SRBT WEBINAR

Infectious Disease and the Infertility Patient:

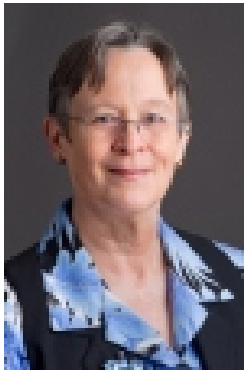
July 14, 2015: Patient Evaluation and Laboratory Safety
July 21, 2015: Fertility Treatment for Infected Patients

Hosts:

SRBT Sue Gitlin, Ph.D.,
Chair, SRBT Education

CRYOPORT Shannon Curiel, Business Development Manager, Cryoport

Erma Z. Drobnis, Ph.D., HCLD



- PhD from U California Davis on the biophysics of sperm-oocyte interaction in mammals
- Post doc training under Drs. John Crowe and Jim Overstreet
- Research faculty member at UCDavis in Ob/Gyn, Vet Medicine
- Currently, director of Andrology at University of Missouri, Columbia School of Medicine.
- Chair SMRU Traveling Scholars program
- In her 35+ years, studied diverse species, published multiple articles. Highlights:
  - Semen quality in urban vs rural areas
  - Sperm glycobiology
  - HIV discordant couples
  - HIV transmission at the endocervix

SOCIETY OF REPRODUCTIVE Biologists & Technologists
ASRM SRBT

Infectious Disease and the Infertility Patient

Part 1. Patient Evaluation & Laboratory Safety

Society of Reproductive Biologists & Technologists (SRBT)
Webinar 07-14-15

Erma Z. Drobnis, Ph.D.

University of Missouri, Columbia – School of Medicine
Missouri Center for Reproductive Medicine & Fertility

Slide Backgrounds: a rendering of the HIV virus by Ukrainian designer Alexey Kashpersky First prize winner of the Computer Graphics Society visualization competition 2012
http://www.cgsociety.org/index.php/CGSFeatures/CGSFeatureSpecial/autopack_challenge_winners

Infectious Disease and the Infertility Patient

- It is exciting to have registrants from 18 countries for this webinar!
- I am most familiar with U.S. regulations for clinical laboratories, and some are included; other regional regulations include NATA in Australia, CPA and HFEA in the UK, EU Tissues and Cells Directive and guidelines from the ISO, WHO and scientific societies that act and standards of clinical practice
- Homework: If you know the web location of regulations for your country (or if I missed relevant U.S. ones), please send them to me after the webinar

Participant Country		
Australia	Greece	Peru
Brazil	India	Saudi Arabia
Canada	Israel	Singapore
Costa Rica	Mexico	South Africa
Denmark	Nigeria	Spain
Germany	Pakistan	United States

Infectious Disease and the Infertility Patient
Part 1. Patient Evaluation & Laboratory Safety

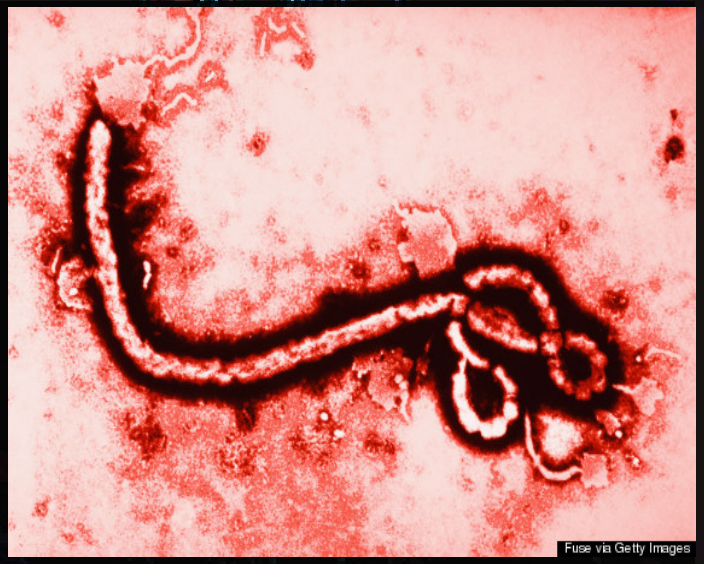
Outline

1. Relevant Infectious diseases
2. Fertility treatments and the potential for disease transmission
   a. Timed intercourse
   b. IUI (intrauterine insemination)
   c. IVF (in vitro fertilization)
   d. Cryopreservation of sperm, embryos and oocytes
   e. Gestational surrogates and carriers
3. Protection of staff
4. Prevention of cross-contamination of patient specimens in the laboratory

Infectious Disease
and the Infertility Patient

1. Relevant infectious diseases

Diseases Relevant to Diagnosis and Treatment of Infertility Patients



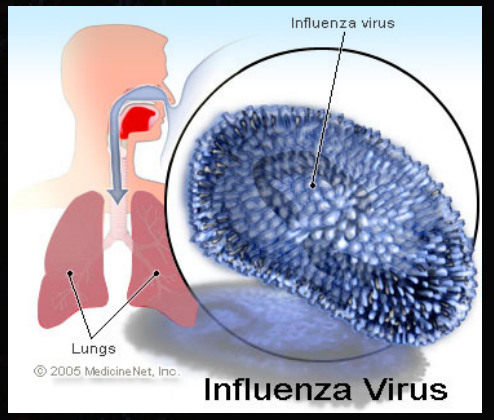
- Interestingly, the reason I was asked to give a webinar on this topic was because it was during the peak Ebola outbreak
- A major concern was that patients in the waiting room and staff could become infected
- SRBT released guidelines on Ebola[1]
- In the meantime, more information became available about Ebola, and interest shifted back to treating patients with STIs in general
- Interestingly, ebola virus persists in semen for several months after all symptoms of the disease have resolved[2] (vs. one month for vaginal secretions); recommended that patients use condoms after recovery

1. SRBT (2014) https://www.asrm.org/SRBT_Offers_Advice_on_Ebola/
2. Mackay IM, Arden KE. Ebola virus in the semen of convalescent men. Lancet Infect Dis 2015;15:149-150.

