Recreating manhood

An update on the generation of artificial gametes

Zsolt Peter NAGY

Reproductive Biology Associates

Why artificial gamete research?

Incidence of infertility

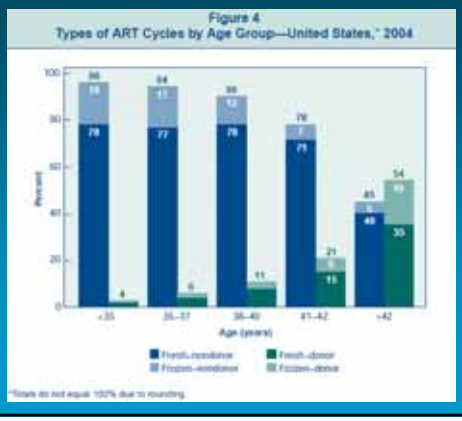
- 15-20% of all couples of reproductive age are infertile
- 30-40% of the infertility due to male factors
- 40-50% of the infertility due to female factors
- 10-15% of the infertility unknown

Why artificial gamete research?

Statistics on ART in US - 2004

Number of ART clinics in the United States in 2004:	461
Number of U.S. ART clinics that submitted data in 2004:	411
Number of ART cycles reported for 2004:	127,977
Number of live-birth deliveries resulting from ART cycles started in 2004:	36,760
Number of live babies born as a result of ART cycles carried out in 2004:	49,458

Why artificial gamete research?



Why artificial gamete research?

Age of Women and Pregnancy rate

PGD/AS – FISH; Diagnostic and not treatment procedure

Why artificial gamete research?

Background, Infertility Treatment Needs

- IVF treatment is increasingly efficient for different indications resulted in the birth of about 2 million children.
- There is a clear need for improvement in cases when no gametes present or gametes are deficient – i.e. ARA, other indications.
- Micromanipulation techniques are evolving:
  - ICSI, nuclear transfer (NT; cloning).
- Stem cell technology advancing:
  - Culture conditions; specific tissue differentiation
- Understanding on Molecular biology of cell cycle, epigenetic effects progressing

How to do ?

Potential Methods to Generate Artificial Gametes:

I. Nuclear transfer

II. Stem cell differentiation

a. Adult stem cells

b. Embryonic stem cells

III. Somatic cells → Stem cells → Gametes

IV. Somatic cells (2n, mitosis) → Haploid cells (1n, meiosis)

How to do ?

I. Nuclear Transfer

What system?

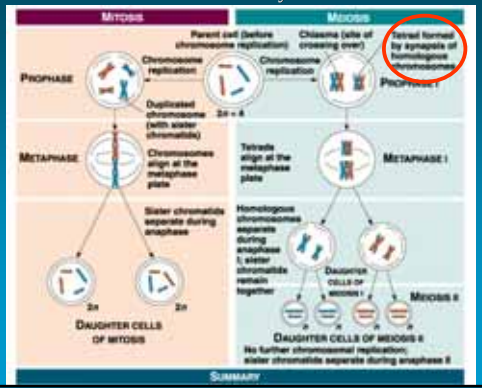
1. Providing sufficient resources for completing meiosis
   - e.g. Spindle/microtubule machinery
2. Easy of manipulation → size: large enough

Oocyte

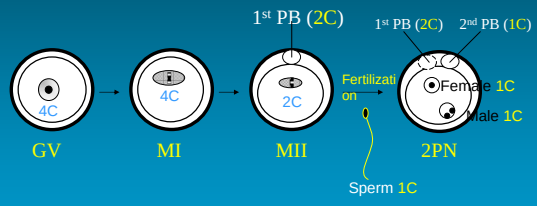
Theoretical considerations

NT

Mitosis and Meiosis – two distinct ways of cell division



Resumption of oocyte meiosis



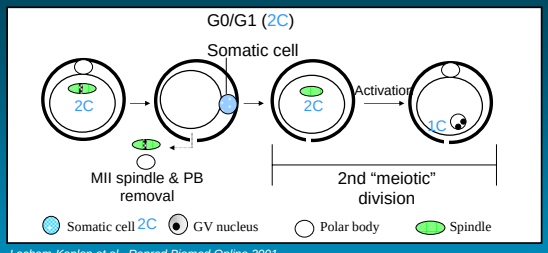
Oocyte cytoplasm retains factors contributing to chromosome reduction.

