# Imprinting diseases and IVF

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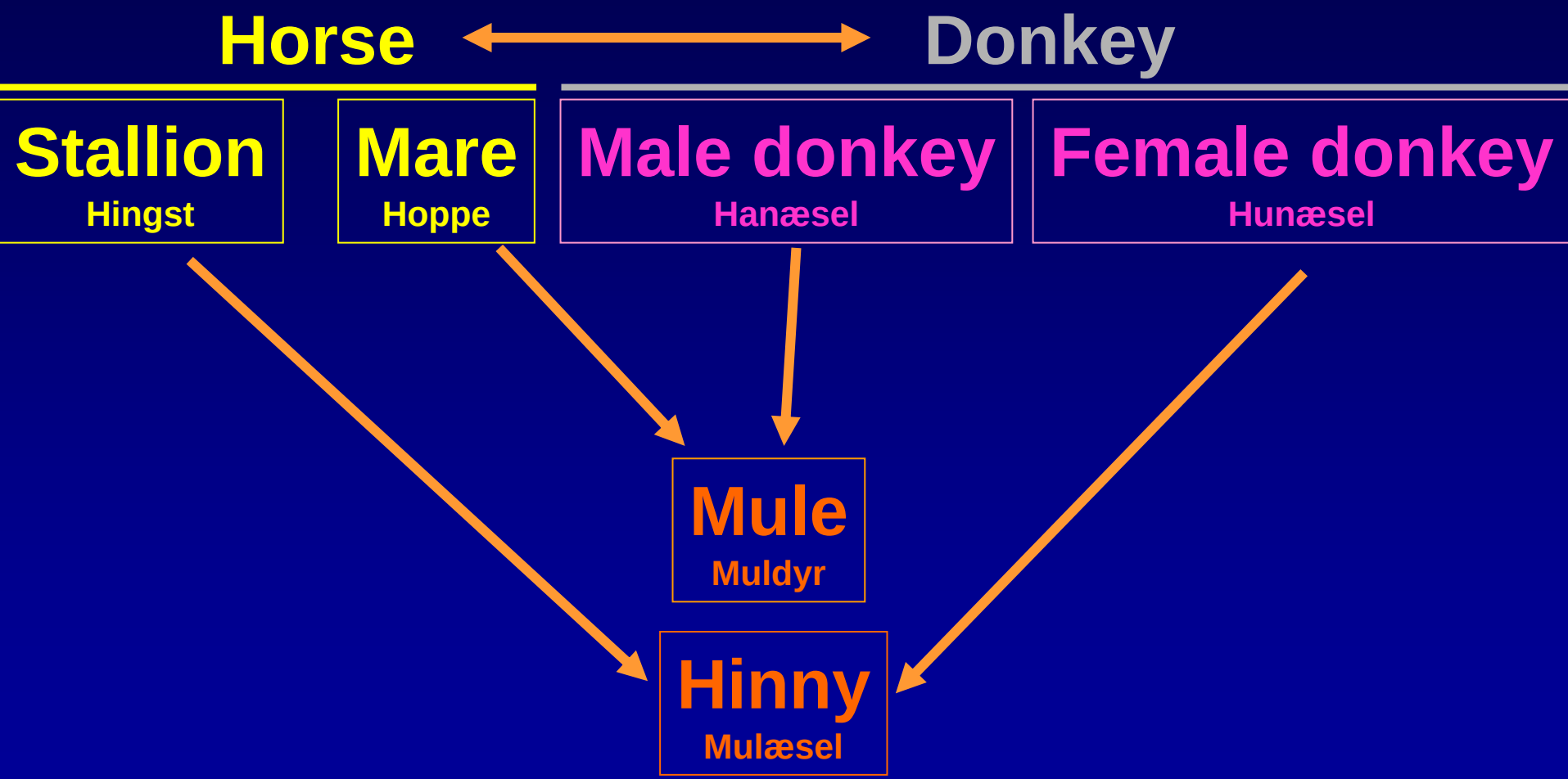
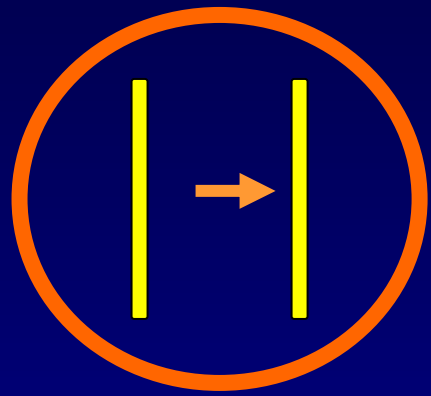
# What is the difference between a mule and a hinny?



