Chromosome abnormality rates in human embryos obtained from in-vitro maturation and IVF treatment cycles

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Introduction

- IVF (in-vitro fertilization): *the most influential breakthrough in reproductive medicine*
  - > 2,000,000 babies born with IVF since 1978
  - Technique improved and pregnancy rates reached 25-50% per started cycle
  - Success depend on # of available oocytes → ovarian stimulation with GnRHa and gonadotropins

Introduction

- Limitation of ovarian stimulation:
  - Inconvenience of daily injections
  - Frequent monitoring scans
  - High cost of medication
  - Risk of ovarian hyperstimulation syndrome

- **Friendly protocol?** – *similar success rate without ovarian stimulation, more convenient and less expensive*

Introduction

- IVM (in-vitro maturation): collection of immature oocytes and fertilizing them following maturation in vitro
  - > 1000 babies born with IVM since 1st live birth reported by Cha in 1991
  - Technique improved over years
  - Eliminates the need for gonadotropin injection, associated cost and inconvenience, and risk of OHSS

Introduction

- The incidence of congenital abnormalities following IVM is comparable to natural conception or IVF.
- Overall embryo implantation rate of IVM are still *lower* than conventional IVF. → NOT widespread use
- Higher incidence of chromosome abnormality in IVM embryos may be a factor limiting implantation potential. (Requena et al., 2009)
- Data on chromosomal constitution of IVM embryos compared with perimplantation IVF embryos are **limited**.

Fluorescence in-situ hybridization (FISH)

- Assess the chromosomal constitution of human cleavage stage embryos produced through ART
  - sex chromosome-linked disease
  - unbalanced translocations in preimplantation embryo
  - screening for chromosomally normal embryos for transfer
- FISH has been successfully used to assess the chromosomal complement of IVM embryos.

  → Controversial whether IVM embryos have higher incidence of chromosomal abnormality limiting their implantation potential as compared with IVF

Aim

- compare retrospectively the incidence of chromosomal abnormality in cleavage-stage IVM and IVF embryos from similar age women undergoing a PGS cycle
- spare embryos (not transferred or cryopreserved) also assessed

Materials and methods

- Participants:

  2004.08 ~ 2008.03 at McGrill Reproductive Center

	IVM (6)	IVF (30)
Recurrent miscarriage or recurrent implantation failure	5/6	27/30
PCO or PCOS	6/6	17/30

- detailed reproductive and genetic counseling before treatment
- informed consent
- spare embryos donated for research

In-vitro maturation treatment

- Follicular development monitored by TVS from D2
- dominant follicle ↑ to 10-12 mm → hCG 10,000 U im
- 35-38 h later → TVOR
- aspirate → initial identification of oocytes → remaining aspirate washed with oocyte wash medium → isolate additional oocytes
- nuclear maturity oocytes: germinal vesicle (GV) stage or germinal vesicle breakdown (GVBD) stage
  - Day 0 (0-6h) → culture in IVM medium → Day 1 (24h) → culture in IVM medium → Day 2 (48h)

- Mature oocyte → ICSI → ET
  - ICSI performed at least 1h after observing first polar body extrusion as suggested previously (Hyun et al., 2007)
  - Fertilization assessed 17-19 h after insemination for the appearance of two pronuclei and two polar bodies
  - ET performed under ultrasound guide on day 4 or 5 post fertilization
  - Luteal support:
    - Progesterone 50 mg / day im
    - Estradiol 2 mg tid

Embryo biopsy

- Fertilization – embryo biopsy on D3 – ET
  - Embryo placed on a droplet containing Ca2+ and Mg2+ free medium
  - → a hole drilled in zona pellucida
  - → single blastomere aspirated gently through the hole
  - → wash and fixation

Spare embryo fixation

- donated spare embryos transferred to a droplet of acid Tyrode solution to remove zona pellucida
- fixation

FISH

- Commercially available multicolor FISH probe for chromosome 13, 15, 16, 18, 21, 22, X, Y
- applied on nuclei of biopsied blastomeres or spare embryos in 2 consecutive rounds
- embryo diagnosed as unaffected were transferred
- interpretation:
  - Diploid normal
  - Haploid, triploid or tetraploid
  - Aneuploid: trisomy, monosomy
  - Chaotic – random loss or gain of chromosomes
  - Mosaic

Statistical analysis

- Aneuploidy rate (per pt) = # of aneuploid embryos / # of embryos biopsied or a successful FISH result x 100%
- t- test
- Mann-Whitney U- test
- chi-squared test
- Fisher's exact test

Results

	IVM-PGS (6)	IVF-PGS (30)
hx of recurrent miscarriage	3/6 (50%)	10/30 (33%)
hx of recurrent implantation failure	2/6 (33%)	17/30 (57%)

Table 1 Patient characteristics and outcomes.

