Effectiveness of semen washing to prevent human immunodeficiency virus (HIV) transmission and assist pregnancy in HIV-discordant couples: a systematic review and meta-analysis

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Objective: To evaluate the effectiveness of semen washing in human immunodeficiency virus (HIV)–discordant couples in which the male partner is infected.
Design: Systematic review and meta-analysis.
Setting: Not applicable.
Patient(s): Forty single-arm open-label studies among HIV-discordant couples that underwent intrauterine insemination (IUI) or in vitro fertilization (IVF) with or without intracytoplasmic sperm injection (ICSI) using washed semen.
Intervention(s): Semen washing followed by IUI, IVF, or IVF/ICSI.
Main Outcome Measure(s): Primary outcome: HIV transmission to HIV-uninfected women; secondary outcomes: HIV transmission to newborns and proportion of couples achieving a clinical pregnancy.
Result(s): No HIV transmission occurred in 11,585 cycles of assisted reproduction with the use of washed semen among 3,994 women. Among the subset of HIV-infected men without plasma viral suppression at the time of semen washing, no HIV seroconversions occurred among 1,023 women after 2,863 cycles of assisted reproduction with the use of washed semen. Studies that measured HIV transmission to infants reported no cases of vertical transmission. Overall, 56.3% of couples (2,357/4,184) achieved a clinical pregnancy with the use of washed semen.
Conclusion(s): Semen washing appears to significantly reduce the risk of transmission in HIV-discordant couples desiring children, regardless of viral suppression in the male partner. There are no randomized controlled studies or studies from low-income countries, especially those with a large burden of HIV. Continued development of lower-cost semen washing and assisted reproduction technologies is needed. Integration of semen washing into HIV prevention interventions could help to further reduce the spread of HIV. (Fertil Steril® 2016;105:645–55. ©2016 The Authors. Published by Elsevier Inc. on behalf of the American Society for Reproductive Medicine. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Key Words: HIV prevention, semen washing, assisted reproduction, serodiscordant, safer conception

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Received September 17, 2015; revised November 16, 2015; accepted November 17, 2015; published online December 11, 2015.
M.Z. has nothing to disclose. H.H. has nothing to disclose. O.M. has nothing to disclose. S.v.d.P. has nothing to disclose. A.E.S. has nothing to disclose. G.R. has nothing to disclose. J.B. has nothing to disclose.
Supported by grants from the Reproductive Health and Research Department of the World Health Organization (J.B.) and from the National Institutes of Mental Health of the National Institutes of Health (NIH/NIMH K01MH100994, J.B.). Dr. van der Poel, a World Health Organization employee, was involved in the writing of the report and the decision to submit the article for publication.
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Fertility and Sterility® Vol. 105, No. 3, March 2016 0015-0282

http://dx.doi.org/10.1016/j.fertnstert.2015.11.028

Approximately 37 million people are living with human immunodeficiency virus (HIV) worldwide (1) and >80% of HIV-infected individuals are of childbearing age (2). A cornerstone of successful HIV prevention campaigns has included the promotion of consistent condom use (3). However, many heterosexual HIV-discordant couples desire pregnancy (4, 5), and consistent condom usage is incompatible with that desire. Couples may risk sexual HIV transmission to achieve pregnancy if they do not have access to safer reproductive methods (4, 6, 7). Semen washing is a safer reproductive strategy that HIV-discordant couples, in which the male is infected, can use to achieve pregnancy (8).

Semen washing removes spermatozoa, which are not vectors for HIV, from the surrounding seminal fluid, and the HIV-negative sperm fractions are used in assisted reproduction (8). The first study, from 1989, of semen washing for HIV-discordant couples, with the use of intrauterine insemination (IUI), found no HIV transmission to 29 uninfected female partners (9). In the two decades following the introduction of semen washing, many more studies have evaluated the effect of this method in conjunction with assisted reproductive technologies, such as IUI, in vitro fertilization (IVF), and IVF with intracytoplasmic sperm injection (ICSI), on HIV transmission in HIV-discordant couples (10–12). We conducted a systematic review and meta-analysis of these studies to estimate the safety and effectiveness of semen washing in reducing HIV transmission in HIV-discordant couples in which the male is infected.

Three systematic reviews addressing prevention of HIV transmission in HIV-discordant couples after semen washing have been completed to date. The first, by Vitorino et al. (10), included 17 observational studies published through December 2007. The second review, by Eke et al. (11), searched for randomized controlled trials published through December 2010 but did not identify any. The third review, by Savasi et al., included 22 observational studies through May 2012 (12). Our systematic review expands on all three reviews by including observational studies, studies of any size, and studies published in any language through December 2014. Barnes et al. (13) published a related systematic review in 2014 that reviewed 24 articles with the primary objective to evaluate reproductive outcomes among HIV-affected couples after IUI and IVF, specifically fecundability, miscarriage rates, and multiple gestation rates. Our systematic review and meta-analysis complements the review by Barnes et al. (13) by evaluating the effectiveness of semen washing in reducing HIV transmission in HIV-discordant couples.

MATERIALS AND METHODS

With the use of Cochrane Collaboration methods, we conducted a rigorous systematic review and meta-analysis. We assessed evidence quality with the Grading of Recommendations Assessment, Development and Evaluation (GRADE) methodology (14). We reported our findings in accordance with Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines (15).

We developed a search strategy to identify studies with abstracts published through December 2014 in four major electronic databases—Medline (via Pubmed), Cochrane Library, Scopus, and the World Health Organization's (WHO) Global Index Medicus. We also searched the International AIDS Conference, British HIV Association Conference, International Conference of Obstetricians and Gynecologists, American Academy of HIV Medicine Conference, American Society for Reproductive Medicine Conference, European Society for Hum Reprod and Embryology Conference, and British Fertility Society Conference for relevant abstracts. In addition to National Library of Medicine Medical Subject Heading (MeSH) terms and other specialized syntax, our search strategies used key terms related to "sperm washing," "assisted reproduction," and "HIV." We also hand searched the references of existing reviews and studies on semen washing. We considered articles regardless of year of publication, language, or sample size.

