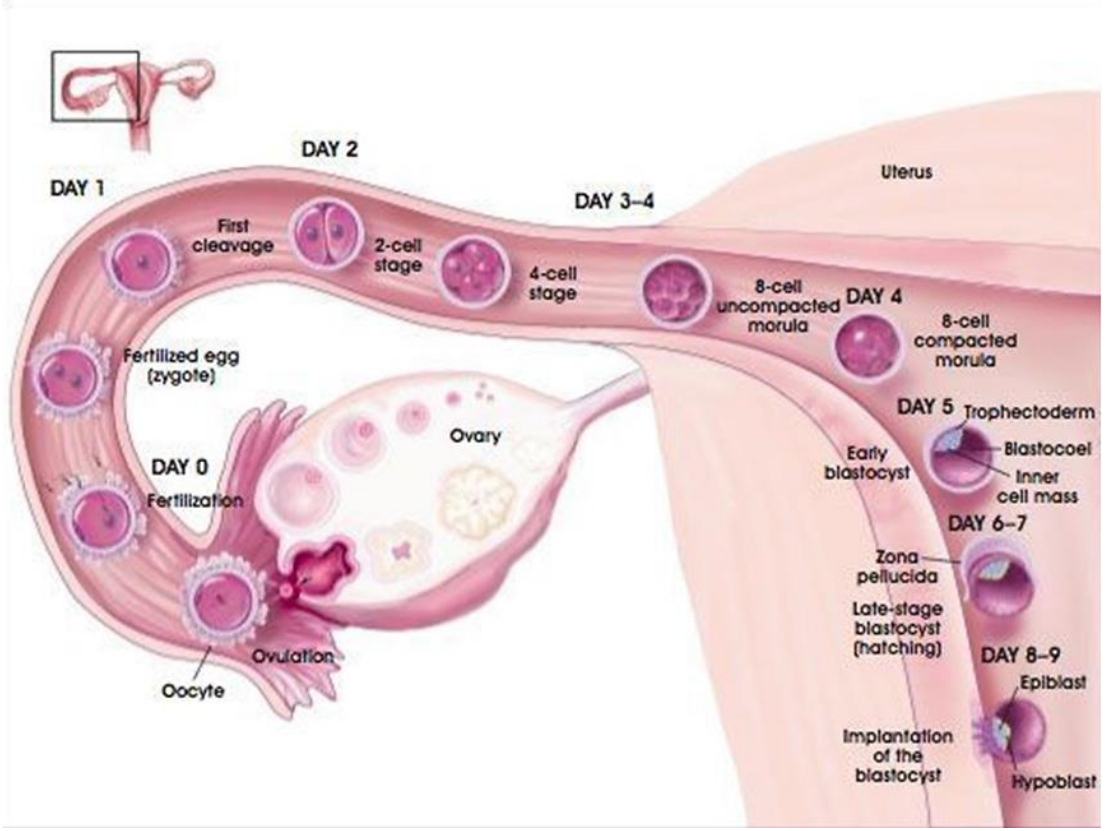

TOGETHER.
ALL THE WAY™
Vitrolife

THE BALANCE BETWEEN SEQUENTIAL AND NON-SEQUENTIAL MEDIA FOR SUCCESSFUL BLASTOCYST CULTURE

Bert Stewart Ph.D

What we attempt to do in the IVF process is to mimic the *in vivo* environment of the Fallopian tube and uterus



Sequential media system developed to mimic the *in vivo* nutrient changes and the change in metabolic requirements of the embryo

Aim is to promote blastocyst development, aiding embryo selection in order to maintain high pregnancy rates while reducing multiple pregnancy

Work by Professor David Gardner and co-workers relied heavily on lessons learned from both embryo and maternal physiology

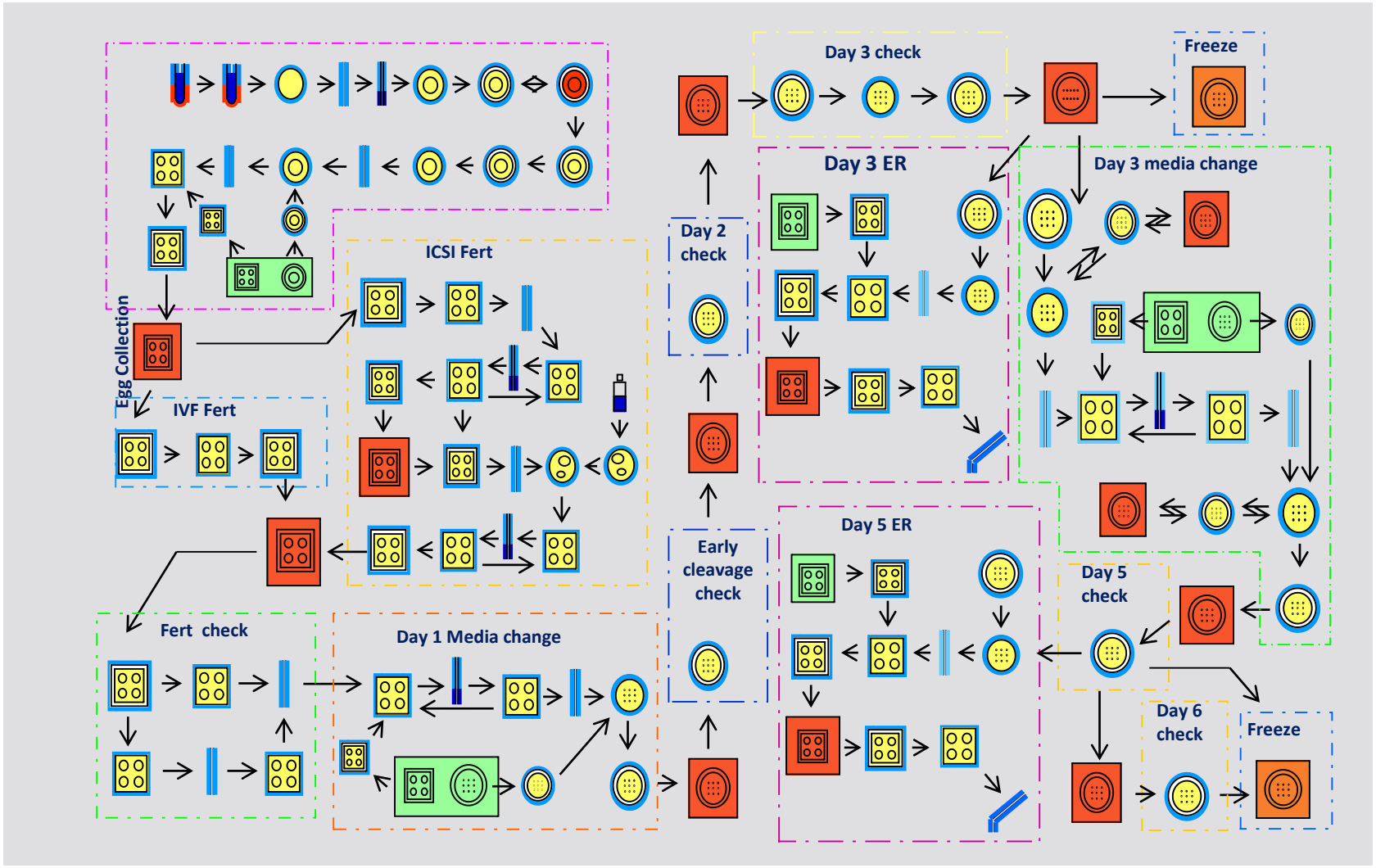
This work led to the formulation of the first sequential culture media system defined as the G-Series, launched in 1998. In short, these media formulations were based on three factors;