Theoretical considerations

Potential for generating gametes from somatic cells and growing oocytes

Kimura and Yanagimachi (1995)	**M2 egg + s. spermatocyte**
Ogura et al. (1998)	**GV egg + p. spermatocyte**
Wakayama and Yanagimachi; 1998	**M2 egg + polar body**
Tesarik, Palermo, Chang	**M2 egg + somatic cell - ?**

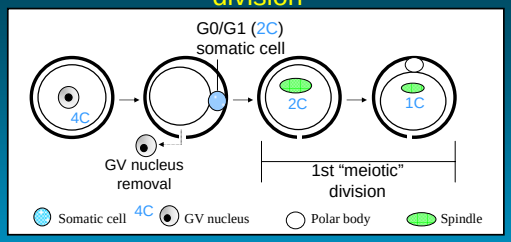
Strategy I : 2 C cell divided by the 2nd meiotic division



Lacham-Kaplan et al., Reprod Biomed Online 2001
Tesarik et al., Reprod Biomed Online 2001
Tateno et al., Fertil Steril 2003
Chen et al., Hum Reprod. 2004
Heindryckx et al., Hum Reprod. 2004
Galat et al., Reprod Biomed Online 2005

Problem: Chromosome separation

Strategy II : 2 C cell divided by the 1st meiotic division



Kubelka and Moor, 1997. Zygote.
Palermo et al., 2002. Reprod Biomed Online.
Fulka et al., 2002. Hum Reprod.
Polanski et al., 2005. Dev Biol.

Problems: Failure to undergo the first meiotic division
& Chromosome separation

NT

NT

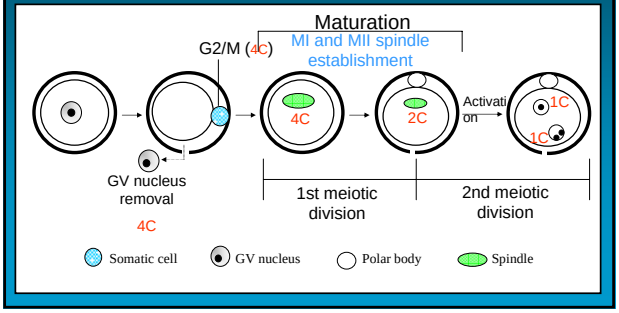
Project 1:

Nuclear and microtubule dynamics of G2/M somatic nuclei during haploidization in GV-stage mouse oocytes

NT

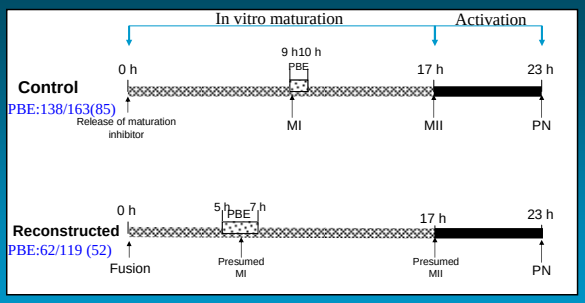
Design

Strategy: 4 C cell divided by 1st and 2nd meiotic divisions



Results

NT



√ *Nuclear status*

√ *Microtubule dynamics*

Results

NT

Fusion efficiency and polar body extrusion rate of control and reconstructed oocytes during in vitro maturation

	Reconstructed oocytes	Control oocytes
No. (%) fused oocytes	150/262 (57.3)	–
No. (%) extruded first PB (17 h IVM)	62/119 (52.1)[a]	138/163 (84.7)[b]
No. (%) oocytes with large-size PB	21/62 (33.9)[a]	5/138 (3.6)[b]
No. (%) oocytes with normal-size PB	41/62 (66.1)[a]	133/138 (96.4)[b]

[b] Values within the same rows with different supercripts differ ($P < 0.05$, χ^2 test).

Chang *et al.*, 2004. *Bio Reprod*

Results

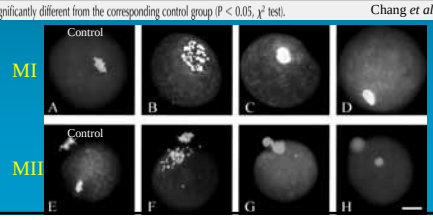
NT

The nuclear morphology of control and reconstructed oocytes during in vitro maturation

Oocytes (h of maturation)	PB extrusion	Expected cell cycle stage	No. of oocytes examined	Nuclear morphology: Condensed chromosomes (%)	Nuclear morphology: Uneven distribution of chromatin (%)	Nuclear morphology: Interphase-like nucleus (%)
Control (9 h)	–	MI	21	21 (100)	0	0
Control (17 h)	+	MII	69	69 (100)	0	0
	–	MI arrest	8	8 (100)	0	0
Reconstructed (4–6 h)	–	MI	31	12 (38.7)[a]	7 (22.6)	12 (38.7)
Reconstructed (17 h)	+	MII	29	16 (55.2)[a]	11 (37.9)	2 (6.9)
	–	MI arrest	30	12 (40.0)[a]	9 (30.0)	9 (30.0)

[a] Significantly different from the corresponding control group ($P < 0.05$, χ^2 test).