The inclusion criteria for studies were: 1) studies that evaluated semen washing; 2) comparative and noncomparative observational and experimental studies, such as clinical trials, cohort studies, and pre-post studies; 3) studies among HIV-discordant couples in which the male was infected and the female partner was attempting pregnancy; and 4) studies that reported the HIV status of the female partner before and after she underwent assisted reproduction with semen washing. Primary outcomes were serologic evidence of HIV infection in female partners after semen washing and virologic evidence of HIV infection in newborns following birth. We excluded studies that did not measure HIV status of the female partner before and after insemination with washed semen. Secondary outcomes included the proportion of women who achieved clinical pregnancy, the proportion of women who had spontaneous abortions/miscarriages, the proportion of infants born with low birth weight, and the proportion of deliveries that were premature. This review followed the "best available evidence approach" (16) and included single-group open-label studies that evaluated all subjects before and after undergoing a single intervention. Although studies using a randomized blinded control group are considered to be the highest quality, the best available evidence approach can be taken when those studies are not available (16).

Two authors (M.Z. and J.B.) independently screened abstracts gathered from electronic database and hand searches. After discussion on discrepancies about abstract inclusion, the authors selected a list of articles for full text review. They independently extracted the following data from included studies and compiled them into prepiloted data tables: 1) study details, including design, period of recruitment, setting, number of couples enrolled, eligibility criteria, method of semen washing and testing, and post-wash semen positivity; 2) time point for HIV testing of women and infants and the number of HIV seroconversions among both groups; 3) other clinical data, including viral loads and CD4 cell counts of male patients and the proportion of male patients on antiretroviral therapy; 4) assisted reproductive techniques used (IUI, IVF, or IVF/ICSI); and 5) reproductive outcomes, including pregnancy, spontaneous abortions, low birth weight, and premature deliveries.

Our systematic review and meta-analysis did not involve human subjects and therefore did not require Institutional Review Board approval.

Statistical Analysis

We pooled data from the studies to derive an estimate of the total reported number of couples who have used semen washing, the total number of semen washing cycles that have been performed, and the total reported number of infants born among couples using this method. We calculated the 95% confidence intervals (CIs) of HIV transmission risk per cycle and per couple for this pooled estimate according to the Jeffreys method (17). We used a one-sided exact binomial test to assess whether the overall probabilities of HIV transmission per cycle and per couple were lower than the historical estimate of per-coital probability of HIV transmission, which is 0.1% (18, 19).

We calculated the proportion of women who acquired HIV for each study and performed a proportion meta-analysis with the use of the random-effects methods of DerSimonian and Laird (20). We tested for heterogeneity in effects with the use of the I^2 statistic.

We calculated the proportion of women achieving clinical pregnancy by dividing the number of reported clinical pregnancies by the total number of cycles initiated, which included cancelled and completed cycles (21). Not all studies in this review reported pregnancy results, and studies that reported pregnancy results did not always report results per couple and per cycle. Moreover, not all studies reported pregnancy results disaggregated by type of assisted reproductive procedure (e.g., IUI vs. IVF or IVF/ICSI). Therefore, numerators and denominators for the pregnancy outcomes vary from the numerators and denominators for the HIV outcomes and do not consistently add up to the total number of events observed.

We also conducted a subgroup analysis among those couples in which the HIV-infected male partner had not achieved viral suppression (determined by plasma viral load) at the time of semen washing. This analysis estimated the independent effect of semen washing on HIV prevention in the absence of viral suppression. The subgroup included men without viral suppression regardless of antiretroviral use. When articles reported the number of men without viral suppression, but not the explicit number of cycles of assisted reproduction performed on their partners, we estimated this number by assuming that the subgroup underwent a similar number of cycles as couples with viral suppression. The definition of viral suppression used by authors of the included studies varied over time from <50 to <400 copies/mL. In studies where neither viral load nor use of antiretroviral medication was reported, the authors contacted corresponding authors to request this information.

We conducted all data analyses with the use of Stata software version 12.0 (Statacorp).

Assessing the Quality of Evidence

To comment on the overall quality of evidence, we assessed the risk of bias in each study. This parameter was our main consideration because it informs how confidently we can accept the results of studies. There is no single generic instrument recommended for assessing bias risk in observational studies (22). To determine the risk of bias in each study, we adapted the GRADE Working Group (23) recommendations to assess the following limitations of observational studies: 1) failure to develop and apply appropriate eligibility criteria; 2) flawed measurement of both exposure and outcome; 3) failure to adequately control confounding; and 4) incomplete follow-up. GRADE is not typically used to assess evidence quality of outcomes reported in single-arm studies; therefore, we modified GRADE to evaluate evidence quality for HIV-related outcomes in our review.

RESULTS

Search Results

The electronic database and conference website searches retrieved 249 relevant abstracts, and hand searching of previous systematic reviews and studies retrieved an additional six abstracts (Fig. 1). Of these 255 abstracts, 12 were duplicates, and after screening the remaining 243 we selected 47 for full-text review. These articles were published in English (n = 40), Hebrew (n = 2), Portuguese (n = 2), Dutch (n = 1), French (n = 1), and Spanish (n = 1). A total of 40 studies (37 published articles and three conference abstracts) met our eligibility criteria and were included in this review (Table 1).

