Dutch Medium trial

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VERENIGING VOOR
KLINISCHE EMBRYOLOGIE

Dutch Medium trial

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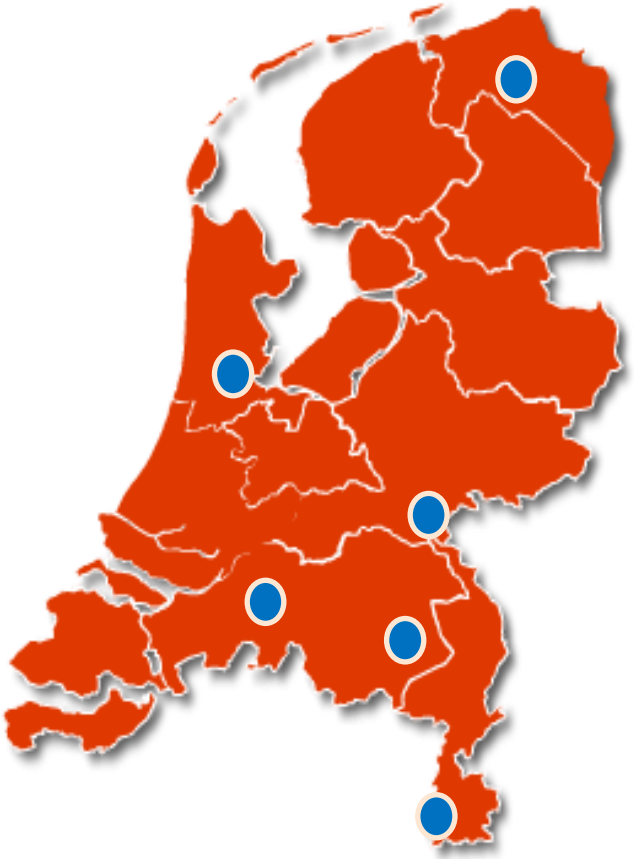
Dutch Medium trial



multi-center trial: 6 IVF-laboratories

umcg — **Groningen**

amc — **AMC Amsterdam**

Radboudumc — **Nijmegen**

St. Elisabeth Ziekenhuis — **Tilburg**

catharina ziekenhuis — **Eindhoven**

Maastricht UMC+ — **Maastricht**

nvog consortium 2.0 — **Dutch Consortium for Healthcare Evaluation and Research in Obstetrics and Gynecology**

Dutch Medium trial

AMC Amsterdam

- PhD student Eleni Mantikou
- Dr. Sebastiaan Mastenbroek
- Prof.dr. Sjoerd Repping

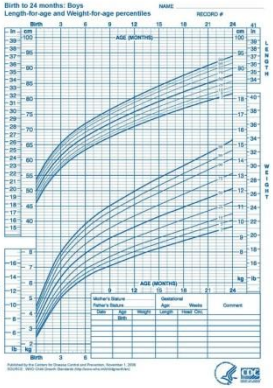
MUMC Maastricht

- PhD student Sander Kleijkers
- Dr. Aafke van Montfoort
- Dr. John Dumoulin

Studies alongside the Dutch Medium trial

Differences in gene expression profiles between human preimplantation embryos cultured in two different IVF culture media

Differences in gene expression profiles in human placentas



Differences in postnatal growth of children (?)

Dutch Medium trial – Introduction 1

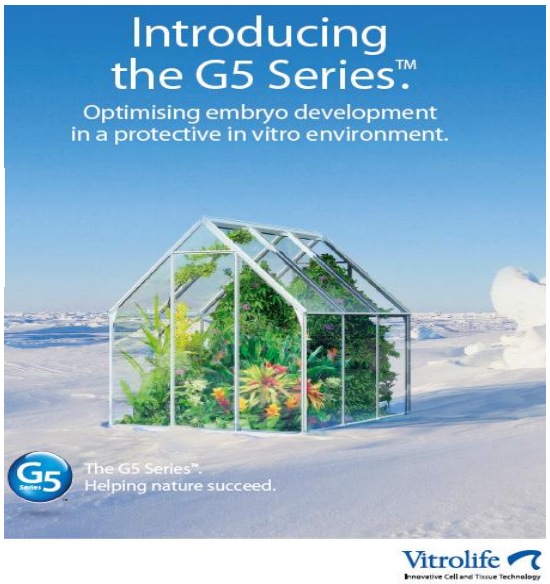
There are many commercial culture media on the market…….

