Does IVF culture medium affect prenatal and postnatal growth in humans?

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KLEM Wetenschapsdag
10-01-2013

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Does IVF culture medium affect prenatal and postnatal growth in humans?

High pregnancy rates are not our only concern

2012: 5 million IVF babies have been born worldwide since the first IVF baby was born in 1978

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Differences in outcome of pregnancies between spontaneous and IVF conceptions

Higher risk of adverse perinatal outcome in IVF singletons when compared with matched controls

- Preterm birth (<37 weeks): RR = **1.5 - 2.0**
- Perinatal mortality: RR = **1.7 - 2.2**
- Low birthweight (<2500 g): RR = **1.6 - 1.8**
- Small for gestational age: RR = **1.4 - 1.6**
- Congenital abnormalities: RR = **1.7**

Helmerhorst, 2004 ; Jackson, 2004 ; McDonald, 2009 ; Pandy, 2012

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Differences in outcome of pregnancies between spontaneous and IVF conceptions

Placental anomalies in IVF pregnancies

- IVF: increased risk of placenta praevia: RR = 2.9 (Jackson, 2004); 5.6 (Romundstad, 2006)
- IVF: larger placentas and higher placental weight/birthweight ratio (Haavaldsen 2012)
- IVF: ultrastructural differences in villi with control placentae (Zhang, 2009)

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Differences in outcome of pregnancies between spontaneous and IVF conceptions

Postnatal outcome in IVF children

- IVF: higher high-density lipoprotein and lower triglyceride levels than controls (Miles, 2007)
- IVF: higher fasting glucose levels (Ceelen, 2008)
- IVF: increases in blood pressure (Ceelen, 2008)
- IVF-conceived girls taller than matched controls (Green, 2010).
- IVF-conceived children display generalized vascular dysfunction (Scherrer, 2012)

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Differences in outcome of pregnancies between spontaneous and IVF conceptions

What could be the underlying cause?

- Patient related factors such as subfertility?
- IVF technique related factors: ovarian stimulation?
- IVF technique related factors: in vitro culture?

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Differences in outcome of pregnancies between spontaneous and IVF conceptions

Patient related factors such as subfertility

Compared with fertile women, subfertile women who conceived by any ART treatment or spontaneously, have an increased risk of:

- perinatal death (Basso, 2005; Thomson, 2005; Wisborg, 2010)
- preterm delivery (Hayashi, 2012; Raatikainen, 2012)
- low birthweight (Thomson, 2005; Hayashi, 2012)
- small for gestational age (Zhu, 2007; Raatikainen, 2012)
- higher rate of congenital malformations (Zhu, 2003)
- higher rate of placenta praevia (Thomson, 2005)

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Differences in outcome of pregnancies between spontaneous and IVF conceptions

IVF technique related factors

IVF / ICSI found to be associated with adverse outcome when children are compared from subfertile women, conceived either spontaneously or after IVF:

- lower birthweight (De Geyter, 2006; Kapiteijn, 2010)
- preterm birth (Kapiteijn, 2010)
- higher risk (OR=2.9) of placenta praevia (Romundstad, 2006)
- higher risk on cerebral palsy (Zhu, 2010)
- lower height and weight at 3 month of age (Ceelen, 2009)
- vascular dysfunction at 11 years af age (Scherrer, 2012)

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The IVF technique seems to be partly responsible for the adverse outcome

Effect of ovarian stimulation

Birthweight in singletons after IVF and:

- standard ovarian stimulation (n=106): 3218 ± 670 g
- modified natural cycle (n=84): 3485 ± 527 g *

Pelinck et al. (Groningen), 2010

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The IVF technique seems to be partly responsible for the adverse outcome

Effect of culture conditions

In animal models: different culture media give rise to differences in:

- Gene expression and DNA methylation of imprinted genes in preimplantation embryo's
- Fetal weight
- Postnatal characteristics

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The IVF technique seems to be partly responsible for the adverse outcome

Effect of culture conditions

In humans: few studies exist

- In vitro culture can affect birthweight (Dumoulin, 2010; Nelissen, 2012)
- No effect (Eaton, 2012)
- No effect (Vergouw, 2012)

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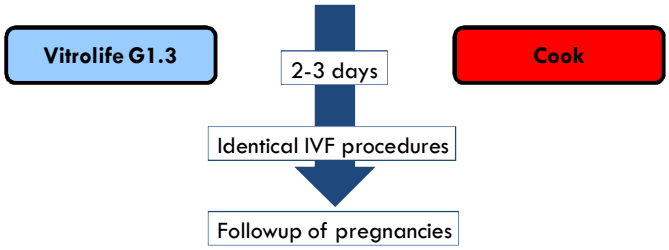
Adverse outcome of IVF conceptions: effect of in vitro culture?

