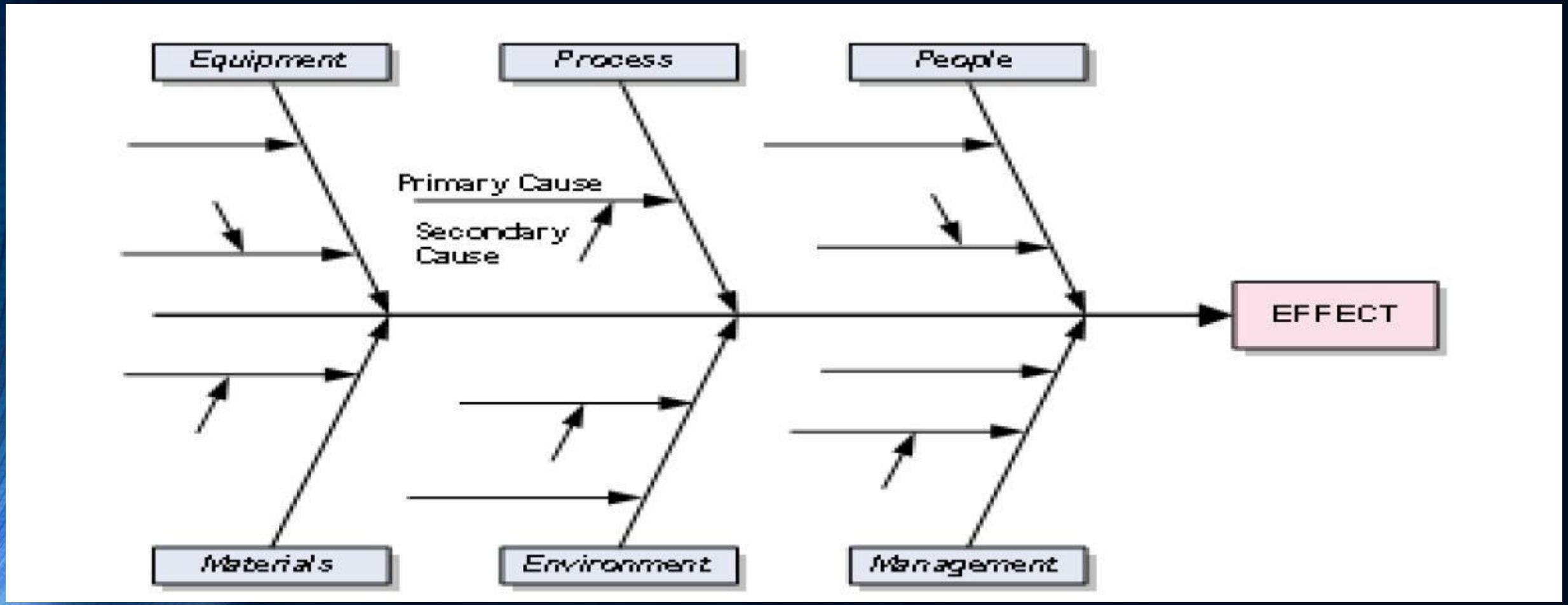
Troubleshooting - Cryopreservation

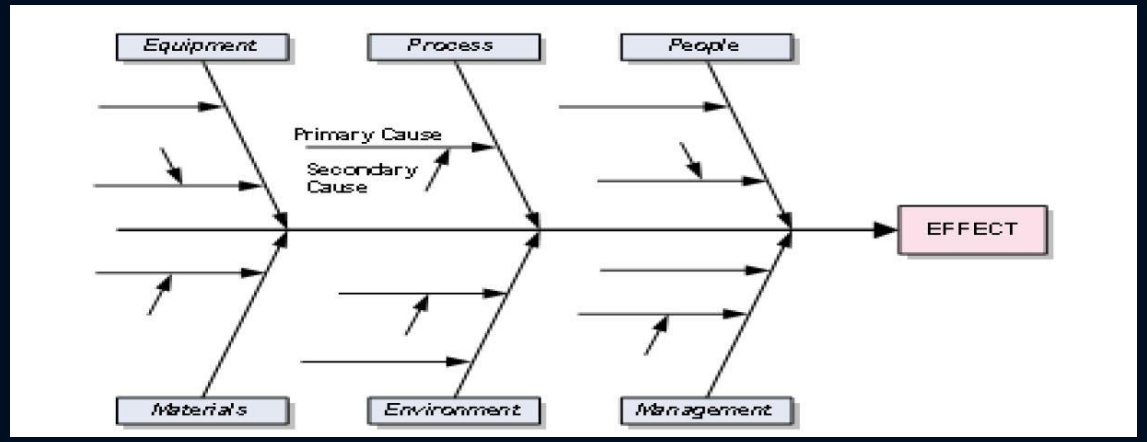
BY KIMBALL O POMEROY, PHD, HCLD
SCIENCE DIRECTOR
THE WORLD EGG BANK

Lab Work Is Like A Rollercoaster

What Has Contributed to My Problem?

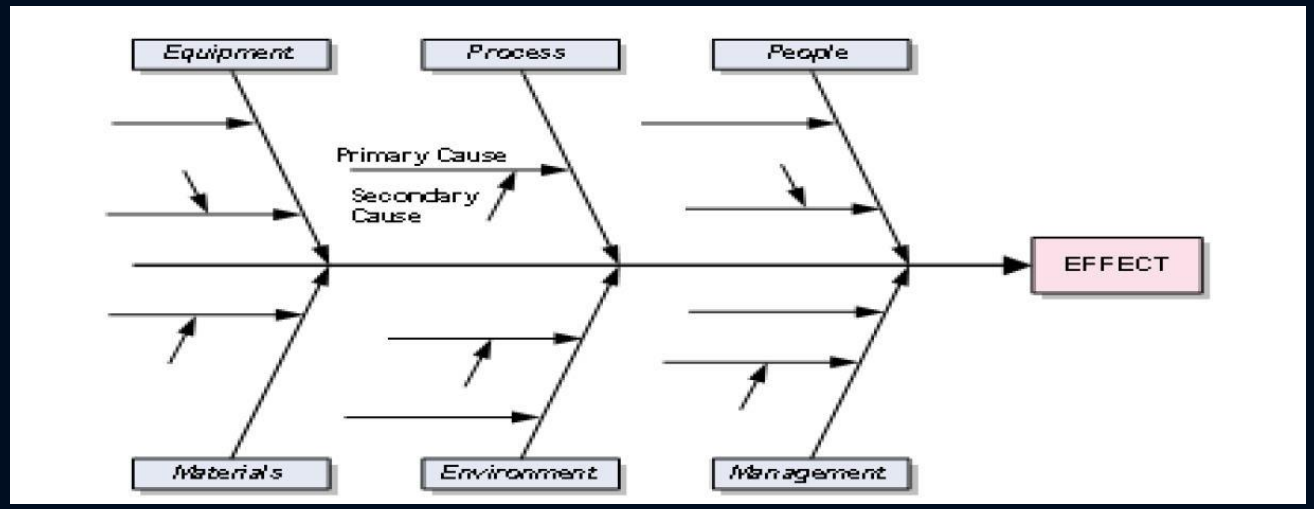


Factors



- Extrinsic
  - Manufacturer changed product without notifying us.
  - Large forest fire in our area.
  - Suite next door decides to renovate with carpeting and painting.
  - Oil is toxic and kills embryos.