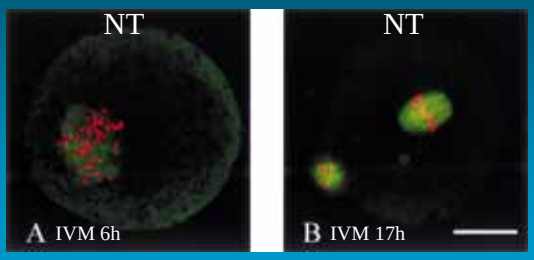
Chang *et al.*, 2004. *Bio Reprod*



Results

NT

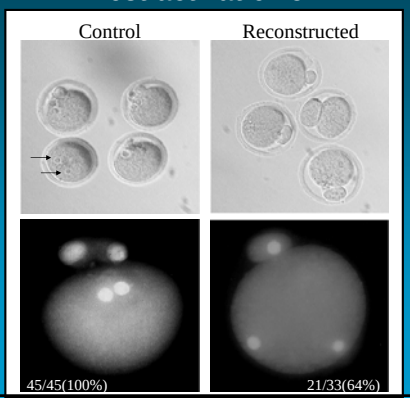
Microtubule Dynamics



Results

NT

Post-activation 6h



Summary

NT

- Cytokinesis of reconstructed oocytes was occurred, as evidenced by PB extrusion.
- The reconstructed oocytes can establish the first and second meiotic spindle for separating a G2/M (4C) somatic nucleus, but misalignment of somatic chromosomes was observed in all occasions.
- After activation, the pronuclear membrane was absent in the reconstructed oocytes, suggesting that the GV materials are important for the formation of the pronuclear membrane.

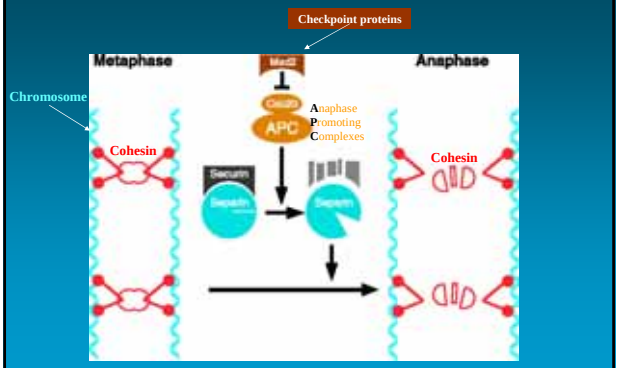
NT

Project 2:

Interactions of the meiotic spindle with mitotic chromosomes in GV mouse oocytes

NT

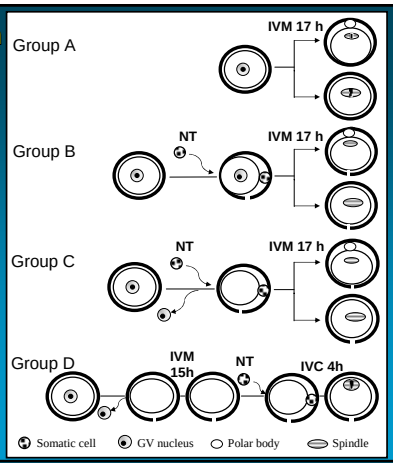
Metaphase–anaphase transition (Mitosis)



Nasmyth et al., (2000) Science

NT

Design



Results

NT

Meiotic spindle formation and chromosome alignment in GV oocytes

Group	Fusion rate (%)	PB extrusion	Presumed cell cycle stage	No. of oocytes examined	Bipolar spindle formation (%)	Complete chromosome alignment (%)
A	–	+	MII	94	93 (98.9)	90 (95.7)
		–	MI-arrest	14	13 (92.9)	12 (85.7)
B	61/92 (66.3)	+	MII	56	53 (94.6)	0 (0) *
		–	MI-arrest	5	5 (100)	0 (0) *
C	67/111 (60.4)	+	MII	48	31 (64.6) *	0 (0) *
		–	MI-arrest	19	10 (52.6) *	0 (0) *
D	14/25 (56.0)	N/A	MII	14	8 (57.1) *	6 (42.9) *

*Significantly different from the corresponding control (Group A) ($P<0.05$, $\chi 2$ test)

