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Comparison of two embryo culture media : a randomized study using sibling oocytes

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Disclosure

The authors have no commercial relationships or other conflicts of interest to declare

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Introduction

Succes rates in assisted reproduction are influenced by a lot of factors, the most important :

- patient's age,
- number of oocytes retrieved
- morphology of the embryos transferred

Morphology of the embryos can be influenced by culture conditions

In vitro development of human embryos for 48 to 72 h can be achieved using a wide variety of media, ranging from simple balanced salt solutions to more complex media

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Transfer of one good morphology embryo (eSET) reduces multiple pregnancies observed in ART

Higher number of good quality embryos
*higher embryo utilization rate :
(embryos transferred + embryos frozen)/total embryos
*more embryos to cryopreserve
*more and better embryos for PGD

Question ?

Are some culture media able to produce more high quality embryos than others?

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University Hospital Ghent :

PGD on day 3 on preferable 8-cell embryo

Embryo culture medium : Cook Cleavage medium (Cook, Australia)

Observation :

high proportion of embryos do not reach 8-cell stage on day 3

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Aim

To compare the effect of two different culture media on the embryo utilization rate on day2/day 3

a prospective, randomized, non-blind study was carried out using sibling oocytes to compare two different media, defined as Cook Cleavage medium (Cook, Australia) and GI™ version 3 (Vitrolife, Sweden)

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Study design

-a randomized sibling oocyte trial
- every patient is the control of herself, so that differences in patient characteristics such as female age, rank of trial, duration and origin of infertility are eliminated

-study was set up as a non-inferiority study
-non-inferiority trials are intended to show that the effect of a new 'treatment' is not worse than that of the control

-the non-inferiority margin was set at 17% because this difference was considered to be embryologically important in our setting

-Embryo ultilization rate is monitored every week with a mean of 58% with culture in Cook medium and for the Vitrolife medium, the lower confidence limit, should be no < 17% below this.
In practice, embryo utilization rate should not be lower than 48%

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Calculate sample size

Two Samples Using Percentage Values

58	**Sample 1 Percentage (%):**	(Value measured from Sample 1 or expected from this sample)
48	**Sample 2 Percentage (%):**	(Value measured from Sample 2 or expected from this sample)
5%	**Alpha Error Level or Confidence Level:**	(Probability of incorrectly rejecting the null hypothesis that there is no difference in the percentage values). An Alpha of 5% corresponds to a 95% Confidence Interval.
10%	**Beta Error Level or Statistical Power (1 - Beta):**	(Probability of incorrectly failing to reject the null hypothesis that there is NO difference in the percentage values – assuming no difference when a real difference exists). A Beta of 50% is used in most simple calculations of sampling error.

Calculate sample size

Sample Size = 425 for both samples!

http://www.dssresearch.com/toolkit/sscalc/size

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-randomization was done at moment of oocyte retrieval
Cumulus oocyte complexes (COC's) were allocated to two seperate oocyte collection dishes by two operators

-oocytes were collected in Oocyte Wash (Cook, Australia) or G-Mops (Vitrolife, Sweden)

-after hyaluronidase treatment and washing, oocytes were put in individual droplets of Fertilization medium (Cook, Australia) or G-Fert (Vitrolife, Sweden)

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-after ICSI, oocytes were cultered in Cook Cleavage (Cook, Australia) or GI –medium (Vitrolife, Sweden)

- embryos were cultured for 48 or 72 hours before transfer

-best quality embryos were selected for transfer based on morpholgy, regardless the type of media used

- primary endpoint : embryo utilization rate (transferred + frozen)

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Population

Inclusion criteria:

- women not older than 36 years old
- ≥ 10 cumulus oocyte complexes
- ICSI
- ejaculated sperm

Exclusion criteria :

- women older than 36 years
- < 10 cumulus oocyte complexes
- IVF
- extreme OAT and surgically retrieved sperm

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Statistics

Statistical analysis was performed with the GraphPad statistical package using contingency table analysis.

Fisher's exact test was used to compare the proportions of fertilized oocytes and embryos frozen + transferred

The difference was considered statistically significant if P was ≤ 0.05

Number of oocytes and female age are presented as mean ± SD

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Results

Patients	57
Cumulus oocyte complexes (COCs)	1002
Mean number oocytes (mean ± SD)	17.6 ± 8.5
Mean female age(years) (mean ± SD)	30.8 ± 3.4

	Cook	Vitrolife	
# COCs	501	501	
# MII	434	430	
# 2PN	345 (79.5%)	323 (75.1%)	NS
# embryos	339	318	
# embryos frozen +ET	197 (58.1%)	175 (55.0%)	NS
# embryos on day 3	157	135	
# 8-cell on day 3	67 (42.7%)	52 (38.5%)	NS

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Conclusion

Within the limits of this study we can conclude that there is no difference in embryo utilization rate between Cook Cleavage and Vitrolife medium

Although the use of sibling oocytes is the best methodology to compare embryo morphology between two culture media, the clinical relevance of the ability for culture media to produce higher quality embryos is debatable and can only be analysed by a per-patient randomized clinical trial on a large serie

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