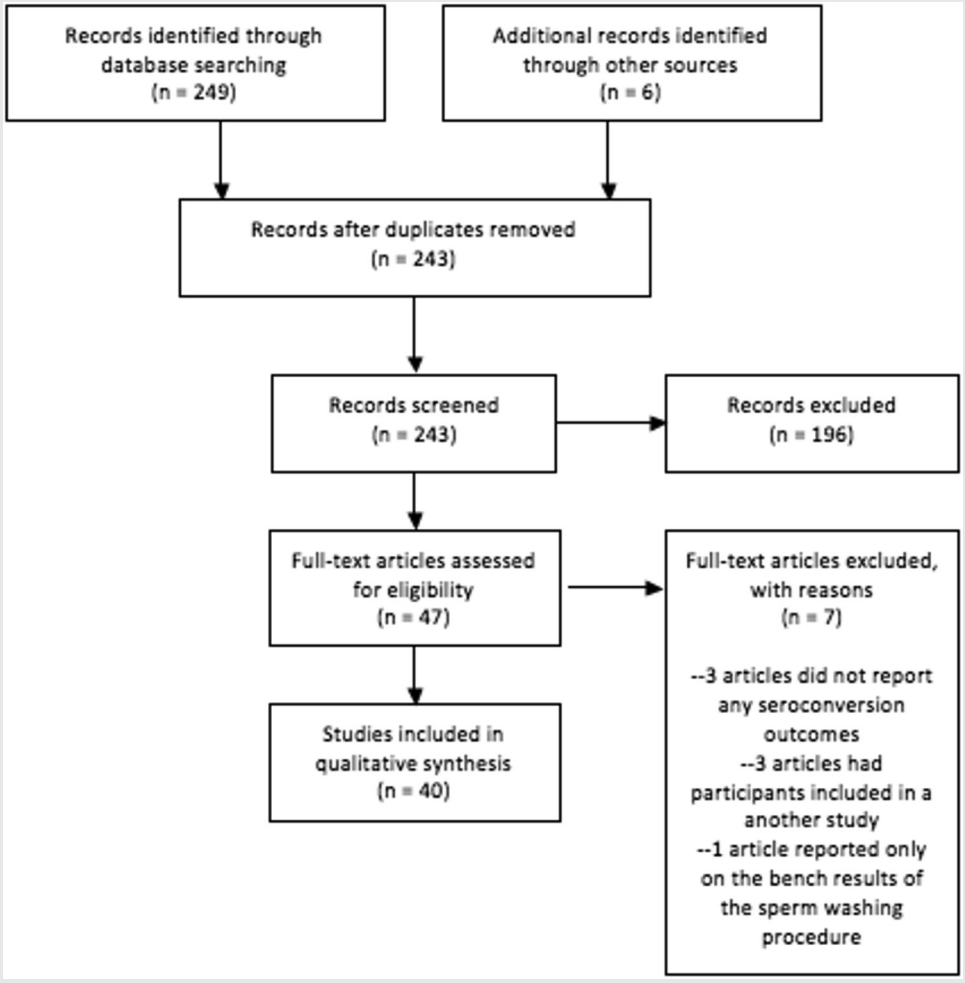
We found no randomized controlled trials. All included studies were single-arm, open-label, pre-post test designs. Eighteen studies were prospective, 21 were retrospective, and one evaluated both retrospective and prospective cohorts. All of the studies took place in high- and upper-middle-income countries representing North American, European, Asian, and Latin American regions.

Population Studied

In the 40 included studies, a total of 4,257 HIV-discordant couples completed 11,915 cycles of assisted reproduction after semen washing (Table 2). Men using assisted reproductive services ranged in age from 29 to 58 years and women from 29 to 40 years (Supplemental Table 1, available online at www.fertstert.org). The vast majority of women (93.8%, 3,994/4,257) and completed cycles (97.2%, 11,585/11,915) had HIV test results available before and after exposure to washed semen (Table 2).

Twenty-one studies reported antiretroviral use among male participants; of the 2,326 men in those studies, 641 (27.6%) were not taking antiretrovirals at the time of semen washing. Twenty-eight studies reported men's plasma viral load; of the 1,890 men in those studies, 985 (52.1%) were not virally suppressed at the time of semen washing. Overall, a minimum of 24% of the couples (1,023/4,257) in the 40 studies were estimated to have not achieved viral suppression at the time of semen washing; this includes men without viral suppression at the time of semen washing (n = 985) and men without a viral load measurement who were known to not be taking antiretroviral medications (n = 38). Among the 21 studies that reported CD4 levels, the average CD4 count of HIV-infected men ranged from 200 to 608 cells/μL.

FIGURE 1



Article selection process with use of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines flowchart.

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Semen Washing Technique

Twenty-nine studies in this review reported washing semen with the use of a technique invented by Semprini et al. in 1989 (8) (Table 1). Some studies used a polymerase chain reaction (PCR) to detect HIV DNA and RNA in the washed semen fractions (62). Five studies reported post-wash semen RNA positivity, ranging from 1.3% to 7.7% (24, 29, 36, 49, 60) (Table 1). Seminal fractions that tested positive for HIV were discarded.

HIV Transmission after Semen Washing

There were no cases of HIV transmission after exposure to washed semen among 3,994 women undergoing 11,585 cycles of assisted reproduction (0/11,585, 95% CI 0–0.0001). This per-cycle HIV transmission risk is significantly lower ($P<.001$) than the historical HIV transmission risk estimate of 0.1% per act of unprotected vaginal intercourse (18, 19). Results of the meta-analysis are presented in Figure 2. Given that there were no cases of HIV transmission in any study, the I^2 score was 0%, indicating no observed heterogeneity.

There were no HIV seroconversions among the subset of 1,023 couples in which the HIV-infected man was not virally suppressed. Those couples underwent an estimated 2,863 cycles of assisted reproduction involving IUI, IVF, and IVF/ICSI (Table 2) and had an estimated per-cycle risk of HIV seroconversion of 0 (0/2863, 95% CI 0–0.0006). This per-cycle HIV transmission risk is significantly lower ($P=.05$) than the historical HIV transmission risk of 0.1% per act of unprotected vaginal intercourse (18, 19).

