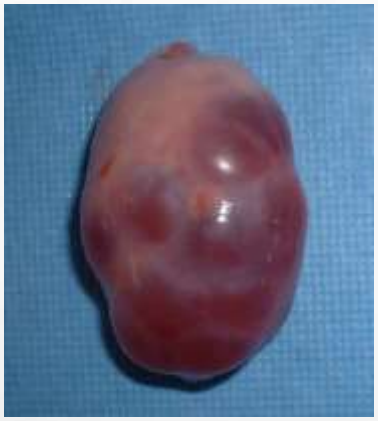
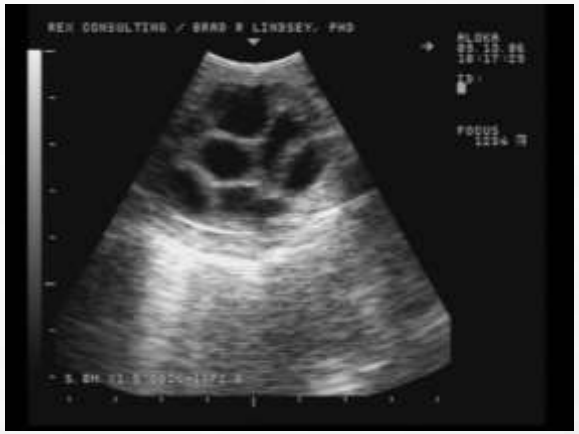
ATEA 2014 CERTIFICATION SESSION

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

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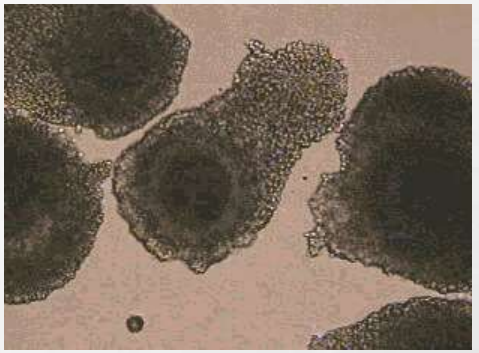
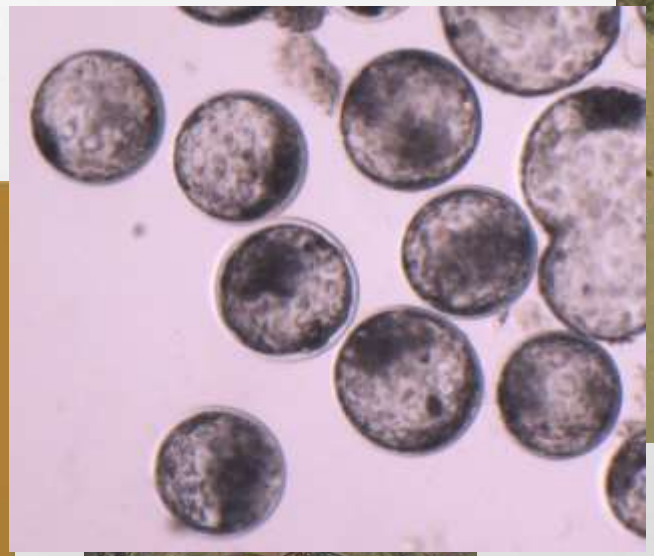
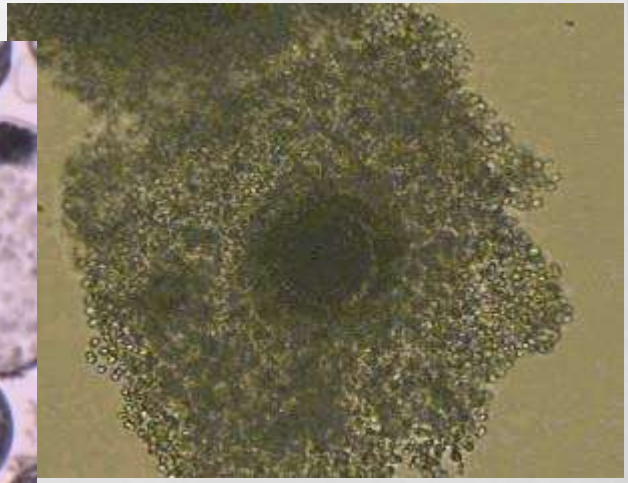
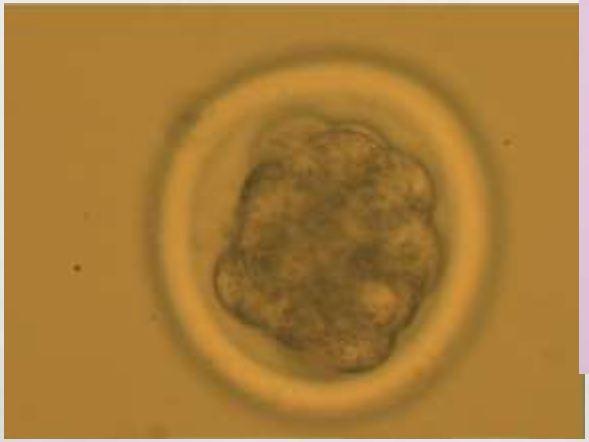








