Cytokines and IVF Embryo Culture – The GM-CSF Experience

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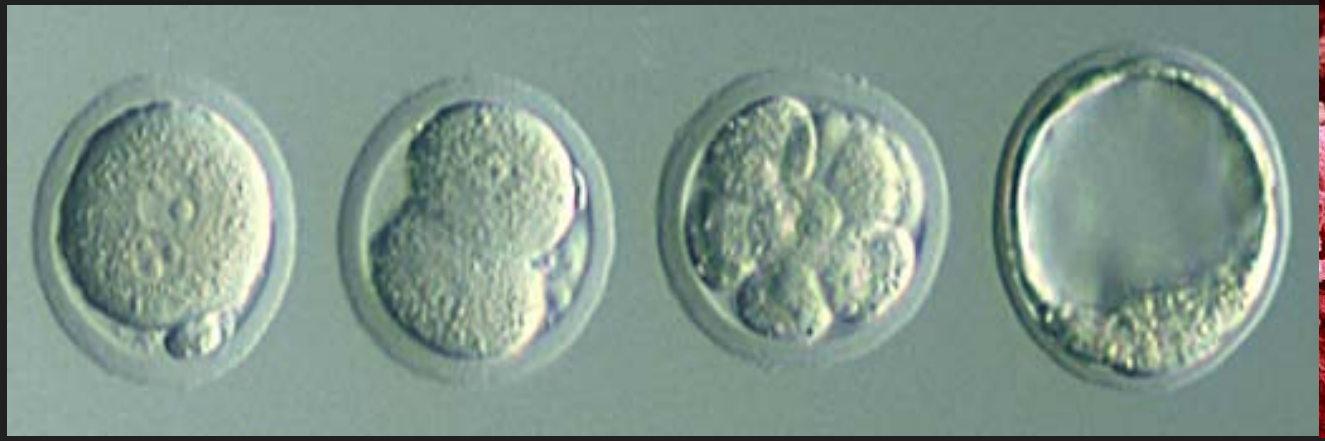
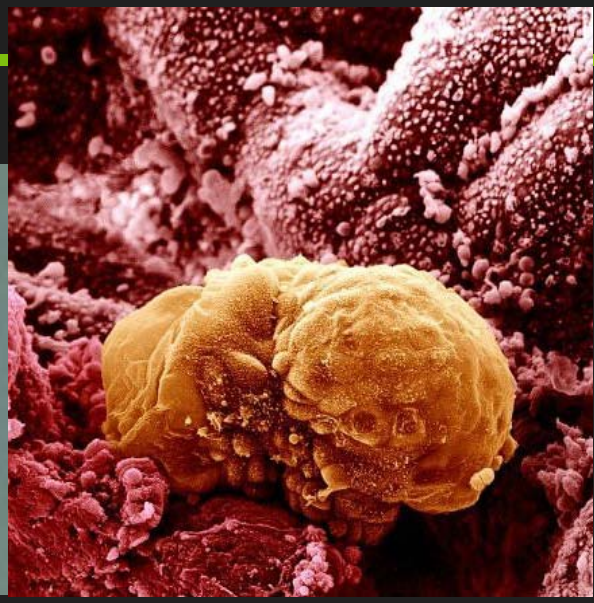
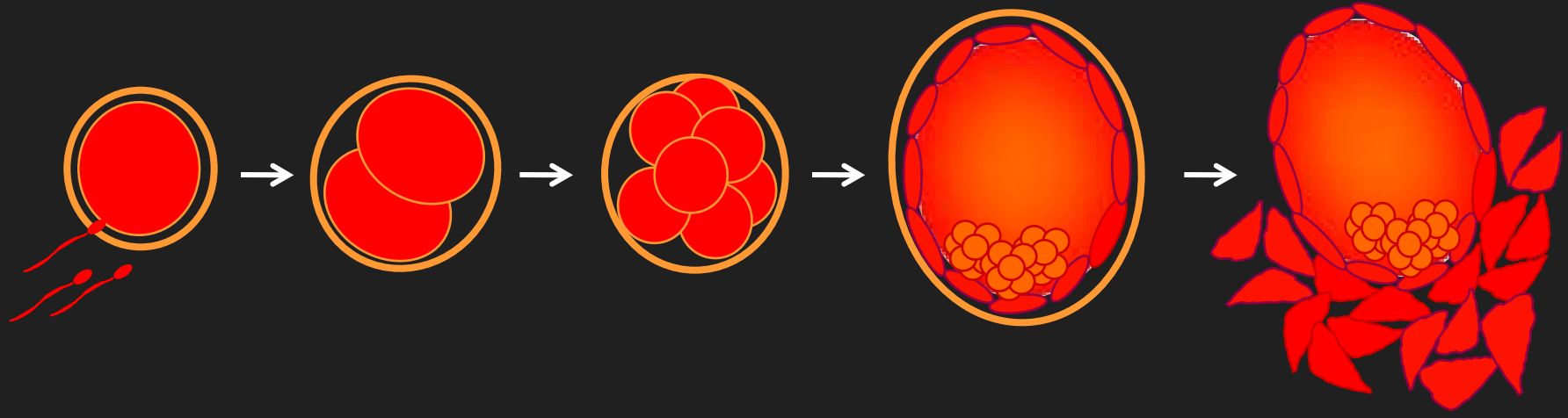
SUB CRUCE LUMEN

first days of life are critical

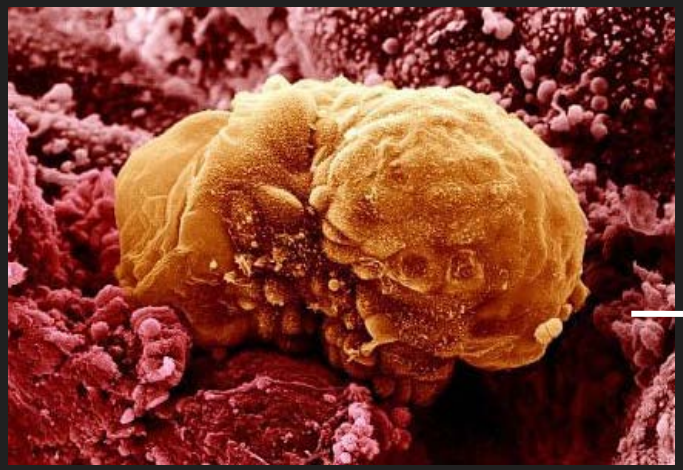


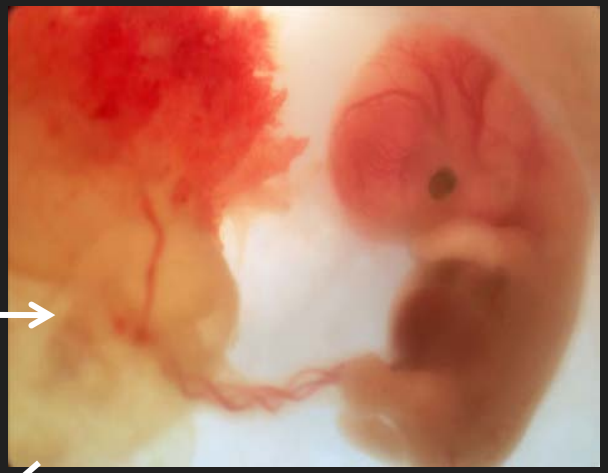


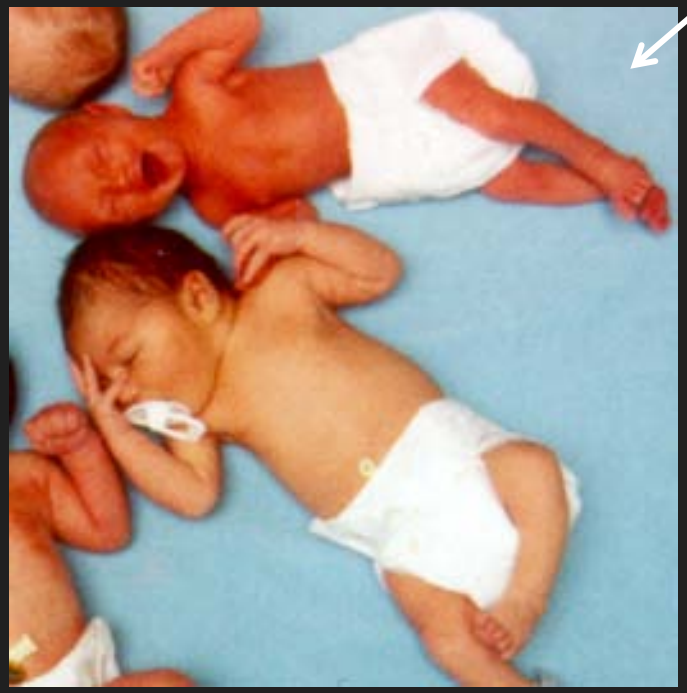
day 1 day 2 day 3 day 4 day 6



peri-conceptual determinants of implantation and fetal growth

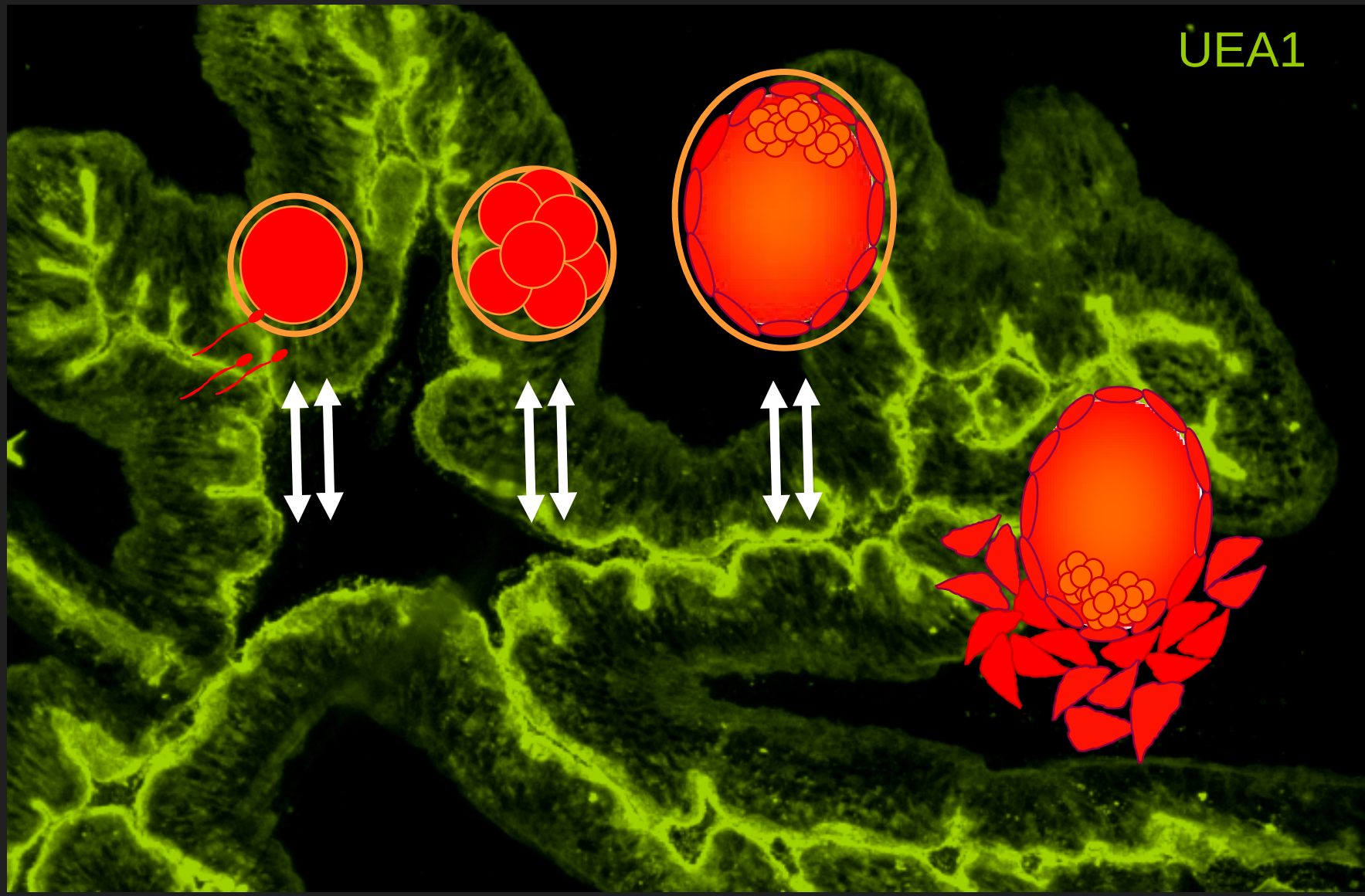




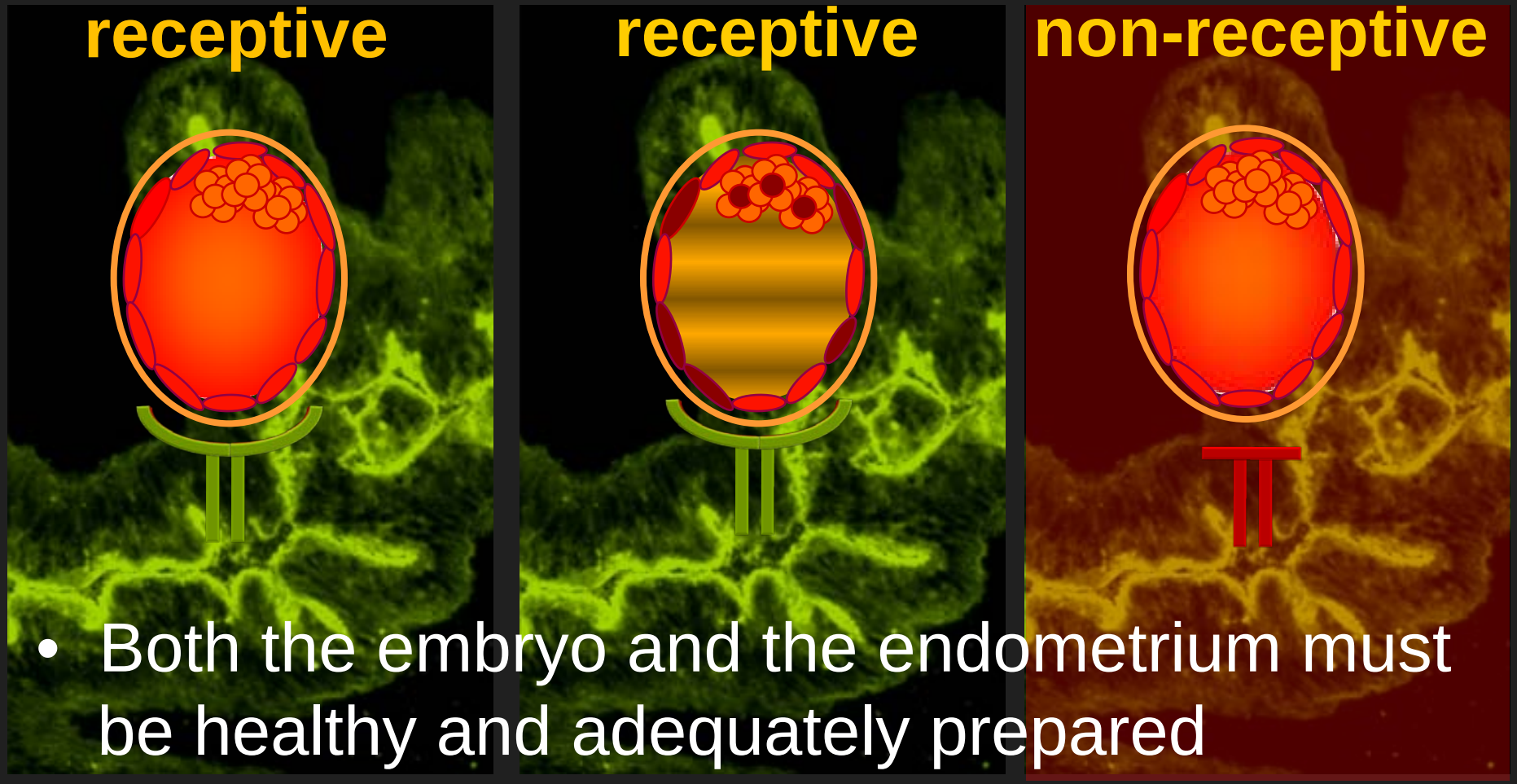


- genetics and epigenetics
- nutrition and obesity
- smoking & lifestyle factors
- environmental stressors
- CYTOKINES