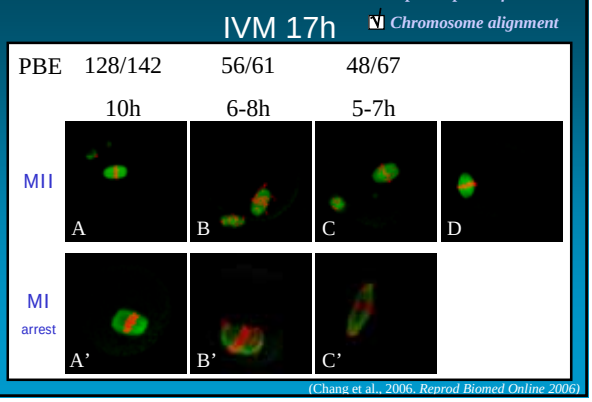
(Chang et al., 2006. *Reprod Biomed Online 2006*)

Results

NT

√ *Bipolar spindle formation*

√ *Chromosome alignment*



(Chang et al., 2006. *Reprod Biomed Online 2006*)

Summary

- The first meiotic division was not blocked nor delayed when mitotic chromosomes were transferred into a maturing ooplast.
- Our data suggests that a spindle checkpoint, providing surveillance of misaligned chromosomes, may be missing, or not fully functional during oocyte maturation in mammals.

Summary

1, Haploidization provides limited success

- Chromosome reduction — Yes
- Spindle formation — Yes
- Correct chr. segregation — No

2, Haploidization technique requires further adjustment

3, Alternative ways to artificial gamete: Stem Cell

How to do ?

II. Stem Cell Differentiation

a. Adult Stem Cells- ?

Derivation of male germ cells from bone marrow stem cells

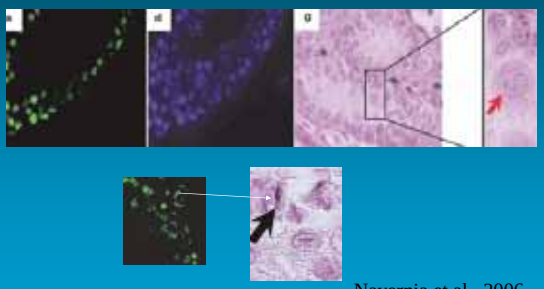
Karim Nayernia[1], Jae Ho Lee[2], Nadja Drusenheimer[1], Jessica Nolte[1], Gerald Wulf[3], Ralf Dressel[3], Jörg Gromoll[4] and Wolfgang Engel[1]

[1]Institute of Human Genetics, University of Göttingen, Göttingen, Germany; [2]Department of Haematology and Oncology; [3]Department of Cellular and Molecular Immunology, University of Göttingen, Göttingen, Germany and [4]Institute of Reproductive Medicine, University of Münster, Münster, Germany

Bone marrow stem cells isolated from Stra8-EGFP transgenic mice

EGFP Positive colonies observed in vitro after the treatment with retinoic acid

In vivo bone marrow stem cells differentiation after transplantation into mouse testis

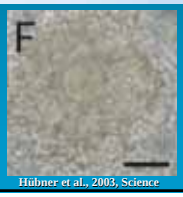


Nayernia et al., 2006

b. Embryonic Stem Cells-

How to do ?

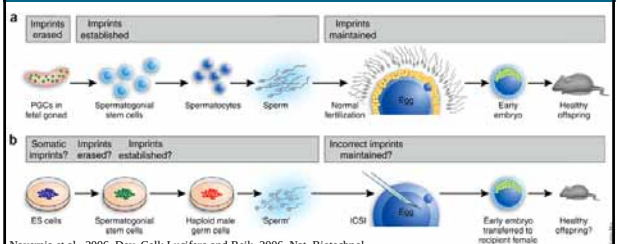
Toyooka,2003, PNAS; Geijsen 2004, Nature



Hübner et al., 2003, Science

How to do ?

b. Embryonic Stem Cells→ Gametes -Live birth



Nayernia et al., 2006. Dev. Cell; Lucifero and Reik, 2006. Nat. Biotechnol.

How to do ?

b. Embryonic Stem Cells→ Gametes -Live birth

Nayernia et al., 2006. Dev. Cell

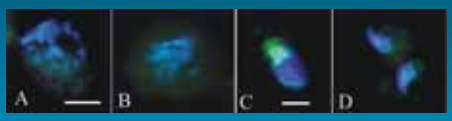
ES cells with XY chromosomes can produce under the same experimental conditions both types of presumptive gametes

Kerkis et al., 2007

Spermatogenesis from ES cells

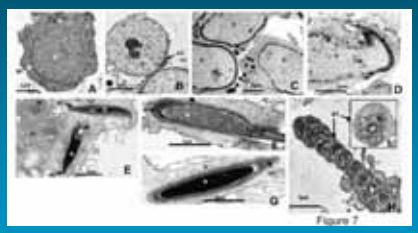
Kerkis et al., 2007

Male GC differentiation.



