TEMPERATUUR BIJ IVF: EEN KRITISCHE PARAMETER?

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Centrum voor Reproductieve Geneeskunde

BE-ART IVF

DISCLOSURE

- Speaker's fee: COOK, Cooper Surgical, Ferring, Hamilton Thorne, Vitrolife
- Consultant: Ferring
- Major Shareholder: BE-ART IVF

- I declare that no commercial or financial interest has influenced the content of this presentation.

Ronny Janssens

CONTENT

I Literature

II How to measure temperature

III Experiments

IV Conclusions

I - LITERATURE

Temperature In Vivo

- Wunderlich, 1868: 37°C (98.6°F) = mean normal body temperature in adults (little red arrow on thermometers)
- Normothermia range
- Cyclic: 36.2°C at rest, at night/morning, 37.5°C in the day
- Varies
  - Sex
  - Menstrual cycle: 0.3-0.5°C rise after ovulation
  - Ethnicity
  - People
  - Environmental conditions
  - Physical activity

I - LITERATURE

Temperature In Vivo

Temperature gradients in female reproductive tract (Grinstead et al. 1985, Hunter et al. 2012)

Table 1 The magnitude of temperature gradients recorded in female reproductive tissues around the time of ovulation.

Organ and location	*Measurement method*	*Reference*	*Species*	*Overall difference in temperature (°C)*	*Comment*
Oviduct: isthmus versus ampulla	Indwelling probes	Bahat et al. (2003)	Rabbit	0.8–1.6	Isthmus always cooler than ampulla
		Hunter and Nichol (1986)	Pig	0.2–1.6	
Ovary: preovulatory follicles versus ovarian stroma	Microelectrodes and/or acute thermosensing	Grinsted et al. (1980)	Rabbit	1.4	Mature follicles always cooler than stroma
		Grinsted et al. (1985)	Human	2.3	
		Greve et al. (1996)	Cow	1.5	
		Hunter et al. (1997, 2000)	Pig	1.3–1.7	

Deviations from physiological temperature during IVF could affect gene expression

I - LITERATURE

Temperature In Vivo

Spermatogenesis

- Testicular functions are temperature-sensitive, therefore, intratesticular temperature should be kept 2–4 °C lower than the rectal temperature. Goldstein et al. 1989
- Varicocele: scrotal hyper- thermia caused by venous blood stasis
- High temperatures impair testicular androgen production via a pathway involving increased oxidative stress damage to the Leydig cells. Shiraishi et al. 2010
- Hot baths and tight insulated underpants reduce sperm count by as much as 90%

I - LITERATURE

Culture temperature

1959: M.C. Chang: IVF in rabbits, Nature

1965: Fertilization of human oocytes. Edwards, Jones

I - LITERATURE

Culture temperature

- Optimal target temperature for IVF is unknown (Higdon et al. 2008)

- Significant differences in temperature between incubators and within incubators (Walker et al. 2013)
- Temperature variations inside commercial IVF incubators (Anifandis et al. 2013)

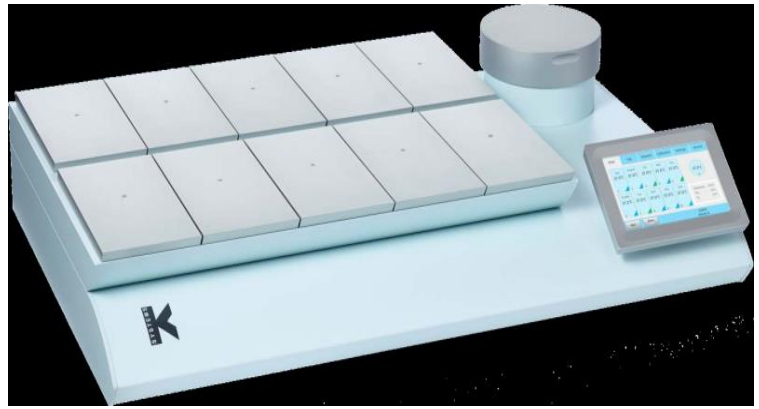
INCUBATORS & TEMPERATURE REGULATION

Culture temperature: 37.0°C ± ΔT?

1983: 0.5°C

2011: 0.3°C

2015: 0.1°C



I - LITERATURE

Temperature and IVF

Metabolism of the viable mammalian embryo: quietness revisited

Henry J. Leese[1,4], Christoph G. Baumann[1], Daniel R. Brison[2], Tom G. McEvoy[3] and Roger G. Sturmey[1]

[1]Department of Biology (Area 3) and Hull York Medical School, University of York, Heslington, York YO10 5DD, UK; [2]Department of Reproductive Medicine, St Mary's Hospital, Manchester M13 OJH, UK; [3]SAC, King's Buildings, West Mains Road, Edinburgh EH9 3JG, UK

- Enzyme activity and embryo metabolisme
- …gametes and early embryos function in vivo at a lower temperature than core body temperature, which could encourage the expression of a quiet metabolism. We call for research to determine the optimum temperature for mammalian gamete/embryo culture.

I - LITERATURE

Temperature and IVF

Journal of Assisted Reproduction and Genetics (2018) 35:643–644
https://doi.org/10.1007/s10815-018-1122-8

COMMENTARY

CrossMark

Whither human IVF? Fertilisable oocytes selected on the basis of follicular temperature

Ronald H. F. Hunter[1,2] · Fernando López-Gatius[3,4]

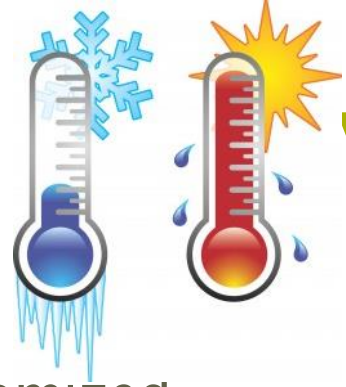
Received: 11 December 2017 / Accepted: 16 January 2018 / Published online: 29 January 2018

Abstract

Bearing in mind specific parallels between cow and human ovarian physiology, as noted in the manuscript, we have measured whether the temperature in a pre-ovulatory follicle is cooler than that in adjacent tissues. Using a novel approach not requiring anaesthetics or surgical procedures, we found that follicular fluid bathing cow oocytes shortly before ovulation is cooler than the neighbouring uterine surface and cooler than deep rectal temperature (the reference body temperature in cattle). By contrast, Graafian follicles of comparable size and ultrasonic image that do not subsequently ovulate do not have a reduced antral temperature. Human pre-ovulatory follicles have previously been reported to be cooler than other ovarian tissues, so the divergence between ovulatory and non-ovulatory follicle temperature suggests a valuable addition to selection procedures currently used in human in vitro fertilisation (IVF) clinics. In future, oocytes to be subjected to IVF might best be those taken from cooler follicles. Follicular antral temperature could become a more sensitive indicator of oocyte potential that a purely morphological assessment.

LITERATURE

Temperature and IVF



Examining the temperature of embryo culture in in vitro fertilization: a randomized controlled trial comparing traditional core temperature (37˚C) to a more physiologic, cooler temperature (36˚C). **Hong et al.** Fertil Steril 2014.

Objective: To determine whether culture at a more physiologically cooler temperature, as suggested by limited human and animal data, would improve blastulation and pregnancy rates in human clinical IVF.
Design: Paired randomized controlled trial.
Setting: Academic.
Patient(s): Infertile couples (n = 52) with a female partner less than 42 years old with eight or more mature oocytes retrieved.
Intervention(s): Mature oocytes obtained from a single cohort of oocytes were randomly divided into two groups; one was cultured at 37°C and the other at 36°C from the time of ICSI to the time of embryo transfer or vitrification. Paired embryo transfers were accomplished by transferring one euploid embryo from each group. DNA fingerprinting was used as needed to determine the outcome for each embryo.
Main Outcome Measure(s): Rate of development of expanded blastocysts suitable for transfer or vitrification (primary outcome), fertilization, aneuploidy, and sustained implantation.
Result(s): A total of 805 mature oocytes were cultured; 399 at 36°C and 406 at 37°C. Paired analysis demonstrated a higher rate of usable blastocyst formation per zygote at 37°C (48.4%) vs. at 36°C (41.2%). Rates of fertilization, aneuploidy, and sustained implantation were equivalent.
Conclusion(s): IVF culture at 36°C does not improve clinically relevant parameters of embryo development or sustained implantation rates.

LITERATURE

Temperature and IVF

Better embryo development @ 37

No difference ongoing pregnancy

Comparing 36.5°C with 37°C for human embryo culture: a prospective randomized controlled trial

Mohamed Fawzy [a,], Mai Emad [a], Mostafa A Gad [a], Mohamed Sabry [b], Hesham Kasem [a], Manar Mahmoud [a], Mohamed A Bedaiwy [c,d]*

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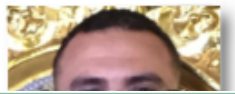
[d] *Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynaecology, University of British Columbia, Canada*



Mohamed Fawzy is an IVF laboratory director at Ibnsina IVF Centre and Banon IVF Centre, Egypt. His area of interest is in improving IVF culture conditions through evidence-based practice by re-evaluating all aspects of IVF procedures.

