Simplifying the IVF procedure: Is it possible?

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Origin of Simplified Culture System

Walking Egg Program---Willem Ombelet, Genk Belgium

WHO—Women's Reproductive Rights

Challenge: Could ART/IVF be effectively introduced into developing countries where much needed but were resource and infrastructure limited?

Primary Goals of IVF Simplification for Low Resource Settings

- Accessibility and affordability
- Reduce costs to start up programs
- Reduce potential for iatrogenic errors, need for highly trained laboratory personnel
- Minimal laboratory infrastructure consistent with successful outcomes similar to those in high resource settings

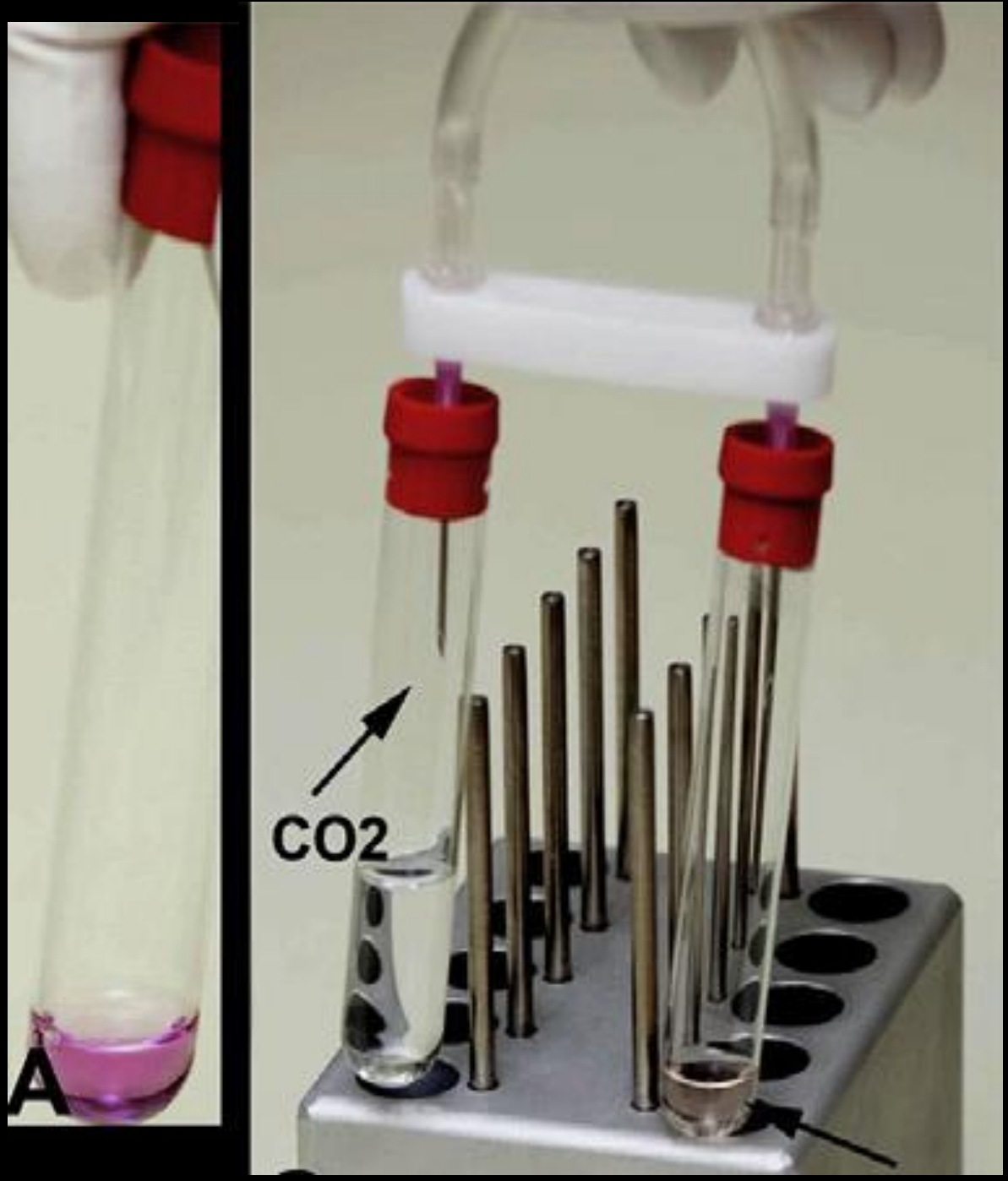
What do we know about what human oocytes and embryos need to fertilize and develop in vitro after nearly 50 years---- a great deal—more if include other species.