In studies that provided data on mother-to-child HIV transmission, there were no cases of vertical transmission among 1,026 newborns, either at birth or at the follow-up evaluations (0/1,026, 95% CI 0–0.0029).

Pregnancy after Semen Washing with IUI, IVF, and IVF/ICSI

Assisted reproduction techniques included IUI with ovarian stimulation or natural cycles (10), IVF, and IVF/ICSI. In studies that reported pregnancy outcomes per women, of

TABLE 1

Description of the 40 studies included in this review, with assisted reproduction technique and semen washing technique used.

Study	Location	Period of recruitment	Study design	Couples (n)	Assisted reproduction technique	Technique used in semen preparation	Technique used in post-wash semen testing	Post-wash semen positivity (%)
Semprini 1992 (9)	Italy	NR	Single-arm open trial	29	IUI	Density gradient and swim-up	Monoclonal HIV antibody	NR
Marina 1998 (24)	Spain	NR	Single-arm open trial	63	IUI	Density gradient and swim-up	PCR, RT-PCR	5.6 (DNA)
Marina 1998 (25)	Spain	NR	Case report	1	IVF ± ICSI	Density gradient and swim-up	PCR, RT-PCR	NR
Veiga 1999 (26)	Spain	02/1997–12/1998	Single-arm open trial	75	IUI, IVF ± ICSI	Density gradient and swim-up	PCR, RT-PCR	NR
Loutradis 2001 (27)	Greece	1999–2000	Case series	2	IVF ± ICSI	Density gradient and swim-up	NR	NR
Weigel 2001 (28)	Germany	1991–1999	Retrospective chart review	54	IUI, IVF ± ICSI	Density gradient and swim-up	PCR	NR
Marina 2002 (29)	Spain	NR	Single-arm open trial	273	IUI, IVF ± ICSI	Density gradient and swim-up	PCR, RT-PCR	2.5 (DNA), 1.3 (RNA)
Quintana 2002 (30)	Argentina	NR	Single-arm open trial	15	IUI, IVF ± ICSI	Density gradient	PCR	NR
Cleary-Goldman 2003 (31)	USA	01/1997–06/2002	Retrospective chart review	25	IVF ± ICSI	Double swim-up	NR	NR
Ohl 2003 (32)	France	01/2001–07/2002	Single-arm open trial	39	IUI, IVF ± ICSI	Density gradient and swim-up	PCR	NR
Pena 2003 (33)	USA	07/1997–07/2002	Retrospective chart review	58	IVF ± ICSI	Density gradient and swim-up	NR	NR
Pena 2003 (34)	USA	08/1997–02/2002	Case series	5	IVF ± ICSI	–	NR	NR
Bujan 2004 (35)	France	12/1999–12/2001	Single-arm open trial	56	IUI	Density gradient and swim-up	PCR, RT-PCR	NR
Garrido 2004 (36)	Spain	08/2001–10/2003	Retrospective chart review	51	IVF ± ICSI	Density gradient and swim-up	Nested PCR, RT-PCR	7.7 (RNA), 2.6 (DNA)
Chu 2005 (37)	USA	07/1997–04/2004	Retrospective chart review	92	IVF ± ICSI	Density gradient and swim-up	PCR	NR
Kowalska 2005 (38)	Poland	05/2002–03/2005	Case series	13	IUI	Density gradient and swim-up	PCR, RT-PCR	NR
Lowenstein 2005 (39)	Israel	NR	Single-arm open trial	2	IUI	Density gradient	RT-PCR	NR
Mencaglia 2005 (40)	Italy	01/2001–12/2003	Single-arm open trial	25	IVF ± ICSI	Density gradient and swim-up	NR	NR
van Leeuwen 2005 (41)	Netherlands	2003–10/2004	Single-arm open trial	20	IUI	Density gradient and swim-up	RT-PCR	NR
Chelo 2006 (42)	Italy	NR	Single-arm open trial	49	IVF ± ICSI	Density gradient and swim-up	RT-PCR	NR
Chu 2006 (43)	USA	08/1997–03/2004	Retrospective chart review	106	IVF ± ICSI	–	NR	NR
Kato 2006 (44)	Japan	NR	Single-arm open trial	43	IVF ± ICSI	Density gradient and swim-up	RT-nested PCR	NR
Manigart 2006 (45)	Belgium	01/2000–06/2005	Single-arm open trial	38	–	Density gradient and swim-up	RT-PCR	NR
Bujan 2007 (46)	Europe	1989–2003	Retrospective multicentre study	1036	IUI, IVF ± ICSI	Density gradient & swim-up	NR	NR
Bujan 2007 (47)	France	06/2000–08/2003	Retrospective chart review	84	IUI	Density gradient and swim-up	PCR, RT-PCR	NR
Bujan 2007 (48)	France	2004	Case report	1	IVF ± ICSI	Density gradient and swim-up	RT-PCR	NR
Savasi 2007 (49)	Italy	01/2002–01/2006	Retrospective chart review	741	IUI, IVF ± ICSI	Density gradient & swim-up	Real-time PCR	4.0 (RNA)
Queiroz 2008 (50)	Brazil	06/2001–05/2007	Retrospective chart review	11	IVF ± ICSI	Density gradient	RT-PCR	NR
Pankam 2008 (51)	Thailand	08/2006–12/2007	Single-arm open trial	43	IUI	Density gradient and swim-up	NR	NR
Garrido 2009 (52)	Spain	08/2007–11/2008	Case report	1	IVF ± ICSI	Density gradient	PCR	NR
Kashima 2009 (53)	Japan	01/2001–07/2007	Single-arm open trial	26	IVF ± ICSI	Density gradient and swim-up	Nested PCR	NR
Sauer 2009 (54)	USA	01/1998–12/2007	Retrospective chart review	181	IVF ± ICSI	Density gradient and swim-up	PCR	NR
Nicopollous 2010 (55)	UK	1999–2008	Retrospective chart review	259	IUI, IVF ± ICSI	Density gradient and swim-up	PCR	NR
Giles 2011 (56)	Australia	2003–06/2010	Single-arm open trial	27	IUI, IVF ± ICSI	Density gradient	PCR, RT-PCR	NR
Schuffner 2011 (57)	Brazil	NR	Case Report	10	IUI	Density gradient and swim-up	RT-PCR	NR
Wu 2011 (58)	Taiwan	2005–2009	Single-arm open trial	14	IVF ± ICSI	Density gradient and swim-up	Real-time PCR	NR

