

REVIEW ARTICLE

Management of Infertility in HIV infected couples: A Review

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Abstract

The HIV epidemic has continued to grow and remains a major challenge to mankind. In the past, ethical considerations about the resulting child and risks of sexual, vertical and nosocomial transmission of HIV prevented practitioners from offering fertility services to people living with HIV. In recent times however, the use of highly active antiretroviral therapy (HAART), has not only improved the life expectancy and quality of life of those infected but also reduced the risk of HIV transmission. The need for fertility services in the HIV-positive population has thus increased and may be employed for management of infertility and protection from transmission or acquisition of HIV infection. As such, preconception counseling, sexual health and fertility screening have become routine in the management of HIV-positive couples. The option of care include adoption, self insemination with husband sperm, embryo donation from couples who have been verified to be HIV negative, insemination with donor sperm, timed unprotected intercourse (TUI) and sperm washing combined with intrauterine insemination (IUI) and assisted reproductive technology (ART) including in-vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI). Access to these fertility services by HIV-positive clients should be facilitated as part of efforts to promote their reproductive health and rights. (*Afr J Reprod Health 2012; 16[4]: 13-20*).

Résumé

L'épidémie du VIH a continué de croître et reste un défi majeur pour l'humanité. Dans le passé, les considérations éthiques concernant le bien-être de l'enfant et le risque de la transmission sexuelle, verticale et nosocomiale du VIH ont limité la dispensation des services de fertilité pour les personnes vivant avec le VIH. Ces derniers temps, cependant, l'utilisation de la thérapie antirétrovirale hautement active (TARHA), a non seulement amélioré l'espérance de vie et la qualité de vie des personnes atteintes, mais aussi de réduire le risque de la transmission du VIH. La nécessité de services de fertilité dans la population séropositive a donc augmenté et peut être utilisées pour le traitement des facteurs d'infertilité et de la protection de la transmission ou l'acquisition de l'infection du VIH. En tant que tel, le Counselling avant la reproduction, le dépistage de santé sexuelle et de fertilité sont nécessaires pour le traitement des couples séropositifs. L'option des soins comprendrait l'adoption, l'auto-insémination avec le sperme du mari, le don d'embryons de la part des couples qui ont été authentifiés comme étant séronégatifs, l'insémination avec le sperme du donneur, les rapports sexuels chronométrés non protégés et le lavage du sperme combiné avec l'insémination intra-utérine (IIU) et les techniques de reproduction assistée (TRA), y compris la fécondation in vitro (FIV) ou l'injection intra-cytoplasmique de spermatozoïdes (IICS). L'accès à ces services de fertilité par les clients séropositifs devrait être facilité comme faisant partie de la santé de reproduction et des droits (*Afr J Reprod Health 2012; 16[4]: 13-20*).

Keywords: HIV, AIDS, Infertility, highly active antiretroviral therapy (HAART), sperm washing, assisted reproductive technology

Introduction

The human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS) epidemic has continued to grow and remains a major challenge to mankind. According to Joint United Nations Programme on HIV/AIDS (UNAIDS) and World Health Organization

(WHO); as at December 2009, about 33.2 million people were living with HIV majority (68%) of which are in sub-Saharan Africa¹. The prevalence of HIV in most African countries has remained high. The 2010 HIV national sentinel survey in Nigeria reported a prevalence of 4.1%². The mode of transmission of HIV in Africa is predominantly heterosexual, the infection rate being more

amongst persons within the reproductive age (15 - 45 years). Until recent times the reproductive health and especially fertility needs of HIV positive clients were largely ignored. This was adduced mainly to ethical considerations of welfare of the child including the risk of transmitting the infection to the child³, reduced life span of the infected parent(s) and the issue of orphans. There were further concern about risk of transmitting infection to the partner, hospital workers and other clients. However, with the advent and increasing access to highly active antiretroviral therapy (HAART) and advancement in scientific/safe practices these concerns have been largely addressed. The use of antiretroviral drugs has thus led to significant improvement in life expectancy and quality of life in HIV infected persons⁴. Similarly antiretroviral drugs used during antenatal, intrapartum and neonatal periods; caesarean section and avoidance of breastfeeding has led to a drop in vertical transmission risk from over 30% to less than 2%^{5,6}. Scientific innovations have also ensured safe procreation for uninfected partners, protection of hospital workers and other uninfected clients. Ethical reasons for restriction of fertility care for HIV positive clients are therefore no longer justifiable^{7,9}.

