MAKING GAMETES AND HELPING EMBRYOS

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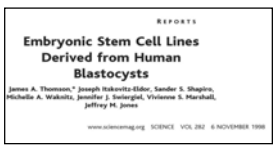
Barcelona. Spain

1.Making gametes
- Embryonic stem cells (ESC).
- Derivation and differentiation
- Gamete differentiation from ESC
  - Derivation of oocytes
  - Derivation of sperm
- Derivation of oocytes from fetal stem cells
- BM stem cells as a source of gametes?

2.Helping embryos
- Assisted hatching
- PGS


REPORTS

Embryonic Stem Cell Lines Derived from Human Blastocysts

James A. Thomson,* Joseph Itskovitz-Eldor, Sander S. Shapiro, Michelle A. Waknitz, Jennifer J. Swiergiel, Vivienne S. Marshall, Jeffrey M. Jones

www.sciencemag.org SCIENCE VOL 282 6 NOVEMBER 1998

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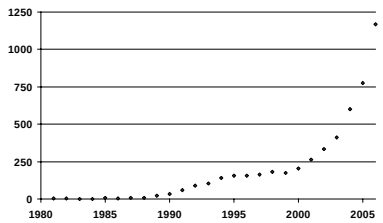
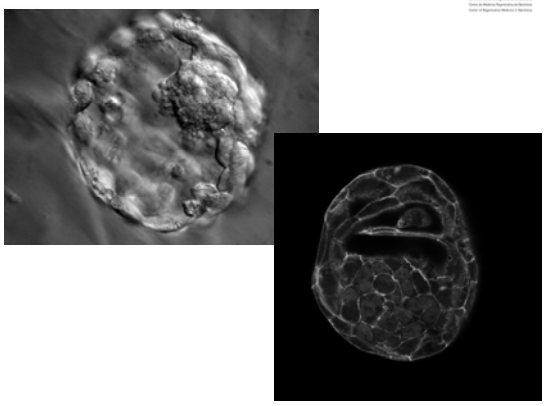
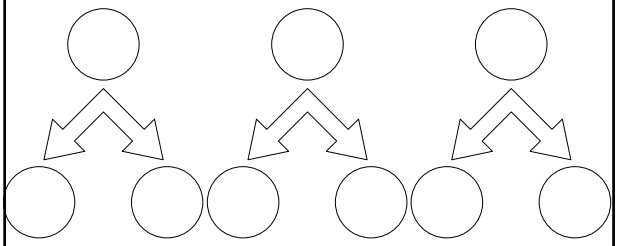




Embryonic Stem Cells: Self Renewal and Differentiation



Asymmetric division | Self-renewal | Differentiation

Differentiation

ADIPOCITO	Dani et al., 1997
ASTROCITO	Fraichard et al., 1995
CARDIOMIOCITO	Doetschman et al., 1985 Maltsev et al., 1993
CONDROCITO	Kramer et al., 2000
HEMATOPOYÉTICAS DEFINITIVAS	Nakano et al., 1996 Nishikawa et al., 1996 Wiles et al., 1991
CÉLULAS DENDRÍTICAS	Fairchild et al., 2000
CÉLULAS ENDOTELIALES	Risau et al., 1988 Yamashita et al., 2000
KERATINOCITOS	Bagutti et al., 1996 Yamashita et al., 2000
PRECURSORES DE LINFOCITOS	Potocnik et al., 1994
MASTOCITOS	Tsai et al., 2000
NEURONAS	Bain et al., 1995 Strubing et al., 1995
OLIGODENDROCITOS	Brustle et al., 1999 Liu et al., 2000
OSTEOBLASTOS	Buttery et al., 2001
ISLOTES PANCREÁTICOS	Lumelski et al., 2001
HEMATOPOYÉTICAS PRIMITIVAS	Doetschman et al., 1985 Nakano et al., 1996
MÚSCULO LISO	Yamashita et al., 2000
MÚSCULO ESTRIADO	Rohwedel et al., 1994
ENDODERMO DEL SACO VITELINO	Doetschman et al., 1985
MESODERMO DEL SACO VITELINO	Doetschman et al., 1985