SAGE® Quinn's Advantage® Cleavage Medium

COOK® MEDICAL Embryo Culture
THE ART OF ONE™ SYSTEM

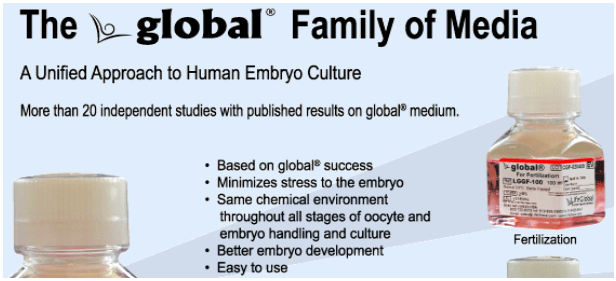
origio MEDICULT MEDIA

InVitroCare incorporated
IVF MEDIA PRODUCTS
... for the Next Generation



Early Cleavage Media™

IrvineScientific®



Dutch Medium trial – Introduction 2

It is yet unknown what culture medium leads to the best success rates in IVF/ICSI….

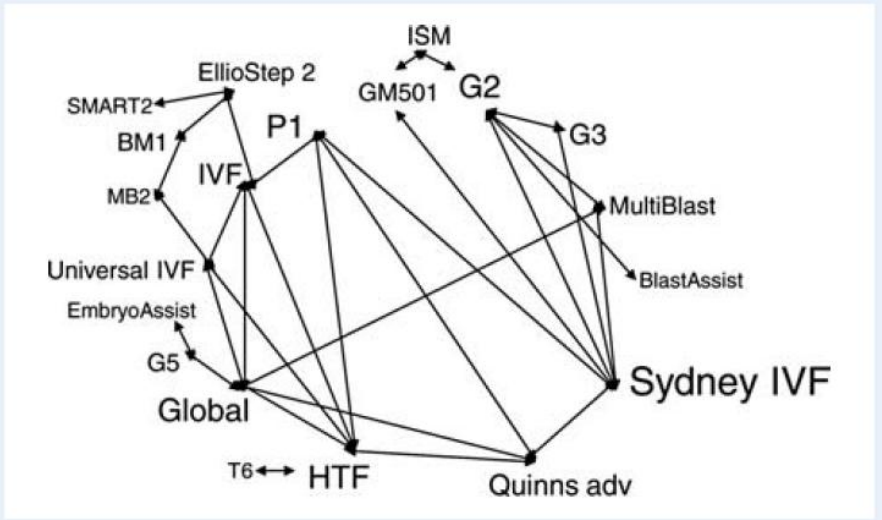


Figure 2 Schematic representation of the comparisons made in the included studies. The font size represents the number of studies on each medium.

Mantikou et al. (2013) Embryo culture media and IVF/ICSI success rates: a systematic review

22 RCTs that evaluated 31 different comparisons. Conventional meta-analysis was not possible for any of the outcomes as nearly all trials compared different culture media.

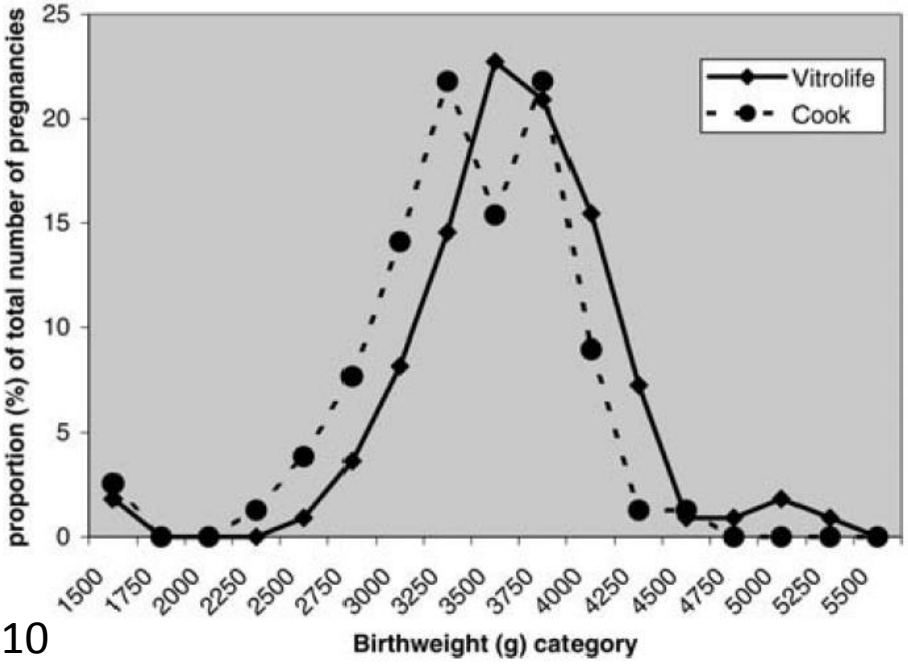
Conclusions: It is yet unknown what culture medium leads to the best success rates in IVF/ICSI.
Rigorously designed RCTs are needed for currently available, as well as newly introduced culture media.