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TABLE 1

Continued.

Study	Location	Period of recruitment	Study design	Couples (n)	Assisted reproduction technique	Technique used in semen preparation	Technique used in post-wash semen testing	Post-wash semen positivity (%)
Leruez-Ville 2013 (59)	France	NR	Case report	1	IVF ± ICSI	Density gradient	Real-time PCR, RT-PCR	NR
Olshtain-Pops 2013 (60)	Israel	NR	Single-arm open trial	22	IUI	Density gradient	PCR	2.9 (DNA)
Semprini 2013 (8)	Italy	07/1989–04/2005	Single-arm open trial and retrospective chart review	635	IUI	Density gradient & swim-up	RT-PCR	NR
Molina 2014 (61)	Spain	11/2005–12/2009	Retrospective chart review	31	IVF ± ICSI	Density gradient	Real-time RT-PCR	NR

Note: HIV = human immunodeficiency virus; IUI = intrauterine insemination; IVF ± ICSI = in vitro fertilization with or without intracytoplasmic sperm injection; NR = not reported in the study; PCR = polymerase chain reaction; RT-PCR = reverse-transcription polymerase chain reaction.

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the 4,184 couples that initiated a cycle of reproduction, 2,357 (56.3%, 95% CI 54.8%–57.8%) had a clinical pregnancy (Supplemental Table 1). Based on reported data, the proportions of couples achieving pregnancy with the use of IUI (56.4%, 95% CI 54.2%–58.5%) and IVF or IVF/ICSI (58.1%, 95% CI = 55.0%–61.1%) were similar (P=.37). In studies that reported pregnancy outcomes per cycle, 19.9% (95% CI 19.1%–20.6%) of initiated assisted reproduction cycles resulted in a clinical pregnancy (Supplemental Table 1). In studies that reported data on spontaneous abortions, 17.0% (95% CI 15.4%–18.6%) of clinical pregnancies ended with a spontaneous abortion. Rates of spontaneous abortions after IUI (15.5%, 95% CI 13.4%–17.7%) and IVF or IVF/ICSI (17.7%, 95% CI 13.9%–22.0%) were similar (P=.32).

Four studies reported birth weight and preterm delivery outcomes (31, 34, 54, 61). Of 259 infants, 115 (44.4%) were born with low (<2,500 g) or very low (<1,500 g) birth weight and 107 (41.3%) were born prematurely (before 37 weeks of gestation). All of the women who experienced premature delivery or gave birth to infants with low birth weight had undergone IVF or IVF/ICSI. The rate of multiple gestations among ongoing/delivered pregnancies was 43.5% (81/186).

Quality of Evidence

All of the 40 included studies enrolled populations of HIV-discordant couples that addressed the study question. Thirty-nine studies were conducted in a controlled manner with rigorous biologic testing for HIV before and after semen washing to measure HIV seroconversion accurately. Individual studies did not calculate effect sizes, nor did they use statistical methods to adjust effect estimates. The risk of missing data was very low overall, with HIV results before and after exposure to washed semen available for 93.8% of women and 97.2% of cycles included in this review. Thirty-nine studies reported no loss to follow-up. Duration of follow-up ranged from 3 months to 12 months, which are appropriate lengths of follow-up to monitor for HIV seroconversion.

DISCUSSION

This is the most comprehensive systematic review and meta-analysis to date evaluating the effect of semen washing on HIV transmission among HIV-serodiscordant couples. We found that semen washing provides a safe and effective method for HIV-serodiscordant couples to become pregnant. There were no instances of HIV seroconversion among HIV-uninfected women inseminated with washed semen from their HIV-infected partners. The estimated per-cycle HIV transmission risk after semen washing is significantly lower than historical estimates of HIV transmission risk per act of unprotected intercourse in both the overall population reviewed and the subgroup of men without viral suppression at the time of semen washing. More than one-half of the couples in this review achieved a clinical pregnancy, and the rate of spontaneous abortions reported was similar to general population estimates (63). There were no cases of vertical transmission. HIV prevention programs that encourage couples to attempt pregnancy with washed semen as an alternative

TABLE 2

Numbers of couples and cycles included in this review, and number of HIV seroconversions.

Parameter	Result
Initiated cycles of assisted reproduction with washed semen	12,079
Completed cycles of assisted reproduction with washed semen	11,915
Couples with at least one completed cycle of assisted reproduction with washed semen	4,257
Women with known HIV results after exposure to washed semen	93.8% (3,994/4,257)
Completed cycles of assisted reproduction among women with known HIV results after exposure to washed semen	97.2% (11,585/11,915)
Men known to be taking antiretroviral therapy at time of semen washing	39.5% (1,685/4,257)
Men who were known to have not achieved viral suppression at time of semen washing (plasma testing)	27.7% (985/4,257)
Completed cycles of assisted reproduction with the use of washed semen among subgroup of couples with a male partner who was not virally suppressed	24.0% (2,863/11,915)
Number of HIV seroconversions (95% CI)	
Per completed cycle of assisted reproduction, overall	0/11,585 (0–0.0001)
Per woman with known HIV outcome, overall	0/3,994 (0–0.0004)
Per completed cycle, among subgroup of couples with a male partner who was not virally suppressed	0/2,863 (0–0.0006)
Per infant	0/1,026 (0–0.0029)

Note: CI = confidence interval; HIV = human immunodeficiency virus.