1.The levels of nutrients within the female reproductive tract
2.The changing requirements of the human embryo
3.The importance of minimising *in vitro*-induced stress as a significant factor for maintaining embryo viability

Gardner DK. Changes in the requirements and utilisation of nutrients during mammalian preimplantation embryo development and their significance in embryo culture. Theriogenology 1998;49:83–102.

But… It's not all about culture media. Handling gametes and embryos can lead to stresses which can lead to reduced viability

Embryo Culture Process



MAJOR STRESS FACTORS

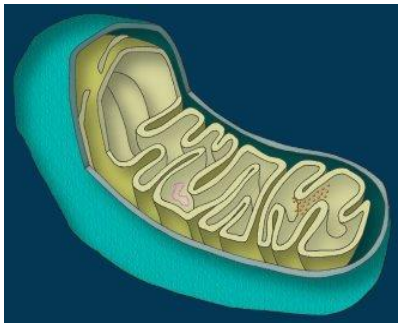
- **Incorrect or fluctuating pH**
- **Incorrect temperature**
- Osmolality fluctuations
- Oxidative stress caused by oxygen radicals
- Metabolic stress
- Cryopreservation
- Infection

IMPORTANCE OF pH

Incorrect pH will lead to

- Reduced cleavage rates
- Pertubations in the regulation of cytoskeletons
- Inability to maintain energy production

REDUCED VIABILITY!



Extracellular pH is a modulator of intracellular pH

Lane et al. Biol Reprod. 1999

pH IN THE G5 SERIES

Physiological pH

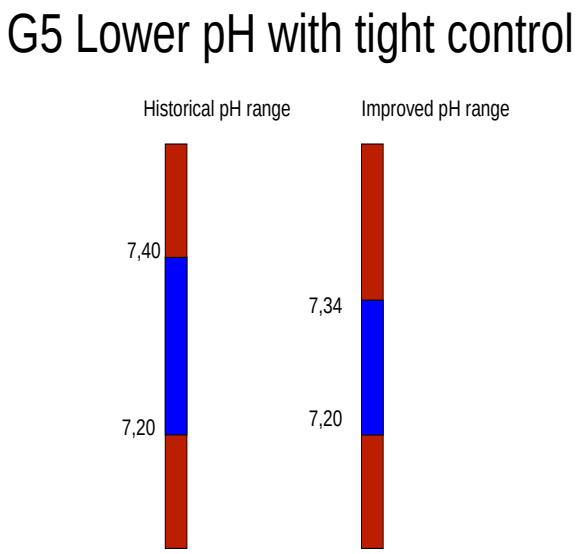
- Intracellular pH is ~7.2

Narrow range

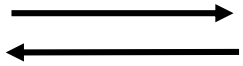
- G5 media set at 7.27+0.07

Increased stability

- Reduced concentration of bicarbonate

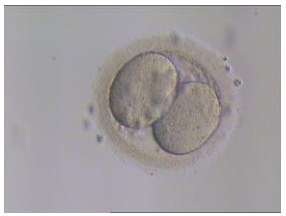


Depending on Clinic protocols embryos must be removed from incubators several times to assess development



Hours post insemination

18



25

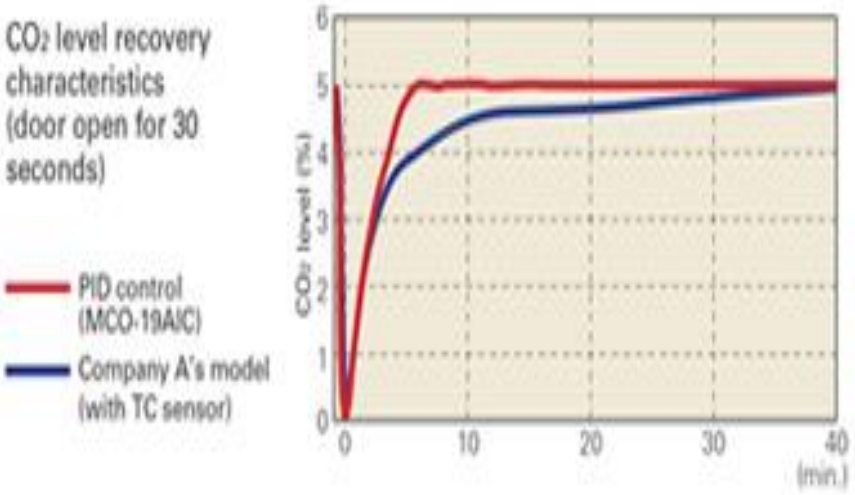
45

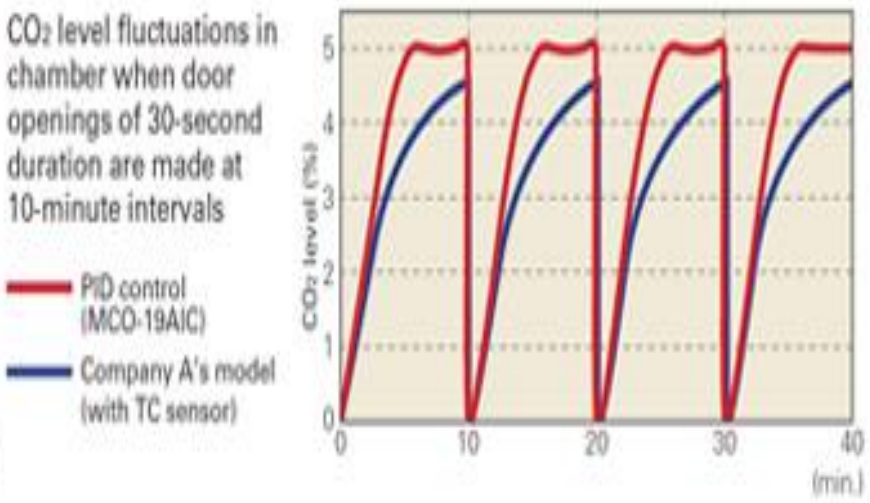
65

120

Opening of incubators alters CO_2 concentrations and recovery times vary dependant on incubator volume and CO_2 sensor type