The strong desires for procreation and child bearing among HIV-infected persons have been documented^{10, 11}. This has led to attempts at natural conception which exposes the partner of HIV positive person to risk of infection or re-infection/super-infection^{12, 13}. The risk of an infected male passing on HIV infection to an uninfected female partner is quoted as 0.1 - 0.3% per act of intercourse and that of infected female to uninfected male partner is 0.03 and 0.09% per act of intercourse, provided the couples are in monogamous relationship; not engaged in any other risky activities, and with absence of genital injury/infections¹⁴. Saracco et al¹⁵ reported an annual HIV transmission rate up to 7.2% in couples engaging in unprotected intercourse in HIV sero-discordant relationship.

Many HIV positive clients may seek fertility services to maximize reproductive potential and/or minimize the transmission risk to their partners and children. Professionals are expected to provide appropriate fertility services and not discriminate

against HIV positive clients¹⁶⁻¹⁹. This article reviews the critical issues in reproduction in the context of HIV infection. The available options of safe procreation and management of infertility in the HIV-positive population is discussed.

Infertility in the General Population

It is estimated that 8-12 percent of couples suffer from infertility²⁰. In the African society where high premium is placed on child bearing, infertile couples are socially ostracized. They face much psychological upheaval and are usually desperate for solution. Infertility may be due to factors in one or both partners. Problems in the male may be quantitative or qualitative abnormalities in the semen. The female causes of infertility may be functional or occlusive tubal/peritoneal factors and endocrine/ovulatory dysfunction. The tubal/peritoneal problems are mainly due to sexually transmitted infections (STIs) / pelvic inflammatory diseases (PID), post partum/post abortal infections, adhesions from previous surgeries and endometriosis. Endocrine/ovulatory dysfunction may be in form of polycystic ovary syndrome (PCOS), hypothalamic dysfunctions and premature ovarian failure. Other contributory factors to infertility include erectile dysfunction, Asherman's syndrome and uterine fibroids²¹.

The management of infertility includes investigations to determine the associated factors and application of appropriate treatment. The standard investigations include seminal fluid analysis in the male. In the female, the tubal/peritoneal factor is assessed using hysterosalpingogram (HSG), or hysterosalpingo contrast sonography (HyCoSy) and/or laparoscopy and dye test. The endocrine / ovulatory status may be assessed by early follicular phase hormone profile (i.e. day 2-5 luteinizing hormone (LH), Follicle stimulating hormone (FSH), estrogen (E₂) prolactin and testosterone) and mid-luteal phase progesterone. Pelvic ultra-sound scan is also necessary to exclude uterine and ovarian pathology. Hysteroscopy may also be performed to evaluate the uterine cavity. The treatment depends on identified problem which may be standard ovulation induction, tubal surgery and partner/donor insemination. The more advanced methods of infertility treatment - assisted

reproductive technology (ART) including in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) may occasionally become indicated.

