Early prediction of genetic disorders in preimplantation embryos based on morphological observations

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PRONATAL Prague

Early prediction of genetic disorders in preimplantation embryos

part of interactive PGS cycle management
…to minimize biological and technical limitations of aneuploidy screening

1. embryology - aneuploidy and mosaicism prediction

2. genetics - FISH aneuploidy detection

Early prediction of genetic disorders in preimplantation embryos

part of interactive PGS cycle management
…to minimize biological and technical limitations of aneuploidy screening

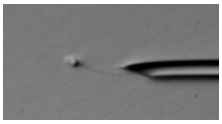
1. embryology - aneuploidy and mosaicism prediction

reevaluation of questionable *(monosomic)* findings
(the 3rd round reanalysis)

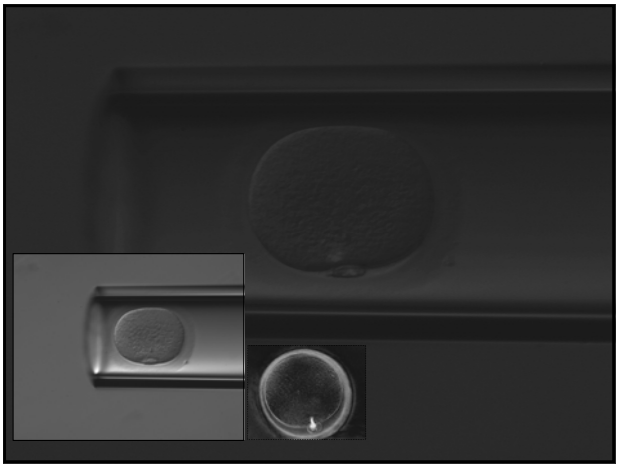
2. genetics - FISH aneuploidy detection

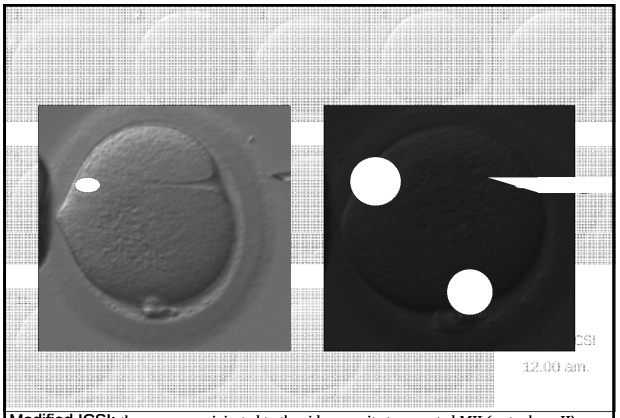
A real example of interactive PGS cycle management

- Patient history: *3 previous IVF attempts*
- PGS indication: *age (41 years) and implantation failure*
- SPG: *normospermy*
- OPU: *15 oocytes (1xGV)*

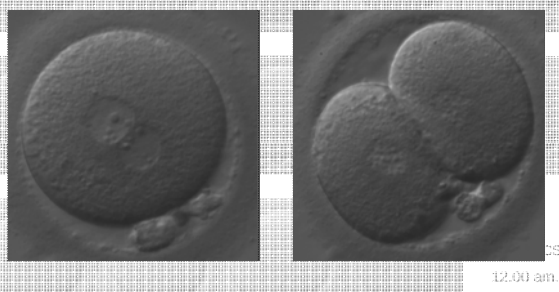


- Expected findings:
  - *Oocyte derived meiotic errors – the main source of aneuploidies affecting all embryonic cells*
  - *Ooplasmic factors deficiency – causing developmental abnormalities (arrests) and mitotic malsegregations leading to mosaic cells occurrence*



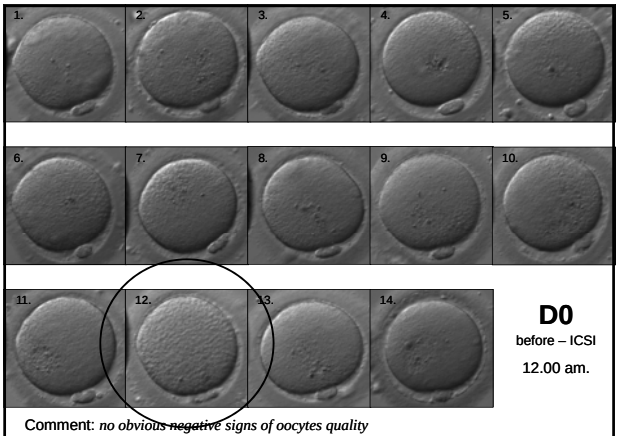


Modified ICSI: *the sperm was injected to the side opposite to expected MII (metaphase II) plate*



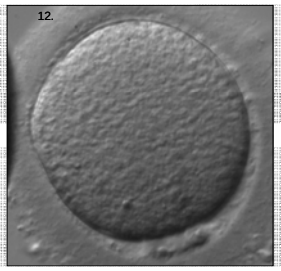
D0
before – ICSI
12.00 am.

Modified ICSI: *the sperm was injected to the side opposite to expected MII (metaphase II) plate to improve an embryonic development*



D0
before – ICSI
12.00 am.

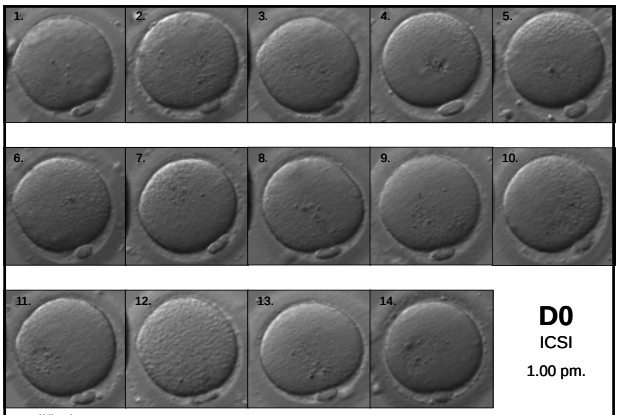
Comment: *no obvious ~~negative~~ signs of oocytes quality*



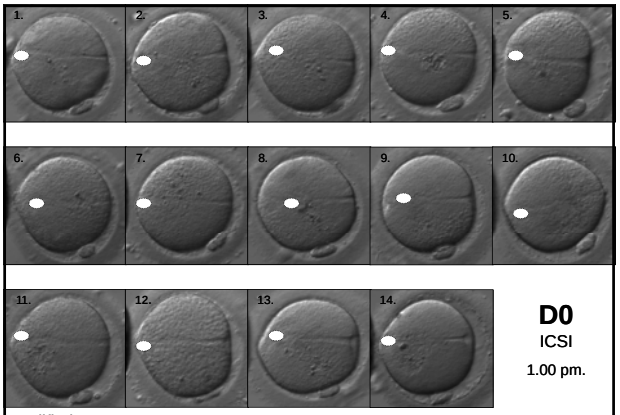
- degeneration
- absence of fertilization
- abnormal development

D0
before – ICSI
12.00 am.