Table 2 – Embryological outcomes in the trial groups.[a]

	Culture group		Odds ratio (95% CI)	P-value
	Test (36.5°C) (n = 205)	Control (37°C) (n = 207)		
Maturation rate: MII oocytes/collected oocytes (%)	2871/3200 (90)	2869/3174 (90)	0.93 (0.79 to 1.10)	NS
Fertilization rate: 2PN oocytes/injected MII oocytes (%)	2017/2871 (70)	2097/2869 (73)	0.87 (0.78 to 0.98)	0.017
Cleavage rate: cleaved embryos/ fertilized oocytes (%)	1985/2017 (98)	2044/2097 (97)	1.61 (1.03 to 2.51)	0.034
Top-quality day-3 embryos/fertilized oocytes (%)	1211/2017 (60)	1497/2097 (71)	0.60 (0.53 to 0.69)	<0.0001
Compaction rate: compacted day three embryos/fertilized oocytes (%)	437/2017 (22)	623/2097 (30)	0.65 (0.57 to 0.75)	<0.0001
Blastocyst formation rate: blastocysts/fertilized oocytes (%)	1193/2017 (59)	1319/2097 (63)	0.85 (0.75 to 0.97)	0.014
High-quality blastocysts/fertilized oocytes (%)	644/2017 (32)	1015/2097 (48)	0.5 (0.44 to 0.56)	<0.0001
Cryopreservation rate: vitrified blastocysts/fertilized oocytes (%)	722/2017 (36)	879/2097 (42)	0.77 (0.68 to 0.88)	<0.0001

[a] Data presented as proportions (odds ratio rate difference and 95% CI).
MII, metaphase II; NS, not statistically significant; 2PN, two pronuclei.

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LITERATURE

Morphokinetics

Oxygen - Kierkegaard et al. Fertil Steril 20013

Culture medium - Ciray et al. J Assist Reprod Genet, 2012

Temperature - ?

LITERATURE

Temperature during handling

Transient cooling to room temperature can cause irreversible disruption of the meiotic spindle in the human oocyte. Pickering S et al, Fertil Steril. 1990

The effect of temperature fluctuations on the cytoskeletal organisation and chromosomal constitution of the human oocyte. Almeida et al. Zygote. 1990

- 2' @ RT = 77% disruption of spindle

Limited recovery of meiotic spindles in living human oocytes after cooling–rewarming observed using polarized light microscopy. Wang et al. Hum Reprod 2001

LITERATURE

Temperature during handling

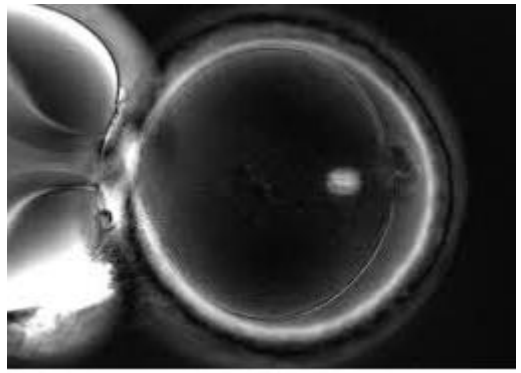
TECHNIQUES AND INSTRUMENTATION

FERTILITY AND STERILITY®
VOL. 77, NO. 6, JUNE 2002

Published by Elsevier Science Inc.
Printed on acid-free paper in U.S.A.

Rigorous thermal control during intracytoplasmic sperm injection stabilizes the meiotic spindle and improves fertilization and pregnancy rates

Wei-Hua Wang, Ph.D.,[a] Li Meng, Ph.D.,[b] Richard J. Hackett, M.Sc.,[c] Rudolf Oldenbourg, Ph.D.,[d] and David L. Keefe, M.D.[c,d]



LITERATURE

Overheating

Zygote

Article Metrics

Volume 12, Issue 1 February 2004 , pp. 65-70

Overheating is detrimental to meiotic spindles within *in vitro* matured human oocytes

Xiao-Fang Sun [a1], Wei-Hua Wang [a2] and David L. Keefe [a3]

DOI: https://doi.org/10.1017/S0967199404002631 Published online: 24 May 2004

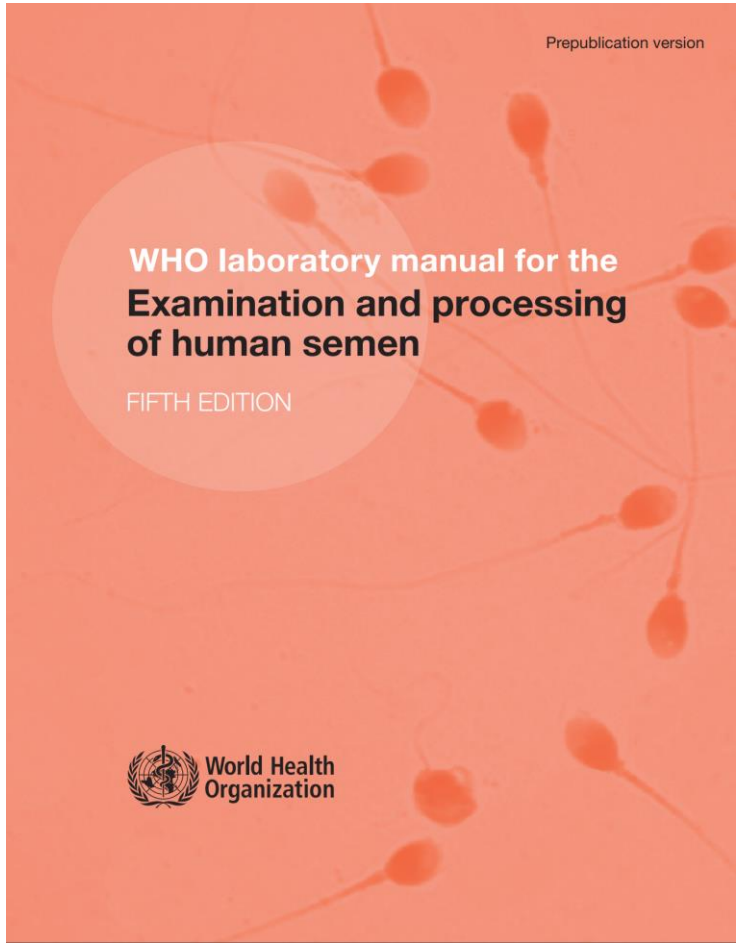
Abstract

The present study was designed to examine the effects of overheating on meiotic spindle morphology within *in vitro* matured human oocytes using a polarized light microscope (Polscope). Immature human oocytes at either germinal vesicle or metaphase I stage were cultured *in vitro* for 24–36 h until they reached metaphase II (M-II) stage. After maturation, oocytes at M-II stage were imaged in the living state with the Polscope at 37, 38, 39 and 40 °C for up to 20 min. After heating, oocytes were returned to 37 °C and then imaged for another 20 min at 37 °C. The microtubules in the spindles were quantified by their maximum retardance, which represents the amount of microtubules. Spindles were intact at 37 °C during 40 min of examination and their maximum retardance (1.72–1.79) did not change significantly during imaging. More microtubules were formed in the spindles heated to 38 °C and the maximum retardance was increased from 1.77 before heating to 1.95 at 20 min after heating. By contrast, spindles started to disassemble when the temperature was increased to 39 °C for 10 min (maximum retardance was reduced from 1.76 to 1.65) or 40 °C for 1 min (maximum retardance was reduced from 1.75 to 1.5). At the end of heating (20 min), fewer microtubules were present in the spindles and the maximum retardance was reduced to 0.8 and 0.78 in the oocytes heated to 39 °C and 40 °C, respectively. Heating to 40 °C also induced spindles to relocate in the cytoplasm in some oocytes. After the temperature was returned to 37 °C, microtubules were repolymerized to form spindles, but the spindles were not reconstituted completely compared with the spindles imaged before heating. These results indicate that spindles in human eggs are sensitive to high temperature. Moreover, maintenance of an *in vitro* manipulation temperature of 37 °C is crucial for normal spindle morphology.

I LITERATURE

Semen

- The procedure may be performed at room temperature or at 37 ˚C with a heated microscope stage, but should be standardized for each laboratory. If sperm motility is to be assessed at 37 ˚C, the sample should be incubated at this temperature and the preparation made with prewarmed slides and coverslips.
- The CASA system must maintain the specimen at 37 ˚C, because sperm motion is sensitive to temperature.
- Improper temperature of stage warmer (e.g. too high temperature will kill spermatozoa



I LITERATURE

Temperature during handling - sperm

Sperm tolerant to long storage at room temperature

IVF: insemination temperature may be important

I LITERATURE

Conclusions

In Vivo

- 37° C body temperature is based on inaccurate measurements and averaged data
- Deep body temperature in mammals is generally but incorrectly regarded as uniform
- Temperature gradients in female reproductive tract
- Gametes and early embryos function in vivo at a lower temperature than core body temperature, which could encourage the expression of a quiet metabolism.

I LITERATURE

Conclusions

In Vitro

- 37° C core body temperature has been the standard for IVF

- Temperature differences within and between incubators
- Leese: impact on enzyme activity and metabilism: lowering incubation temperature?
- Cooling affects microtubules and spindle organization – what is too low?
- Rigourous thermal control (during ICSI) improves fertilization and pregnancy rates
- Overheating is detrimental – what is too high?