Effect of HIV on Fertility

Higher rates of infertility have been reported among the HIV-positive group compared to the general population. This has been attributed to many factors. Infertility in the HIV-positive couple may be voluntary as they might be using condom regularly during sexual intercourse. This is particularly relevant in the context of sero-discordant couple, in order to avoid transmitting the infection to the HIV-negative partner. The use of condom has remained the most effective means of preventing transmission of HIV in sexually active persons²². Reduction in the frequency of sexual activity as a result of chronic ill health and for psychological reasons may also be contributory. HIV-positive females are more prone to tubal/peritoneal factors from increased susceptibility to infections and PID²³. Adverse effects on the hypothalamo-pituitary ovarian axis and reduced ovarian reserve have also been suggested²⁴. HIV-positive men tend to have reduced seminal parameters (including count, motility and morphology)²⁵. A recent report also suggested that the success rates in IVF/ICSI may be reduced in the HIV-positive clients due primarily to poor ovarian response²⁶. Early recourse to fertility screening and treatment is therefore necessary to optimize chances of success.

Management of Infertility in HIV-Positive Patients

While it is generally recommended that HIV-positive clients should be allowed access to fertility services, there is yet no generally accepted protocol for their management. However, interest in the subject is developing rapidly. When indicated, standard investigations, medical / surgical treatment modalities and assisted reproductive technology used in the management of infertility in the general population applies. The main principles in the management include reduction of the level of infectiousness in infected

partner(s) and reduction of exposure and susceptibility especially in uninfected partner. Pre-conception reproductive counseling, sexual health and fertility screening are recommended in the determination of the preparedness and type of fertility services needed^{17, 18, 27}. It is essential that the HIV-positive couples who contemplate pregnancy should be on an appropriate antiretroviral therapy to suppress viral load whilst maintaining a satisfactory health state and thus reduce the risk of transmission to the partner and/or baby. Hence the need to commence antiretroviral treatment and/or ensure adherence should be emphasized. Counseling and education on safe sex practices should be provided and condom should be used throughout the period of fertility treatment, pregnancy and the postpartum. The care of the HIV positive persons contemplating pregnancy should be multidisciplinary, comprising of HIV physician, fertility specialist and obstetrician with a special interest in HIV.

Pre-conception Reproductive Counseling

Preconception reproductive counseling should be provided to the partners individually and together before commencement of fertility treatment. It entails provision of information on available reproductive options, the inherent risks, costs, likely chance of success and failure of each option. Counseling message should be able to differentiate between risk free and risk reduction methods of fertility management. It should address issues of welfare of the child when one or both partners are sick and or die and the potential social and psychological impact of parental HIV status on the offspring. The counseling should explore the risk of sexual and vertical transmission of HIV. The chance of mother - to - child transmission of HIV (MTCT) if the female is infected and the available strategies of prevention should be discussed. This would enable clients to make informed choice and give consent for treatment

Sexual Health Screen

The presence of Chlamydia, gonorrhoea, syphilis, herpes genitalis, trichomonas vaginalis, bacteria vaginosis, Hepatitis B virus, Hepatitis C virus and

other sexually transmitted infections increases the risk of acquisition or transmission of HIV virus²⁸. Screening and treatment of these conditions is a necessary step prior to infertility management.

Fertility Screen

The aim of the fertility screen is to identify any potential fertility factor which may delay conception and hence increase exposure period. It will also assist in defining the optimum mode of treatment. Seminal fluid analysis is undertaken in the male. In the female, tests include early follicular phase (day 2 - 5) endocrine profile - follicle stimulating hormone, luteinizing hormone, oestrogen, and prolactin; mid-luteal serum progesterone for detection of ovulation; pelvic ultrasound scan for uterine and ovarian factors; hysterosalpingogram (HSG), or hysterosalpingo contrast sonography (HyCoSy) and/or laparoscopy and dye test for tubal patency test. Diagnostic hysteroscopy may also be undertaken to evaluate the uterine cavity.

Option for Conception in the Context of HIV Infection

The available options for achieving pregnancy depends on if the couples are HIV sero-discordant or sero-concordant for HIV. The various options are shown in Table 1. These include- adoption, self insemination of husband sperm, insemination with donor sperm, timed unprotected intercourse (TUI) and sperm washing combined with IUI, IVF or ICSI.