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to intercourse without condoms may help to prevent the incidence of sexual transmission of HIV (6).

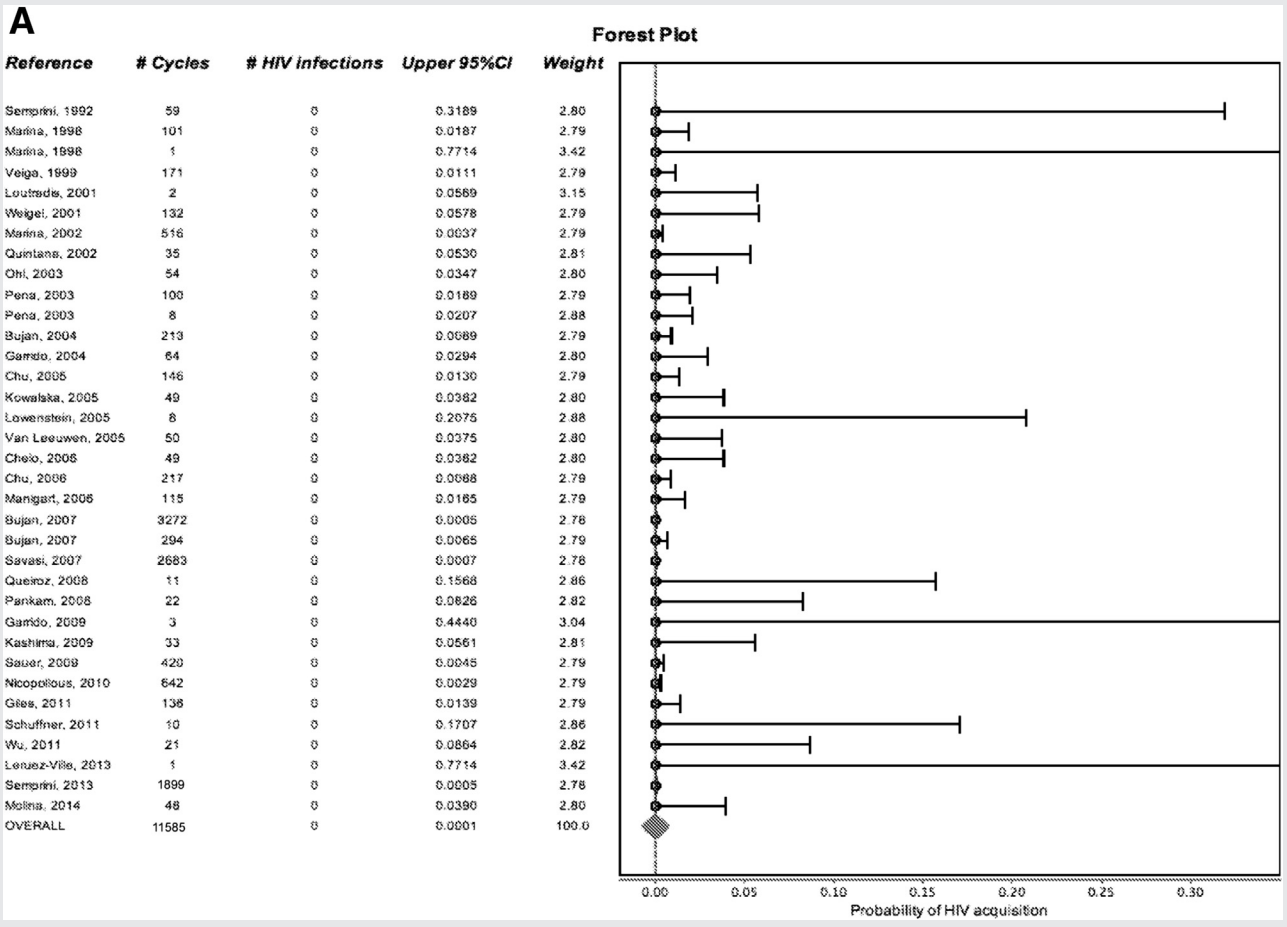
Approximately one-half, 52.1%, of the men in studies that reported viral loads of participants were not virally suppressed at the time of semen washing. This number is an underestimate of the true number of men who were virally unsuppressed, because 12 of studies we reviewed did not report on viral load and 19 did not report on antiretroviral use. The absence of HIV transmission in this subgroup of men without viral suppression suggests that semen washing may be a safer and more effective reproductive method in settings where men are unable or unwilling to initiate antiretroviral therapy, are nonadherent, or are not virally suppressed. For example, in sub-Saharan Africa it is estimated that at least one-third of individuals who have been taking antiretrovirals for 12 months do not attain viral suppression (64). Furthermore, up to 48% of men taking effective antiretroviral therapy with undetectable viral load in blood plasma samples have detectable virus in their semen (65, 66). Semen washing may be relevant in such settings where men can access antiretroviral therapy and are highly adherent to it but remain at risk of transmitting HIV to their partners. Semen washing may offer a safer alternative to intercourse without condoms to prevent HIV transmission to the uninfected female partner.