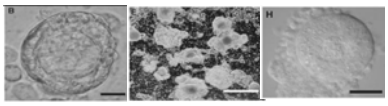
GERM CELLS - GAMETES

Derivation of Oocytes from Mouse Embryonic Stem Cells

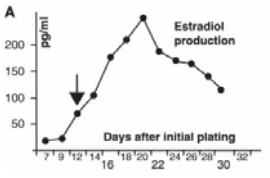
Karin Hübner,[1] Guy Fuhrmann,[3] Lane K. Christenson,[4] James Kehler,[1] Rolland Reinbold,[1] Rabindranath De La Fuente,[2] Jennifer Wood,[4] Jerome F. Strauss III,[4] Michele Boiani,[1] Hans R. Schöler[1]*

www.sciencemag.org SCIENCE VOL 300 23 MAY 2003

Formation of follicular structures



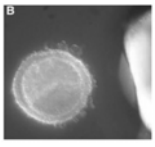
After 2 weeks in culture, structures similar to advanced follicle-like structures appear. Most of them degenerate but around 20% give rise to "oocytes"> 40 µm.



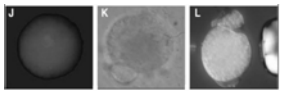
- The functional activity of the follicles is evidenced by estradiol production

Oocyte characterisation

- At D26 of culture, oocyte-like cells (50-70 µm) are released from surrounding somatic cells and float freely in the supernatant.



- All ZP markers are + except ZP1 (accounting for fragile ZP structure)



Addition of gonadotrophins to isolated follicles results in extrusion of oocytes and formation of a polar body-like structure

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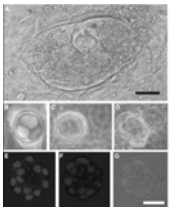
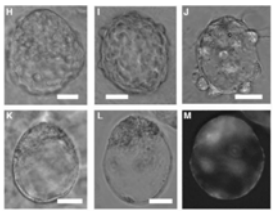
CMR[B]

Blastocyst-like structures derived from ES cells



- At about day 43, structures similar (morphology and markers) to preimplantation embryos are found.



These blastocyst-like structures represent parthenotes (probably) induced by culture conditions

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CMR[B]

Differentiation (2007) 75:902–911 DOI: 10.1111/j.1432-0436.2007.00181.x

ORIGINAL ARTICLE

Tingting Qing · Yan Shi · Han Qin · Xin Ye · Wei Wei · Haisong Liu · Mingxiao Ding · Hongkui Deng

Induction of oocyte-like cells from mouse embryonic stem cells by co-culture with ovarian granulosa cells

Our results demonstrate that granulosa cells were effective in inducing the differentiation of ES cell-derived PGCs into oocyte-like cells through direct cell-to-cell contacts. Our method offers a novel *in vitro* system for studying oogenesis; in particular, for studying the interactions between PGCs and granulosa cells.

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CMR[B]

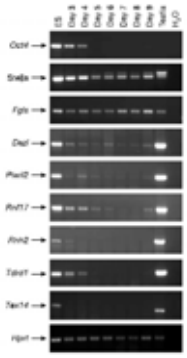
Derivation of embryonic germ cells and male gametes from embryonic stem cells

Nieis Geijsen[1,2], Melissa Horoschak[1,3], Kitai Kim[1,3], Joost Gribnau[1], Kevin Eggan[4] & George Q. Daley[1,3]

[1]*Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, Massachusetts 02142, USA*
[2]*Center for Regenerative Medicine and Technology, Massachusetts General Hospital, Boston, Massachusetts 02114, USA*
[3]*Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, and Division of Pediatric Hematology/Oncology, The Children's Hospital and Dana Farber Cancer Institute, Boston, Massachusetts 02115, USA*
[4]*Department of Molecular and Cellular Biology, Harvard University, 7 Divinity Avenue, Cambridge, Massachusetts 02138, USA*