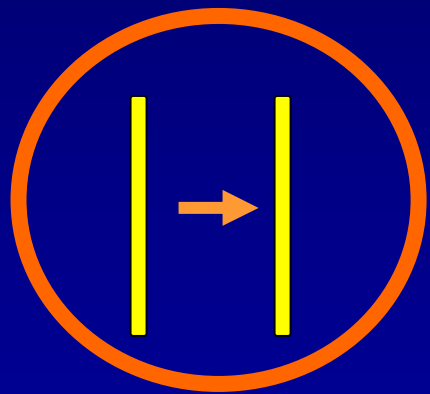
# Uniparental-disomia

Egg without nucleus, fertilised by one sperm. Duplication of sperm genome



Complete Mole

Duplication of egg genome without fertilisation



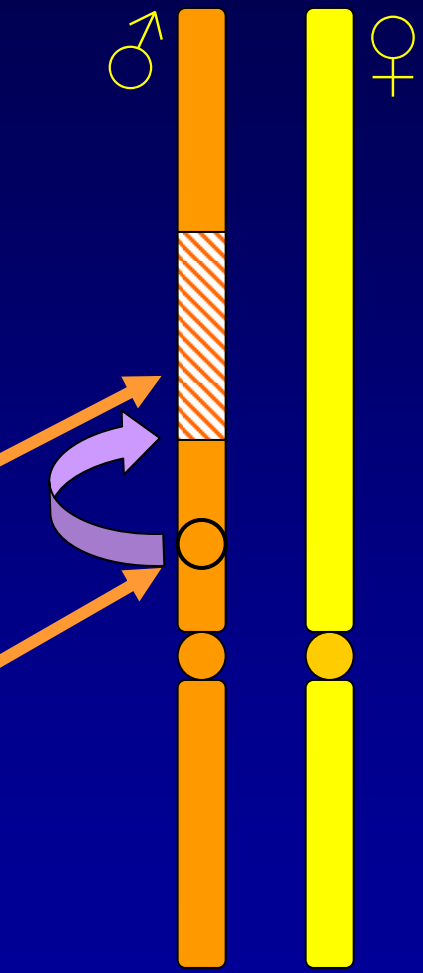
Teratoma

**Conclusion:** Total uniparental disomy has always fatal consequences for the embryo

# Genomic imprinting

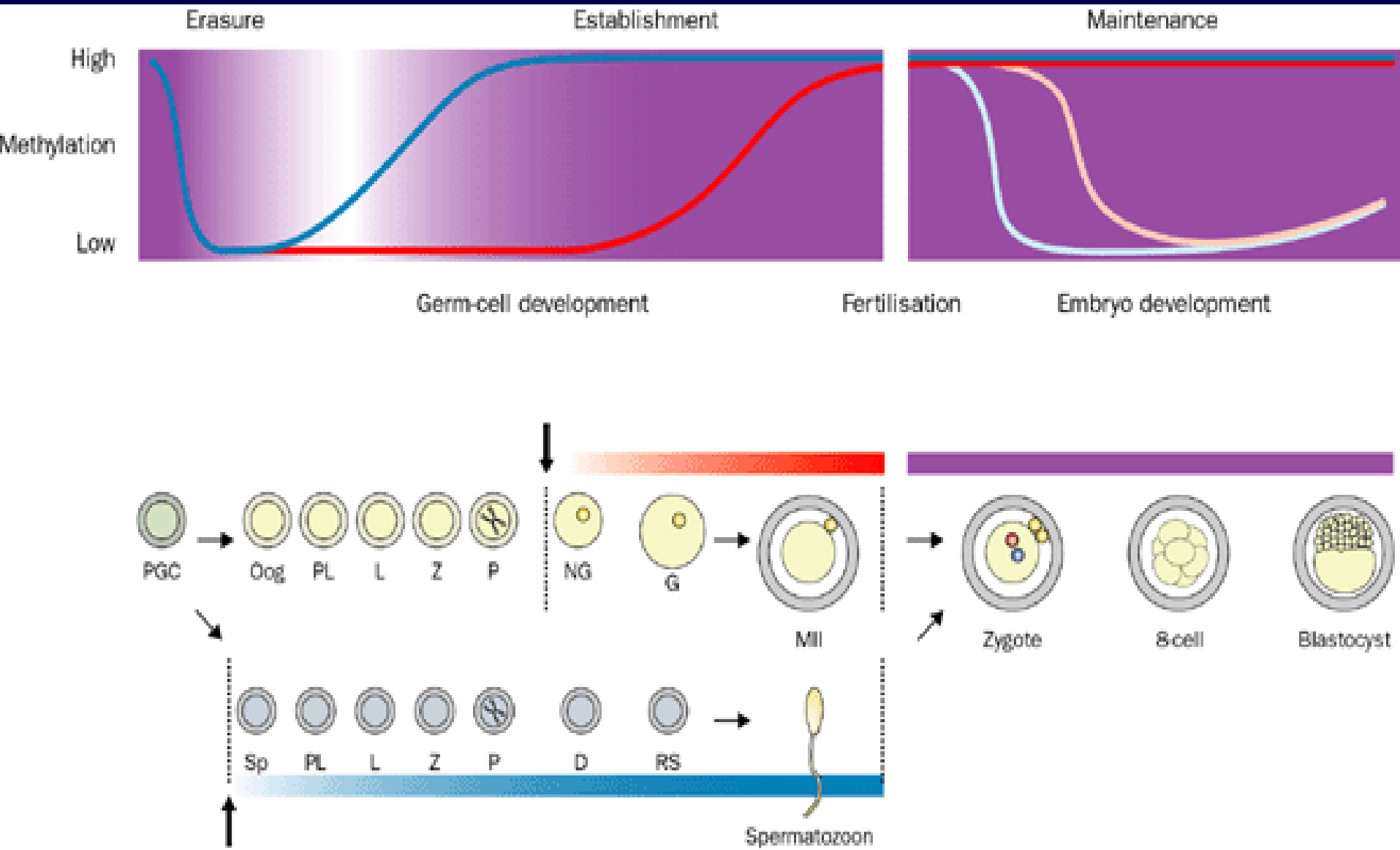
**Definition:**

An epigenetic modification of the genome, in which some genes in the allele from one of the parents is ”closed” down (methylated)




Imprinting is controlled by imprinting centers (IC) located nearby the imprinted areas on the same chromosome

# Imprinting in gameto and embryogenesis




Gosden et al. Lancet 2003; 361: 1975-77

# Principal imprinting and modification

**Gametogenesis: Principal imprinting process**

**Day 1: Fertilisation**

**Day 1-5: Modification of imprinting**

**Day 5-7: Implantation**

**Day 5+: Differentiation**

**Principal:** determined by the parental origin.
**Modification:** controlled by the physical environment during early stages of cleavage. Could be a mechanism by which the embryo adapts to the prevailing physical environment

Rycke et al. Hum Reprod 2002; 10: 2487-94.

# Imprinting versus differentiation

| Imprinting | Differentiation |
| --- | --- |
| Mono allelic | Bi- or mono allelic |
| Gametogenesis | Gametogenesis |
| Early stages | All stages |

**Both:** The genes are closed by methylation and blocked by histones.

Rycke et al. Hum Reprod 2002; 10: 2487-94.

