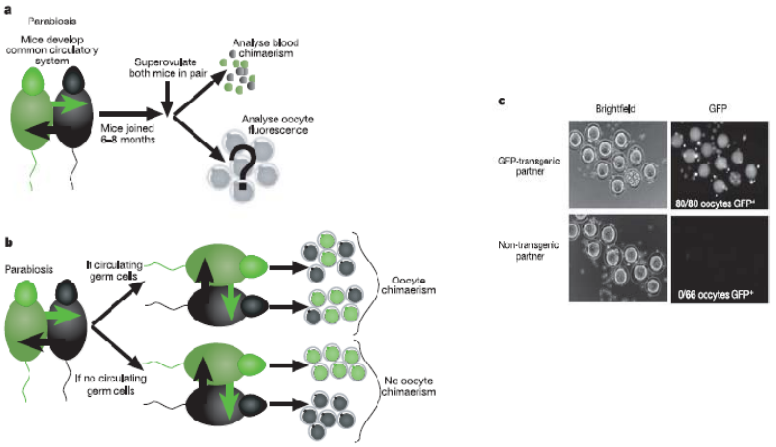
Ovulated oocytes in adult mice derive from non-circulating germ cells

Kevin Eggan[1], Sara Jurga[2], Roger Gosden[3], Irene M. Min[2] & Amy J. Wagers[2]

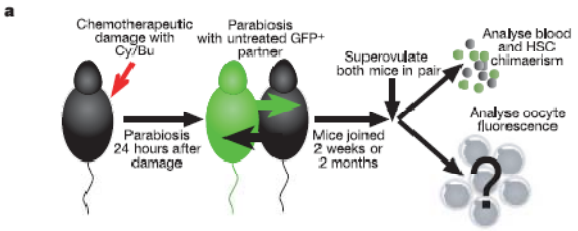
2006

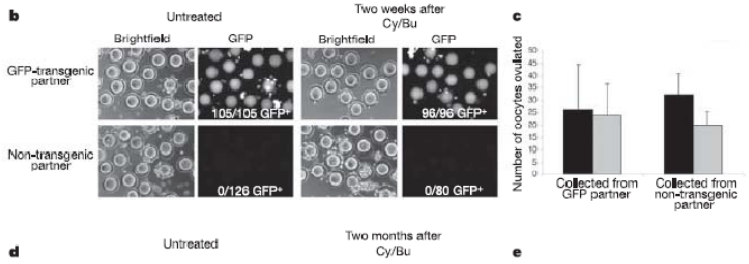
Transplantation and parabiotic mouse model.

Capacity of circulating BM to generate ovulated oocytes?



- Circulating cells do not give rise to mature ocytes in long term parabionts
- Circulating cells do not give rise to mature oocytes in injured parabionts