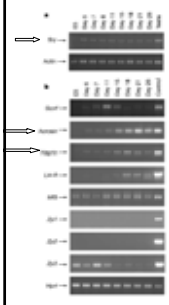
NATURE | VOL 427 | 8 JANUARY 2004 | www.nature.com/nature

- Culture conditions: Embryoid body differentiation
- Marker expression
- pluripotency (Oct4).
- GC development markers (*Stella ,Fragilis)*
- Genes exclusively expressed in GC line (*Dazl, Piwil2, Rnf17, Rnh2, Tdrd1 y Tex14)*



During embroid body formation pluripotency markers expression decreased with some expression of GC specific genes

PGC differentiation to functional gametes

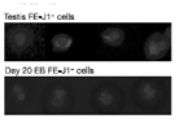


- Upregulation of genes associated with male germ cell development (Sry, Acrosin, Haprin, markers of Sertoli cells)
- No expression of Zp proteins.
- Within the context of EB differentiation, the default programm of female gametogenesis is suppressed.

Meiosis

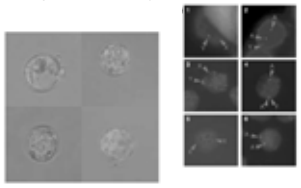
EB microenvironment is permissive for male germ cell development and meiotic maturation, even though highly inefficient

Sperm morphology



EB derived cells have a similar morphology to testis derived haploid cells. Round spermatids?

Biological functionality



- ICSI with haploid cells into recipient oocytes
- 50% of the embryos cleaved to 2 cell stage
- 20% reach the blastocyst stage.
- FISH: normal for sex chromosomes

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CMR[B]

Embryonic stem cells can form germ cells *in vitro*

Yayoi Toyooka, Naoki Tsunekawa, Ryuko Akasu, and Toshiaki Noce*

Mitsubishi Kagaku Institute of Life Sciences, 11 Minamiooya Machida-shi, Tokyo 194-8511, Japan

PNAS | September 30, 2003 | vol. 100 | no. 20 | 11459

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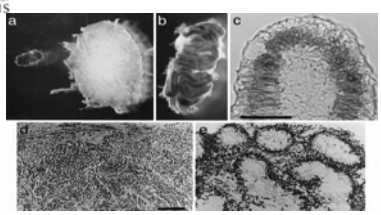
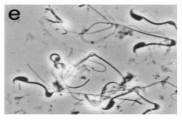


- Germ cell in vivo production from mESC.
- Transplantation of ESC aggregates to testicular capsule.
- ESC derived cells can participate in spermatogenesis and produce functional germ cells *in vitro*



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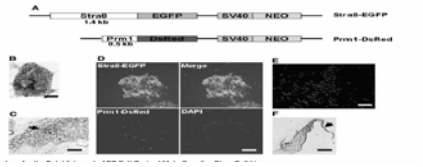
CMR[B]

In Vitro-Differentiated Embryonic Stem Cells Give Rise to Male Gametes that Can Generate Offspring Mice

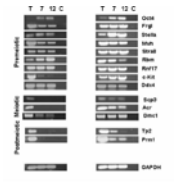
Short Article

Nayernia et al, Developmental Cell 11, 125-132, July 2006

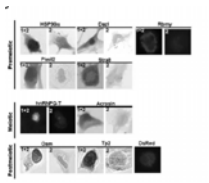


- Establishment of two cell lines (Spermatogonial stem cells- SSCs) with patterns of differentiation towards male germ cells .
- Derived from mESC (directed gene expression for premeiotic and haploid male germ cells)
- Motility of cells
- Formation of tail-like structures

- Positive for PGCs, premeiotic (Oct 4, Fragilis, Stella,..),, meiotic (Scp3, Acr) and postmeiotic male germ cells markers



- Formation of sperm structures.
- Acrosome like structures
- Condensation of the nucleus



- Disruption of methylation imprints?
- ICSI of in vitro-generated cells (haploid cells) into oocytes of wildtype females.
- Polar body extrusion, PN formation and normal features of embryo development.
- Transfer into the oviducts of pseudopregnant females

