Transferring blastocysts in the poor responder patient
Pros & Cons

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What do we know?

• Following natural conception, the embryo is in cleavage stage while traversing the fallopian tube, but blastocyst stage in the uterus for implantation
• Embryo development to cleavage stage occurs under maternal genomic control
• To reach blastocyst stage, embryos need to develop under their own genomic control
What do we think we know?

- Implantation rates of blastocysts tend to be higher than those for cleavage stage embryos.
- Culturing to blastocyst may identify the embryos with higher quality and allow transfer of fewer embryos to reduce the multiple pregnancy rate but at the same time to maintain the pregnancy rate.
Why need to consider an alternative to cleavage stage embryo transfer

• It is physiologically premature to expose early-stage embryos to the uterine environment
• The uterus provides a different nutritional milieu from the fallopian tube
• Cleavage embryo transfer may cause homeostatic stress on the embryo, resulting in a reduced implantation potential
• Acknowledges shortcomings of the morphological criteria used for selection of cleavage-stage embryos. A large proportion of morphologically normal Day 3 embryos are chromosomally abnormal
Why need to consider an alternative to cleavage stage embryo transfer

- In order to achieve acceptable pregnancy rates, two or more embryos had to be replaced.
- However, pressure on the ART industry to reduce the multiple birth rate has seen a steady decline in the number of embryos to be transferred. Elective single embryo transfer for selected patients is the now the trend.
What do we not know?

• Whether a policy of blastocyst culture offers genuine advantages
• Which couples may benefit from blastocyst culture and how to identify them
Criticism with blastocyst culture

- Having no embryos to transfer
- Having no extra embryos for cryopreservation for future use
- Monozygotic twinning
- Altered sex ratio in births
Day 3

Day 5
Blastocyst vs Blastomere Transfer

- Meta-analysis on 1944 couples in 15 RCT from 16 publications (including studies with same and different no. of blastocysts/ cleavage embryos being transferred and patients with different prognosis):
  - No difference in: Life birth rate per couple
    Pregnancy rate per couple
    Multiple pregnancy rate per couple
    Miscarriage rate per couple
  - Increase chance of no embryos for transfer with blastocyst culture
  - Increase rates of embryo freezing per couple with cleavage embryo transfer
  - Monozygotic twining???

N Johnson, D Blake, C Farquhar 2007
Blastocyst vs Blastomere Transfer

- When at least 4 good-quality embryos are available on Day 3 of embryo culture and then to proceed with blastocyst culture:
  - blastocyst-stage transfer resulted in:
    - Higher ongoing pregnancy rate [51.3% Vs 27.4%]
      (OR 2.78; 95% CI 1.45-5.34)
    - Higher live birth rate [47.5% Vs 27.4%]
      (OR 2.4; 95% CI 1.25-4.59)
  - Higher twin birth rate observed in both blastocyst and cleavage-stage embryo transfer (36.38% Vs 30.4%; P >0.05)

Papanikolaou EG, et al. 2005
Blastocyst vs Blastomere Transfer

- Meta-analysis for IVF outcome after transfer of equal number of blastocysts or cleavage-stage embryos
- 1654 patients, from 8 studies:
  - higher life-birth rate with blastocyst transfer
    (OR 1.39; 95% CI: 1.10-1.76; P=0.005)
  - higher clinical pregnancy rate with blastocyst transfer
    (OR 1.27; 95% CI: 1.03-1.55; P=0.02)
  - Multiple pregnancy rate not significantly different
    (OR 0.86; 95% CI: 0.58-1.29; P=0.46)
Blastocyst vs Blastomere Transfer

Higher cancellation rate in patients randomized to have a blastocyst stage embryo transfer

\[(\text{OR } 2.21; \text{ 95\% CI: } 1.47-3.32; \text{ P}=0.0001)\]

Lower cryopreservation rate in patients who had a blastocyst stage transfer

\[(\text{OR } 0.28; \text{ 95\% CI: } 0.14-0.55; \text{ P}=0.0002)\]

