Genetics of Early Development

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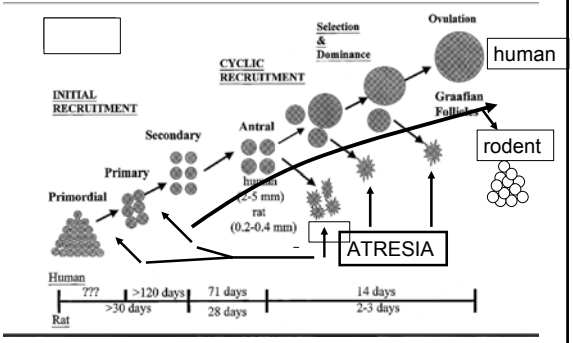
What is 'Genetics'?

1. Chromosome complement – aneuploidies; PGD; mosaicism
2. Mitochondrial (mt)DNA - little human data
3. Epigenetics - ????

Need for more information about frequencies and etiologies; effects of infertility, patient age, ART procedures, **and ovarian stimulation**

Follicular recruitment in humans and rodents



McGee and Hsueh, Endocr. Rev. 2000

Consequences of Superovulation

- > normal number of dominant follicles
- some destined for atresia, rescued
- heterogeneous cohort of oocytes, some will be defective
- defective oocytes can be fertilized, and
- some embryos will be defective but perhaps appear normal; unable to develop to term
- problem much greater with higher primates (human, rhesus monkey) than with rodents

The "Superovulation Coefficient"

Rodents: natural cycle 10-12 ovulatory follicles, highly efficient, litter almost as large.
E.g., hamster: no. of blastocysts per natural cycle = no. of CLs
"Superovulation Coefficient" [30-50 oocytes or embryos/10-12] = **3-5**

Humans: natural cycle 1 (2) oocyte or embryo
"Superovulation Coefficient" [20-25 oocytes] = **20-25**!

Comparison of ovarian stimulation outcomes in rodents vs. humans

No. of follicles/oocytes

Species	Natural cycle	Ovarian stimulation	"super-ovulation coefficient"
rodent	10 - 12	30 - 50	3 - 5
human	1 (2)	20 - 25	>20

Relationship between ovarian stimulation and oocyte quality

no. of oocytes ↑ oocyte quality ↓

Large cohorts of heterogeneous oocytes create serious problems for the clinical embryologist!

Problems using human oocytes or embryos to derive data on frequency and source of genetic anomalies

- Oocytes/embryos in cohort are heterogeneous; best embryos selected for transfer; mosaicism can complicate interpretation
- There are no in vivo (IVO) fertilized embryos for comparative database

Animal models for human follicle recruitment and pregnancy

Species	No. ovulatory follicles	Cycle length (days)	Type of cycle	Gestation length (months)
Human	1	28	menstrual	9
Rodents	10 - 12	4 - 5	estrous	0.5 - 0.75
Dairy cattle	1	21	estrous	9
Rhesus monkey	1	28	menstrual	5.5

Advantages of embryo research with rhesus monkeys

Closely related to humans: share >90% DNA homology (both are primates!)

Embryology, endocrinology and blastocyst/implantation very similar

IVF/embryo culture technology well established – numerous offspring born

Can obtain in vivo produced embryos for comparison!

"Petri" – first IVF monkey

Data using the rhesus monkey as a translational model

Experimental design

Four regimens:

A. FSH + hCG (human recombinant) } same female
B. FSH only, IVM } same female
C. No/low FSH (clomiphene?); hCG to drive oocyte maturation and fix timing of collection
D. No stimulation, excised ovaries, IVM*

Compare oocyte and embryo quality/defects

Brenner and Bavister Labs

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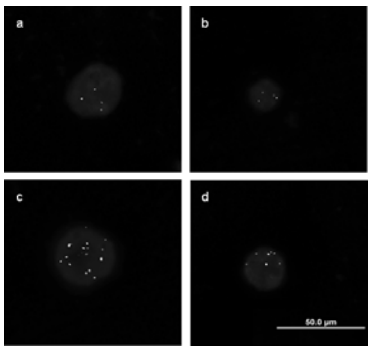
Mosaicism in primate embryos

- 25 - 62% of human preimplantation embryos are mosaic; the average percentage of aneuploid blastomeres within mosaic embryos is 54 - 60%
- Following data from 'good quality day 3' rhesus macaque embryos - ≥ 70% of their blastomeres were cytogenetically analyzed using BAC-FISH probes for macaque chromosomes X, Y, 17, 18 and 20 (homologous to human chromosomes X, Y, 13, 18 and 16 respectively (Dupont et al., submitted – Brenner lab, WSU)

Five-color FISH assay on rhesus macaque blastomere nuclei using pooled BAC probes.