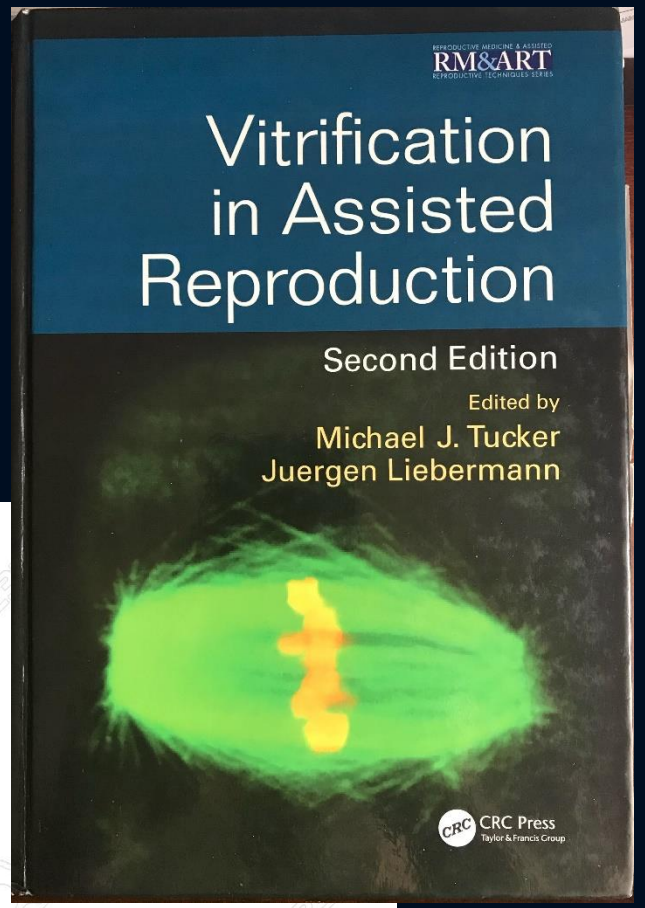
Factors



- Intrinsic
  - Protocol drift
  - Failure to check the levels of liquid nitrogen in storage tanks frequently (or not turn alarm back on)
  - Use a newer media to improve blast development but when used for fertilization, fertilization rates decrease due to lower glucose levels.

Troubleshooting

- Understand the Science Behind the Process
- Define the Process – Where to Look First
- Define Exactly What the Problem Is
- Collect Data
- Design Experiments



The Theory of Vitrification (Ultra Rapid Cooling)

- Osmotic Damage – Moving Water or Vitrificants Too Rapidly
- Toxic Damage – Vitrificants – Time and Temperature

The Theory of Vitrification (Ultra Rapid Cooling)

- Liquid to Glass State – No Intracellular Ice Crystals
- Warming Glass to Liquid Avoid Recrystalization
- High Concentration of Vitrificants
- High Cooling and Warming Rates
- Small mass to Cool or Warm
- Warming Rate More Critical