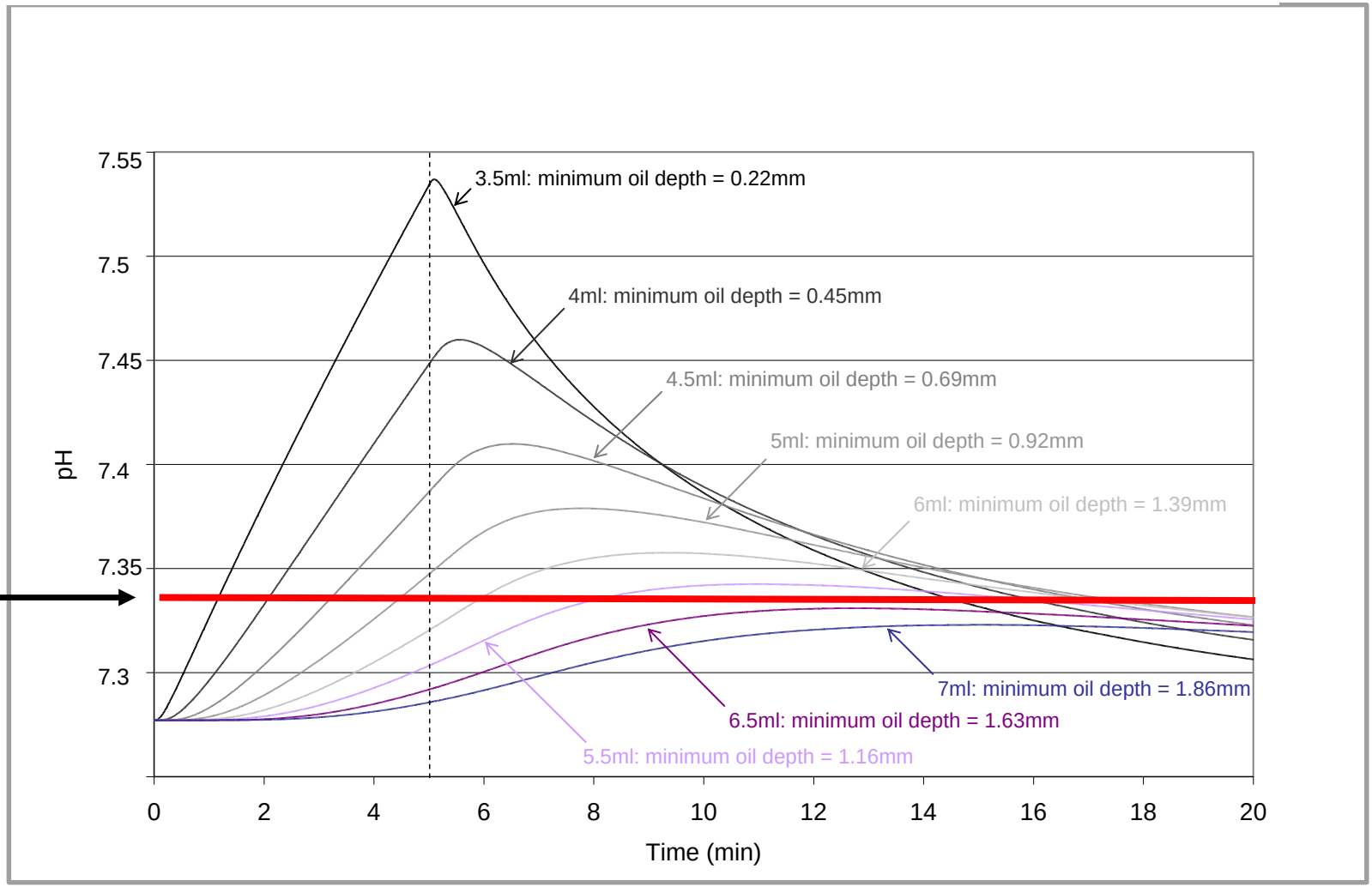
Repeated openings will have a cumulative effect on CO_2 levels