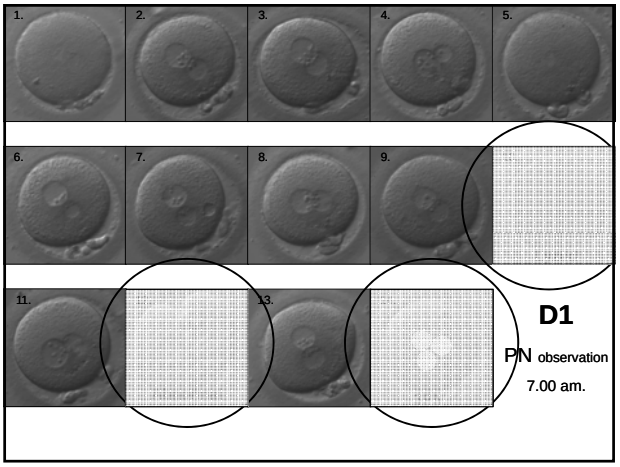
Comment: *except of the oocyte No. 12 – atretic cytoplasm*

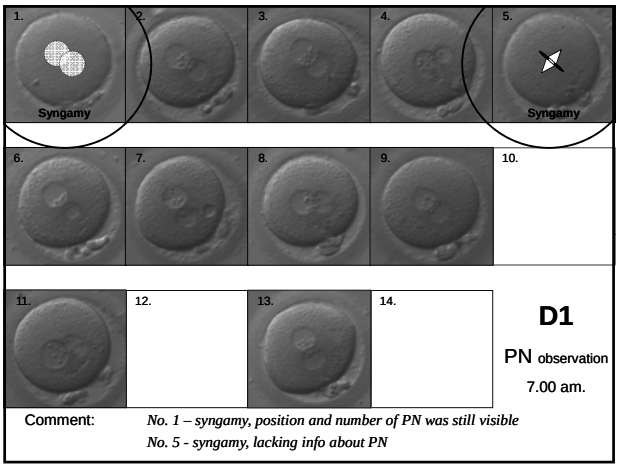


Modified ICSI: *the sperm was injected to the side opposite to expected MII (metaphase II) plate*

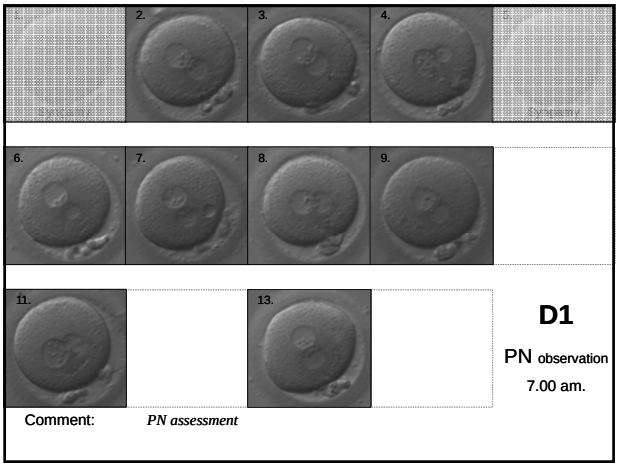


Modified ICSI: *the sperm was injected to the side opposite to expected MII (metaphase II) plate*

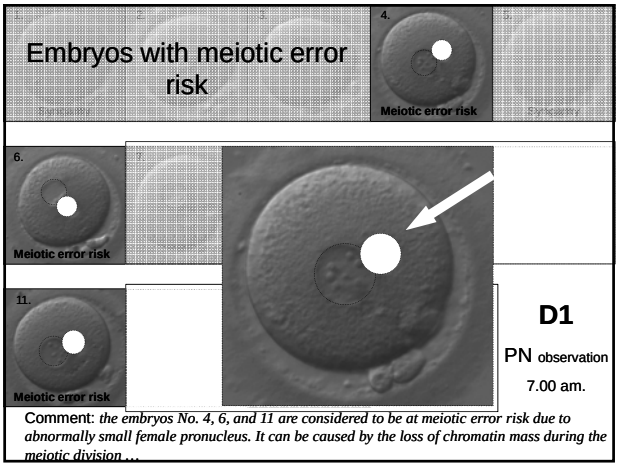




Comment: *No. 1 – syngamy, position and number of PN was still visible*
No. 5 - syngamy, lacking info about PN

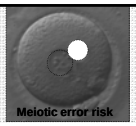


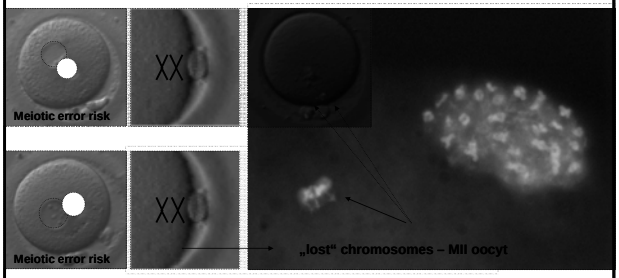
Comment: *PN assessment*



Comment: *the embryos No. 4, 6, and 11 are considered to be at meiotic error risk due to abnormally small female pronucleus. It can be caused by the loss of chromatin mass during the meiotic division …*

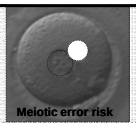
Embryos with meiotic error risk

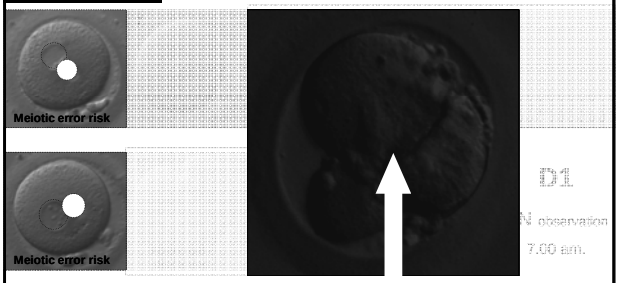




….the loss of chromosomes during the 1st and 2nd meiotic division can affect all embryonic cells and substantial chromatin mass loss can affect the size of female pronucleus

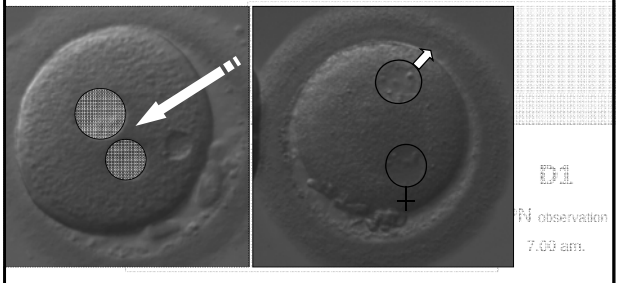
Embryos with meiotic error risk or abnormal development





