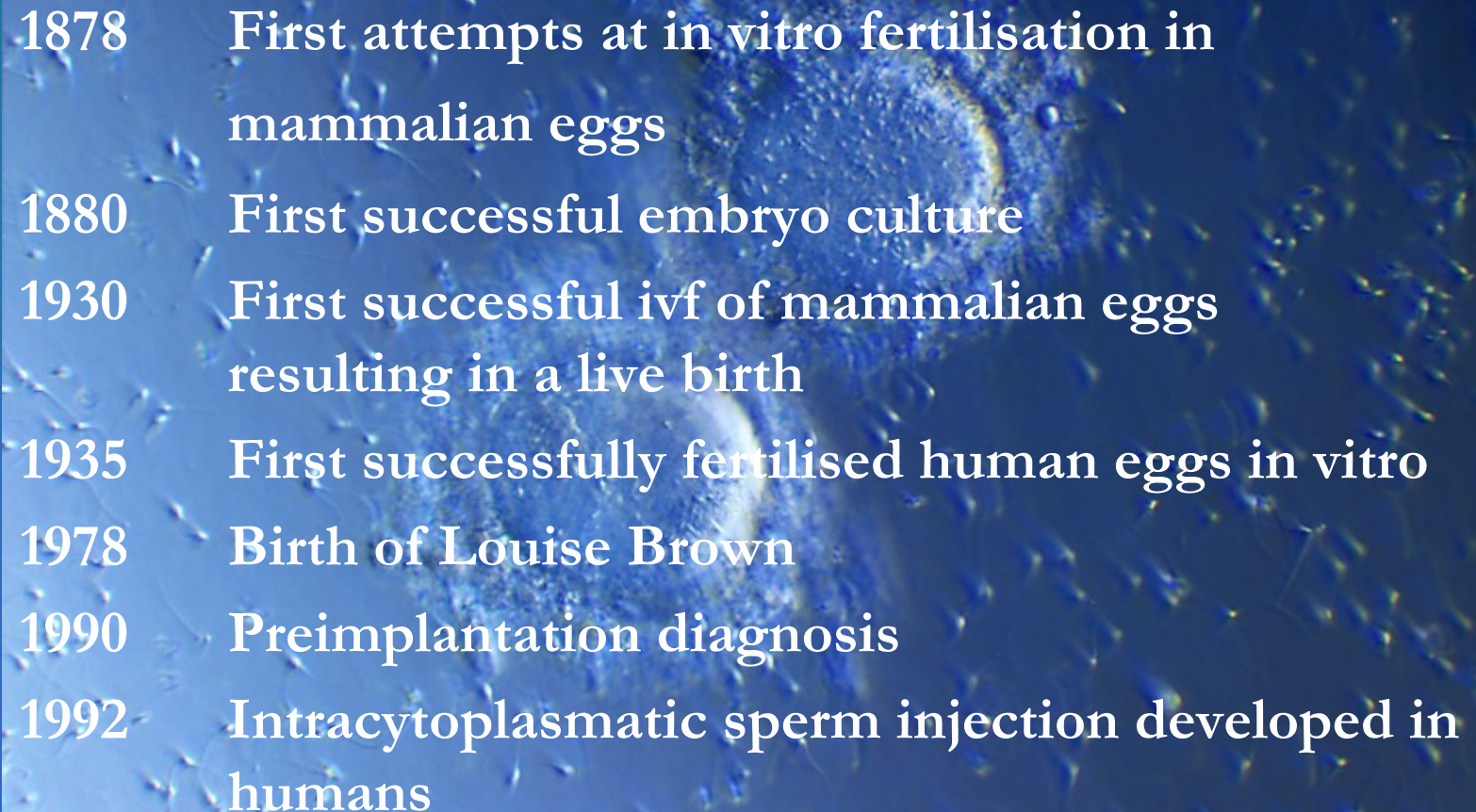


Genetics and Counselling in Reproductive Medicine

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Bertinoro 5-5-2004

Landmarks of in vitro fertilisation technology (IVF)

- 
- 1878 First attempts at in vitro fertilisation in mammalian eggs
 - 1880 First successful embryo culture
 - 1930 First successful ivf of mammalian eggs resulting in a live birth
 - 1935 First successfully fertilised human eggs in vitro
 - 1978 Birth of Louise Brown
 - 1990 Preimplantation diagnosis
 - 1992 Intracytoplasmic sperm injection developed in humans