Diseases Relevant to Diagnosis and Treatment of Infertility Patients (continued)

As far as transmission by casual contact

- important for protection of patients in the clinic and staff with patient contact
- Generally covered by governmental and/or hospital regulations for medical clinics



- Counsel patients to see their primary care provider for annual exam and vaccination review
- Influenza A ("the flu"): staff should be vaccinated annually; include signs, masks, hand sanitizer station in waiting room
- Tuberculosis (TB): staff should be tested annually
- Other Staff Immunizations for those without immunity: Measles, Mumps & Rubella (MMR); varicella; hepatitis B; Tetanus, Diphtheria, Pertussis (Tdap)

Diseases Relevant to Diagnosis and Treatment of Infertility Patients (continued)

For infertility patients, we are most concerned with STIs that may:

- Contribute to the patients' infertility
- Can be passed:
  - Between partners (horizontal transmission),
  - To the patient, embryo or gametes during fertility treatments (nosocomial)
  - To the child (vertical transmission)
- Infect staff members
- Cross-contaminate reproductive tissues (sperm, eggs, embryos) during processing and storage

Diseases Relevant to Diagnosis and Treatment of Infertility Patients (continued)

Routine screening of infertility patients can be done during a history and physical exam:

- If the woman has not had a PAP smear in the last year, she can be referred to her primary care provider for a pelvic exam with screening for infectious diseases as appropriate based on her history and physical exam
- The man should also be referred to his PCP or the urologist for annual exam

Treatable STIs	
Chlamydia	Can reduce fertility by causing tubal disease. may increase susceptibility to other STIs; PTL, PROM, LBW infant
Gonorrhea	Miscarriage, premature birth, PROM, LBW infant
Syphilis	Premature birth, stillbirth, sick infant
Trichomoniasis	increases susceptibility to other STIs

Diseases Relevant to Diagnosis and Treatment of Infertility Patients (continued)

Of most concern in the fertility clinic are bloodborne pathogens that are sexually transmitted

- These viruses are present in semen and vaginal secretions
- Testing will depend on patient risk factors and the fertility treatment to be used

Untreatable Viral Infections – Bloodborne Pathogens	
CMV (Cytomegalovirus)	HTLV 1&2 (human T-lymphocyte virus)
HIV (human immunodeficiency virus)	HPV (human papilloma virus)
HBV (hepatitis B virus)	HSV-2 (herpes simplex virus-genital herpes)
HCV (hepatitis C virus)	Viral hemorrhagic fevers (e.g., Ebola)

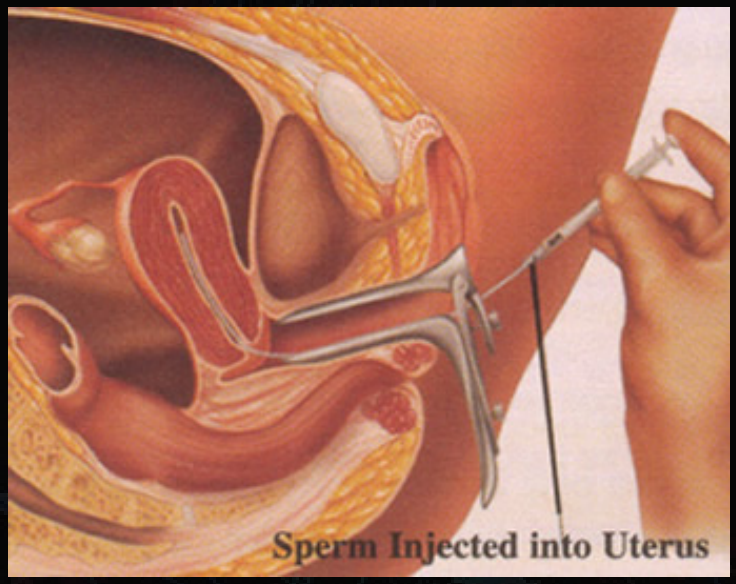
Infectious Disease
and the Infertility Patient

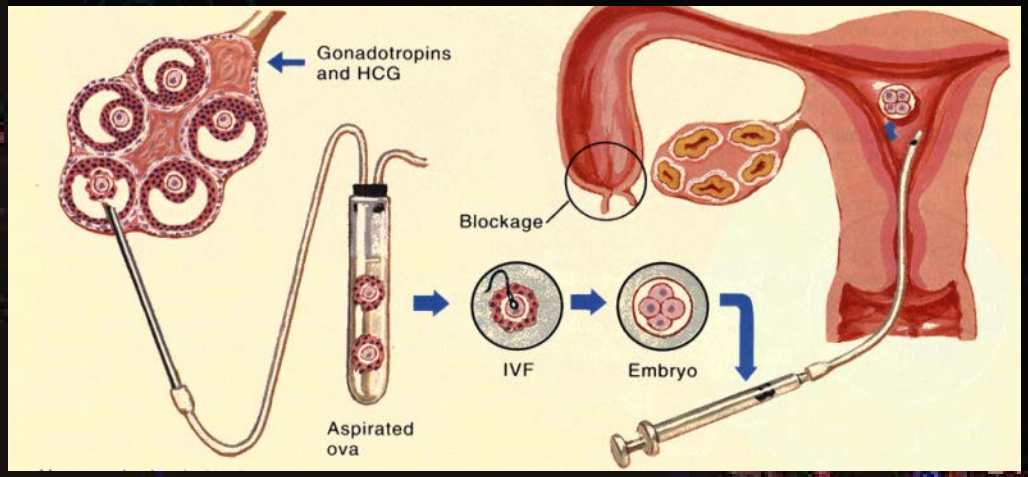
2. Fertility treatments and the potential for disease transmission

Fertility Treatments and the Potential for Disease Transmission

Common treatment strategies for infertility patients:

