SPERM ONLY PLEASE: Prevention of infections in an artificial insemination program

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UNIVERSITY OF PRETORIA

ESHRE Campus workshop
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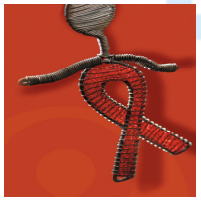
CONTENT

I. INTRODUCTION



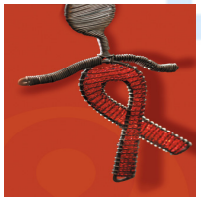
'Semen contains vitality & heredity, not germs': seminal discourse in the AIDS era[1].

Infection and contamination control in ART laboratory[2-4]**:**

- **Environment** (inside & outside of lab e.g. *Pseudomona* spp. in water, fungal spores in air)
- **Staff** (individuals & techniques)
- **Facilities & supplies** (equipment, eg. cryostorage tanks; media)
- **Patients & specimens**

1. Khan *et al*. *J Health Popul Nutr* 2006; **24**:195-200;
2. Englert *et al*. *Hum Reprod Update* 2004; **10**:149-162; 3. Elder, Baker & Ribes. Infections, Infertility and Assisted Reproduction 2005; 4. Magli *et al*. *Hum Reprod* 2008; **23**:1253-1262

I. INTRODUCTION



Infection and contamination control in ART laboratory[1]:

Patients & specimens (*semen*)

- Prevalence of pathogens (origin)
- Risk reduction (prevent)

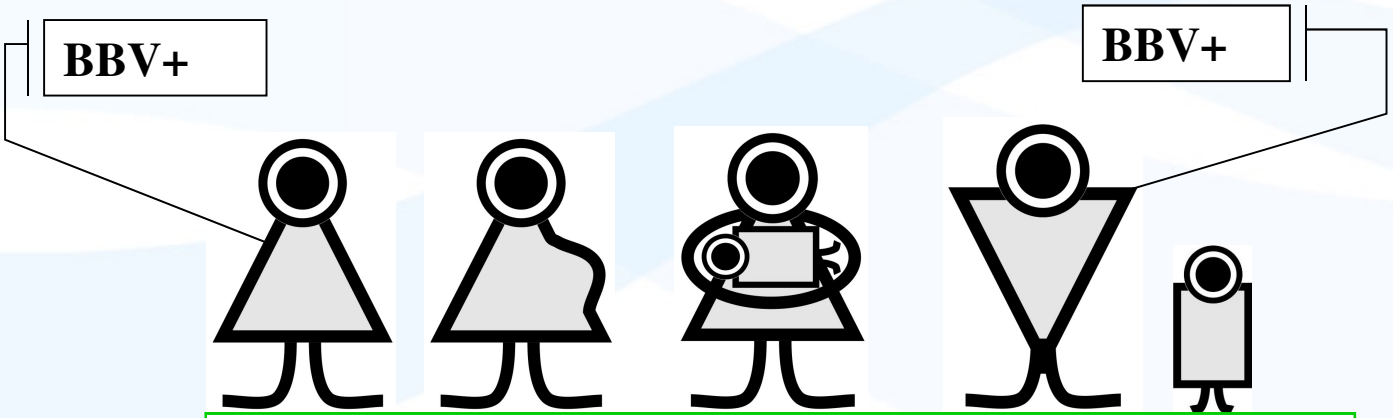


Fig 1: Prediction of reproductive potential

1. Elder, Baker & Ribes. Infections, Infertility and Assisted Reproduction. 2005

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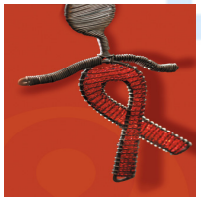


Infection and contamination control in ART laboratory[2]:

Patients & specimens (*semen*)

- Prevalence of pathogens (origin)
- Risk reduction (prevent)

Fig 1: Prediction of reproductive potential

1. Elder, Baker & Ribes. Infections, Infertility and Assisted Reproduction. 2005

II. AIM

- The prevalence of pathogens *(origin)*[1] and methodologies to prevent infections *(risk reduction)* from ***male** → **female*** in an ART program[2]:

***Male: HIV-seropositive and the female HIV-seronegative**

through:

- **Screening of patients**, thorough counselling, appropriate treatment and applying appropriate **semen decontamination** & **ART (IUI) procedures**

1. Dejucq-Rainsford & Jégou. *Curr Pharm Des* 2004; **10**:557-75
2. Englert *et al*. *Hum Reprod Update* 2006; **10**:149-161

CONTENT

III. PREVALENCE - Bacteria

Prevalence of micro-organisms in semen samples[1]:

Coagulase negative staphylococci

Ureaplasma sp.

α-Haemolytic streptococci

Escherichia coli

Enterococcus faecalis

Enterococcus sp.

Mycoplasma sp.

Staphylococcus aureus

1. Fourie *et al. Andrologia* (in preparation); **2.** Radhouane *et al. BMC Infec Dis* 2007; **7**:129-138

III. PREVALENCE - STI's

Besides HIV, the most common STIs reported include[1-2]:

chancroid,
human papiloma virus,
herpes simplex,
trichomoniasis and candidiasis,
while **gonorrhoea**,
syphilis and **chlamydia** also contribute
to damages of Fallopian tubes.

1. Bambra *Hum Reprod Update* 1999; **5**:1-20; **2.** Ochsendorf *Andrologia 2008;* **40***:72-75*

III. PREVALENCE – Interaction STD & HIV

- Association of sexually transmitted diseases (STDs) and HIV[1]
  Epidemiologic synergy –
  Transmission: *Susceptibility* & *Infectiousness*
  Duration of infectiousness:
  Rate of progression/recovery, recurrence of STDs
- STDs seems to have a stronger effect on *susceptibility* to HIV than on *infectiousness* of HIV; **treatment of STDs** in HIV+ patients should be targeted[1]
- **Impact on:**
  - **seminological parameters**[1-2],
  - **leukocytospermia** and/or seminal **viral load**[1,3] and
  - **shedding**[4] (e.g. cytomegalovirus[5], *Herpes simplex*, *Candida* or *Trichomonas* infections[6])

1. Røttingen *et al*. Sex Transm Dis **28**:579-597; 2. Bezold *et al*. *Fertil Steril* 2007; **87**:1087-1097;
3. Xu *et al*. *J Infect Dis* 1997; **176**:941-7; 4. Gupta *et al*. *J Infect Dis* 2000; **78**:1321-1323;
5. Sheth *et al*. *J Infect Dis* 2006; **193**:45-48; 6. Reichelderfer *AIDS* 2000; **14**:201-7