LITERATURE

Cairo Consensus on IVF Culture conditions – submitted RMB Online

"There is only one thing that is truly important in an IVF lab: everything" Cairo Consensus Guidelines on IVF Culture Conditions

Abstract

This proceedings report presents the outcomes from an international Expert Meeting to establish consensus guidelines on IVF culture conditions. Topics reviewed and discussed were: embryo culture – basic principles and interactions; temperature in the IVF lab; humidity in culture; CO2 control and medium pH; O2 tension for embryo culture; workstations – design and engineering; incubators – maintaining the culture environment; micromanipulation – maintaining a steady physical-chemical environment; handling practices; assessment practices; culture media – buffering and pH, general composition and protein supplementation, sequential or single-step media for human embryo culture, use and management – cold chain and storage; test equipment calibration and certification; and laboratory equipment and real-time monitoring. More than 50 consensus guideline points were established under these general headings.

Table 1: Consensus Meeting participants and contributors

Name	Affiliation
Jacques Cohen[1]	ART Institute of Washington and Althea Science, USA
David Mortimer[1]	Oozoa Biomedical Inc, Canada
Sharon Mortimer[2]	Oozoa Biomedical Inc, Canada
Mohamed Fawzy[3]	Ibnsina and Banon IVF Centers, Egypt
Mina Alikani	Reproductive Science Center of New Jersey, USA
Alison Campbell	CARE Fertility Group, UK
James Catt	Optimal IVF, Australia
Ronny Janssens	Centre for Reproductive Medicine, UZ Brussel, Belgium
Ragaa Mansour	The Egyptian IVF Center, Egypt
Sebastiaan Mastenbroek	Center for Reproductive Medicine, University of Amsterdam, The Netherlands
Marius Meintjes	Frisco Institute for Reproductive Medicine, USA
Dean E Morbeck	Fertility Associates, New Zealand
Catherine Racowsky	Brigham and Women's Hospital, Harvard Medical School, USA
Don Rieger	Life Global LLC, Canada
Jason Swain	CCRM IVF Network, USA

LITERATURE

Cairo consensus

Temperature: When measuring the temperatures of incubators or other highly sensitive environments, a tolerance limit of no more than ±0.1˚C is recommended. Thermometers should be calibrated against a NIST- traceable reference device at least twice per year

Temperature

Available evidence supports the maintenance of human oocytes and embryos at 37˚C during culture. It could be that there is a range of acceptable temperatures, but this range has not yet been determined. If the available equipment cannot provide a tight temperature control, then a slightly lower temperature is likely better than a slightly higher one.

CONTENT

I Literature

II How to measure temperature

"You cant' control what you cannot measure"

III Experiments

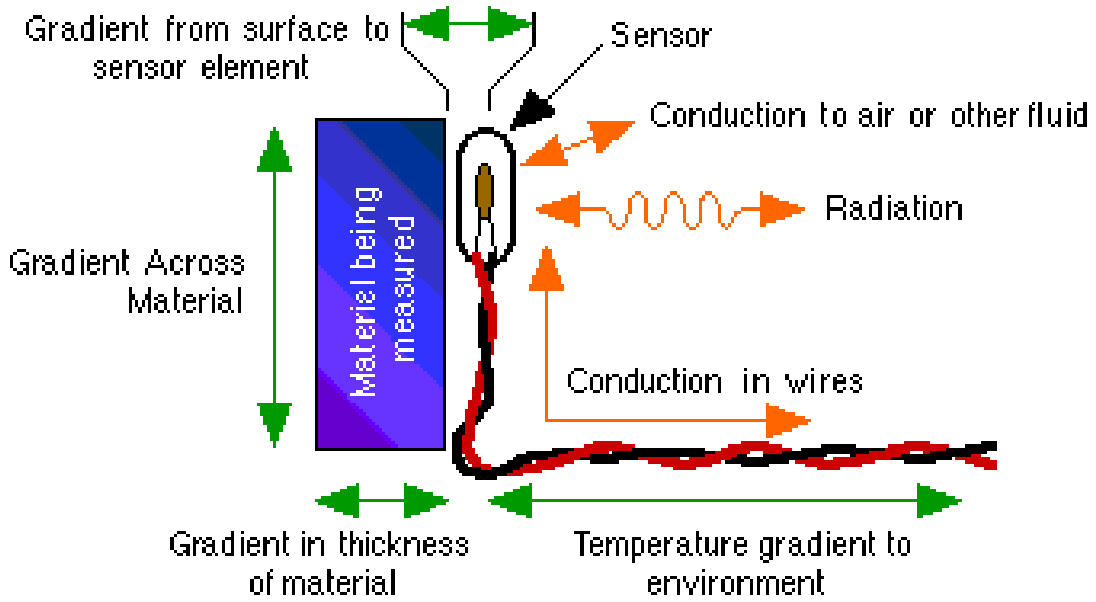
IV Conclusions

II HOW TO MEASURE TEMPERATURE

Introduction

Is temperature measurement difficult?

Temperature	Accuracy Required			
	±5°C	±1°C	±0.5°C	±0.1°C
-200°C	care needed	difficult	difficult	very difficult
0°C to 50°C	easy	care needed	difficult	very difficult
1000°C	care needed	very difficult	extremely difficult	almost impossible
2000°C	very difficult	extremely difficult	almost impossible	don't even try



Thermal Flows To and From a Temperature Sensor

II HOW TO MEASURE TEMPERATURE

Temperature sensors

Negative Temperature Coefficient thermistor (NTC)

- Small, accurate, fast

Resistance Temperature Detector

- Pt100 - Pt1000
- Very accurate, large, slow

Thermocouple

- Less accurate, small, fast

Semiconductor

Typical Temperature Sensor Characteristics

Typical Characteristics	Thermistors General Purpose	Resistance Temperature Devices (RTDs)	Thermocouples (TCs)	Semiconductor Temperature Sensors
Temperature Range	- 55°C to + 125°C	- 200°C to + 850°C	-600°C to +2000°C	-50°C to +150°C
Linearity	Exponential	Fairly linear	Fairly Linear	Best
Sensitivity	High	Low	Medium	Highest
Response Time	Fast	Slow	Fast to Slow (depends on construction)	Slow
Excitation or power	Needed	Needed	Not Needed	Needed
Long-Term Stability	Low	High	High	Medium
Self-heating	Yes	Yes	No	Yes
Cost	Low	Low (film) High (wire wound)	Moderate to High: (depends on construction)	Low to Moderate

II HOW TO MEASURE TEMPERATURE

Temperature sensors

Heated stages: Thermocouple

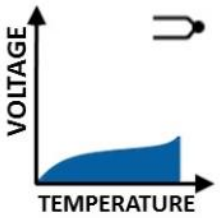
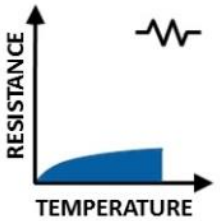
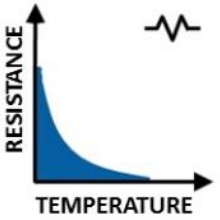
- Very small (inside dish)
- Very fast (detection of hot spots)

Large box incubators: RTD (Pt 100 – Pt 1000)

- Size not important
- Slow response
- Precise

Desktop Incubators: Thermistor

- Small size, direct heat - fast response

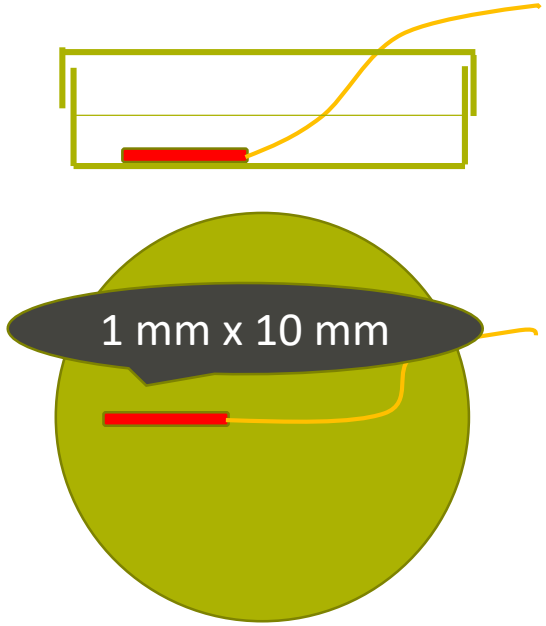
	Advantages	Disadvantages
THERMOCOUPLES 	✓ Simple ✓ Rugged ✓ Inexpensive ✓ No external power ✓ Wide temperature range ✓ Variety of styles	× Nonlinear response × Small sensitivity × Small output voltage × Requires CJC × Least stable
RTD 	✓ Most stable ✓ Good Linearity ✓ Most accurate	× Low sensitivity × Externally powered × Costly × Small output resistance × Self-heating error
THERMISTOR 	✓ Fast ✓ High output ✓ Minimal lead resistance error	× Limited temperature range × Externally powered × Nonlinear × More fragile × Self-heating error

II HOW TO MEASURE TEMPERATURE

Digital thermometer - thermistor

YSI 4610 precision thermometer with MEAS 451 1.3mm Tubular Probe (NTC thermistor)