1. Timed intercourse
2. IUI (intrauterine insemination)
3. Donor insemination
4. Semen cryopreservation
5. IVF (in vitro fertilization)





6. ICSI (intracytoplasmic sperm injection
7. Oocyte/embryo cryopreservation
8. Surrogacy
9. Gestational Carrier

Fertility Treatments and the Potential for Disease Transmission

Timed Intercourse

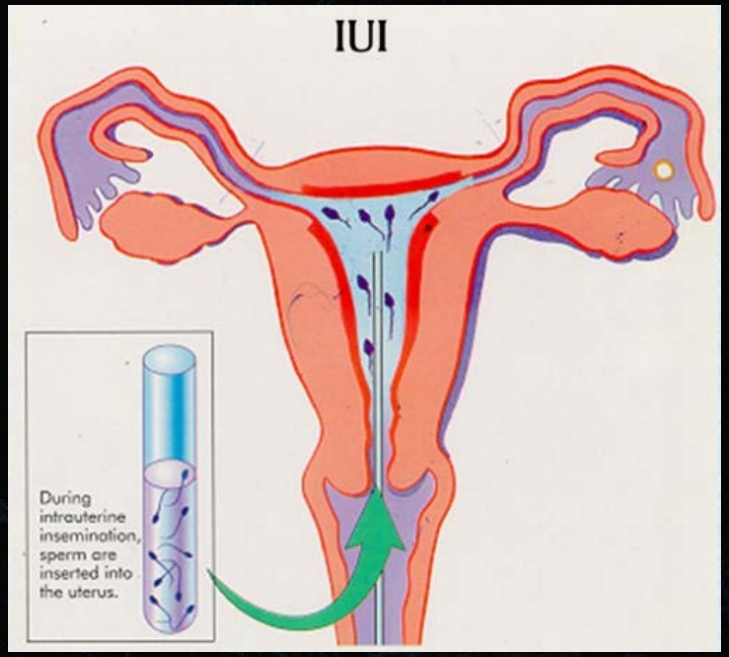
Used for idiopathic infertility or when both partners have findings consistent with potential fertility

- Staff is not involved in the reproduction
- The couple is already sexually intimate
- Concern is primarily for the offspring
- Prenatal counseling should include risks of vertical virus transmission

Fertility Treatments and the Potential for Disease Transmission

IUI (intrauterine insemination)

During intercourse, many organisms are introduced into the vagina; the immune system and cervical barrier protect the upper reproductive tract



IUI Involves breaching some of the female reproductive tract's defenses to disease

- Infection is the primary risk of IUI
- In some cases, the couple are serodiscordant for viral infections

Because semen is handled in the lab, it is possible for:

- Staff to become infected
- Cross-contamination of specimens from another couple

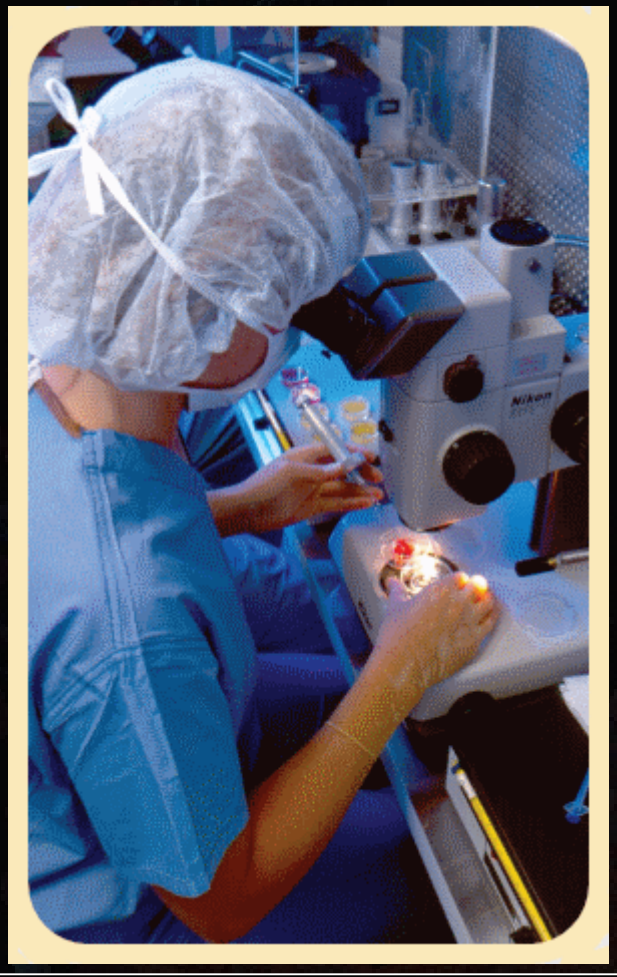
Clinics vary in whether they require patient infectious disease testing for IUI; there are no available guidelines suggesting that this be done

Fertility Treatments and the Potential for Disease Transmission

IVF (in vitro fertilization)

IVF and IVF/ICSI

- As for IUI, gametes are removed from the patient and processed in the laboratory
- Cross-contamination of specimens between patients is a concern
  (prevention later in talk)
- ASRM guidelines require that both partners of a couple undergoing IVF be screened for infectious diseases



1. ASRM Practice Committee (2013) Recommendations for gamete and embryo donation: a committee opinion. Fertil Steril 99:47-62.