Landmarks II

- Partial zona dissection (PZD)
- Sub Zonal Insemination (SUZI)
- Intracytoplasmic sperm injection (ICSI)
- Rounded spermatid nucleus injection (ROSNI)



Testicular sperm aspiration (TESA) or biopsy (TESE)

- Microsurgical Epididymal Sperm Aspiration (MESA)

The «ICSI times»

- The advent of *in-vitro fertilisation (IVF)* and *intracytoplasmic sperm injection (ICSI)* has opened the door to reproduction for many couples for whom there was no reasonable prospect of conception

Skipping evolutionary barriers? Is it going to affect the future?

“Bad genes tend to end up in bodies that die young or without reproducing
(R.Dawkins,1995)

“What form of male infertility are we left to cure? (Silber, 1995)

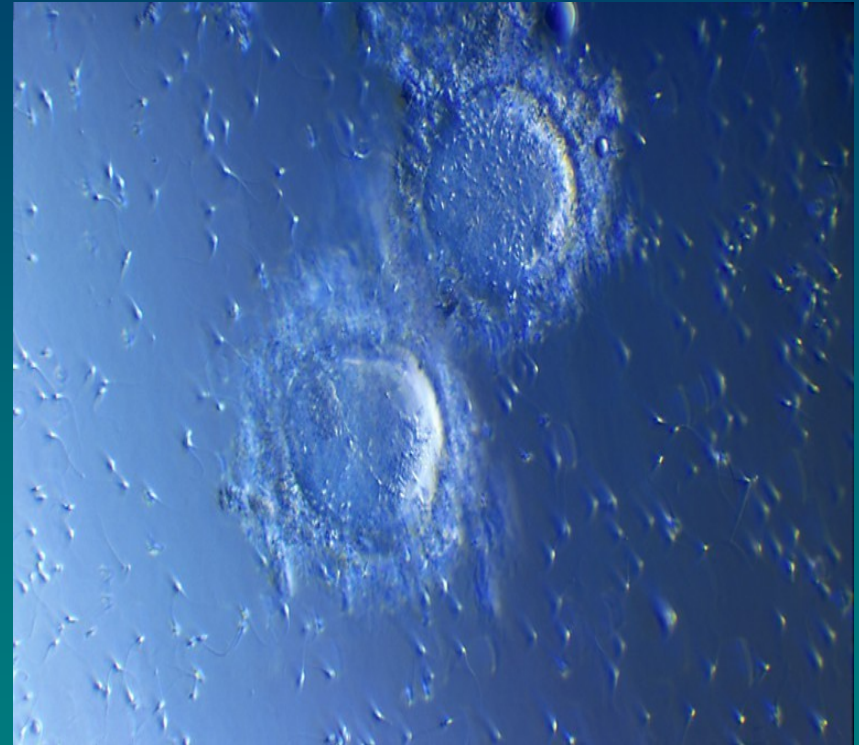
Do risks exist ?

*Risk of transmitting
(parental) chromosomal
aberrations ?*

*Risk of de novo, mainly sex
chromosome, anomalies ?*

*Risk of transmitting fertility
problems to offspring ?*

*Increased incidence of
congenital malformations ?*



Issues for genetic counselling

- Age of partners
- Infertility in the context of an abnormal phenotype
- Infertility as only « symptom »

Decline of fertility

- Females of most mammalian species, including humans experience reproductive decline with age ending in complete loss of fertility by mid-life, even though there are still primary oocytes within the ovary.
- Demographic, anthropological, historical and clinical studies of diverse populations agree that the female fecundity declines with age.

Decline of fertility II

- Age (chronological and biological)
- Fecundability and fecundity
 - older women need a longer exposure to pregnancy but exposure can decline on behavioural grounds (i.e. duration of marriage)

❖ *Frank O. Bianchi PG and Campana A.
J Biosoc Sci 26, 349-368,1994*

Where does the aging process start?

- Endocrine mechanisms
- Ovarian reserve
- Oocyte quality
- Implantation

Changes

- Already 10 years before menopause slight changes in menstrual cyclicity can be discerned.
 - ❖ Follicular phase shortens and hence does the menstrual cycle.
- A progressive **rise in FSH** secretion has been described throughout reproductive life and accelerates approximately a decade before menopause and therefore coincides with a phase of accelerated follicle depletion.