Adoption

In a well adjusted relationship, adoption can serve as means of fulfilling the desire for a child in the family. It is a risk free measure and may be useful in the context when one or both partners are HIV infected. The child is however, not a biological product of either partner. The health situation of the couple(s) could be an obstacle in the adoption process. Adoption is yet to be popularly accepted in the African society. The potential social and psychological impact of parental HIV status on the adopted child should also be considered.

Self Insemination with Partner's Semen

Timed self insemination of partner's semen by an infected woman at the time of her ovulation has been used as a risk free means of achieving pregnancy when the male partner is uninfected.²⁹

Insemination with Donor Sperm

This involves the use of sperm from an HIV-negative donor for artificial insemination. This practice effectively removes the risk of HIV seroconversion in the uninfected female partner as the donor will have been confirmed to be HIV negative before the sperm is used. This option of fertility treatment is applicable when the male partner is HIV-positive but the female partner is HIV-negative. It however, removes the chance of genetic parenting in the male.

Timed Unprotected Intercourse (TUI) - Natural Conception

This involves the practice of engaging in unprotected sexual intercourse during the fertile period of the woman's menstrual cycle. There is likelihood that overtime the uninfected partner may acquire the infection. It is also possible for infected partner to acquire another variant or drug resistant virus. Its use prior to the period of HAART was associated with high risk of transmission. The use of HAART is effective in reducing plasma levels of HIV RNA and expectedly results in reduction in risk of HIV sexual transmission³⁰. Although the risk of HIV transmission through unprotected intercourse when the patient is on HAART is not yet fully quantified recent reports indicate that transmission risks is significantly reduced if the viral load is undetectable. Recently some authors³¹ advocated the use of unprotected intercourse limited to the fertile window period in the woman's cycle when the infected partner is on HAART and viral load is undetectable or less than 1000 copies /ml as an option for couples with no access to other safer methods of achieving pregnancy. A prospective cohort study of 453 HIV sero-discordant couples reported a dose effect for infected patients with no transmission in cases where the infected partner had plasma viral loads of less than 1000 copies/ml³².

Table 1: Management Options in HIV- Positive Couples

HIV Status	Management Options
Female Sero-discordant Couple (Male +ve, Female –ve)	Timed unprotected intercourse (infected partner should be on antiretroviral treatment) Insemination with donor sperm Sperm wash + intrauterine insemination Sperm wash + In-vitro fertilization Sperm wash + Intracytoplasmic sperm injection Adoption
Male Sero-discordant Couple (Male –ve, female +ve)	Timed unprotected intercourse (infected partner should be on antiretroviral treatment) Self-insemination of partners semen Adoption
Sero-concordant Couple (Male +ve, Female +ve)	Any of the above

Barreiro et al³³ in their study found no seroconversion in 62 discordant couples who became pregnant when the viral load was undetectable in the infected partner. However, under certain conditions viral shedding in semen persists even in men with fully suppressed plasma viral load because of different compartmentalization of HIV in plasma and semen³⁴. Unprotected intercourse in the setting where one or both partners are HIV-infected is potentially unsafe.

Sperm Wash

Sperm wash is an established, safe and effective risk-reduction method of obtaining HIV free spermatozoa (free of seminal plasma and semen cells such as leucocytes) for intra uterine insemination (IUI) and other methods of assisted reproductive technology. The method of sperm washing was pioneered by Semprini et al³⁵. The technique is based on the observation that HIV is present free in seminal fluid and as cell-associated virus in leucocytes and non-spermatozoa cells (NSC) but is not capable of attaching to, or infecting spermatozoa. Sperm wash involves the process of sperm migration on density gradient centrifugation, repeated washing of the migrated pellet followed by spermatozoa swim up procedure. In practice sperm obtained after 3-7 days of abstinence is centrifuged in a 40-80% colloidal, silica density gradient to separate progressively motile HIV-free sperm from