IVF EMBRYO TIMELINE

- 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:

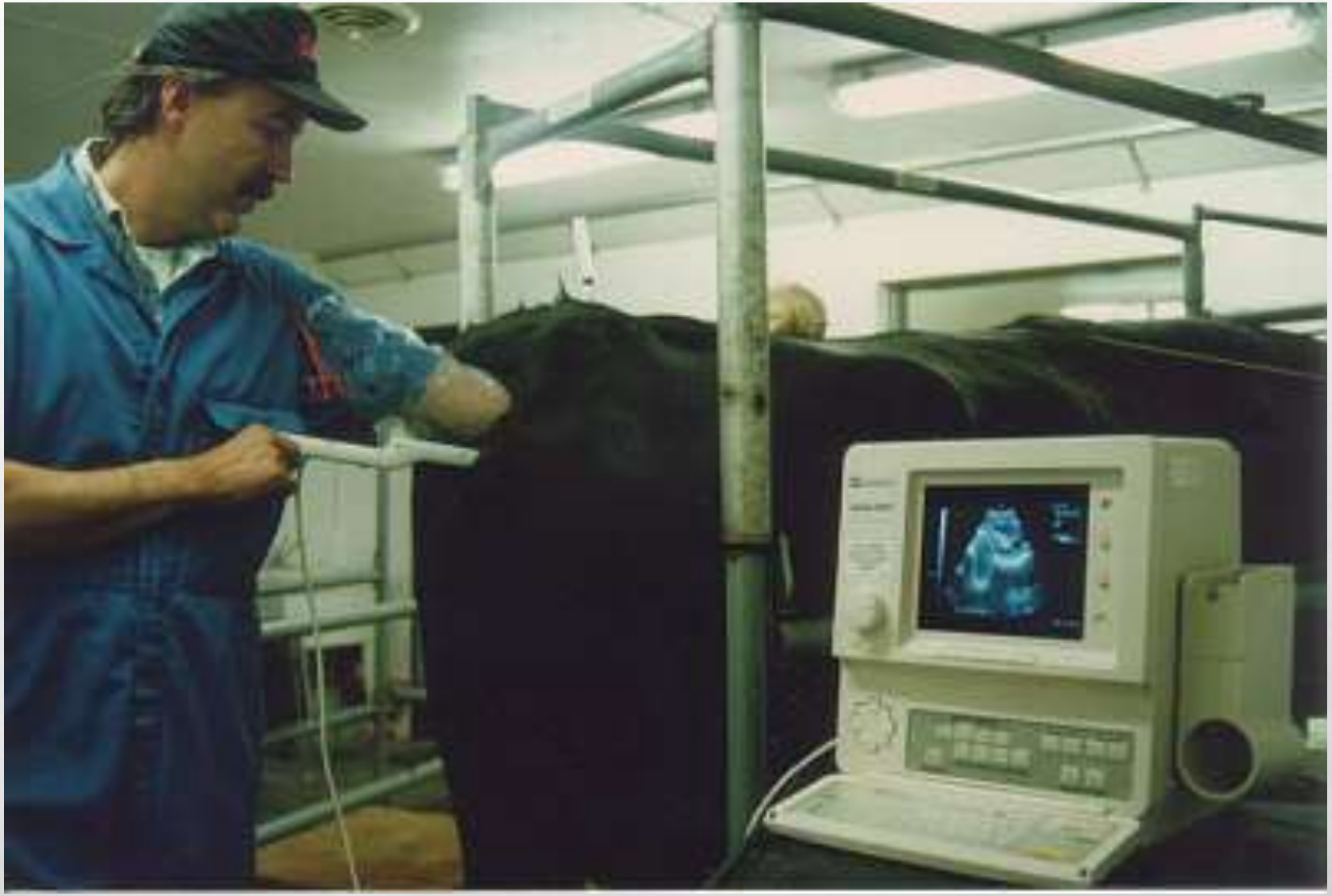
(1) ZP of intrafollicular oocytes may have different resistance to adherence / penetration of pathogens than ovulated oocytes