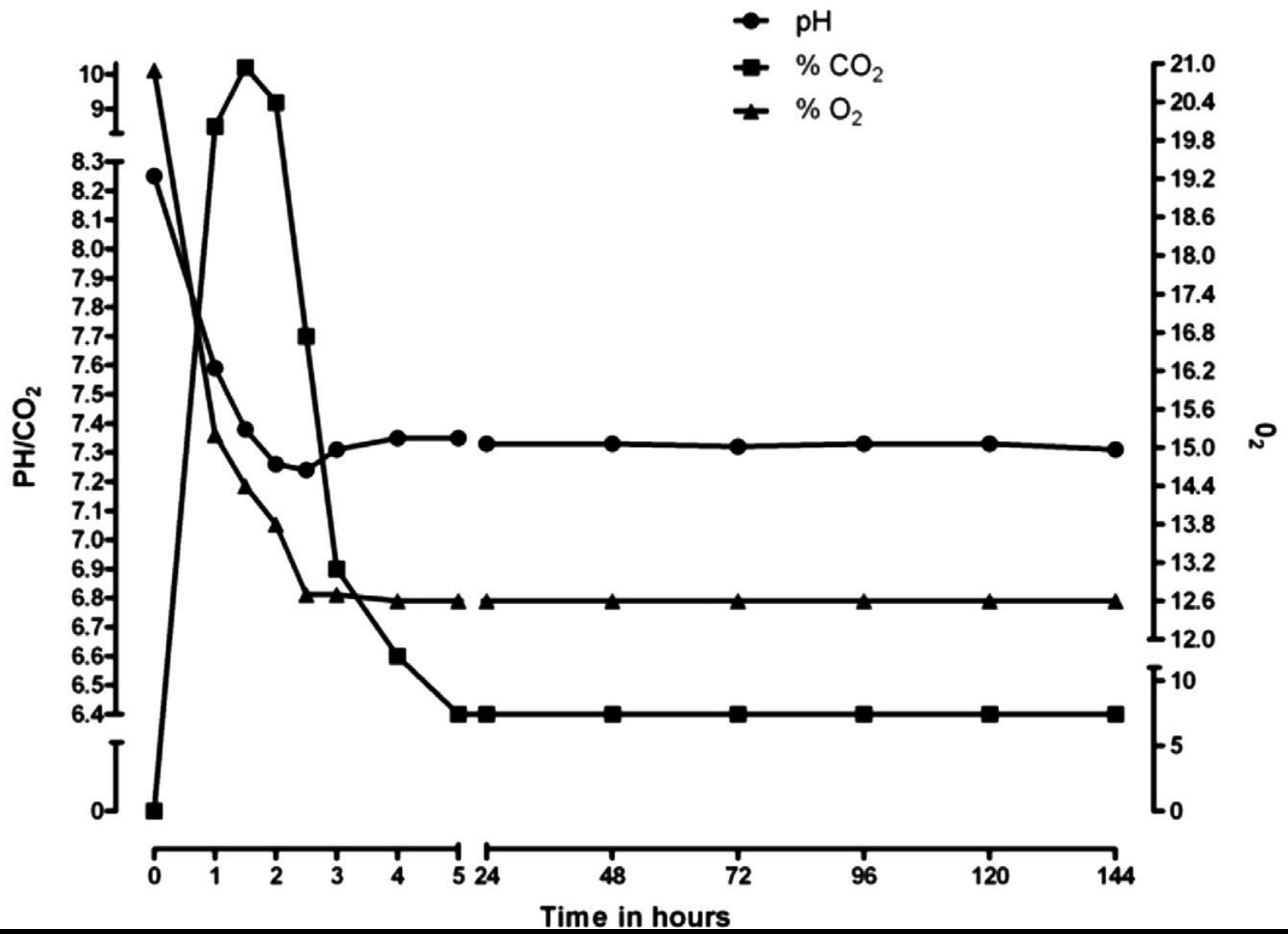
Recognize factors that determine developmental competence that are largely uncontrollable (aneuploidy)

With this knowledge, IVF can be reduced to its essentials: media, pH, temperature and to be largely left undisturbed.