Macaque chromosomes X (green), Y (orange), 17 (red), 18 (blue) and 20 (yellow) each are identified by dot-like or double-dot signals. 2A; haploid female blastomere. 2B; haploid male blastomere. 2C; chaotic blastomere. 2D; monosomy X.

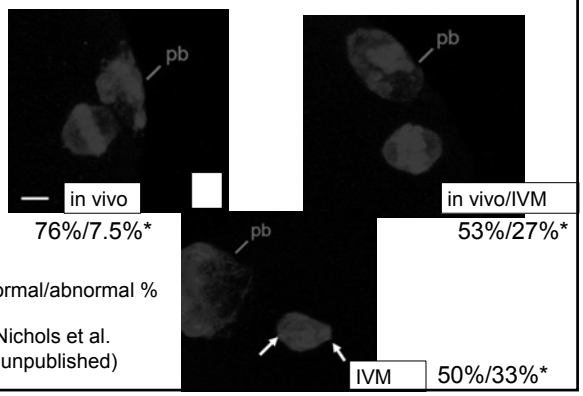
Dupont et al., Fertil. Steril. 2008

Chromosomal complement of IVP Rhesus macaque preimplantation embryos

Embryo classification	No. embryos	No. blastomeres abnormal	normal	total
Normal	41 (51%)		311	311
Completely abnormal*	15 (19%)	107 (100%)		107
Partially abnormal (mosaic)	24 (30%)	116 (52%)	108 (48%)	224

* Completely abnormal embryos represent haploid, polyploid, aneuploid and chaotic embryos

MII spindle defects in IVM rhesus oocytes



*normal/abnormal %

Nichols et al. (unpublished)

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Anomalies in human IVP embryos

mtDNA anomalies in oocytes; increase with age of patient

>50% of IVP human embryos are from women >35 years old (CDC 2003)

Numerous laboratories have detected a particular mtDNA mutation, the "common deletion," ΔmtDNA4977, in human oocytes at a frequency of 30% to 50%

Expect significant frequency of mtDNA defects in human embryos (and ES cell lines); but not examined to date

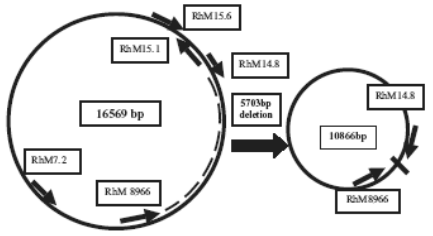

Figure 1. Nested PCR strategy used to detect the rhesus common deletion in the mitochondrial DNA (mtDNA) genome. Primers RhM15.1 (inside arrow) and RhM15.6 (outside arrow) were used as an internal control for the presence of mtDNA. RhM15.6 (outside arrow) and RhM7.2 (inside arrow) were used in the first PCR amplification. Primers RhM14.8 (outside arrow) and RhM8966 (inside arrow) were used for the nested PCR to identify the 5703 bp deletion. The dashed inside line represents the 5703 rhesus common deletion which is removed in the nested PCR if mtDNA mutations are present in the sample. Black bar indicates deleted base pairs.

Data from Gibson et al. (2005) Mol. Hum. Reprod. 11:785-789

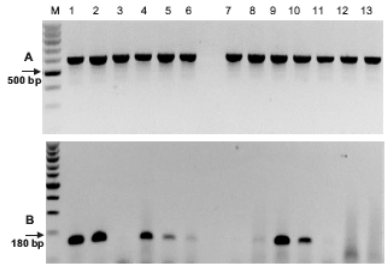

Figure 3. Rhesus control mitochondrial DNA (mtDNA) and common deletion in rhesus gonadotrophin-stimulated oocytes and day 3 embryos. Lane M, 100 bp ladder. **A**, lanes 1–6 demonstrate the internal control region amplified with primers RhM15.6 and RhM15.1 showing a 599 bp band in stimulated oocytes. Lanes 7–13 in **A** show a 599 bp band control amplicon in IVF day 3 embryos. **B**, lanes 1, 2 and 4–9 show the rhesus common deletion or 180 bp amplicon generated in stimulated oocytes or day 3 embryos using primers RhM15.1 and RhM7.2 followed by RhM14.8 and RhM8966.