establishing pregnancy… a partnership

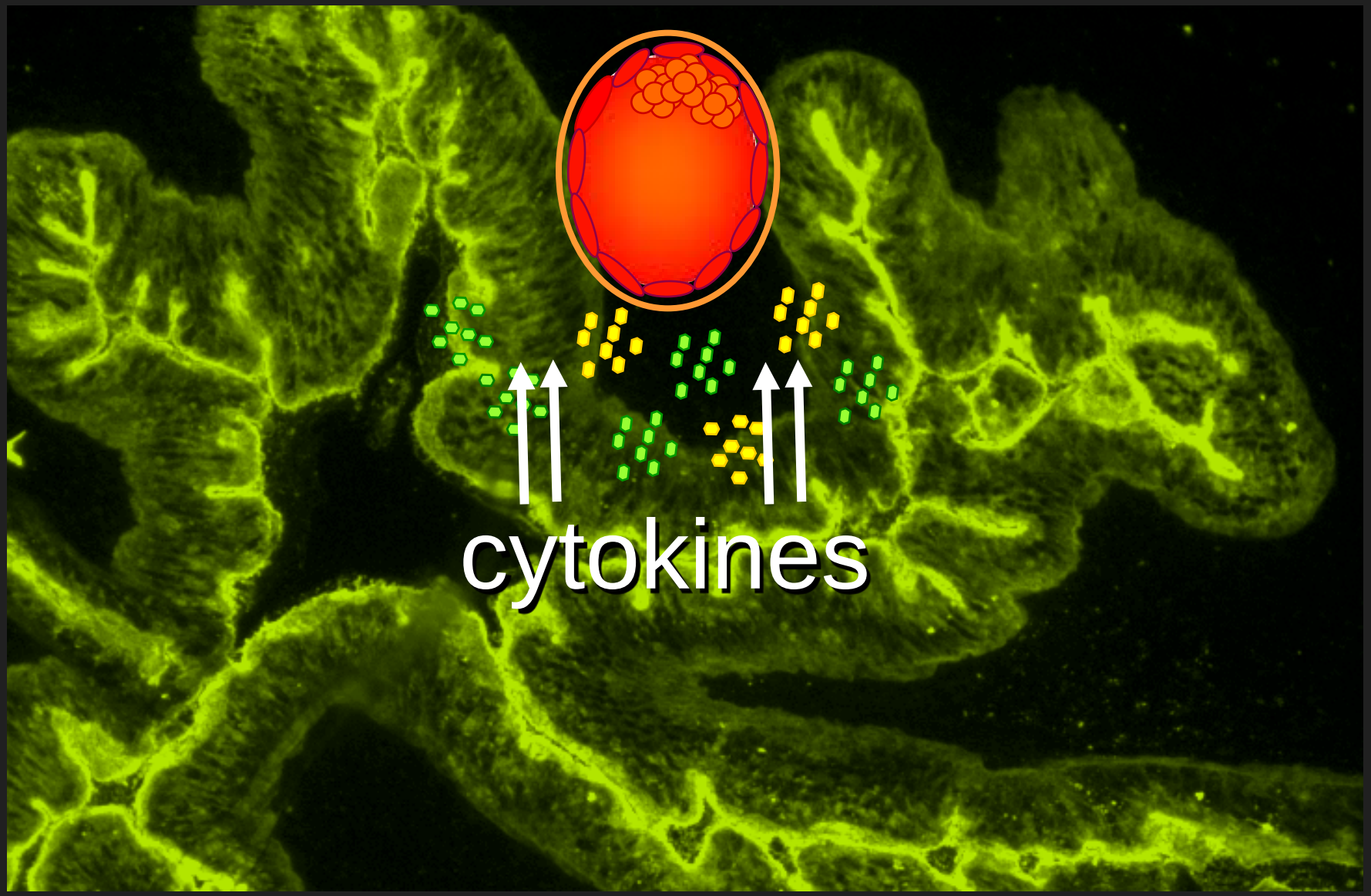


establishing pregnancy… a partnership

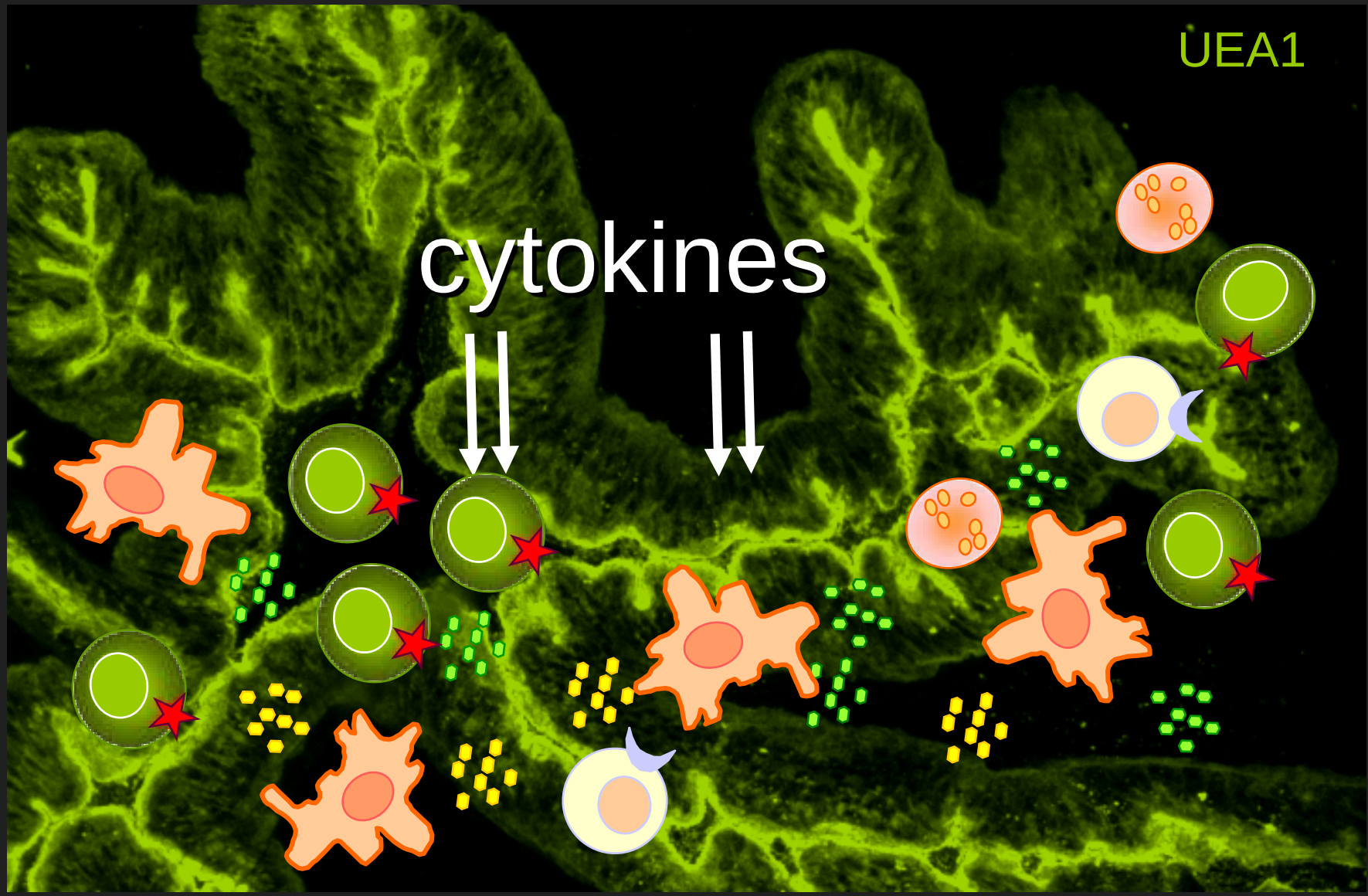


- Both the embryo and the endometrium must be healthy and adequately prepared
- Disruption → infertility, miscarriage, IUGR, preeclampsia

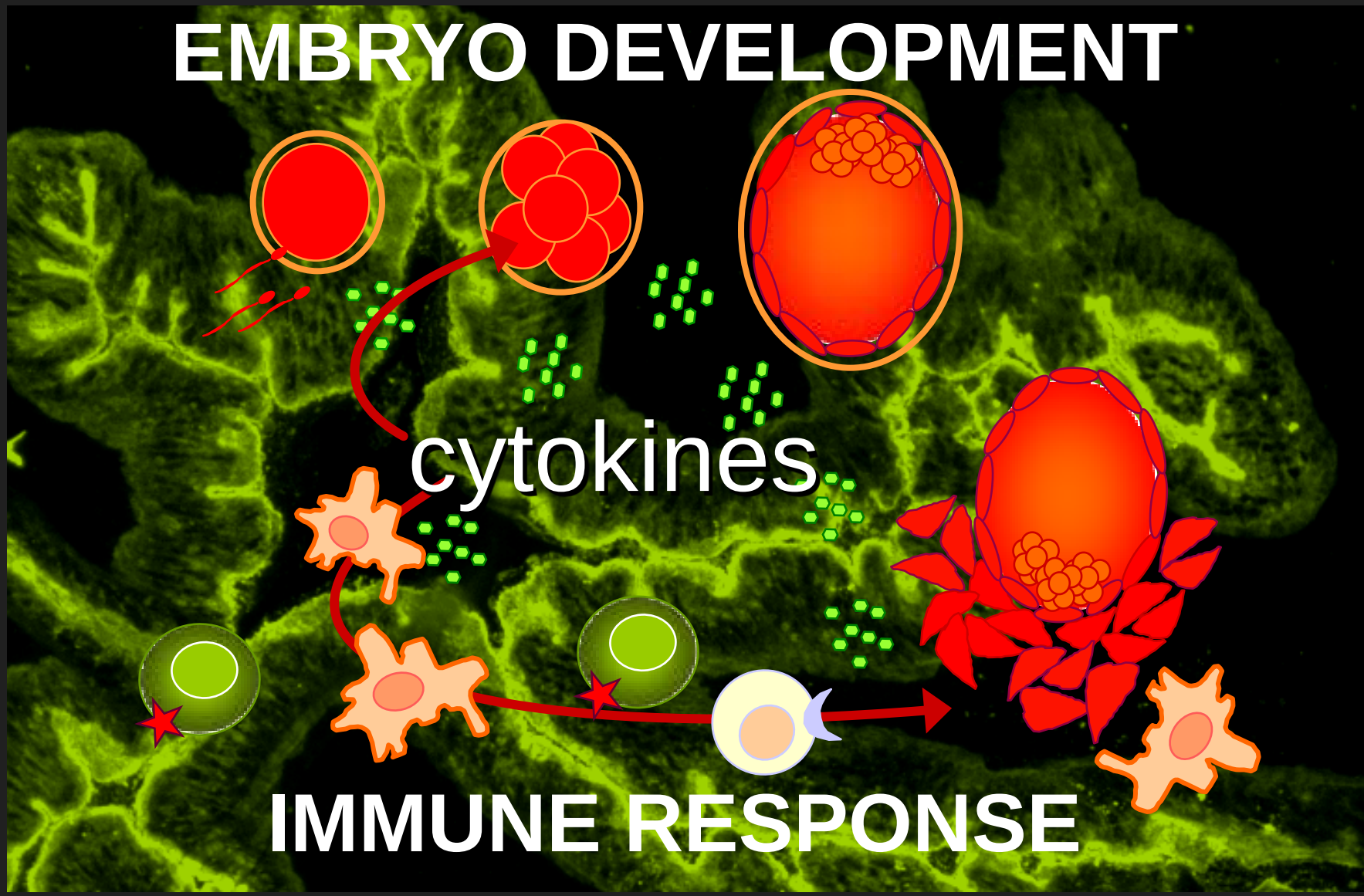
epithelial cells → embryo communication



epithelial cells → leukocyte communication



two-way communication → optimal outcome



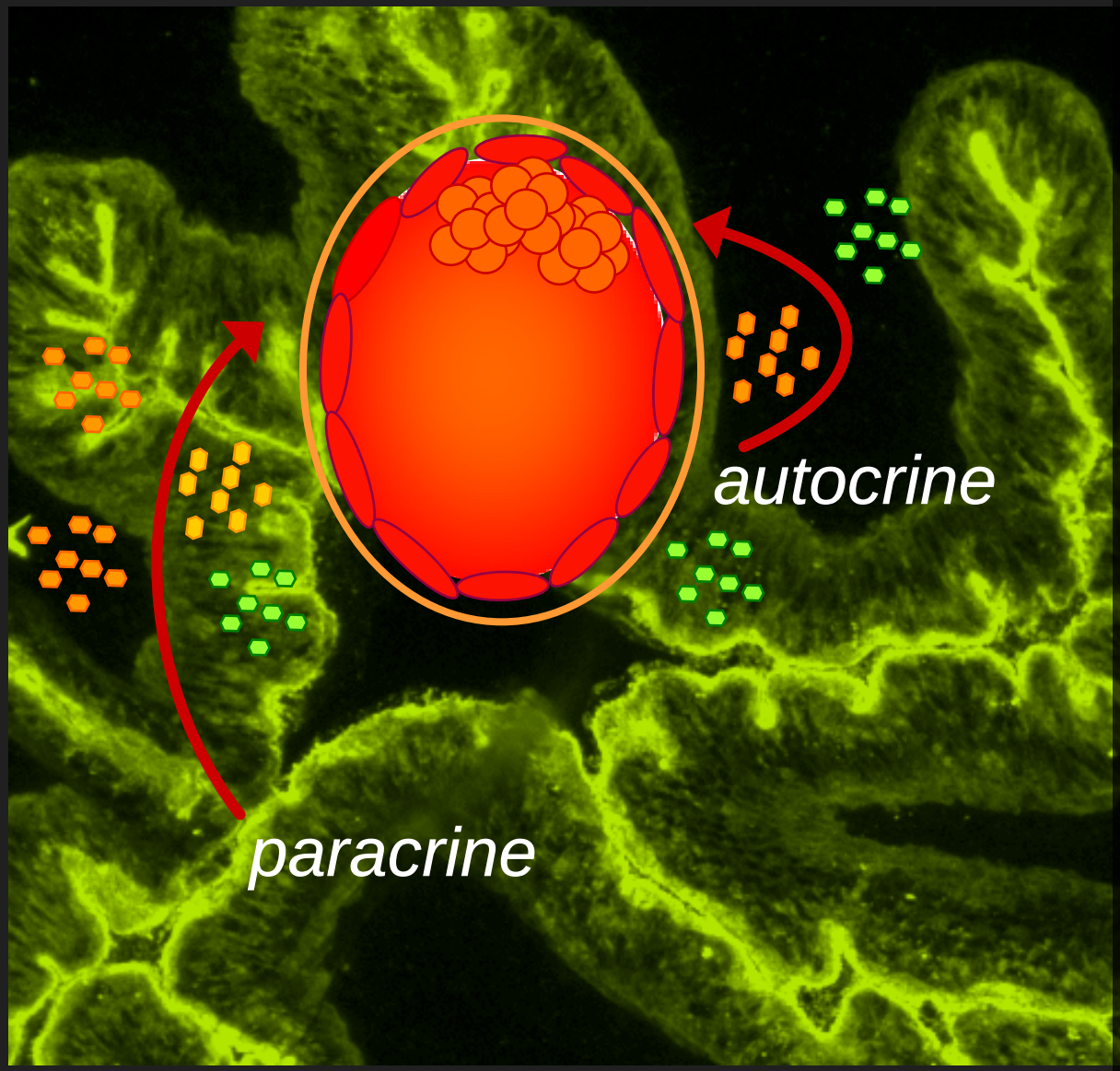
peri-conceptual cytokines are a key factor in pregnancy success

infertility
miscarriage
IUGR
preeclampsia

CYTOKINES

healthy pregnancy

cytokine regulation of pre-implantation embryos



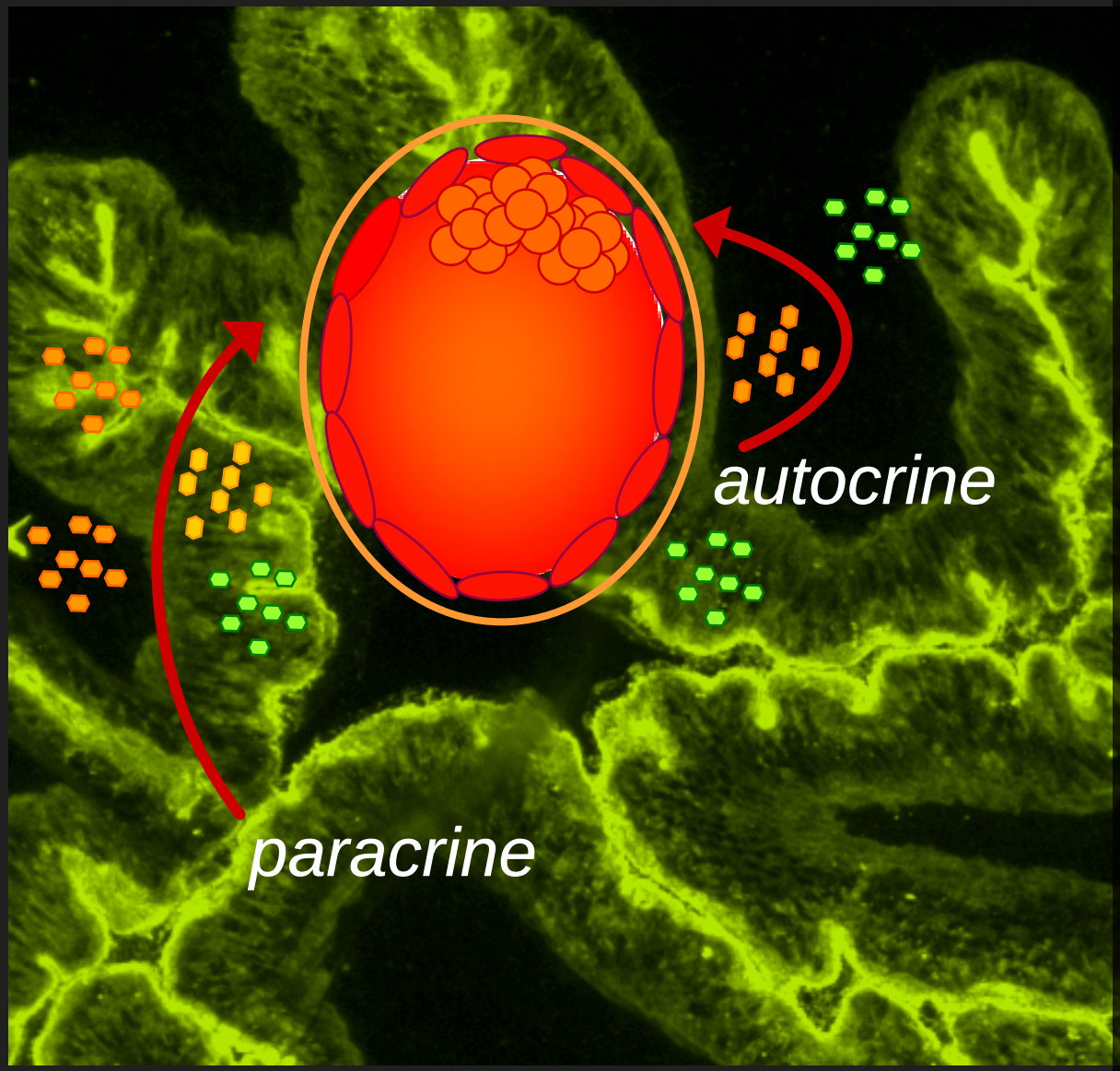
GM-CSF
LIF
PAF
GH
IGF-I
IGF-II
EGF
TGFα
TGFβ

↑

TNFα
IFNγ

↓

many (but not all) cytokines are autocrine



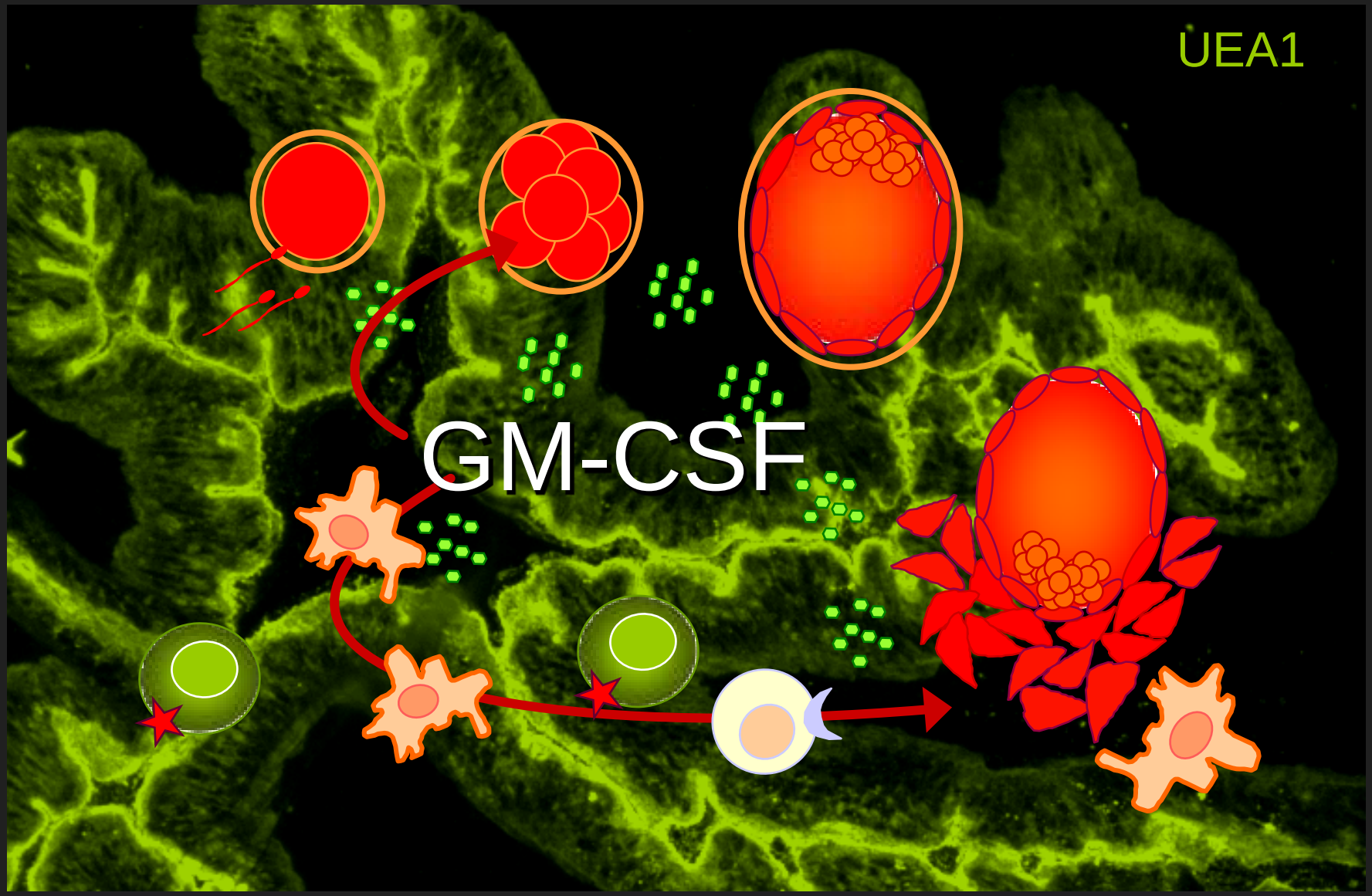
GM-CSF
LIF
PAF
GH
IGF-I
IGF-II
EGF
TGFα
TGFβ

TNFα
IFNγ

effects of cytokines on pre-implantation embryos

	Mo	Hu
• % zygote development to blastocyst	?	✓
• speed of development to blastocyst	✓	✓
• cell number & allocation to ICM and TE	✓	✓
• cell viability and apoptosis	✓	✓
• gene expression profile	✓	✓
• metabolism	✓	✓
• stress response	✓	✓
• implantation & developmental competence	✓	✓
• developmental programming in fetus	✓	?