- 65 embryos transferred
- 12 animals born.
- Larger or smaller offspring
- Premature death (5 days to 5 months after birth).
- Abnormal methylation patterns and phenotypic abnormalities

⇨ **interference with the essential reprogramming events in gametogenesis**

CLONING AND STEM CELLS
Volume 9, Number 4, 2007
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DOI: 10.1089/clo.2007.0031

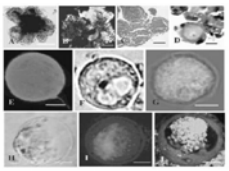
In Vitro Differentiation of Male Mouse Embryonic Stem Cells into Both Presumptive Sperm Cells and Oocytes

ALEXANDRE KERKIS,[1] SIMONE A.S. FONSECA,[1*] RUI C. SERAFIM,[1] THAIS M.C. LAVAGNOLLI,[1,2] SORAYA ABDELMASSIH,[1] ROGER ABDELMASSIH,[1] and IRINA KERKIS[2*]

Spermatogenesis: ES cells + "hanging drop" + EB with RA, between 9 and 11 days:
Migration of presumptive PGC (Fig. 1) from EB in culture medium, formation of aggregates composed by different cell types, such as spermatogonia, spermatocytes, spermatids, sperm cells (Figs. 3, 4, and 6).
Differentiation was confirmed by immunofluorescent analysis with GC specific antibodies (Fig. 3), RT-PCR analyses (Figs. 2 and 5) and DAPI (DNA binding dye) staining (Fig. 3, 4, and 6). FISH analysis indicate meiosis event (Fig. 8).

Oogenesis: ES cells + "hanging drop" + EB with RA and floating EB for 10–15 days. Total time of differentiation, approx. 25 days.
After 9–11 days floating EB start to form aggregates, which underwent differentiation within 10–15 days into follicular-like structures (∅ 37–134 μm), presumptive GV, oocytes, and blastocysts.
Differentiation was confirmed by morphological, immunofluorescent, histological (Fig. 7) and RT-PCR (Figs. 2 and 5) analysis and DAPI staining (Fig. 7).

Female GC differentiation



Male GC differentiation

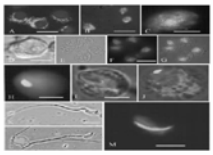
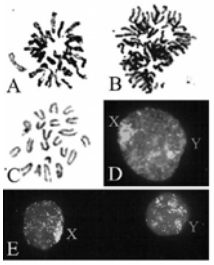


FIG. 3

Reduction in chromosome number



Kerkis et al, 2008

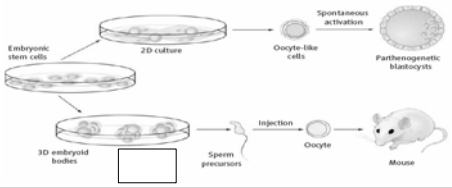
REVIEW

Gametes from Embryonic Stem Cells: A Cup Half Empty or Half Full?

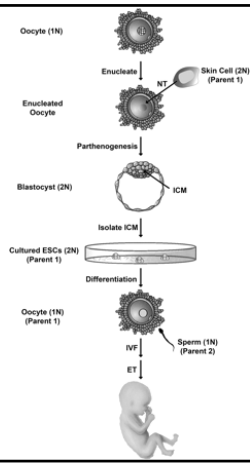
George Q. Daley

When first reported 4 years ago, gametogenesis from embryonic stem (ES) cells promised an accessible in vitro model to facilitate molecular analysis of the germ lineage. Formation of primordial germ cells is robust, but terminal gametogenesis remains inefficient and doubts about gamete function persist. Although useful for research, clinical use of ES cell–derived gametes appears a distant prospect.

