EXCITING CREATIVE TECHNIQUES IN BOVINE EMBRYOLOGY Hands-on Workshop – lectures and practices St Luke's College, Ji-Paraná, Rondônia, Brasil (just one step from Sao Paulo) 8-13 September, 2025.

MONDAY (8th)

Morning 9-12

Formal Opening and Introduction (30 min)C

Keynote lectures (30 min ea)

- Are we on the right track? GV
- OPU/IVF in the Amazon Region CF
- OPU/IVF in Eastern and Western Europe AB
- Application fields of cloning in science, medicine and agriculture LL

Afternoon 13-18

- Transvaginal Ovum Pick-up (OPU) in cattle: videos and on-site presentation CF
- Bovine in vitro embryo production: step by step procedure AB

IVM and IVF including sperm preparation Sperm preparation

TUESDAY (9th)

Morning 9 - 12

Alternativ ways for embryo culture GV

- The actual situation full of problems
- Individual vs. group: The Well of the Well (WOW)
- Humidity issues: the Humdish
- The Submarine Incubation System (SIS)

The idea and the realization

Experiences with exclusive use of SIS in a commercial embryo laboratory CF

- Pipetting issues: including the POP

Debate:

- <u>Traditional way vs the above techniques</u> – <u>pro and contra arguments</u> (<u>comments from LL, AB, CF)</u>

Afternoon 13-18

Practice with the above techniques GV, AB, CF

- SIS
- WOW
- Humdish
- Glass capillary preparation and the delicate way of embryo handling by mouth pipetting

WEDNESDAY (10th)

Morning 9 - 12

Slow freezing: principles, protocols, and practical applications AB

Vitrification GV

- theoretical summary
- If yes, why not? Progress and setbacks
- The original, the best: The Open Pulled Straw (OPS)
- Direct Transfer (DT) a unique feature of the OPS
- -Rapid Rehydration after warming

Debate:

- Traditional freezing vs. vitrification – pro and contra arguments (comments from LL, AB, CF)

Afternoon

- All aspects of OPS vitrification (equilbration, cooling, storage, warming, direct transfer) GV, AB

THURSDAY (11th))

Morning 9 - 12

Somatic Cell Nuclear Transfer (SCNT) GV

- Background
- What is it good for?
- Mission unaccomplished?
- Foreseeable breakthroughs

Legalization of cloning in Brazil LR

How intellectual property protection can impact the advancement of research in cloning AM

Traditional cloning LL

Somatic cell preparation LL

Afternoon 13 - 19

- Somatic cell preparation (demonstration only) LL (GV)
- Zona digestion of IVM oocytes GV
- Mouth pipetting practices GV
- (- Random bisection of zona-free oocytes by a handheld microblade) if time allows! GV

FRIDAY (12th)

Morning 9 - 12

Handmade Cloning (HMC) VG

- The idea and realization
- Past achievements and future perspectives
- HMC video

Debate:

- Traditional vs. handmade cloning – pro and contra arguments (comments from LL, AB, CF)

Afternoon 13 - 19

Handmade cloning procedure (GV)

- Random bisection of zona-free oocytes by a handheld microblade GV
- Oriented bisection GV
- Pairing with somatic cells
- Fusion (mostly demonstration, limited practice)

SATURDAY (13rd)

Morning 9 - 13

Parthenogenetic activation of oocytes and reconstructed embryos GV

Perspectives opened by the presented techniques in the Amazon region and worldwide

Discussion about techniques and collaborations ALL INCLUDING STUDENTS AND GUESTS!!!

FORMAL CLOSING OF THE WORKSHOP, PROVIDING CERTIFICATES OF ATTENDANCE

Afternoon - as long as required

Practice in the lab again -

- parthenogenetic activation of oocytes
- repeated practice of all steps of HMC

INFORMAL FAREWELL - Kiss and Bye...