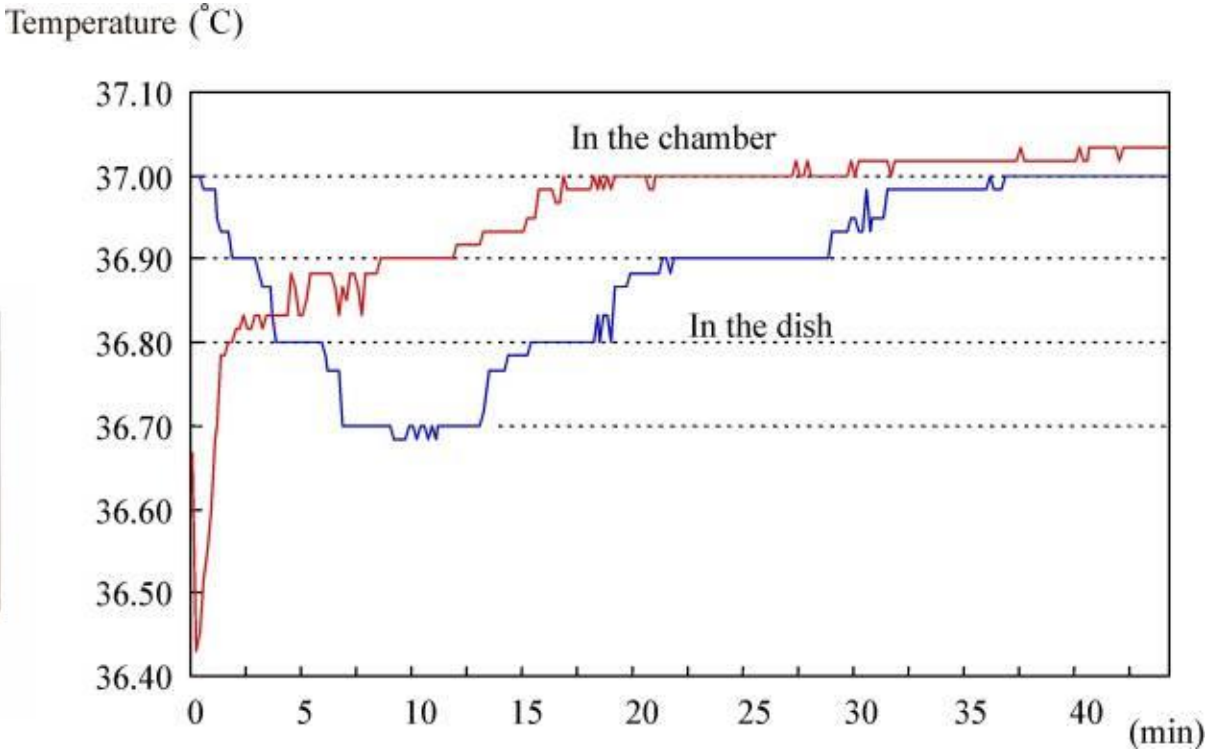


pH at point of embryo removed from incubator for 5 minutes then returned

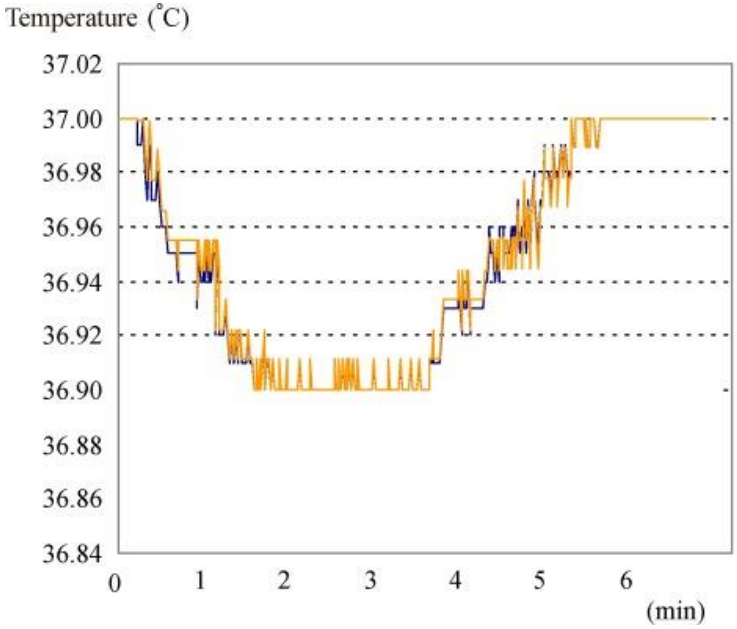
Different volumes of oil overlay modulate rate of change (60mm Petri)



Temperature recovery of 50 litre box-type incubator

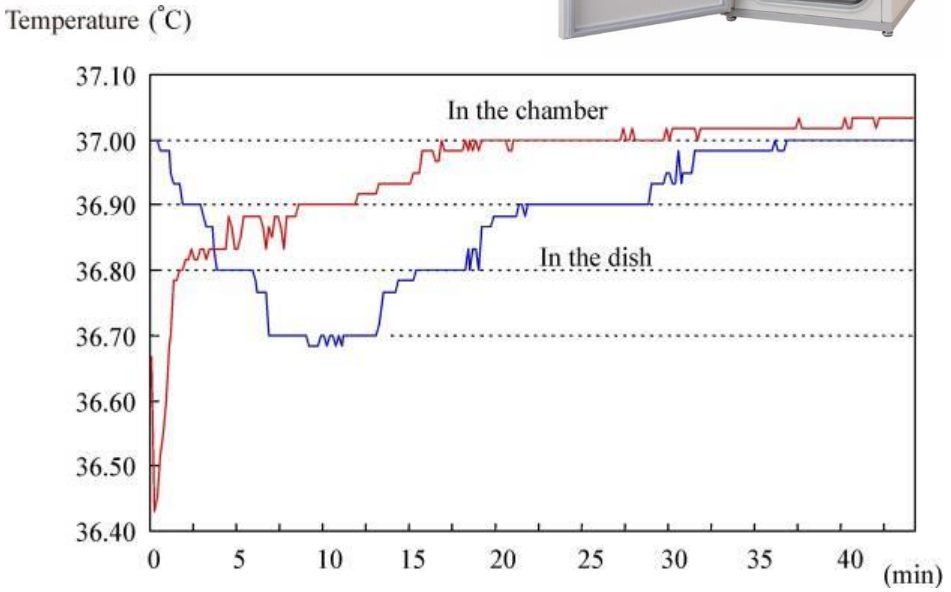


Recovery time



In the dish

In the chamber



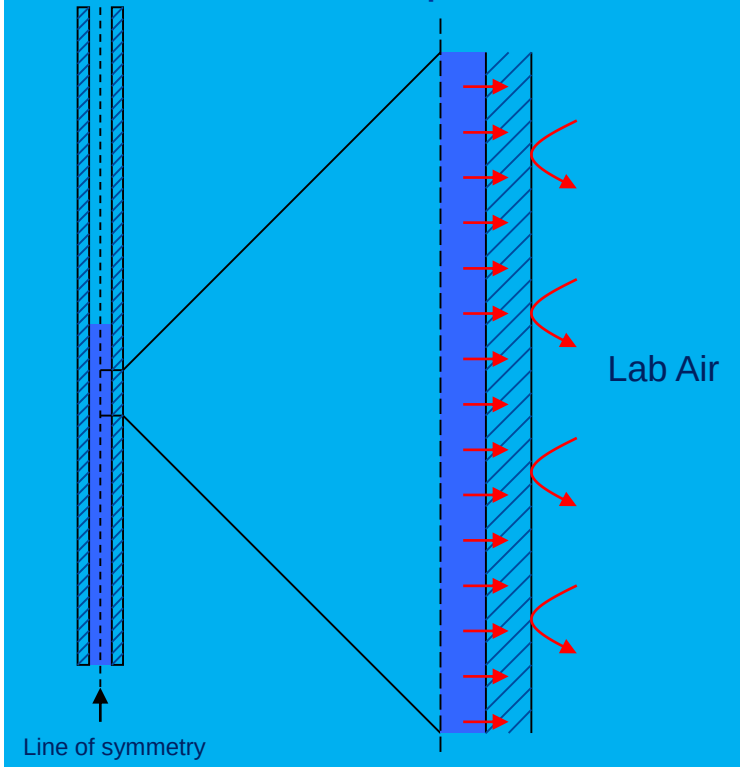
Embryo formation rate and blastocyst formation rate

	Top-load mini-incubator	Front-load conventional incubator
Early-stage embryo formation rate (%) (good embryos/Fertilized eggs)	40.3* (75/186)	28.4 (42/148)
Blastocyst formation rate (%) (good blastocysts/total embryos)	15.1* (25/166)	7.2 (10/139)

* $_P$ <0.05 (mini-incubator vs. conventional incubator)

Manipulation of embryos outside incubators can also lead to temperature fluctuations