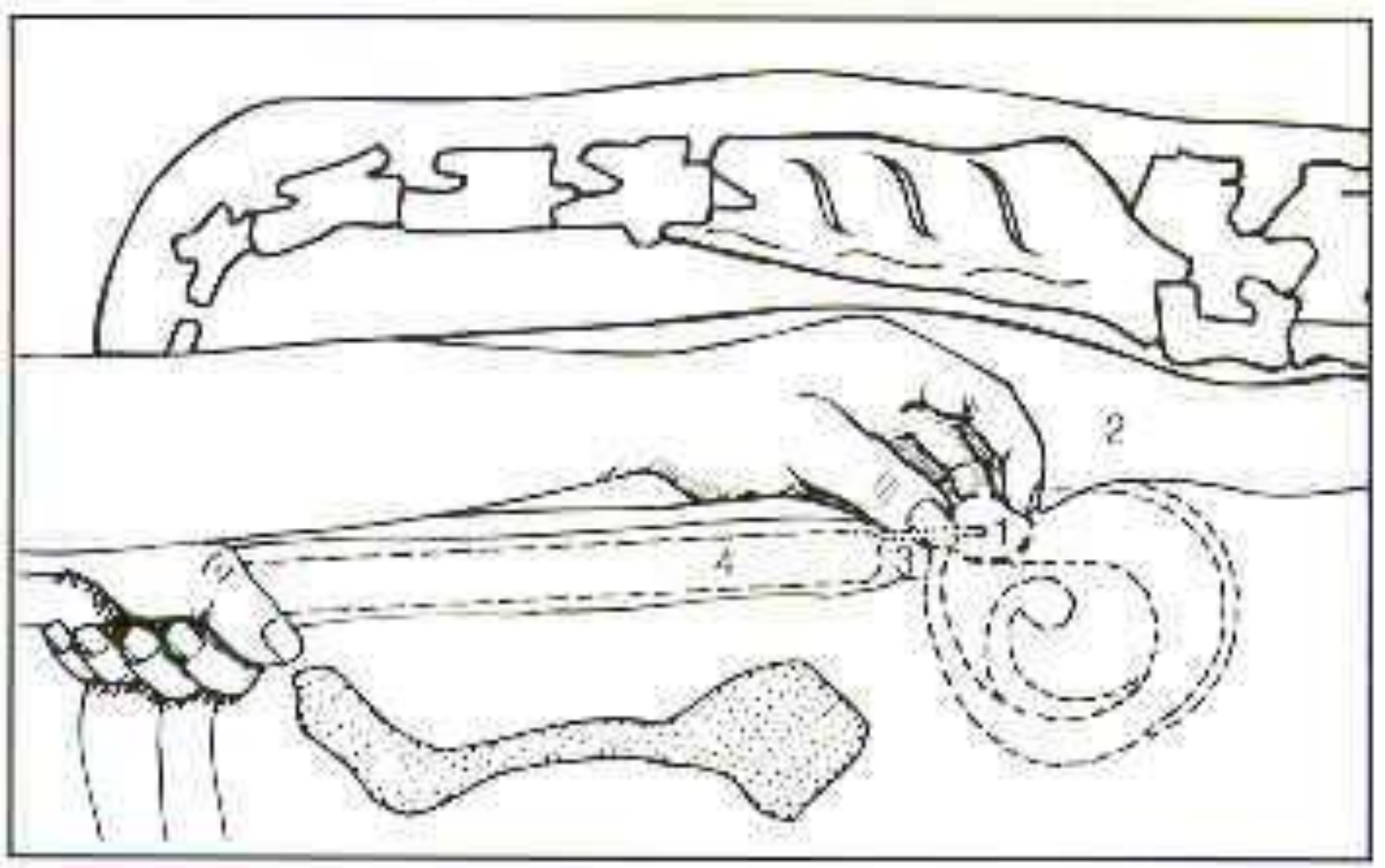
(2) Post-mortem/slaughter-house/abbatoir ovaries are usually pooled and thus, lose traceability