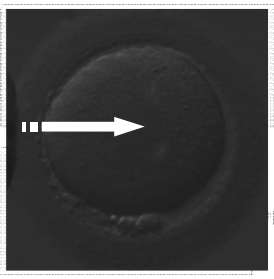
…. small female pronucleus is frequently observed in asynchr ous PN disappearance what leads to abnormal embryonic development

Embryos with meiotic error risk or abnormal development

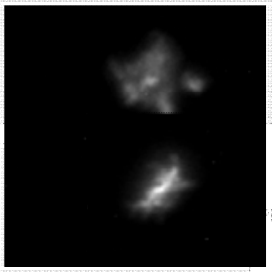


…. also distinct pronuclei (No.7.) can indicate the occurrence of abnormalities

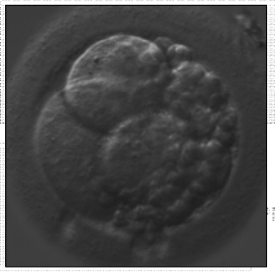
Embryos with meiotic error risk
or abnormal development



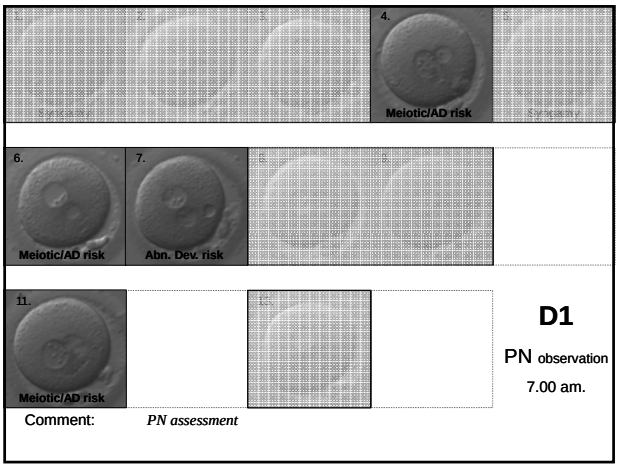
Embryos with meiotic error risk
or abnormal development

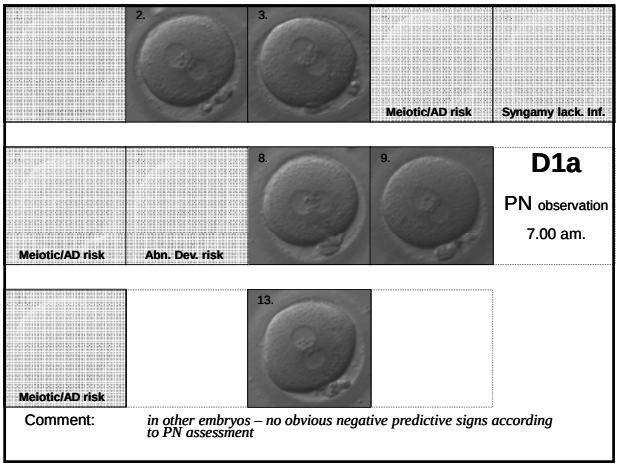


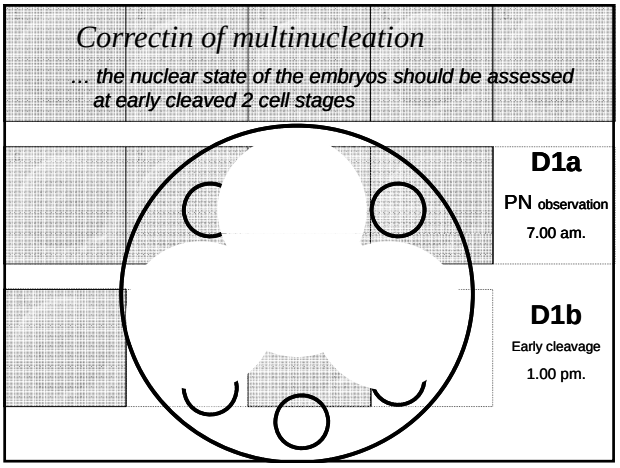
Embryos with meiotic error risk
or abnormal development



uniparental segregation

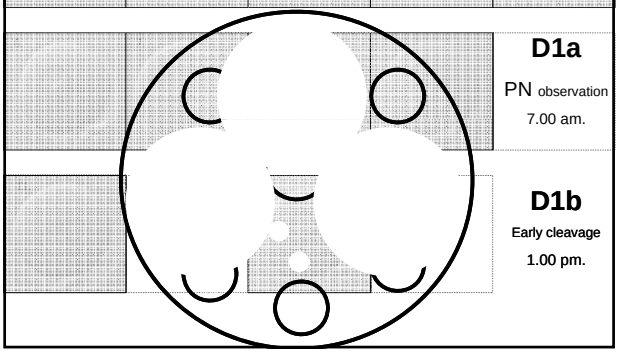

4.
Meiotic/AD risk
6.
Meiotic/AD risk
7.
Abn. Dev. risk
11.
Meiotic/AD risk
D1
PN observation
7.00 am.
Comment:
PN assessment


2.
3.
Meiotic/AD risk
Syngamy lack. Inf.
8.
9.
Meiotic/AD risk
Abn. Dev. risk
D1a
PN observation
7.00 am.
13.
Meiotic/AD risk
Comment:
in other embryos – no obvious negative predictive signs according to PN assessment


Correctin of multinucleation
... the nuclear state of the embryos should be assessed at early cleaved 2 cell stages
D1a
PN observation
7.00 am.
D1b
Early cleavage
1.00 pm.

Correctin of multinucleation

… the nuclear state of the embryos should be assessed at early cleaved 2 cell stages



D1a

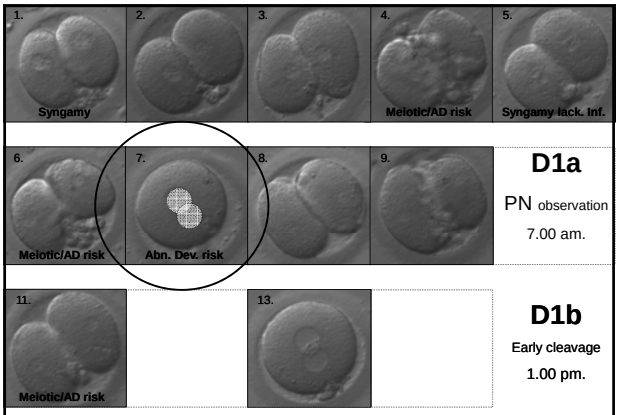
PN observation

7.00 am.

D1b

Early cleavage

1.00 pm.