# Genomic imprinting

**Creation of a healthy embryo demands**

- A successful meiotic process in parents
- Imprinting of gamete specific genes
- A fertilisation of egg with one spermatozoa
- Paternal as well as a maternal genome
- Epigenetic preservation of imprinted genes during early stages of embryogenesis (first days)
- >1000 other things

# Genomic imprinting

- By now we have identified 75 imprinted genes.

*These genes are of significance for*

- growth regulation
- placental growth
- embryonic and postnatal development
- brain function
- behaviour, psychological traits
- neoplastic transformation

Li/03

Walter & Paulsen. Sem Cell Develop Biol 2003; 14: 101-10

# Imprinting diseases 1

Dysregulation of imprinted genes are now described in several human diseases, which are characterised by:

- growth abnormalities
- placental abnormalities
- mental retardation, abn. Psychological traits
- abdominal wall defects
- increased risk of early cancers

Walter & Paulsen. Sem Cell Develop Biol 2003; 14: 101-10 Li/03

# Imprinting diseases 2

**Specific imprinting diseases in humans**

- Beckwith-Wiedemann syndrome (BWS)

  *Imprinting disorder on chromosome 11p*
- Prader-Willis syndrome (PWS)

  *Imprinting disorder on chromosome 15q*
- Angelman syndrome (AS)

  *Imprinting disorder on chromosome 15q*
- Childhood cancers

Li/03

Walter & Paulsen. Sem Cell Develop Biol 2003; 14: 101-10

# Imprinting diseases 3

## Childhood cancers

- Wilms tumour
- Neuroblastoma (m1p and p2)
- Acute myeloblastic leukaemia (p7)
- Rhabdomyosarcoma (m11p)
- Osteosarcoma (m13)

All these diseases are rare; 1-10/10,000 born

Li/03

Walter & Paulsen. Sem Cell Develop Biol 2003; 14: 101-10

# Growth media and imprinted genes in mouse

- Small changes in physical composition of growth media after in vitro fertilisation have consequences for the embryo
- These consequences are at least partly mediated through an altered imprinting
- These changes during first days after fertilisation are irreversible.

Khosla et al. Biol of Reproduct 2001; 64: 918-26

# Imprinting diseases and IVF

- Several case-reference studies have suggested a higher proportion of IVF in children with imprinting disorders as compared with a reference population
- The studies are small, insufficiently matched
- No consensus whether ICSI implies a differential risk as compared with conventional IVF

# Imprinting disease and IVF

## Danish National IVF cohort study

## "DaNIC"

Lidegaard Ø, Pinborg A, Nyboe Andersen A

## Aim of the study

- To assess the frequency of imprinting diseases in IVF children as compared with normally concipated children
- To screen IVF children for developmental diseases and compare it with the frequency in normally concipated children

Lidegaard et al. Human Reproduction 2005; 20: 950-4

# Danish National IVF cohort study
## Material & methods

- All singleton children born in DK 1995 - 2001
- Stratification into three groups:

  No IVF, IVF without ICSI, and IVF with ICSI
- IVF children identified in National IVF registry
- Follow up until July 2003 (or 5 years)
- Diseases identified in National Register of Patients (LPR) and Central Register of Psychiatric Diseases (CRPD)

Lidegaard et al. Human Reproduction 2005; 20: 950-4

# Danish National IVF cohort study
## Methodological problems

- Many children with imprinting diseases are not diagnosed with such a disease, and are therefore hidden in unspecified groups of syndromes or developmental diseases.
- Some of these syndromes are rare. Therefore inclusion of even all births over seven years may not bring enough IVF children to detect differences in frequency between IVF and non-IVF children

Lidegaard et al. Human Reproduction 2005; 20: 950-4

# Included diagnosis codes (ICD 10)

Imprinting diseases are expected to be coded in one of five main diagnosis groups.