Heat Transfer in Pipettes



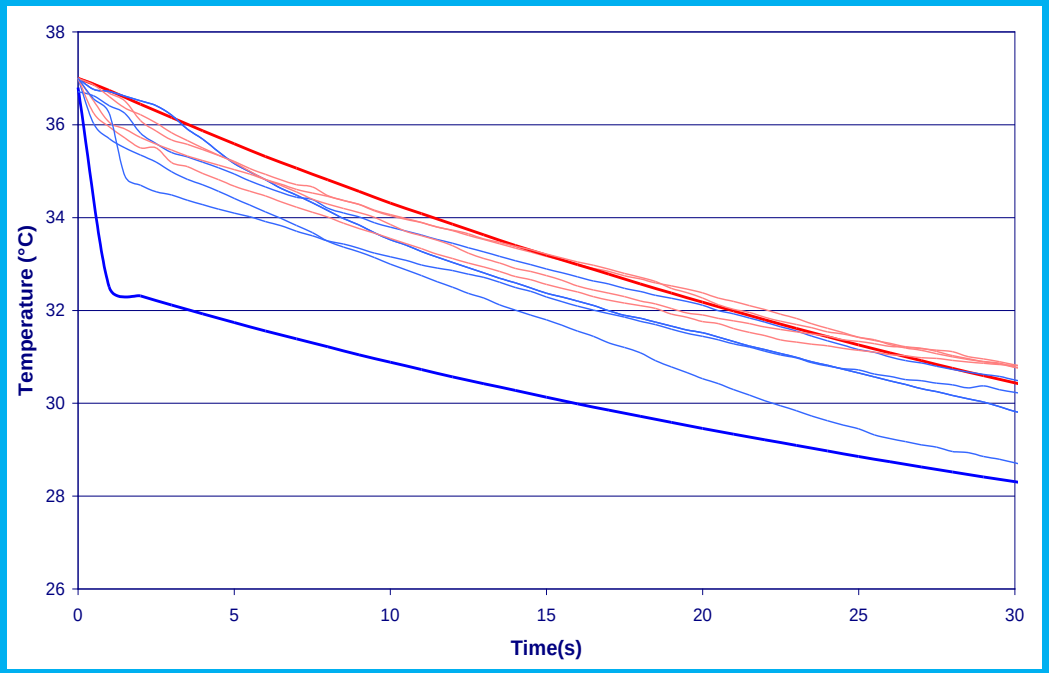
(1) Liquid (37°C) into pipette

(2) Immediately heat is lost to the walls the pipette

(3) The surface of the pipette loses heat to the surrounding air

Heat loss of a glass manipulation pipette is both rapid and significant

Heat loss in Pasteur (glass) Pipettes



The introduction of time-lapse technology to continuously monitor the development of an embryo inside the incubator substantially reduces the need to handle the embryo outside the incubator.

Widely used sequential media, which are optimised to minimise metabolic stress, still require embryos to be removed from incubation to change the media, with or without time-lapse monitoring.

UNDISTURBED CULTURE

The access to time-lapse technology has given a unique possibility to balance metabolic stress against less handling stress during embryo culture.

Benefits of time-lapse

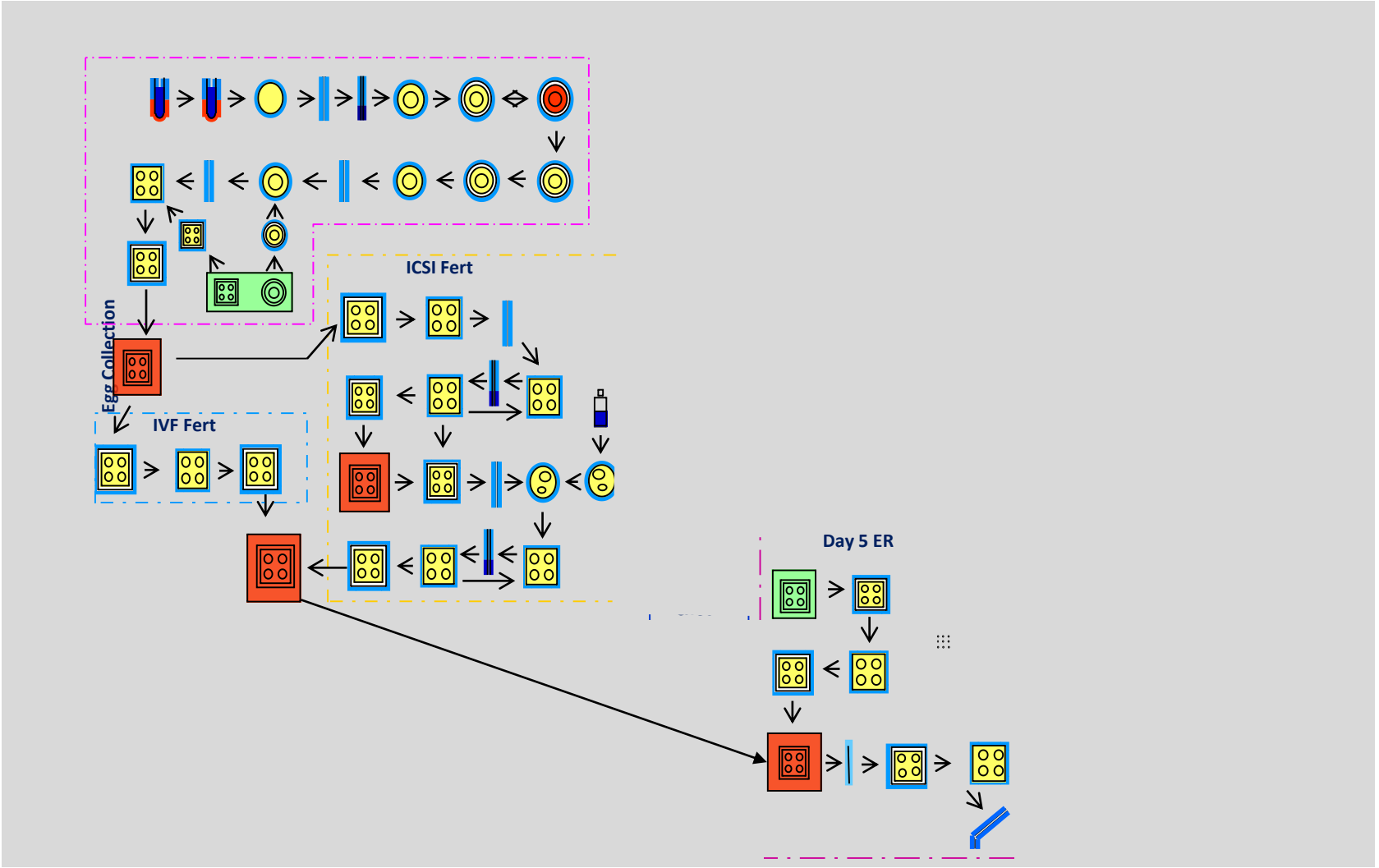
- Less handling [1]
- Embryos in a stable environment
- Supports better embryo development [2,3]
- Less light exposure than standard evaluation [4]