Follicular/oocyte development and oocyte quality

- In the human oocytes enter meiosis early in fetal life (~ 12 weeks).
- Oocytes progress to the diplotene stage of meiosis I (GV stage).
- Oocytes stay in this state until just before they resume ovulation
- This event is gonadotropin-dependent and triggered off by the mid-cycle LH surge

Data on apoptosis in the oocyte

- Does apoptosis play a role in relation to «the age factor » in the human oocyte?
- Maturation rates in vitro of oocytes was lowest in the age group 41 to 50.
- The rate of apoptosis in immature oocytes in vitro was also higher in this group.

Wu et al. Fertil Steril 74, 1137-41, 2000

Data from IAC

- *Campana et al.*

Hum Reprod, 11,732-736, 1996

- review of 1115 cycles of IUI (332 couples).
- outcome was affected by the woman's age (>39 years old) and by total motile sperm count
- No pregnancy after the age of 44

Data from IAD

Kang BM and Wu TC Obstet Gynecol 88, 93-8, 1996

The cumulative probability of pregnancy after 7 cycles was 88% for women 35 years old or younger compared to 65% and 42% in women 35-40 and over 40 years old

Data from IVF cycles

- «Embryo quality and developmental potential is compromised by age»

Ziebe S., Loft A., Petersen J.H. et al.

Acta Obstet Gynecol Scand 80, 169-174, 2001

- Decrease

- ❖ in number of oocyte recovery (1 oocyte per 2.3 years)

- ❖ Number cleaved oocytes (1 oocyte per 3.7 years)

- Due to decreased number of retrieved oocytes

Results II

- The percentage of fragmented oocytes increased 3% per year of age
- The number of transferred embryos decreased significantly from 2.1 at the age of 25 to 1.8 at the age of 40.
- In the selected subgroup of «good quality embryos» implantation rates decreased significantly with age
- The decrease in the ongoing pregnancy rate was almost linear (~ 1.5 per year)

Effects of maternal age on the oocyte developmental competence

- Meiotic incompetence (effects on fertilisation)
- Errors in meiosis (genetic abnormalities)
- Cytoplasmatic deficiencies (anomalies at different stages of development (before or after fertilisation))

❖ *Armstrong DT Theriogenology 55, 1303-22, 2001*

Data from oocyte donation

■ *Borini et al. Fertil Steril 65, 94-7, 1996*

Retrospective data analysis of cases where recipients of different ages shared oocytes from a single donor

- 114 women undergoing a cycle of oocyte donation
- Group A : = or <39 / Group B : 40 to 49
- Pregnancy rates of 47,3% and 24,5% respectively
- Abortion rates 14,8% and 7% respectively

Risk of miscarriage and age

- Fetal loss is the possible destiny of 13 % of clinical pregnancies.
- At 42 more than half of pregnancies result in fetal loss.
- The risk of spontaneous abortion is 8.9% in the age group 20-24 and 74% in those aged 45 or more
 - *Andersen et al. BMJ 320,1780-1712, 2000*

Evidence for declining fecundity in older men

- *Hum Reprod 15 ,1703-1708, 2000
Ford et al.*
- *All couples in the Avon Health District expecting a baby between April 91 and December 92 were eligible.*
- *Questionnaires were completed at 18 weeks.*
- *Of 12 106 couples 70.7% had planned the pregnancy and 99.5% stated the time to conception*

Male fertility declines with age? II

- *Specific and non specific fertility factors*
- *Parity, paternity, cohabitation, oral contraception.*
- *educational achievements, housing, cigarette smoking, alcohol consumption, obesity.*

Results I

- Of the 8515 planned pregnancies
- 74% were conceived at 6 months,
14% in the second 6 months
12% after more than a year.
- after adjustment for variables (including age of the mother)
the likelihood of conception within 6 or 12 months was lower in older men

Results II

- Adjusted odd ratios for conception in ≤ 12 months compared to men ≤ 25 years old were
 - 0.62 (30-34 y)
 - 0.50 (35-39y)
 - 0.51 (≥ 40 y)
- There is a larger decline in male fecundity with advancing age than previously reported

Male fertility declines with age? I

- *No*
- Retrospective analysis for 558 oocyte donation cycles (441 couples) in an oocyte donation program
 - Am J Obstet Gynecol 2001
 - ❖ Paulson RJ, Milligan RC, Sokol RZ »
 - Negative correlation between male age and total sperm count
 - No association between male age and fertilisation rates, pregnancy or live birth rates