Vitrolife vs Cook study: Methods

July 2003 – December 2006

Lab: randomisation of all consecutive cycles by alternating between the two media
Clinic: planned OPUs, unaware of randomisation



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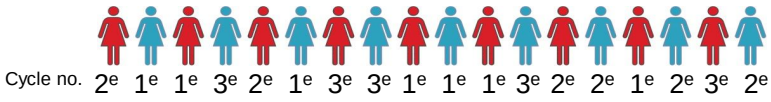
Vitrolife vs Cook study: Methods



Total n=715
First cycle n=414

Vitrolife G1.3

Total n=717
First cycle n=412

Cook

Dumoulin *et al.* 2010: perinatal followup of singleton pregnancies resulting from the 826 first cycles after fresh ET

Nelissen *et al*. 2012: perinatal followup of all pregnancies (fresh and cryo) resulting from all 1432 treatment cycles

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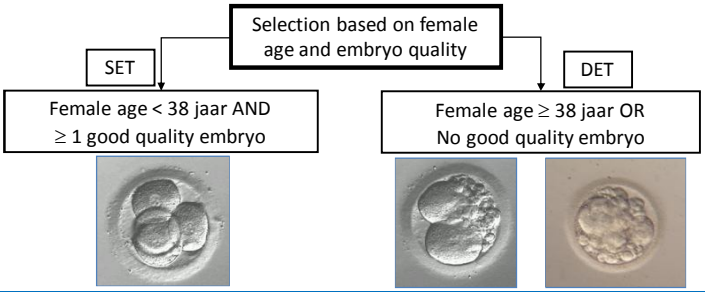
Adverse outcome of IVF conceptions: effect of in vitro culture?

Vitrolife vs Cook study: Methods - Laboratory

- Culture: 5% O_2, 6% CO_2, 89% N_2
- Transfer on day 2 or 3
- Transfer policy:



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Adverse outcome of IVF conceptions: effect of in vitro culture?

Pregnancy results after fresh ET

	Vitrolife	Cook
1432 treatment cycles	**715**	**717**
Clinical pregnancies (fetal heart activity)	**210 (29.4)**	**168 (23.4)***
Pregnancy losses before 20 weeks	**10**	**10**
Stillbirths after 20 weeks	**2**	**3**
2nd pregnancy of couples during study period	**9**	**3**
Lost to followup	**0**	**1**
Live births	**189**	**151**
Triplet pregnancies	**1**	**1**
Singletons	**168**	**126**
Twins	**20**	**24**

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Perinatal results of singletons after fresh ET

	Vitrolife (n=168)	Cook (n= 126)	*P*-value
Gestational age (GA) at birth	39.6 ± 0.1	39.4 ± 0.2	NS
Preterm birth (<37wks)	6 (3.6)	8 (6.4)	NS
Birthweight (g)	3436 ± 44	3253 ± 50	0.006
Z-score	0.05 ± 0.08	-0.265 ± 0.08	0.007
Low birthweight (<2500g)	4 (2.4)	12 (9.5)	0.006
Low birthweight with GA ≥ 37 wks	2 (1.2)	8 (6.4)	0.015
High birthweight (>4500g)	5 (3.0)	0	NS

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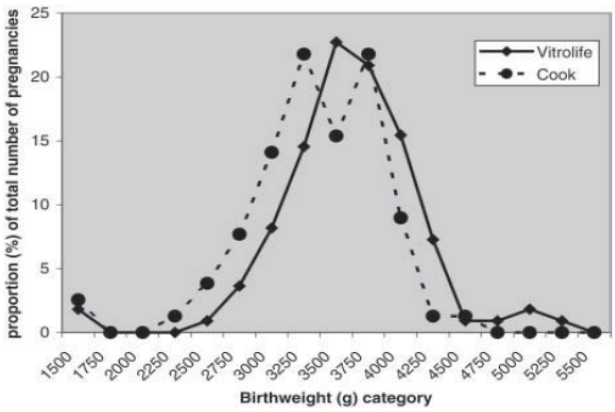
Percentage of singletons per birthweight category