Dutch Medium trial – Introduction 3

Does the culture medium affect perinatal outcome ….?

Maastricht UMC - Vitrolife vs Cook study (2003 – 2006)

Percentage of singletons per birthweight category



No. of singletons:

Vitrolife: 168
Cook: 126

Dumoulin *et al*., 2010

Dutch Medium trial – Introduction 4

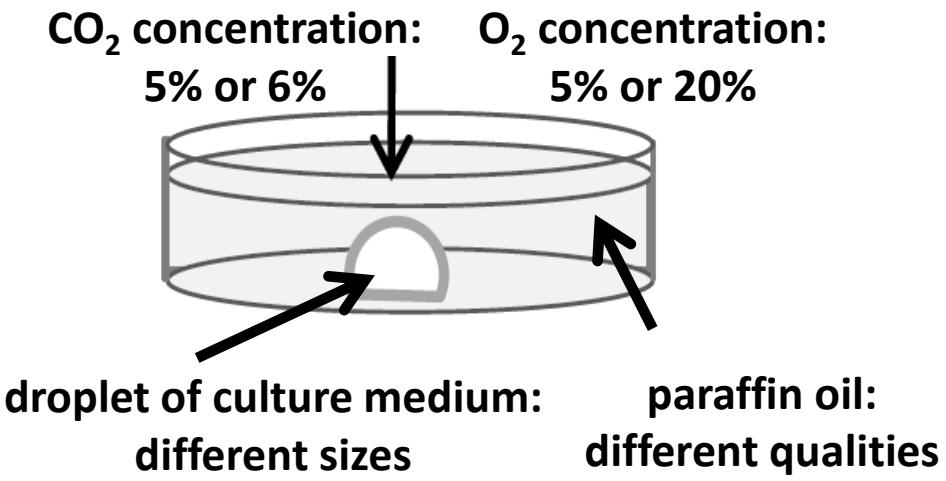
Culture media? Complex culture systems!

Composition

- water
- ions
- bufferingsystem
- energy source
- protein (albumin)
- antibiotics
- amino acids
- vitamins
- growth factors
- undescribed components

- pH
- temperature
- osmolality

- flushing media
- sperm washing media
- cumulus removal media
- ICSI (PVP) media
- culture for 2-6 days:



- sequential or single step media
- embryo transfer media

Dutch Medium trial – Methods 1

- 836 patients randomised over 2 culture media: Vitrolife version 5 versus HTF (Lonza)
- CCMO approval NL3o44r.ooo.o9 ; Dutch Trial Register NTR1979
- Inclusion started in January 2011, completed in May 2012
- Study period completed in May 2013, all babies born in March 2014
- Data collection completed in October 2014

Primary outcome parameter:

- Percentage of liveborn babies after 1 year of treatment

Secundary outcome parameters:

- Fertilization rate
- Utilization rate of embryos for transfer and/or cryopreservation
- Implantation rate
- Foetal growth
- Perinatal outcome (birthweight)

Dutch Medium trial – Methods 2

- Randomisation: centrally, one day before oocyte retrieval of the first cycle
- Stratification: maternal age (<38 and ≥ 38 years), IVF/ICSI, centre
- All clinical and laboratory treatment procedures were performed according to the routine IVF/ICSI procedures in each particular center

- Main differences between the media:

	G5 Vitrolife	HTF Lonza
Single step vs sequential culture	Sequential	One step
Glucose	2.5 and 0,5 mM	2.8 mM
Antioxidants	Yes	No
Amino acids	Yes	No
Albumin source	HSA included	GPO added
CO_2 %	6%	5%

Results: fertilization and embryo development

	Vitrolife	HTF	Signif.
Patients	**417**	**419**	
Fertilization rate per cycle	**63%**	**69%**	**<0.001**
Utilizable embryos per cycle	**2.8 ± 2.3**	**2.3 ± 1.8**	**<0.001**
Embryos transferred per fresh ET	**1.5 ± 0.6**	**1.5 ± 0.6**	**NS**
Embryos cryopreserved per cycle	**1.4 ± 2.4**	**0.8 ± 1.8**	**<0.001**
Implantation rate (fresh embryos)	**20%**	**15%**	**<0.001**
Implantation rate (cryo embryos)	**11%**	**11%**	**NS**