NSC/leucocytes and seminal plasma, which remain in the supernatant. The sperm pellet at the bottom is resuspended in fresh medium and centrifuged twice before final swim up. Polymerase chain reaction (PCR) test for HIV RNA or DNA is performed on aliquot of the final sample. This is recommended as part of quality control to confirm that the final product is free of HIV particles. While this method is effective in separating spermatozoa from the free virus and other components of seminal fluid which could harbor the virus, detectable viral load have been reported in up to 8% of samples tested after sperm wash procedure^{14,36}. The washed sperm can be used for IUI, IVF and ICSI. A multicentre study from the Centre for Reproductive Assistance Techniques for HIV in Europe (CREAThE) showed that sperm wash procedures is safe and effective in preventing sexual transmission of HIV to uninfected partners³⁷. In this report, there were no seroconversion in the partners who had 2840 IUI, 107 IVF, 397 ICSI and 49 frozen embryo transfers cycles, followed up for over 6 months after assisted reproduction attempt. The safety of washed sperm has also been attested to by other workers^{38,39}.

Sperm Wash combined with Intrauterine Insemination (IUI), In-Vitro Fertilization (IVF) and Intracytoplasmic Sperm Injection (ICSI)

Some authorities advocate that washed sperm be used primarily for IUI in a normal (unstimulated)

cycle at the time of ovulation. Sperm wash combined with IUI appears to be simple, easy and of low cost. Due to increased presence of infertility factors in HIV-positive clients, sperm washing may be combined with ovulation induction, IVF or ICSI. This is the recommended practice in many European countries and United Kingdom^{14,27,40}.

Other experts prefer sperm washing combined primarily with ICSI as the treatment of choice even in the absence of any fertility factor^{41,42}. This is based on the premise that IUI requires the insemination of large numbers of sperm to be effective and typically multiple cycles of insemination to achieve a pregnancy. In ICSI only one sperm is used per oocyte thereby providing a theoretical advantage by minimizing oocyte contact with potentially contaminated seminal plasma and semen cells such as leucocytes. Because the semen analysis is often abnormal in HIV-positive men, the use of ICSI in infertility management become even more relevant in this group of clients. The theoretical risk of injecting spermatozoa contaminated with HIV viral particle is a limitation to this practice. ICSI is costly, more labor intensive, less accessible and may be associated with higher risk of obstetric complications than IUI. However, in a 10 year review of 420 consecutive cycles of sero-discordant couples where ICSI with washed sperm was the procedure, the authors recorded no case of maternal or perinatal HIV transmission⁴³.

Safety Issues in Infertility Management of HIV-Positive Clients

Handling and freezing gametes and embryos from patients who are HIV-positive carries risk of cross-contamination to samples from HIV-negative patients and health workers. Contamination with HIV, Hepatitis B and C has been documented in ART settings^{44,45}. This is one reason why some centres are reluctant in offering fertility treatment to HIV positive clients. Recently some guidelines and recommendations on laboratory and patient care have been put in place to minimize this risk^{14,46,47}. The key issues in the recommendation includes-

- a) *Screening of all clients undergoing ART for HIV and hepatitis B and C.*
- b) *Handling of potentially infected samples in separate laboratory or laboratory area with dedicated equipment (eg. incubators, flow hoods), alternatively if space and cost are issues, to schedule HIV-positive cases to be last in work list or at a different time to HIV-negative ones.*
- c) *Cryopreservation in separate cryostorage tanks for each infection and infection combination*
- d) *The use of "double bagging" or sealing techniques to prevent the direct contact of cryocontainers with liquid nitrogen*
- e) *The storage of samples in liquid nitrogen vapour instead of in liquid nitrogen itself*
- f) *The use of sperm washing techniques to decrease the viral load before freezing semen samples*
- g) *Strict observance of universal safety precautions including adequate decontamination process (1% hypochloride or other suitable chemicals) and single-use disposable materials.*
- h) *Quality management systems with detailed and regular risk assessment / audit*

Uncertain Issues in Infertility Management in HIV-Positive Couples

Although much can now be offered the HIV positive couples there are issues yet to be resolved. In the setting of an HIV-positive woman, the process of ART treatment involves many invasive procedures (including IUI, oocyte retrieval and embryo replacement) that can contaminate the gamete or embryo and may result in HIV transmission⁴⁸. There is the need to monitor patients and babies conceived through these procedures.