III. PREVALENCE – Viruses (origin)

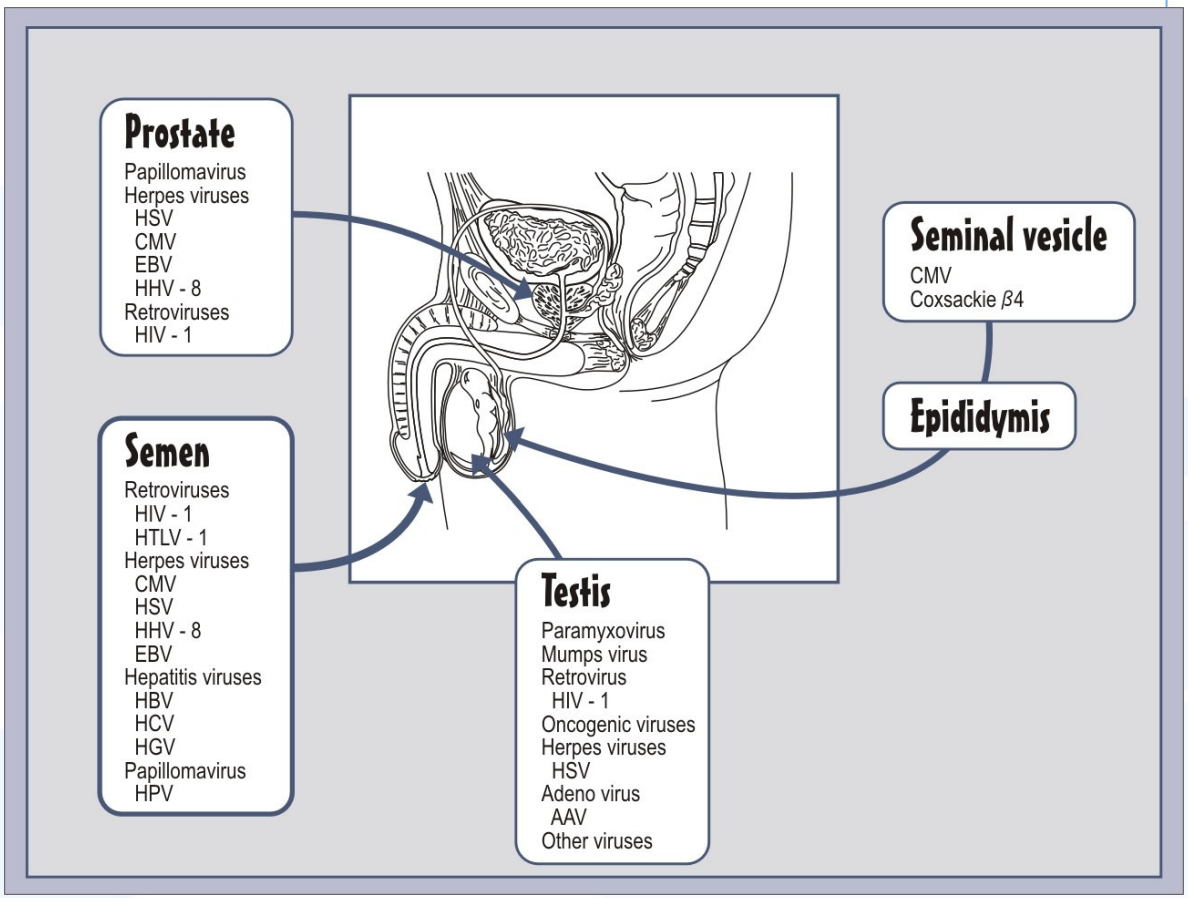


Fig 2: Summary of viruses identified in the genital tract of the male

Adapted from Dejucq-Rainsford & Jégou. *Curr Pharm Des* 2004;**10**:557-75

III. PREVALENCE – Viruses (blood-semen loads)

Table 1: HIV-1 RNA detection before and after preparation according to viral load in blood[1]

Blood viral load	Semen	%	Sperm sample
<50	7/41	17	0/41
>50-<1,000	7/20	35%	0/20
>1,000-<10,000	4/8	50%	0/8
>10,000	15/16	94	**6/16**
Total:	33/85	39	6/85

- **Blood** viral load impact on **seminal** viral load[1]
- **Seminal viral load** impact on efficiency of **sperm washing**[2]

  Washed sperm samples (DGC + SU) contaminated with HIV-1 RNA, when seminal plasma **>1 x 10^6 copies/ml**

1. Englert *et al. Hum Reprod Update* 2004; **10**:149-162;
2. Fiore *et al. Fertil Steril* 2005; **84**:232-234

CONTENT

IV. RISK REDUCTION

Screening & counselling of male patient

Decisions:

- **Diagnostic**
  - **Infectious disease tests**
    - Swab – bacterial infections
    - Blood - viral load/CD4 count
    - Semen – spermiogram
    - - bacteria culture
      - viral load - DNA/RNA
- **Treatment**
  - Antibiotics/HAART
  - Semen decontamination technique
    - Density gradient centrifugation (DGC)
    - Washing cycles/Swim-up/Inserts
  - Viral validation - DNA/RNA
- **Appropriate ART procedure** – **IUI** - IVF/ICSI