- DC Childhood cancers: Wilms, retinoblastoma
- DF  Mental retardation diagnoses
- DG Neurological disease (cerebral palsy)
- DQ Syndromes
- DR Developmental abnormalities

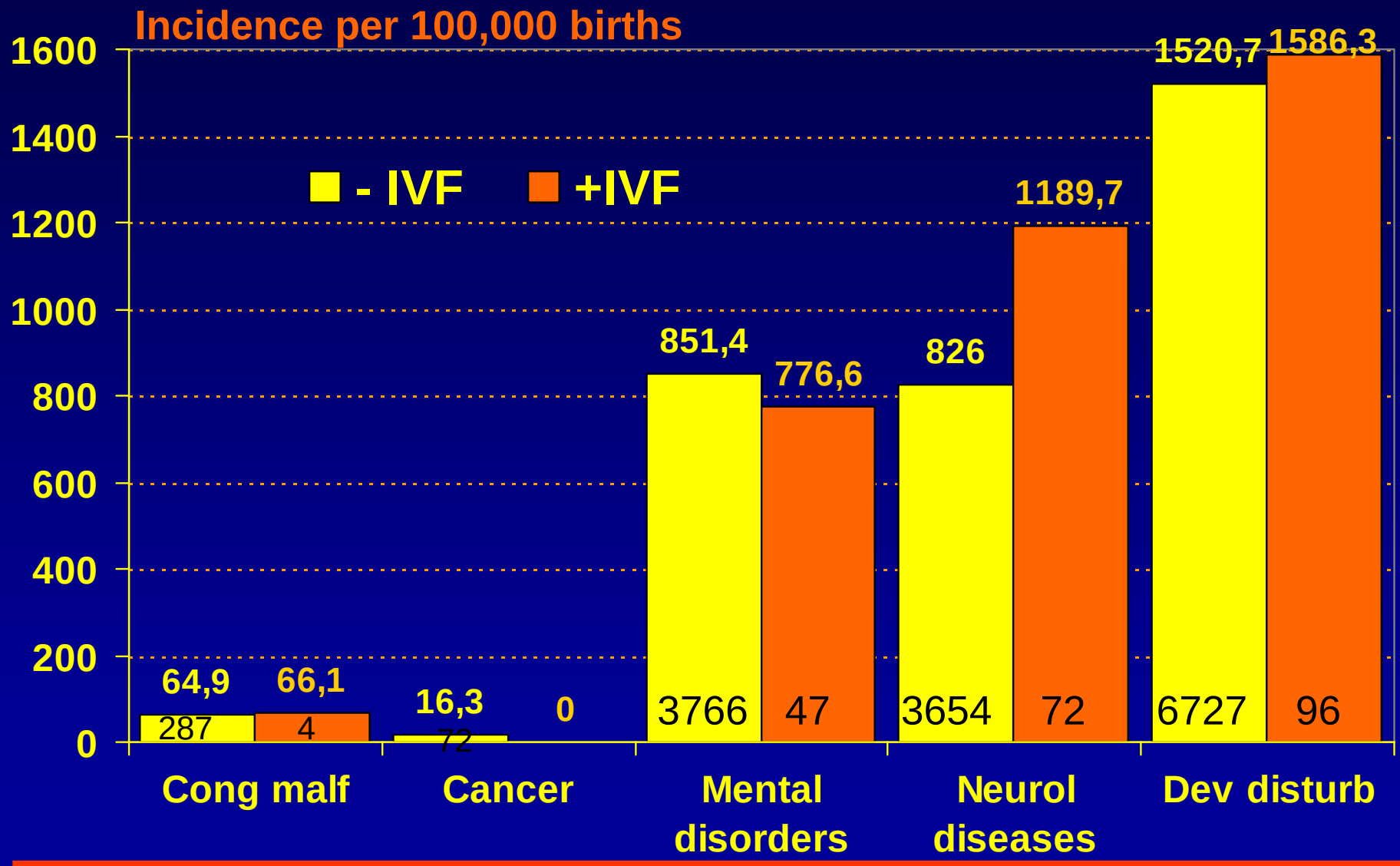
Lidegaard et al. Human Reproduction 2005; 20: 950-4