System accuracy ±0.05˚C from 20˚C to 50˚C with 4610-series probes



II HOW TO MEASURE TEMPERATURE

Desktop incubators

Thermistor probe

Inside culture dish/oil

II HOW TO MEASURE TEMPERATURE

Thermocouples

Measurement range

Accuracy

Measurement site design

Reaction time

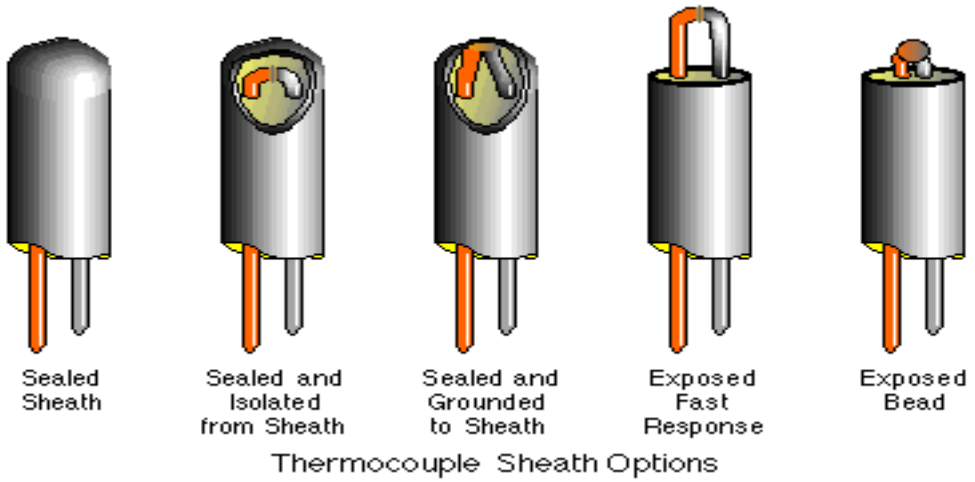
Durability

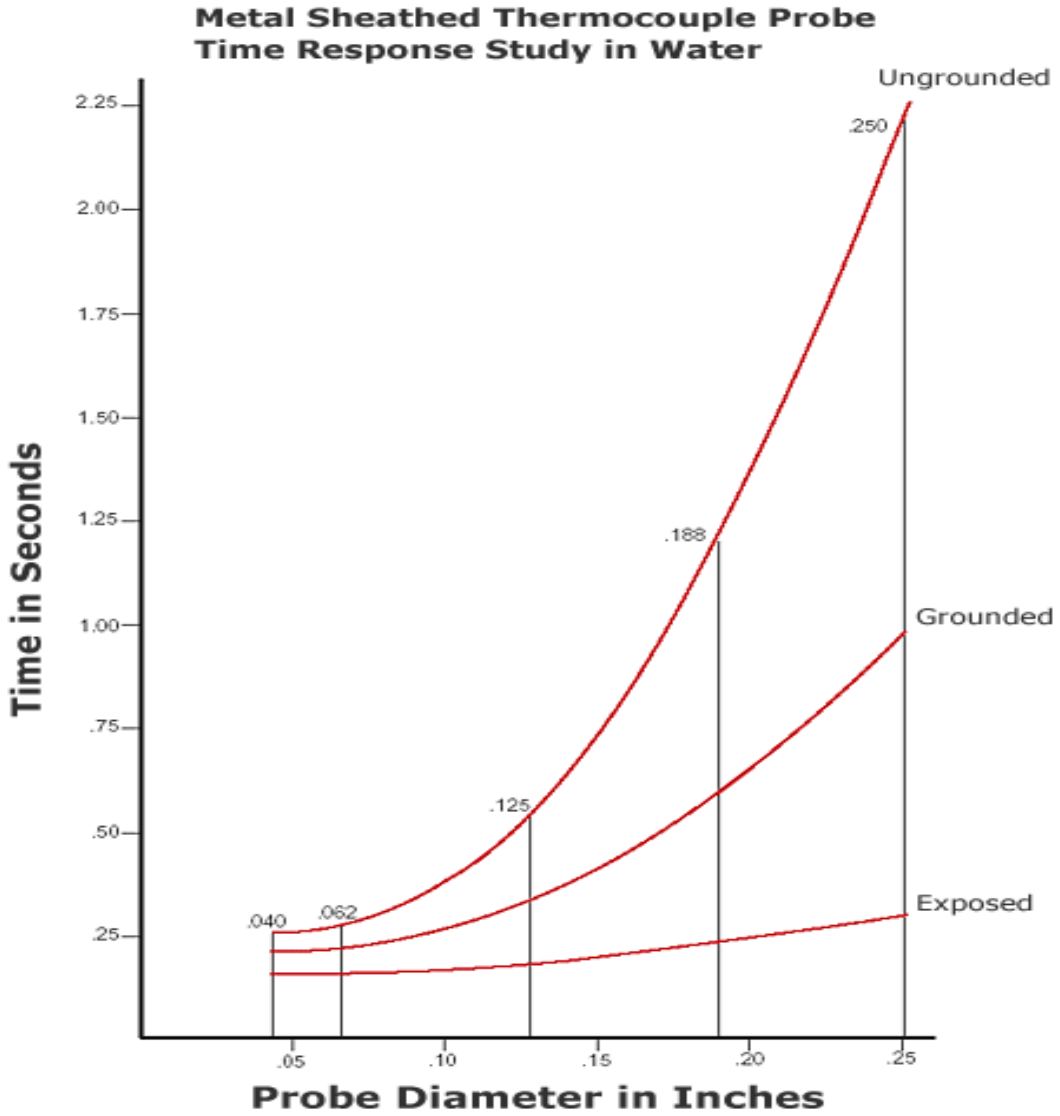
Type	Conductor Combination	Temperature Range	
		°F	°C
B	Platinum 30% Rhodium / Platinum 6% Rhodium	2500 to 3100	1370 to 1700
E	Nickel-chromium / Constantan	32 to 1600	0 to 870
J	Iron / Constantan	32 to 1400	0 to 760
K	Nickel-chromium / Nickel-aluminum	32 to 2300	0 to 1260
N	Nicrosil / Nisil	32 to 2300	0 to 1260
R	Platinum 13% Rhodium / Platinum	1600 to 2640	870 to 1450
S	Platinum 10% Rhodium / Platinum	1800 to 2640	980 to 1450
T	Copper / Constantan	-75 to +700	-59 to +370

II HOW TO MEASURE TEMPERATURE

Thermocouple reaction time

Response time vs mass





II HOW TO MEASURE TEMPERATURE

Digital thermometer – thermocouple

Heated stages – ICSI rig

Thermocouple type K

Non shielded, fast response

- Detection of “hot spots”
- Dont’ expect stable measurements

II HOW TO MEASURE TEMPERATURE

Tips and tricks

Ambient temperature should be stable: 21 – 23°C

Mimic each step in the process

Specific dish for specific procedure: type, volume, lid or not, air-flow on?

Validate testprotocol

Leave heating equipment on at all times

II HOW TO MEASURE TEMPERATURE

Measuring frequency

It depends on your equipment - risk assessment - validation

- Is it stable over time?
- Is it critical?

Egg collection heating block: each time

Heated stages: 2 times/year

- Know the hot spots
- establish trend

Incubators: real-time monitoring and alarming

II HOW TO MEASURE TEMPERATURE

Do you know exact temperatures at…?

Manipulations

- Follicular aspiration
- Transport to laboratory
- Examination of follicular fluid
- Oocyte/embryo handling
- Embryo culture
- Transfer

Equipment

- Laboratory (OR) equipment
- ICSI rig
- Incubator

Dont take anything for granted!!!

II HOW TO MEASURE TEMPERATURE

Checklist

Daily verification of setpoints

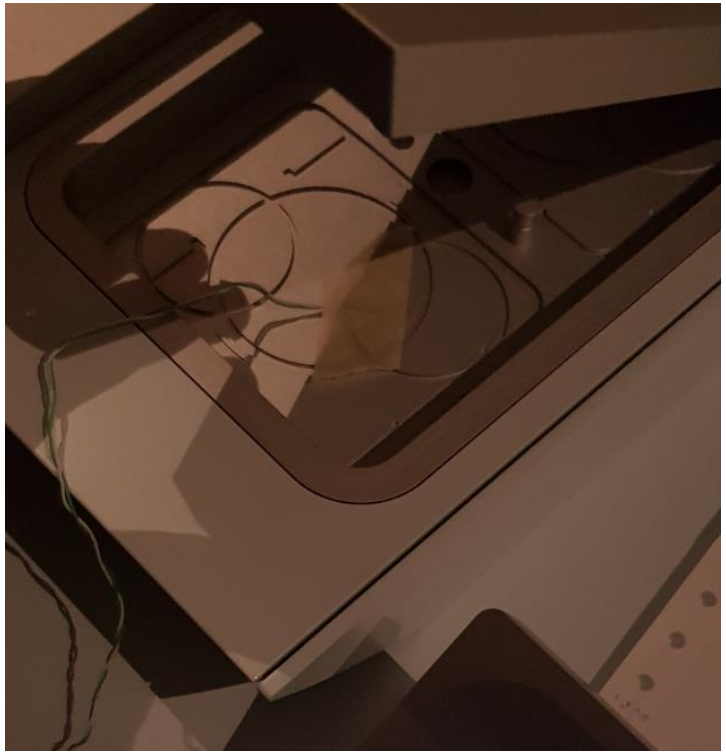
- Changes by cleaning staff
- Unintended changes (power failure)