Fertility Treatments and the Potential for Disease Transmission

Donor Gametes – 3rd Party Reproduction

For donor sperm, cryopreservation is required

- FDA regulations[1,2] and ASRM guidelines[3] require that sperm donors be screened for infectious diseases, the sperm cryopreserved, and the donor be retested 6 months later before release of specimens for use
- For embryo and oocyte donation, quarantine is not required. For oocytes, the donor must be tested within 30 days before oocyte collection.
- Cryopreservation and storage can allow cross-contamination of tissue
- FDA also has requirements covering processing, labeling and storage of frozen tissues,[1,3] and specify the laboratory conditions to be used[4]

1. FDA. Human cells, tissues, and cellular and tissue-based products; final rule. Fed Register. 2007(April 1):714-17. [21CFR1271]
2. FDA, Center for Biologics Evaluation and Research (2007) Guidance for Industry: eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps).
3. ASRM Practice Committee (2013) Recommendations for gamete and embryo donation: a committee opinion. Fertil Steril 99:47-62.
4. FDA, CBER (2009) Guidance for Industry: Current Good tissue Practice (CGTP) and Additional Requirements for Manufacturers of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps).

Fertility Treatments and the Potential for Disease Transmission

Donor Gametes (continued)

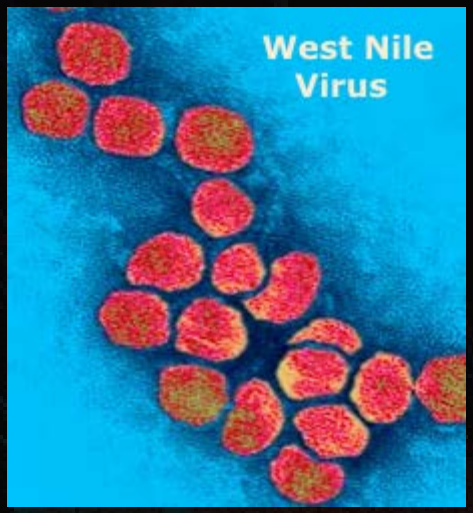
Disease screening for All Donors

- HIV 1 & 2 antibodies
- HBV surface antigen
- HBV core antibody
- HCV antibody
- HIV 1 & HCV antigen (NAT)

Defer donors with risk factors for CJD (Creutzfeldt-Jakob disease)

Additional disease screening for Sperm Donors

- Syphilis
- Chlamydia
- HTLV-I & II antibody
- CMV antibody
- Gonorrhea
- HSV 1 & 2*
- HPV*



Defer donors with confirmed or suspected WNV infections[1]

Defer donors recently receiving smallpox vaccine[2]

* Not required but performed by many sperm banks

1. ASRM/SART (2008) Position statement on West Nile virus. Fertil Steril (2008) 90:S270-S271.
2. SART/ASRM (2008) Position statement on donor suitability of recipients of small pox vaccine (vaccinia virus). Fertil Steril 90:S572-S273.

Fertility Treatments and the Potential for Disease Transmission

Gestational Surrogates and Carriers

As with donor gametes, these involve third party reproduction

- Must prevent infection of the third party
- FDA requires that for surrogacy, the male infertility patient be screened as a sperm donor[1,2]
- For a gestational carrier, both male and female patients must be screened
- No screening of the surrogate or gestational carrier is required

1. FDA. Human cells, tissues, and cellular and tissue-based products; final rule. Fed Register. 2007(April 1):714-17. [21CFR1271]
2. FDA, Center for Biologics Evaluation and Research (2007) Guidance for Industry: eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps).

Infectious Disease
and the Infertility Patient

3. Protection of staff

Infection Control Plans

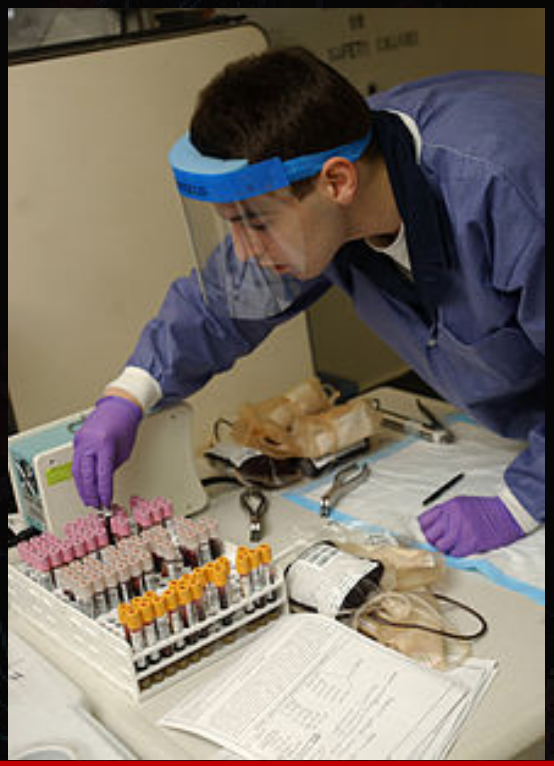
- Nosocomial infections include illnesses contracted by health care personnel following occupational exposure to pathogens carried by patients
- OSHA in the USA established regulations to protect laboratory personnel from contracting diseases spread through contact with blood and other body fluids
  - Laboratories must implement the Universal Precautions (developed by the CDC)
  - Laboratories must implement an infection control plan: written SOPs that cover all aspects of infection control

1. Department of Health and Human Services, Centers for Disease Control and Prevention (CDC) and National Institutes of Health (NIH) Biosafety in Microbiological and Biomedical Laboratories. Fifth Ed. 2007. http://www.cdc.gov/OD/ohs/biosfty/bmbl5/BMBL_5th_Edition.pdf
2. Department of Labor, Occupational Health & Safety Administration (OSHA) Bloodborne Pathogens Standard (29 CFR 1910.1030), Hazard Communication Standard (29 CFR 1910.1200), Occupational Exposures to Hazardous Chemicals in Laboratories (29 CFR 1910.1450), Exit Routes Standards (29 CFR Subpart E 1910.35, 1910.36, 1910.37, 1910.38 and 1910.39), and Electrical Standards (Subpart S - Electrical 29 CFR 1910.301 to 1910.399).

Protection of Staff

What are Universal Precautions?