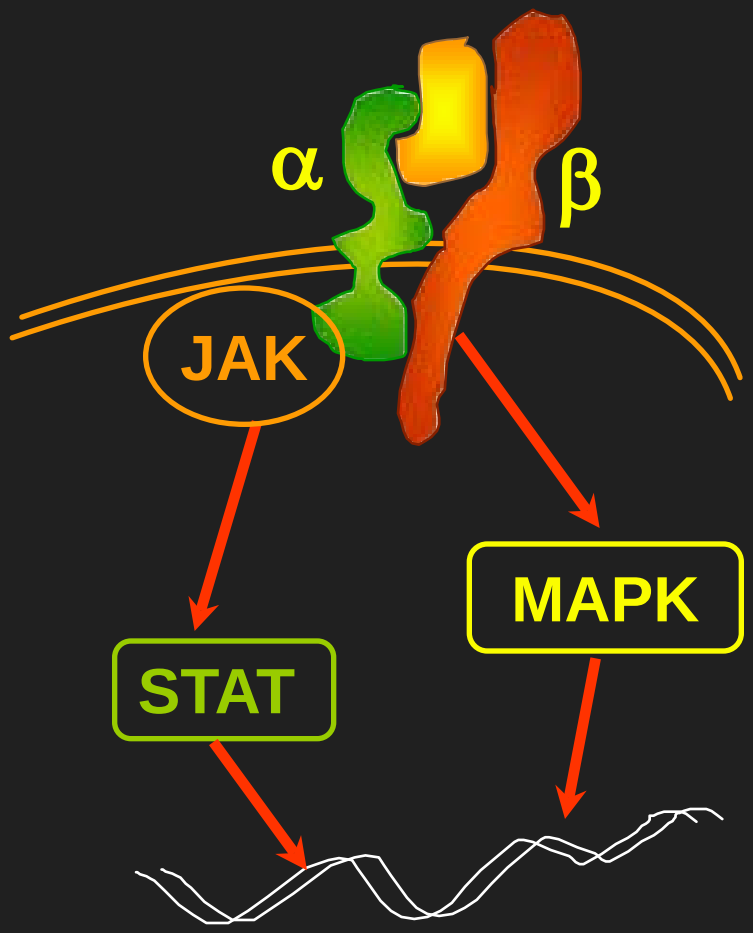
GM-CSF – a pivotal cytokine in early pregnancy



Pathway from laboratory to clinic

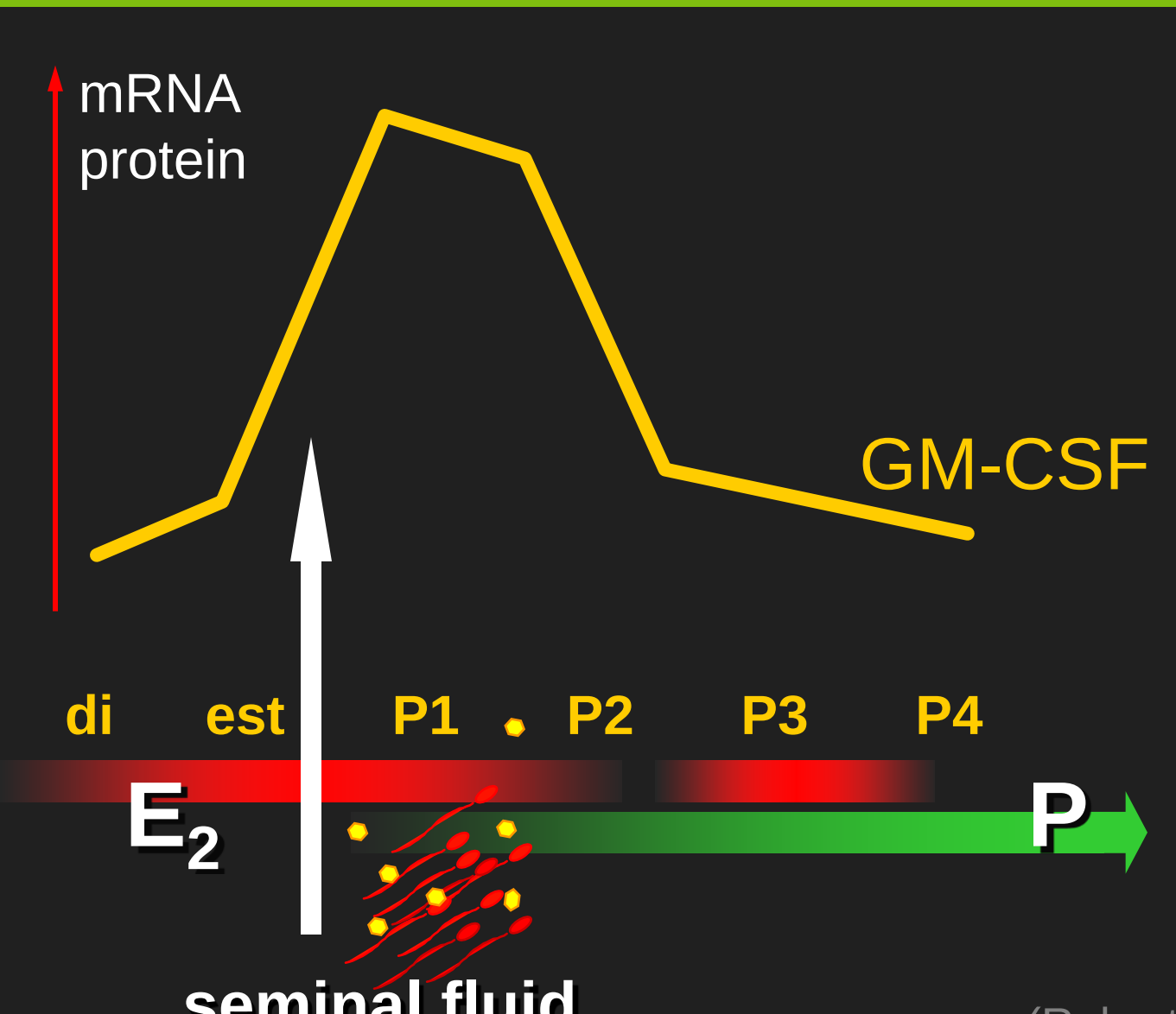
- Investigate fundamental reproductive biology
- Devise rational, evidence-based clinical intervention
- Rigorously evaluate and prove safety and efficacy

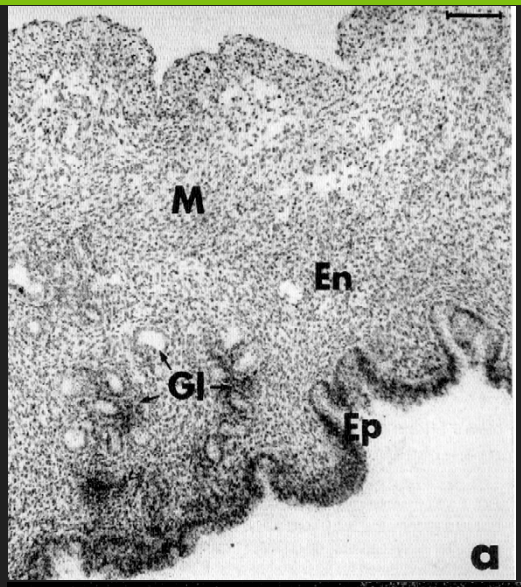
GM-CSF (CSF2) = granulocyte-macrophage colony-stimulating factor

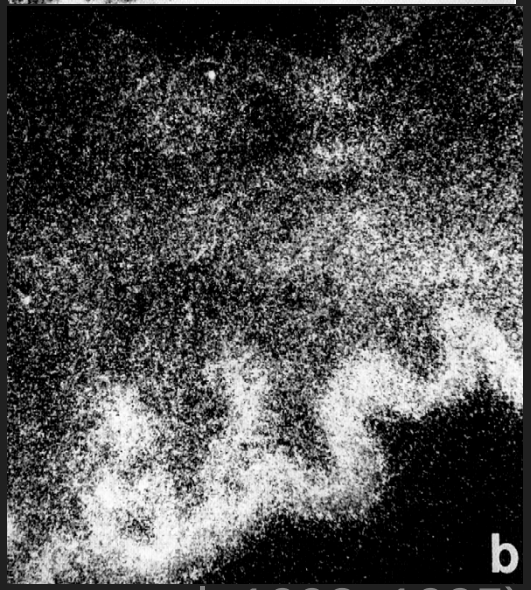


- 23 kD glycoprotein secreted / ECM-associated
- binds GM-CSF Rα / βc to signal via JAK/STAT & MAPK
- monocyte/macrophages, dendritic cells, granulocytes
- proliferation of progenitors, cell survival, differentiation
- endothelial cells, trophoblasts

GM-CSF expression in mouse uterus

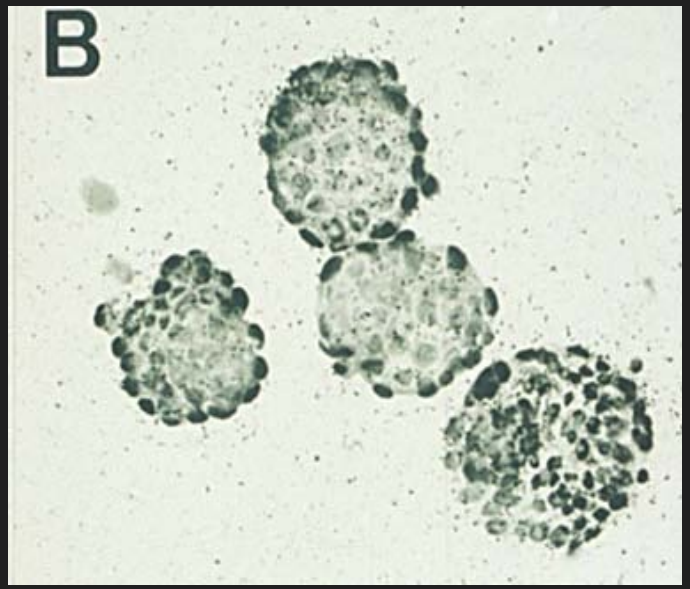


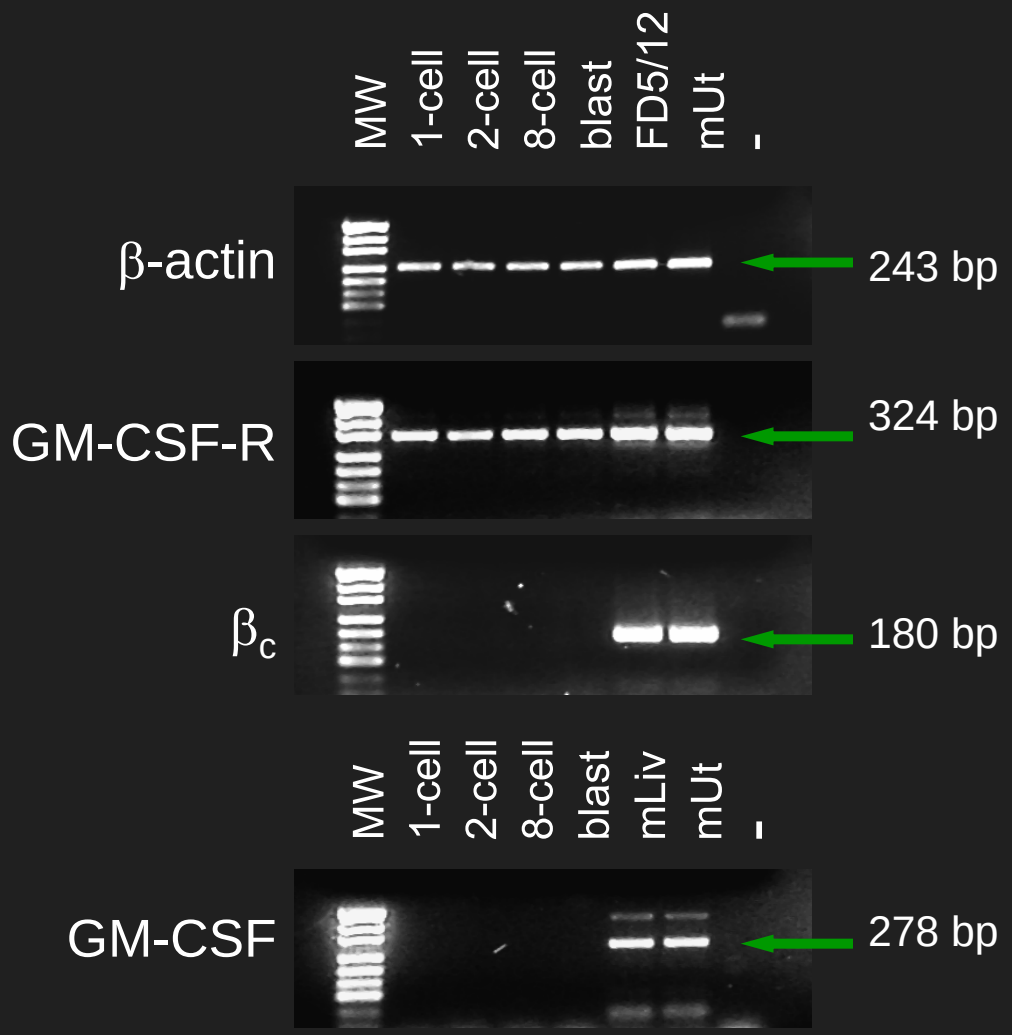




(Robertson et al. 1992, 1995)

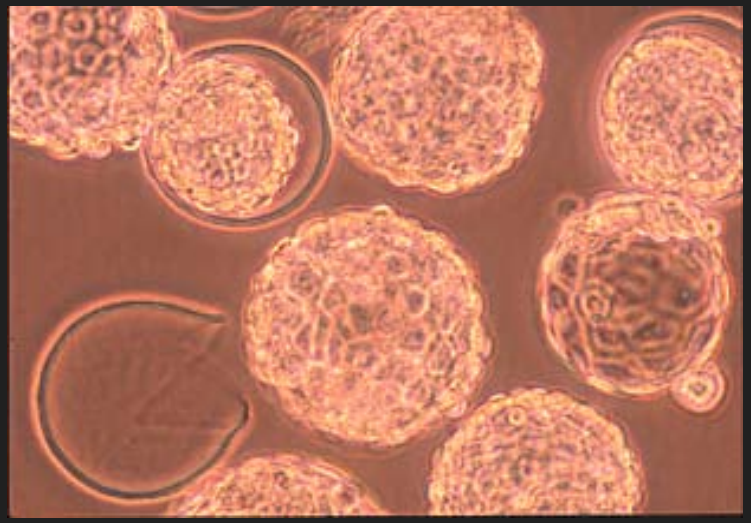
Expression of GM-CSF receptors in embryos

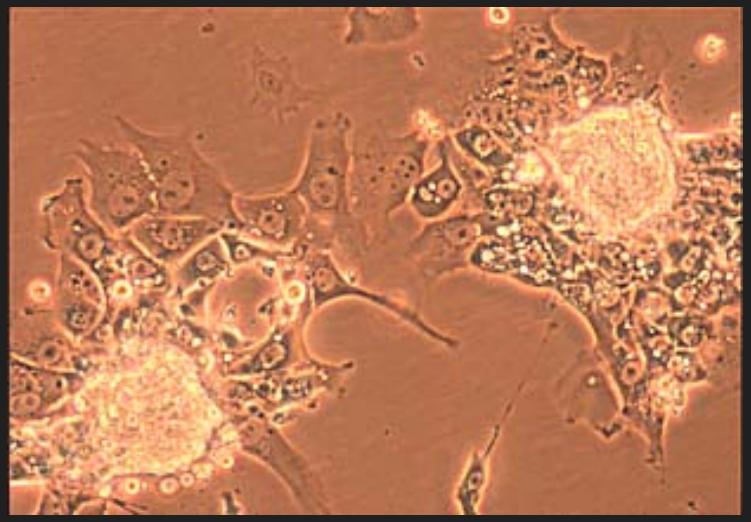




(Robertson et al. *Biol Reprod* 2001)