The Simplified IVF Culture System

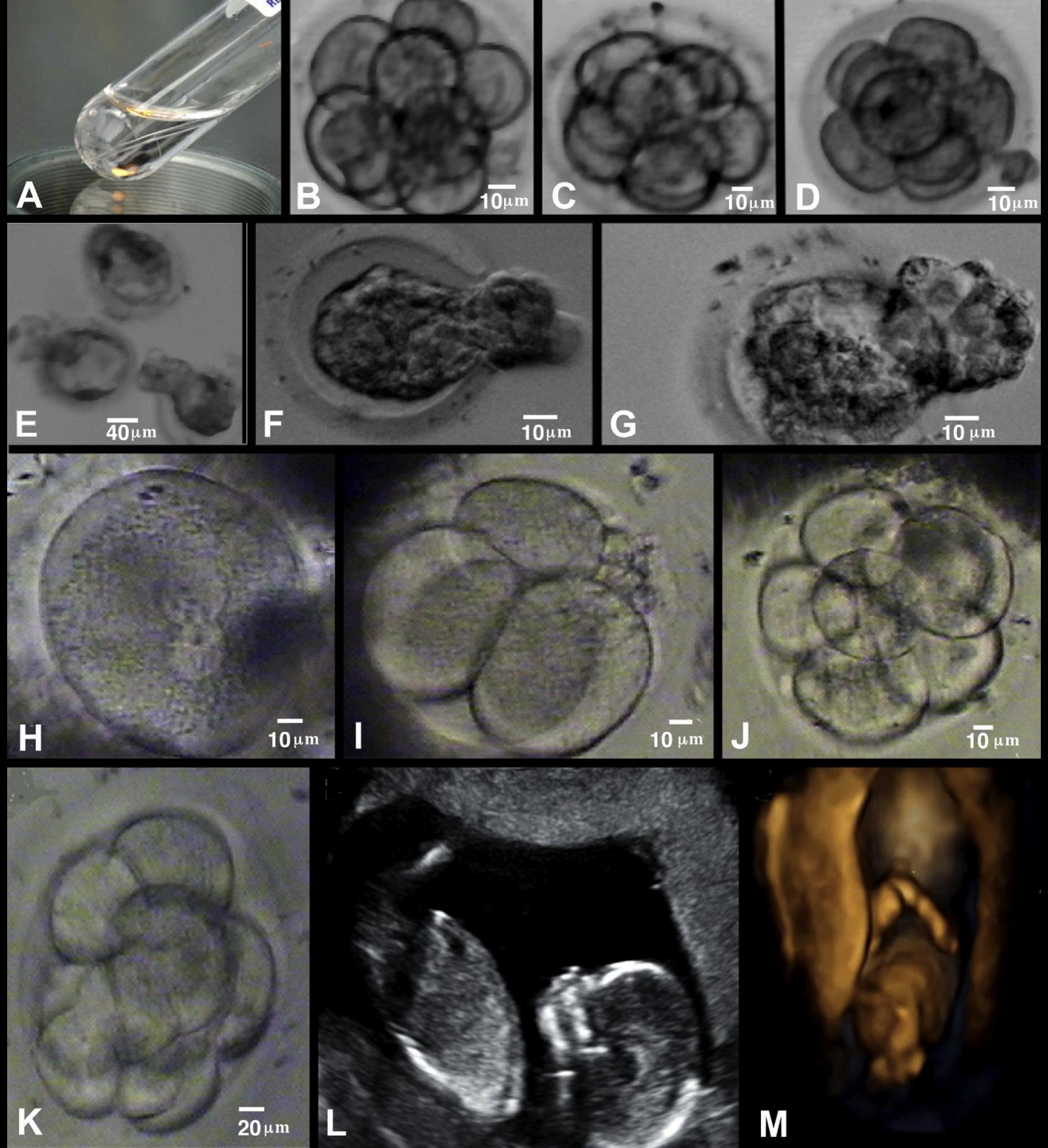
Van Blerkom et al, 2014, RBMO




pH
% CO_2
% O_2
PH/CO_2
O_2
10
9
8.3
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0
6.9
6.8
6.7
6.6
6.5
6.4
0
21.0
20.4
19.8
19.2
18.6
18.0
17.4
16.8
16.2
15.6
15.0
14.4
13.8
13.2
12.6
12.0
10
5
0
0 1 2 3 4 5 24 48 72 96 120 144
Time in hours