The Process

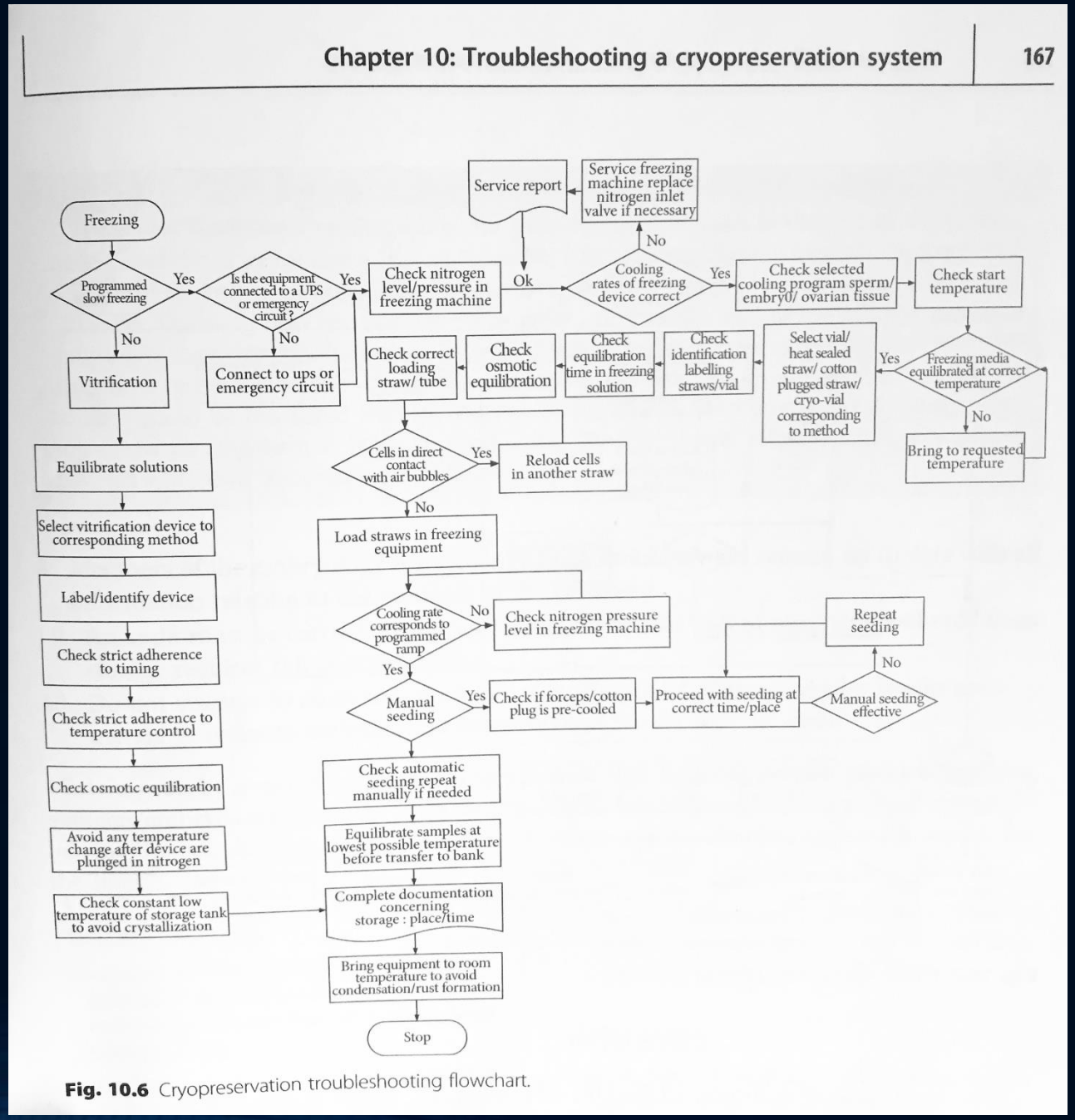
