OVERVIEW OF IVF AS IT RELATES TO INTEGRATION INTO CERTIFICATION
RATIONALE

• IVF IS MORE PREVALENT & DEEPLY INTEGRATED IN ET INDUSTRY

• CERTIFIED PRACTITIONERS SHOULD HAVE A GENERAL KNOWLEDGE-BASE OF IVF INFORMATION, WHETHER OR NOT THEY WILL DIRECTLY UTILIZE IT IN THEIR PRACTICE

• THERE ARE CONTINUALLY EMERGING OPPORTUNITIES AND VARIOUS ENTRY-POINTS FOR THE ET PRACTITIONER & HIS CUSTOMERS TO INTEGRATE IVF TECHNOLOGY
IVF EMBRYO TIMELINE

- **GAMETE RECOVERY = DAY -1**
  - TRANSVAGINAL OOCYTE RECOVERY (TVOR) / FOLLICULAR ASPIRATION (ASP) / OVUM PICK-UP (OPU)
  - IN-VITRO MATURATION OF OOCYTES (IVM)

- **IN-VITRO FERTILIZATION OF OOCYTES (IVF) = DAY 0**
  - STD. TIMELINE FOR ET: DAY 0=ESTRUS / DAY 1=OV. & FERT.

- **IN-VITRO CULTURE OF ZYGOTES (IVC) = DAY 1 TO DAY 6 - 7**

- **TRANSFER OR CRYOPRESERVATION OF IVF EMBRYOS = DAY 7**
IVF DONOR ANIMALS

- Initially used for open, dry donors w/ infertility issues

- Commonly used now in all classes of donors including: virgin heifers, pregnant cows in 1st trimester, dry, lactating, b/t conventional ET, etc.

- Donor set-up protocols vary
  - Non-stimulated (random day of cycle or w/ follicular wave mgmt. like DFR, GNRH, etc.)
  - Stimulated w/ FSH-priming (usually in conjunction with pre-synchronizing estrus, follicular wave mgmt. or both)
LOGISTICAL & MANAGEMENT CONSIDERATIONS FOR IVF PRODUCTION

Pros:
- Simpler protocol for donors, no AI
- Wider range of donor utility
- More frequent opportunity to produce embryos

Cons:
- Demanding logistics (more steps, different days for donors & recipients)
- Greater recipient demands and/or less efficient recipient utilization
- Lower pregnancy rates, esp. w/cryopreserved IVF embryo
- Higher early embryonic mortality
- Some gestational abnormalities? Associative dystocia?
EXPORT ELIGIBILITY???

Sanitary procedures cannot be extrapolated from ET to IVF:

1. ZP of intrafollicular oocytes may have different resistance to adherence / penetration of pathogens than ovulated oocytes

2. Post-mortem/slaughter-house/abbatoir ovaries are usually pooled and thus, lose traceability
Equipment for Oocyte Retrieval
OOCYTE RECOVERY EQUIPMENT

• CONVEX LINEAR TRANSDUCER & ULTRASOUND CONSOLE
• STABLE CART OR GURNEY
• VACUUM PUMP W/FILTERED VACUUM LINE
  • ADJUST PRESSURE TO REGULATE FLOW RATE TO ~10 ML H2O/MIN
• NEEDLES, TUBING, & COLLECTION VESSEL AND/OR FILTER
• COLLECTION MEDIA (TL HEPES W/BSA & HEPARIN*)
• MEANS TO KEEP MEDIA, TUBES WARM
• SUITABLE COLLECTION CHUTE AREA, NEAR LAB SPACE
IVF LABORATORY DESIGN
IVF LABORATORY DESIGN

• RESTRICTED ACCESS/ENTRY TO ROOMS THAT ARE ORGANIZED & SEGREGATED W/SPECIFIC FUNCTIONS & SANITARY LEVEL

• FLOW OF PERSONNEL SHOULD BE REDUCED FROM OUTSIDE TO INNER-MOST ROOMS, ACCORDING TO SANITARY LEVEL

• FILTERED VENTILATION W/POSITIVE AIRFLOW IS NECESSARY TO PREVENT BACK-DRAFT OF AIRBORNE CONTAMINANTS
OOCYTE SEARCHING LAB

• SUITABLE MICROSCOPE, PREFERABLE W/HEATED STAGE

• OOCYTE RINSING/WASHING MEDIA (TL HEPES W/BSA, WITH/ AND W/O HEPARIN)

• OOCYTE MATURATION MEDIA - TCM199 BASIS W/FSH, LH, FBS, ETC.
  • NA-BICARB- (FOR CO2) OR ZWITTERIONIC-BUFFERED (FOR NON-CO2)

• MEANS TO KEEP MEDIA, DISHES WARM (~36-38C)

• PORTABLE INCUBATOR?