# Results: Main diagnosis groups

| Group | - IVF | + IVF |
|---|---|---|
| Number of births | 442,349 | 6,052 |
| DC Cancer | 72 | 0 |
| DF Mental retardation | 3,766 | 47 |
| DG Neurological dis. | 3,654 | 72 |
| DQ Congenital syndromes | 287 | 4 |
| DR Development. Disturb | 6,727 | 96 |
| All included diagnoses | 14,506 | 219 |

Lidegaard et al. Human Reproduction 2005; 20: 950-4

# Results: Main diagnosis groups



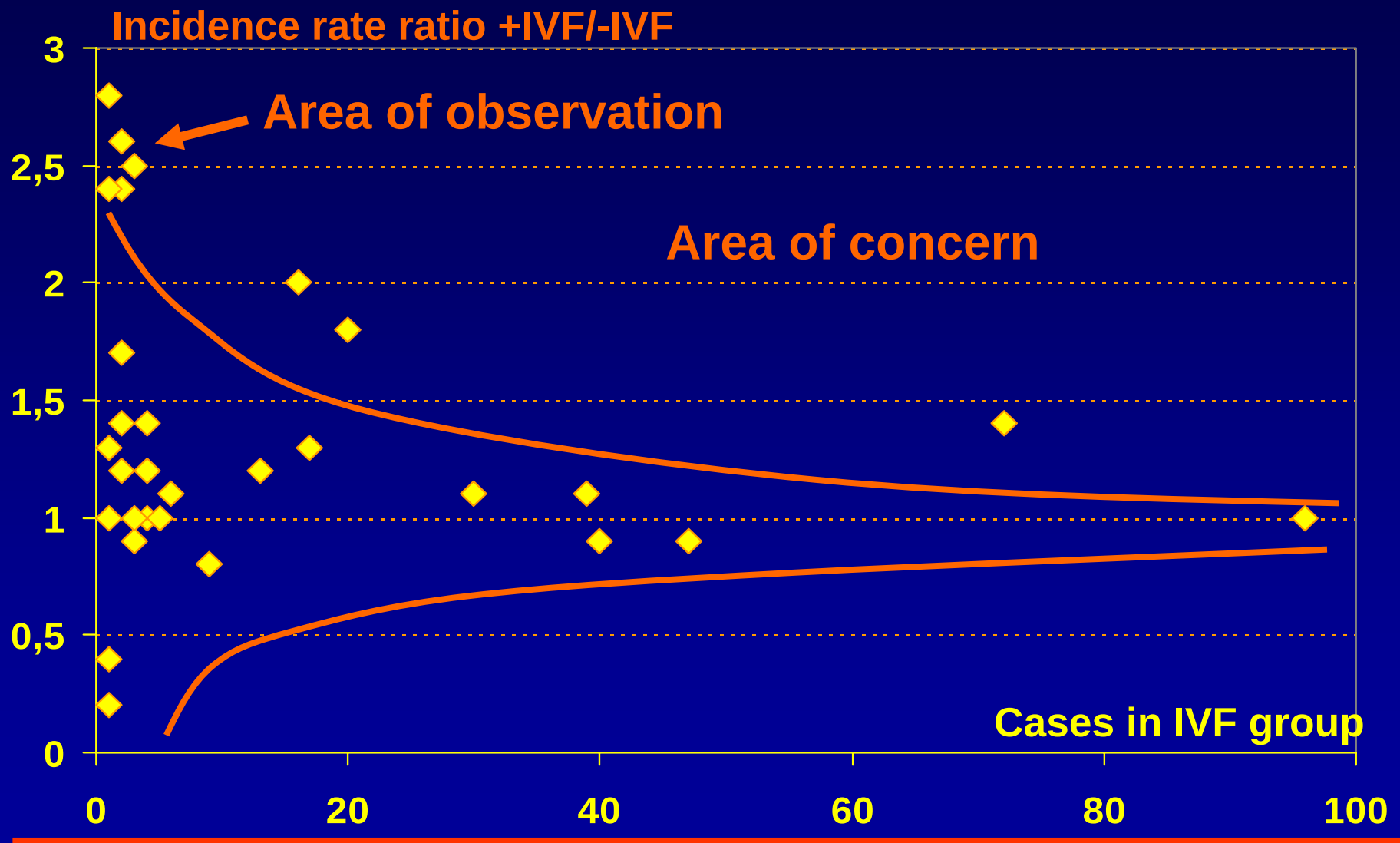

Danish National IVF cohort study

# Results: Specific syndromes/diagnoses

A clinical meaningful difference between non-IVF and IVF group in incidence rate of a specific syndrome demands