D1a

PN observation

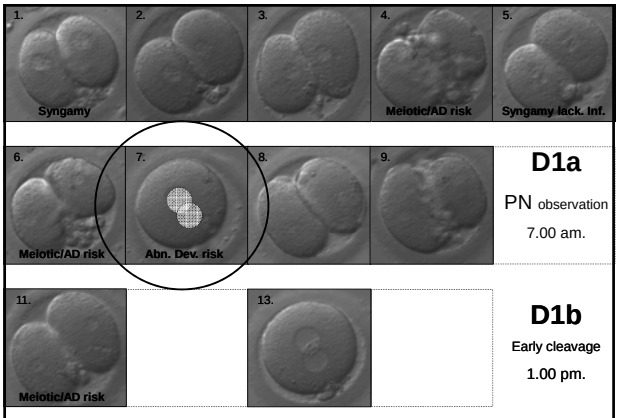
7.00 am.

D1b

Early cleavage

1.00 pm.

Comment: *No. 7–normal apposition of PN was noticed*



D1a

PN observation

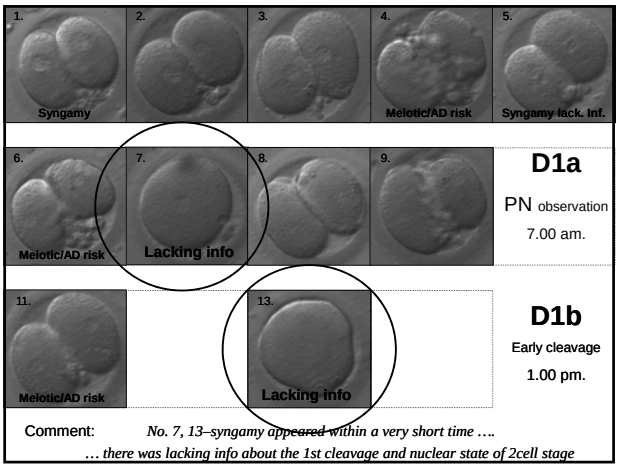
7.00 am.

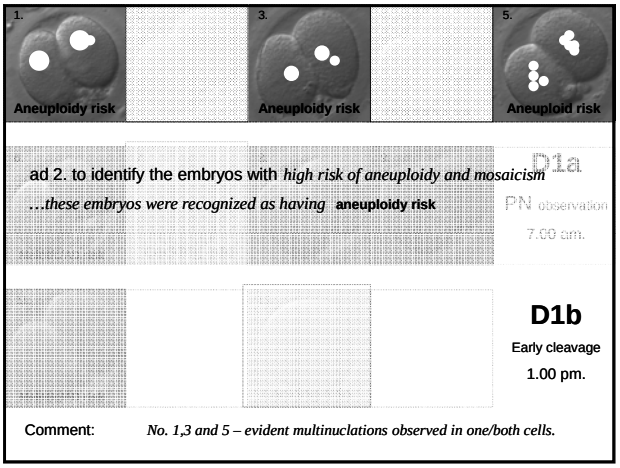
D1b

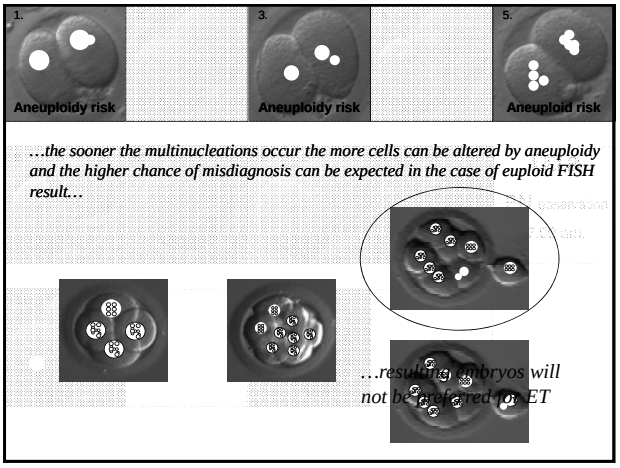
Early cleavage

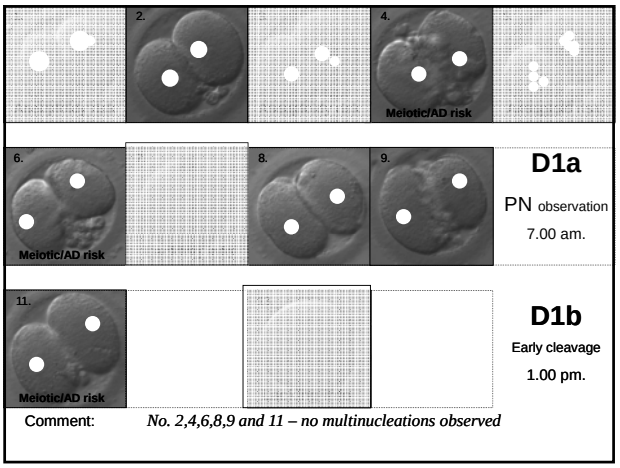
1.00 pm.

Comment: *No. 7–normal apposition of PN was noticed and our prediction was revised*

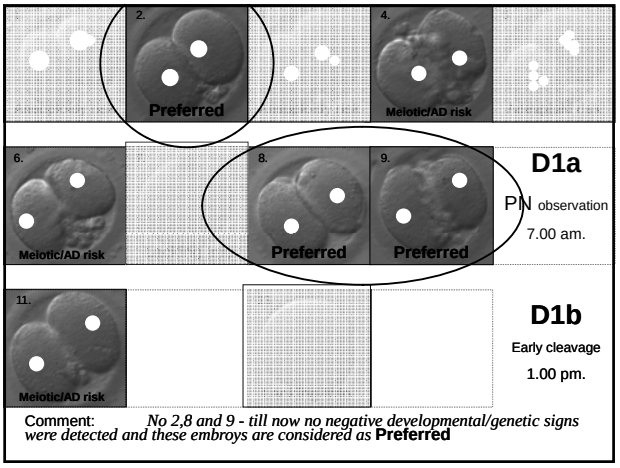

1.
Syngamy
2.
3.
4.
Meiotic/AD risk
5.
Syngamy lack. Inf.
6.
Meiotic/AD risk
7.
Lacking info
8.
9.
D1a
PN observation
7.00 am.
11.
Meiotic/AD risk
13.
Lacking info
D1b
Early cleavage
1.00 pm.
Comment:
No. 7, 13–syngamy appeared within a very short time ….
… there was lacking info about the 1st cleavage and nuclear state of 2cell stage


1.
Aneuploidy risk
3.
Aneuploidy risk
5.
Aneuploid risk
ad 2. to identify the embryos with high risk of aneuploidy and mosaicism
…these embryos were recognized as having aneuploidy risk
D1a
PN observation
7.00 am.
D1b
Early cleavage
1.00 pm.
Comment:
No. 1,3 and 5 – evident multinuclations observed in one/both cells.