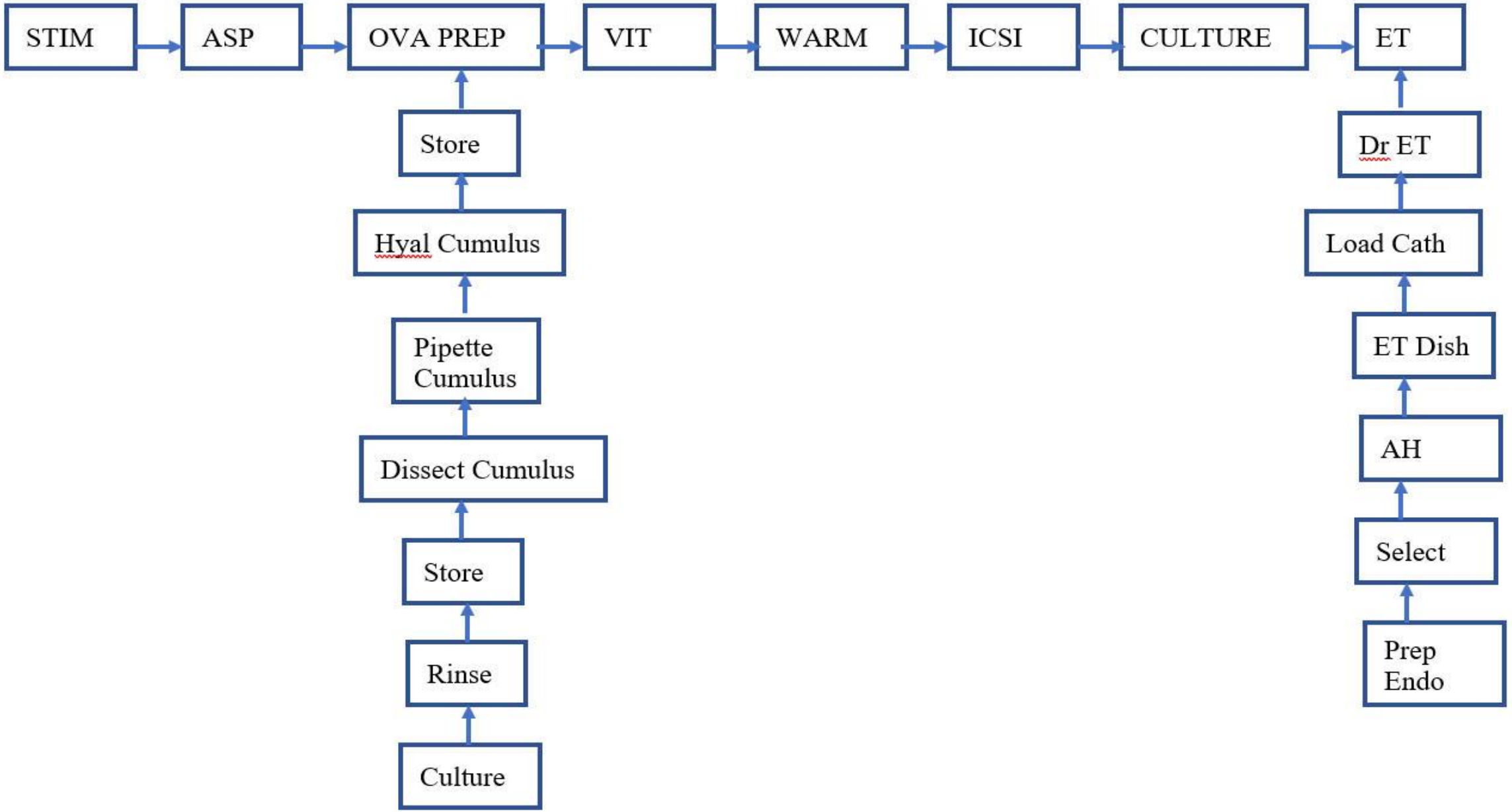