- A rate ratio (+IVF/-IVF) well above 1
- A minimum number of cases in IVF group

# Results: Specific diagnosis groups




Danish National IVF cohort study

# Results: Specific syndromes/diagnoses

| Diagnosis | +IVF | - IVF | Ratio |
|---|---|---|---|
| DG47 Sleeping disturb. | 16 | 572 | 2.0 |
| DG80 Cerebral palsy | 20 | 819 | 1.8 |
| DG total (neurol. Diseas) | 72 | 3.654 | 1.4 |
| **Observational group:** | | | |
| DG81 Hemiparesis | 3 | 87 | 2.5 |
| DG90 Dis of auton.nerv sys | 1 | 30 | 2.4 |
| DQ271 Dwarf growth | 2 | 60 | 2.4 |
| DR620C Motor retardation | 1 | 26 | 2.8 |
| DR630 Anorexy | 2 | 57 | 2.6 |

Danish National IVF cohort study

# Results: Specific imprinting diseases

| Diagnosis | - IVF | +IVF | Expect |
|---|---|---|---|
| DQ871E Prader Willi Syn | 3 | 0 | 0.041 |
| DQ871G Russel Silver S | 2 | 0 | 0.027 |
| DQ873A Beckwith Wiedem | 0 | 0 | 0 |
| DC54 Kidney canc. Wilms | 44 | 0 | 0.603 |
| DC692A Neuroblastoma | 5 | 0 | 0.068 |
| Total | 54 | 0 | 0.740 |

Conclusion: Misclassification of many of the specific imprinting syndromes. No indication, however, of a many fold increased risk of imprinting diseases in IVF children.

# Discussion: IVF versus other children

- Childhood cancers (DC) are not more frequent in IVF children.
- Mental disturbances/retardation (DF) are not more frequent in IVF children.
- There might be an increased risk of cerebral palsy (DG80) in IVF children as compared with non-IVF children. This was found also in Sweden by Strömberg et al 2002 (OR 2.8)
- The increased frequency of sleeping disturbances (DG47) could be influenced by the higher age of IVF-parents.

# Discussion: IVF versus other children

- Syndromes (DQ codes) overall are not more frequent in IVF children.
- Developmental disturbances (DR) are not more frequent in IVF children.

**Proposal:**

- Systematic follow-up of IVF children according to the same logistics
- Establishment of codes for all imprinting diseases, common to all European countries.

# Imprinting & ART: conclusion

**In vitro cultured embryos may have their imprinting influenced in a way which may**

- **Increase the risk of imprinting diseases**
- **Leave its stamp on imprinting with consequences for**
  - **fetal development**
  - **psychical traits of fetus**

# Imprinting & ART: conclusion

**In vitro cultured embryos may have their imprinting influenced in a way which may**

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  - **psychical traits of fetus**

# Imprinting: Perspectives

- Could be an important adaptive function in phylogenesis and ontogenesis
- More attention to interaction between culture medium and imprinting in future
- Probably a small quantitative impact in IVF
- Potential invalidating influence on stem cell cultures and in vitro maturation
- Could be a new approach to artificial modification of psychological traits in human beings

# Imprinting: Actions

- More research on the influence of culture media on fetal outcomes
- Systematic follow-up of IVF children
- Common ICD codes for all imprinting diseases (European)
- Specific attention on IVM outcomes and embryos transferred as blastocysts

Slides on www.lidegaard.dk/slides