Handle all body fluids as if they contain bloodborne pathogens



This includes

- Labeling of areas, materials & containers considered infectious
- Hand hygiene
- Protective barriers (PPE)
- Care in the use of sharps

These precautions are used for every patient, regardless of whether they are known to have an infectious disease or not

Department of Labor, Occupational Health & Safety Administration (OSHA) Bloodborne Pathogens Standard (29 CFR 1910.1030)

Infection Control SOPs

Each laboratory should have written standard operating procedures (SOPs) covering every aspect of infection control, including:

- Personnel training
- Facility design
- Sanitation of facility & equipment
- Air quality
- Personal protective equipment (PPE)
  - Gloves
  - Hair covers, shoe covers
  - Lab coat, gown, jumpsuit that cover arms & wrists
  - Goggles/Face masks
  - Splash shields
  - Biosafety hood
- Hand hygiene
- Use of sharps

- Prevention of aerosolization
- Storage of biohazardous materials
- Disposal of biohazardous materials
- Spill cleanup

Infection Control SOPs (continued)

The main biohazards in Andrology Laboratories are semen, sperm suspensions and unfixed smears on slides

- Train each employee with regular re-training and assessment; ensure that custodial staff are trained
- Sharps SOP – what sharps are permissible in the laboratory; how to use and dispose of sharps

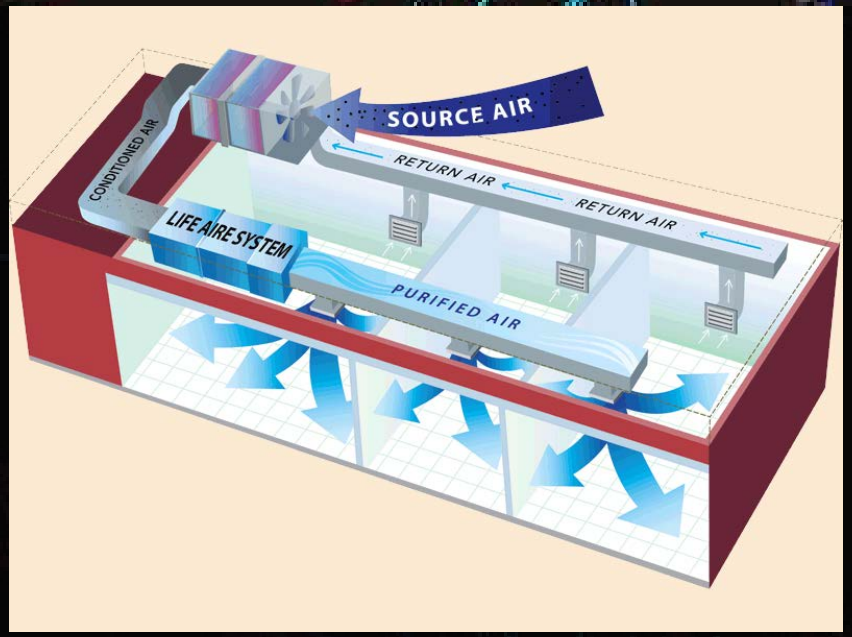
- Prevent aerosolization of semen and sperm suspensions – during centrifugation, use tightly sealed tubes that pass the 95 kPa pressure test and/or screw top caps on centrifuge cups

- Storage of biohazards – Biohazard in refrigerators /freezers ; biohazardous waste
- Disposal of biohazardous waste – according to local and national regulations

Facilities – Hygiene – Air Quality

Laboratory air quality is important in infection control; Ideally:

- Air entering the lab is filtered to protect specimens from contamination
  (positive pressure also supports this)
- Air leaving the lab is filtered to protect outside air from contamination
  (negative pressure also supports this)

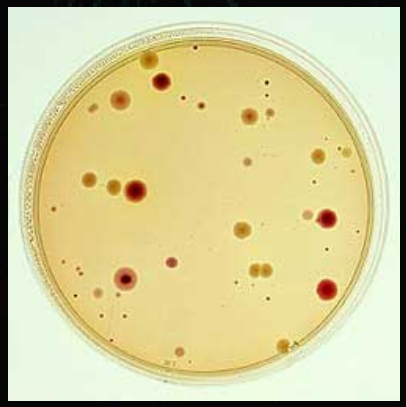


- Air quality can be monitored by settle plate testing (next slide)

Note: although the tests described on upcoming slides are for non-pathogenic microorganisms, routine monitoring helps insure that microorganisms in general are not being transferred from specimens to surfaces, media and patient samples

Air Quality – Settle Plate Testing

- There are a variety of methods for monitoring microbial contamination in air
- Settle plate testing-simple and measures the microorganisms that fall passively onto a culture plate
- Microorganisms in air are generally associated with skin cells or other particulates and deposit at about 1 cm/sec
- Sensitivity depends on plate surface area and exposure time
- Vacuum-assisted systems increase the volume of air falling on the plate but this may not replicate the contamination process



- Generally, the plate is placed in the work area during routine processing of patient specimens and closed when processing is complete
- After appropriate incubation, contamination is measured in colony forming units (CFU) per unit area per unit time

Settle Plate Testing for Microbial Contamination in Air

1. Warm 3 TSA (trypticase soy agar) plates to room temperature for each sampling location. Label each plate with the location.
2. Include a negative control (e.g. biosafety hood) and a positive control (e.g., high traffic area outside the laboratory)
3. Pace the plates in the sample areas and remove the lids during a routine process (e.g., semen processing; passing embryos into the lab), then replace the lids.
4. Incubate plates media-side-up, within a zip-lock bag with a damp paper towel.
5. Incubate 2 plates for 48 – 72 hours at 35° ± 1°C to detect bacterial contamination
6. Incubate 1 plate at room temperature for at least 5 days for mold detection
7. When incubation is complete, count the colonies. Record growth greater than 100 colonies as TNTC (too numerous to count). Record results on a QC-Microbial Monitoring Form.
8. The negative control plate should have no growth. Negative Control Remedial Action
9. The positive control should exhibit growth of both bacteria and mold. Positive Control Remedial action
10. If more than 5 colonies grow from any of the work counter plates, this is evidence of contamination. Work Area Remedial Action