Fig. 10.6 Cryopreservation troubleshooting flowchart.

Process for Troubleshooting Poor PG Rates Using Vitrified Donor Ova



Example of Troubleshooting a Problem With Vitrification - Embryologist had no survival of two blastocysts

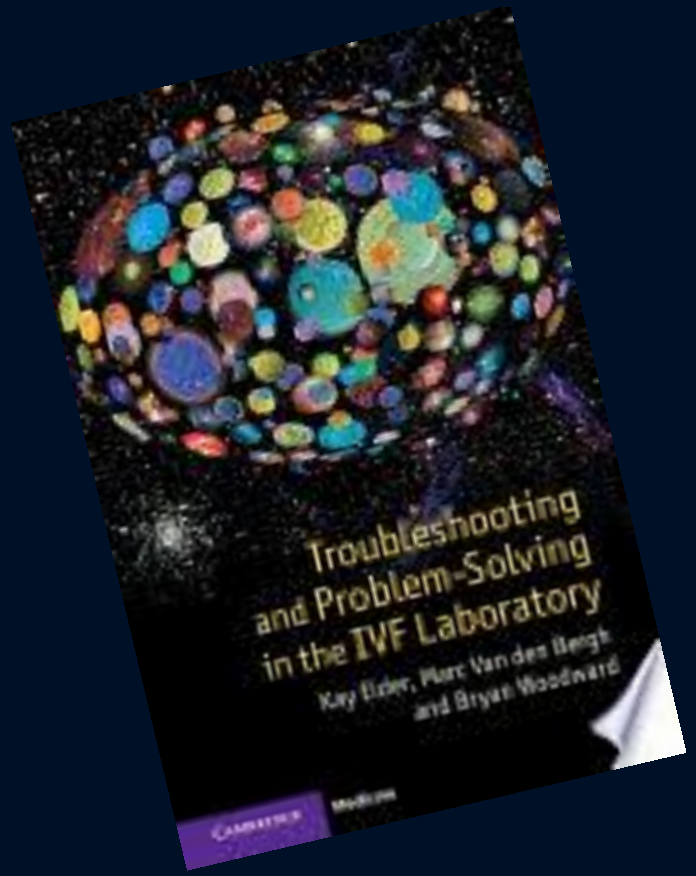
- Assumed – Knowledge of Theory
- Process Mapping - SOPs
- Gather the Facts