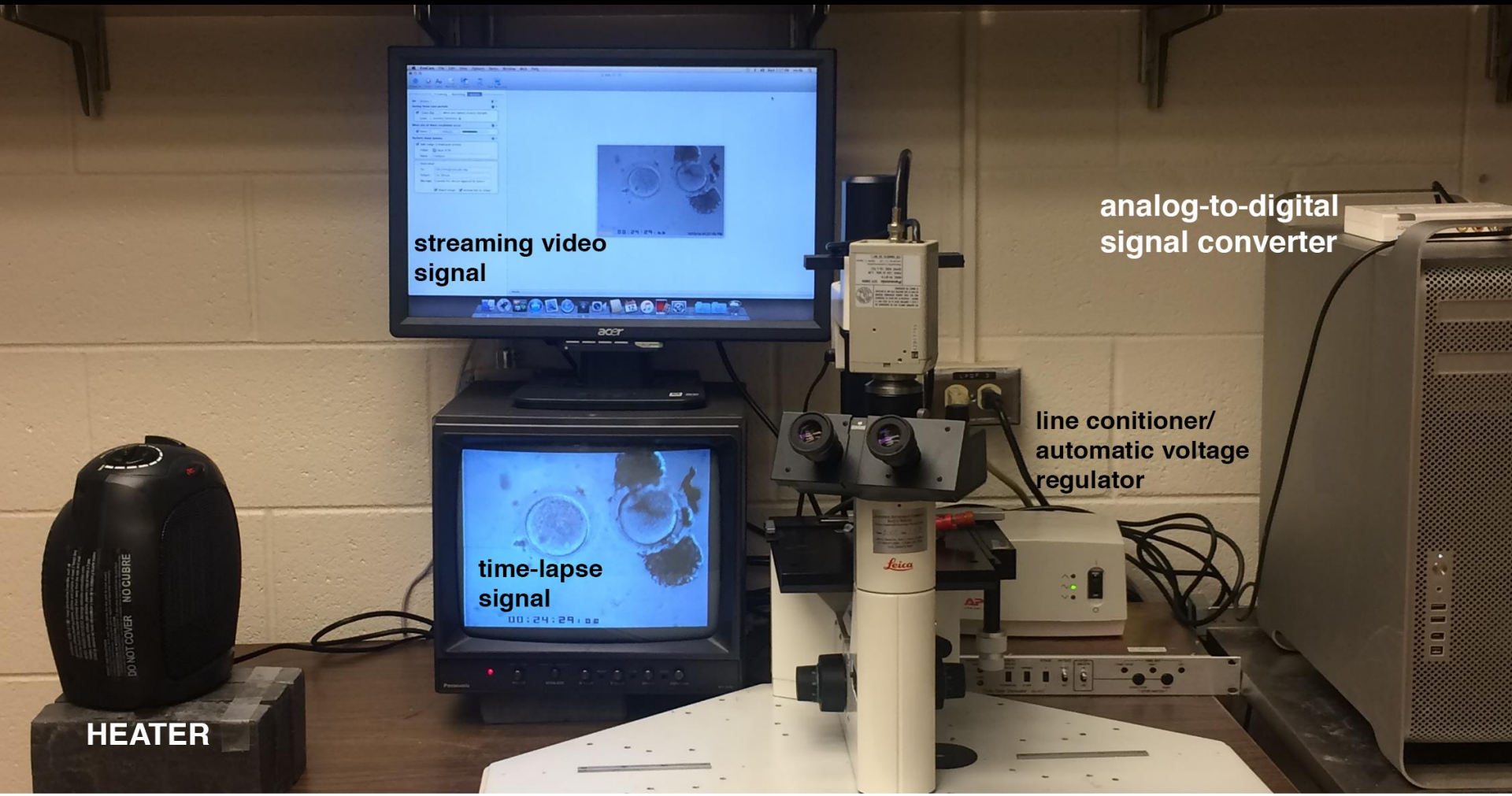
F

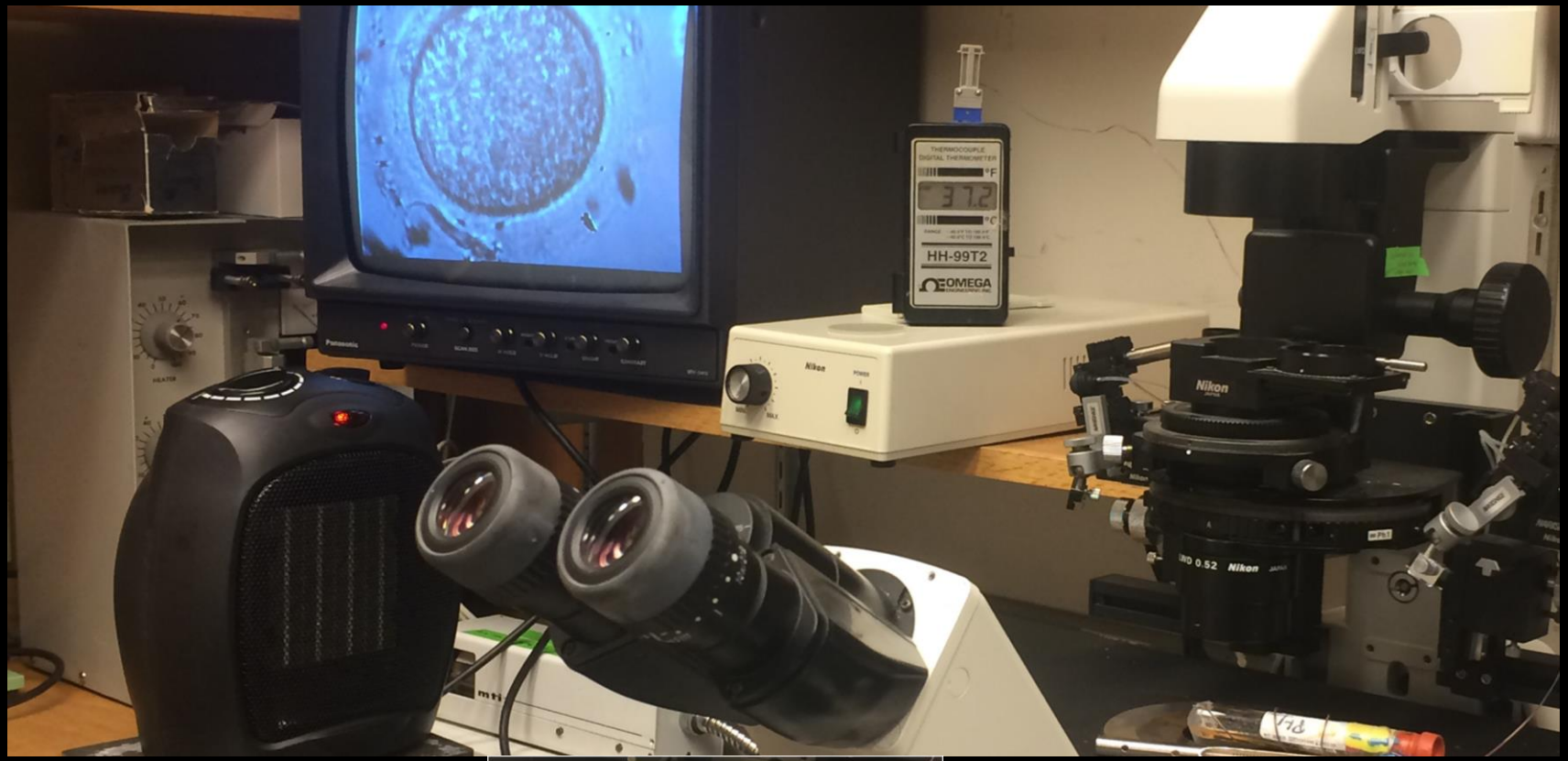

Van Blerkom et al, 2014, RBMO


A
B
10μm
C
10μm
D
10μm
E
40μm
F
10μm
G
10 μm
H
10μm
I
10 μm
J
10 μm
K
20μm
L
M

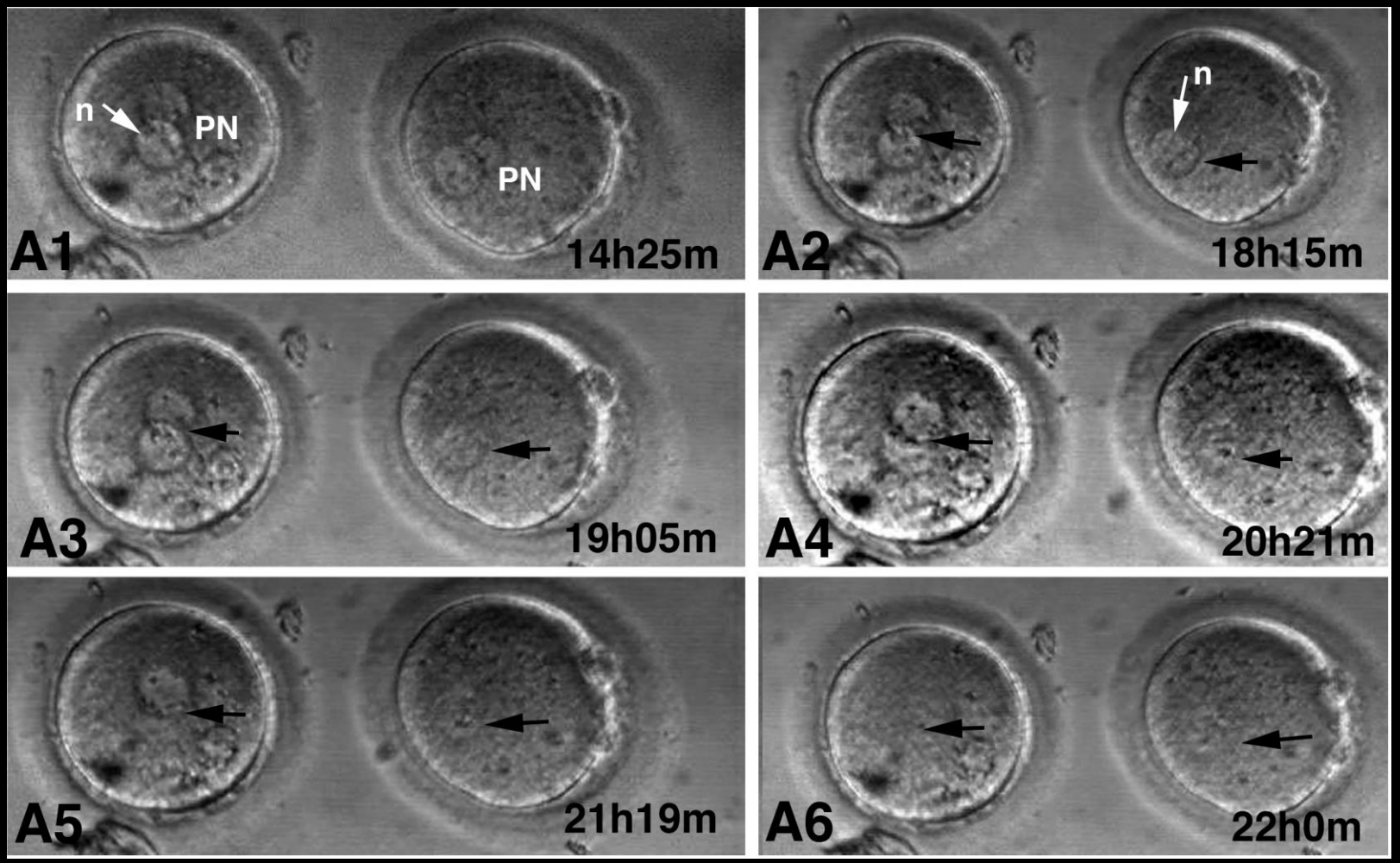
But wait----there's more!