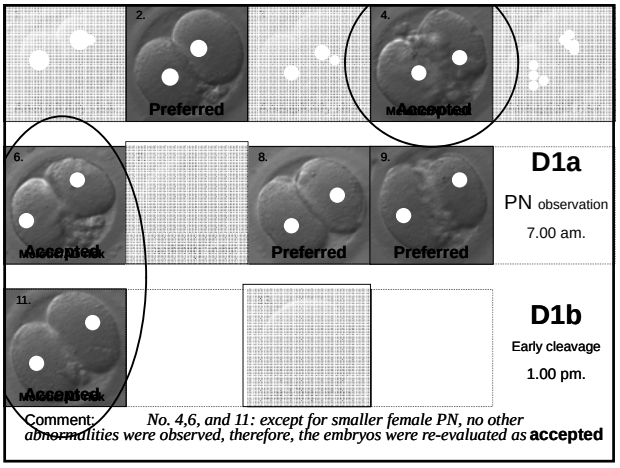

1.
Aneuploidy risk
3.
Aneuploidy risk
5.
Aneuploid risk
…the sooner the multinucleations occur the more cells can be altered by aneuploidy and the higher chance of misdiagnosis can be expected in the case of euploid FISH result…
…resulting embryos will not be preferred for ET



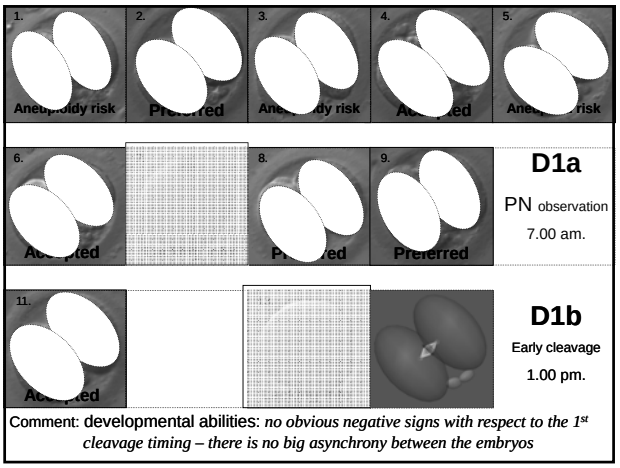
Comment: *No. 2,4,6,8,9 and 11 – no multinucleations observed*



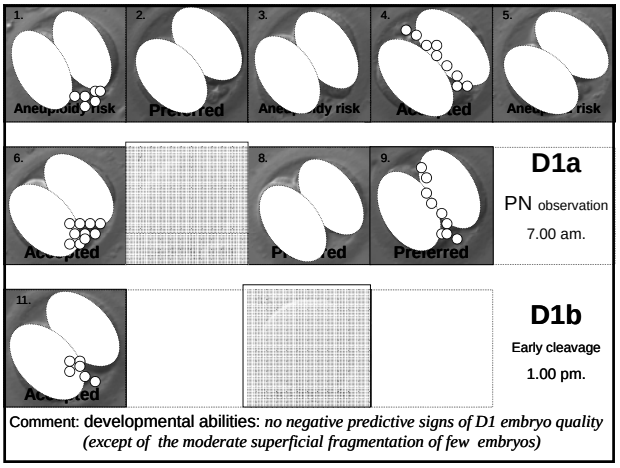
Comment: *No 2,8 and 9 - till now no negative developmental/genetic signs were detected and these embroys are considered as* **Preferred**



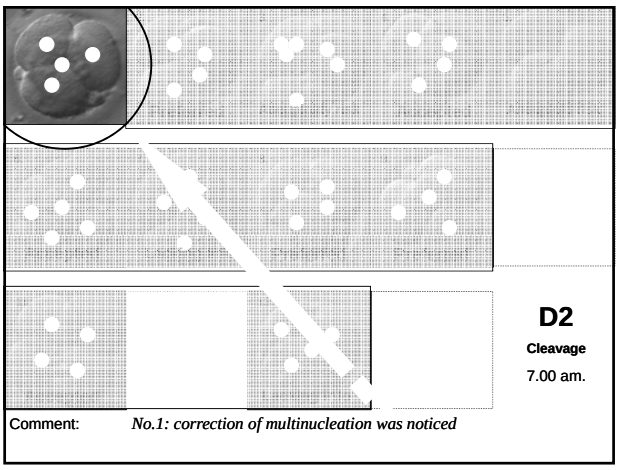
Comment: *No. 4,6, and 11: except for smaller female PN, no other abnormalities were observed, therefore, the embryos were re-evaluated as* **accepted**



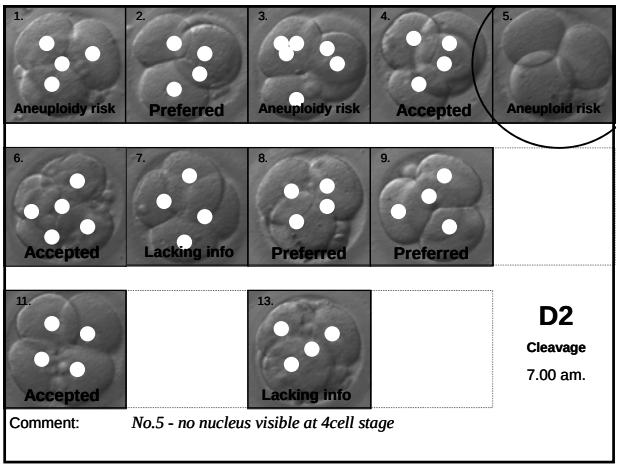
Comment: developmental abilities: *no obvious negative signs with respect to the 1st cleavage timing – there is no big asynchrony between the embryos*



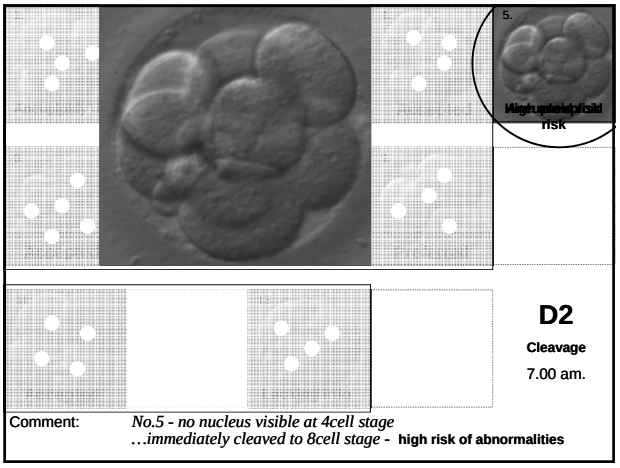
Comment: developmental abilities: *no negative predictive signs of D1 embryo quality (except of the moderate superficial fragmentation of few embryos)*