Papanikolaou EG, et al. 2008
Blastocyst vs Blastomere Transfer

Cumulative pregnancy rate from fresh + frozen embryo transfers

- 478 couples with eSET (n=243) and eSBT (n=235)
- Higher clinical implantation rate in fresh cycles of eSBT
  - (43.6% Vs 29.6%, P<0.004)
- Higher delivery rate per oocyte retrieval with fresh cycles of eSBT
  - (34.0% Vs 25.1%, P<0.05)
- After failed fresh transfer:
  - eSET group: 216 of 253 frozen cleavage embryos & 59 of 71 frozen blastocysts were thawed; total of 154 cleavage embryos and 34 blastocysts were transferred
  - eSBT group: 138 of 178 frozen blastocysts were thawed and 82 were transferred
<table>
<thead>
<tr>
<th></th>
<th>eSET group</th>
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<th>SBT group</th>
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<td>Day 2 embryo thawing</td>
<td>Day 5/6 blastocyst thawing</td>
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<td>Number of thawed cycles</td>
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<td>Number of transfer cycles</td>
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<td>Number of clinical pregnancies</td>
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<td>2 3</td>
<td>6 3</td>
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<td>Rate per transfer cycle</td>
<td>20.0% 21.1%</td>
<td>12.5% 33.3%</td>
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<tr>
<td>Number of gestational sacs</td>
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<td>2 3</td>
<td>6 3</td>
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<td>Clinical implantation rate</td>
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<td>12.5% 16.7%</td>
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<tr>
<td>Number of deliveries</td>
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<td>2 3</td>
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<td></td>
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<tr>
<td>Rate per transfer cycle</td>
<td>12.0% 21.1%</td>
<td>12.5% 33.3%</td>
<td>15.0% 14.3%</td>
<td></td>
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<tr>
<td>Number of multiple deliveries (%)</td>
<td>1 (16.7) 2 (18.2)</td>
<td>0 0</td>
<td>0 0</td>
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</tbody>
</table>

E Tr, Day 2 embryo transferred; B Tr, Day 5/6 blastocyst transferred.
Blastocyst vs Blastomere Transfer

- In the eSET group, 26 additional delivered were achieved following frozen embryo transfers, whereas only 9 were achieved in the eSBT group.
- The cumulative delivery rate per cycle was similar in both groups: 34.2% in eSET group Vs 37.9% in eSBT group.
- No difference was observed in the cumulative multiple delivery rate between both groups.

Blastocyst vs Blastomere Transfer

eSBT vs eDET at cleavage stage

• In 173 IVF/ICSI cycles
• Patients with at least 2 available embryos on D3 were asked to consider eSBT
• Patients who requested to have DET were encouraged to do so at the cleavage stage
• Clinical pregnancy rate in eSBT was higher than eDET at cleavage stage (42.9% vs 39.8%)
• No multiple pregnancy in eSBT group but 25.7% multiple pregnancy rate (all twins) in eDET group

Saunders PA, et al. 2013
Blastocyst Transfer and Monozygotic Twining

Monozygotic twinning (MZT)

- May be the result of an abnormal hatching event, induced by a breach in the integrity of the zona pellucida (ZP), herniation of the blastomeres, and splitting of the embryo
- Such a breach may be caused by AR procedures such as ICSI and/or assisted hatching
- The damaged ZP may stimulate the splitting of the ICM
- MZT is associated with increased morbidity of miscarriage, structural congenital abnormality (including conjoined twins, acardiac state, microsomia, limb reductions), growth discordancy and twin-to-twin transfusion syndrome
Blastocyst Transfer and Monozygotic Twining

Meta-analysis of 9 studies

• 7 studies higher risk of MZT after blastocyst transfer, but a statistical significance achieved in only 4 trials