Ingangsdatum: 07/01/2009 Controleblad ochtenddienst Embryologie

Datum:		Toegestane afwijking tov.setpoint: ± 0.5°C		
		setpunt	controle	Aktie!
Werkpost 1	Verwarmtafel L micr (16122)	42.0	T°	
IVF	Tafelincubator OPU (18443)	36.6	T°	
	Verwarmblok OK OPU (14158)	39.0	T°	
Werkpost 2	Verwarmtafel R micr (17897)	40.1	T°	
IVF	Tafelincubator K-Systems (18445)	36.6	T°	
	Verwarmblok OPU (18840)	39.4	T°	
	Verwarmplaat (00307)	41.6	T°	
Werkpost 3	Tafelincubator K-Systems (14420)	36.9	T°	
evaluatie	Microscooptafelverwarming (24044)	37.0	T°	
Werkpost 4	Microscooptafelverwarming linkam(50021)	42.0	T°	
evaluatie	Tafelincubator K-Systems (24421)	37.2	T°	
Werkpost 18	Microscooptafelverw Hunter (21616)	38.2	T°	
ICSI flow	Tafelincubator K-Systems (24417)	39.5	T°	
Werkpost 17	Microscooptafelverw Minitub (50020)	40.1	T°	
ICSI				
Werkpost 8	Tafelincubator K-Systems (24440)	38.0	T°	
Cryo1	Verwarmplaat cryo (12998)	43+/-1°C	T°	
	Waterbad (08207) eerst aanzetten!	29.9	T°	
Werkpost 9	Waterbad (11702)	42.3	T°	
Cryo2	Tafelincubator K-Systems (24441)	43.1	T°	
Werkpost 10	Verwarmtafel L micr (17898)	38.9	T°	
ICSI flow	Tafelincubator K-Systems (18417)	36.8	T°	
Werkpost 11	Verwarmtafel R micr Hunter (21615)	38.2	T°	
ICSI flow	Tafelincubator K-Systems (14436)	36.5	T°	
Werkpost 12	Microscooptafelverw Tokai (17899)	38.5	T°	
ICSI flow	Werktafelincubator (24442)	37.6	T°	
Werkpost 13	Microscooptafelverw inj R (0117)	39.5	T°	
ICSI				
Werkpost 14	Microscooptafelverw Tokai (21502)	42.6	T°	
ICSI	Tafelincubator K-systems (18419)	37.7	T°	
Werkpost 15	Microscooptafelverw RI (24035)	38.4	T°	
ICSI				