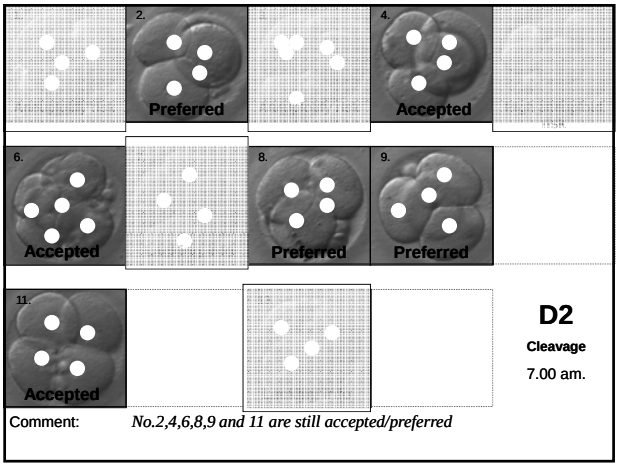
Comment: *No.1: correction of multinucleation was noticed*



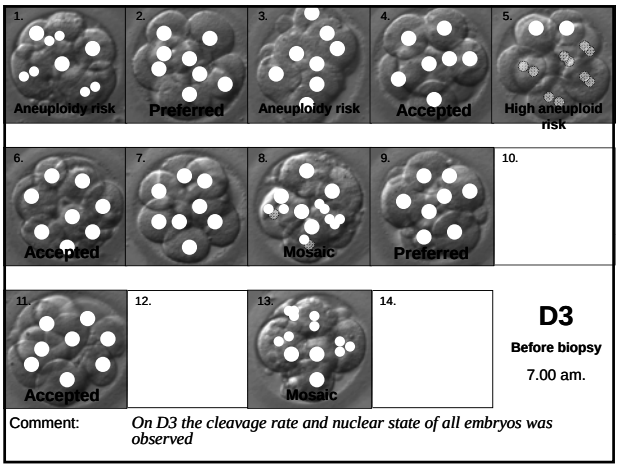
Comment: *No.5 - no nucleus visible at 4cell stage*



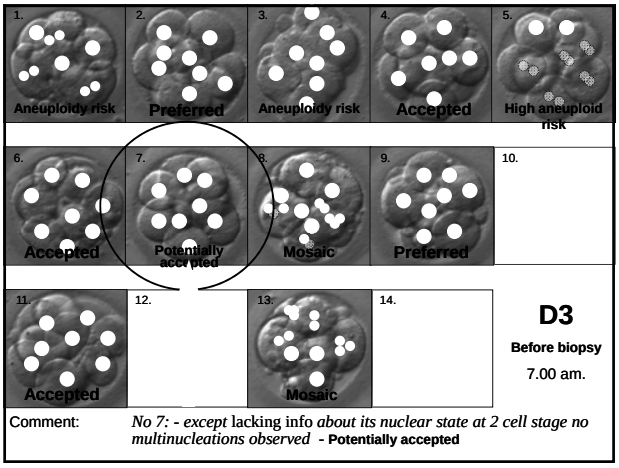
Comment: *No.5 - no nucleus visible at 4cell stage*
…immediately cleaved to 8cell stage - **high risk of abnormalities**



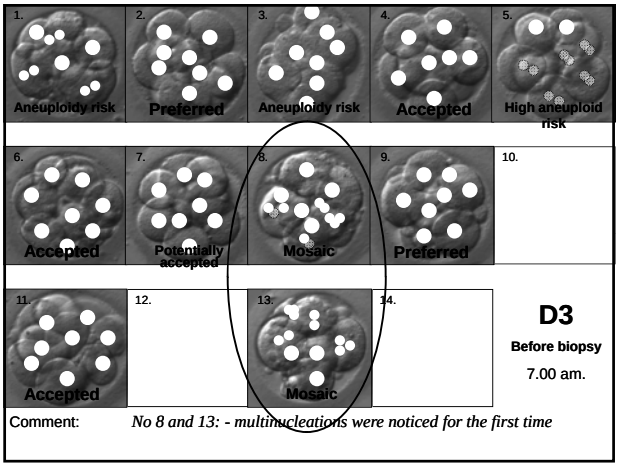
Comment: *No.2,4,6,8,9 and 11 are still accepted/preferred*



Comment: *On D3 the cleavage rate and nuclear state of all embryos was observed*



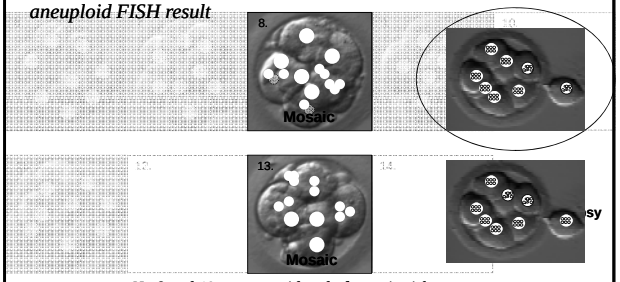
Comment: *No 7: - except* lacking info *about its nuclear state at 2 cell stage no multinucleations observed* **- Potentially accepted**



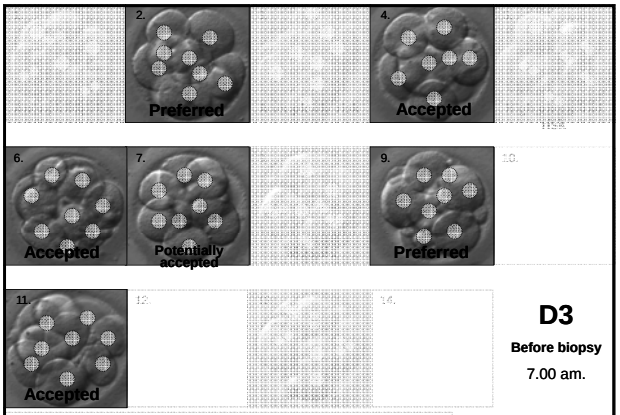
Comment: *No 8 and 13: - multinucleations were noticed for the first time*

ad 2. to identify the embryos with *high risk of aneuploidy and mosaicism*

…the latter they appear the less cells are altered by aneuploidy and higher chance of misdiagnosis can be expected in the case of aneuploid FISH result



Comment: *No.8 and 13 were considered of mosaic risk*



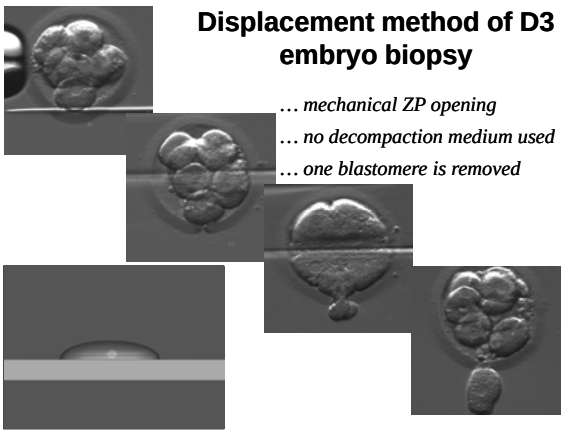
D3

Before biopsy

7.00 am.