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Many factors affect birthweight

- ☐ Singleton or part of a multiple birth (only singletons)
- ☐ Gestational age at birth (z-score)
- ☐ Fetal gender (z-score)
- ☐ Parity
- ☐ Maternal height and weight
- ☐ Age of the mother
- ☐ Pregnancy related factors: gestational diabetes and hypertension
- ☐ Lifestyle factors such as smoking
- ☐ History of subfertility (duration of subfertility)
- ☐ Number of transferred embryos (vanishing twins)
- ☐ Paternal height and weight

Lineair Regression analysis

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Table IV Results of multiple regression analysis among live born singletons

Variable	Birthweight (grams)		
	Adjusted		
	β^a	95% CI	*P*-value
Vitrolife (versus Cook)	112	11 to 214	0.031
Maternal height (per cm)	12	3 to 21	0.008
Secondary subfertility (versus primary)	220	95 to 345	<0.001
Gestational age at birth (per week)	171	142 to 199	<0.001
Child's gender (male versus female)	177	73 to 281	<0.001

95% CI, confidence interval; NS, not significant.
[a]β is the regression coefficient. With birthweight as an outcome, a beta of 112 for Vitrolife versus Cook means that neonates conceived in medium from Vitrolife weighed an estimated 112 g more than neonates conceived in medium from Cook. A β of 12 for maternal height (per cm) means that each additional cm adds an estimated 12 g to neonatal birthweight.

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Perinatal results of twins after fresh ET

	Vitrolife (n=20)	Cook (n= 24)	*P*-value
Gestational age (GA) at birth	36.3 ± 0.4	35.5 ± 0.3	NS
Preterm birth (<37wks)	9 (45.0)	16 (66.7)	NS
Birthweight (g)	2400 ± 81	2284 ± 68	NS
Low birthweight (<2500g)	23 (58)	31 (65)	NS
Mean birthweight disparity (g)	259 ±32	465 ± 93	0.045
Birthweight discordance(>25%)	0	6 (25)	0.019

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Perinatal results of singletons after cryo ET

	Vitrolife (n=22)	Cook (n= 45)	*P*-value
Gestational age (GA) at birth	39.3 ± 0.3	39.4 ± 0.2	NS
Preterm birth (<37wks)	1	2	NS
Birthweight (g)	3465 ± 107	3394 ± 77	NS
Z-score	0.18 ± 0.21	-0.04 ± 0.14	NS
Low birthweight (<2500g)	0	0	

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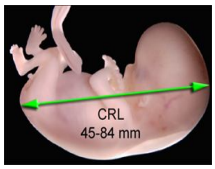
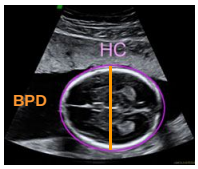
Fetal development of 294 singletons after fresh ET

- Ultrasound examination at 8, 12 and 20 weeks' gestation
- First-trimester serum markers (fβ-hCG, PAPP-A)

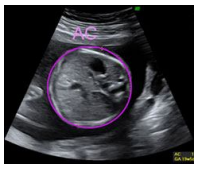


CRL: crown-rump length

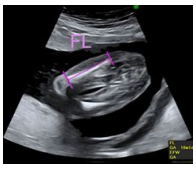


BPD: biparietal diameter ("from ear to ear")

HC: head circumference



AC: abdominal circumference



FL: femur length

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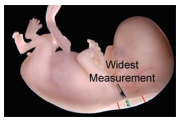
Fetal development 10-12 weeks of pregnancy

- Nuchal translucency (NT) and serum markers
- Expressed as multiples of the median (MoM) for gestational age with corrections for maternal weight



	Vitrolife group (n=45)	Cook group (n=38)	*P*-value
NT (MoM)	0.78 ± 0.10	0.65 ± 0.33	NS
PAPP-A (MoM)	1.03 ± 0.85	1.05 ± 0.92	NS
fβ-hCG (MoM)	1.55 ± 0.19	1.06 ± 0.10	0.031

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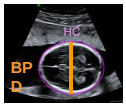
Fetal development 20 weeks of pregnancy