IV. RISK REDUCTION – semen washing

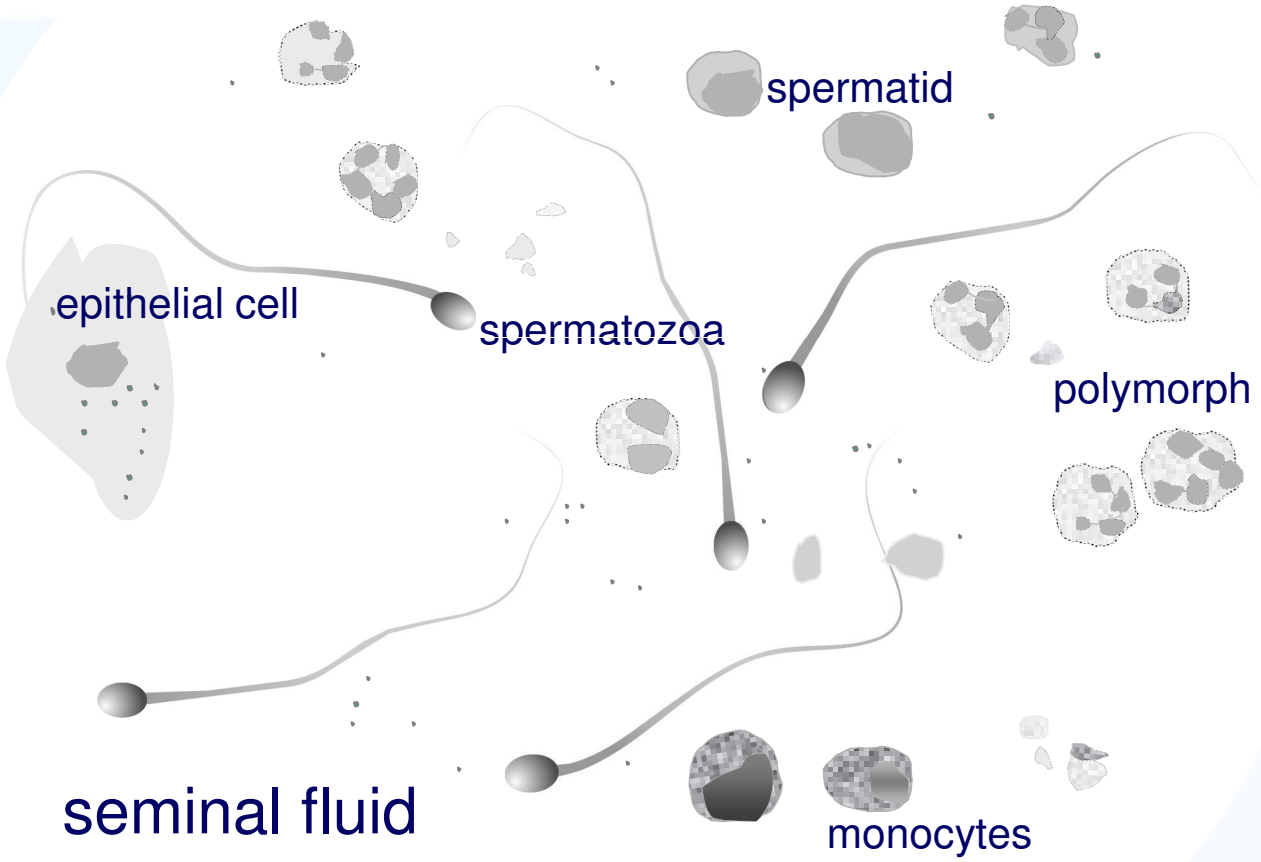


Fig 3.1: Schematic presentation of spermatozoa and non-sperm cells
(not according to scale)

IV. RISK REDUCTION – semen washing

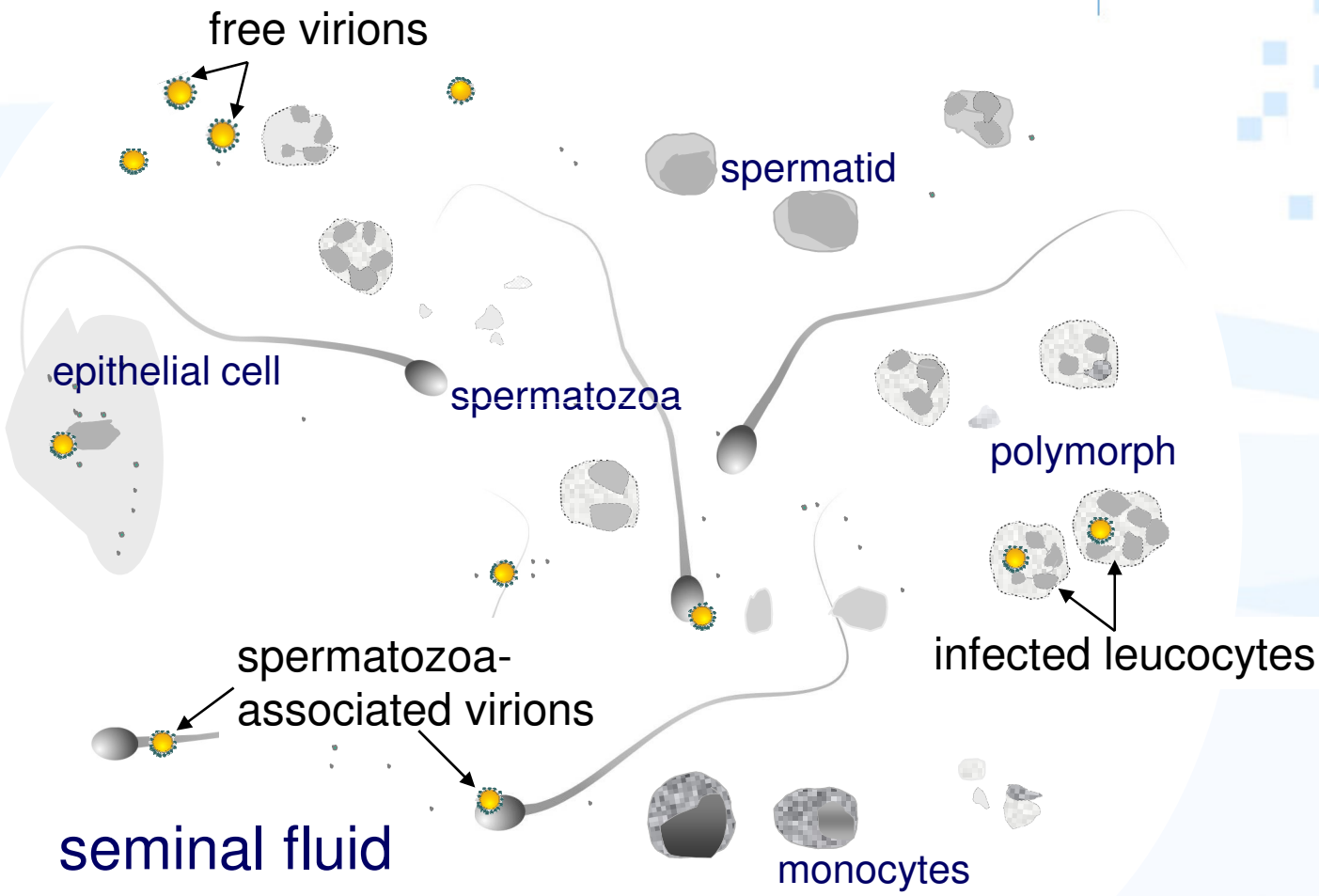


Fig 3.2: Schematic presentation of spermatozoa and non sperm cells, with viral particles (not according to scale)