The Addition of A Fully Functional Low Cost, Simplified Time Lapse and Video Streaming Capacity

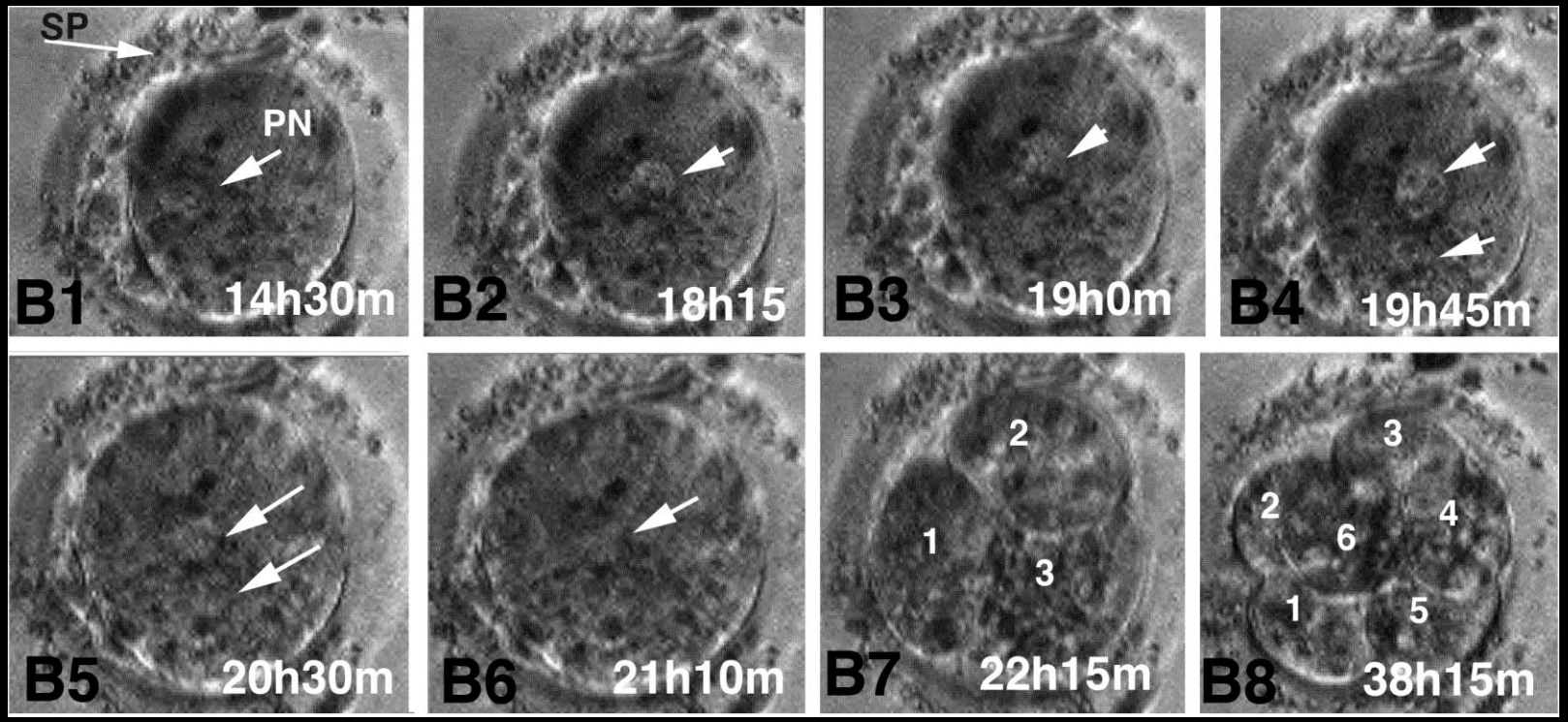

streaming video signal
analog-to-digital signal converter
line conitioner/ automatic voltage regulator
time-lapse signal
HEATER


THERMOCOUPLE
DIGITAL THERMOMETER
°F
37.2
°C
HH-99T2
OMEGA
Panasonic
Nikon
Nikon
Nikon