Results: pregnancy outcome

	Vitrolife	HTF	Signif.
Patients	**417**	**419**	
Women with at least 1 pos. preg.test	**236 = 57%**	**211 = 50%**	**0.06**
Abortion rate per pos. preg. test	**22%**	**25%**	**NS**
Twin pregnancy rate	**9%**	**13%**	**NS**
Live birth rate	**184 = 44%**	**159 = 38%**	**0.08**

Results: neonatal outcome of live born singletons

	Vitrolife	HTF	Signif.
Live born singletons	**163**	**137**	
Gestational age at birth (wk)	**39.2 ± 0.1**	**39.4 ± 0.1**	**NS**
Preterm birth (<37 wk)	**8.6%**	**2.2%**	**0.02**
Birthweight (g)	**3299 ± 46**	**3480 ± 44**	**0.005**
Z-score	**-0.13 ± 0.08**	**0.17 ± 0.08**	**0.008**
Low birthweight (<2500 g)	**9.8%**	**2.9%**	**0.020**
Small for gestational age (<10 pct)	**8.6%**	**3.6%**	**NS**
High birthweight (>4500 g)	**0.6%**	**2.9%**	**NS**
Large for gestational age (>90 pct)	**9.2%**	**11.7%**	**NS**

Results: neonatal outcome of live born singletons

Multiple regression analysis for singletons.

Variable	β*	95% CI*	P Value
Culture medium (G5 versus HTF)	-116	-212 to -20	0.019
Child's gender (male versus female)	144	47 to 241	0.004
Gestational age at birth (per week)	188	159 to 218	<0.001
Pregnancy complications (yes versus no)	-23	-158 to 111	0.731
No. of transferred embryos (1 versus >1)	-30	-128 to 69	0.556
Embryo transfer (fresh versus cryo)	70	-61 to 200	0.293
Parity (primiparous versus multiparous)	134	28 to 240	0.013
Maternal height (per cm)	9	1 to 16	0.025
Maternal weight (per kg)	5	1 to 10	0.013
Maternal smoking (<10 versus ≥10 cig/day)	-262	-420 to -105	0.001
Paternal height (per cm)	2	-5 to 9	0.552
Paternal weight (per kg)	2	-2 to 6	0.274

* β denotes regression coefficient. CI denotes confidence interval.

Does the type of culture medium used influence birthweight of children born after IVF?

Study	Culture media compared	Singletons (N)	Signif.?
Dumoulin /Nelissen, 2010-12	Cook vs Vitrolife G3	168 vs 126	Yes
Eaton, 2012	Vitrolife G3, G5, Global	102, 53, 43	No
Vergouw, 2012	HTF vs Sage/Quinn	99 and 259	No
Lin, 2013	Vitrolife G5, Global, Sage/Quinn	596, 460, 145	No
Ziebe, 2013	Medicult EmbryoAssist +/- GM-CSF	133 and 118	No
Carrasco, 2013	RCT: Vitrolife G3 vs Cook Not randomized: G3, Cook, ISM1	49 vs 49 154, 172, 197	No No
Eskild, 2013	Medicult Universal, ISMI, Vitrolife G1	1584, 402, 449	Yes
Hassani, 2013	Medicult ISMI vs Vitrolife G5	86 vs 78	Yes
Zhu, 2014	Vitrolife G5 PLUS vs HSA added	608 vs 489	Yes
Wunder, 2014	Vitrolife G5 vs Cook	352 and 173	No
Lemmen, 2014	Medicult EmbryoAssist vs Cook	977 vs 147	No
De Vos, 2015	Medicult Univ./Emb.Assist vs Vitrolife G3/G5	1388 vs 710	No
Dutch Medium Trial	Vitrolife G5 vs HTF	163 vs 137	Yes

Adapted from Zandstra, van Montfoort and Dumoulin (2015)

Does the type of culture medium used influence birthweight after IVF? Evidence from animal studies

Table I Selection of animal studies reporting on effect of culture (medium) on birthweight.