	In-vitro maturation	*In-vitro fertilization*
Couples	6	30
Female age (years) (range)	33.00 ± 1.55 (32–35)	32.17 ± 2.88 (24–35)
Embryos biopsied	10.33 ± 5.96	11.73 ± 3.79
Embryos with FISH result	8.00 ± 4.40	10.20 ± 3.61
Chromosome abnormality rate per biopsied embryo on day 3	45.56 ± 24.78	50.23 ± 15.40
Chromosome abnormality rate per successful FISH result	58.73 ± 31.58	57.36 ± 13.76
Embryos transferred	2.83 ± 1.33	2.93 ± 0.91
Implantation rate (median, interquartile range)[a]	20.83 (0–50)	33.33 (0–50)[b]
Clinical pregnancy rate[c]	50.0 (3/6)	53.3 (16/30)
Live birth rate[c]	50.0 (3/6)	53.3 (16/30)

Table 2 Specific chromosome abnormality in IVM embryos categorized according the maturation status of the oocyte.

Maturation status	**A** *Embryos from in-vivo matured oocytes*		**B** *Embryos from oocytes that reached metaphase II 24 h after follicle aspiration*		**C** *Oocytes that reached metaphase II 48 h after follicle aspiration*	
	Day 3	*Spare*	*Day 3*	*Spare*	*Day 3*	*Spare*
Chromosome abnormality rate per biopsied embryo[a]	60.00 (3/5)	NA	46.94 (23/49)	NA	87.50 (7/8)	NA
Chromosome abnormality rate per successful FISH result[a]	75.00 (3/4)	NA	62.16 (23/37)	NA	100 (7/7)	NA
No. of embryos	3	3	23	22	7	2
Aneuploid[b]	33.3 (1)	0 (0)	47.83 (11)	27.27 (6)	57.14 (4)	0 (0)
Haploid[b]	0 (0)	0 (0)	8.70 (2)	0 (0)	0 (0)	0 (0)
Polyploid[b]	33.3 (1)	33.33 (1)	26.09 (6)	13.64 (3)	0 (0)	0 (0)
Chaotic[b]	33.3 (1)	66.67 (2)	17.39 (4)	40.91 (9)	42.86 (3)	100 (2)
Mosaic[b]	NA	0 (0)	NA	18.18 (4)	NA	0 (0)