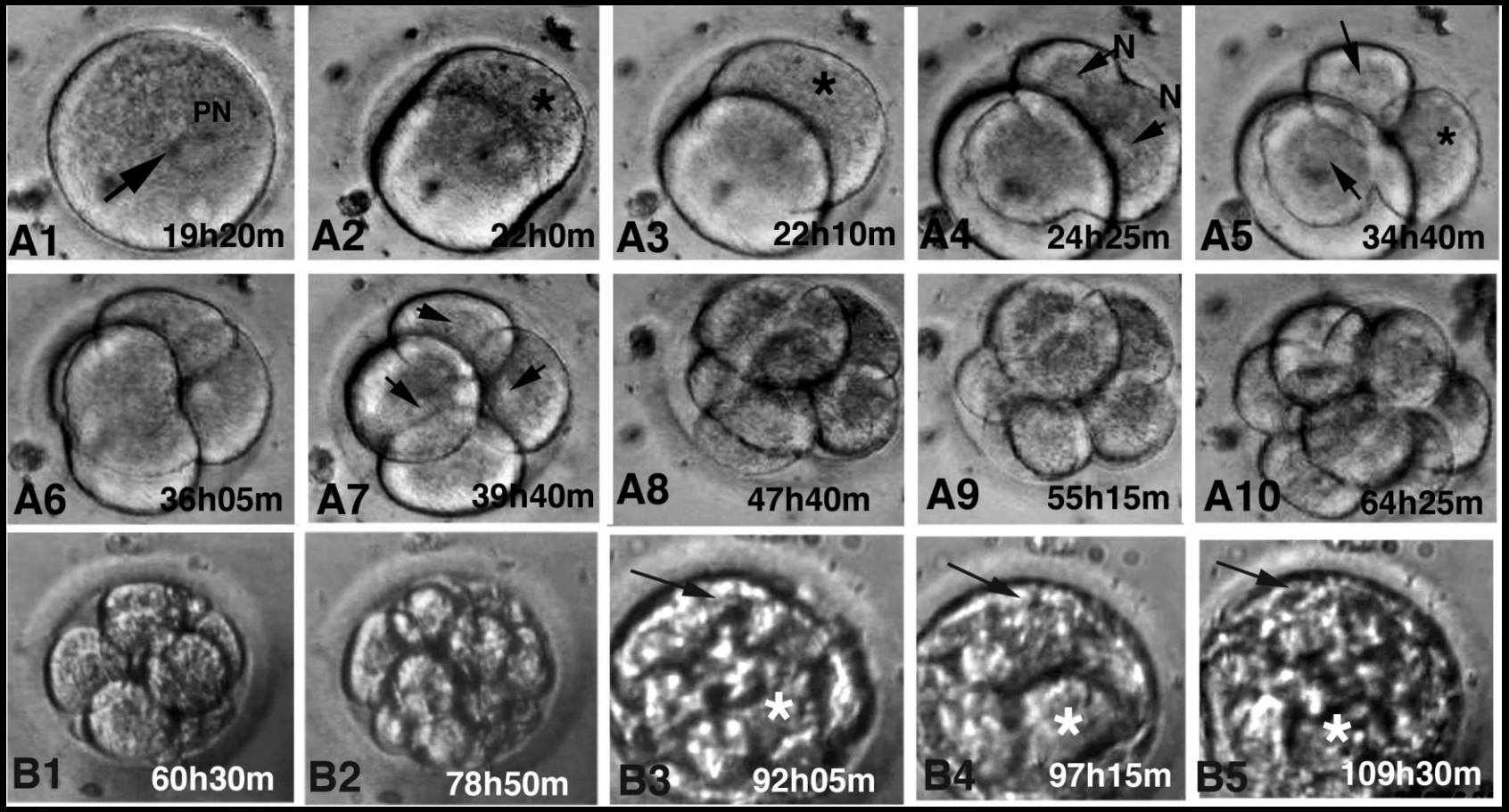
Normal Pronuclear Formation and Breakdown