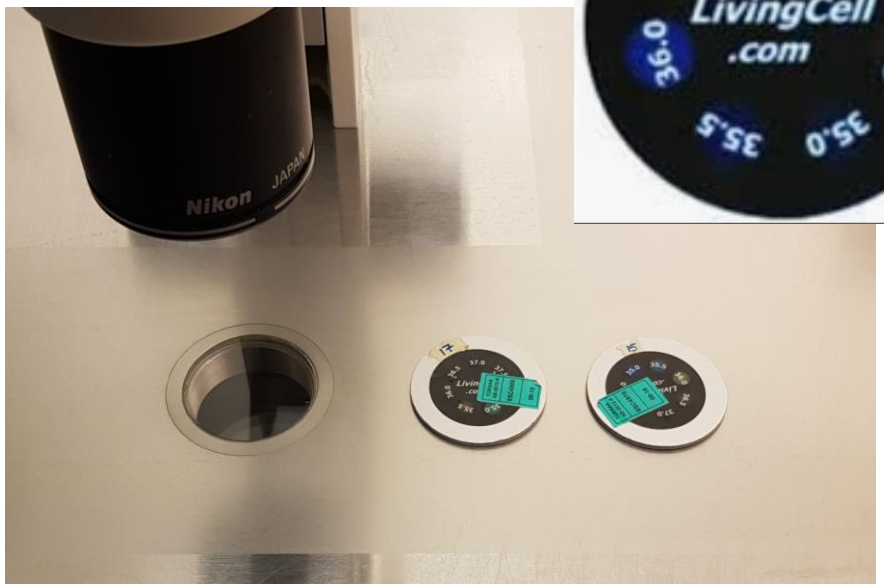
II HOW TO MEASURE TEMPERATURE

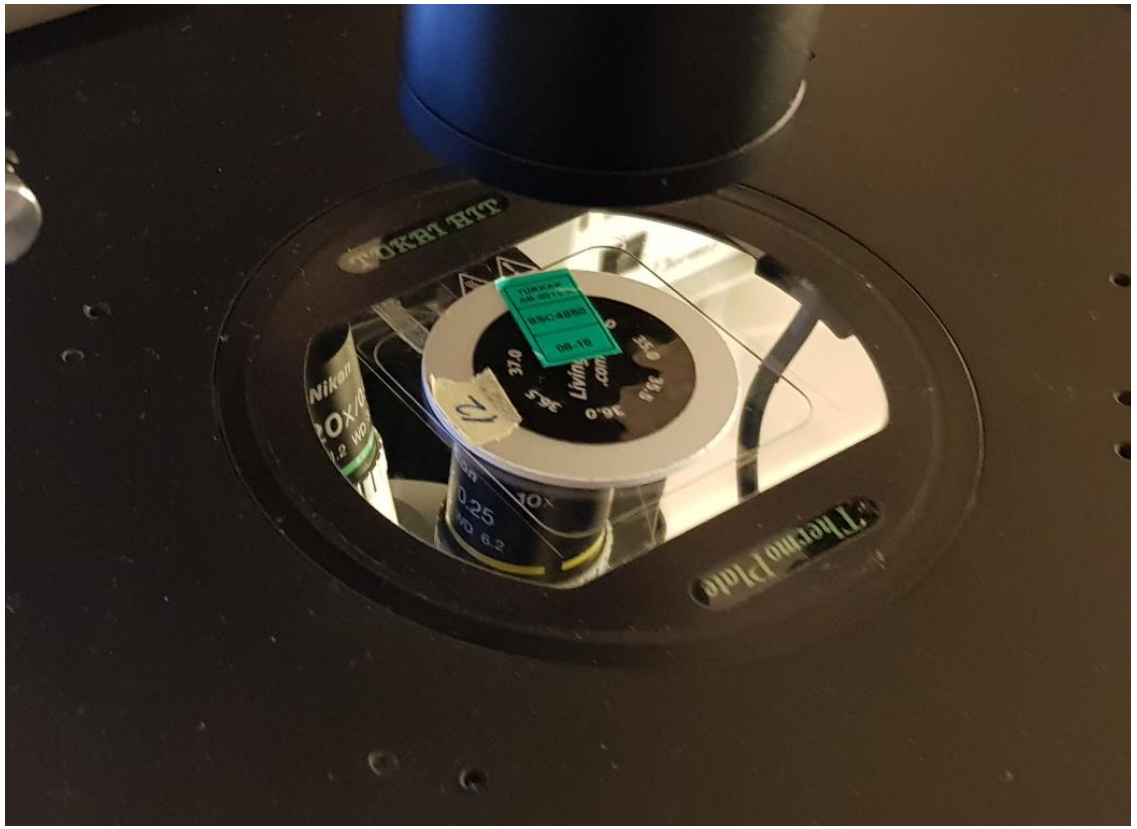
Wrong use of thermocouple



II HOW TO MEASURE TEMPERATURE

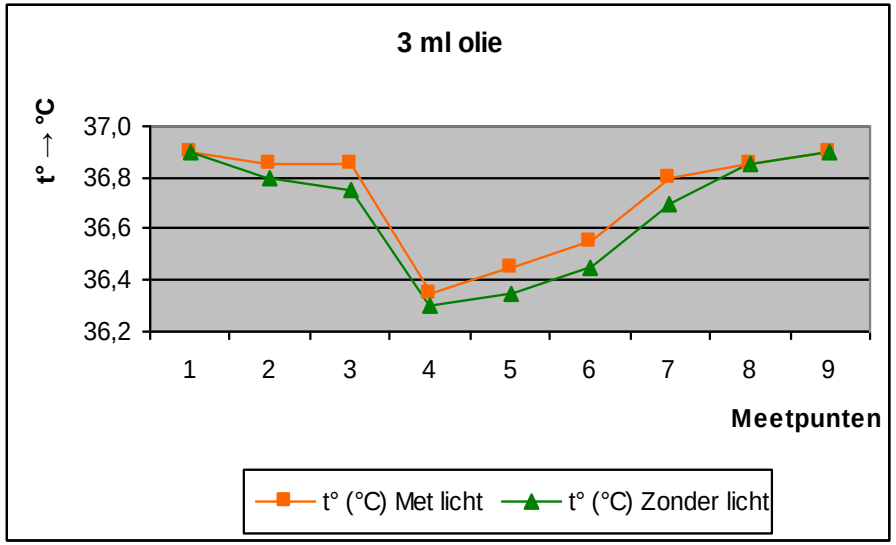
Inaccurate measurement

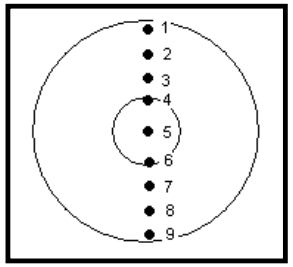




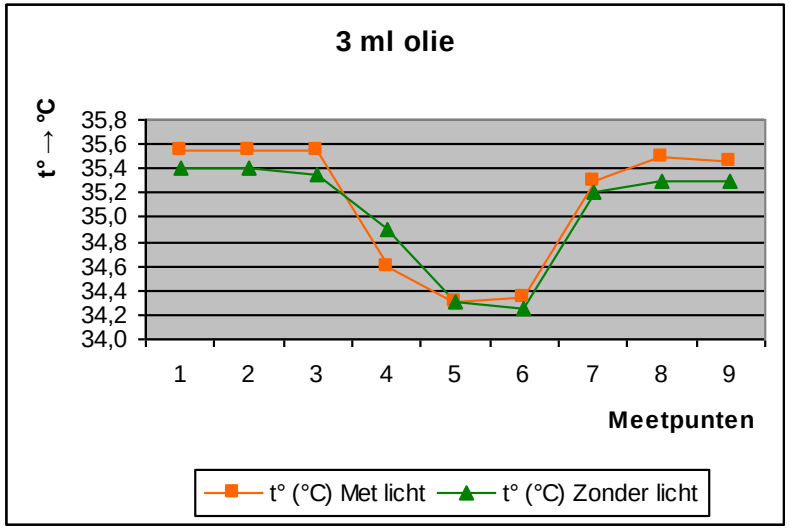
II HOW TO MEASURE TEMPERATURE

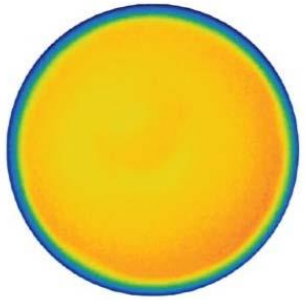
Glas heated stage



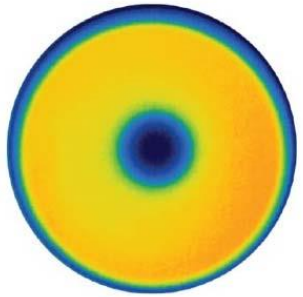


Linkam heated stage (metal)

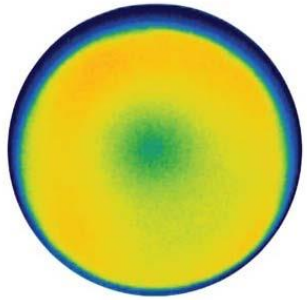




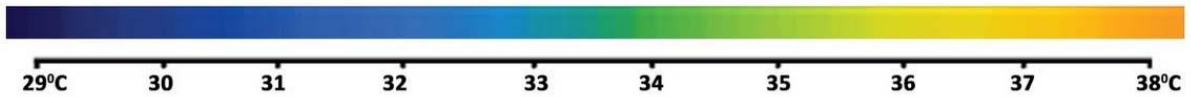
Thermal image of Petri dish surface when placed on heated metal plate **WITH THERMOSAFE™**



Petri dish surface when placed on heated metal plate **WITHOUT THERMOSAFE™**



Petri dish surface when placedon market leading glass ITO **WITHOUT THERMOSAFE™**



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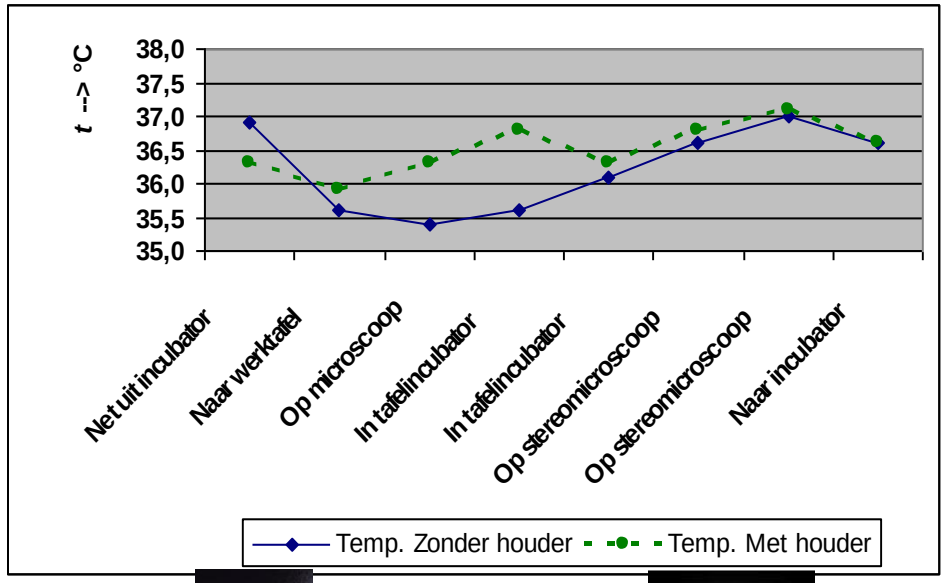
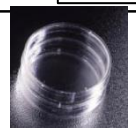
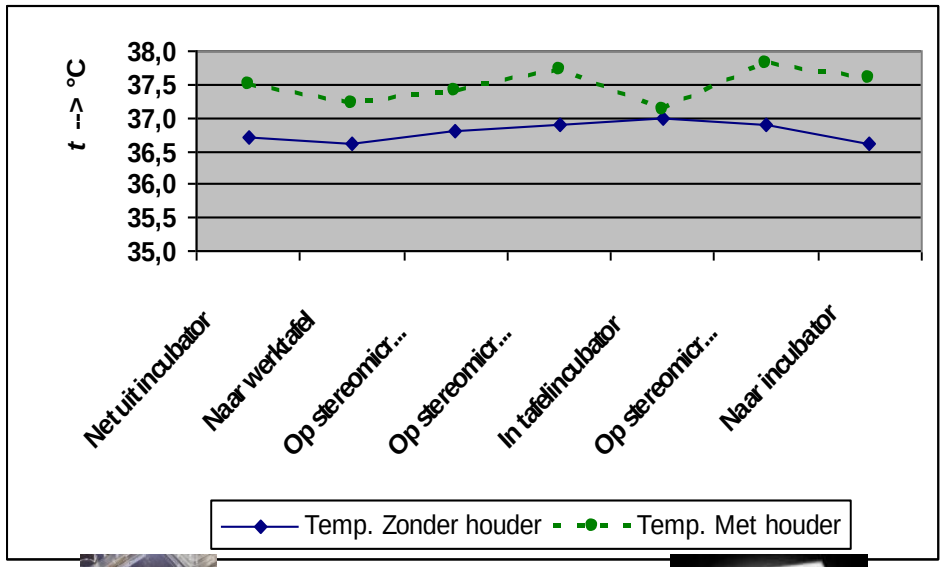
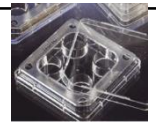
II HOW TO MEASURE TEMPERATURE









- *fast cooling during transport*
- *Protective potential of metal tray holders??*

II HOW TO MEASURE TEMPERATURE

Equipment specifications

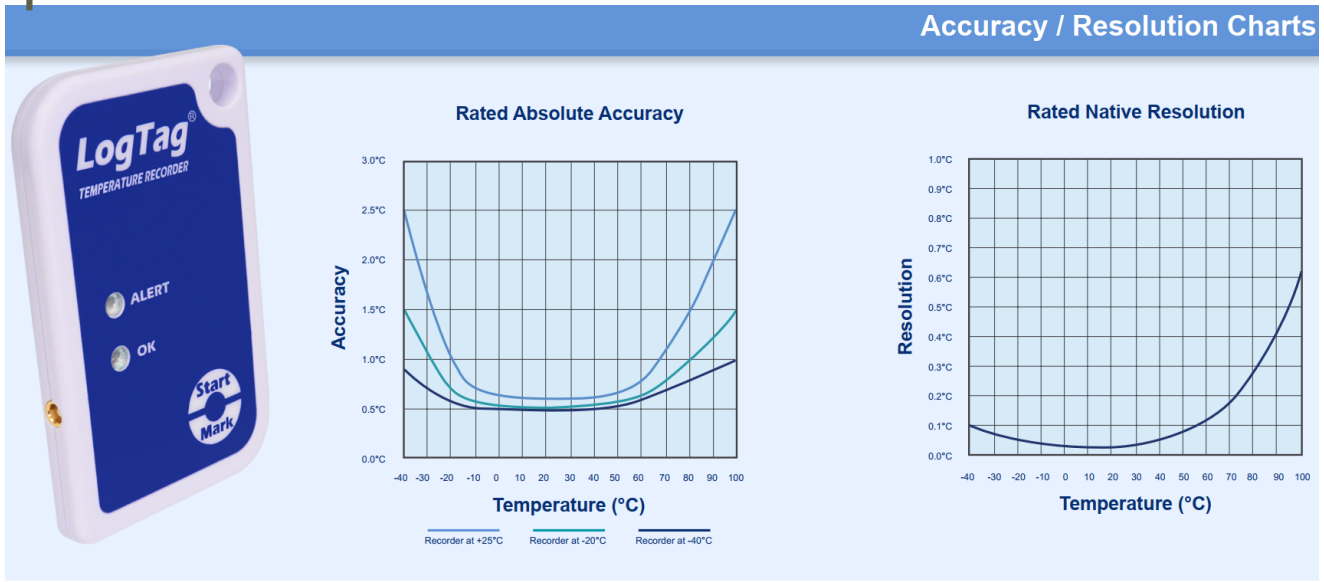
Risk of overheating $\geq 37.2°C$

	Stability	Homogeneity	Accuracy of regulation	Max sp
Labotect Hot Plate A4	± 0.2°C	± 0.3°C		36.7°C
Laboteckt blockthermostat	± 0.2°C	± 0.2°C		36.8°C
Labotect Cell transporter	?	?	± 0.3°C	?
Labotect CO2 incubator Labo C201	± 0.1°C	± 0.3°C		36.8°C
Minitube Embryo and oocyte transport	?	?	± 0.5°C	?
Cooper surgical G95 incubator	?	?	± 0.2°C	?
Modern desktop incubator	< 0.1°C	0.1°C		37.0°C