IV. RISK REDUCTION – semen washing

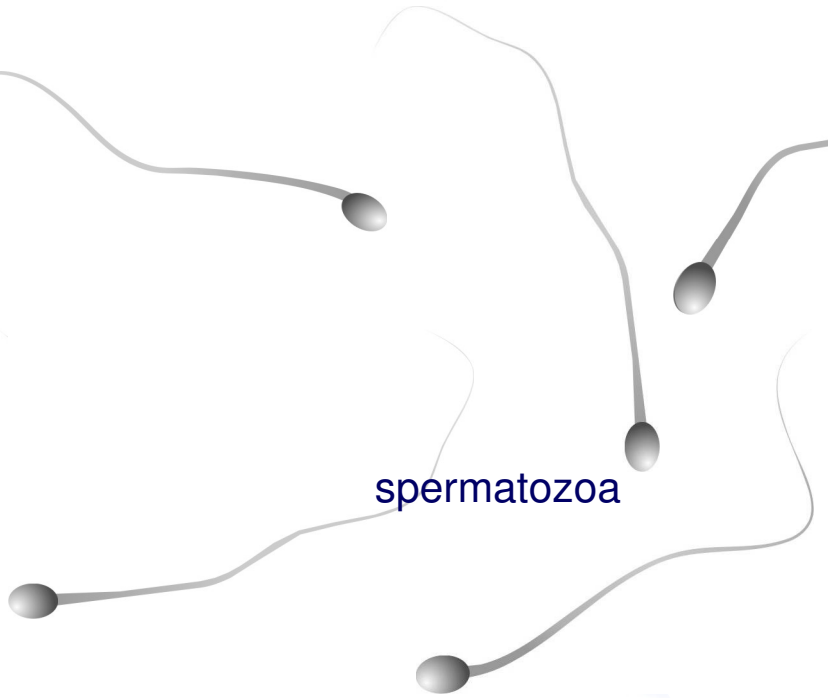


Fig 3.3: Schematic presentation of spermatozoa without contaminating cells (not according to scale)

IV. RISK REDUCTION - (DGC + tube insert 2)

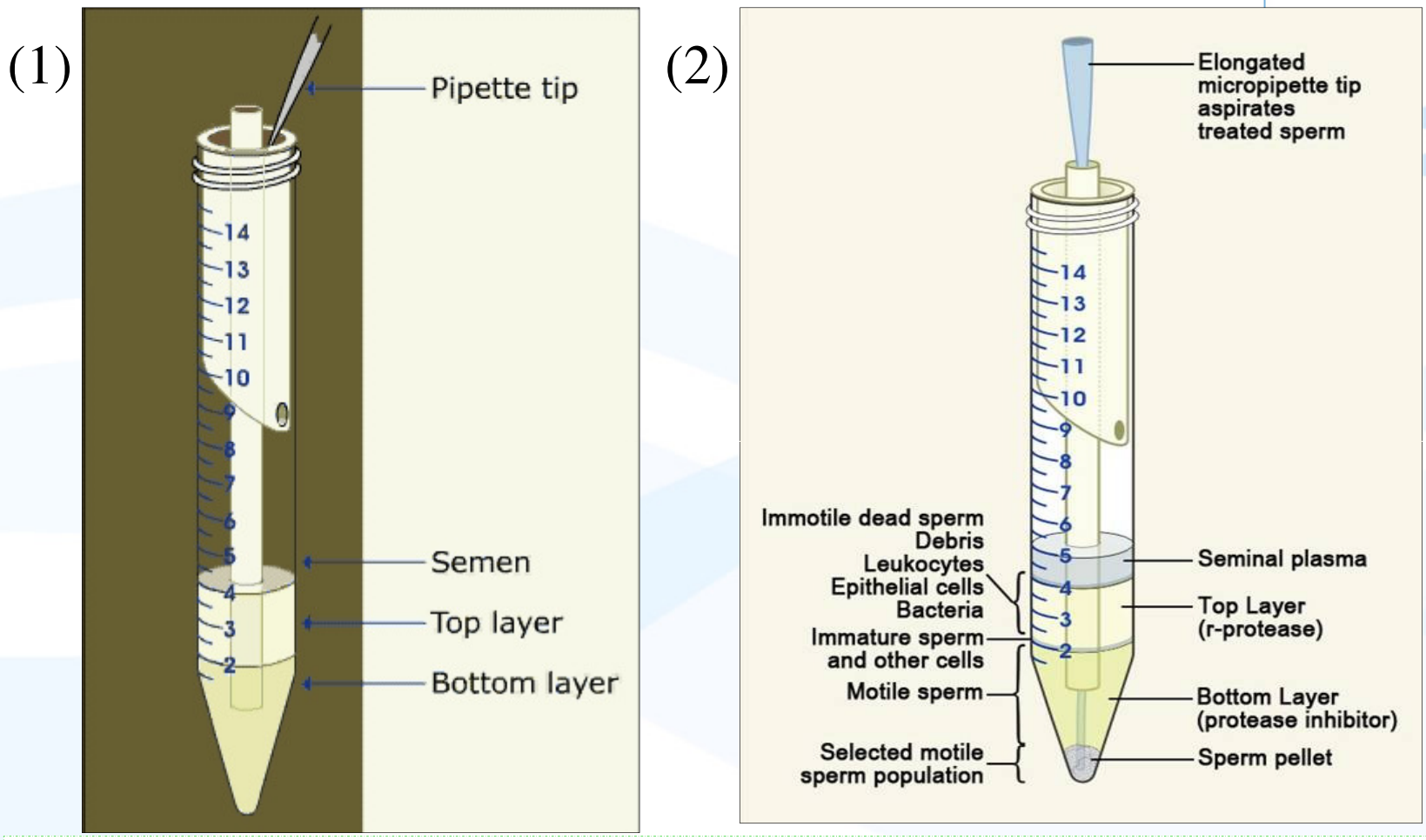
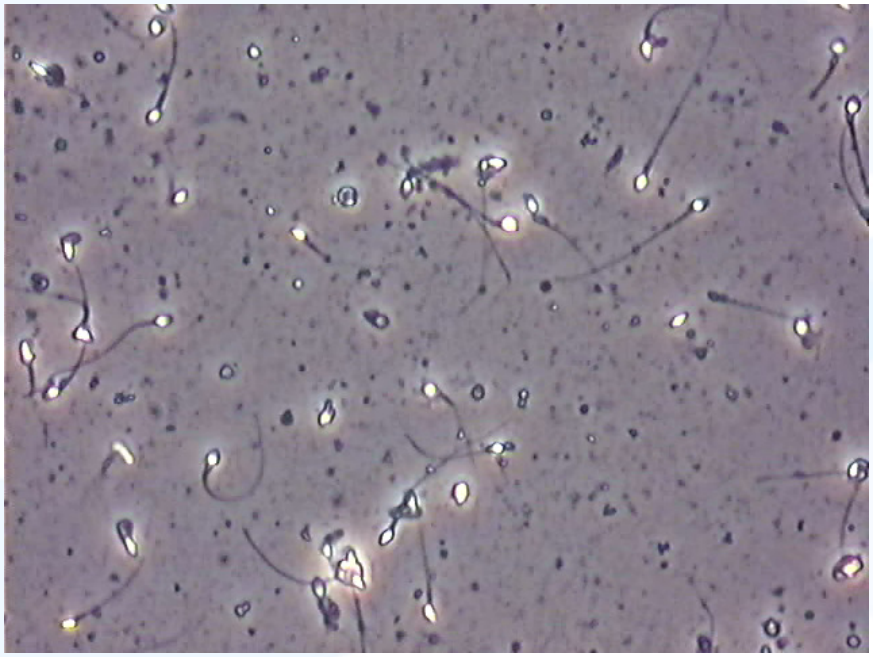