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 H20/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

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1
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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/OUT HEPARIN)
- OOCYTE MATURATION MEDIA – TCM199 BASIS W/FSH, LH, FBS, ETC.
  - NA-BICARB- (FOR CO2) OR ZWITTERIONIC-BUFFERED (FOR NON-C02)
- MEANS TO KEEP MEDIA, DISHES WARM (~36-38C)
- PORTABLE INCUBATOR?
- IVM AT 38.5^{O} 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

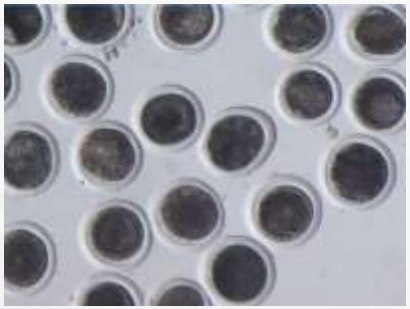
SEMEN FOR IVF EMBRYO PRODUCTION

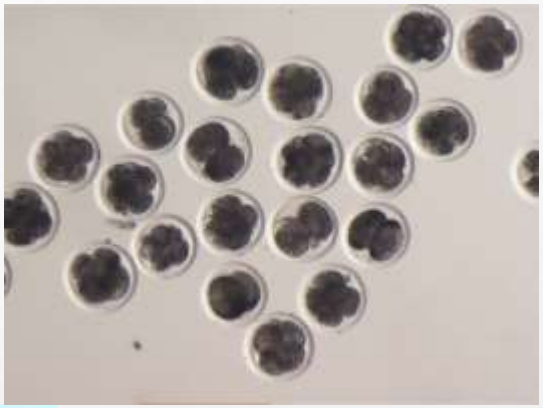
- TREMENDOUS INHERENT VARIATION IN EMBRYO DEVELOPMENT RATES BY BULL USED IN IVF
- CONVENTIONAL SEMEN – PRE-SCREEN FERTILITY?
- GENDER OR SEX-SORTED SEMEN:
  - SORTED, FROZEN IN 2.1X OR $5.0X10^6$
  - CONVENTIONAL, POST-THAW SORTED (“REVERSE SORT”)
  - FRESH-COLLECTED SEMEN, SORTED