II HOW TO MEASURE TEMPERATURE

Temperature during transport

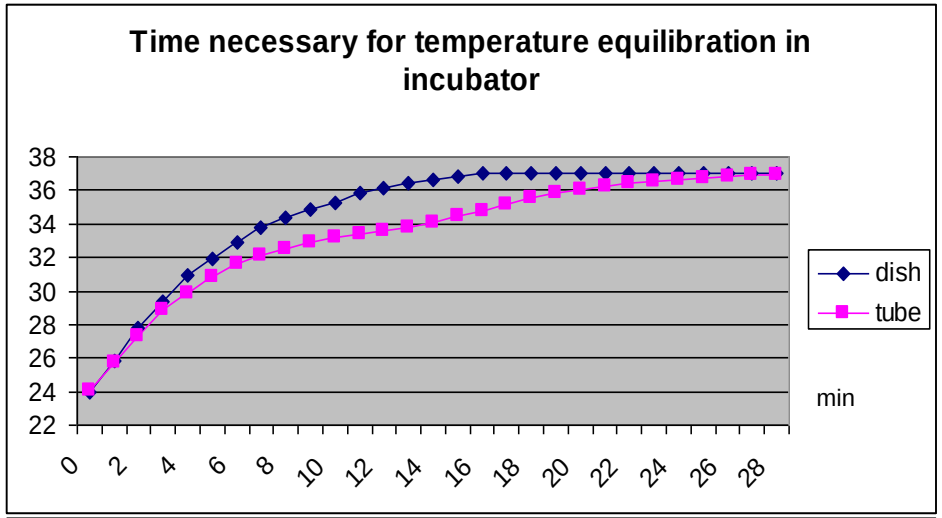
Temperature logger

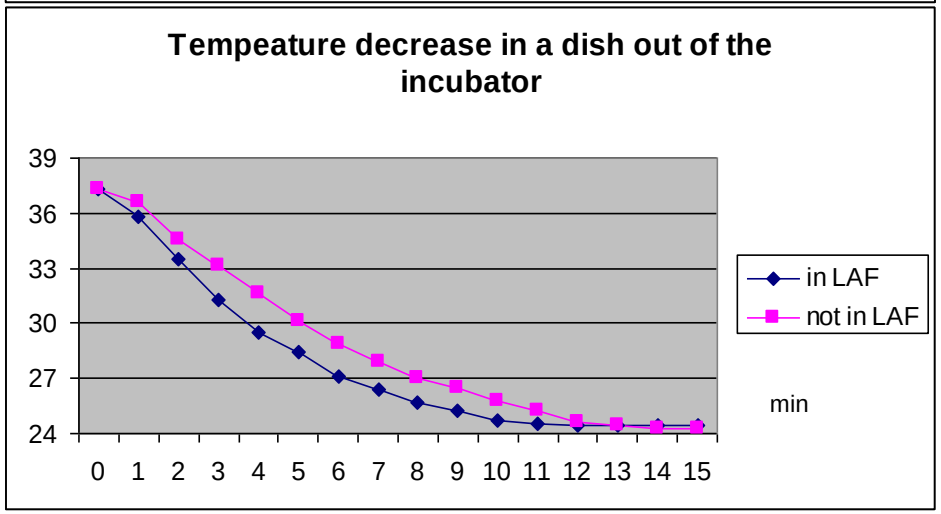


Product Specifications

Product Model	TREX-8
External Temperature Sensor Measurement Range	-40°C to +99°C (-40°F to +210°F).
Operating Temperature Range	-40°C to +85°C (-40°F to +185°F).
Storage Temperature Range	-10°C to +55°C (14°F to +131°F).
Rated Temperature Reading Accuracy	Better than ±0.5°C for -10°C to +40°C. Better than ±0.7°C for -10°C to -30°C & +40°C to +60°C. Better than ±0.8°C for -30°C to -40°C & +60°C to +80°C. Better than ±1.0°C for +80°C to +99°C. *Actual performance is typically much better than the rated values. Please see the Rated Absolute Accuracy chart above.* *Accuracy figures can be improved by recalibration.*
Rated Temperature Reading Resolution	Less than 0.1°C for -40°C to +40°C. Less than 0.2°C for +40°C to +80°C. Less than 0.6°C for +80°C to +99°C. *Please see the Rated Native Resolution chart below. LogTag Analyzer® currently displays to one decimal place of °C or °F.*
Sensor Reaction Time	Typically less than 2 minutes (T90) in moving air (1m/s) for ST100T, ST100H & ST100S types.
Recording Capacity	8031 temperature readings. 53 days @ 10min logging, 80 days @ 15min logging.
Sampling Interval	Configurable from 30 seconds to 18 hours.

II HOW TO MEASURE TEMPERATURE





Heating: 20 min

Cooling:

- 0.5°C/min

37.0 ± 0.5°C = 1 min!!!

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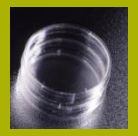
II HOW TO MEASURE TEMPERATURE

Heating and cooling

	Optimal T° after (min)	Complete cooling after (min)
3,5 cm culture dishes (3 ml oil) 	~ 20	~ 20
Centre Well (500 µl medium + 1 ml oil) 	~ 20	~ 15
Centre Well (500 µl medium) 	~ 30	~ 15
Nunc (500 µl medium + 400 µl oil) 	~ 30	~ 25
Nunc (500 µl medium) 	~ 40	~ 20

II HOW TO MEASURE TEMPERATURE

Conclusions

Accurate temperature measurement is very difficult

Many laboratories do not have accurate/calibrated equipment/probes

High uncertainty of measurement

Exact methodology/accuracy/uncertainty of measurements in literature: ?

CONTENT

I Literature

II How to measure temperature

III Experiments UZ Brussel

IV Conclusions

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RBMO

ELSEVIER

ARTICLE

The effect of different temperature conditions on human embryos *in vitro*: two sibling studies

BIOGRAPHY

Neelke De Munck has been working as a Clinical Embryologist at the Centre for Reproductive Medicine at UZ Brussel where she obtained her PhD on safety and efficiency of oocyte vitrification. She is currently the scientific advisor at the IVI RMA Fertility Clinic of Abu Dhabi.

De Munck Neelke[1,*], Janssens Ronny[1], Santos-Ribeiro Samuel[2], Tournaye Herman[1], Van de Velde Hilde[1], Verheyen Greta[1]

KEY MESSAGE

Circadian temperature rhythm does not improve fertilization and utilization rate, although other circadian temperature rhythm limits could be more favourable. No difference was found in development between culture at 36.6°C or 37.1°C

ABSTRACT

Research question: What temperature is optimal for human embryo development up to day 5 or 6?

Design: Two prospective sibling oocyte studies on culture temperature were conducted in a university-based tertiary referral centre. Eligibility critera for both studies: Study 1: 50 cycles between August and October 2015, with culture at a stable temperature (37.0°C ± 0.3°C) or culture using a circadian temperature rhythm (CTR) (1 am to 6 am: 36.6°C, gradual increase to 37.5°C; 11 am to 9 pm: 37.5°C; gradual decrease to 36.6°C); study 2: 99 cycles between April and November 2016, with stable culture at 36.6°C or 37.1°C. Primary outcome measures: fertilization and embryo development (top and good quality) up to day 5 or 6, and utilization rate (number of embryos transferred and cryopreserved per zygote). Secondary outcome measure: clinical pregnancy (number of pregnancies with at least one gestational sac).

Results: An incubator with CTR was used for culture. An effect was found on embryo development (utilization rate: 42.1% versus 32.6%; $P = 0.014$), but not on clinical pregnancy rate (60.0% versus 45.5%; $P = 0.670$). Stable culture at 36.6°C or 37.1°C did not affect embryo development (utilization rate: 40.0% versus 40.4%; $P = 0.905$); clinical pregnancy rate was improved by culture at 37.1°C (46.4% versus 74.2%; $P = 0.036$).

Conclusion: Culture in an incubator with CTR does not improve fertilization rate or embryo quality. Embryo culture at 36.6°C or 37.1°C showed similar embryo development.

III – THE EXPERIMENTS

Questions

Is there an optimal culture temperature?

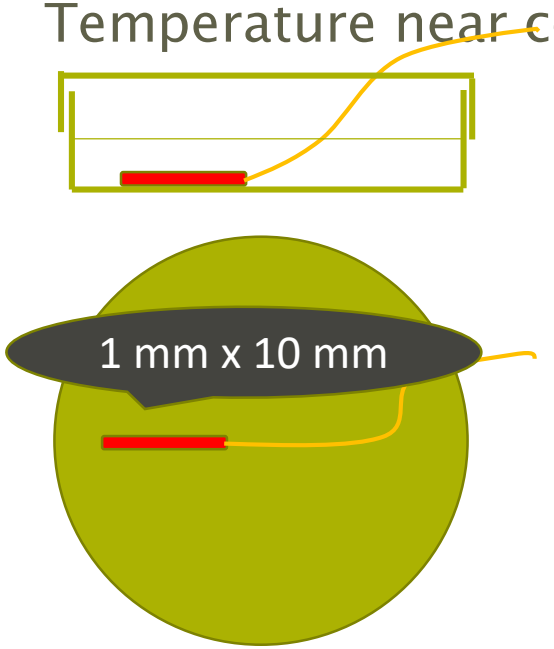
Is there an acceptable range?