Sonographic markers	Vitrolife group (n=115)	Cook group (n=91)	Adjusted mean difference	*P*-value
BPD (biparietal diameter)	50.2	49.8	0.5	0.07
HC (head circumference)	177.3	175.9	1.8	0.03
AC (abdominal circumference)	152.1	151.2	0.8	0.43
FL (femur length)	32.7	32.8	-0.1	0.81



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Fetal development 20 weeks of pregnancy

- ΔGA (days) = difference between actual GA and GA calculated with 3 different BPD-dating formulae

	Vitrolife group (n=115)	Cook group (n=91)	Adjusted mean difference in days	*P*-value
Mull *et al.* 1996 #1	+3.28	+2.10	1.14	0.04
Mull *et al.* 1996 #2	+2.28	+1. 10	1.14	0.04
Selbing & Kjessler, 1985	+6.18	+4.77	1.36	0.048

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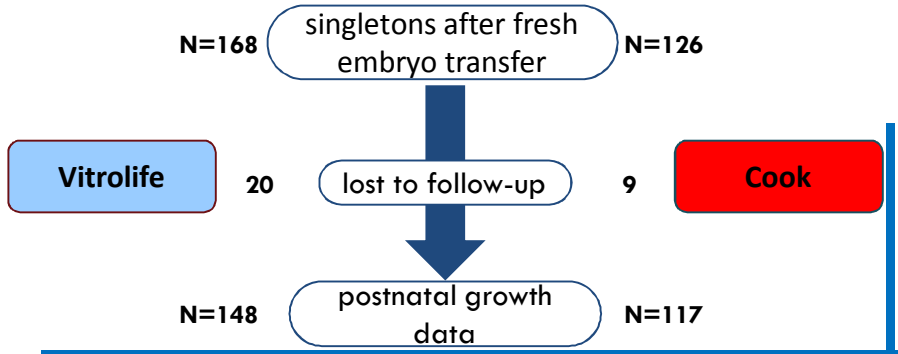
Postnatal development up to 2 years

weight, height & head circumference measurements from infant welfare centres around 1, 2, 3, 4, 6, 7.5, 9, 11, 14, 18, and 24 months of age



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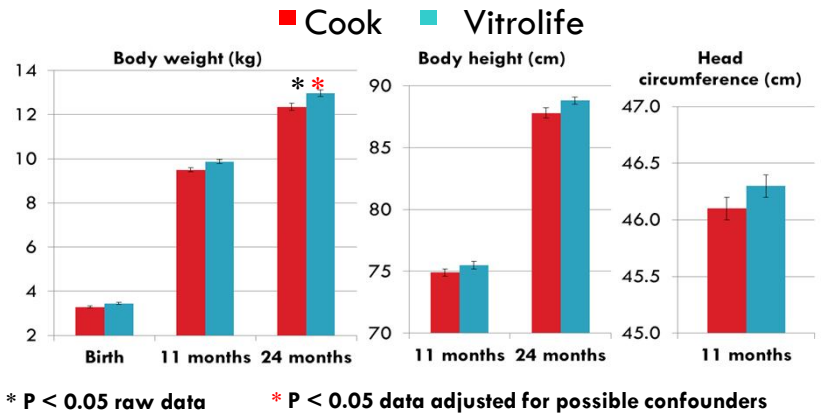
Postnatal development up to 2 years



* P < 0.05 raw data * P < 0.05 data adjusted for possible confounders

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Conclusions Vitrolife vs Cook study

- At birth: higher birthweight in Vitrolife group
- Differences in fetal development between the two media groups are apparent already during the 2nd trimester
- Higher fβ-hCG in Vitrolife group
- Effect of culture medium still present after the first 2 years of life

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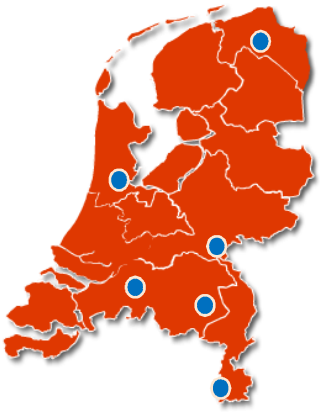
Dutch multi-center culture medium trial



792 patients randomised over 2 culture media:
Vitrolife version 5 versus HTF (Lonza)
Inclusion completed in May 2012

Outcome parameters:
- IVF results after 1 year of treatment
- Foetal growth
- Perinatal outcome (birthweight)
- Postnatal outcome and growth

- Epigenetic effects

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