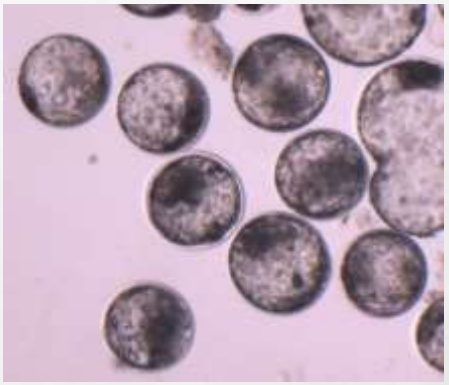
PREPARATION FOR CULTURE

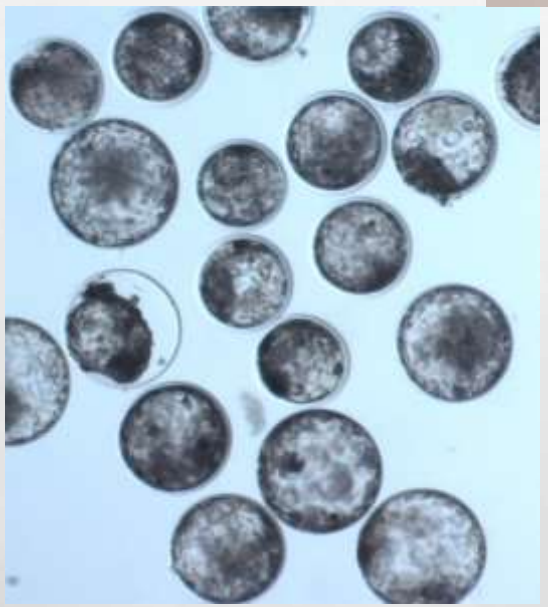
- PRESUMPTIVE ZYGOTES (FOLLOWING CO-INCUBATION W/SPERM FOR 8-22 HOURS) ARE TYPICALLY "STRIPPED" OF REMAINING CUMULUS CELLS (NOW COLLAPSED) & EXCESS ADHERENT SPERM CELLS BY:
  - MANUAL STRIPPING WITH SMALL BORE PIPETTOR
  - MANUAL STRIPPING AFTER EXPOSURE TO ENZYME SUCH AS HYALURONIDASE
  - MECHANICALLY ASSISTED STRIPPING WITH BENCHTOP VORTEXER

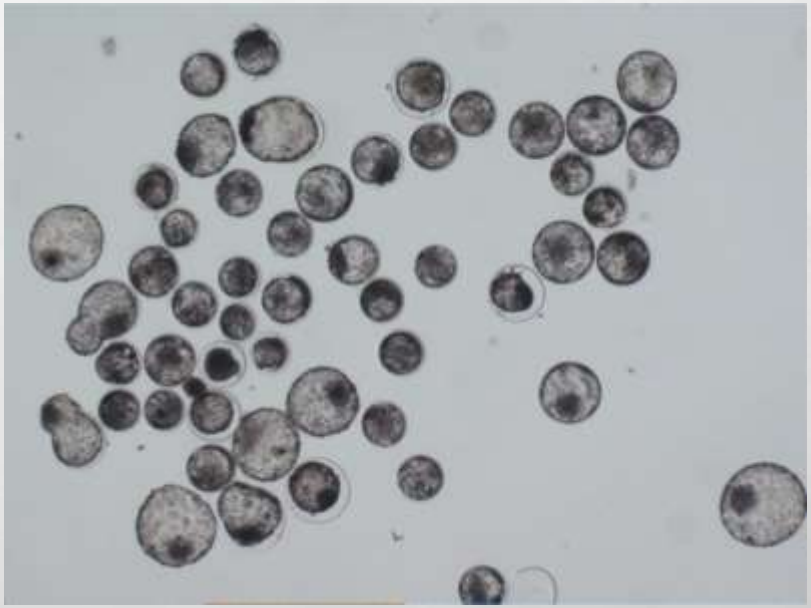
- CULTURE MEDIA HAVE EVOLVED IN TYPE & COMPOSITION, BUT NOW IS TYPICALLY A MODIFIED SOF OR KSOM WITH THE ADDITION OF HIGH QUALITY BSA SUCH AS PROBUMIN
  - IVM AT 38.5^{O} C IN 5:5:90 CO_2:O_2:N_2 GAS MIXTURE
  - IVM AT 38.5^{O} C IN 5% CO_2 IN AIR











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???