Effect of GM-CSF in culture medium on blastocyst and post-blastocyst development



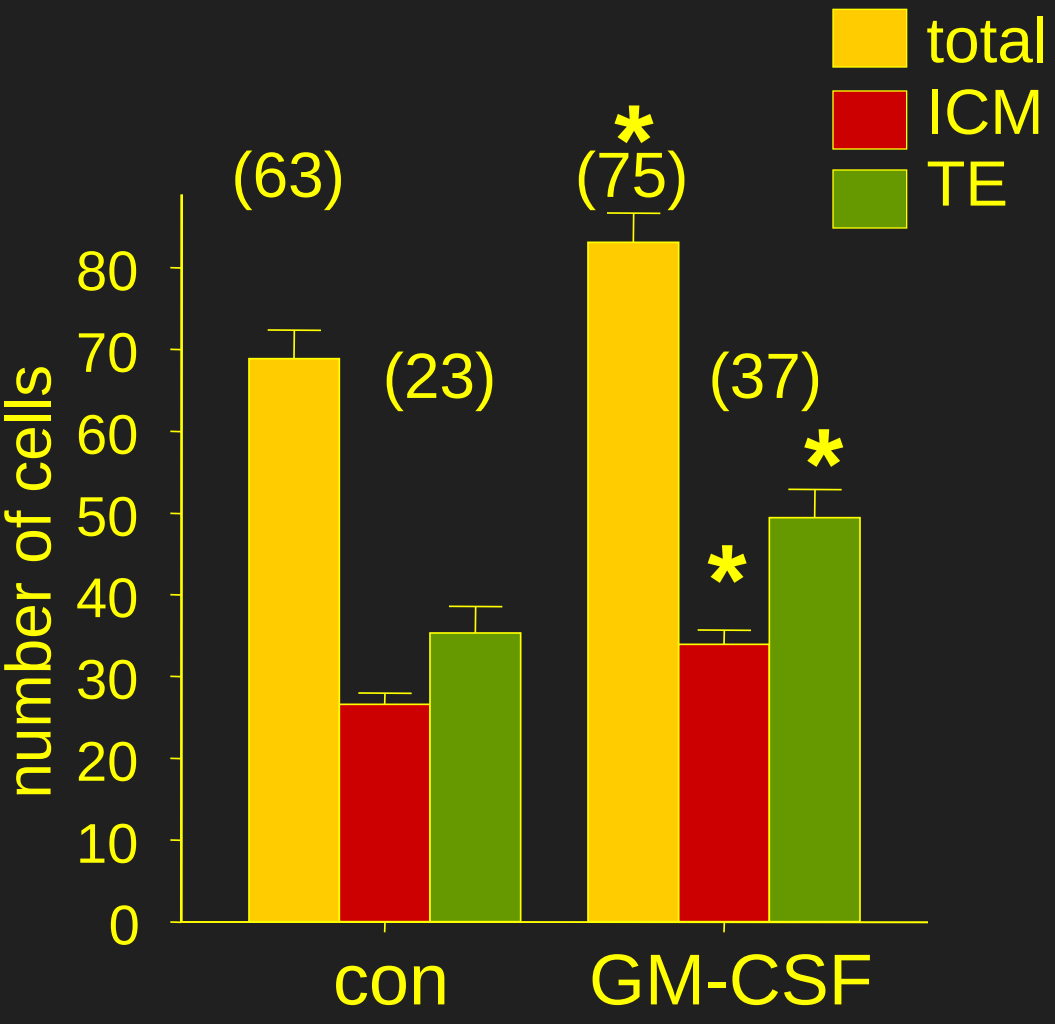


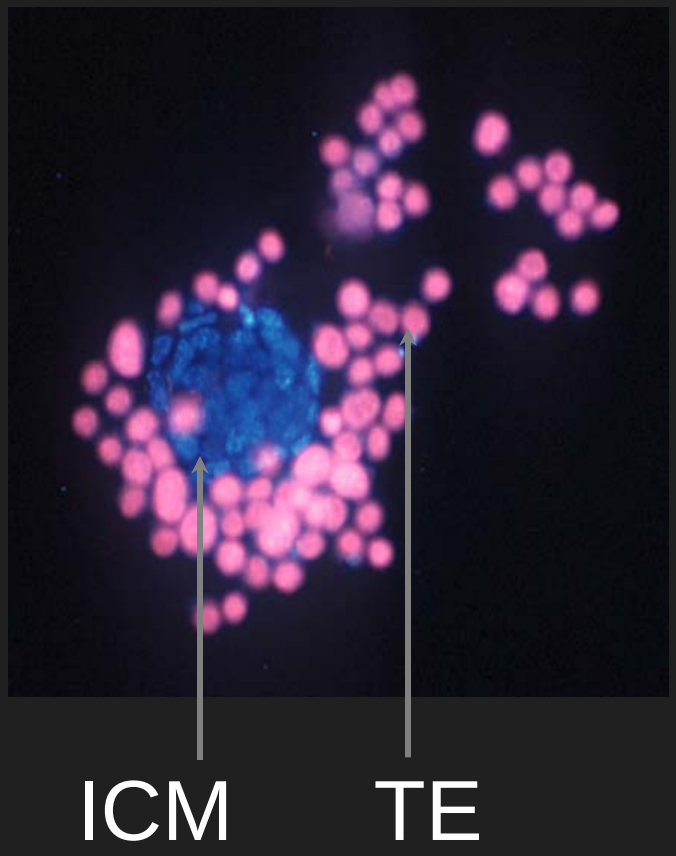
	Control (N)	%	+ rGM-CSF (N)	%
% 8-cell → Bϕ	(696)	92	(538)	89
% Bϕ Hatch	(330)	78	(294)	82
% Bϕ Attach	(492)	79	(267)	92*

*p < 0.001

(Robertson et al. *Biol Reprod* 2001)

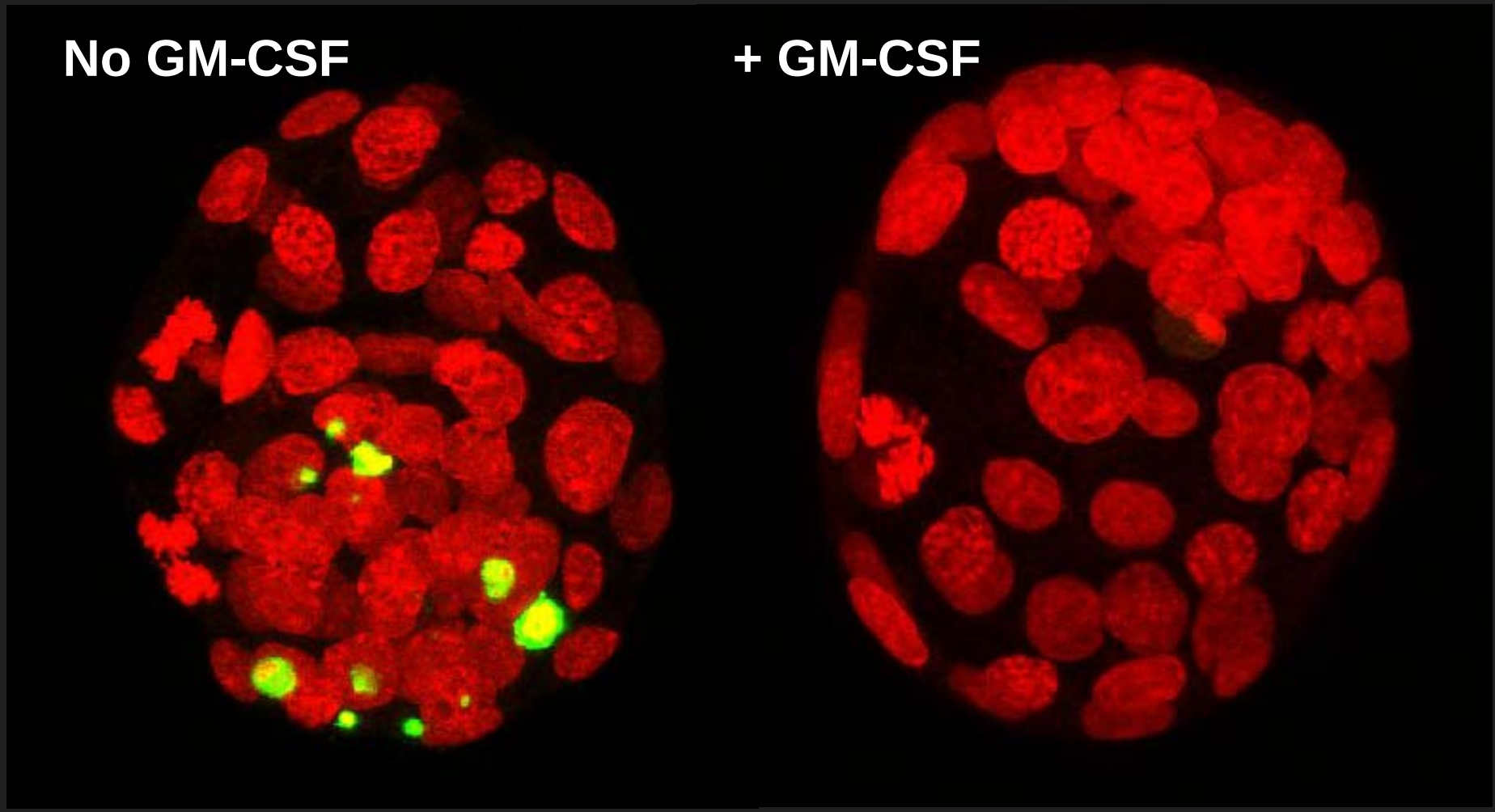
Effect of GM-CSF on cell number in blastocysts



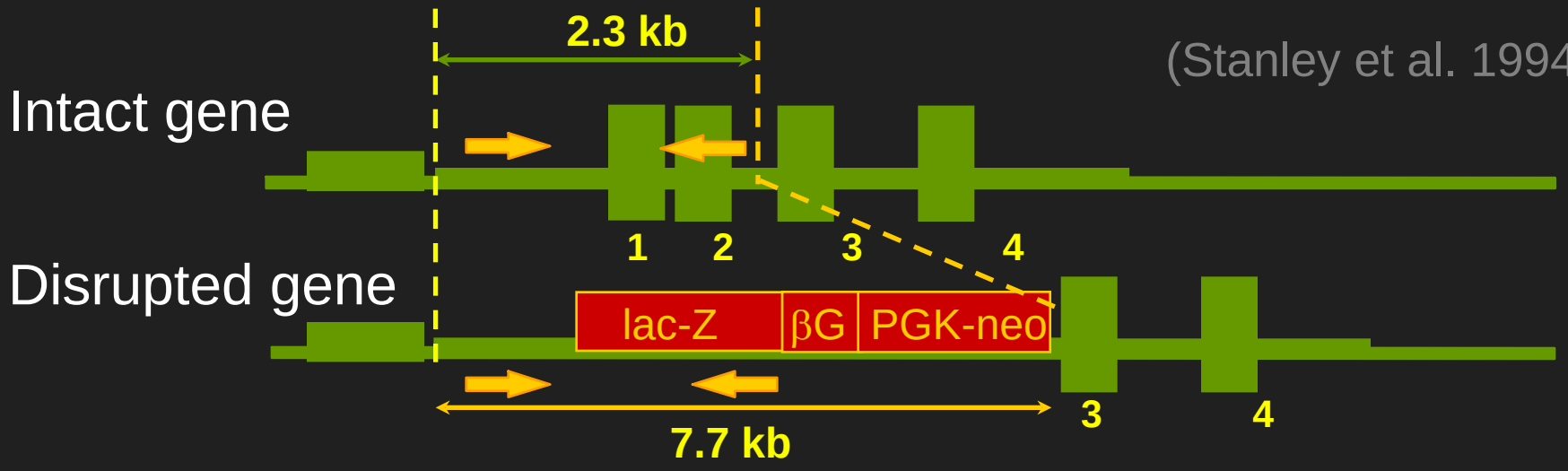


(Robertson et al. *Biol Reprod* 2001)

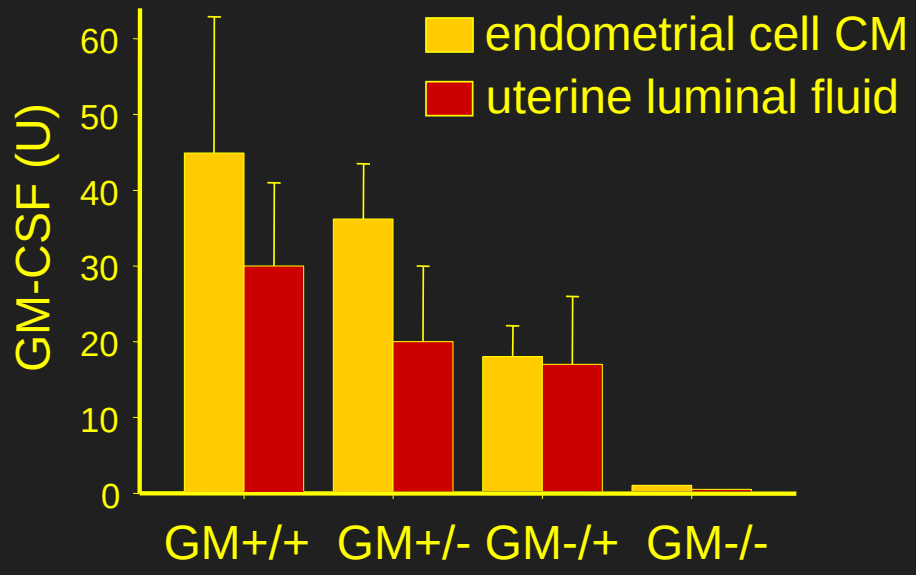
GM-CSF deficiency & apoptosis in blastocysts



GM-CSF null mutant mice



(Stanley et al. 1994)

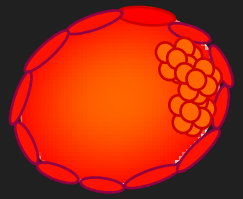


Summary: effects of GM-CSF deficiency

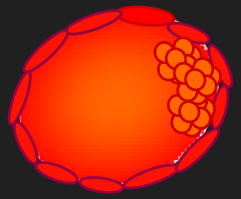
- litter sizes are 25% smaller at weaning due to late gestation and early postnatal loss
- miscarriage is increased 2-fold
- fetal malformation is increased 2-fold
- IUGR is increased 9-fold
- males are more adversely affected
- placental structure is altered

(Robertson et al. *Biol Reprod* 1999)

Microarray to analyse GM-CSF regulation of blastocyst gene expression

A. 

in vitro
control

B. 

in vitro
+ 2 ng/ml GM-CSF

Affymetrix microarray

→ candidate gene families / genes

Microarray Results: Pathway Express

KEGG Pathway name	Impact	p-value
Focal adhesion	35.6	0.04
MAPK signalling pathway	31.8	0.14
Adherens junction	17.7	0.006
Tight junction	15.3	0.25
Calcium signalling pathway	12.5	0.33
Wnt signalling pathway	12.2	0.03
Apoptosis	9.9	0.04
Toll-like receptor signalling pathway	5.6	0.28
Phosphatidylinositol signalling	5.7	0.08
Notch signalling pathway	4.9	0.08

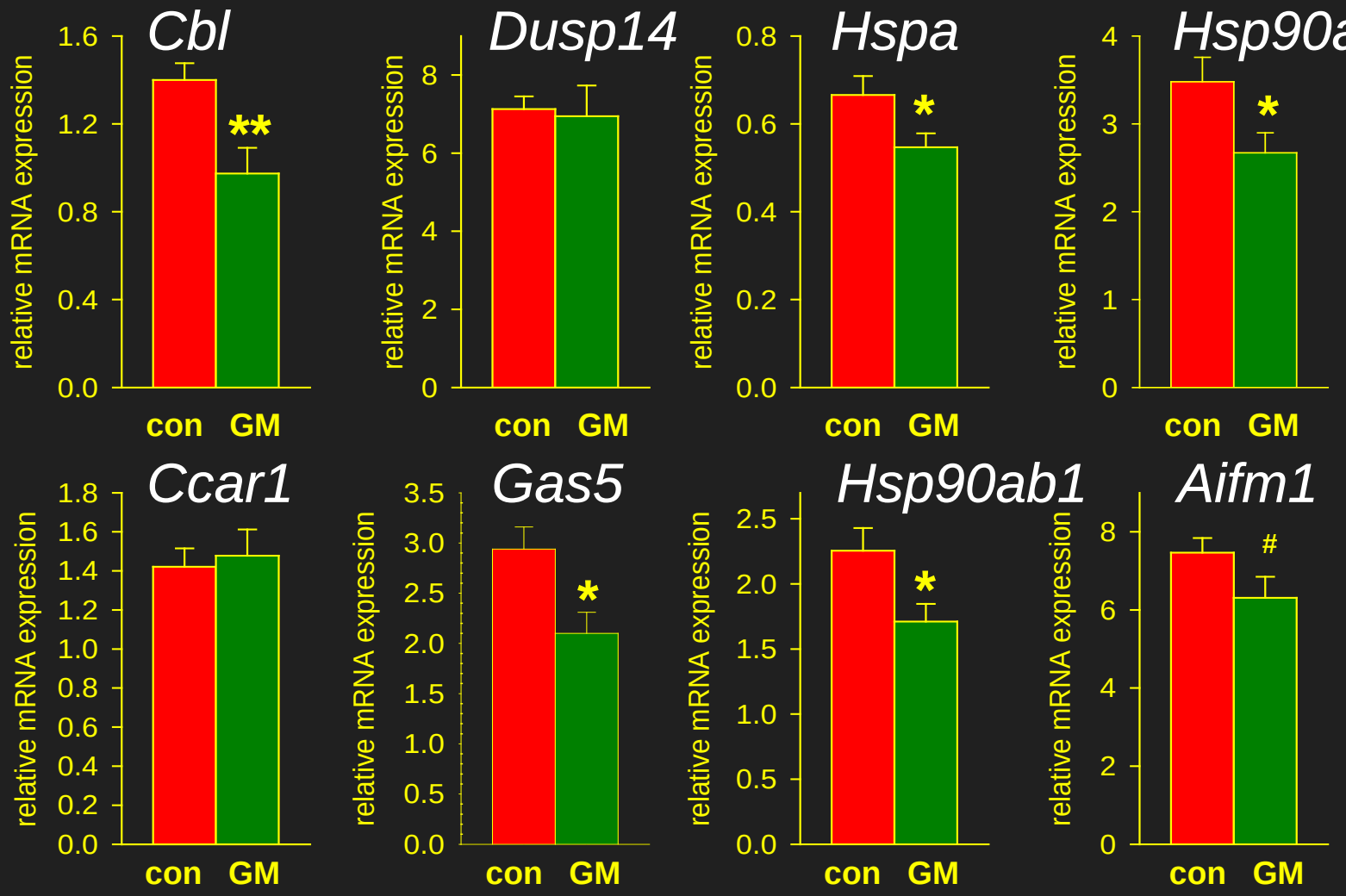
(Chin et al *Human Reproduction* 2009)

Microarray Results: GM-CSF-regulated genes

Apoptosis and cell survival:		fold Δ + GM-CSF
Cbl	casitas B-lineage lymphoma	-20.8
Ccar1	cell division cycle & apoptosis regulator 1	-4.13
Gas 5	growth arrest specific 5	-3.92
Pik3c2a	phosphatidylinositol 3-kinase, alpha	-2.77
Heat shock proteins:		
Hspa5	heat shock 70kD protein 5	-6.37
Hsp105	heat shock protein 105	-3.26
Hspa4	heat shock protein 4	-2.60
Stress response genes:		
Hif1a	hypoxia inducible factor 1, alpha	-2.76