• After using the random effect model, MZT risk was demonstrated higher after blastocyst transfer then after early cleavage-stage embryo transfer (OR 3.04; 95% CI 1.54-6.01)

• Criticism – substantial heterogeneity among the MZT trials
  – with subgrouping based on the study period, MZT rates difference was observed during the earlier study period before 2002 (OR 4.05; 95% CI 3.16 – 5.18), but no difference in studies after 2002 (OR 1.00; 95% CI 0.43-2.32)

Chang HJ, et al. 2008
Blastocyst Transfer and Monozygotic Twining

Other Mechanisms suggested for increased MZT with blastocyst transfer:
• Extended time in culture
• Culture media composition
• Experience of the embryology laboratories

With improvement in culture medium and experience of embryologist, the increased rate of MZT in blastocyst culturing might not be observed
Blastocysts Transfer and Monozygotic Twining

- 233 of 9272 clinical pregnancy (2.5%) had MZT
- The prevalence was higher following blastocyst then cleavage embryo transfer (1.9% Vs 3.0%, P=0.005)
- After grouping by maternal age, blastocyst and cleavage embryo transfer had equivalent MZT
- Grouping by mean cell number of the developing cohort of embryos on D3 showed increasing MZT as the quality of the overall cohort improved (P<0.0001)
- Grouping by mean cell number of entire cohort on D3 showed equivalent MZT rates following blastocyst or cleavage embryo transfer
- The highest risk for MZT was in those with supernumerary embryos for cryopreservation, reflecting good cohort quality. While high, blastocyst and cleavage embryo transfers were equivalent

Hong KH, et al. 2012
Does Blastocyst transfer alter sex ratio

• Meta-analysis of 4 studies
• M/F ratios were consistently higher after blastocyst transfer the and after cleavage-stage transfer in all studies, but statistical significance was achieved in only one report
• Of the 1102 infants born from blastocyst transfer, 626 were male and 476 were female, giving an M/F ratio of 1.32
• M/F ratio in cleavage-stage ET was 1.04
• OR 1.29; 95% CI 1.10-1.51)

Chang HJ, et al. 2008
Neonatal Outcome Blastocyst Transfer

Neonatal outcomes among singleton births

- Significantly higher odds of preterm births after blastocyst transfer in 4 studies (including 54,795 cleavage stage and 20,724 blastocyst stage births, OR 1.32, 95% CI 1.19-1.46)
  
  But no difference in the adjusted odds of VPTB (AOR 1.18, 95% CI 0.93-1.49)

- No difference in LBW in 4 studies (54,109 cleavage stage and 20,392 blastocyst births, AOR 1.06; 95%CI 0.9-1.15)

- No difference in VLBW in 3 studies (22,088 cleavage stage and 5,772 blastocyst births, AOR 1.01; 95% CI 0.73-1.38)

- Significantly higher odds of congenital anomalies in 2 studies (including 22,068 cleavage stage and 4,517 blastocyst stage births, OR 1.18, 95% CI 1.03-1.62)

Dar S, et al. 2014
Key Issues

• What is the rationale for extending culture for blastocyst transfer (D5/6)?

• What are the downsides to extending culture?
Effect of prolonged culture

- Up genes for apoptosis, oxidative stress
  - (Lonergran 2003 RBM online)
- Affects epigenetic reprogramming, imprinted genes (Calle Theriogenology 2012)
- Increase monozygotic twins
- Affects gender ratio
Pros of extended culture

- Superior selection of most viable embryos
  - Identify embryos that are capable of developing to the Blastocyst stage
- Modest selection against aneuploid embryos
- Allows transfer of fewer higher quality embryos
- Uterine receptivity issues
  - Uterine environment is likely to be more favorable for blastocyst transfer

Theoretically, higher implantation rates and lower multiple birth rates will result
Cons of extended culture

- Increased risk of transfer cancellation
- Is the culture system as good as the uterus?
- Pregnancy rates may be decreased in poor prognosis patients
  - Study: Day 5 ET decreases the chance of pregnancy in cases of poor day 3 embryo quality (Racowsky et al Fertil Steril 2000)
- Increased risk of monozygotic and monochorionic twins after day 5 transfers
Cons of extended culture

- **Embryo cryopreservation issues**
  - Because of the attrition of viable embryos from day 3 to day 5, fewer embryos are available for freezing on day 5.