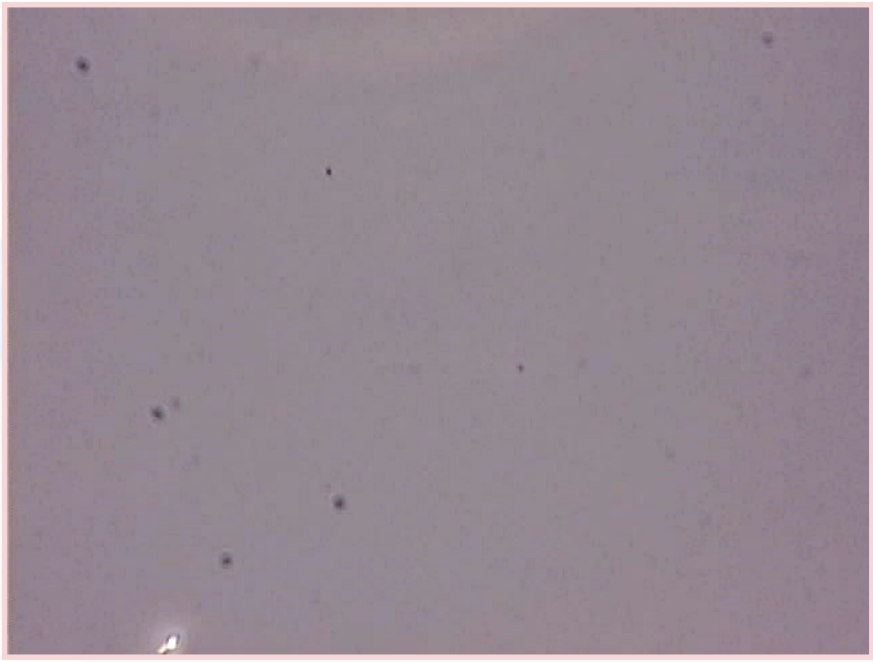
Fig 5: Schematic presentation of sperm processing using a tube insert: 1) Layering; 2) Prevention of recontamination using an elongated micro-pipette to bypass contaminated layers

Loskutoff *et al. Fertil Steril* 2006; **81**:440-447; Huyser *et al. Hum Reprod* 2006; **21**(Suppl 1)i58

IV. RISK REDUCTION - (DGC + tube insert 2 - **video**)

Raw semen	Processed and washed sperm





CONTENT:

IV. DISCUSSION – ART procedure

To test or not to test[1-3]:

- **Type of test:**
- **Various tests** with different thresholds of detection:
  - Nuclisense Kit RNA extraction; NASBA, bDNA, RT-PCR, nPCR
  - False-negative results due to inhibitors of PCR extraction or dilution below detection limit[1]

1. Persico *et al*. *Hum Reprod* 2006;**21**:1525-30; **2.** Nakhuda & Sauer. Chapter 25. In: Oehninger & Kruger: Male Infertility. 2007; **3.** Gilling-Smith *Curr Obstet Gynaecol* 2006;**16**:299-305

IV. DISCUSSION – ART procedure

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- **Type of test:**
- **Various tests** with different thresholds of detection:
  - Nuclisense Kit RNA extraction; NASBA, bDNA, RT-PCR, nPCR
  - False-negative results due to inhibitors of PCR extraction or dilution below detection limit[1]
- **What to test:**
  - **Semen** or seminal compartments
  - Purified **sperm** sample (post-processing)
  - **DNA** and/or **RNA**
- **When to test:**
  - Prior to procedure, same-day evaluation/freeze-thaw processing
  - Follow-up female partner & child

1. Persico *et al*. *Hum Reprod* 2006;**21**:1525-30; **2.** Nakhuda & Sauer. Chapter 25. In: Oehninger & Kruger: Male Infertility. 2007; **3.** Gilling-Smith *Curr Obstet Gynaecol* 2006;**16**:299-305

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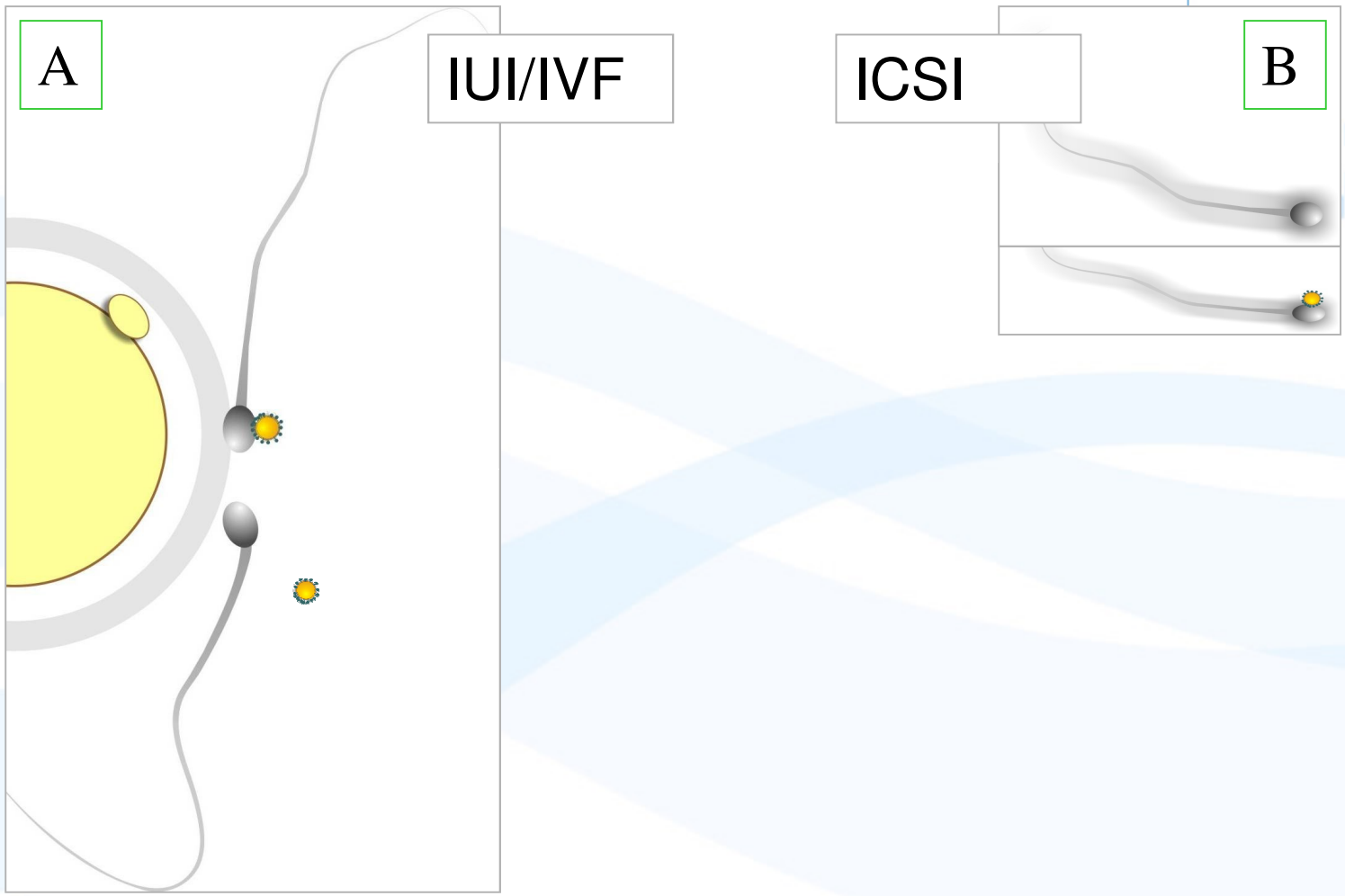