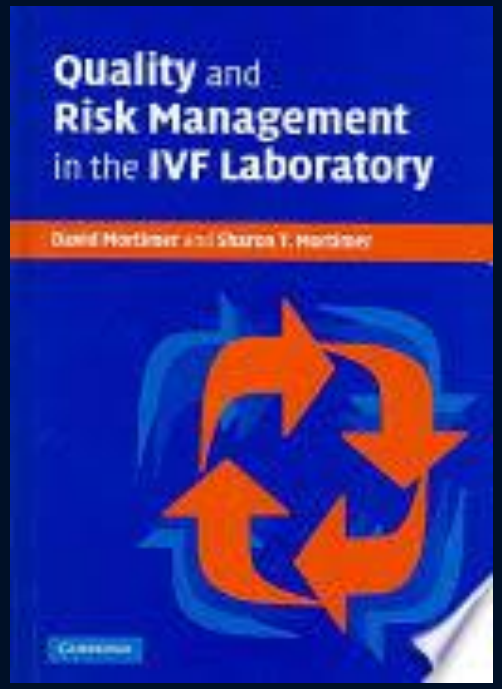
Vit Flowsheet

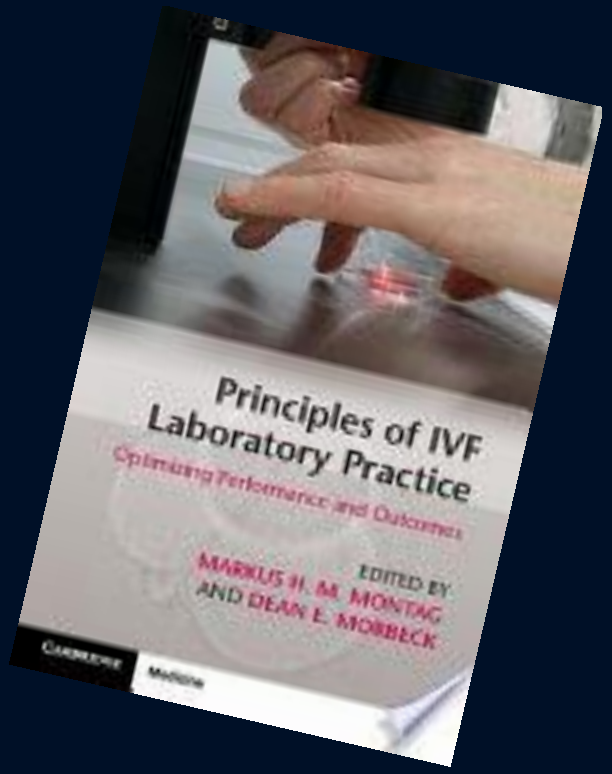
The Facts

- Interview Embryologist
  - Seemed Normal
  - Noticed Dense Media Column
  - Flash
  - Blastocyst Blebbed/Shredded
  - New Lots of Warming Media (TS DS WS)

Vit Flowsheet


Troubleshooting and Problem-Solving in the IVF Laboratory
Kay Elder, Marc Van den Bergh and Bryan Woodward


Quality and Risk Management in the IVF Laboratory
David Mortimer and Sharon T. Mortimer


Principles of IVF Laboratory Practice
Optimizing Performance and Outcomes
EDITED BY
MARKUS H. M. MONTAG
AND DEAN E. MORBECK

The End

BUT YOU ARE STILL ON THE ROLLER COASTER!