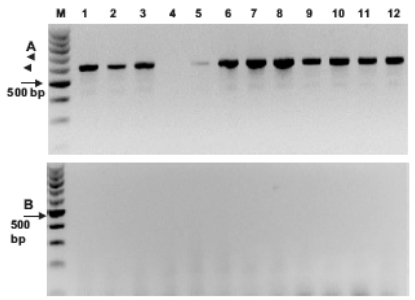

Figure 4. Rhesus mitochondrial DNA (mtDNA) control and common deletion in non-stimulated germinal vesicle (GV) oocytes. Lane M, 100 bp DNA ladder. **A**, lanes 1–12 demonstrate 590 bp mtDNA amplified with primers RhM15.1 and RhM15.6. **B**, lanes 1–12 show that no PCR amplicons were generated in the same oocytes using primers RhM15.1 and RhM7.2 followed by RhM14.8 and RhM8966.

mtDNA defect data in rhesus monkeys

Frequency of the mitochondrial DNA (mtDNA) rhesus common deletion in stimulated oocytes and embryos versus non-stimulated oocytes

	Number amplified	mtDNA deleted	mtDNA non-deleted	Frequency %
Stimulated	70	50	20	**71.4**
Non-stimulated	127	27	100	**21.3***

A ratio of the number of rhesus common deletions amplified compared to the number of samples amplified indicates the amplification frequency.
*Statistically significant difference ($P < 0.001$) by 2 ×2 *G*-test distribution.

Data from Gibson et al. (2005) Mitochondrial DNA deletions in rhesus macaque oocytes and embryos. Molec. Hum. Reprod.11:785–789.

Conclusions (mtDNA study)

1. Mitochondrial replication must occur during IVM (resumption of meiosis)
2. FSH stimulation increases the frequency of mtDNA errors in oocytes and embryos derived from them; likely detrimental to embryo viability; implications for current patient stimulation regimens

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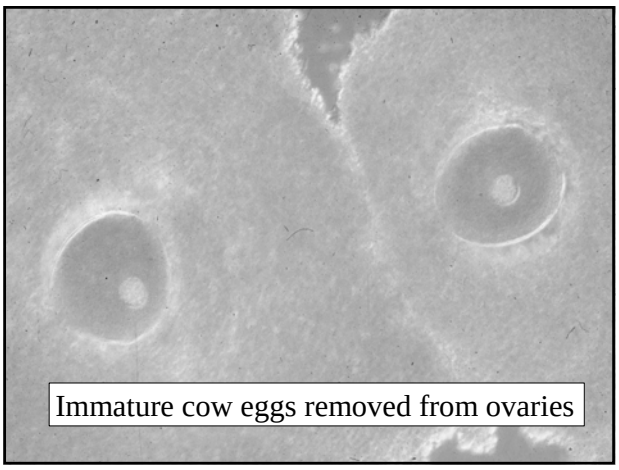
Gene expression defects in IVF embryos: animal models

differential protein production in different culture media

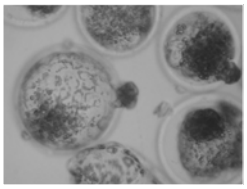
altered gene expression **compared with in vivo produced** bovine **embryos** (C. Wrenzycki data)

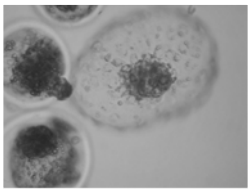
"IVP" of bovine embryos using culture technology

Bovine ovaries from slaughterhouse

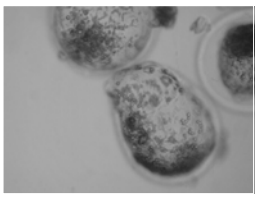


Immature cow eggs removed from ovaries





Expanded and "hatching" bovine blastocysts from IVM/IVF oocytes



Advantages of bovine embryos for genetic studies

- Large numbers of IVP embryos produced from slaughterhouse ovaries by IVM/IVF; high rates of development to blastocyst (~50% of MII oocytes)
- IVO embryos (morulae/blastocysts) readily available by superovulation, AI and uterine flushing

Comparison of Pregnancy Rates with IVP vs. in vivo-produced blastocysts (all day 7)

Blastocyst source	No. of transfers	Pregnancy Rate (%)
IVP (fresh)	4606	54[a]
in vivo	599	67[b]

Data from Hasler JF (1998) J. Anim. Sci. 76(Suppl. 3):52-74

Quality of the embryo?