Male fertility declines with age? II

■ *Yes*

- ASRM 2000, San Diego California
- 2 groups communicated data suggesting the effect of the male partner's age
 - ❖ Kentucky Center for Reproductive Medicine
Retrospective study on 800 sperm samples showed a decrease of TFSF (total functional sperm fraction) from 107.1 million to 35.5 million from the age of 20 to 50 years old

Male fertility declines with age? III

- French group found a negative correlation between the fertilisation rate and the age of the husband on a oocyte donor program
- Fertilisation rate for men less than 39 years old was 60,2 % and only 51,3% for men over 39.

A review of the literature

- Kidd SA, Askenazi B and Wrombek AJ
 - Fertil Steril 2001 75(2), 237-48
 - The weight of the evidence suggests that increased male age is associated with a decline in semen volume, sperm motility and sperm morphology but not with sperm concentration.

Daddy's time bomb?

- «*Mom may have her biological clock but dad may be harboring a time bomb*»
- Study on 90 000 Israeli children found that advancing paternal age accounts for 1 out of every four schizophrenia cases.
- The older the man was at the time of conception the higher the risk after the age of 40.
- The age of the mother did not matter
Harlap et al. Arch Gen Psy, May 2001

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Reproductive Impairment in Gonosomal aneuploidy

47,XXY: Klinefelter

azoospermia, small testes, some will have some immature gametes in the testis

47,XYY:

lower sperm production, variable degrees of impairment of gametogenesis

47,XXY

Rarer, but often associated with normal sperm analysis

X-linked disorders and male infertility

- Kallmann syndrome

Kalig -1 Gene mutation (Xp22.3)

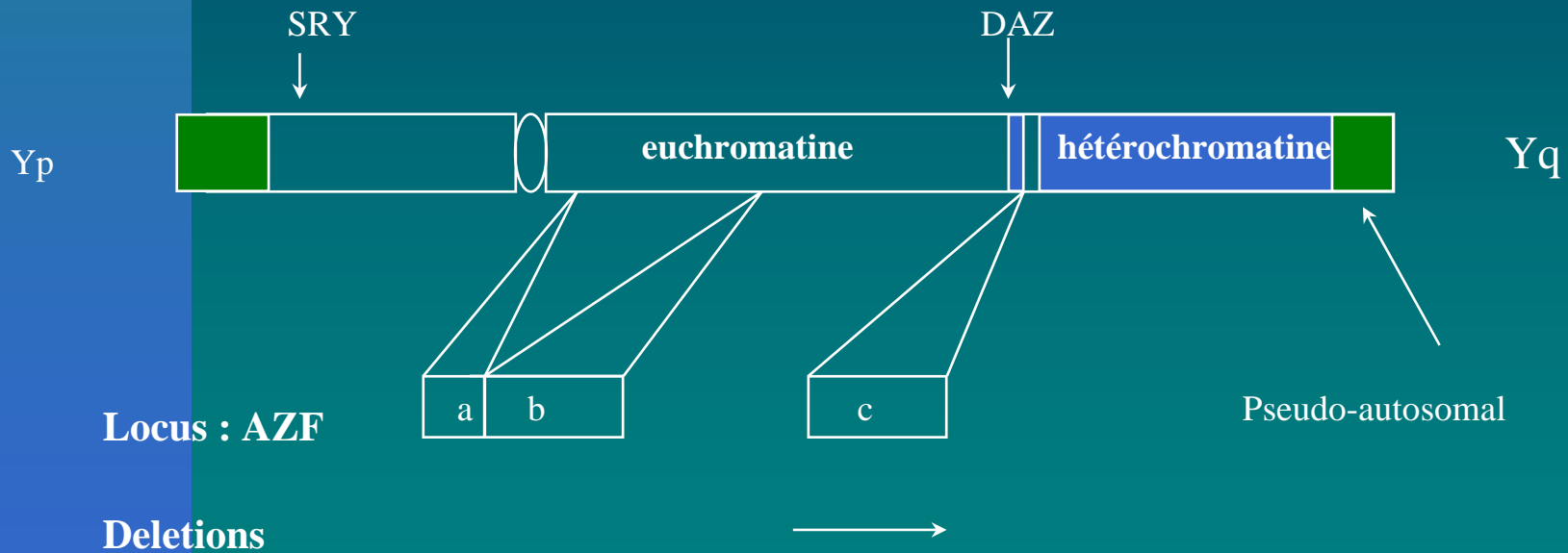
Function in cell adhesion and axonal path finding