Science,2007



Customised gamete derivation by Nuclear Transfer



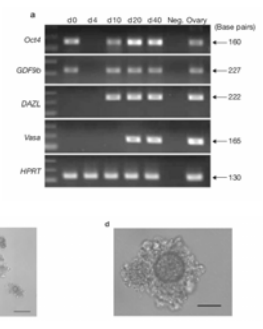
nature cell biology

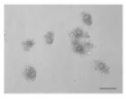
2006

In vitro germline potential of stem cells derived from fetal porcine skin

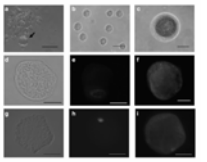
Paul W. Dyce[1], Lihua Wen and Jiuhang Li[1,2]

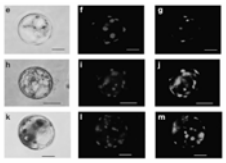
- Stem cells isolated from the skin of porcine fetuses have the intrinsic ability to differentiate into oocyte-like cells.
- When differentiation was induced, a subpopulation of these cells expressed markers (Oct4, GDF9b,*DAZL* and Vasa), consistent with germ-cell formation.
- On further differentiation, these cells formed follicle-like aggregates that secreted oestradiol and progesterone a responded to gonadotropin stimulation.

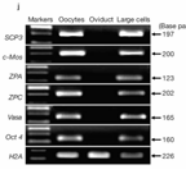




- Some of these aggregates extruded large oocyte-like cells that expressed oocyte markers, such as ZP, and meiosis markers,(SCP3).
- Some of these oocyte-like cells spontaneously developed into parthenogenetic embryo-like structures.







Laboratory Investigation (2006) 86, 654–663

Derivation of male germ cells from bone marrow stem cells

Karim Nayernia[1], Jae Ho Lee[1], Nadja Drusenheimer[2], Jessica Nolte[1], Gerald Wulf[2], 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

Recent studies have demonstrated that somatic stem cells have a more flexible potential than expected, whether put into tissue or cultured under different conditions. Bone marrow (BM)-derived stem cells can transdifferentiate into multilineage cells, such as muscle of mesoderm, lung and liver of endoderm, and brain and skin of ectoderm origin. Here we show that BM stem cells are able to transdifferentiate into male germ cells. For derivation of male germ cells from adult BM stem (BMS) cells, we used the Stra8-enhanced green fluoresence protein (EGFP) transgenic mouse line expressing EGFP specifically in male germ cells. BMS cell-derived germ cells expressed the known molecular markers of primordial germ cells, such as fragilis, stella, Rnf17, Mvh and Oct4; as well as molecular markers of spermatogonial stem cells and spermatogonia including Rbm, c-Kit, Tex18, Stra8, Piwil2, Dazl, Hsp90α, β1- and α6-integrins. Our ability to derive male germ cells from BMS cells reveals novel aspects of germ cell development and opens the possibilities for use of these cells in reproductive medicine.

Laboratory Investigation (2006) **86**, 654–663. doi:10.1038/labinvest.3700429; published online 1 May 2006

Conclusions

- Functional gamete-like cells can be obtained from embryonic and fetal stem cells.
- BM stem cells give rise to male gametes
- Customised gametes may be obtained from ESC using NT techniques.
- Derivation of gametes from SC brings new insights into germ cell development , epigenetic reprogramming and germline gene modification

* Stem cell derived gametes can become a valuable resource for research. The use of such gametes in ART remains a “distant prospect"

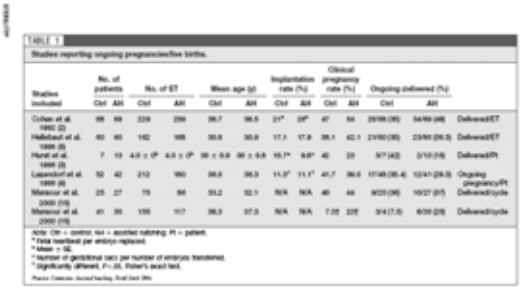
Assisted Zona Hatching

What is the evidence?