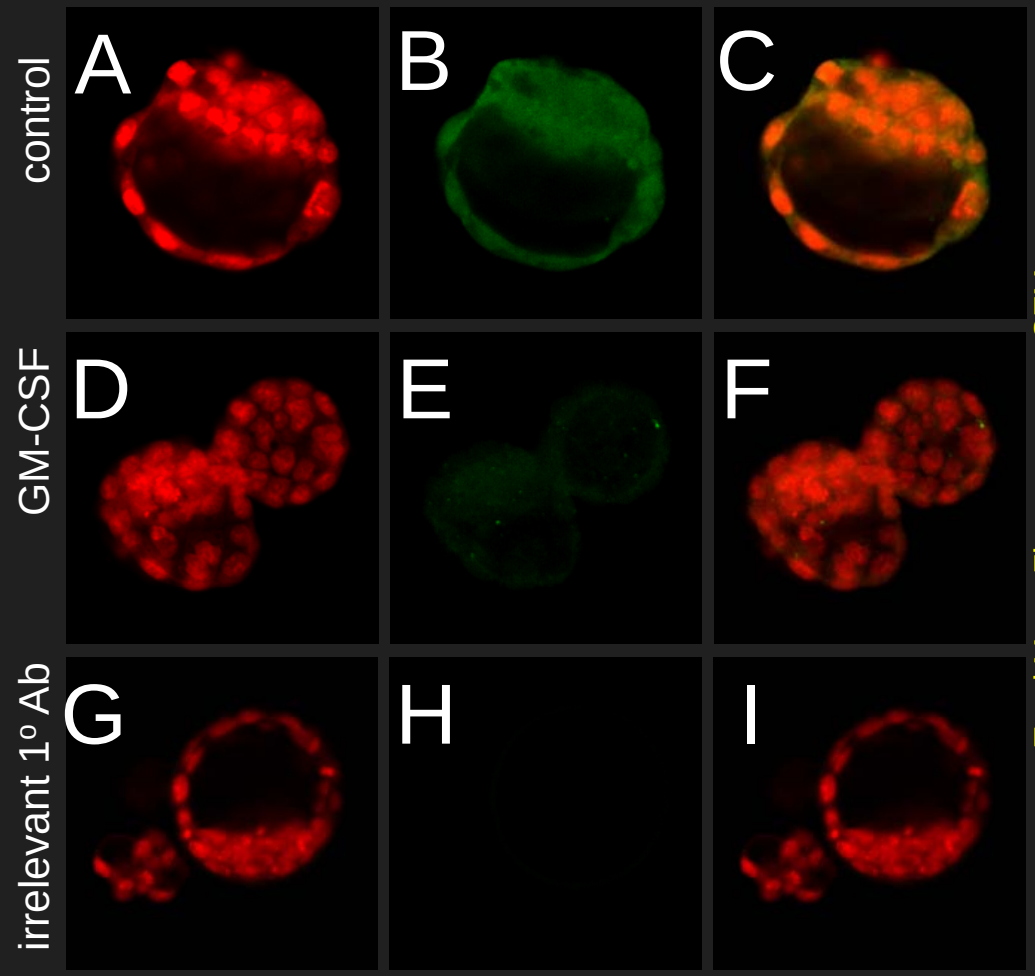
(Chin et al *Human Reproduction* 2009)

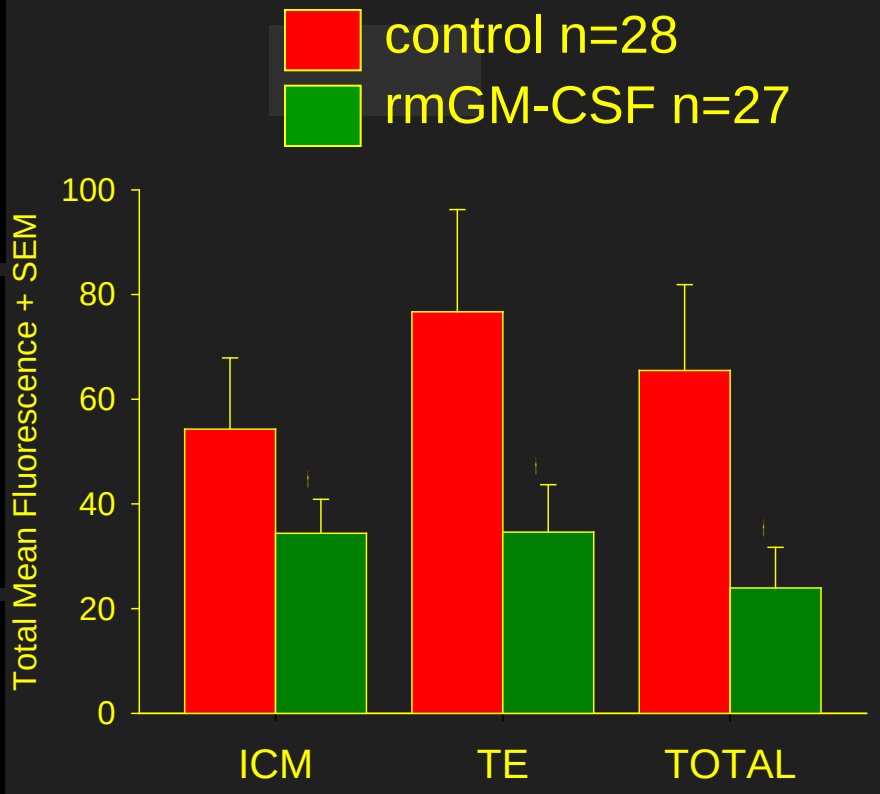
qRT-PCR analysis of stress response genes



Mann-Whitney, * P < 0.05, **P < 0.005, #P = 0.091

Effect of GM-CSF on HSPA1A/1B in blastocysts

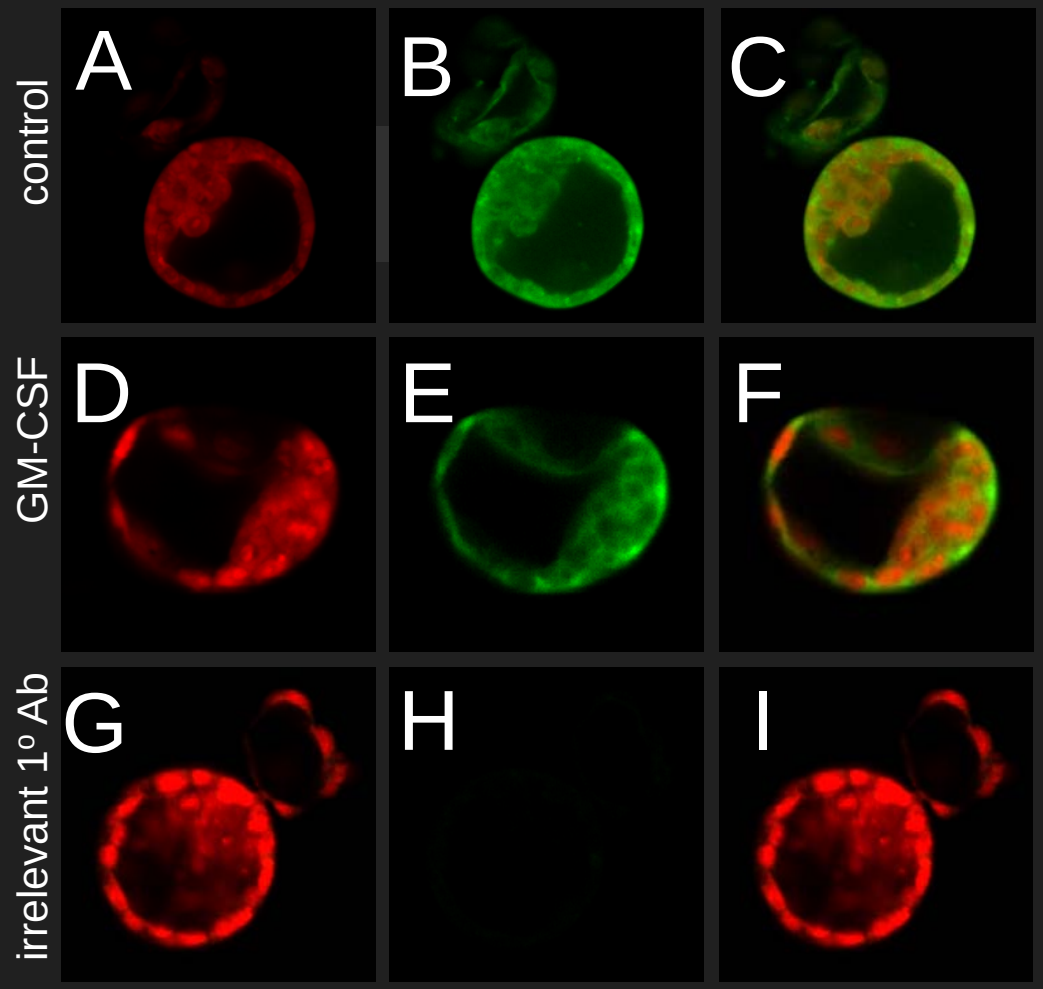


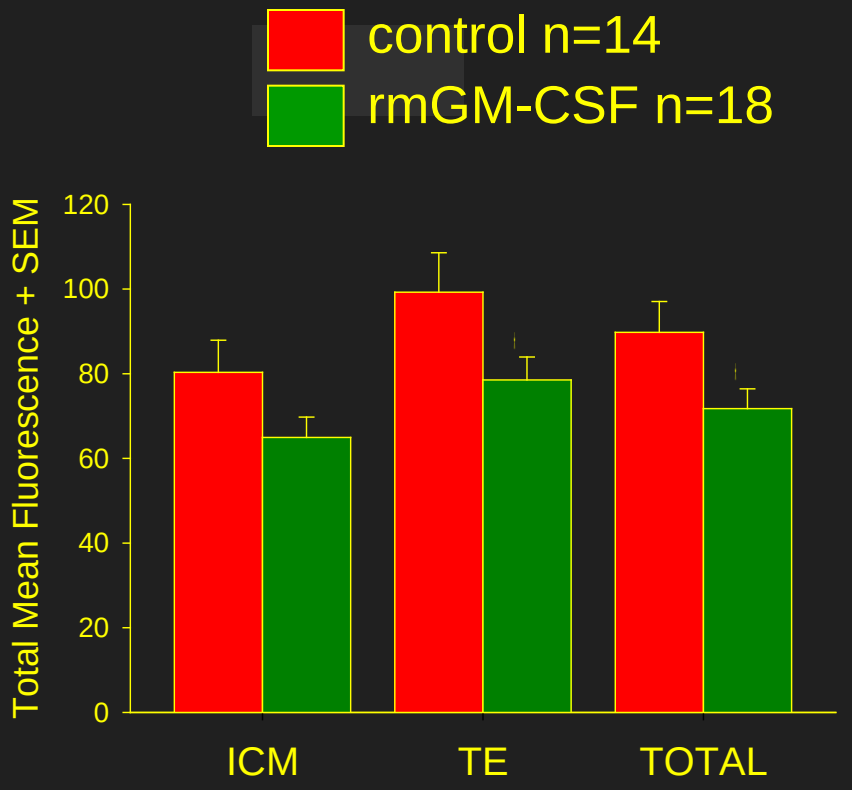


independent t-test, * P ≤ 0.03

(Chin et al *Human Reproduction* 2009)

Effect of GM-CSF on Bcl2 protein in blastocysts

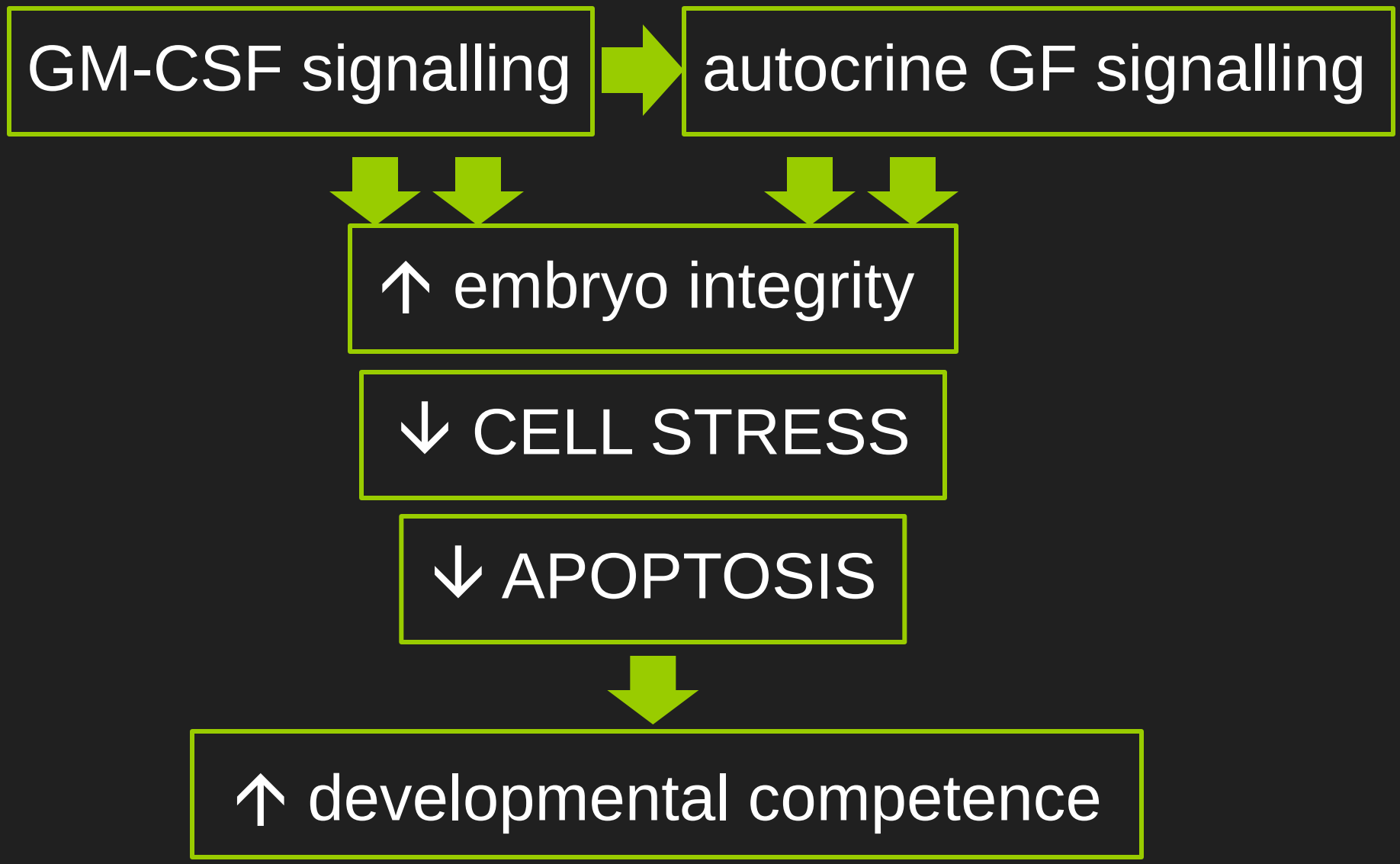




independent t-test, * P ≤ 0.05

(Chin et al *Human Reproduction* 2009)

Gene pathways influenced by GM-CSF



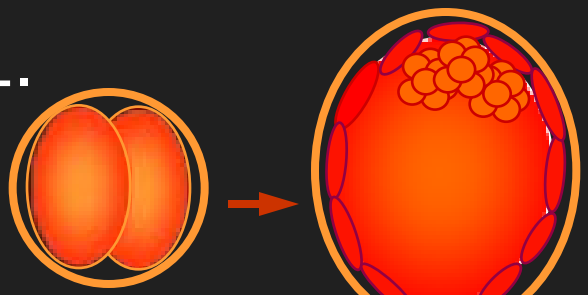
Research Question

How important is early embryo exposure to GM-CSF in later fetal development?

Could GM-CSF act to ‘program’ the embryo for late fetal and post-natal health?

Effect of embryo exposure to GM-CSF on later fetal and placental development

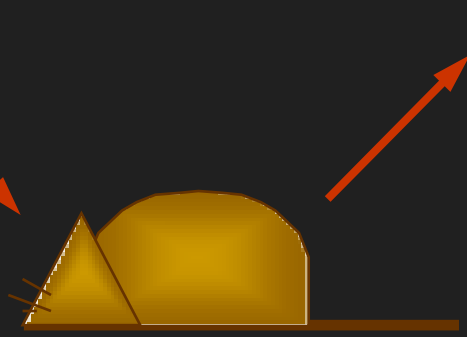
1.



A. In vitro:
control medium
(n=415)

B. In vitro:
+ GM-CSF (n=483)

C. In vivo (n=383)



2. embryo transfer

3. late gestation outcome E18

(Sjöblom et al. *Endocrinology* 2005)

Effect of GM-CSF on fetal and placental weights

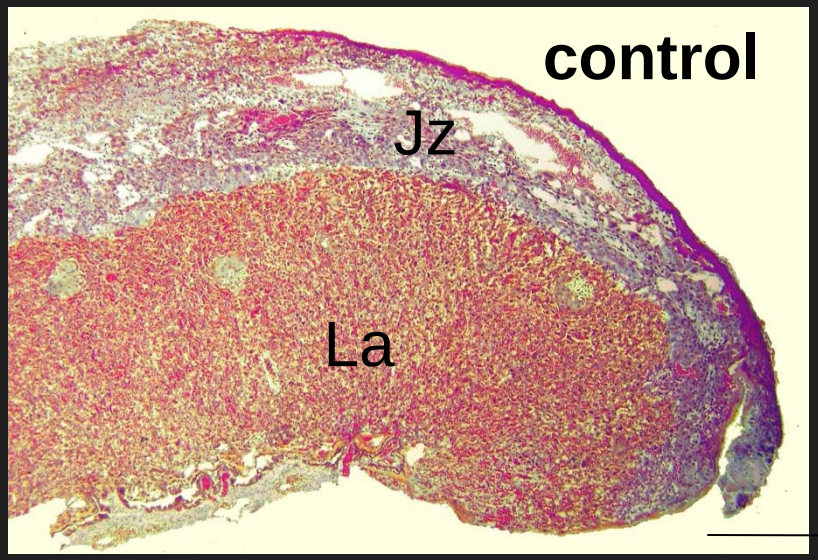
	in vivo	control	+ GM-CSF
n	280	317	316
# pregnant	29/29	29/32	29/32
fetal weight	1291 ± 13	1160 ± 10†	1206 ± 9*
placental weight	123 ± 2	123 ± 2	124 ± 2
fetal:placental ratio	10.9 ± 0.2	9.7 ± 0.1†	10.0 ± 0.1*

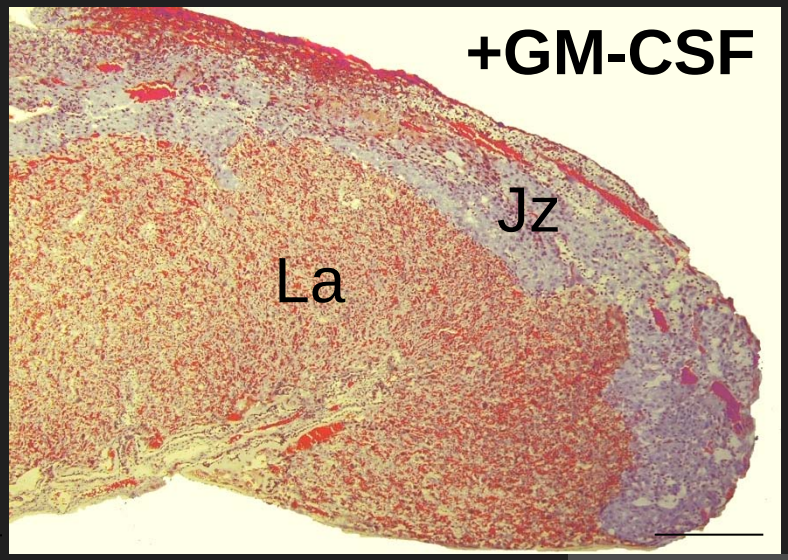
† p <0.05 vs. in vivo group * p <0.05 vs. medium only group