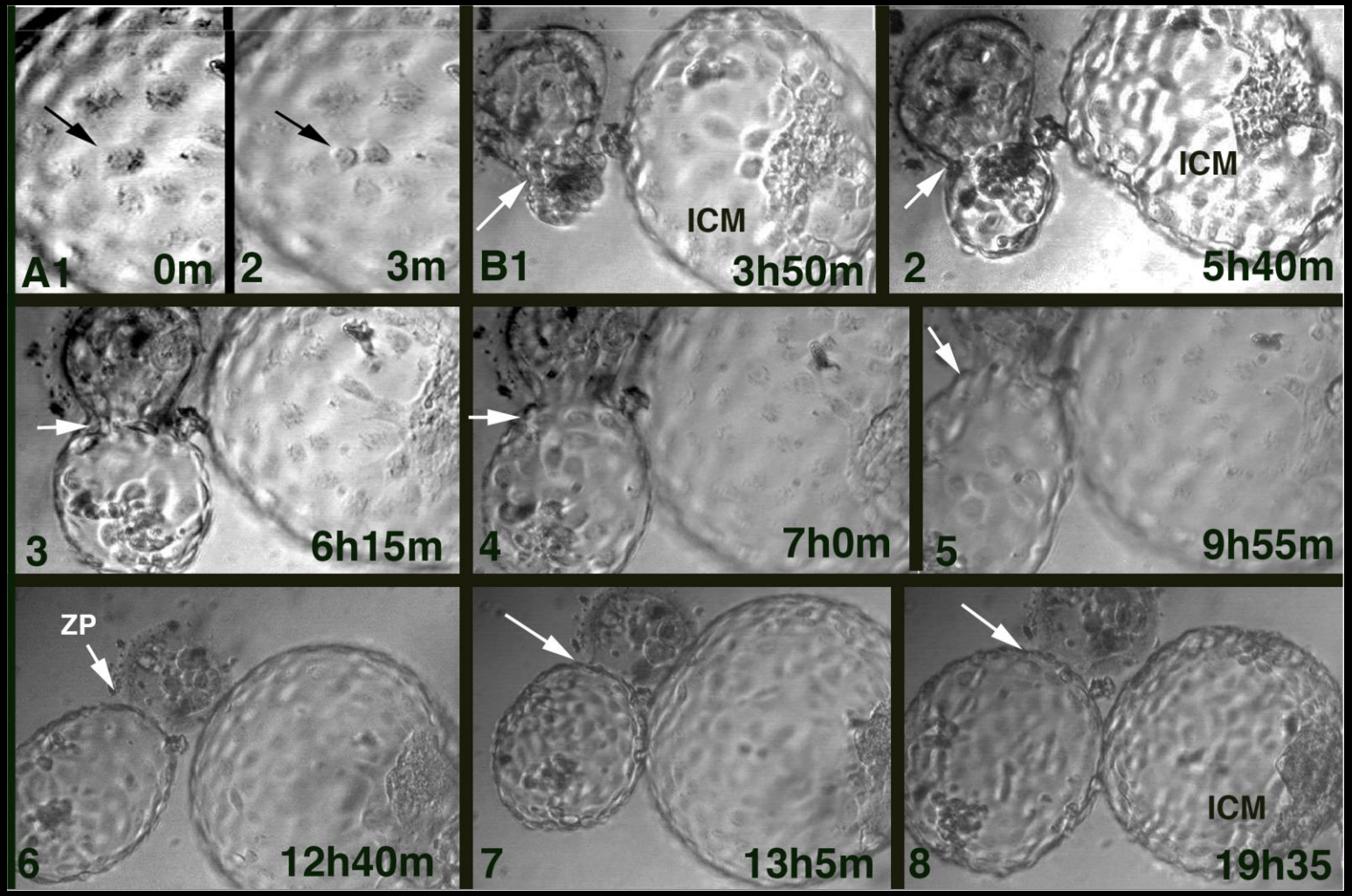
Abnormal First Cleavage Division



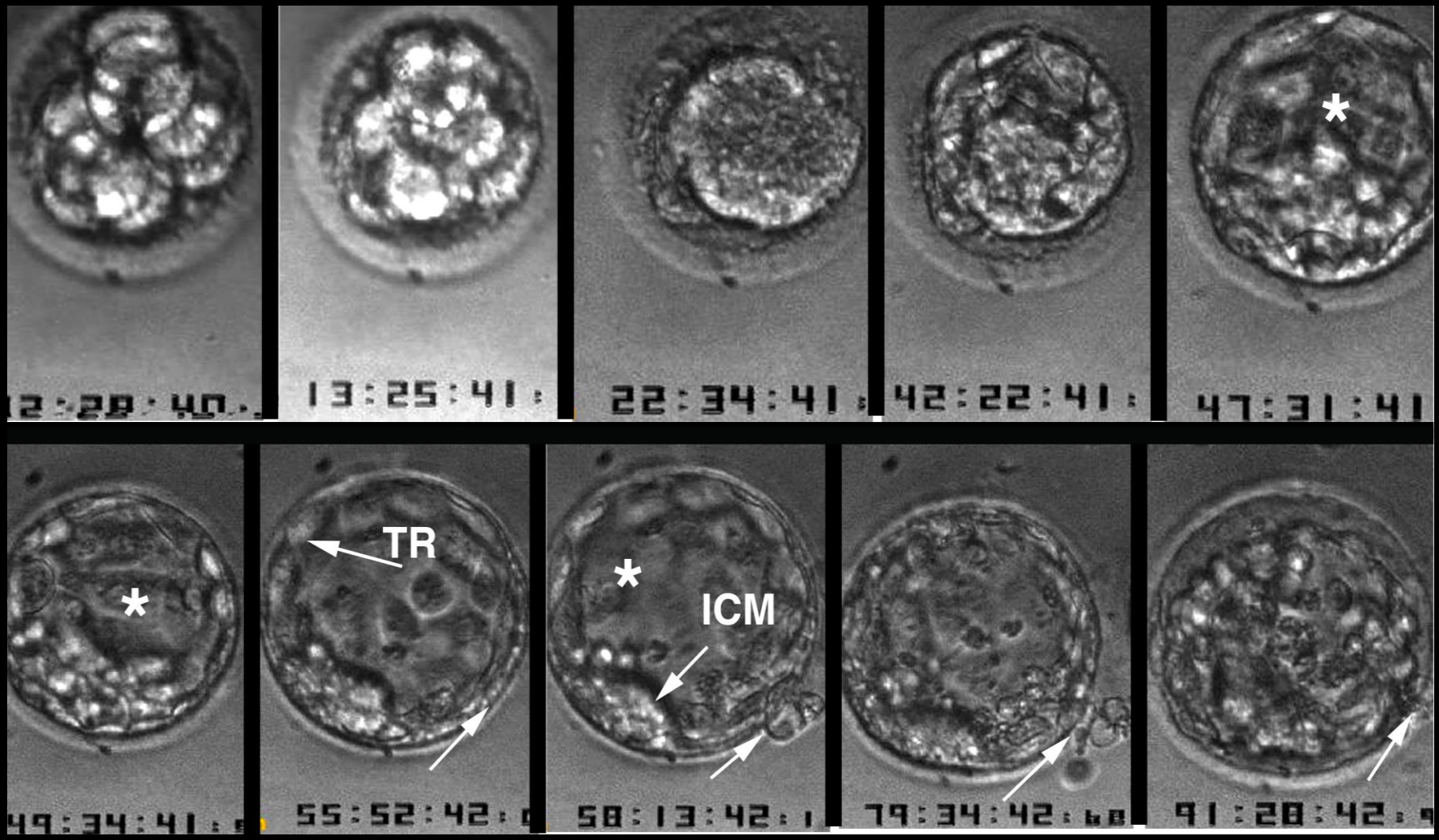
Abnormal Second Cleavage Division



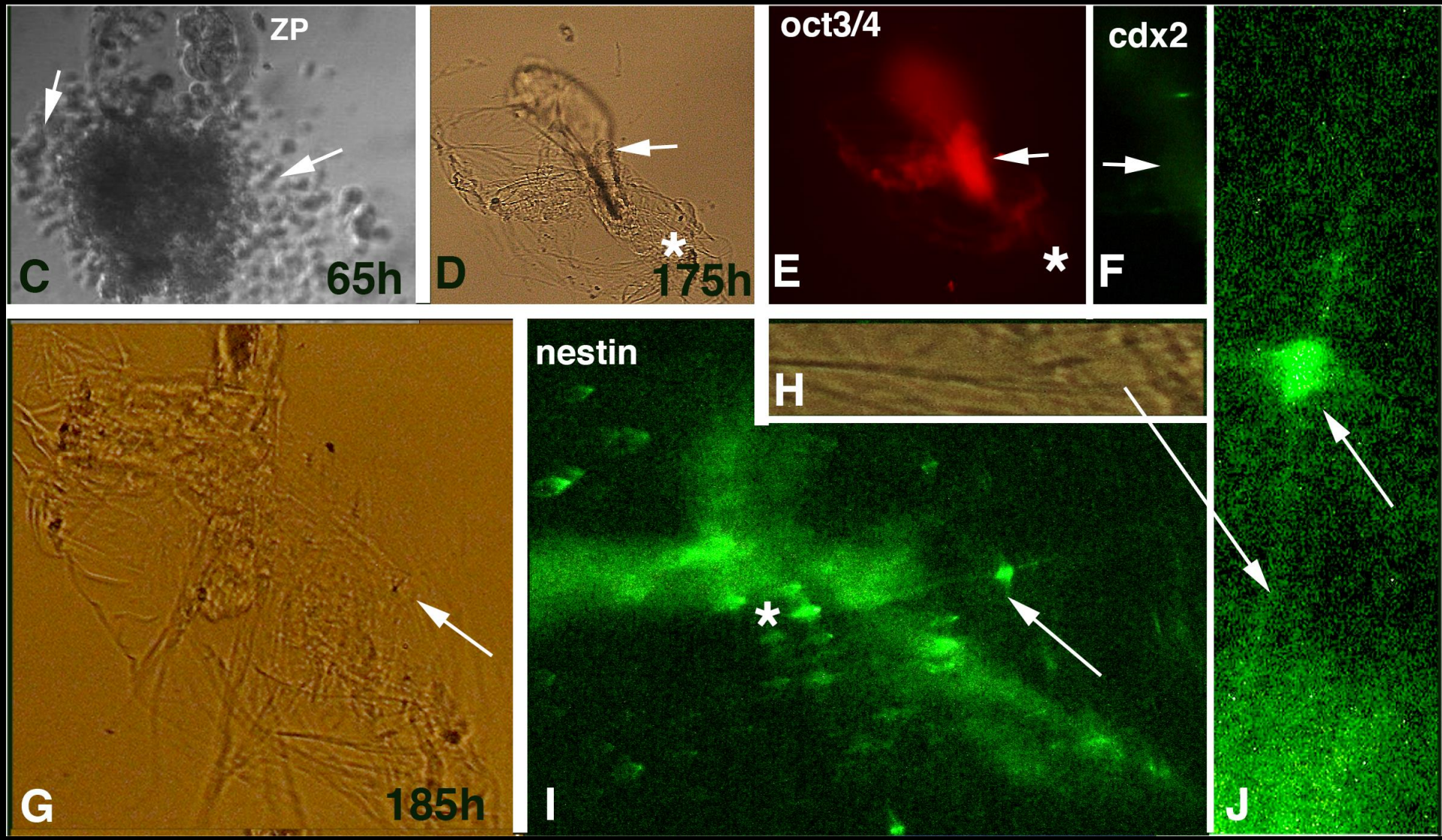
Normal Blastocyst Hatching



Failed Hatching



Spontaneous Post-Hatching "Differentiation" —putative nestin*-positive neurite growth



*neuroectodermal stem cell marker– neural cell —specific -intermediate filament protein"

Clinical Applications

- **Training and remote viewing for simultaneous video and voice discussions for quality control, selection/deselection, research opportunities.**

- **Detection of cytokinetic abnormalities likely contributing to in aneuploidy that may be missed in static observations if occurring at in opportune times post-insemination or fertilization**

Basic Research Applications

- Sampling of media for secreted bioactive molecules that may be viability markers
- Visualizing effects of ‘activators’ (e.g., calcium ionophores) or metabolic inhibitors (e.g., FCCP) or sperm
- Studying molecular and cellular aspects of post hatching development and putative hES cell formation and differentiation
- Effects of CRISPR/Cas 9 gene editing at earliest stages of embryogenesis to post hatching-day 14?

Current Directions

- **Charging vacutainers/culture tubes with precise pre-mixed atmosphere that may be required for culture of embryos from different species. Atmosphere lasts indefinitely in both self- generating and premixed instances. Lower O2 concentrations (2-4% currently being tested).**
- **For bovine: IVM-IVF-development to blastocyst in same culture tube with transport to recipients in thermos for ET—with embryos withdrawn directly from tube. Same for embryos thawed at central facility but but transported to recipients at some distance.**

Auto-contrast and focusing with simultaneous image processing to maximize resolution and image quality in real time for recording and streaming.

Acknowledgements

- Christine Hennigan
- Sarah Zimmerman

Normal Hatching

Failed Hatching