Conclusion

Increasing demand for fertility services among HIV positive couples have been documented in many countries. The current trend is mainly attributable to the availability and use of HAART which has made HIV infections more of a chronic but controlled medical condition. Fertility care for the HIV positive is now regarded as a right that should not be denied. Cumulative data suggest that

sperm washing protocols is safe and effective in significantly reducing or eliminating HIV from semen sample. Washed semen can be safely used with IUI, IVF and ICSI. These procedures have potential to reduce sexual HIV transmission in our society with predominant heterosexual transmission. It is essential to make these services accessible to HIV positive clients.

References

- UNAIDS and WHO. AIDS epidemic update: December 2010: Geneva: UNAIDS. 2010
- Federal Ministry of Health (FMOH). Technical Report 2010. National HIV sero-prevalence sentinel survey among pregnant women attending antenatal clinics in Nigeria. Abuja. FMOH, 2010.
- Minkoff H, Santoro N. Ethical considerations in the treatment of infertility in women with human immunodeficiency virus infection. *N Engl J Med* 2000; 342: 1748-50
- Pallela FJ, Delaney KM, Moorman AC, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. *N Engl J. Med* 1998; 338: 853-60
- Johnson F. HIV and Pregnancy: National and international perspectives. In: O'Brien PMS(ed), Yearbook of Obstetrics and Gynaecology, Volume 8. Royal College of Obstetricians and Gynaecologists (RCOG) Press, London 2000; 143-153.
- Townsend C, Cortina-Borja M, Peckham C, Lyall H, de Ruiter A, Tookey P. Very low risk of mother-to-child transmission of HIV in women on HAART achieving viral suppression in the UK and Ireland. *AIDS* 2008; 22: 973-981
- Englert Y, Van Vooren JP, Place I, Liesnard C, Laruelle C, Delbaere A. ART in HIV-infected couples. Has the time come for a change in attitude? *Hum Reprod* 2001; 16: 1309-15
- Sauer MV. Providing fertility care to those with HIV: time to re-examine healthcare policy. *Am J Bioeth* 2003; 3: 33-40
- Gilling-Smith C, Smith JR, Semprini AE. Infertility and HIV: time to treat. There's no justification for denying treatment to parents who are HIV positive. *Br Med J* 2001; 322: 566-7
- Chen JL, Phillips KA, Kanose DE, Collins RL, Miu A. Fertility desires and intentions of HIV-positive men and women. *Fam Plann Perspect* 2001; 33: 144-152
- Klein J, Pena JE, Thornton MH, Sauer MV. Understanding the motivations, concerns, and desires of human immunodeficiency virus 1 -serodiscordant couples wishing to have children through assisted reproduction. *Obstet Gynecol* 2003; 101: 987-94