Sanitizing the Work Area

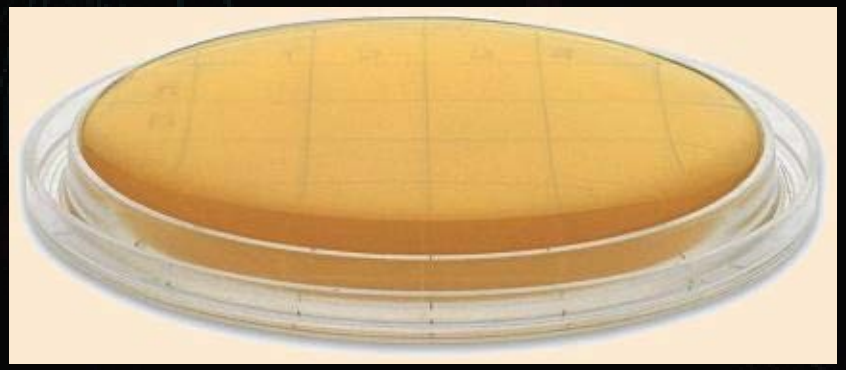
Aseptic conditions are required when sperm are processed for insemination and when oocytes/embryos are processed

- The agents used to sanitize surfaces must have low cytotoxicity or be removed completely
- A germicide may be used (some recommend Roccal II), followed by extensive rinsing with sterile water
- 70% ethanol can be used, but it has relatively low toxicity to microorganisms. Hydrogen peroxide increases sterilization activity.
- After cleaning the surface, saturate with 70% ethanol and allow to dry
- All surfaces in the laboratory must be cleaned on a regular schedule
- Liquid waste not autoclaved or incinerated within a container can be poured into 10% bleach for sterilization before discarding via the sewer

Surveillance of Surface Contamination

- Touch samples can be used to assess the contamination of surfaces
- RODAC (replicate organism detection and counting) plates have the agar in a convex bump on the surface that can be pressed directly onto the surface to be evaluated

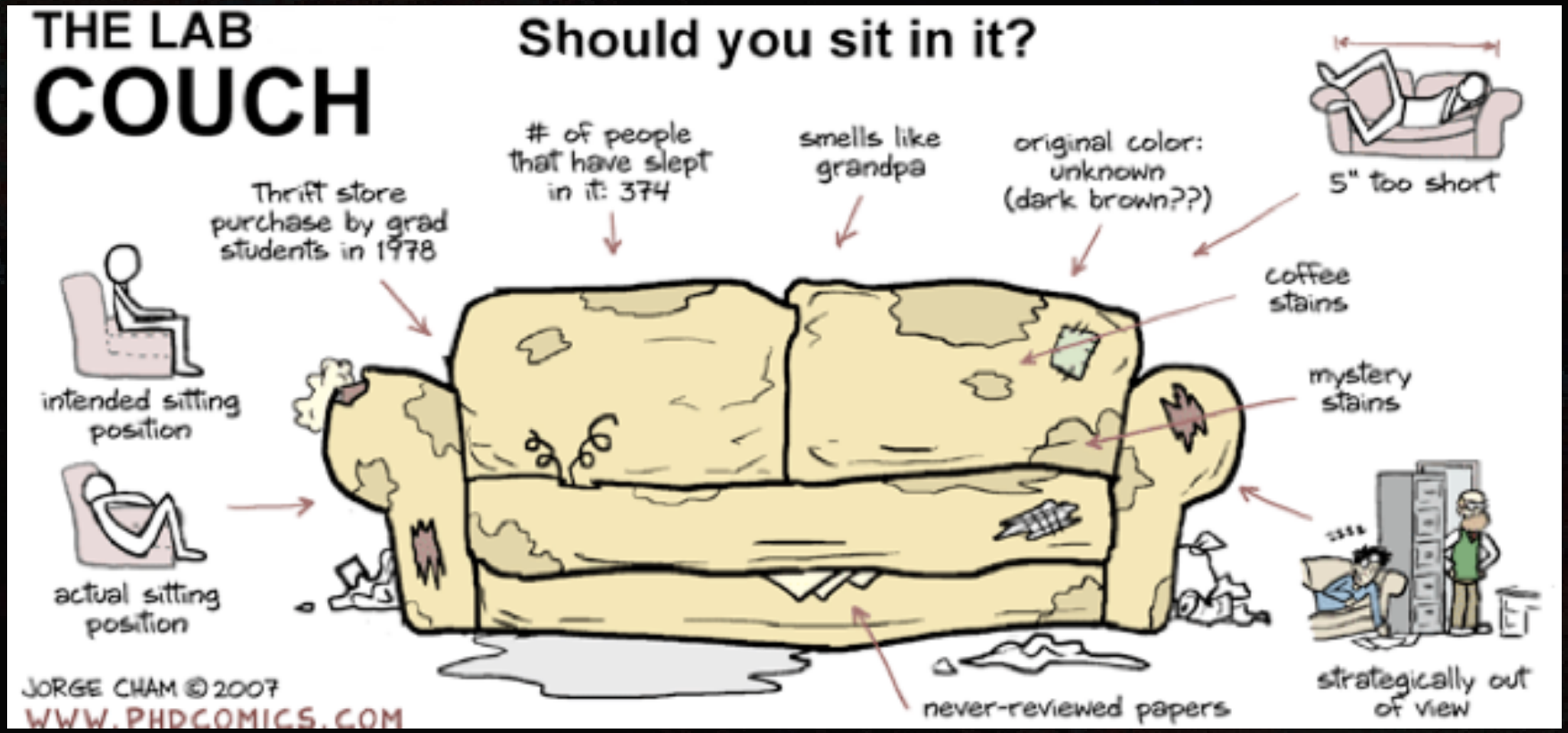
- For most laboratory surfaces, the test area can be touched firmly while wearing a sterile glove. The gloved fingers are then touched to the agar surface of a TCA plate for incubation and counting of CFUs.