Fig 6: Conventional insemination (A) versus ICSI (B) – hypothetical risk to introduction external particles. Adapted from: Kvist U. ESHRE Campus Symposium 1-3 Oct 2009. http: www.eshre.com; See also Semprini & Fiore *Curr Opin Obstet Gynecol* 2004; **16**:257-262

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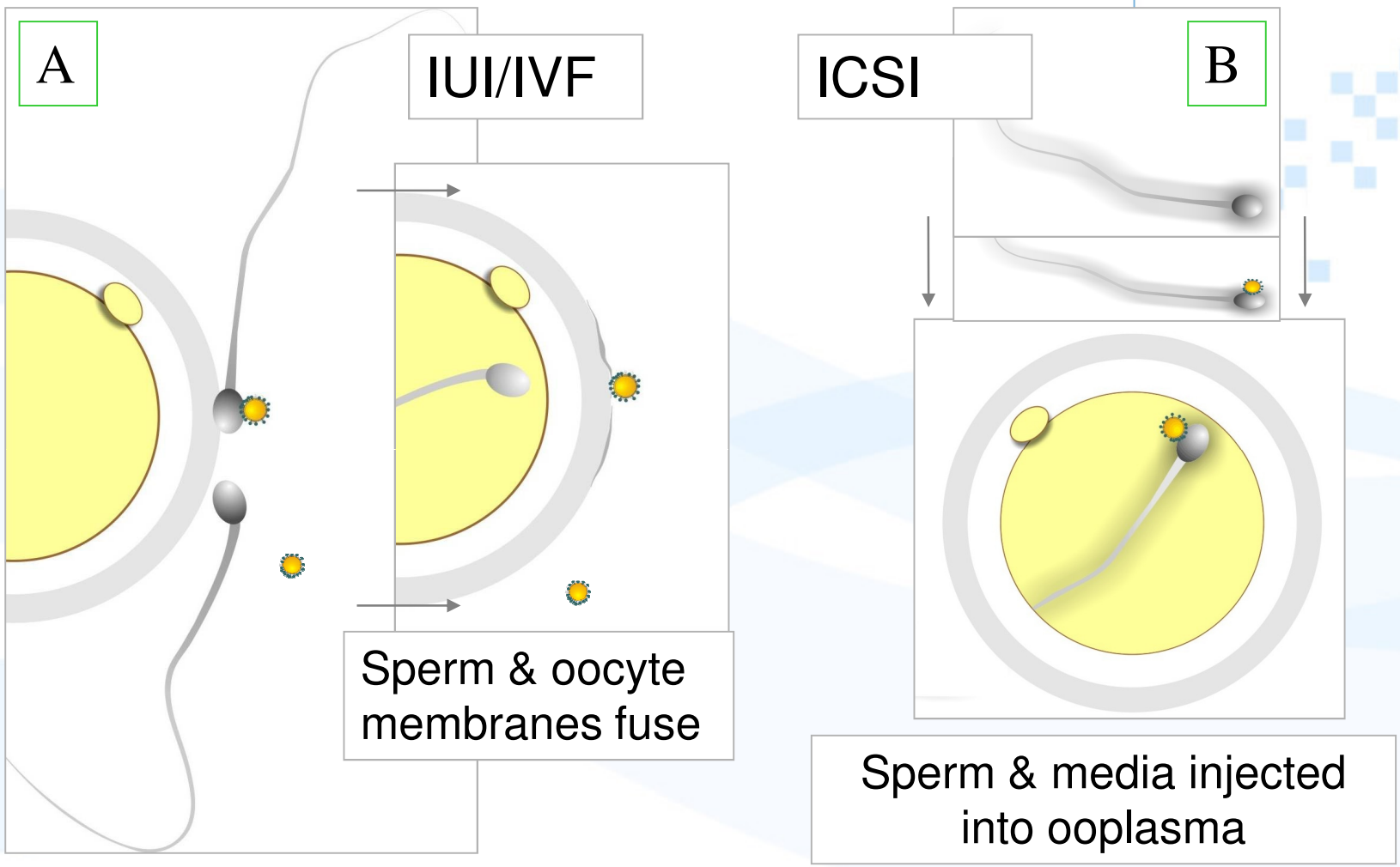

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IV. DISCUSSION – validation – HIV-1 DNA in spermatozoa

418 *Current HIV Research*, 2009, 7, 418-424

Presence of HIV-1 DNA in Spermatozoa from HIV-Positive Patients: Changes in the Semen Parameters

Walter Cardona-Maya[*,1], Paula Velilla[2], Carlos Julio Montoya[2], Ángela Cadavid[1] and María T. Rugeles[2]

[1]*Reproduction Group, University of Antioquia, Medellin, Colombia;* [2]*Immunovirology Group, University of Antioquia, Medellin, Colombia*

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Journal of Reproductive Immunology
41 (1998) 41–67

The debate on the presence of HIV-1 in human gametes

Baccio Baccetti [a,*], Arrigo Benedetto [b], Giulia Collodel [a], Antonino di Caro [b], Anna Rosa Garbuglia [b], Paola Piomboni [a]

[a] *Institute of General Biology, University and Center for the Study of Germinal Cells, C.N.R., Via T. Pendola 62, 53100 Siena, Italy*
[b] *Center of Virology, L. Spallanzani Hosp. IRCCS, Rome, Italy*

Spermatozoa capture HIV-1 through heparan sulfate and efficiently transmit the virus to dendritic cells

Ana Ceballos,[1] Federico Remes Lenicov,[1] Juan Sabatté,[1] Christian Rodríguez Rodrigues,[1] Mercedes Cabrini,[1] Carolina Jancic,[2] Silvina Raiden,[2] Mónica Donaldson,[3] Rodolfo Agustin Pasqualini Jr.,[3] Clara Marin-Briggiler,[4] Mónica Vazquez-Levin,[4] Francisco Capani,[1] Sebastián Amigorena,[5] and Jorge Geffner[1,2]