Requirements on culture media

- Avoiding metabolic stress without renewal
- Stable formulation for 5-6 days in 37ºC
- Performance comparable to sequential media
- Function just as good with other G-Series media

1. Zhang et al (2010); Reprod Biomed Online 20 (4):510-515 2. Rubio et al (2014); Fertil Steril 102 (5):1287-1294 3. Kahraman et al (2013); J Reprod Stem Cell Biotechnol;3:55-61 4. Li et al (2014); J Assist Reprod Genet 31 (7):795-801

Embryo Culture Process

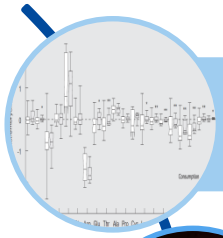


Challenges with development of a non-sequential medium for extended culture

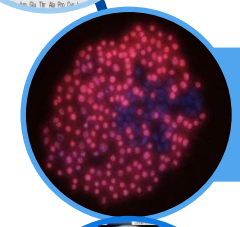
During the development of a single culture medium for blastocyst culture, specific attention has to be paid to the composition, concentration and utilisation of specific nutrients, as well as the build-up of metabolites since no media is added or removed.

Special focus has to be placed on energy substrates like lactate, pyruvate and glucose, as well as the amino acid composition since these are among the major sources for ammonium formation in a medium.