- Smith DM, Wong JK, Hightower GK et al, HIV drug resistance acquired through superinfection. *AIDS* 2005; 19: 1251-1256
- Gottlieb GS, Nickle DC, Jensen MA et al. Dual HIV-1 infection associated with rapid disease progression. *Lancet* 2004; 363: 619-622.
- Fakoya A, Lamba H, Mackie N, Nandwani R, Brown A, Bernard EJ, Gilling-Smith C, Lacey C, Sherr L, Claydon P, Wallage S, Gazzard B. British HIV Association, BASHH, FSRH guidelines for the Management of the sexual and reproductive health of people living with HIV infection 2008. *HIV Medicine* 2008; 9: 681-720
- Saracco A, Mussico M, Nicolosi A, Angarano G, Arici C, Gavazzeni G, et al. Man - to woman sexual transmission of HIV: longitudinal study of 343 steady partners of infected men. *J Acquir Immune Defic Syndr* 1993; 6: 497-502
- Frodsham LCG, Boag F, Barton S, Gilling-Smith C. Human immunodeficiency virus infection and fertility care in the United Kingdom - demand and supply. *Fertil Steril* 2006; 85: 285-9
- The ESHRE Ethics and Law Task Force. Taskforce 8 ethics of medically assisted fertility treatment for HIV positive men and women. *Hum Reprod* 2004; 19: 2454-6
- Ethics Committee of the American Society for Reproductive Medicine. Human immunodeficiency virus and infertility treatment. *Fertil Steril* 2002; 77: 212-22
- Agboghroma CO. Gynaecological and reproductive Health issues in HIV-positive women. *WAJM* 2010; 29(3): 135-42
- World Health Organization. Infertility: a tabulation of available data on prevalence of primary and secondary infertility, Geneva. WHO Programme on maternal and child health and family planning. Division of family health, 1991
- Giwa-Osagie OF, Ogunyemi D, Emuveyan EE, Akinla O. Etiologic classification and socio medical characteristic of infertility in 250 couples. *Int J Fertil* 1984; 29: 104-8
- Holmes KK, Levine R, Weaver M. Effectiveness of condoms in preventing sexually transmitted infections. *Bull World Health Organ* 2004; 82: 454-461.
- Irwin KL, Moorman AC, O'Sullivan MJ, Sperling R, Koestler ME, Soto I, et al. Influence of human immunodeficiency virus infection on pelvic inflammatory disease. *Obstet Gynecol* 2000; 95: 525-33
- Clark R, Mulligan K, Stamenovic E, Chang B, Watts H, Andersen J, et al. Frequency of anovulation and early menopause among women enrolled in selected AIDS clinical trials group studies. *J Infect Dis* 2001; 184: 1325-7.
- Duloust E, Le Du A, Costagliola D, Gulbert J, Kunstmann JM, Heard I, et al. Semen alterations in HIV-1 infected men. *Hum Reprod* 2002; 17: 2112-18
- Coll O, Fiore S, Florida M, et al. Pregnancy and HIV infection: a European consensus on management. *AIDS* 2002; 16(Suppl. 2):1-18