- Alternatively, a drop of sterile water can be applied to the surface, then collected and spread on the agar surface

Sanitizing the Work Area (continued)

- Patient facilities in the semen collection room that contact the patient should be sanitized after each patient visit
- This Includes the Couch! all surfaces, including under the cushions, should be made of materials that can be routinely sanitized

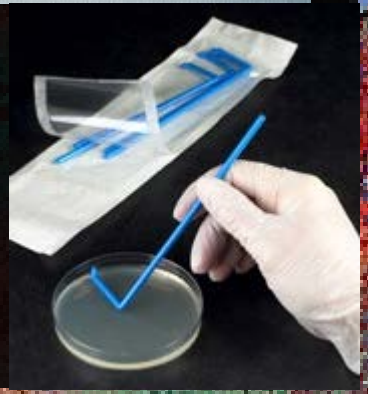


Surveillance of Media Contamination

- The phenol red, which is contained in (or can be added to) some media, is a pH indicator, which will turn more orange-yellow when the medium is contaminated

- Turbidity (lack of transparency) can also be routinely monitored
- Products are available that involve dipping a strip in the medium, incubation for a set time and counting of CFUs

- TSA plates can be used by sampling the media with a sterile swab and streaking it on the agar or by spreading on the surface of the agar before standard incubation for bacteria and mold



Infectious Disease
and the Infertility Patient

4. Cross-contamination of specimens in the laboratory

Cross-Contamination of Specimens in the Laboratory

Prevention of cross-contamination is based on:

- Cleanliness
- Separation of specimens from different patients
- One person conducts procedure (hand-offs are critical processes)

Important Note: hair and skin cells contribute significantly to microbial contamination

Lab coat/gown should be covered by gloves

cross-contamination of Specimens in the Laboratory

Separation in Space and Time

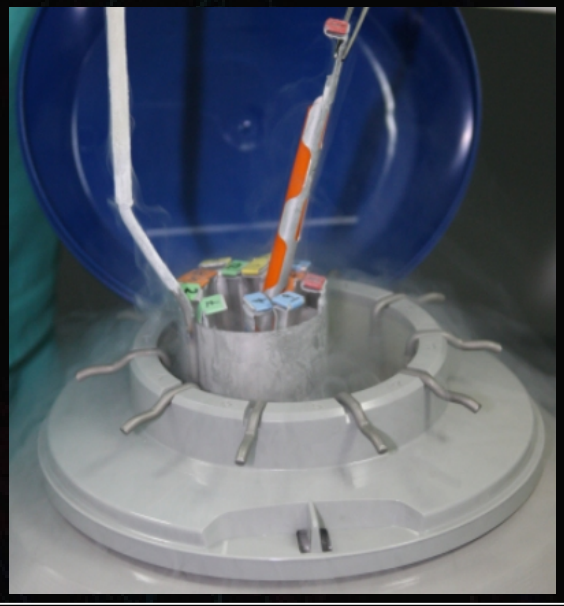
- Insemination procedures (particularly those involving semen from virus-positive patients) should be separated in "space and time" from those for other patients to reduce risk of cross-contamination
- Disposable, sterile contact materials
- Separate facilities/instruments/equipment
- A physically separate area for different patients
- Scheduling virus-positive patients at a different time allows:
  - Undivided attention of personnel
  - Time to sanitize completely, the collection room, work area and equipment before handling specimens from other patients
- Process critical samples within a biosafety cabinet

cross-contamination of Specimens in the Laboratory

Cryopreserved Storage

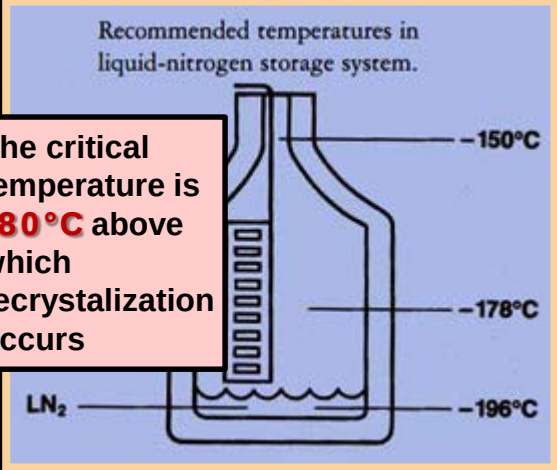
Some reproductive tissues require cryopreservation

- FDA regulations and ASRM guidelines cover processing, labeling and storage of frozen tissues, and specify the laboratory conditions to be used
- Regulations/guidelines are intended primarily to prevent cross-contamination of specimens
- It is well-known that liquid nitrogen (LN_2) in Dewars can become contaminated resulting in cross-contamination of cryovial /straw contents and disease transmission[1-6]



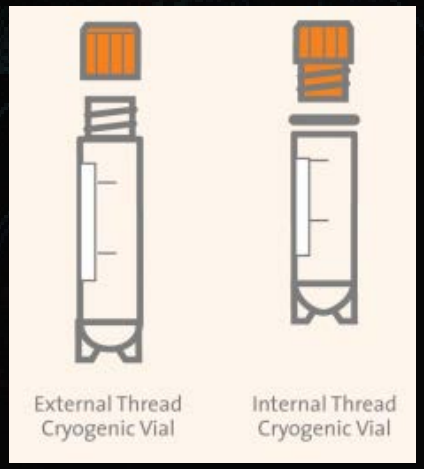
1. Bielanski, A. (2005) Experimental microbial contamination and disinfection of dry (vapour) shipper Dewars designed for short-term storage and transportation of cryopreserved germ plasm and other biological specimens. Theriogenol 63:1946-1957.
2. Bielanski, A. Bergeron, H. Lau, P.C.K. and Devenish, J. (2003) Microbial contamination of embryos and semen during long term banking in liquid nitrogen. Cryobiol 46:146-152.
3. Bielansk, A. and Vajta G. (2009) Risk of contamination of germplasm during cryopreservation and cryobanking in IVF units. Hum Reprod 24:2457-2467.
4. Chen, H.-I., Tsai, C-D., Wang, H-T. and Hwang, S-M. (2006) Cryovial with partial membrane sealing can prevent liquid nitrogen penetration in submerged storage. Cryobiol 53: 283-287.
5. Morris, G.J. (2005) The origin, ultrastructure, and microbiology of the sediment accumulating in liquid nitrogen storage vessels. Cryobiol. 50:231-238.
6. Pomeroy, K.O., Harris, S., Conaghan, J., Papadakis, M., Centola, G., Basuray, R. and Battaglia, D (2009) Storage of cryopreserved reproductive tissues: evidence that cross-contamination of infectious agents is a negligible risk. Fertil Steril 94:1181-1188.