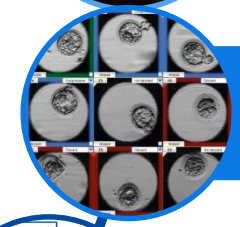
DEVELOPMENT BY THE BOOK



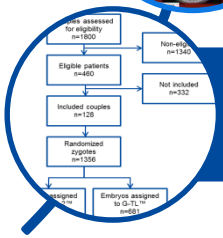
Design Phase



Pre-clinical animal studies

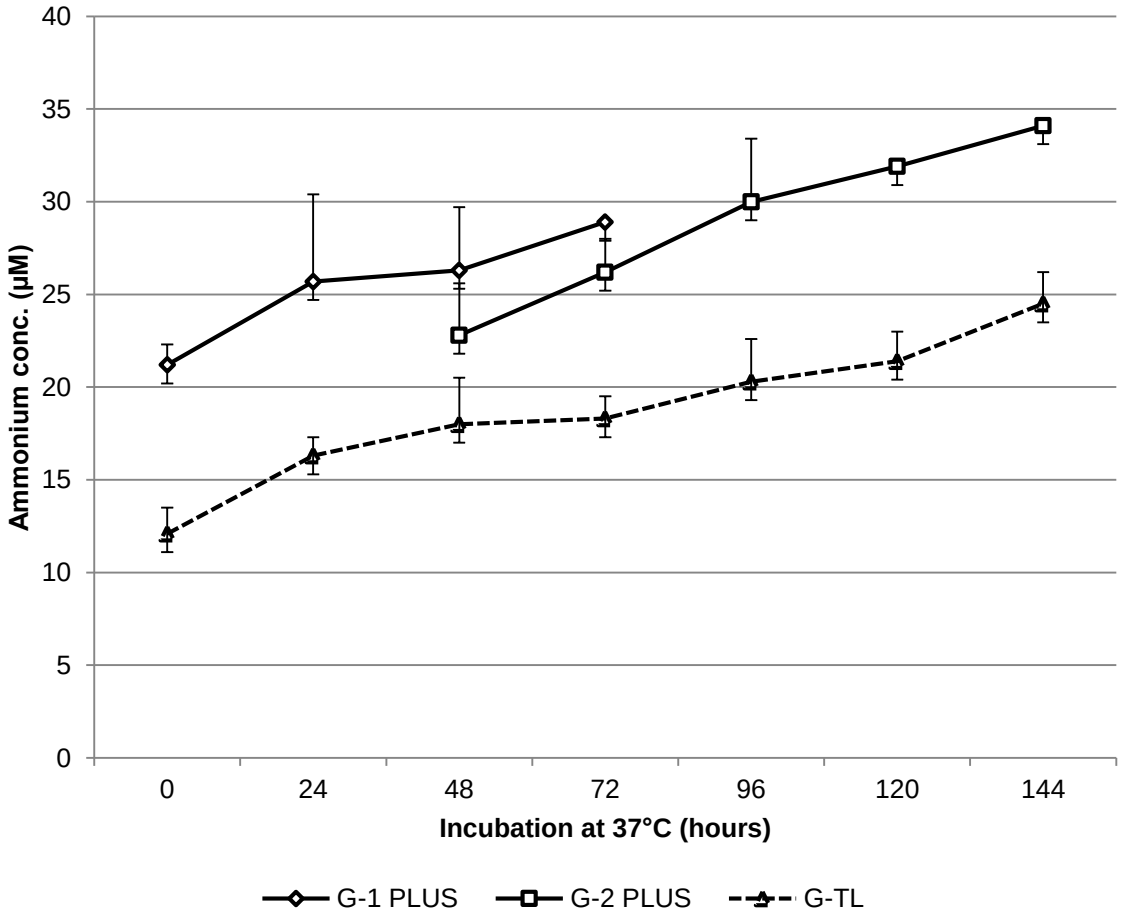


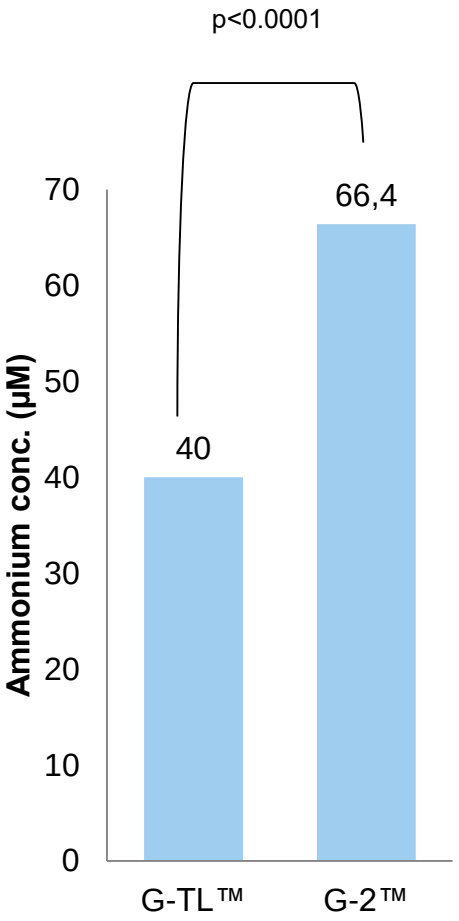
Pre-clinical human studies



Randomized clinical multicenter trial

AMMONIUM MEASUREMENTS



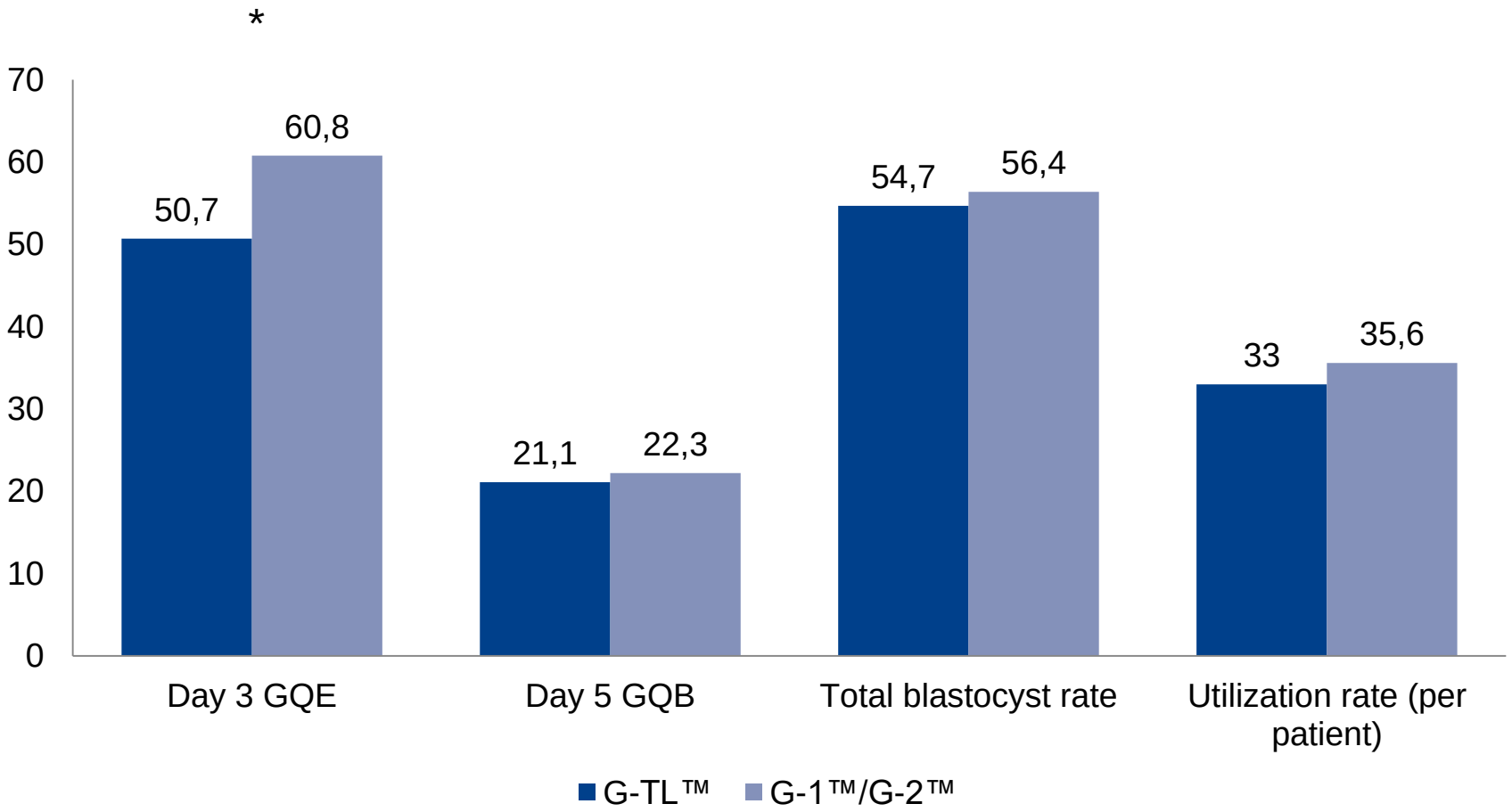


Hardarson, et al. A non-inferiority, prospective, randomized controlled trial comparing embryo development .Fertility and Sterility In press 2015.

Vitrolife

EMBRYO DEVELOPMENT (MULTI-CENTRE RCT)

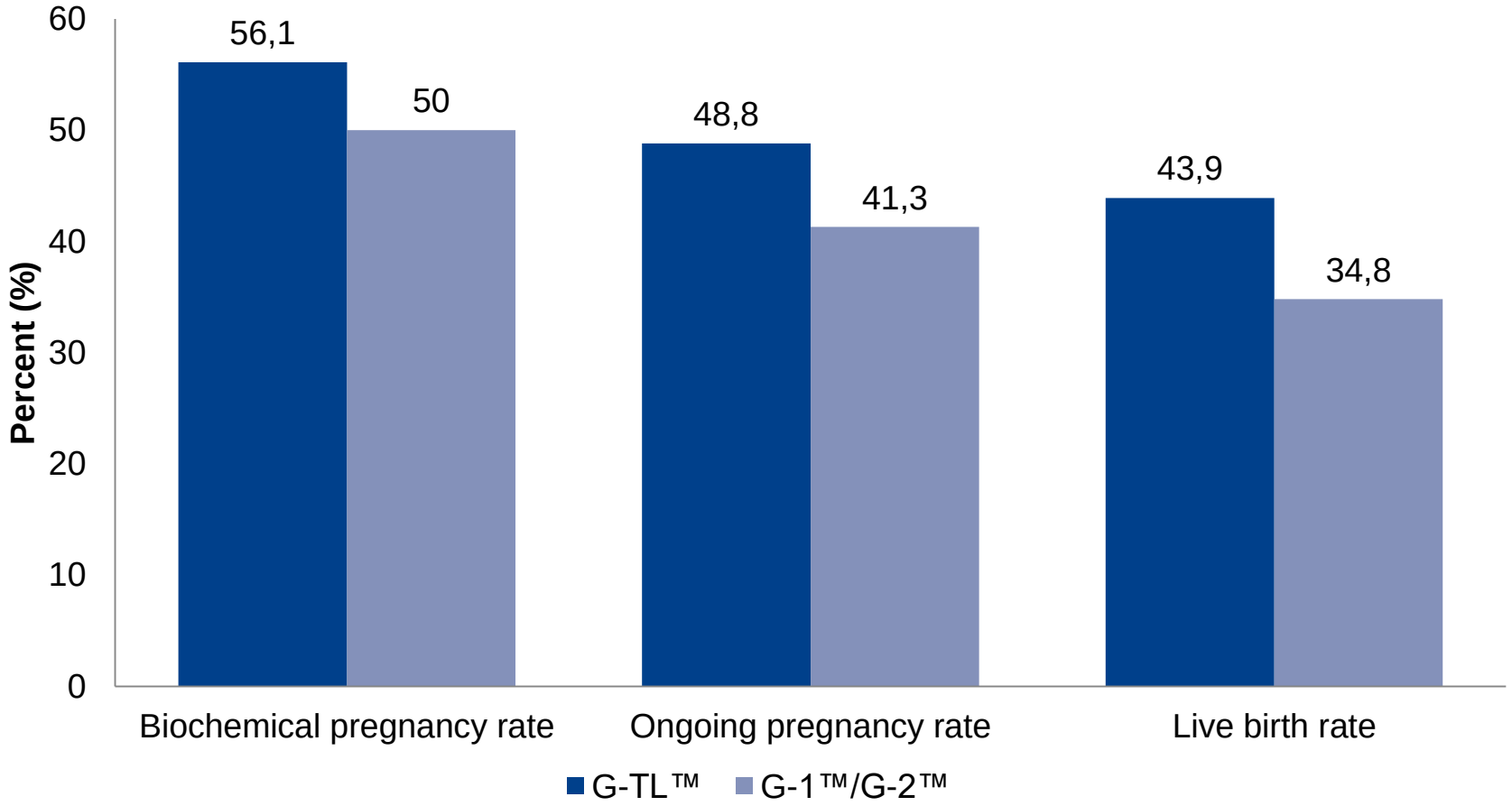
Calculated from 2PN stage and scored according to ALPHA/ESHRE consensus scoring system