Comment: *No 2,4,6,7,9 and 11: - still preferred or potentially accepted*

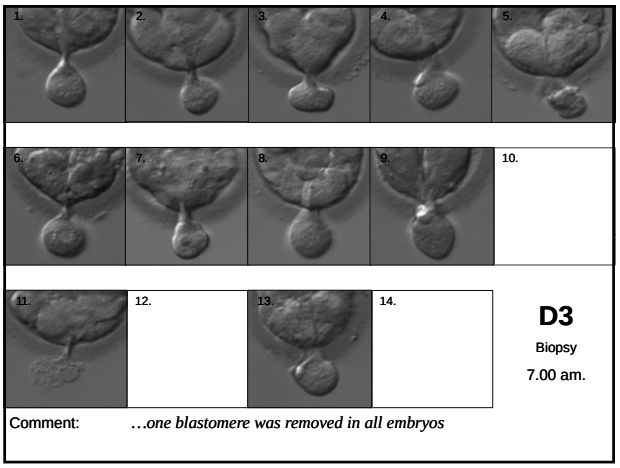
Displacement method of D3 embryo biopsy



… mechanical ZP opening

… no decompaction medium used

… one blastomere is removed

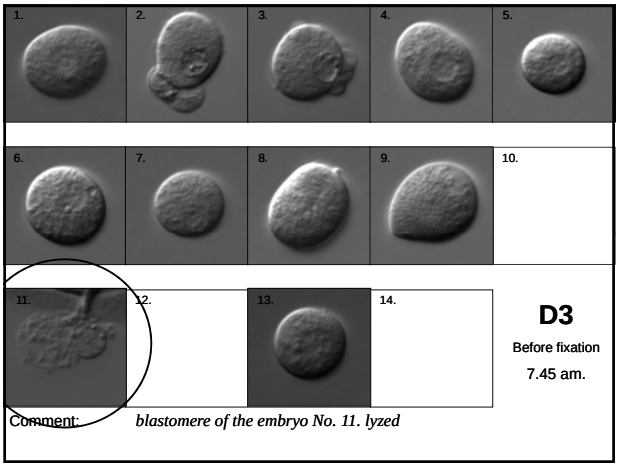


D3

Biopsy

7.00 am.

Comment: *…one blastomere was removed in all embryos*

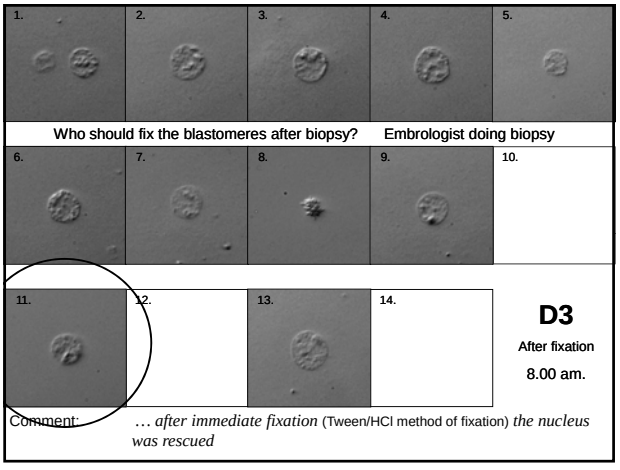


D3

Before fixation

7.45 am.

Comment: *blastomere of the embryo No. 11. lyzed*

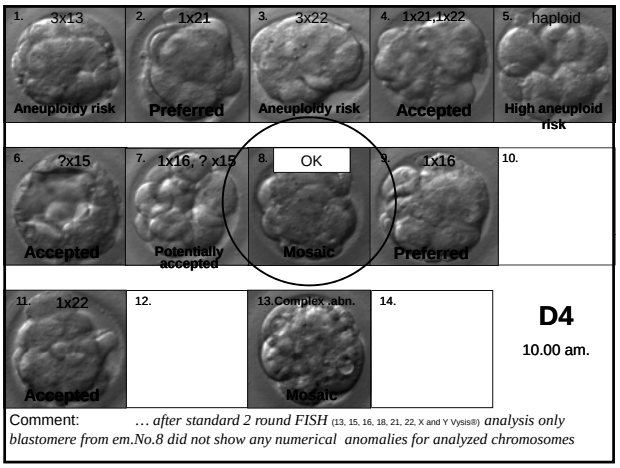


D3

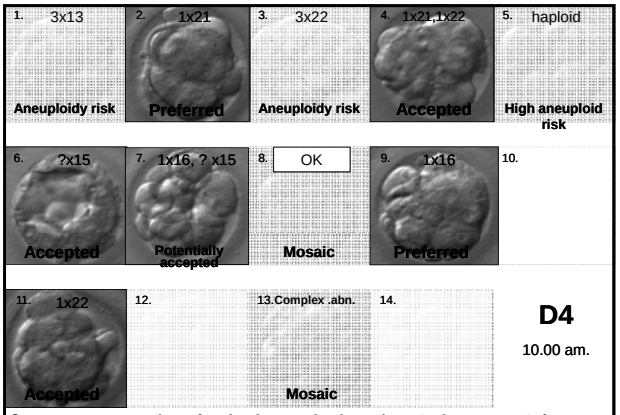
After fixation

8.00 am.

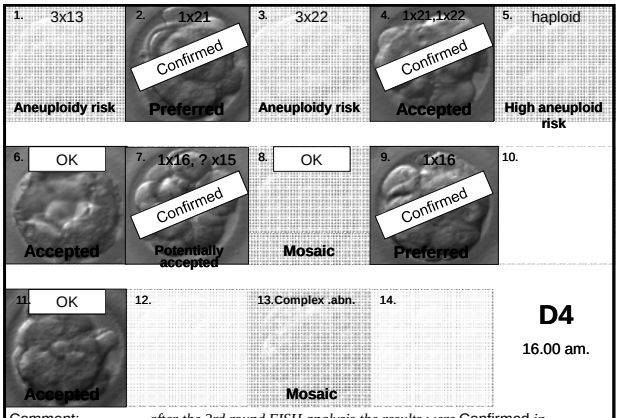
Comment: *… after immediate fixation* (Tween/HCl method of fixation) *the nucleus was rescued*



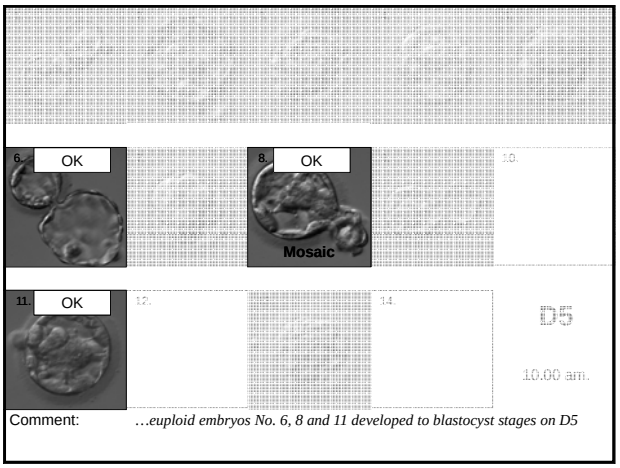
Comment: *… after standard 2 round FISH* (13, 15, 16, 18, 21, 22, X and Y Vysis®) *analysis only blastomere from em.No.8 did not show any numerical anomalies for analyzed chromosomes*