Data are mean ± SEM

(Sjöblom et al. *Endocrinology* 2005)

Effect of GM-CSF on placental structure at E18



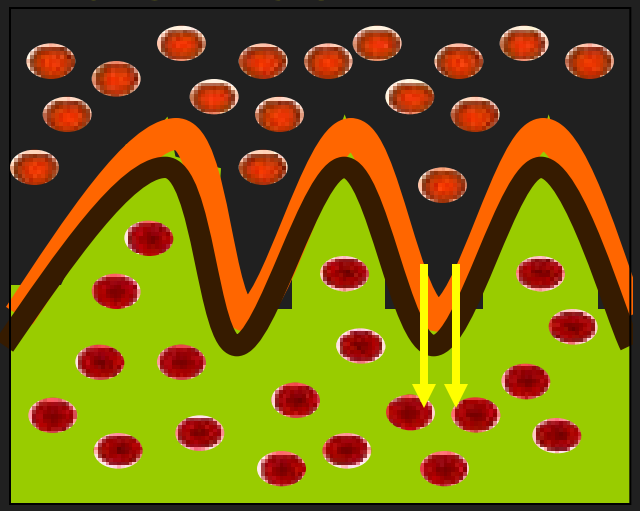


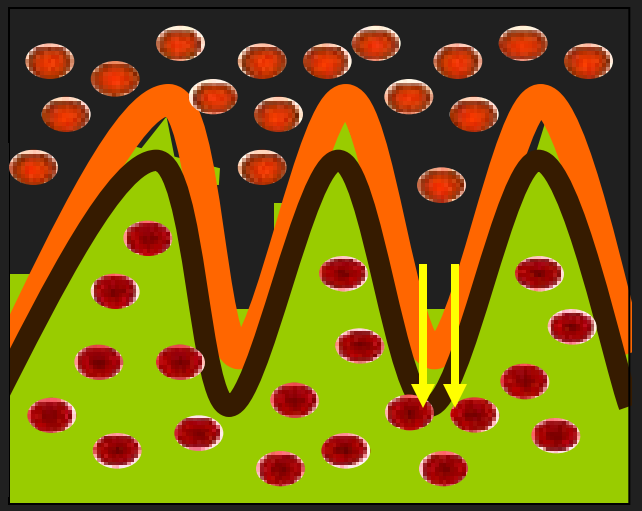
	in vivo	control	+GM-CSF
junctional zone	49 ± 0.2	52 ± 1.4 †	48 ± 0.6 *
labyrinth	51 ± 0.2	48 ± 1.4 †	52 ± 0.6 *
La : Jz	1.04	0.95† (↓27%)	1.07 *

† p <0.05 vs. in vivo group, * p <0.05 vs. GM-CSF group

(Sjöblom et al. *Endocrinology* 2005)

Effect of GM-CSF on placental exchange function



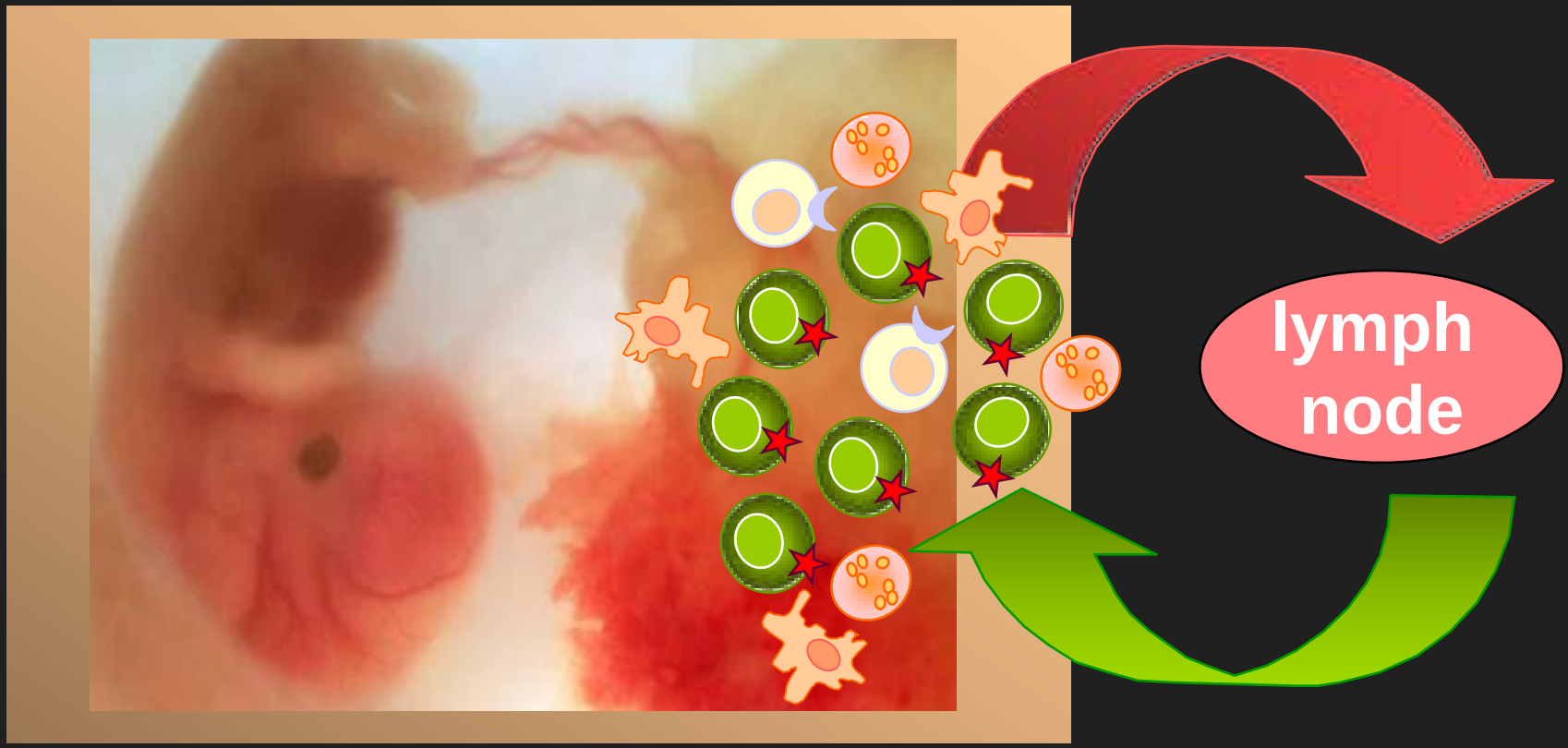


SURFACE AREA	++	++++
LABYRINTH MASS		
NUTRIENT TRANSPORT	++	++++

GM-CSF ↑ placental function

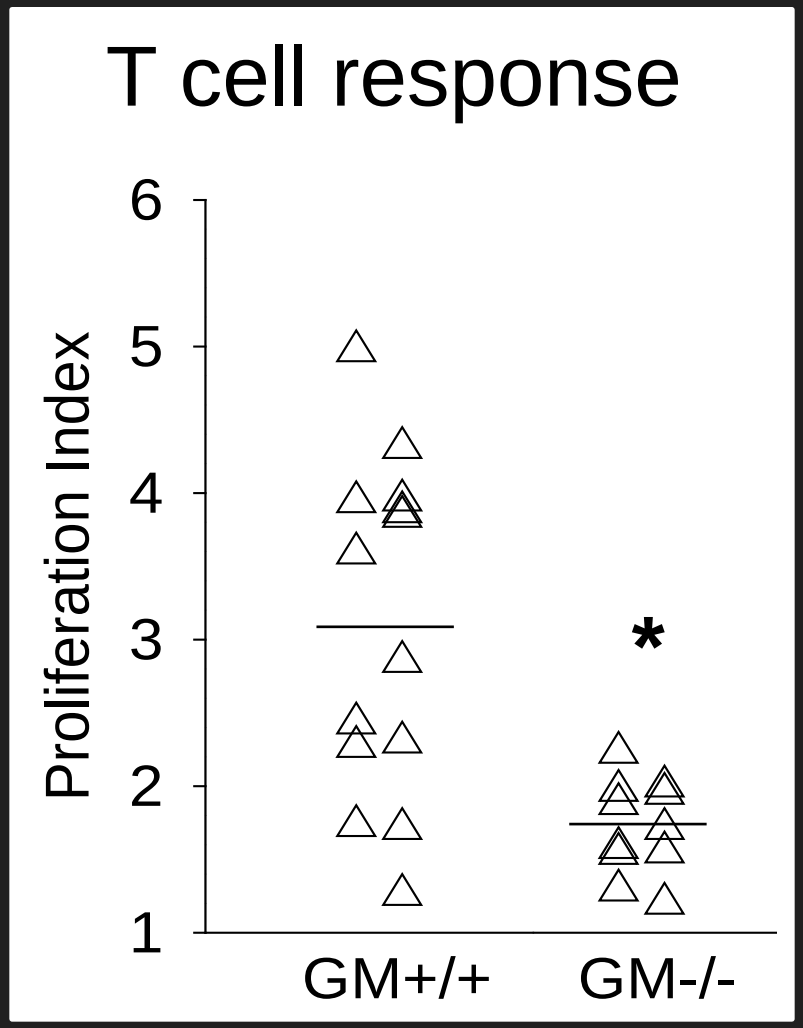
(Sjöblom et al. *Endocrinology* 2005)

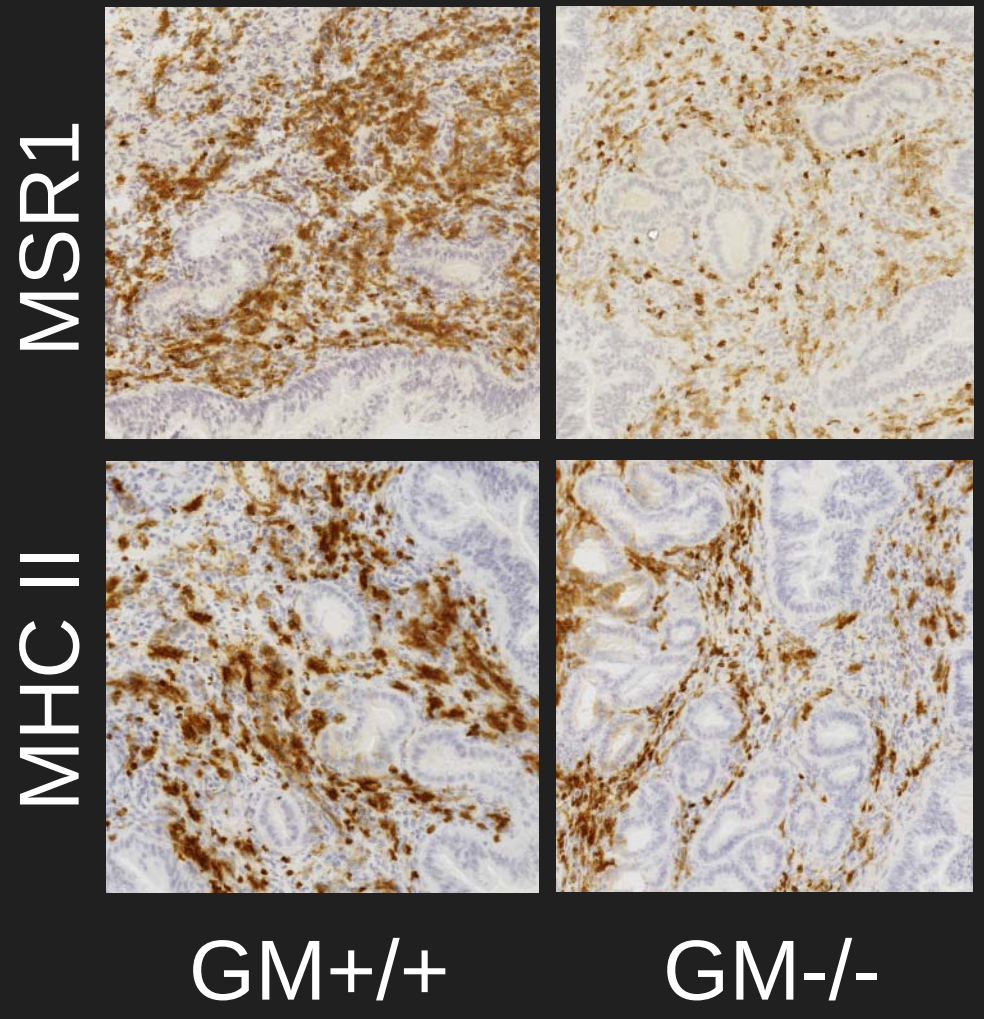
GM-CSF and the immune response to pregnancy



Immune quality control of implantation

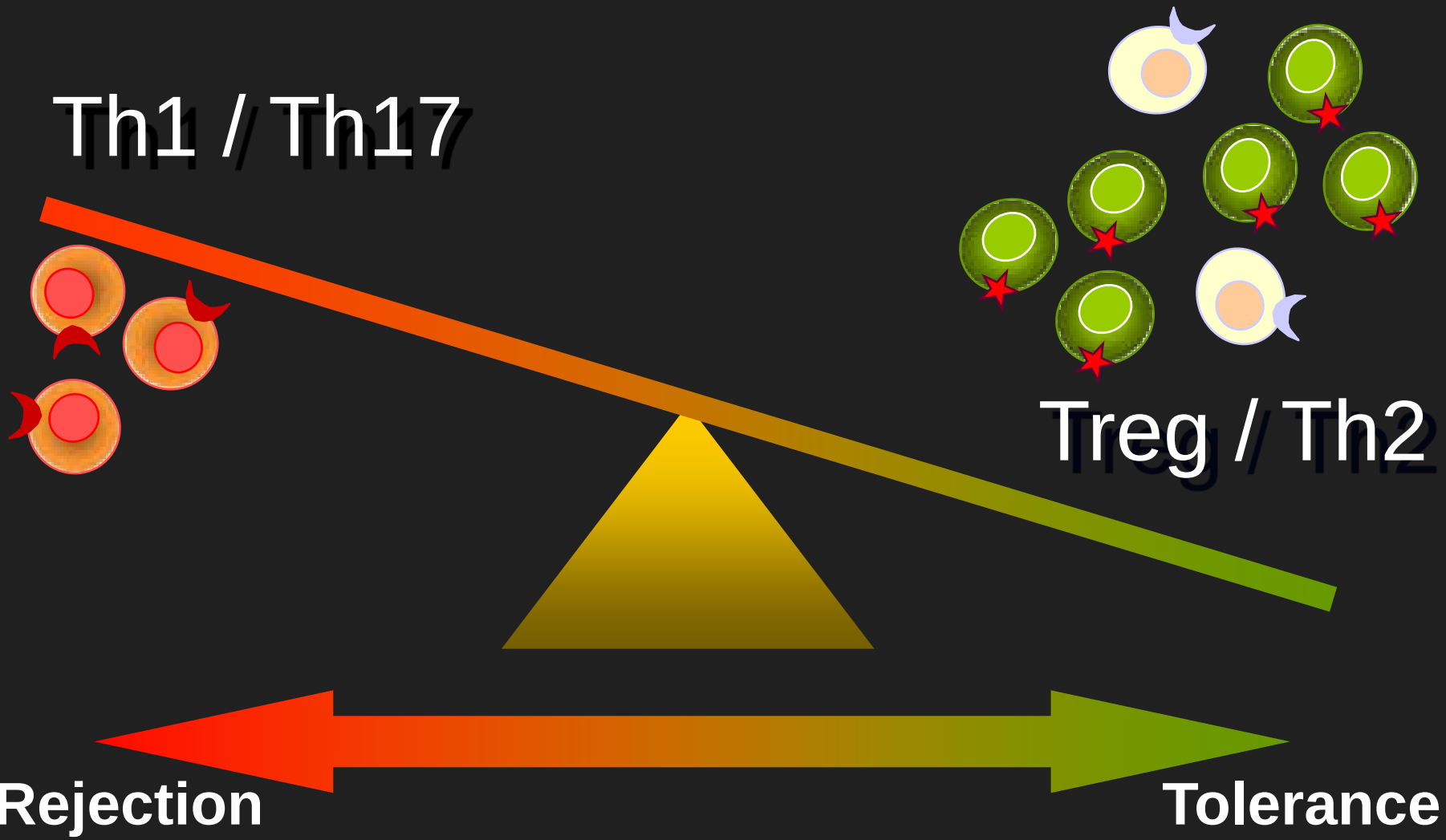
Effect of GM-CSF null mutation on antigen presentation and T cell activation





(Moldenhauer et al. *J Immunology* 2010)
(Robertson et al. *J Reprod Immunol* 1994)

Immune balance and implantation success

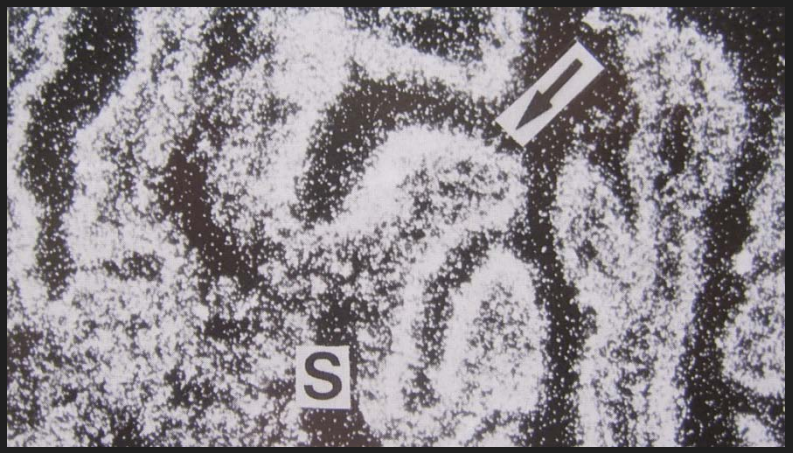


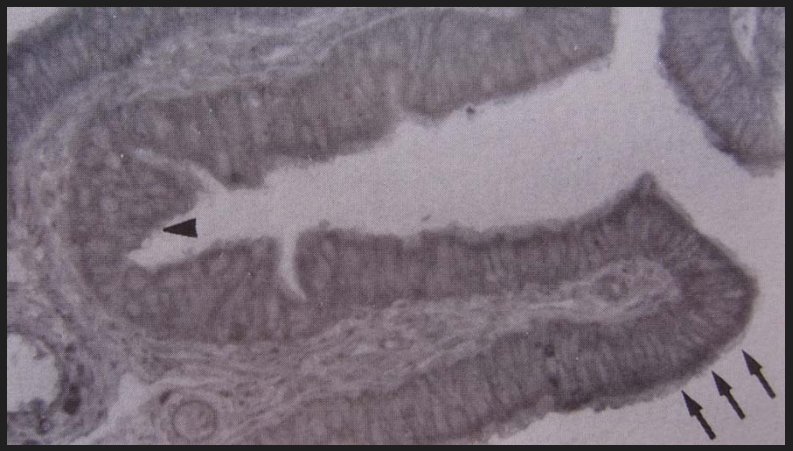
Conclusion

Peri-conceptual GM-CSF assists implantation success and pregnancy outcome through:

1. Promoting robust embryo development, reducing cellular stress and inhibiting apoptosis
2. Programming developmental trajectory, resulting in optimal placental development and function
3. Stimulating immune system to promote quality control to ensure only healthy embryos implant

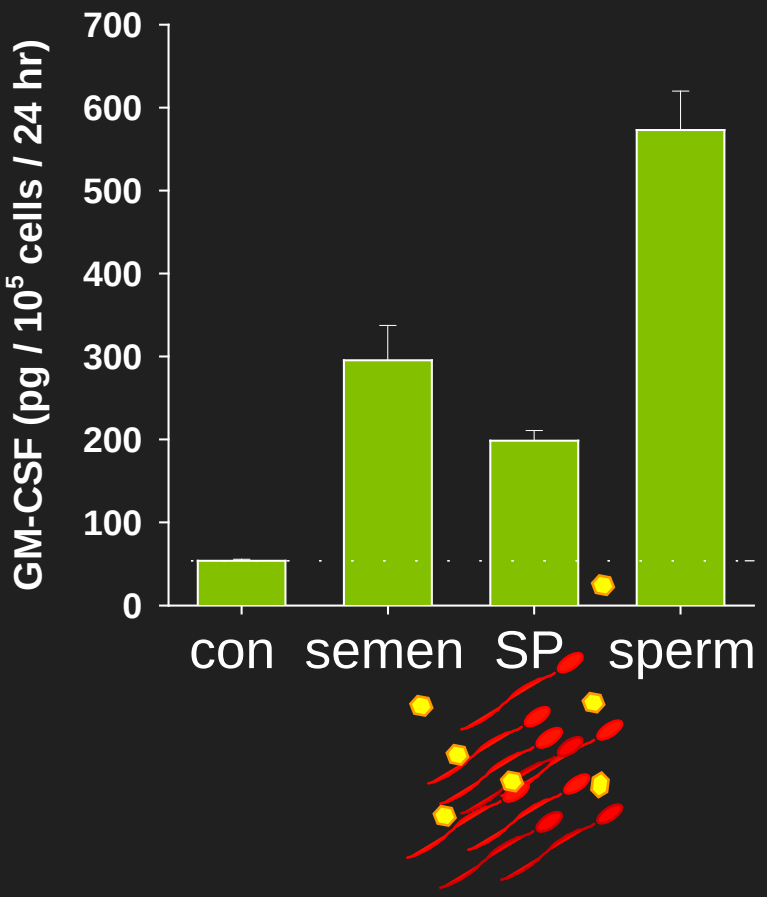
GM-CSF expression in human uterus and oviduct





- GM-CSF is expressed in epithelial cells of oviduct - maximal in early secretory phase
  (Zhao and Chegini, JCEM 1994)
- GM-CSF is expressed in epithelial cells of uterus - maximal in mid-secretory phase
  (Giacomini et al., Hum Reprod 1995; Chegini et al., MHR1999)
- GM-CSF is abundant in uterine luminal fluid
  (Paiva et al., Hum Reprod 2011)

Regulation of GM-CSF in human FRT cells

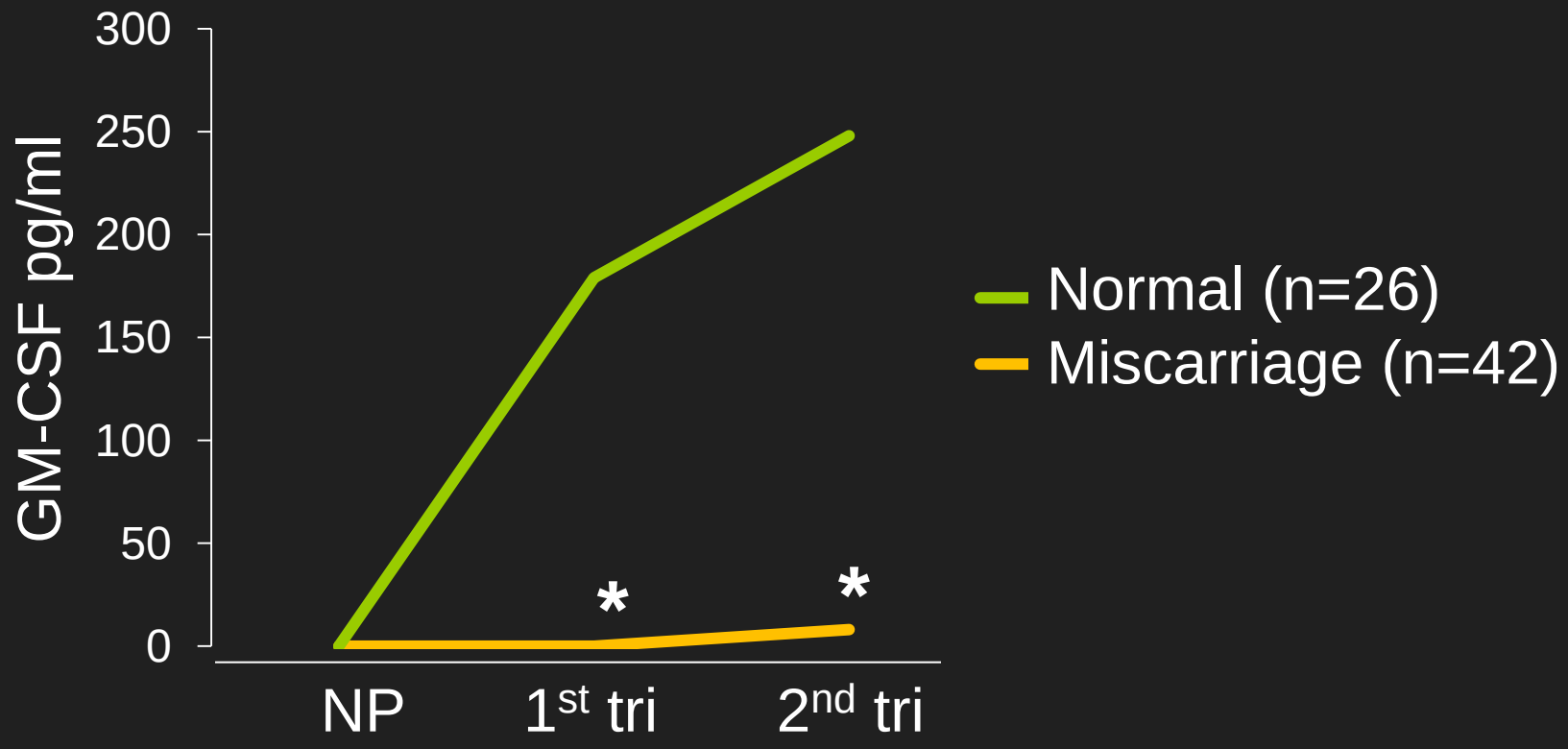


- GM-CSF is regulated by E and P & induced by seminal plasma and sperm
  (Sharkey et al., MHR 2007; Sharkey & Robertson, unpub)
- GM-CSF is induced by TLR ligands and suppressed by IFN□
  (Sharkey & Robertson, unpub)
- GM-CSF is induced by hCG
  (Paiva et al., Hum Reprod 2011)

GM-CSF and reproductive dysfunction in women

- Serum GM-CSF in pregnancy is reduced in women with recurrent miscarriage

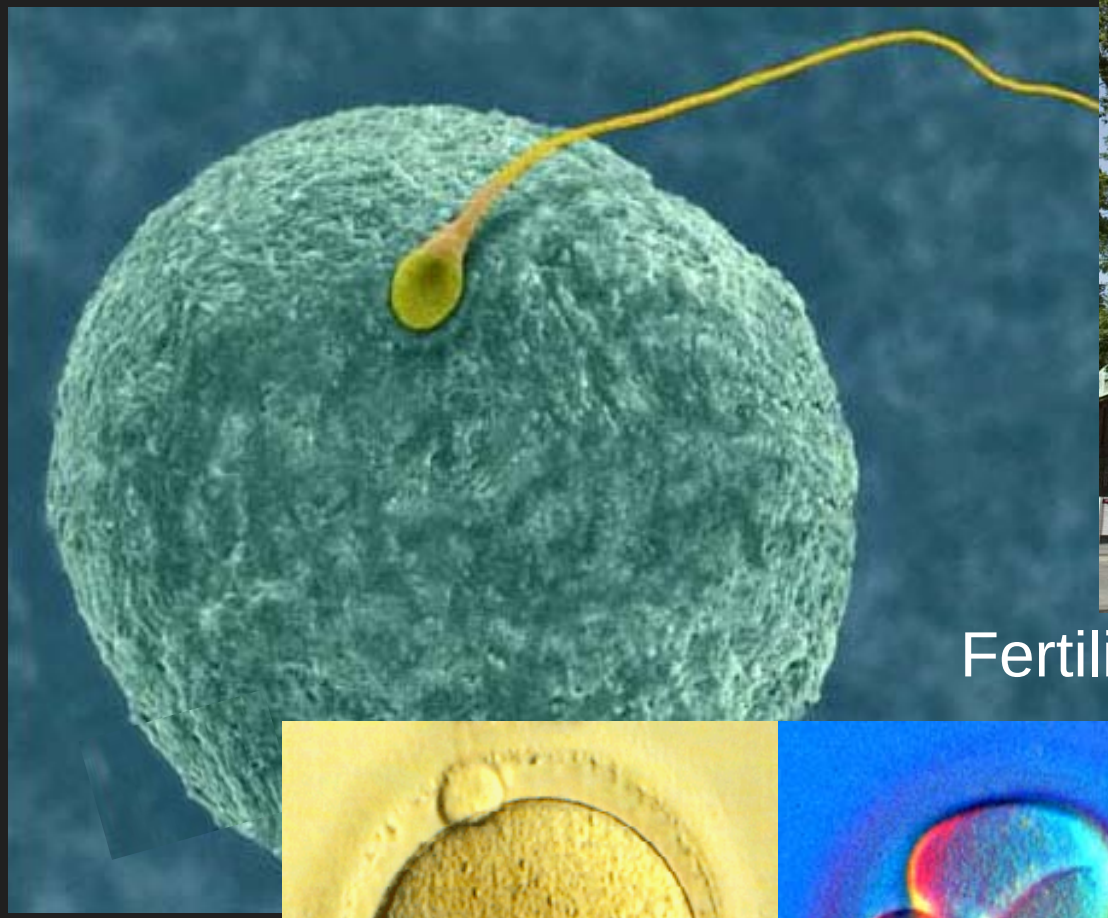
  (Perricone et al., Am J Reprod Immunol 2003)



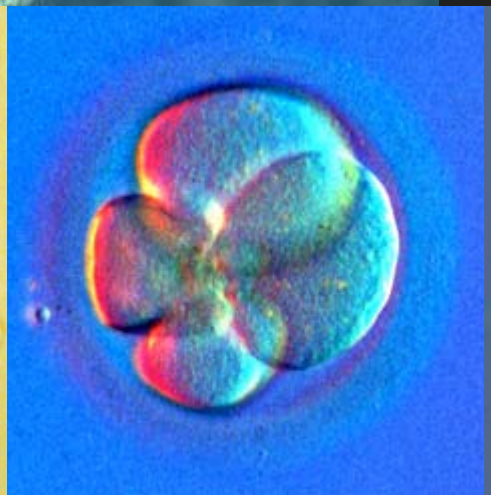
GM-CSF and reproductive dysfunction in women

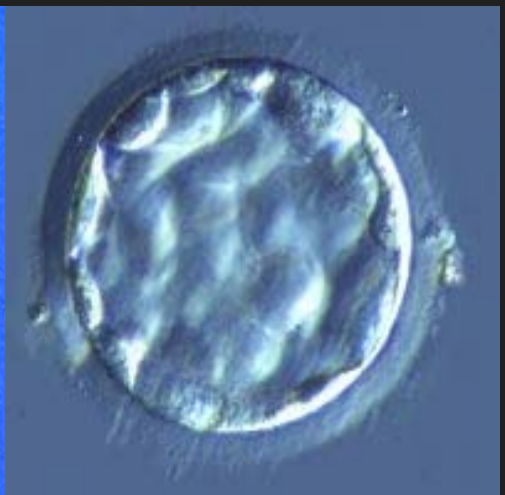
- GM-CSF synthesis by endometrial epithelial cells is associated with IVF success
  (Spandorfer et al., Am J Reprod Immunol 2008)
- Follicular fluid GM-CSF is reduced in women experiencing unexplained infertility
  (Calogero et al., Cytokine 1998)
- Trend to reduced endometrial GM-CSF mRNA expression in cohort of women with recurrent miscarriage (Jasper et al., J Reprod Immunol 2007)

GM-CSF in human IVF?

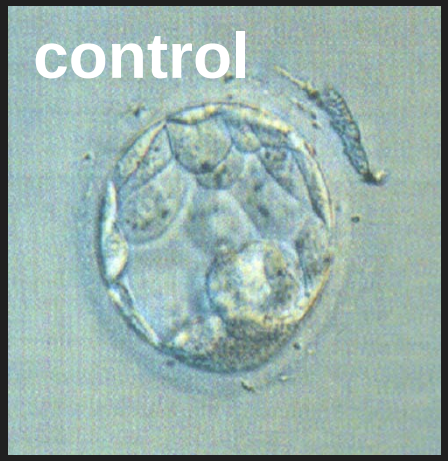


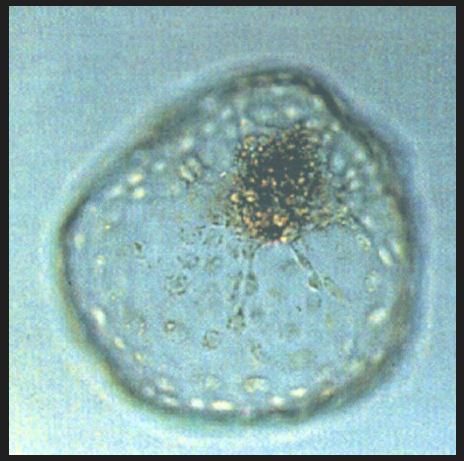
Fertilitetcentrum, Gothenburg

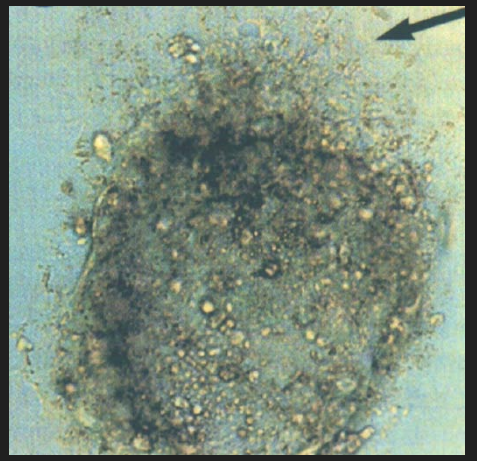




Effect of GM-CSF on human embryo development







	Control	+ GM-CSF
n	50	49
blastocyst	31%	76% *
hatch	47%	78% *
attach	0%	43% *

*P< 0.01

(Sjöblom et al. *Hum Reprod* 2000)

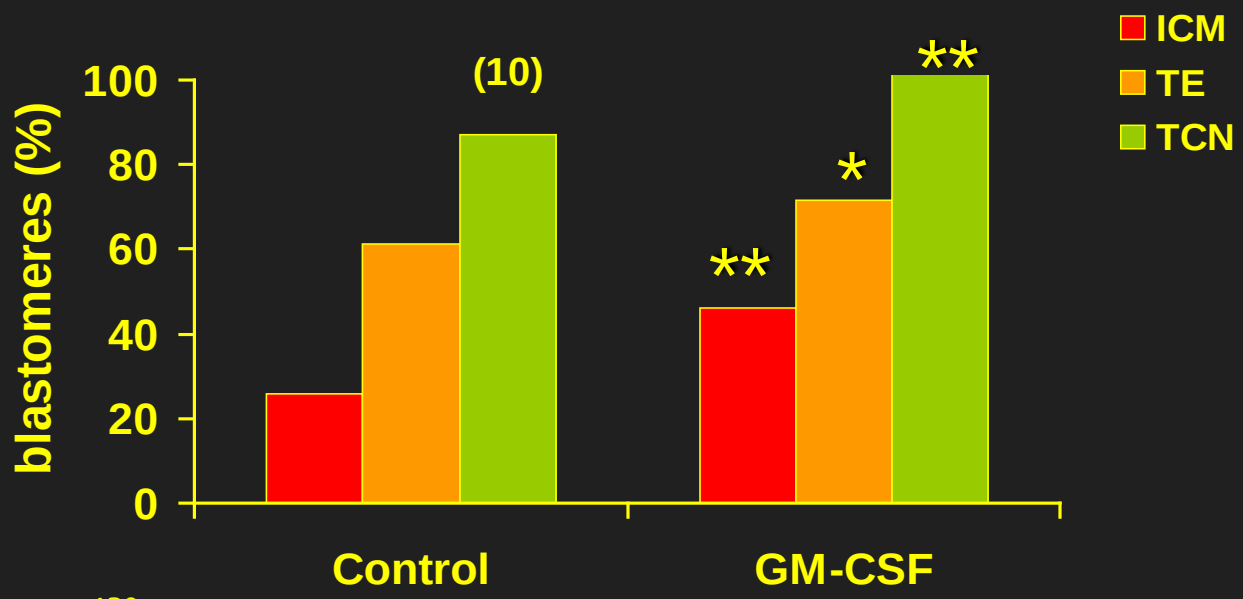
Effect of GM-CSF is not dependent on culture system

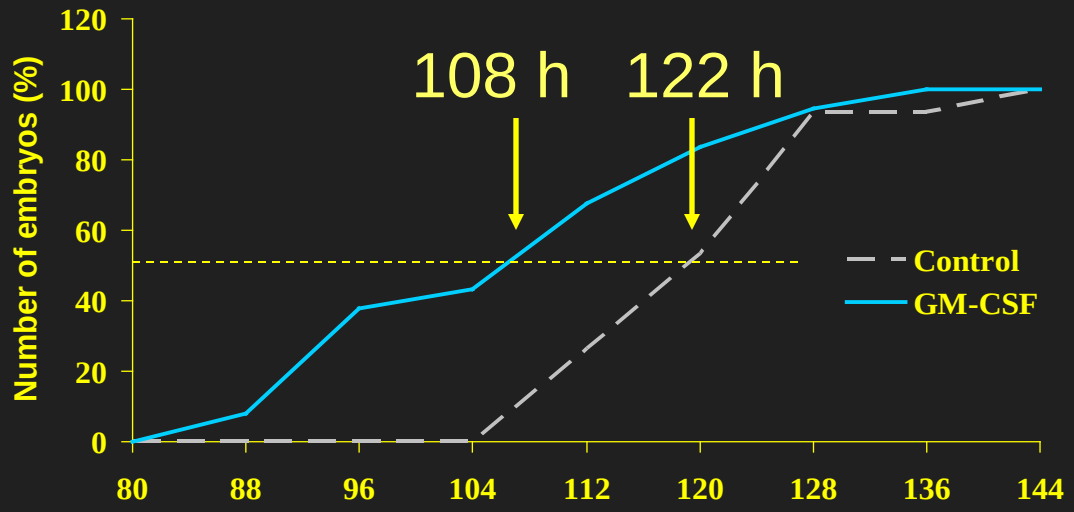
Culture system		n	% blast
Scandinavian IVF Science			
IVF-50 / S2	con	38	14 (37%)
	+GM-CSF	38	30 (79%)***
G1.2 / G2.2	con	23	7 (30%)
	+GM-CSF	21	15 (71%)**
Cook IVF			
Sydney IVF cleavage / blastocyst medium			
	con	80	29 (36%)
	+GM-CSF	82	58 (71%)***