Hardarson, et al. A non-inferiority, prospective, randomized controlled trial comparing embryo development. Fertility and Sterility In press 2015. *p<0.0005

Vitrolife

CLINICAL OUTCOME (86% SINGLE EMBRYO TRANSFERS)



Hardarson, et. al. A non-inferiority, prospective, randomized controlled trial comparing embryo development. Fertility and Sterility In press 2015

Vitrolife

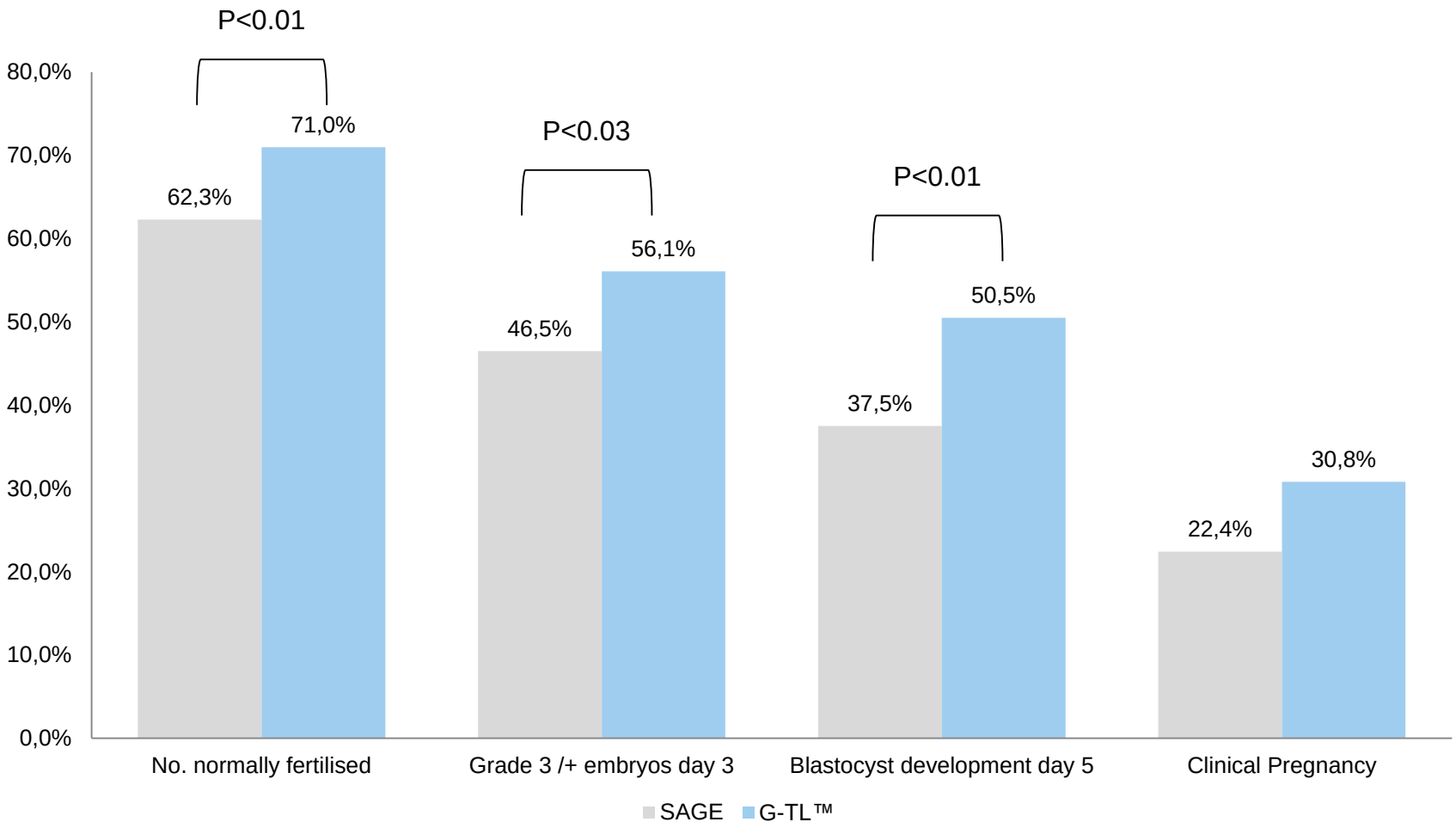
CONCLUSIONS FROM RANDOMIZED CLINICAL MULTICENTER TRIAL

- G-TL and G-1/G-2 are equivalent in relation to blastocyst quality and rate
- Ammonium levels for G-TL are low in continuous and extended culture
- Functions well in a time-lapse culture setting

Vitrolife

Using G-TL in standard incubators

IMPROVEMENTS IN EMBRYO DEVELOPMENT



Shaw L.A., Leary, C., Maguiness S.D, Robinson J, A retrospective comparison, The 9th conference of the Association of Clinical Embryologists, U.K., 2015

Vitrolife

WORK FLOW AND COSTS

Practical and financial incentives for the clinical laboratory for standard embryo culture

Less work for the embryologists

- Single dish set-up for D1-D5 culture

Consumable cost reductions

- Fewer culture dishes
- Less oil
- Fewer transfer pipettes
- Fewer seriological pipettes
- Fewer micro-pipette tips

SUMMARY

- Developed by the book for a safe introduction into clinical use
- Excellent results compared with sequential in a time-lapse setting
- Functions well with other G-Series media
- Formulation optimized for undisturbed culture:
  - Amino acids and energy substrates tuned to embryo needs
  - Low ammonium build-up, with and without embryos
  - Stable and consistent

Vitrolife

TOGETHER. ALL THE WAY™