Some have argued that IVF and IVF/ICSI have better fertility outcomes than IUI (31); however, the overall pregnancy success rates were similar between the methods in the HIV-discordant couples reviewed. Additionally, some have argued that IVF or IVF/ICSI poses less risk of HIV transmission than IUI because it uses a single spermatozoon (67). However, neither women nor newborns in the reviewed studies acquired HIV after IUI with the use of washed semen. Additionally, the vast majority of assisted reproduction cycles performed used IUI. Although lower-cost IVF procedures are being developed and evaluated, the method is currently at least ten times costlier than IUI in most settings (68, 69). Moreover, IVF is more invasive, carries some surgical risk, and requires additional clinic and laboratory capacity that may not exist in many low-resource settings. Therefore, IUI with washed semen may offer an effective, affordable, feasible, and safe strategy for preventing HIV transmission among HIV-discordant couples desiring children (70).

Traditionally, semen washing followed by assisted reproduction has been used to meet the needs of couples with infertility or subfertility. A 2012 WHO study reported that infertility affects one in four couples in developing countries (71). That study estimated that infertility globally affected 48.5 million heterosexual stable couples that had been attempting pregnancy for ≥ 5 years, of which 10 million lived in sub-Saharan Africa, the region most affected by HIV (71). Safer conception strategies in these areas must consider not only the risk of HIV transmission but also underlying infertility. Assisted reproduction with washed semen may help both fertile and infertile couples achieve pregnancy, while simultaneously reducing the risk of HIV transmission to the woman and her newborn. However, the availability of semen washing must be considered. Semen washing is currently provided by only a limited number of fertility or reproductive health centers worldwide. Establishing capacity for semen washing in any part of the world will depend on the availability of financial and clinical resources and expertise of clinical staff (70). Efforts to scale up capacity for semen washing and the development of lower-cost procedures are warranted, particularly in HIV endemic settings.

Despite a comprehensive search of the scientific literature, without language restrictions, limitations to include only published studies, or sample size constraints, we did not find any published randomized control trials or cohort studies with an internal comparison group to test the effect of semen washing on HIV prevention in HIV-discordant couples. Without a matched and untreated comparison group, it is difficult to determine whether there would be a significant difference in the rate of HIV transmission between women inseminated with washed semen compared with those who were not. Given the absence of a direct comparison group, studies have evaluated their results against historical estimates of the overall risk of HIV transmission during unprotected intercourse. This comparison has its limitations (72). Furthermore, the lack of studies without a comparator group affects the quality of our evidence. The GRADE approach to

FIGURE 2



Proportion meta-analysis plot for human immunodeficiency virus (HIV) transmission probability and upper 95% confidence interval (CI) after sperm washing (**A**) per cycle and (**B**) per woman.

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assessing evidence quality by outcome denotes data from observational studies with comparators as "low-quality evidence" (23). Evidence quality can be graded down to very low owing to a lack of internal comparators in the studies evaluated. Because our included studies are single-arm observational trials, we assigned them a high risk of bias and graded the evidence quality down to very low (Supplemental Table 2, available online at www.fertstert.org).

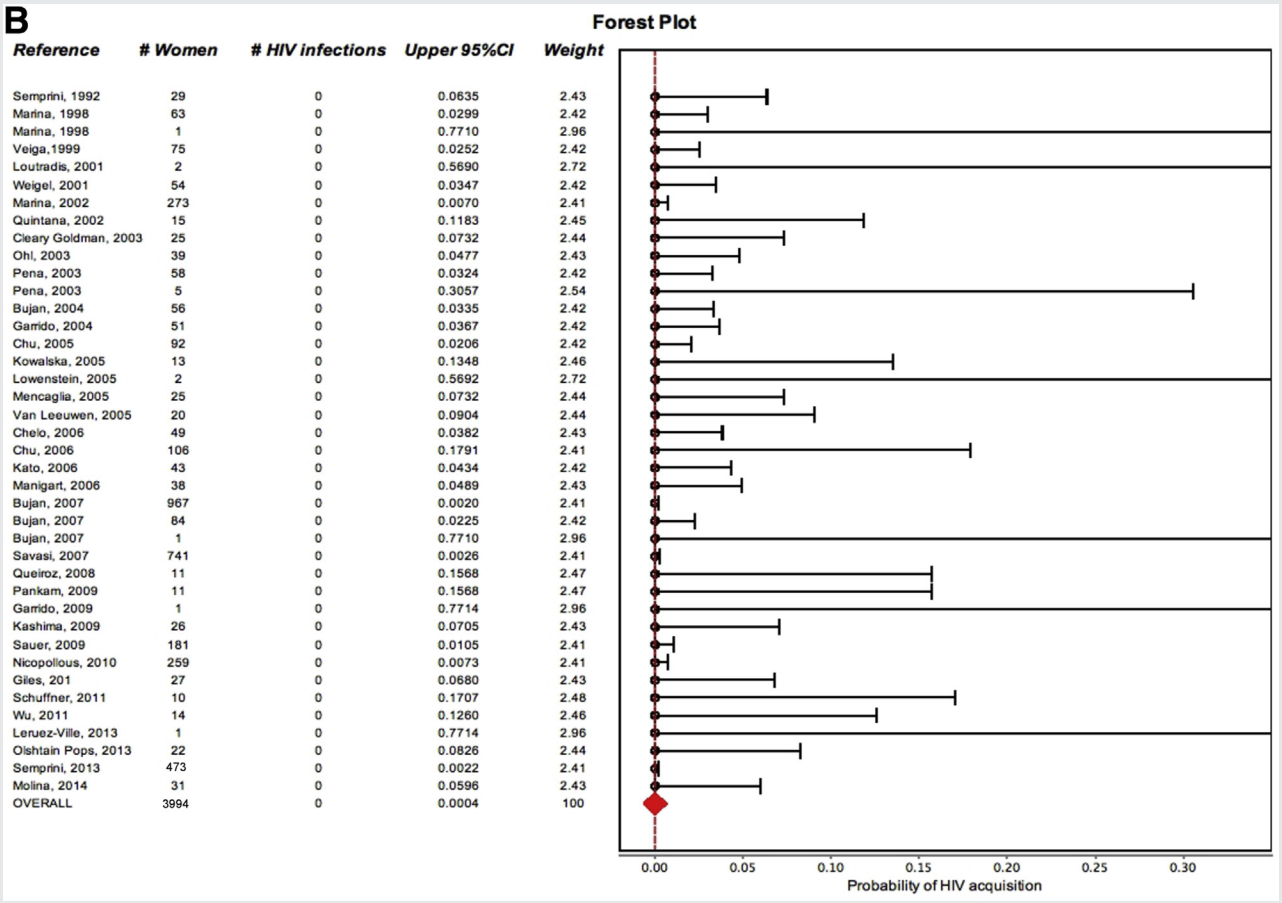
The majority of studies included in this review did not report on differences in semen quality between patients with or without previous viral suppression. However, one study by Nicopollous et al. (55) noted that semen parameters were not significantly different between men with detectable and undetectable viral loads, despite significantly lower CD4 counts among unsuppressed men. There is evidence in the literature that although highly active antiretroviral treatment impairs semen parameters, resulting in lower ejaculate volume and sperm with less motility (73), HIV parameters, such as CD4 cell count, viral load, and duration of antiretroviral therapy, are not significantly correlated with semen quality (74).