What is an acceptable minimum- maximum value?

III – THE EXPERIMENTS

Temperature Calibration procedure

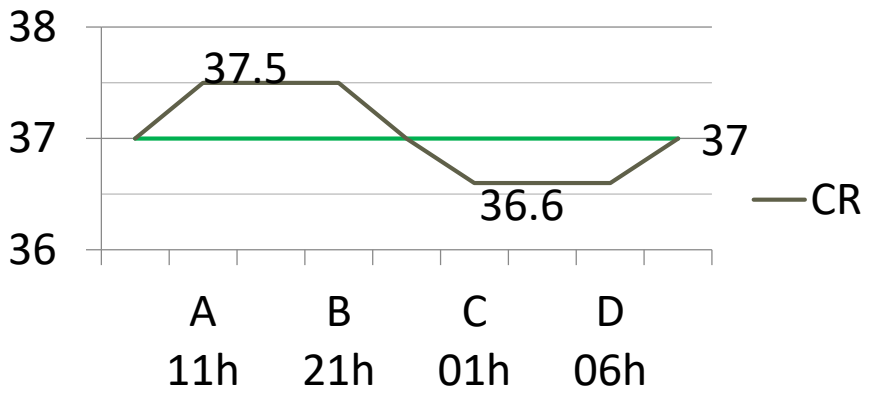
Temperature near cells



- Thermometer: Measurement specialties 4610 precision thermometer with YSI 4611 probe
- Accuracy ± 0.05°C (20°C – 50°C)
- Incubator temperature calibration function ± 0.1°C to adjust “real” temperature

III – THE EXPERIMENTS

1. Circadian Temperature Rhythm (CTR)



III THE EXPERIMENTS

CTR study 36.6 – 37.5

- 36.6°C (1 – 6) tot 37.5°C (11 – 21h)
- Sibling oocytes, 50 patients, d5/6

Temperature	37.0 G-185	CTR G-210	
MII	337	337	
Fertilized (%)	283 (84.0)	26 (78.9)	0.11
D3			
Excellent (%)	153 (54.1)	131 (49.2)	0.76
Good (%)	70 (24.7)	73 (27.4)	
Moderate (%)	36 (12.7)	39 (14.7)	
Poor (%)	24 (8.5)	23 (8.6)	
D5	250	241	
Excellent (%)	51 (20.4)	39 (16.2)	0.20
Good (%)	97 (38.8)	87 (36.1)	
Moderate (%)	41 (16.4)	52 (21.6)	
Poor (%)	61 (24.4)	63 (26.1)	
Transferred	31	26	
Cryopreserved D5	77	47	
Cryopreserved D6	34	37	
Per MII	144/337 (42.7)	112/337 (33.2)	0.01

III THE EXPERIMENTS

CTR study 36.6 – 37.5 - Conclusions

1 CTR

- Embryos can be cultured in a range from 36.6 to 37.5˚C
- Maintaining a Circadian Temperature Rhythm (36.6 to 37.5˚C) during culture does not improve fertilization rate and embryo development
- Embryo utilisation rate is lower –37.5˚C might be too high

III – THE EXPERIMENTS

1. CTR 36.6 – 37.5
2. Sibling 36.6 vs 37.1°C

III THE EXPERIMENTS

Sibling 36.6 vs 37.1°C

Does stable incubation temperature at 36.6°C or 37.1°C affect the embryo quality after ICSI?

- Prospective sibling oocyte study (may – nov 2016)
- 100 ICSI d5/6 cycles, min 6 MII, ejaculated semen,no PGD
- One incubator @ 36.6°C or 37.1°C - 6.0% CO_2 and 5.0% O_2
- Origio Sequential Series
- Main outcome measures:
  - >Fertilisation rates
  - >Embryo quality D3 - D5/6
  - >Utilisation rates

III THE EXPERIMENTS

Sibling 36.6 vs 37.1˚C

Incubator Temperature calibration

Chambers 1 to 5 as close as possible to 37.10 °C and chambers 6 to 10 to 36.60°C - 6.0% CO_2 – 5.0 % O2

Inc 40	Chamber 1	Chamber 2	Chamber 3	Chamber 4	Chamber 5	**Average**
	37.11	37.05	37.15	37.15	37.07	**37.11**
	Chamber 6	Chamber 7	Chamber 8	Chamber 9	Chamber 10	
	36.66	36.61	36.66	36.59	36.62	**36.63**

THE EXPERIMENTS

Culture temperature	36.6°C	37.1°C
Cycles	99	
# COC/ # MII	1432 / 1153 (80,5%)	
# injected	572	581
2PN (%)	460 (80.4)	455 (78.3)*
Embryo Quality D3		
Grade 1 (%/2PN)	238 (51.7)	261 (57.4)*
Grade 2 (%/2PN)	122 (26.5)	101 (22.2)
Embryo Quality D5		
Grade 1 (%/2PN)	101 (23.8)	104 (24.3)*
Grade 2 (%/2PN)	127 (30.0)	135 (31.5)

* NS

	36.6°C	37.1°C
Transfers (SET)	59	
Transfer (DET)	11	
No transfer (EQ)	2	
Freeze all	26	
No Cryo (EQ)	1	

	36.6°C	37.1°C
Vitrified D5	120	144
Vitrified D6	72	47
Transfered D5	37	44
Utilisation rate/MII (%)	229/572 (40.0)	235/581 (40.4)*

No significant differences in fertilisation – embryo quality – utilisation rate

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III THE EXPERIMENTS

Sibling 36.6 – 37.1˚C - Conclusions

1 CTR

- Embryos can be cultured in a range from 36.6 to 37.5˚C
- Maintaining a Circadian Temperature Rhythm (36.6 to 37.5˚C) during culture does not improve fertilization rate and embryo development
- Embryo utilisation rate is lower –37.5 might be too high

2 Sibling 36.6 – 37.1˚C

- Incubation at 36.6 or 37.1˚C results in identical fertilisation rates, embryo quality on day 3 or day 5 and embryo utilisation rate
- The quiet embryo metabolism hypothesis cannot be confirmed

III – THE EXPERIMENTS

1. CTR 36.6 – 37.5
2. Sibling 36.6 vs 37.1˚C
3. RCT 36.6 vs 37.1˚C˚

III THE EXPERIMENTS

RCT 36.6 vs 37.1˚C

	36,6°C	37,1°C
No. of Cycles	33	38
Age (y)	32.4 ± 3.1	31.4 ± 4.5
No. Of COCs	11.9 ± 4.4	11.7 ± 3.6
# COCs	392	444
No. Of MII	9.9 ± 3.4	9.8 ± 3.0
# MII (%)	326 (83.2)	374 (84.2)
Fertilization		
Fertilized	7.6 ± 3.3	7.9 ± 3.0
# Fertilized (%)	251 (77.0)	300 (80.2)
Day 3 Embryo Quality		
Excellent Quality (%)	126 (50.2)	195 (77.7)
Good Quality (%)	72 (28.7)	67 (26.7)
Moderate Quality (%)	41 (16.3)	26 (10.4)
Poor Quality (%)	12 (4.8)	12 (4.8)
Day 5 Embryo Quality		
No. of Embryos to Day 5 Culture	238	289
Excellent Quality (%)	27 (11.3)	39 (13.5)
Good Quality (%)	76 (31.9)	100 (34.6)
Moderate Quality (%)	58 (24.9)	65 (22.5)
Poor Quality (%)	77 (32.4)	85 (29.4)
Utilization		
No. Of Embryos Transferred on Day 5	33	38
No. Of Embryos Cryopreserved on Day 5+6	34 + 35	62 + 43
Per No. Of Mature Oocytes (%)	102/326 (31.3)	143/374 (38.2)
Per No. Of Fertilized Oocytes (%)	102/251 (40.6)	143/300 (47.7)

III THE EXPERIMENTS

RCT 36.6 vs 37.1°C (update 10/07/2018)

	36.6°C	37.1°C
day 5 transfers	33	38
unknown outcome	0	1
positive hCG (%)	23 (69.7)	24 (63.2)
clinical (FHB + echo 1)	19 (57.6)	19 (50.07)
ongoing clinical (some miscarried)	19	16
biochemical	2	2
negative hCG	10	13

III THE EXPERIMENTS

RCT 36.6 – 37.1˚C - Conclusions

- No effect on clinical outcome
- More blastocyst frozen after culture in 37.1˚C

- Effect of faster cleavage @ 37.1˚C?
  - Morphokinetics?

CONTENT

I Literature

II How to measure temperature

III Experiments UZ Brussel

IV Conclusions

IV- CONCLUSIONS

Questions

Is there an optimal culture temperature?

- Maybe not but data not conclusive yet

Is there an acceptable range?

- YES

What is an acceptable minimum- maximum value?

- 36.6 to 37.1 is acceptable
- Minimum – maximum ?

No effect of incubation temperature on clinical outcome

IV CONCLUSIONS

Discussion

Future?

- Other incubation temperatures?
- Static versus dynamic culture systems?

Thanks

- Dr Neelke De Munck
- Dr Samuel dos Santos Ribeiro

THANK YOU

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Centrum voor Reproductieve Geneeskunde

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