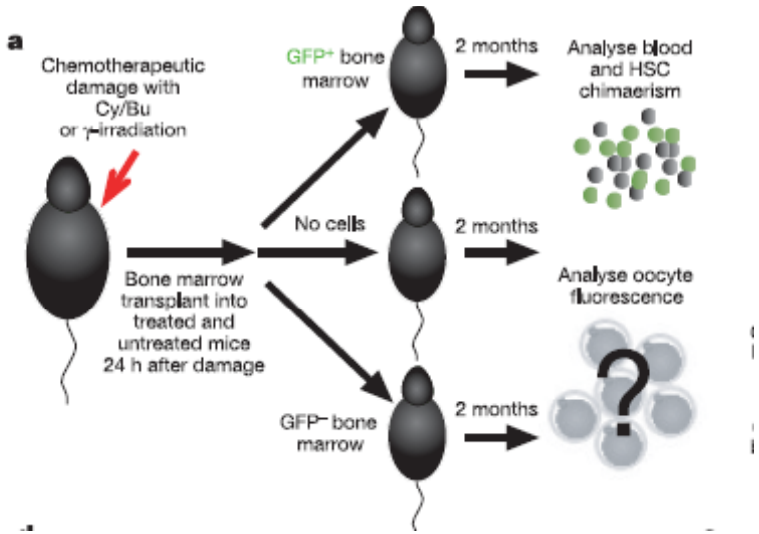


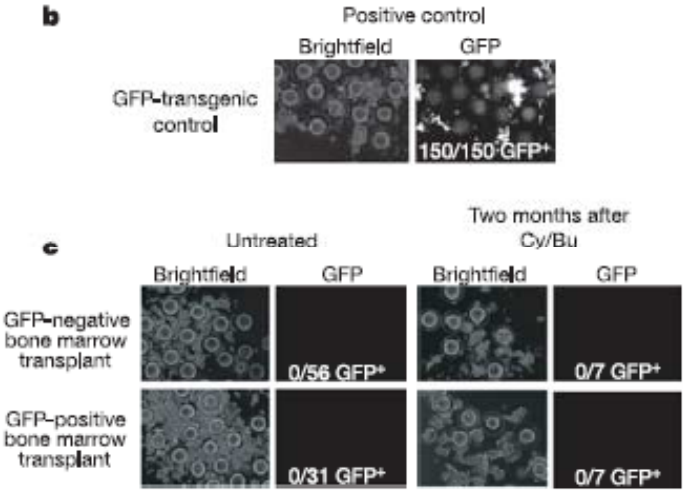
Ovulated oocytes in adult mice derive from non-circulating germ cells

Kevin Eggan[1], Sara Jurga[2], Roger Gosden[3], Irene M. Min[2] & Amy J. Wagers[2]

2006

- Bone marrow cells do not give rise to oocytes and do not enhance ovulation of endogenous oocytes in transplanted mice



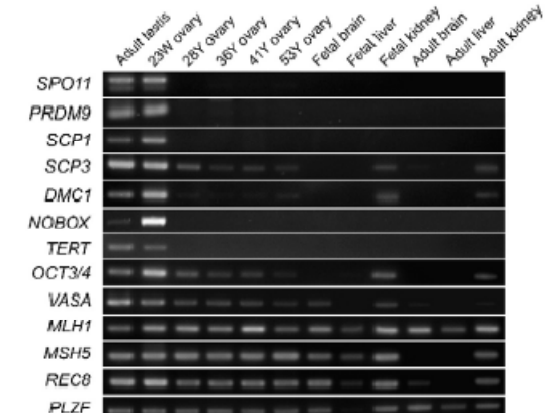


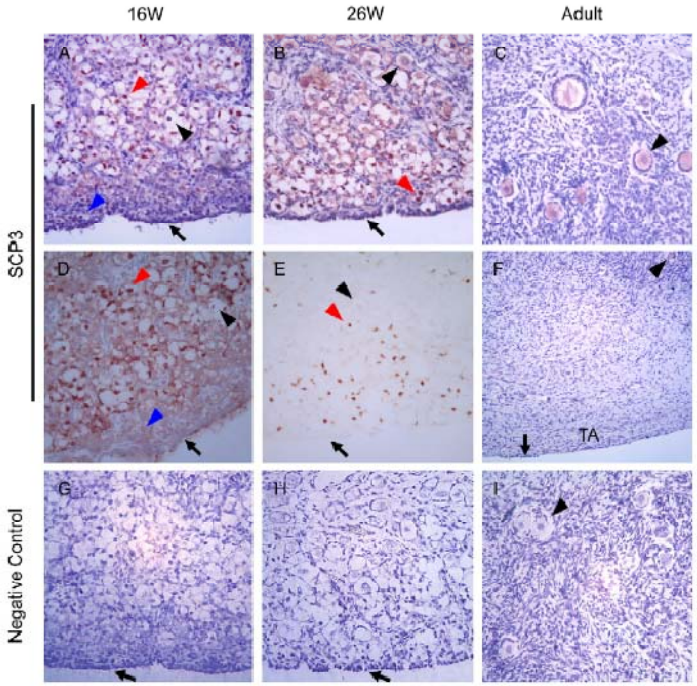
Germline stem cells and neo-oogenesis in the adult human ovary

Yifei Liu [a], Chao Wu [a], Qifeng Lyu [a], Dongzi. Yang [b], David F. Albertini [c], David L. Keefe [d,*], Lin Liu [a,d,*]

2007

Expression of meiotic and GC proliferation markers in adult human ovaries (12 women, 28-53 years)





- No early meiotic specific or oogenesis associated mRNAs exist in normal adult human ovaries
- Absence of early meiocytes and proliferating germ cells

Recovery of Female Fertility After Chemotherapy, Irradiation, and Bone Marrow Allograft: Further Evidence Against Massive Oocyte Regeneration by Bone Marrow-Derived Germline Stem Cells

REINER A. VEITIA,[a,b,c,d,e] ELIANE GLUCKMAN,[f] MARC FELLOUS,[a,b,c,d,e] JEAN SOULIER[g]