Studies included in this review did not report whether there were significant differences in pregnancy rates in the different techniques (IUI, IVF, or IVF/ICSI) between patients with and without viral suppression. However, one study by Savasi et al. (49) calculated the rates of clinical pregnancy after IUI among participants taking antiretroviral therapy and those not. The rate of clinical pregnancy per cycle in the group taking antiretroviral medication (17.4%, 332/1,902) was not significantly different ($P=.105$) from the rate of clinical pregnancy in the group not taking antiretroviral medication (25%, 124/498). Because IUI and IVF procedures typically wash sperm to prepare it for insemination regardless of the HIV status of the patient, this review is unable to comment on rates of birth outcomes after assisted reproduction without semen washing.

A future direction of research may explore the utility of conducting post-wash semen HIV testing. Very few (n = 5) of the studies included in this review reported on this method. Additionally, no study compared rates of HIV transmission after assisted reproduction with or without post-wash semen HIV testing. Therefore, we are unable to comment on the utility of conducting post-wash semen HIV testing before IUI or IVF in this context.

This review has numerous strengths, including an exhaustive search strategy, inclusion of 20 years of multinational and

FIGURE 2 Continued



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different-language studies, and the consistency of results across the included studies. Additional strengths of the studies included in this review include the use of serologic and virologic testing of women before and after semen washing and extremely high rates of participant retention. Finally, a major strength of this review is its large sample size; this review includes 11,585 completed cycles of assisted reproduction with known HIV outcomes and 2,863 cycles in which HIV-infected men had not attained viral suppression.

CONCLUSION

The absence of HIV seroconversion in the reviewed studies suggests that semen washing prevents HIV transmission in HIV-discordant couples attempting pregnancy where the man is infected. There is a lack of studies on semen washing from low-income and lower-middle income countries, including countries in sub-Saharan Africa with a high HIV prevalence. Efforts to develop lower-cost semen washing and assisted reproduction technologies that can be used in settings with fewer resources are therefore warranted. Integration of semen washing into HIV prevention protocols may help to curb the incidence of sexual HIV transmission.

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SUPPLEMENTAL TABLE 1

Age of participants and pregnancy outcomes.

Variable	IUI	IVF or IVF/ICSI	Overall
Age range in y			
Men	31–38	29–58	29–58
Women	29–38	30–40	29–40
Pregnancy outcomes, % (95% CI), (n)			
Proportion of initiated cycles of assisted reproduction with the use of semen washing that resulted in pregnancy	17.9 (17.0–18.9), (1,184/6,599)	32.3 (30.0–34.6), (545/1,687)	19.9 (19.1–20.6), (2,331/11,742)
Proportion of couples that achieved pregnancy	56.4 (54.2–58.5), (1,162/2,062)	58.1 (55.0–61.1), (593/1,020)	56.3 (54.8–57.8), (2,357/4,184)
Proportion of pregnancies ending in spontaneous abortion (per couple)	15.5 (13,4–17.7), (182/1,177)	17.7 (13.9–22.0), (65/367)	17.0 (15.4–18.6), (376/2,215)

Note: The number of pregnancies, couples, cycles, and spontaneous abortions for IUI, IVF, or IVF/ICSI may not add up to the number of total events observed. This is because not all studies reported pregnancy outcomes and not all studies provided data disaggregated by IUI, IVF, or IVF/ICSI. ICSI = intracytoplasmic sperm injection; IUI = Intrauterine insemination; IVF = In vitro fertilization.

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SUPPLEMENTAL TABLE 2

GRADE evidence quality assessment: Is semen washing effective in preventing HIV seroconversion in women and their infants? (observational studies; n = 40)

HIV seroconversion	Bias risk	Inconsistency	Indirectness	Imprecision	Evidence quality
Per completed cycle of assisted reproduction, overall	Very serious risk[a]	No serious risk	No serious risk[b]	No serious risk[c]	Very low
Per woman with known HIV outcome, overall	Very serious risk[a]	No serious risk	No serious risk[b]	No serious risk[c]	Very low
Per completed cycle, among subgroup of couples with a male partner who was not virally suppressed	Very serious risk[a]	No serious risk	No serious risk[b]	No serious risk[c]	Very low
Per infant	Very serious risk[a]	No serious risk	No serious risk[b]	No serious risk[c]	Very low

Note: HIV = human immunodeficiency virus.
[a] Single-arm studies, no internal comparator.
[b] Without a comparator, not direct, but this problem is covered under bias risk.
[c] No events, but owing to lack of comparator this problem is covered under bias risk.

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