Study	Study group	Culture media used	Control group	Transferred at	Studied culture component	Significant effect of IVC
Mouse studies						
Le *et al.* (2013)	OS + IVF	HTF	OS + IVD 2-cell	2-cell	IVC	Increased BW in IVC versus IVD
Hemkemeyer *et al.* (2014)	OS + IVD 1-c	KSOM(AA), WM, ISM1/2, HTF/Multiblast	OS + IVD to fetus	B stage	IVC Culture media	Increased BW in all IVC media versus IVD. No differences between IVC media
Delle Piane *et al.* (2010)	OS + IVF	WM or KSOM(AA)	OS + IVD B's	B stage	IVC Culture media	Reduced FW in all IVC versus IVD, Reduced FW in WM versus KSOM-AA
Scott *et al.* (2010)	OS + IVF	CZB	IVD to fetus	2-cell	IVC	Increased BW in IVC versus IVD
Zander *et al.* (2006)	OS + IVD 1-c	G1.2/G2.2 w/wo added ammonium	OS + IVD B's	B stage	IVC Ammonium	Reduced FW in IVC versus IVD, but only in the presence of ammonium during the first day of IVC
Sjöblom *et al.* (2005)	NO + IVD 2-c	SQC w/wo GM-CSF	NO + IVD B's	B stage	IVC GM-CSF	Reduced FW in IVC versus IVD as well as in medium without versus with supplementation of GM-CSF
Thouas *et al.* (2003)	OS + IVD 1-c	MTF + BSA	OS + IVD B's	B stage	IVC	Increased FW in IVC versus IVD
Khosla *et al.* (2001)	OS + IVD 1-c	M16 w/wo serum	OS + IVD B's	B stage	IVC Serum	Reduced FW in IVC versus IVD as well as in medium with versus without supplementation of serum
Lane and Gardner (1997)	OS + IVD 1-c	mMTF w/wo AA	OS + IVD B's	B stage	IVC AA	Reduced FW in all IVC groups versus IVD as well as in medium without versus with AA. FW also dependent on AA composition
Gardner and Lane (1996)	OS + IVD 1-c	CZB or DM1/DM2	OS + IVD B's	B stage	IVC Culture media	Reduced FW in all IVC groups versus IVD. FW also dependent on medium type
Mehta and Kiessling (1990)	OS + IVF	EBSS w/wo EDTA, AA, serum	OS + IVD 2-cell	4-cell (IVC), 2-cell (control)	IVC EDTA, AA, serum	Reduced FW in all IVC groups versus IVD. FW also dependent on EDTA concentration and presence of AA
Han and Kiessling (1988)	OS + IVF	EBSS or Ham's F10 w/wo serum	OS + IVD 2-cell	4-cell (IVC), 2-cell (control)	IVC Serum	Reduced FW in all IVC groups versus IVD. FW also dependent on medium type and on the presence of serum
Bowman and McLaren (1970)	NS + IVD 8-c	Brinster medium wo AA	NS + IVD B's	B stage	IVC	Reduced FW in all IVC groups versus IVD. FW also dependent on time in culture
Ovine studies						
Rooke *et al.* (2007)	OS + IVD 1-c	SOF w/wo serum	OS + IVD B's	B stage		Increased FW in medium supplemented with serum versus IVD when serum is present during the first 48 h of IVC
Sinclair *et al.* (1999)	OS + IVD 1-c	SOF w/wo serum	OS + IVD D6 embryos	B stage		Increased FW in medium with serum versus IVD
Thompson *et al.* (1995)	OS + IVD 1-c	SOF w/wo AA and serum or BSA	OS + IVD to birth	B stage		Increased BW in medium supplemented with serum versus IVD as well as in medium with versus without supplementation of serum
Walker *et al.* (1992)	OS + IVD 1-c	—	OS + IVD B's	B stage		Increased BW in IVC versus IVD

BW, birthweight; FW, fetal weight; IVC, *in vitro* culture; IVD, *in vivo* developed; OS, ovarian stimulation.

Zandstra, van Montfoort and Dumoulin (2015)

Dutch Medium trial – Future: medium-2

	G5 Vitrolife	CSCM Irvine
Single / sequential	Sequential	One step
Glucose	2.5 and 0.5 mM	0.5 mM
Amino acids	Yes	Yes
Albumin source	HSA included	HSA included
CO_2 %	6%	6%

- Coordinating investigator: Dr. Aafke van Montfoort
- 2000 patients will be randomised, only the first treatment cycle
- Sample size calculation: 1980 patients needed to detect 6% difference
- CCMO approval NL50712.000.14
- Priority “lack of evidence”-topic by NVOG Consortium
- Inclusion will start medio 2015
- Primary outcome parameter: % of liveborn babies after 1 year of treatment
- Secundary: fertilization rate, utilization rate of embryos, perinatal outcome

Dutch Medium trial - conclusions

Primary outcome parameter:

- Live birth rates not significantly different (44% vs. 38%; P=0.08)

Secundary outcome parameters:

- Fertilization rate: significantly higher in HTF
- Utilization rate of embryos for ET and cryopreservation: sign. higher in G5
- Implantation rate: significantly higher in G5
- Foetal growth: no differences
- Birthweight significantly higher in HTF

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