2007

Woman who gave birth to a child after allogenic BMT (after 8 years)

- DNA analysis of the mother, child and BM donor
- No relationshionship was established between the child and the donor

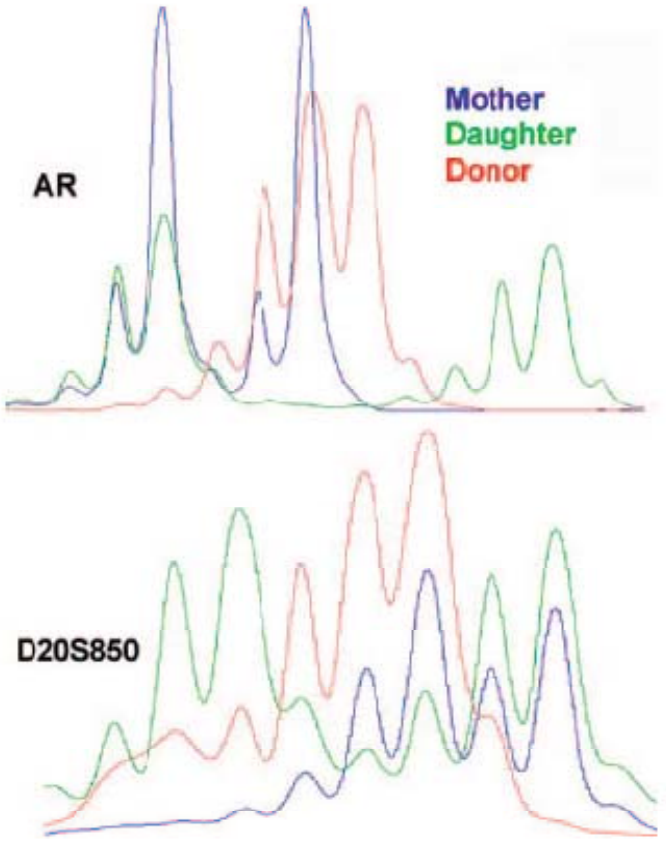


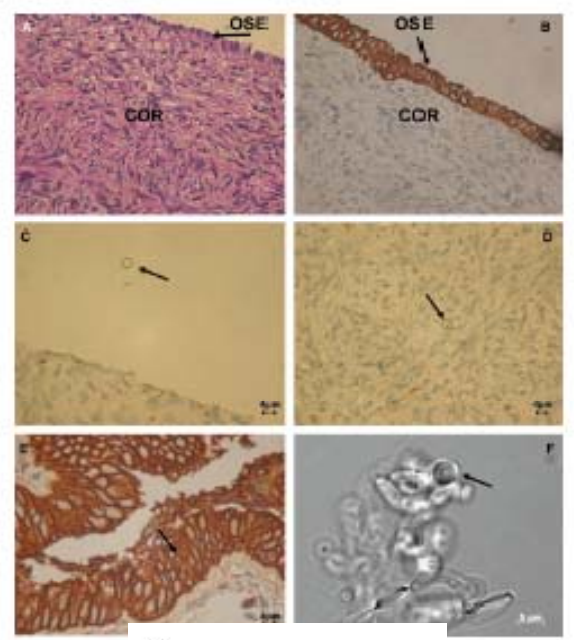
Figure 1. Geretic analysis of the DNA of the mother, the daughter, and

Irma Virant-Klun · Nicolas Zech · Primož Rožman ·
Andrej Vogler · Branko Cvjetičanin · Polona Klemenc ·
Elvira Maličev · Helena Meden-Vrtovec

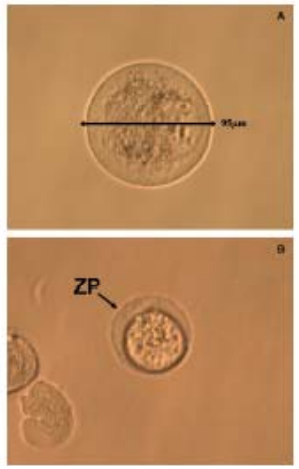
Putative stem cells with an embryonic character isolated from the ovarian surface epithelium of women with no naturally present follicles and oocytes

2008

Isolate putative SC from the ovarian surface epithelium



- Putative ovarian stem cells from the ovarian cell epithelium express early embryonic developmental markers
- They grow in vitro
- Oocyte-like cells develop