Embryo Assisted hatching

- Assisted hatching (AH) involves the artificial thinning or breaching of the ZP.
- Has been proposed as one technique to improve implantation and pregnancy rates following *in vitro* fertilization (IVF).
- An increased implantation rate following mechanical opening of the ZP (partial zona dissection-PZD) was first reported in 1990 *(Cohen et al 1990)*
- The assisted hatching procedure can be performed on day 3 or day 5 after fertilization.
  - drilling with acidified Tyrode's solution
  - PZD with a glass microneedle
  - Laser photoablation
  - Use of a piezomicromanipulator

Embryo Assisted hatching



Studies reporting ongoing pregnancies/live births.

Studies included	No. of patients		No. of ET		Mean age (y)		Implantation rate (%)		Clinical pregnancy rate (%)		Ongoing (delivered (%))		
	Ctrl	AH	Ctrl	AH	Ctrl	AH	Ctrl	AH	Ctrl	AH	Ctrl	AH	
Cohen et al. 1992 (3)	69	69	229	259	36.7	36.5	21*	19*	47	54	25/69 (36)	34/99 (49)	Delivered/ET
Hellebaut et al. 1996 (9)	60	60	162	168	30.8	30.8	17.1	17.8	36.1	42.1	21/60 (35)	23/60 (38.3)	Delivered/ET
Hurst et al. 1999 (5)	7	10	4.0 ± 0[a]	4.0 ± 0[a]	30 ± 0.8	30 ± 0.8	10.7*	9.6*	42	33	3/7 (43)	2/10 (14)	Delivered/Pt
Lazendorf et al. 1998 (6)	52	42	212	160	35.5	35.3	11.3*	11.1*	41.7	36.0	17/49 (36.4)	12/41 (29.3)	Ongoing pregnancy/Pt
Mansour et al. 2000 (10)	25	27	75	90	33.2	32.1	N/A	N/A	40	44	9/25 (36)	10/27 (37)	Delivered/cycle
Mansour et al. 2000 (10)	41	39	155	117	36.3	37.3	N/A	N/A	7.35	23[f]	3/4 (7.5)	6/39 (23)	Delivered/cycle

Note: Ctrl = control; AH = assisted hatching; Pt = patient.
[a] Fetal heartbeat per embryo replaced.
[b] Mean ± SE.
[c] Number of gestational sacs per number of embryos transferred.
[d] Significantly different, P<.05, Fisher's exact test.

Practice Committee. Assisted hatching. Fertil Steril 86.

The role of assisted hatching in in vitro fertilization: a review of the literature. A Committee opinion
The Practice Committee of the Society for Assisted Reproductive Technologya and the Practice Committee of the American Society for Reproductive Medicine
Fertil Steril Vol 86, Suppl 4, Nov 2006

Embryo Assisted hatching

Conclusions

•The available published evidence does not support the routine or universal application of assisted hatching in all IVF cycles at this time.

• Assisted hatching may be clinically useful in patients with a poor prognosis, including those with 2 failed IVF cycles and poor embryo quality and advanced age women (38 years of age)

• Higher clinical pregnancy and implantation rates have been observed after assisted hatching. However, delivery rates have not significantly improved, possibly because the small sample sizes in studies reporting delivery rates have lacked sufficient power to detect a difference.

Individual ART programs should evaluate their own patient populations to determine which subgroup may benefit from AH

Preimplantation genetic screening (PGS) Is it useful for advanced age patients?

Preimplantation genetic screening for abnormal number of chromosomes (aneuploidies) in in vitro fertilisation or intracytoplasmic sperm injection (Review)

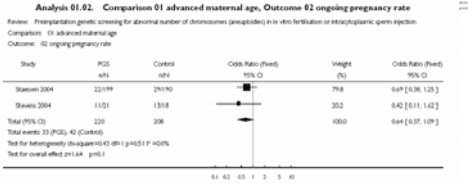
Twisk M, Mastenbroek S, van Wely M, Heineman MJ, Van der Veen F, Repping S

This record should be cited as:
Twisk M, Mastenbroek S, van Wely M, Heineman MJ, Van der Veen F, Repping S. Preimplantation genetic screening for abnormal number of chromosomes (aneuploidies) in in vitro fertilisation or intracytoplasmic sperm injection. *Cochrane Database of Systematic Reviews* 2006, Issue 1. Art. No.: CD005291. DOI: 10.1002/14651858.CD005291.pub2.