**p < 0.01, *** p< 0.005

(Sjöblom et al. *Hum Reprod* 1999)
(Sjöblom et al. *Biol Reprod* 2002)

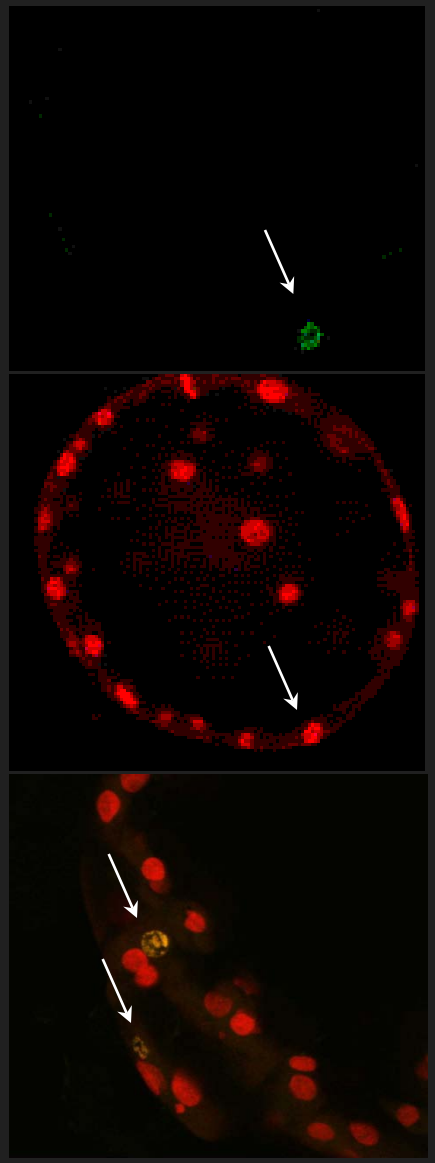
Effect of GM-CSF on human embryo quality





(Sjöblom et al. *Hum Reprod* 2000)

Effect of GM-CSF on apoptosis in human blastocysts



	Control	GM-CSF
n	29	32
total apoptosis	4.9%	2.1%**
ICM apoptosis	6.3%	1.5%**
TE apoptosis	4.2%	2.6%*

**p < 0.01, * p< 0.05

(Sjöblom et al. *Hum Reprod* 2000)

Summary: effects of culture with GM-CSF on human embryo development

- embryos express GM-CSF receptors
- GM-CSF doubles number of embryos reaching blastocyst stage, and increases hatching and attachment *in vitro*
- Effect is not dependent on culture media system
- GM-CSF accelerates blastocyst development by 14h
- GM-CSF increases cell number by 35%
- GM-CSF reduces apoptosis by 50%

Translation of GM-CSF to the IVF clinic

- GM-CSF is a necessary component of an ‘optimal’ environment for pre-implantation embryos
- Human trials using GM-CSF addition to IVF embryo culture media were warranted
- In 2005, we formed a commercial partnership with ORIGIO a/s (Denmark) to evaluate efficacy of GM-CSF in human IVF

GM-CSF does not adversely affect embryo karyotype

	medium	+GM-CSF
number embryos	32	24
number FISH	27	23
overall normal	50%	67%
uniformly normal	28%	33%

All chromosomes in all cells in all embryos assessed

Agerholm, Ziebe et al. *Reprod Biomed Online 2010*

Clinical trial to evaluate GM-CSF in human IVF

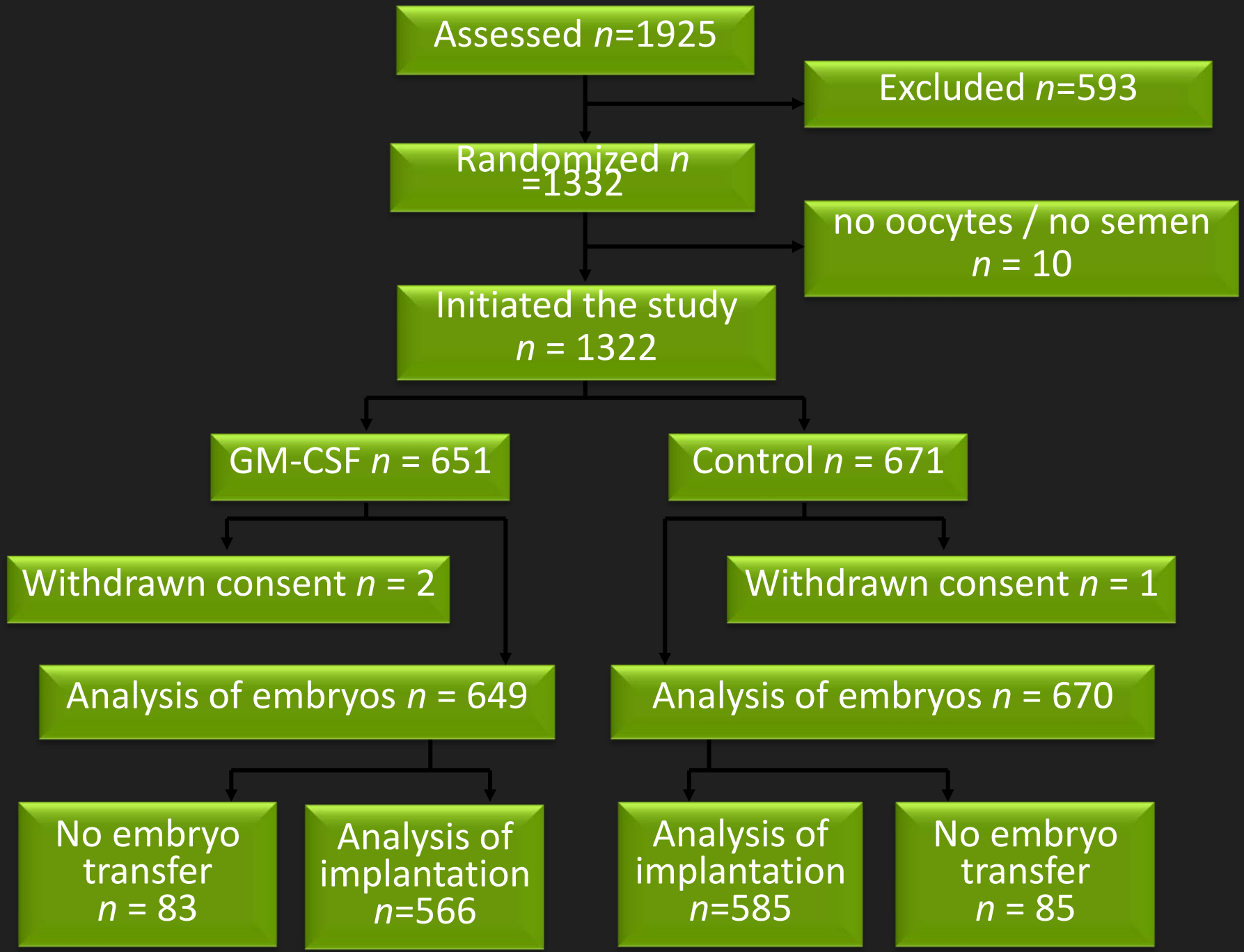
- Multicentre, placebo-controlled, randomised, double-blinded trial to evaluate effect of GM-CSF on IVF outcomes completed with ORIGIO a/s and Soren Ziebe (University Hospital of Copenhagen)
- 1332 IVF patients, 14 IVF clinics in Denmark and Sweden
- day 3 transfers, 1-2 embryos transferred
- 2 ng/ml GM-CSF in fertilisation, culture and transfer medium
- primary endpoint = ongoing implantation rate at week 7

A randomized clinical trial to evaluate the effect of granulocyte-macrophage colony-stimulating factor (GM-CSF) in embryo culture medium for in vitro fertilization

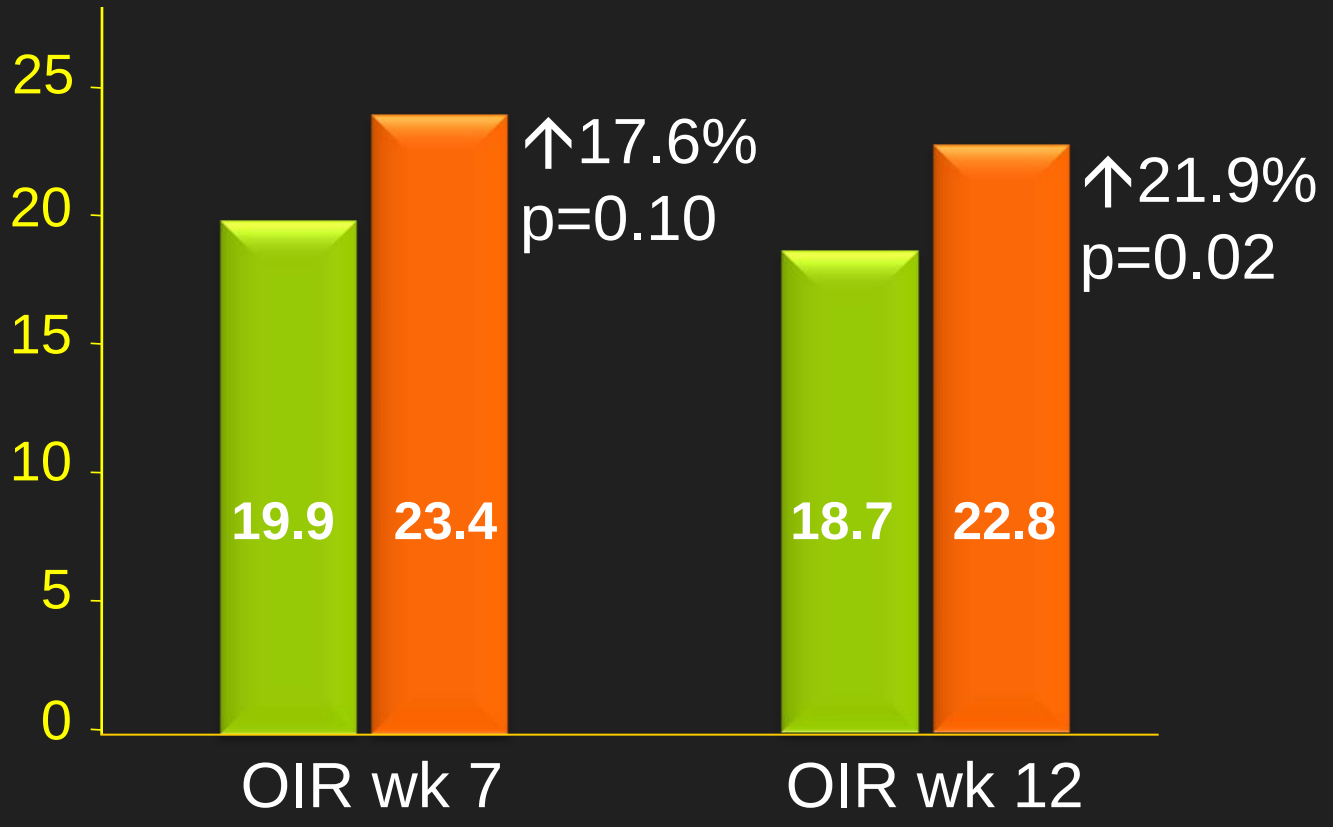
Søren Ziebe, M.Sc., D.Sc.,[a] Anne Loft, M.D.,[a] Betina B. Povlsen, M.Sc.,[b] Karin Erb, M.Sc.,[c] Inge Agerholm, Ph.D.,[d] Michael Aasted, M.D.,[e] Anette Gabrielsen, M.Sc.,[f] Christina Hnida, Ph.D.,[g] Dorit P. Zobel, Ph.D.,[h] Bibi Munding, M.Sc.,[h] Susanne H. Bendz, Ph.D.,[h] and Sarah A. Robertson, Ph.D.[i]

[a] Fertility Clinic, Rigshospitalet, University Hospital of Copenhagen, Copenhagen, Denmark; [b] Fertility Clinic, Skive Regional Hospital, Skive, Denmark; [c] Fertility Clinic, Odense University Hospital, Odense, Denmark; [d] Fertility Clinic, Braedstrup Hospital, Braedstrup, Denmark; [e] Fertility Clinic Dronninglund, Aalborg University Hospital, Dronninglund, Denmark; [f] Ciconia Aarhus Private Hospital, Aarhus, Denmark; [g] Fertility Clinic, Herlev University Hospital, Copenhagen, Denmark; [h] ORIGIO, Måløv, Denmark; and [i] Robinson Institute, School of Paediatrics and Reproductive Health, University of Adelaide, Adelaide, South Australia, Australia

Ziebe et al. *Fertility and Sterility* May;99(6):1600-1609, 2013

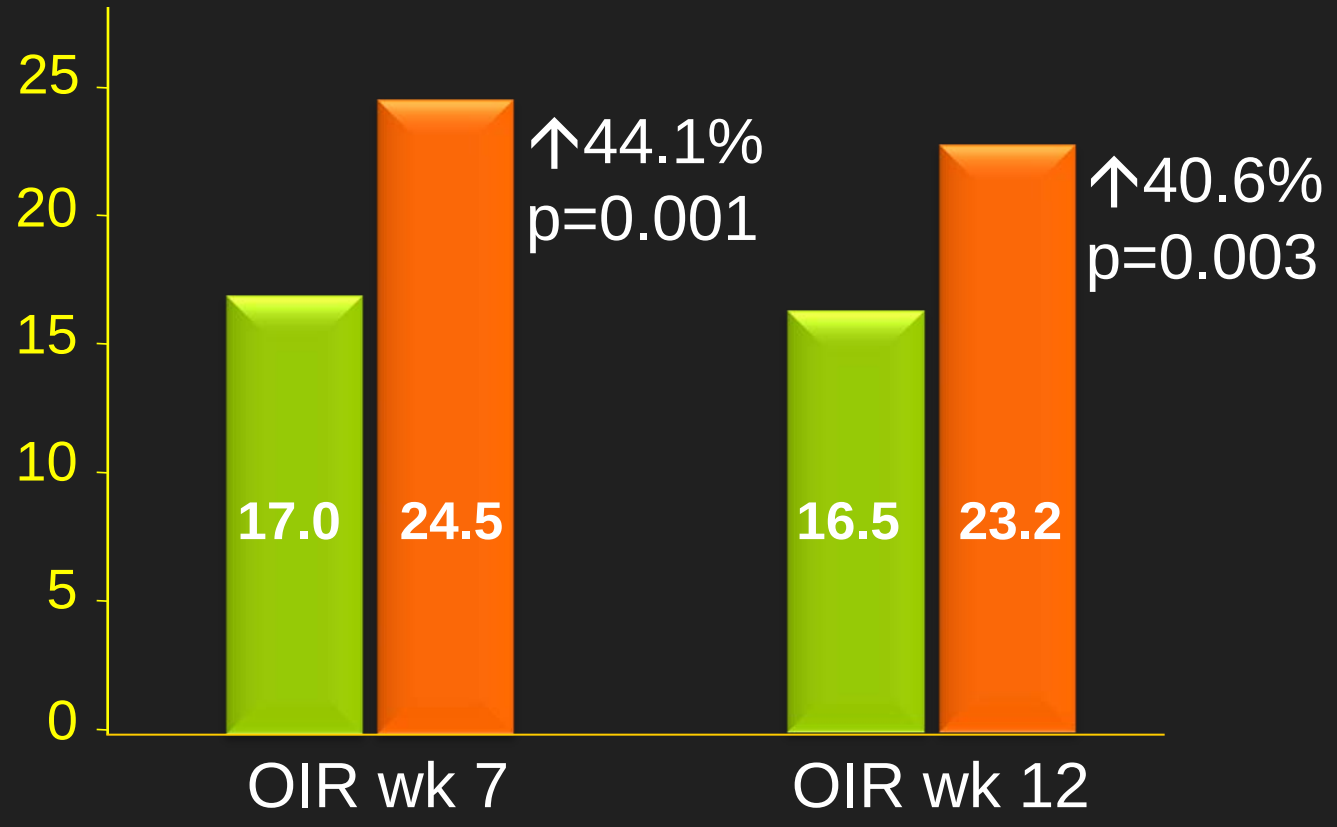

Assessed n=1925
Excluded n=593
Randomized n =1332
no oocytes / no semen n = 10
Initiated the study n = 1322
GM-CSF n = 651
Control n = 671
Withdrawn consent n = 2
Withdrawn consent n = 1
Analysis of embryos n = 649
Analysis of embryos n = 670
No embryo transfer n = 83
Analysis of implantation n=566
Analysis of implantation n=585
No embryo transfer n = 85

Effect of GM-CSF on implantation rate (all women)



OIR = ongoing implantation rate
(viable embryos / embryos transferred)

Effect of GM-CSF on implantation rate (women with previous miscarriage)



OIR = ongoing implantation rate
(viable embryos / embryos transferred)

Effect of GM-CSF on perinatal endpoints

- 18.5% increase in children born (p=0.042)
- no effect on gestational age at delivery, perinatal death
- no effect on fetal abnormality
- no effect on rate of multiple pregnancies
- no effect on fetal weight

Effect of GM-CSF on pregnancy progression

	GM-CSF	Control	*P value*
No. of women with transfer	564	585	
Positive hCG (N, % cycles)	214 (37.9)	218 (37.3)	0.46
Early pregnancy loss ≤12wk (N, % positive hCG)	49 (22.9)	73 (33.5)	0.02
Biochemical pregnancy	29 (13.6)	44 (20.2)	0.07
Ectopic pregnancy	4 (1.9)	2 (0.9)	0.45
Miscarriage (wk 7→12)	16 (7.5)	27 (12.4)	0.11
Live birth	163	141	
Children born	194	164	

Take-home message

- GM-CSF is present in human reproductive tract and may be dysregulated in fertility disorders
- GM-CSF is essential for embryo protection from stress and optimal development
- GM-CSF promotes implantation success and developmental competence in embryos
- providing GM-CSF to embryos supports robust placental development and fetal health

Embryogen: Product Launch at ESHRE 2011

- New treatment option for women with previous miscarriage (IVF or natural conception)

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Claire Roberts
Michelle Lane
Anne Macpherson
Loretta Chin

Robinson Institute
University of Adelaide
AUSTRALIA

Cecilia Sjoblom
Mats Wikland

Fertilitetscentrum, Goteborg
SWEDEN

Soren Ziebe
Sussi Bendz

University Hospital of Copenhagen
ORIGIO a/s, Måløv, DENMARK

National Health and Medical Research Council

Robinson REPRODUCTIVE HEALTH Institute

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SUB CRUCE LUMEN