- Production of live and healthy offspring
- Morphology (colour, compaction, perivitelline space, cell number)
- Developmental competence (blastocyst rate, timing of blastocyst formation)
- Metabolism
- Incidence of chromosome anomalies
- Ultrastructure
- Cryotolerance
- **Gene expression patterns**

Analyzed gene transcripts (1)

Function	Gene
Ion transport	Na^+/K^+-ATPase
Cell adhesion proteins	Gap junction proteins Desmosomal proteins und glycoproteins Cell adhesion proteins Tight junction proteins
Growth factors/ receptors	Insulin-like growth factors Basic fibroblast-growth factor
Metabolism	Glucose transporter-1, -2, -3, -4, -5, -8 Phosphoglycerate kinase Glucose-6-phosphate dehydrogenase

Analyzed gene transcripts (2)

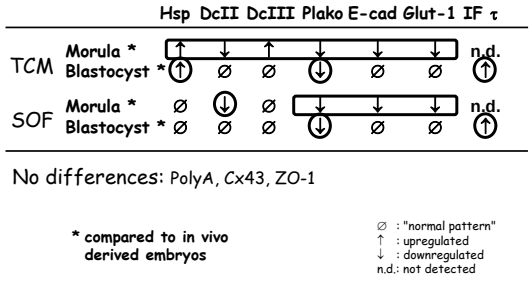
Function	Gene
Stress indicators	Heat shock proteins Superoxid dismutases Sulfhydryl oxidases
Trophoblastic function	Interferon τ Mash2
Chromatin structure	Histones
Transcription	RNA polymerase 1 Upstream binding factor Poly(A) polymerase
Methylation	DNA methyltransferases
X-Inactivation	X-inactive specific transcript (Xist)

Analyzed gene transcripts (3)

Function	Gene
Apoptosis	Bax (Pro-apoptotic protein) Bcl-xL (Anti-apoptotic protein) X-linked inhibitor of apoptosis
Differentiation	Leukaemia inhibitory factor and its receptors

Eckert and Niemann, 1998; Knijn et al., 2002; 2004; Lazzari et al., 2002; Lequarre et al., 2001; Lonergan et al., 2003; Miller et al., 2003; Rizos et al., 2003; Watson et al., 1999; Wrenzycki et al., 1996; 2001; 2003

Culture system:
Tissue Culture Medium (TCM) vs Synthetic Oviduct Fluid (SOF)



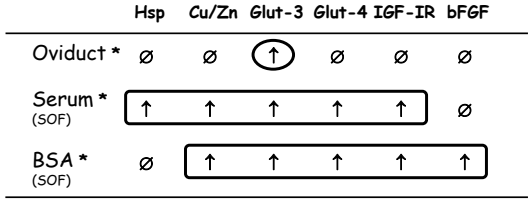
No differences: PolyA, Cx43, ZO-1

*** compared to in vivo derived embryos**

∅ : "normal pattern"
↑ : upregulated
↓ : downregulated
n.d.: not detected

Wrenzycki et al. (2001), Hum. Reprod. 16, 893-901

Culture system:
In vivo culture (ligated sheep oviduct)



No differences: Glut-1, Glut-2, Igf2r, H4

*** compared to in vivo derived embryos**

∅ : "normal pattern"
↑ : upregulated

Lazzari et al. (2002), Biol. Reprod. 67, 767-775

Effects of IVP on gene expression patterns - Summary -

- Profound alterations on the relative amounts of specific transcripts due to the basic culture system; only weak effects of the "protein source"
- Significant differences between in vivo and in vitro derived embryos, especially at the morula stage
- Nearly identical expression patterns between IVP embryos cultured in the ligated sheep oviduct and their in vivo counterparts
- Pertubed dosage compensation for X-linked gene transcripts

General conclusions (1)

- Preimplantation embryos possess an enormous degree of flexibility and can compensate suboptimal environmental conditions to a large extent.
- Analysis of the relative abundance of developmentally important gene transcripts provides a useful tool to assess the developmental competence and viability of oocytes and embryos at the molecular level.
- Persistent deviations in expression patterns of developmentally important genes are proposed to be causally involved in (LOS) and low viability.

General conclusions (2)

- Observed deviations from the normal gene expression pattern may reflect epigenetic changes, specifically in the pattern of methylation.
- The differences still found between in vivo and manipulated embryos emphasize the need for further research to optimize in vitro culture systems and manipulation methods.
- The findings made in the bovine model call for a critical assessment of the effects of human "Assisted Reproductive Technologies (ART)".

What is 'Genetics'? Conclusions (1)

- Chromosome complement: ovarian stimulation regimens likely contribute to high rates of aneuploidies in primate IVP embryos
- Mitochondrial (mt)DNA errors are common, increase with female age, and also are caused in part by ovarian stimulation with FSH
- Epigenetics: animal models suggest IVP embryos have frequent, multi-gene differences compared with IVO embryos

What is 'Genetics'? Conclusions (2)

- Accurate information on mechanisms of genetic defects in IVP embryos needs to come from animal models – destructive analysis of all oocytes or embryos in cohort
- Monkey and bovine oocytes/embryos are best translational models; young, fertile animals can be used
- Data obtained could lead to radical alterations in standard ovarian stimulation protocols

Relationship between ovarian stimulation and oocyte quality

no. of oocytes ↑ oocyte quality ↓

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