fertilization rate	78.6%	96.7%	**71%**
cleavage rate	90.9%	96.6%	**77.3%**

- small # of embryos in group A & C

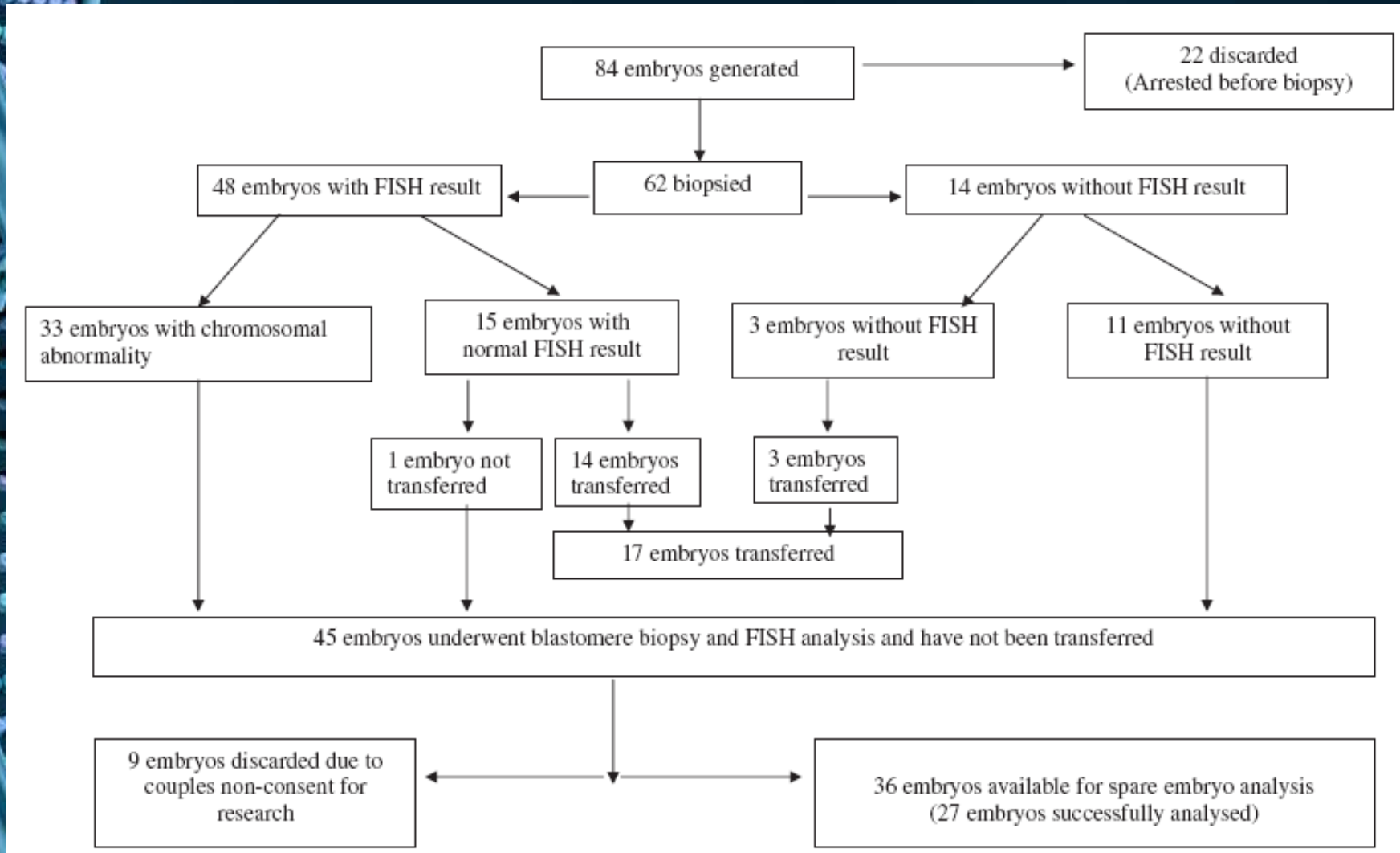

Figure 1 In-vitro maturation embryo flow chart. FISH = fluorescence in-situ hybridization.

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13/27 (48%)

4/27 (14.8%)

fertilization rate	78.6%	96.7%	71%
cleavage rate	90.9%	96.6%	77.3%

Table 3 Final diagnosis based on spare embryo analysis.

	IVM (n = 27)	*IVF (n = 53) (day-3 diploids excluded)*
Diploid	0 (0)	1 (1.9)
Aneuploid	6 (22.2)	11 (20.8)
Mosaic[a]	4 (14.8)	20 (37.7)
Haploid	0 (0)	1 (1.9)
Polyploidy	4 (14.8)	3 (5.7)
Chaotic	13 (48.1)	17 (32.1)

Values are number (%).
IVF = in-vitro fertilization; IVM = in-vitro maturation.
[a]$P = 0.03$.

- in mosaic embryos:
  - 11/20 (55%) and 5/20 (25%) had majority and minority of nuclei with the same abnormality pattern as diagnosed on day 3
  - 4/20 (20%) was not concordant with day 3 diagnosis

Summary

- The incidence of chromosomal abnormality per FISH result was similar in IVM and IVF embryos.
- Embryos derived from oocytes matured 48 h after collection had a higher chromosomal abnormality rate compared with embryos derived from in-vivo matured oocytes and matured in first 24 h after collection.

Discussion

- The chromosomal abnormality rate observed in IVF embryos in the control group is slightly higher than previously prospective studies involving women of similar age groups (Blockeel et al., 2008; Staessen et al., 2008; Verpoest et al., 2008).
  - Screening panel included **8** chromosomes
  - Different method to calculate aneuploidy rates: multiplicity associated with one woman having several embryos

- The data on chromosome status of IVM embryos are limited.
  - aneuploidy rate of 60% (12/20) in IVM embryos (Requena et al., 2009)
  - similar to current study (58.7%), FISH panel included the same chromosomes
  - Differences in population and IVM protocol:
    - older women with fertility problems (miscarriage, IVF failure or PCOS)
    - hCG injection
    - Small sample

- Benkhalifa et al. (2009) analyzed the chromosomal complement of 188 IVM embryos that were arrested at the cleavage stage.
  - exclusively included PCOS and hCG given 36 h before oocyte collection
  - FISH for chr 13, 18, 21, X, Y
  - 86 arrested embryos derived from oocyte matured in the first 28h and 61 from oocyte matured at 48h after collection
  - Chomosome abnormality rate was 32.6% and 49.2%

Maturation status	*Embryos from in-vivo matured oocytes*		*Embryos from oocytes that reached metaphase II 24 h after follicle aspiration*		*Oocytes that reached metaphase II 48 h after follicle aspiration*	
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Limitations

Small sample size

- aneuploidy rate in IVM group should be higher ??
  - 1 patient (1/6) only had 2 embryos available for biopsy and both tested normal → aneuploidy rate = 0
  - Aneuploidy rate in remaining five is 69.4%. (still not significantly different from IVF group)

Confounding uncontrolled variables

- prospective RCTs

- There is a strong correlation between the maternal age and the risk of aneuploidy.
  - similar aneuploidy rate in IVM and IVF embryos can be explained by similar maternal ages in both groups
  - different types of chromosome abnormality
    - a trend toward a higher % of chaotic embryos in IVM
    - incidence of mosaic higher in IVF
    - "chaotic" more frequently observed in arrested embryos (Bielenska et al., 2002), irrespective of age
    - in IVM group, 44% (12/27) of embryos were developmentally arrested compared with 23% (11/53) of embryos in IVF group

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- embryos derived from 24h matured oocytes should be preferentially transferred in IVM cycles (Zhang et al.)
- Currently, implantation rate of IVM is lower than IVF.
  - ↑ incidence of chromosome abnormality in IVM suggested as a possible reason (current study not support)
- FISH of certain # of chromosome does not rule out all possible chromosome abnormalities or problem at genetic level.

- Embryo implantation is a complex process.
  - multiple events at the molecular level
  - cross-talk between embryo and endometrium
- Further research should address other factors as well as possible differences in genetic constitution of IVM and IVF embryos.


THANK YOU
Have a nice day !