Kerkis et al., 2007

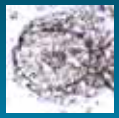
Transmission electron microscopy of "sperm cells" obtained in vitro



Kerkis et al., 2007

Female GC differentiation from mouse ES cells.

Ovary-like structure



Single oocyte surrounded by granulose cells

Single oocyte, Hemoxylin-eosin (HE)

Blastocyst – like structure

Kerkis et al., 2007

III. Somatic cells → Stem cells → Gametes

a. Combining NT and Stem cell differentiation

How to do ?

WHEN ?

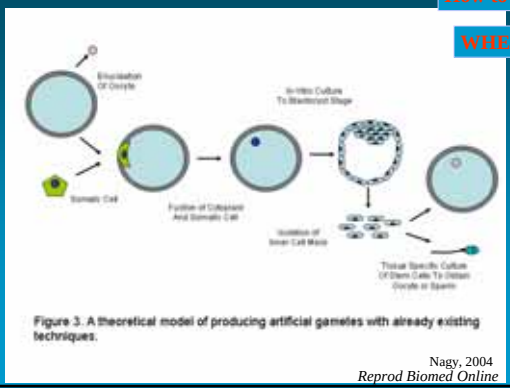
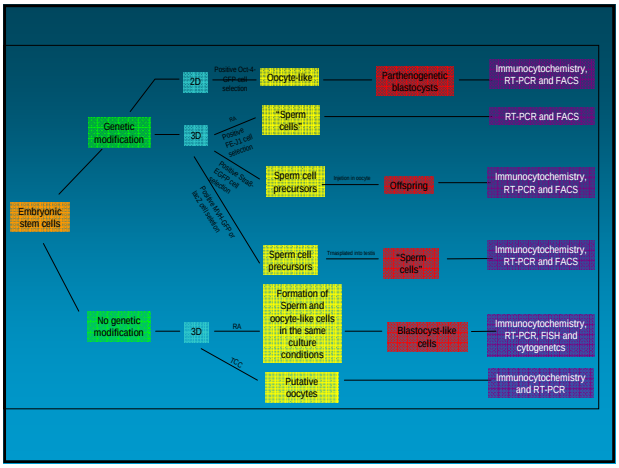


Figure 3. A theoretical model of producing artificial gametes with already existing techniques.

Nagy, 2004
Reprod Biomed Online



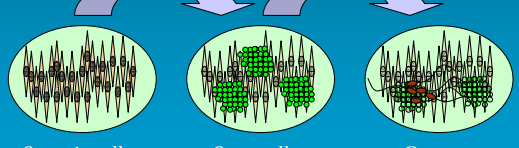
How to do ?

III. Somatic cells → Stem cells → Gametes

b. Combining somatic cell reprogramming and stem cell differentiation

In vitro reprogramming: *In vitro differentiation:*

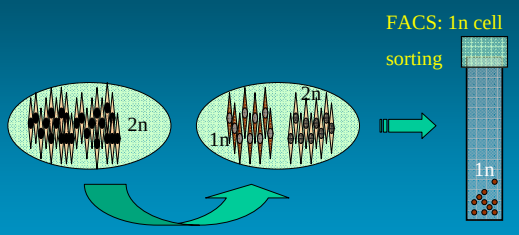
Reprogramming factors *Stem cells*



Somatic cells Stem cells Gametes

How to do ?

IV. Somatic cells (mitosis) → Haploid cells (meiosis)



Converting mitosis into meiosis by:

Adding “meiosis factors”

SUMMARY

- There is a clear clinical need for the production of artificial gametes in IVF
- Current approaches of haploidization do not work; or it provides extreme low efficiency
- Using oocyte/cytoplast machinery for somatic cell haploidization is associated with a high level of spindle and cytogenetic defects
- Alternative approaches, through stem cell or in-vitro meiosis may be investigated in the future; biological safety (epigenetic alterations); ethical concerns need to be addressed

Acknowledgements

Jeremy Chang

Advisory Committee

Dr. Yang

Dr. Tian

Dr. Riesen

Dr. Rasmussen

Dr. Zhang

Dr. Nagy

Clínica e Centro de Pesquisa em Reprodução Humana Roger Abdelmassih, São Paulo, Brasil

Dr. Roger Abdelmassih

University of Connecticut

Marina Julian

Jilong

Dr. Suzuki

Dr. Michele Barber

All lab members