27. Gilling-Smith C, Nicopoulos JD, Semprini AE, Frodsham LC. HIV and reproductive care - a review of current practice. *BJOG* 2006; 113: 869-878
28. Fleming DT, Wasserhelt JN. From epidemiological synergy to public health policy and practice: the contribution of other sexual transmitted diseases to sexual transmission of HIV infection. *Sex Transm Infect* 1999; 75: 3-17
29. Savasi V, Lanzani C, Persico T, et al. Pregnancy by self insemination in women infected by HIV-1 (Abstract). 57th Annual meeting of the American Society for Reproductive Medicine. 20-25 October 2001, Orlando FL. *Fertil Steril* 2001, 76(Suppl 3) 349.
30. Castilla J, Del Romero J, Hernando V, Marincovich B, Garcia S, Rodriguez C. Effectiveness of highly active antiretroviral therapy in reducing heterosexual transmission of HIV. *J Acquir Immune Defic Syndr* 2005; 40: 96-101.
31. Vernazza PL, Hollander L, Semprini AE, Anderson DJ, Duerr A. HIV -discordant couples and parenthood: how are we dealing with the risk of transmission? *AIDS* 2006; 40(4):635-636
32. Quinn TC, Wawer MJ, Sewankambo N, et al. Viral load and heterosexual transmission of human immunodeficiency virus type 1. Rakai Project Study Group: *N Engl J Med* 2000; 342: 921-929
33. Barreiro P, Del Romero J, Leal M, Hernando V, asencio R, De Mendoza C, Labarga P, Nunez M, Ramos JT, Gonzalez-Lahoz, J. and Soriano V. Natural Pregnancies in HIV-serodiscordant couples receiving successful antiretroviral therapy. *J Acquir Immune Defic Syndr* 2006; 43: 324-326.
34. Luizzi G, Chirianni A, Clementi M, et al. Analysis of HIV -1 load in blood, semen and saliva : evidence for different viral compactments in a cross-sectional and longitudinal study. *AIDS* 1996; 10: 51-56
35. Semprini AE, Levi-Setti P, Bozzo M, Ravizza M, Taglioretti A, Sulpizio P, Giuntelli S, Pardi G and Brechot C. Insemination of HIV-negative women with processed semen of HIV-positive partners. *Lancet* 1992; 340: 1317-1319.
36. Hanabusa H, Kuji N, Kato S, Tagami H, Kaneko S, Tanaka H et al. An evaluation of semen processing methods for eliminating HIV-1. *AIDS* 2000; 14: 1611-6
37. Bujan L, Hollander L, Coudert M, Gilling-Smith C, Vucetich A, Guibert J, Vernazza P, Ohl J, Weigel M, Englert Y, Semprini AE, for the CREAThe network. Safety and efficacy of sperm washing in HIV-serodiscordant couples where the male is infected: results from the European CREAThe network. *AIDS* 2007; 21: 1909-1914.
38. Savasi V, Fernazzi E, Lanzani C, Oneta M, Parrilla B, Persico T. Safety of sperm washing and ART outcome in 741 HIV-1 sero-discordant couples. *Hum Reprod* 2006; 22: 772-777.
39. Sauer MV. Sperm washing techniques address the fertility needs of HIV-seropositive men: a clinical review. *Reprod Biomed Online* 2005; 10" 135-140
40. Marina S, Marina F, Alcolea R et al. Human immunodeficiency virus type 1 - serodiscordant couples can bear children after undergoing intrauterine insemination. *Fertil Steril* 1998; 70: 35-39
41. Sauer MV, Chang PL, Establishing a clinical program for human immunodeficiency virus 1-seropositive men to father seronegative children by means of in vitro fertilization with intracytoplasmic sperm injection. *American journal of obstetrics and gynaecology* 2002; 186: 627-33
42. Mencaglia L, Falcone P, Lentini GM, Consigli S, Pisoni M, Lofiego V, Guidetti R, Piomboni P, De leo V. ICSI for treatment of human immunodeficiency virus and hepatitis C virus -serodiscordant couples with infected male partner. *Hum Reprod* 2005; 20: 2242-2246
43. Sauer MV, Wang JG, Douglas NC, Nakhuda GS, Vardhana P, Jovanovic V, Guarnaccia MM. Providing fertility care to men seropositive for human immunodeficiency virus: reviewing 10 years of experience and 420 consecutive cycles of in vitro fertilization and intracytoplasmic sperm injection. *Fertil Steril* 2009; 91(6): 2455-60
44. Clarke GN. Sperm cryopreservation : Is there a significant risk of cross contamination? *Hum Reprod* 199; 14: 2941-3
45. Blank S, Simonds RJ, Weisfuse I, Rudnick J, Chiasson MA, Thomas P, Possible nosocomial transmission of HIV. *Lancet* 1994; 344: 512-4
46. Gilling-Smith C, Emiliani S, Almeida P, Liesnard C, Englert Y. Laboratory safety during assisted reproduction in patients with blood-borne viruses. *Human Reprod* 2005; 20: 1433-1438
47. The Practice Committee of the American Society for Reproductive Medicine. Guidelines for reducing the risk of viral transmission during fertility treatment. *Fertil Steril* 2008; 90: S156-162
48. Frodsham LCG, Cox AD, Almeida A, Rozis G, Gilling-Smith C. In vitro fertilization in HIV-positive women: potential mother-to-embryo viral transmission risk. *Hum Reprod* 2004; 9: 138.