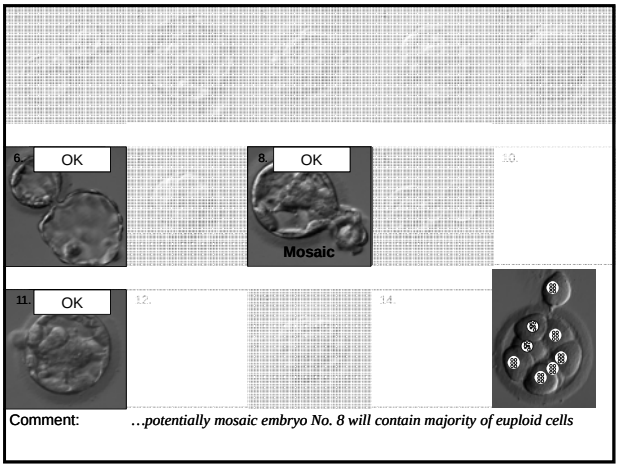
Comment: *… the preferred and accepted embryos determined as monosomic for some chromosome(s) were subjected to 3rd round FISH analysis using alternative* (eg. subtelomeric – Kreatech instead of centromeric – Vysis set of dg. sonds)



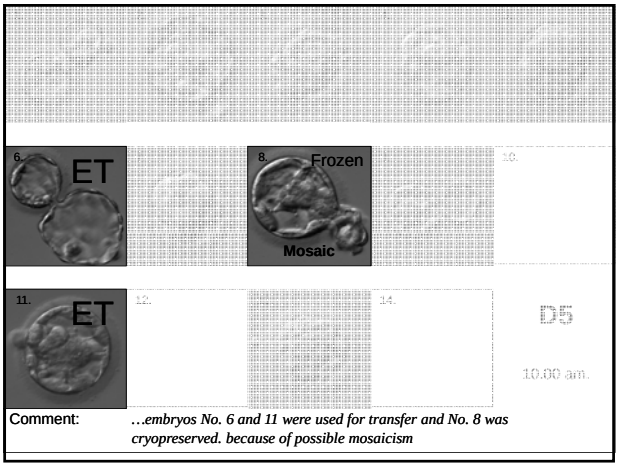
Comment: *…after the 3rd round FISH analysis the results were* Confirmed *in embryos No. 2,4,7 and 9 and* Revised *in No. 6 and 11*



Comment: *…euploid embryos No. 6, 8 and 11 developed to blastocyst stages on D5*

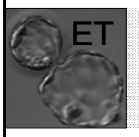


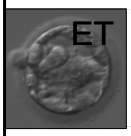
Comment: ***…potentially mosaic embryo No. 8 will contain majority of euploid cells***



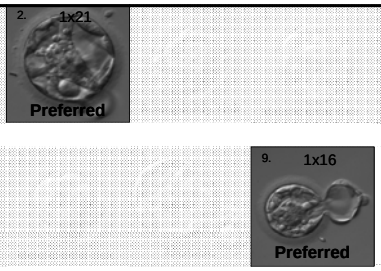
Comment: ***…embryos No. 6 and 11 were used for transfer and No. 8 was cryopreserved. because of possible mosaicism***

8th week of pregnancy - singleton





D5



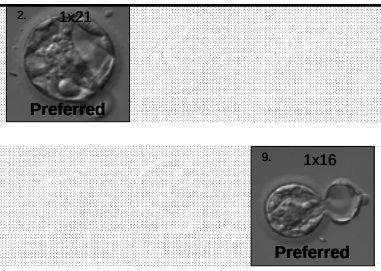
… in this case none of morphologicaly preferred embryos was determined as genetically competent what underlines the importance of PGS

D5

developmental competency and genetic constitution are to some extent independent properties of early embryo

… the embryos predicted „risky" for aneuploidy and mosaic were confirmed by FISH to be aneuploid

… the 3rd round FISH reanalysis rescued some embryos which were determined as monosomic for some chromosome and otherwise would be discarded



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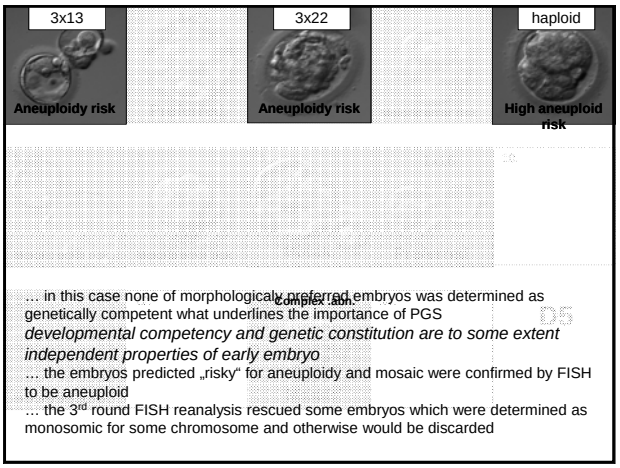

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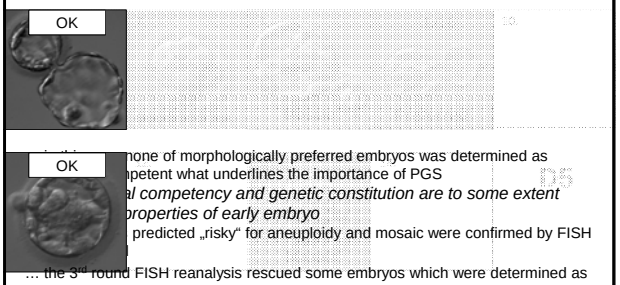

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Conclusion:

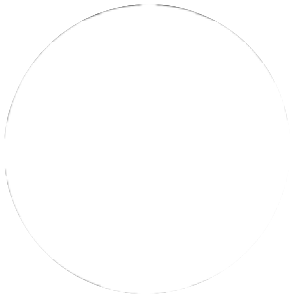
… an interactive embryologist/genetic approach in PGS cycles with 3rd round reanalysis can rescue some developmentally competent embryos (diagnosed as monosomic for one chromosome)

… prediction of genetic disorders by morphological observation plays an important role in this system and can also be effectively used in all IVF cycles

Conclusion:

…a real benefit of aneuploidy screening is that the method is forcing us to deeper understanding of developmental processes and it gives the chance to improve our knowledge

…finally they got it…



…an embryo sexing…

…finally they got it…

Thank you for your attention