[1]Centro Nacional de Referencia para el SIDA, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires C1121ABG, Argentina
[2]Instituto de Investigaciones Hematológicas, Academia Nacional de Medicina, Buenos Aires C1425ASU, Argentina

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Journal of Reproductive Immunology
41 (1998) 161–176

JOURNAL OF REPRODUCTIVE IMMUNOLOGY

Analysis of human immunodeficiency virus in semen: indications of a genetically distinct virus reservoir

Randal A. Byrn [a,*], Ann A. Kiessling [b]

[a] *Vertex Pharmaceuticals, 130 Waverly Street, Cambridge MA 02139-4242, USA*
[b] *Department of Surgery, Division of Urology, Beth Israel Deaconess Medical Center, Harvard Institutes of Medicine, Room 353, 4 Blackfan Circle, Boston, MA 02115, USA*

Human Reproduction Vol.22, No.11 pp. 2868–2878, 2007 doi:10.10
Advance Access publication on September 12, 2007

HIV-1 viral DNA is present in ejaculated abnormal spermatozoa of seropositive subjects

B. Muciaccia[1], S. Corallini[1], E. Vicini[1], F. Padula[1], L. Gandini[2], G. Liuzzi[3], A. Lenzi[2] and M. Stefanini[1,4]

[1]*Department of Histology and Medical Embryology, Sapienza University of Rome, Rome, Italy;* [2]*Department of Medical Sapienza University of Rome, Rome, Italy;* [3]*National Institute for Infectious Disease Spallanzani (INMI), Rome, Italy*

IV. DISCUSSION – ART procedure & viral validation

The adherence of viral particles & presence of HIV in spermatozoa has been a matter of debate[1]

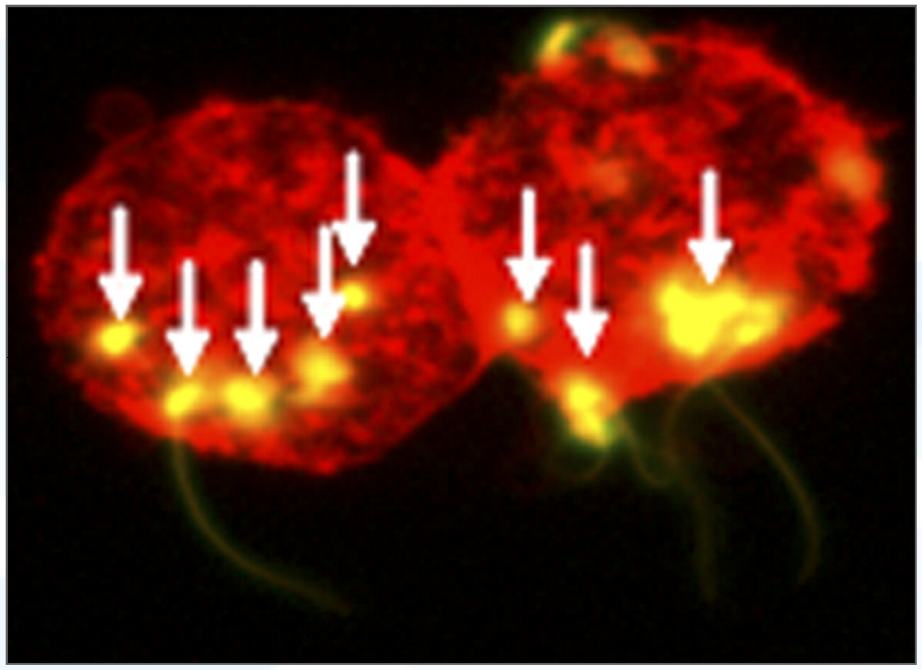

Fig 6: Spermatozoa (arrows) transmit HIV-1 when they attach to dendritic cells (DC - red)[2] Spermatozoa acted as HIV carrier; attachment increase at pH 6-7, modulating the function of mucosal DC

1. Muciaccia *et al.*, *Hum Reprod* 2007; **22**:2868-2878'
2. Cebellos *et al.*, *J Exp Med* 2009; doi:10.1084/jem.20091579 (Reproduced with permission from: Jorge Geffner, IIHEMA, Academia Nacional de Medicina Buenos Aires, Argentina)

IV. Conclusion – Current treatment, ART & sperm washing

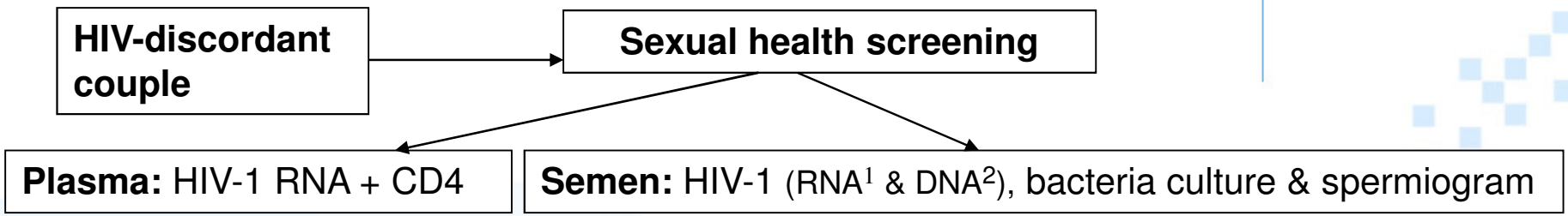

Fig 7: Flow chart for the ART management (adapted from: Ombelet *et al.*, ESHRE Monograph p64-72 2008.) **1.** RNA – quantitative Cobas Ampliprep/Cobas Taqman HIV-1, v 2, LLD 40 copies/ml;
2. DNA qualitative - Amplicor HIV-1 DNA, v 1.5, Roche Diagnostics