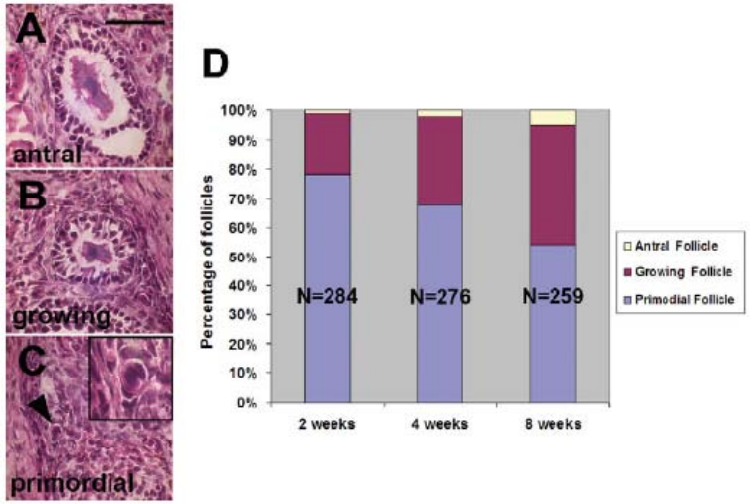
The oocyte population is not renewed in transplanted or irradiated adult ovaries

2008

S. Begum[1], V.E. Papaioannou[1,3] and R.G. Gosden[2]

Ovaries from adult mice transplanted to sterilised transgenic GFP mice

819 oocytes examined in 30 grafts



- No oocytes expressed GFP at 2, 4 and 8 weeks of transplantation
- Growing follicles were survivors of the original population

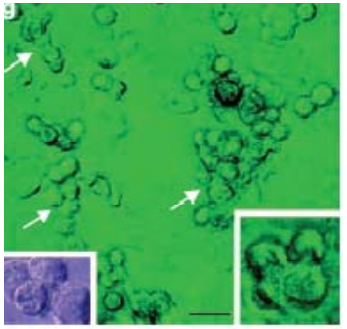
No *de novo* oogenesis from systemic GC

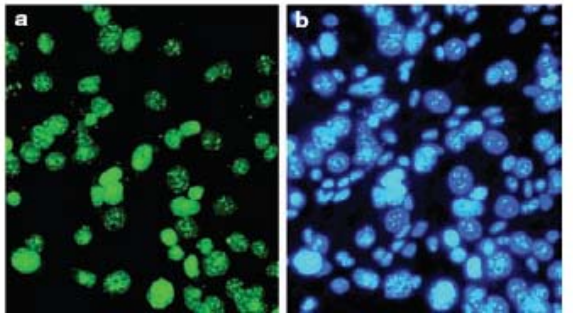
Production of offspring from a germline stem cell line derived from neonatal ovaries

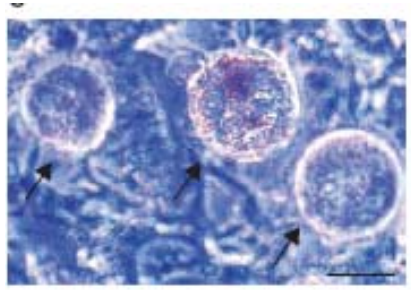
Kang Zou[1], Zhe Yuan[1], Zhaojuan Yang[1], Huacheng Luo[1], Kejing Sun[1], Li Zhou[1], Jie Xiang[1], Lingjun Shi[1], Qingsheng Yu[1], Yong Zhang[1], Ruoyu Hou[1] & Ji Wu[1,2]

2008

- Establishment of a neonatal mouse FGCS line with normal karyotype, high telomerase activity. 15 months in culture ((adult mice for 6 months)
- Similar characteristics of SSC







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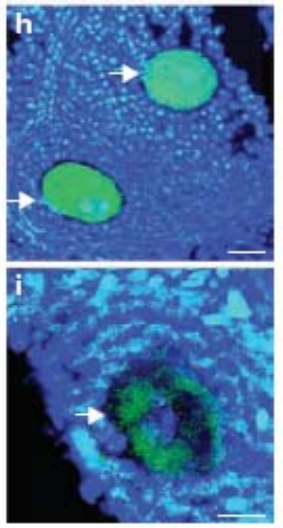
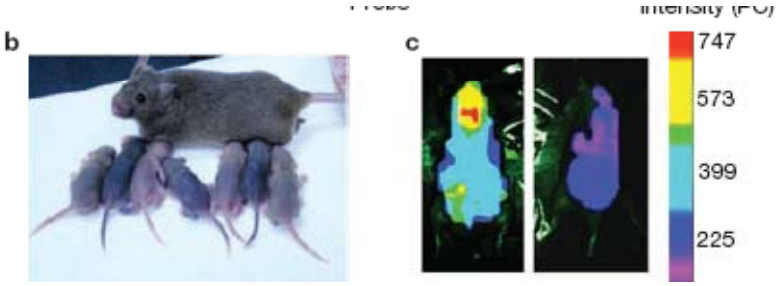
2008