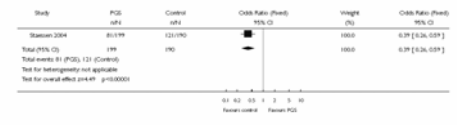
Dexeus

CMR[B]

Analysis 01.02. Comparison 01 advanced maternal age, Outcome 02 ongoing pregnancy rate



Analysis 01.03. Comparison 01 advanced maternal age, Outcome 03 proportion of women reaching embryo transfer



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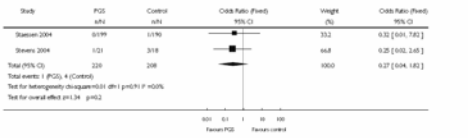
CMR[B]

Analysis 01.07. Comparison 01 advanced maternal age, Outcome 07 miscarriage rate per clinical pregnancy



Analysis 01.01. Comparison 01 advanced maternal age, Outcome 01 live birth rate

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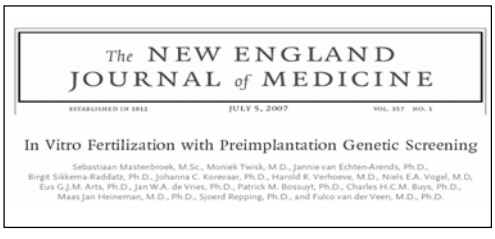
CMR[B]



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In Vitro Fertilization with Preimplantation Genetic Screening

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Table 2. Outcomes in Women Who Underwent Preimplantation Genetic Screening and in Controls.

Outcome	Women Who Underwent Preimplantation Genetic Screening (N=206)	Controls (N=202)	Rate Ratio (95% CI)*	P Value
Women with an ongoing pregnancy — no. (%)	52 (25)	74 (37)	0.69 (0.51–0.93)	0.01
Women with ≥1 biochemical pregnancy — no. (%)	81 (39)	106 (52)	0.75 (0.60–0.93)	0.008
Total no. of biochemical pregnancies	94	118		
Women with ≥1 clinical pregnancy — no. (%)	61 (30)	88 (44)	0.68 (0.52–0.88)	0.003
Total no. of clinical pregnancies	67	92		
Women with ≥1 miscarriage — no. (%)	37 (18)	36 (18)	1.01 (0.67–1.53)	0.97
Total no. of miscarriages	43†	44‡		
Women with ≥1 live birth — no. (%)	49 (24)	71 (35)	0.68 (0.50–0.92)	0.01
Total no. of live births	59§	85¶		

* CI denotes confidence interval.
† One miscarriage occurred at 18 weeks of gestation; all other miscarriages occurred before 12 weeks of gestation.
‡ All miscarriages occurred before 12 weeks of gestation.
§ There were 39 singleton and 10 twin births; one woman underwent elective termination of pregnancy, one pregnancy ended in an intrauterine death, and one premature delivery resulted in the postpartum death of a twin.
¶ There were 57 singleton and 14 twin births; two women underwent elective termination of pregnancy, and one pregnancy ended in an intrauterine death.

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- Multicentre trial of preimplantation genetic screening reported in the New England Journal of Medicine: an in-depth look at the findings.
  Cohen J, Grifo JA.
- Substandard application of preimplantation genetic screening may interfere with its clinical success.
  Munné S, Gianaroli L, Tur-Kaspa I, Magli C, Sandalinas M, Grifo J, Cram D, Kahraman S, Verlinsky Y, Simpson JL.
- IVF with preimplantation genetic screening, a promising new treatment with unexpectedly negative health outcomes: the Hippocratic role of Data Monitoring Committees.
  Ankum WM, Reitsma JB, Offringa M; Hippocratic role of Data Monitoring Committees.