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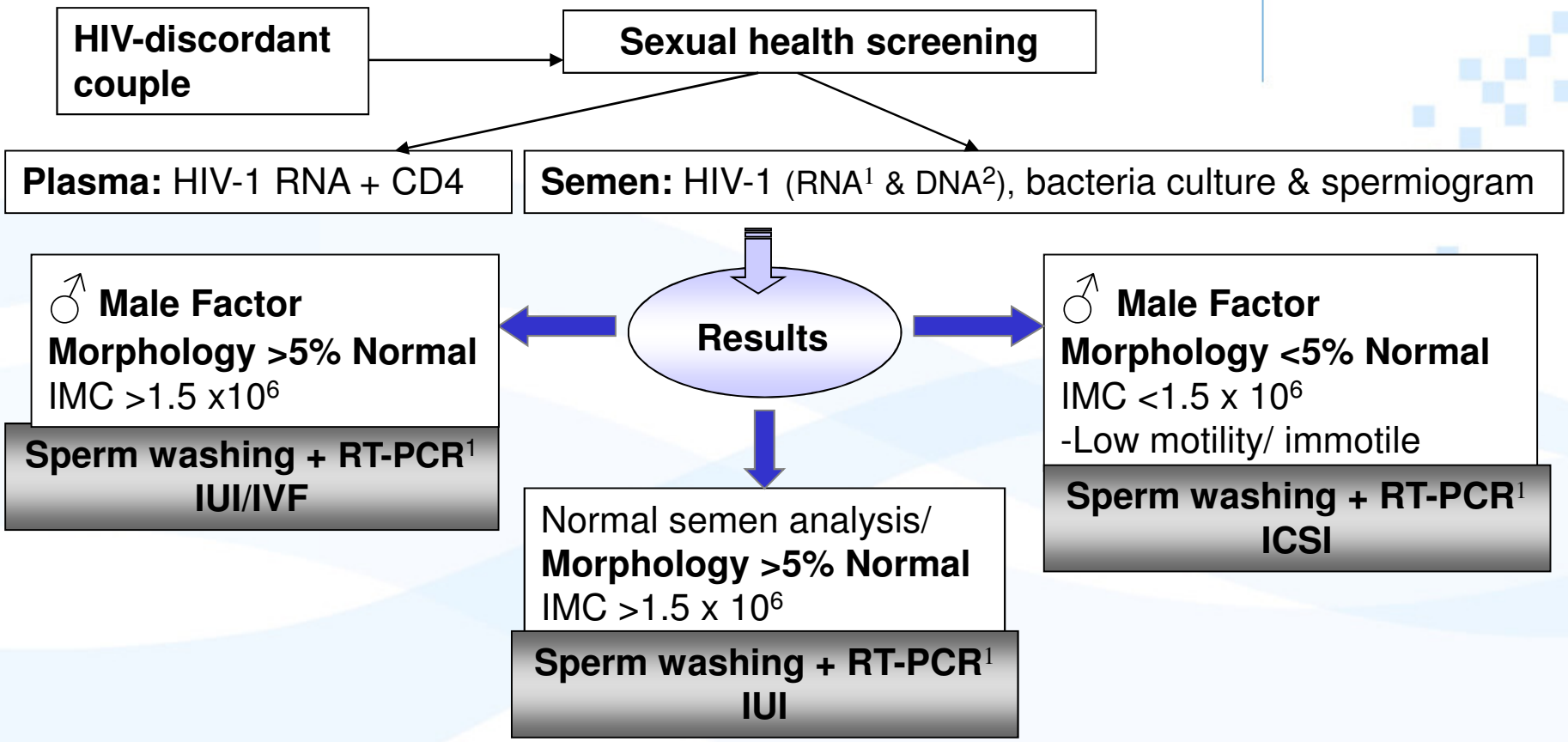

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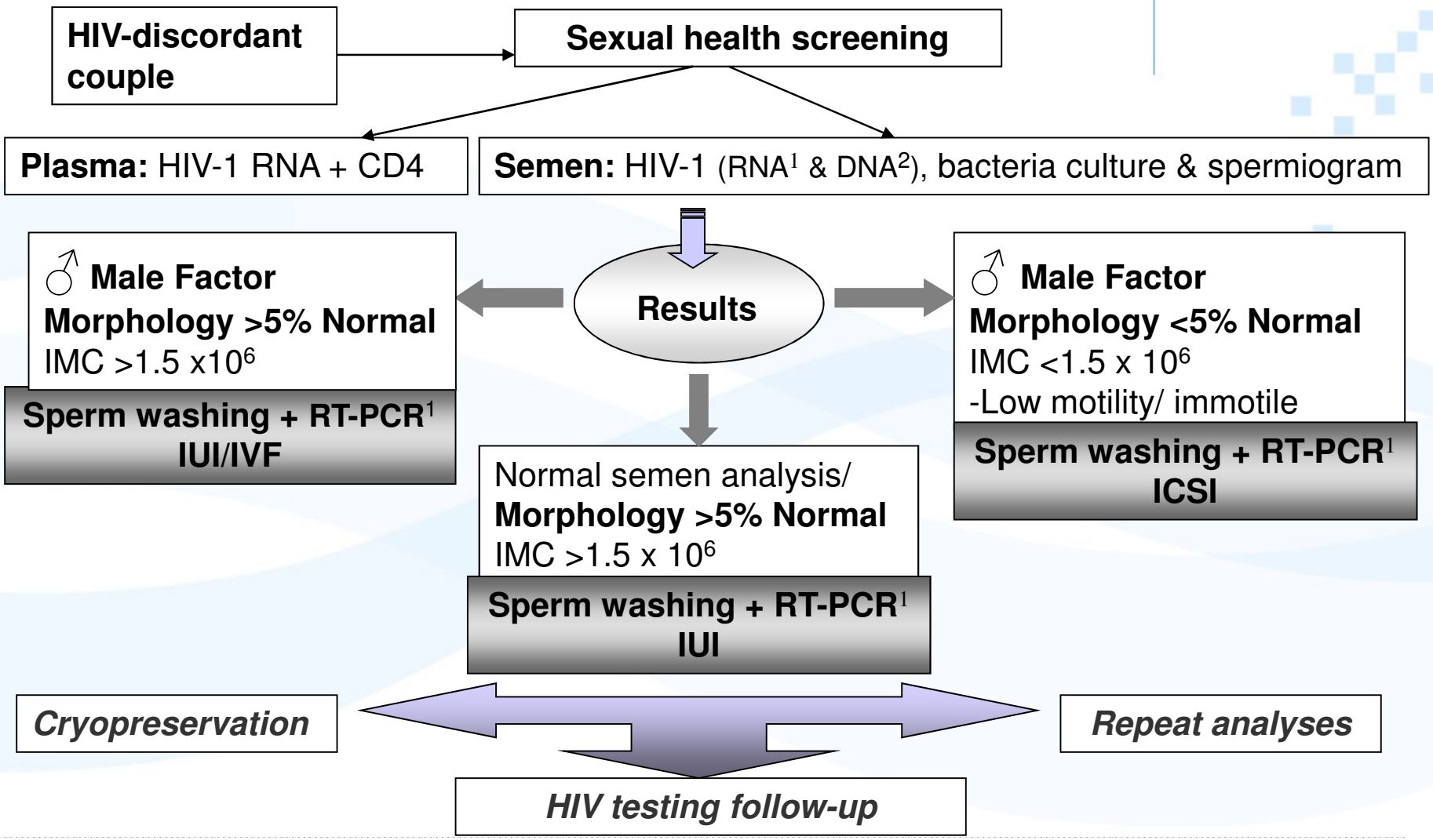

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