- **Decreased cumulative PR**
  - Fewer embryos frozen on day 5/6 = Decreased cycle efficiency (i.e. Cumulative PR).

- **Imprinting disorders**
  - IVF babies have an increased risk for some syndromes associated with loss of imprinting (e.g. Beckwith Weidemann’s and Angelman’s).
Cons of extended culture

• Increase in adverse neonatal outcomes?
•  three times risk in birth defects (Huang 2013)
• Skewing of Male offspring
  – Male embryos are 2.6 times more likely to produce a grade 5 or 6 blastocyst compared with female embryos (Alfarawati et al 2011)
• Lab issues
  – Requires more incubator space and more embryology time
  – Low oxygen tension required?
Blastocyst transfer for all

- Risk of no transfer – 10% vs 2%
- (IF there are blastocysts)
- Reduced cryopreservation potential
- May be higher pregnancy rate/transfer fresh
- But significant difference cumulative PR favouring cleavage transfer (cochrane review 2012)
Patient’s View

• May not choose blastocyst culture given the 50-60% blastocysts rate, especially if number of day 3 embryos are low?
• May prefer to be informed on Day 5 if their embryos have low viability and not to have an embryo transfer, rather than to suffer the disappointment of failed implantation; and to reduce cost for embryo transfer/ cryopreservation?
• Want to start a fresh cycle earlier rather than to waste time for FET cycles with poor chance of conception?
• May wish to have embryo transfer irrespective of embryo quality because does not want to “lose any chance”?
• Little research into the feelings of women given such choices
Patient’s View (Poor responders)

• Every cycle is an asset, one additional chance
• May not accept blastocyst culture as usual cleavage embryos number small (1-2), and extended culture no guarantee transfer
• In third party paid countries different mindset?
• Expect service providers to decide by individual evidence with honesty
Is this the ultimate

• 1 egg
• 1 embryo – 1 blastocyst
• 1 singleton pregnancy
• (1 PGS?)
Criteria for selective blastocyst culture and transfer

- 10 eggs collected
- 8 to cleavage stage
- 4 good quality D3 embryos
- No previous failures

How does poor responders stood up to this test?
Conclusion

1. Blastocysts have advantages over cleavage embryos in higher implantation rate and lower miscarriage rate
2. Blastocyst culture also may have undesirable effects like reduced number of embryos, MZT, sex and imprinting problems
3. In patients with a lot of oocytes, blastocyst culture and transfer is desirable
4. For poor responders, culture blastocysts and transfer may lead to more despair, increase in cost, and therefore not the optimal choice
Organizing Committee:
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Speakers:
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Dr. Yoshiharu Morimoto, Dr. Zeev Blumenfeld, Dr. S.L. Tan, Dr. Johan Smitz, Dr. Jie Qiao, Dr. PC Ho,
Dr. Yanping Kuang, Dr. Ernest Ng, Dr. Peter Leung, Dr. Marc-Andre Sirard, Dr. John Yovich, Dr. Ben Tsang,
Day 2/3 vs Day 5/6

• DET cleavage = SET blastocyst
• Lower fresh cycle PR
• Better cumulative PR
  – ? Previous poor blast freezing
• Less monozygotic twinning
• Less cancellation rate
Day 2/3 vs Day 5/6

- Poor responders – Day 2/3 better than Day 5
- Less laboratory personnel cost
- No need for patient return
Cons against Day 2/3 transfer

- Decrease embryo selection
- Decrease selection aneuploidy
- Reduced uterine receptivity