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Hands On Portion

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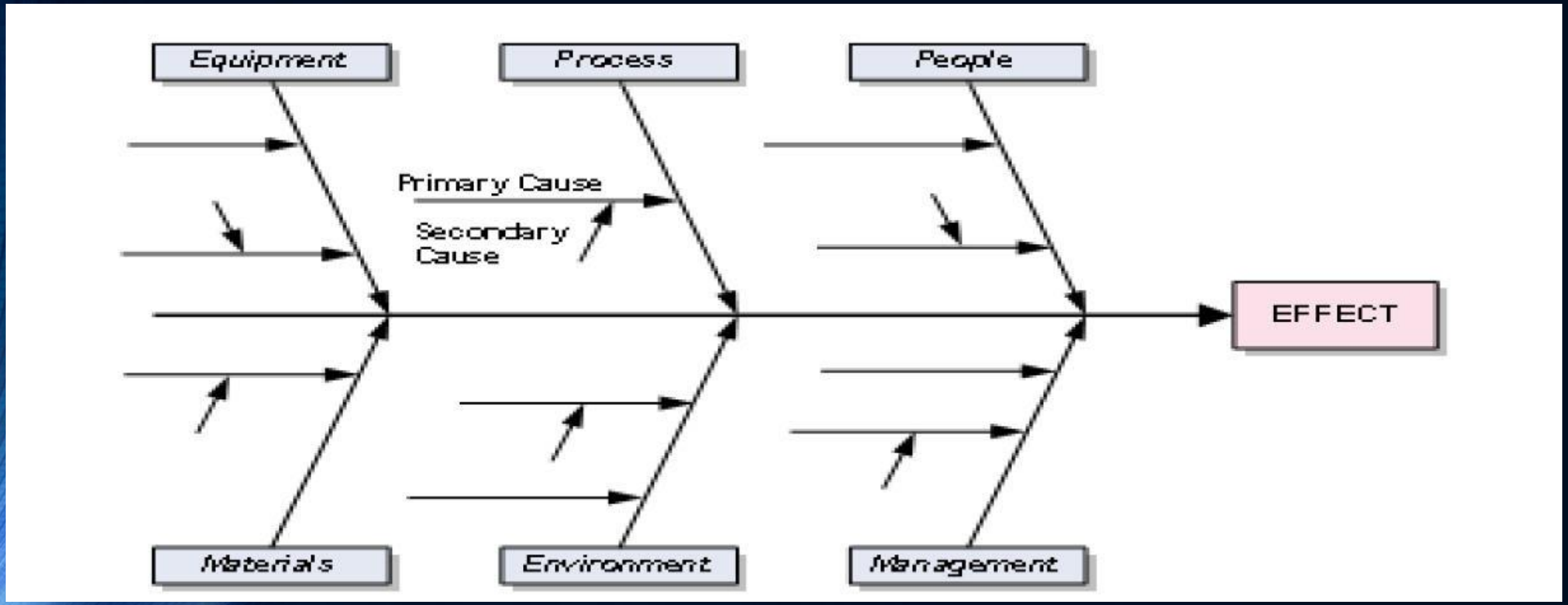
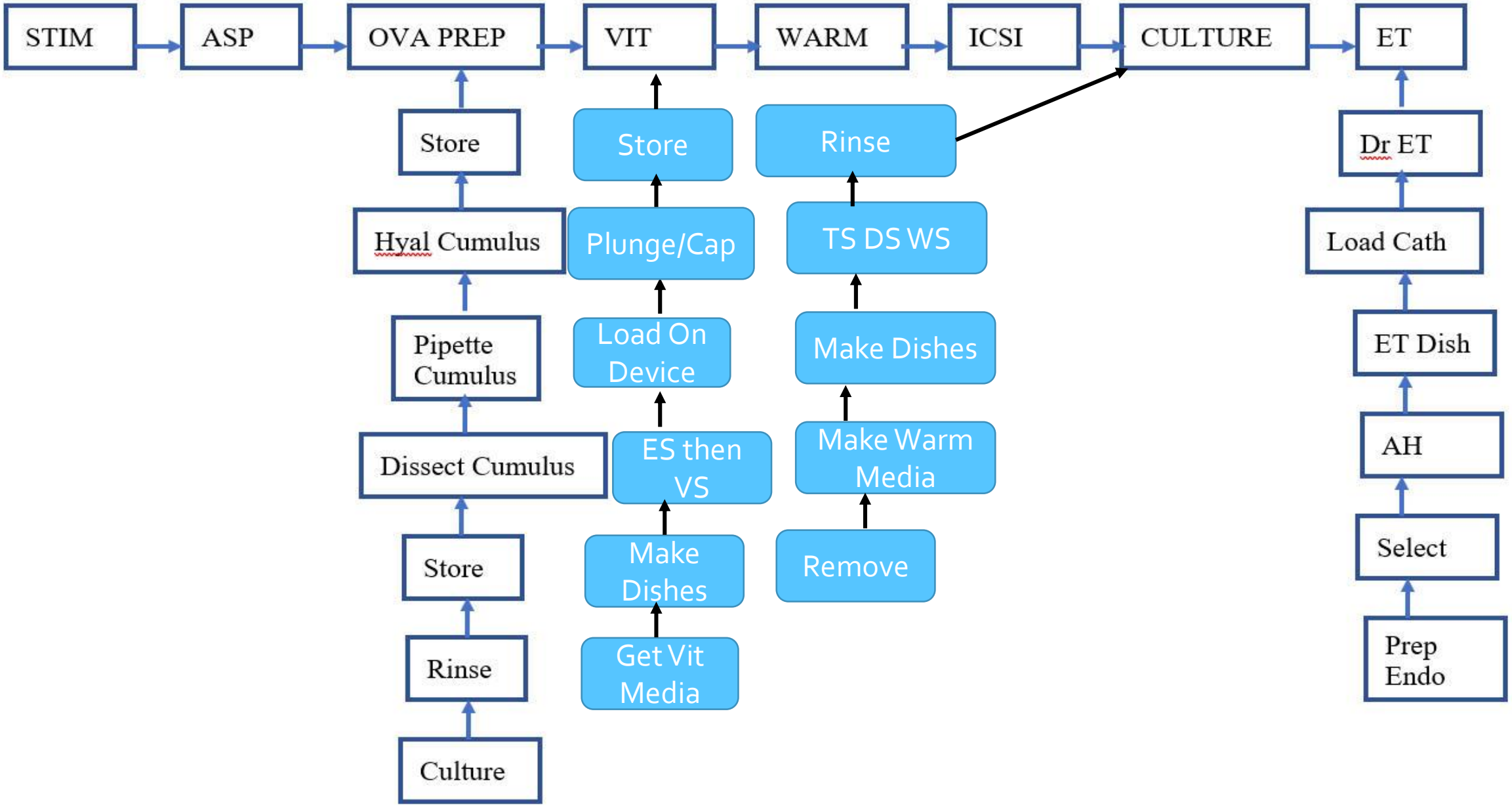


Warming Blastocyst Flow



Scenario 1 – Missing Embryos

- Six Tanks – 1 to 6
- Eight Racks – 1 to 8
- Cryolocks in goblets in Canes
- Labelled Top – ID#, Lname, Date
- Schedule – Geena Davis; #1923; 1 device; 2 Blastocysts
- NOT THERE!
- Initial Plan
- What Data Do You Want?

Scenario 2 – Poor Fertilization Donor Vitrified Ova

- Six Donor Ova from Bank
- Pt Mary Brown #2457
- Donor # 1576 – Rtr 5/8/16
- New Warming Media
- 5 of 6 Survived
- 1 of 5 Fertilized
- Observation @ ICSI "a little grainy"
- Initial Plan
- What Data Do You Want?

Scenario 3 – Poor PG Rate FETs

- Last 6 FETs With 2 PG

- Initial Plan
- What Data Do You Want?

Scenario 4 – Poor Survival Vit Patient's Ova

- 0 of 6 Survived
- "Kitasato" Method
- What Data Do You Want?

The End

BUT YOU ARE STILL ON THE ROLLER COASTER!

Warming Method