- FGSC infected with GFP and transplanted into ovaries of infertile mice

- Tranplanted cells underwent oogenesis and produced offspring with GFP transgene





- Existence of circulating germ cell progenitors?
- Potential contribution to fertility preservation (*helper follicles* that promote maturation)

- Germline stem cells (ovarian stem cells) may reside in the mammalian ovary
- Role in oogenesis under normal physiological conditions or as a stress response of the ovary

Salud de la mujer Dexeus

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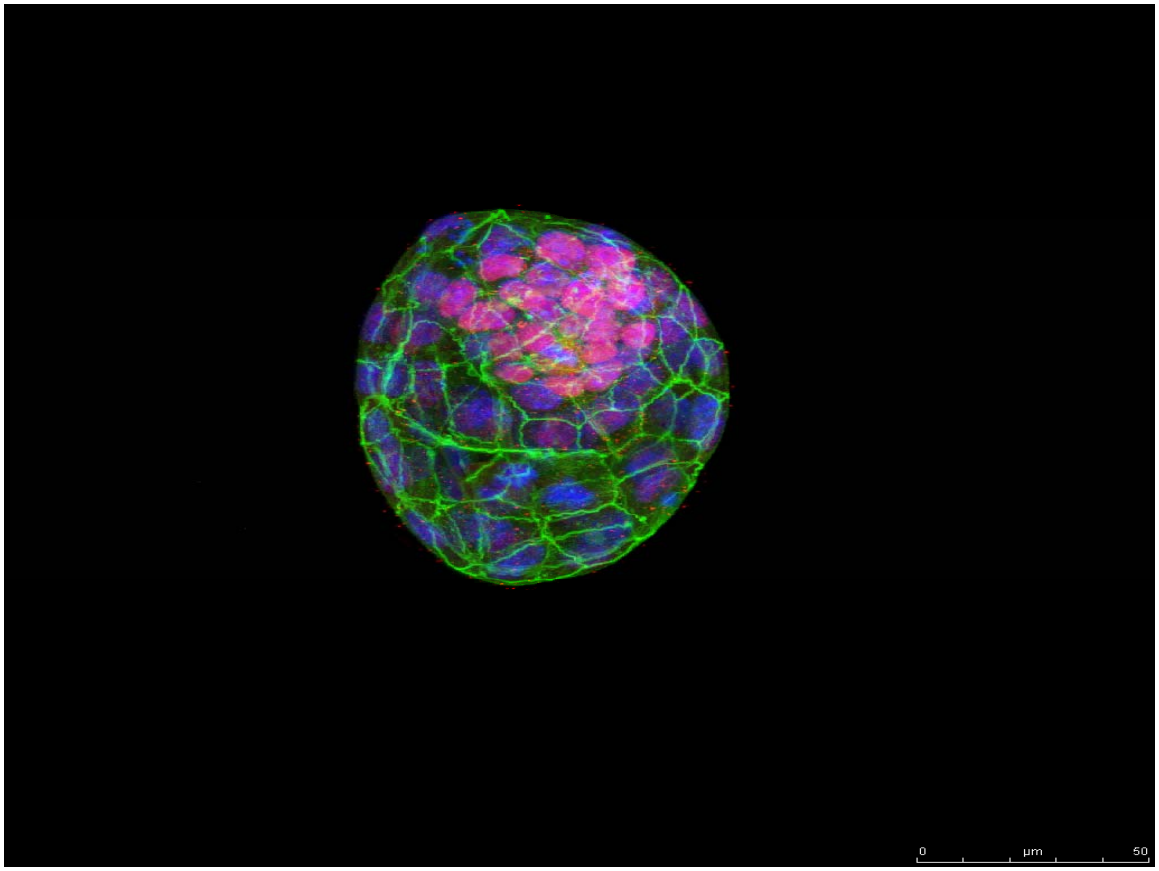

Thank you for your attention

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