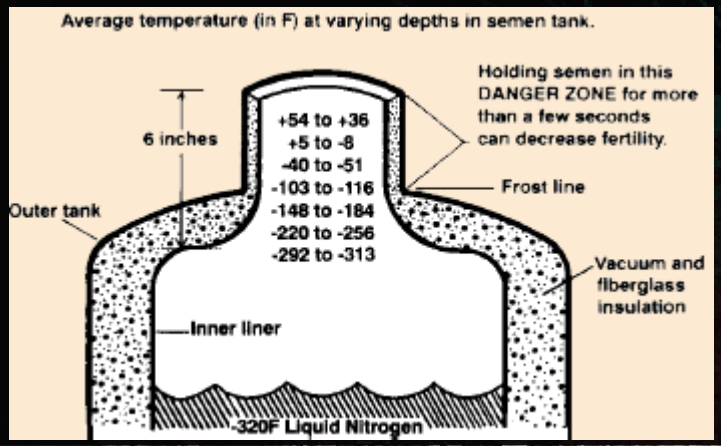
Current methods to prevent cross-contamination during liquid nitrogen storage include:



The critical temperature is -80°C above which recrystalization occurs

- Internal threaded cryovials – not 100% hermetic seal
- Specialized tubing covering cryovials – requires heat sealer; inconvenient to remove a single vial
- Specialized straws – straws are not widely used for semen in the U.S.; but hermetically sealed straws are gaining popularity for oocytes and embryos
- Storage in nitrogen vapor – may not preserve low temperature during transfer and removal of units





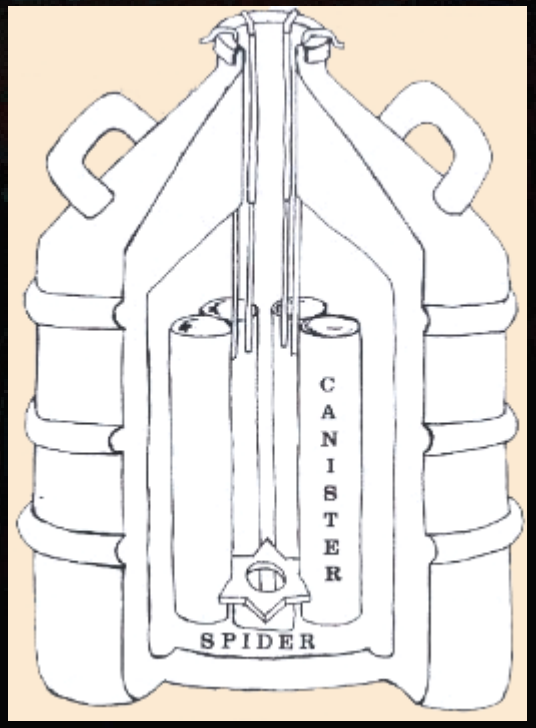
Testing Dewars for Microbial Contamination

Working in the sterile hood, label one 50 mL conical tube and two room temperature TSA plates for each Dewar to be tested and 2 plates each for the negative and positive controls.
Open Dewar and use measuring stick to stir up sediment from the bottom of the tank.
Remove the tube lid. Using long forceps, lower the tube into the liquid nitrogen until the tube contains approximately 50 mL.
Replace the lid loosely on the tube and transfer it rapidly to a tube rack in the sterile hood.
Allow the nitrogen to evaporate completely.
Within the sterile hood, remove tube lid, add 500 µL of sterile water, replace the lid firmly.
Use a vortex mixer and inversion to allow the water to rinse the entire inside of the tube.
Remove the tube lid and dip a sterile swab into the water until saturated.
Remove TSA plate lid and swipe the surface of the agar with the cotton swab without disturbing agar surface. Alternatively, pipet 0.5 mL of liquid onto the agar surface, spread in a thin film across the entire surface of the agar with a sterile cell spreader Replace the plate lid.
As a negative control, use 500 µL sterile water to rinse and sample a sterile tube. As a positive control, use contaminated water to rinse and sample a sterile tube.
Incubate plates media-side-up, within a zip-lock bag with a damp paper towel.
Incubate 1 plate for 48 to 72 hours at 35° + 1°C for bacteria growth. Incubate the remaining plates at room temperature (20-25°C) for at least 5 days for mold detection.
When incubation is complete, count the colonies. Record on QC-Microbial Monitoring Form.
Test failure: the sterile water grows more than 5 colonies (negative control failure) or the contaminated water shows no growth (positive control failure).
If more than 5 colonies grow from any Dewar, decontaminate, evaluate contamination sources .

Sanitizing Liquid Nitrogen Dewars

LN_2 Dewars should be sanitized if they fail microbial monitoring and on a regular schedule

- Empty and allow Dewar to warm



- Can remove central “spider” on some tanks
- If size of tank neck permits, inner surfaces can be sanitized by hand following laboratory’s SOP for sanitizing surfaces
- For small diameter necks, fill the tank with sanitizing solution, then rinse extensively with sterile water

That's All Folks!

Thank You....