• IVM AT 38.50 C IN 5% CO2 IN ATMOS. AIR FOR 20-22H
  • ATMOSPHERE = 78% N2 / 21% O2 / 0.04% CO2
FERTILIZATION STEP

• SEMEN IS PREPARED FOR IVF BY
  • SWIM-UP PREPARATIONS
  • SILICA-GEL SEPARATION GRADIENTS SUCH AS PERCOL

• DEVELOPMENT RATES MAY BE OPTIMIZED BY
  • FINAL CONCENTRATION OF SPERM CO-INCUBATED W/EGGS
  • CONCENTRATION OF HEPARIN (CAPACITATION AGENT)
  • ADDITION OF OTHER ADDITIVES, SUCH AS CAFFEINE
SEMEN FOR IVF EMBRYO PRODUCTION

• TREMENDOUS INHERENT VARIATION IN EMBRYO DEVELOPMENT RATES BY BULL USED IN IVF

• CONVENTIONAL SEMEN - PRE-SCREEN FERTILITY?

• GENDER OR SEX-SORTED SEMEN:
  • SORTED, FROZEN IN 2.1X OR 5.0X10⁶
  • CONVENTIONAL, POST-THAW SORTED (“REVERSE SORT”)
  • FRESH-COLLECTED SEMEN, SORTED
PREPARATION FOR CULTURE

- **Presumptive Zygotes** (following co-incubation w/sperm for 8-22 hours) are typically “stripped” of remaining cumulus cells (now collapsed) & excess adherent sperm cells by:
  - Manual stripping with small bore pipettor
  - Manual stripping after exposure to enzyme such as hyaluronidase
  - Mechanically assisted stripping with benchtop vortexer

- **Culture media** have evolved in type & composition, but now is typically a modified SOF or KSOM with the addition of high quality BSA such as Probumin
  - IVM at 38.5°C in 5:5:90 CO2:O2:N2 gas mixture
  - IVM at 38.5°C in 5% CO2 in air
TRANSFER / CRYOPRESERVATION

• EVALUATION CRITERIA FOR TRANSFER DAY 6 VS. DAY 7
  • LOAD TUBES FOR TRANSPORT IN PORTABLE INCUBATOR DAY 6, TRANSFER D7
  • LOAD STRAWS FOR TRANSFER LATE DAY 6/EARLY DAY 7 FOR TRANSFER
  • TRANSFER STAGE 4 EMBRYOS? DELAYED, VERY LOW PREGNANCY RATE

• CRYOPRESERVATION DAY 7
  • CONVENTIONAL SLOW-FREEZING IN GLYCEROL / EG FOR DT
  • VITRIFICATION IN VARIOUS PACKAGING (OPS, CRYO-TOP, CRYO-LOOP, CRYO-HOOK, 1/4CC STRAW, ETC.)
  • STD. APPROACH FOR CONV. ET - CRYO. BEST / TRANSFER WORST
  • STD. APPROACH FOR IVF - TRANSFER BEST / CRYO. BEST
INTEGRATION OF IVF

• MANY SHORT-COURSES, TRAINING OPPORTUNITIES

• IVF IN ON-GOING RESEARCH & COMMERCIAL EFFORTS
  • DATA REPORTING IN PUBLICATIONS, MEETINGS

• LOGICAL INTEGRATION INTO PRACTICE:
  • TRANSFER OF IVF EMBRYOS ON DAY 7
  • TRANSPORT OF IVF EMBRYOS ON DAY 6 / TRANSFER DAY 7
  • OPU DAY -1 / LOAD IN MAT / SHIP TO CENTRALIZED LAB / REC. D6?
  • INTEGRATE FULL-BLOWN IVF LAB???