Anosmia, facial asymmetry, cleft palate, colour blindness, undescended testis

Androgen insensitivity

- Defect of the androgen receptor gene (Xp11-12)
- Complete range of phenotypes from testicular feminisation to apparently normal but with infertility problems

Microdeletions of the Y chromosome



Depending on tested loci and inclusion criteria, 5-20% of the infertile males show a deletion

Cystic fibrosis and infertility

- Autosomal recessive disorder
- 1/25 carriers in Caucasians
- Family of gene mutations
- Short arm of ch 7
- Membrane protein that functions as a ion channel
- CBAVD : frequently CF carriers
- Most frequent mutation delta F 508
- Involved also in cases of abnormal spermatogenesis

Is there an increased risk for the offspring of infertile men ?

- Concerns may originate from :
 - Extent of hereditary conditions to the offspring, derived from an affected, till then infertile parent.
 - Possible consequences of the ART itself
 - Possible adverse consequences of the use of sperm that in many cases would not have been fit or mature enough to achieve fertilisation

OAT men at increased risk of abnormal offspring?

- Peripheral blood chromosomal abnormalities are more common in infertile male seeking ICSI treatment
- Survey on 11 publications reporting on 9766 infertile men
- *Martini et al. 1996*
 - Incidence of chromosomal abnormalities
5.8%

Effects of sperm damage

- Fertilisation
- Cell division
- Embryo development
- Pregnancy outcome
- Congenital anomalies in babies
- Future health issues

**“Atypical decondensation of the sperm nucleus,
delayed replication of the male genome,
and sex chromosome positioning following
intracytoplasmic human sperm injection (ICSI)
does ICSI itself introduce chromosomal anomalies?”**

Terada et al. Fertil Steril 2000

Risks for offspring and assisted reproductive technology.
Questions are now being asked...

What do we know?

■ **To this day**

- still insufficient offspring follow up in published series
- because of major differences in the way offspring is followed up and malformations classified among different countries
- because of statistical difficulties

Technical limitations

- To detect a change in the risk of a birth defect if its risk is 2% (doubling of risk) 1000 ICSI birth and 1000 controls are needed
- Special registers have only recently been established

Offspring - potential risks?

- Several papers show a
 - slightly increased sex chromosome aneuploidy (1%)

Bonduelle et al. 1996

In't Veld et al. 1995

■ Van Steirteghem et al. 1996

– prenatal diagnosis on 585 babies
conceived by ICSI: 2% of abnormal
karyotypes

❖ 1% de novo, 1% paternal transmission

■ Loft et al 1999

- Danish cohort study -730 newborns-
 - ❖ 2.7% of major birth defects, 1.2% minor birth defects

Congenital birth defects I

- *Birth defects in infants conceived by intracytoplasmic sperm injection: an alternative interpretation*

J Kurinczuk et C Bower. BMJ 1997

reanalysed the Bonduelle data by reclassifying the birth defects (British Paediatric Association ICD-9) and comparing them to a control group

Congenital birth defects II

- ❖ 420 liveborn infants born after intracytoplasmic sperm injection and 100 454 liveborn infants in Western Australia delivered during the same period
- ❖ Estimates of birth prevalence of birth defects and comparison of odds ratios between cohorts

Congenital birth defects III

– *Results*

- ❖ Seven point thirty eight % of major birth defects, 0.78% minor birth defects
- ❖ Infants born after ICSI were twice as likely as Western Australian infants to have a major birth defect and nearly 50% more likely to have a minor defect
- ❖ Excess of cardiovascular, genitourinary and gastrointestinal defects (in particular cleft palate and diaphragmatic hernia)

Risks for offspring and assisted reproductive technology.
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What do we know?



Seeds of doubt

Powell K Nature 17 April 2003

Twice as high a risk of a major birth defect as naturally conceived infants?

Hansen et al. NEJM: 346,725. 2002

The course of pregnancy or the outcome after ICSI are not affected by origin or number of sperm in the ejaculate

Ludwig M et al. Hum Reprod: 18, 351. 2003

ART is associated with a human overgrowth syndrome (Beckwith-Wiedemann)

DeBaun M R et al. Am J Hum Genet 72, 156. 2003

