Human Reproduction, Vol.25, No.10 pp. 2507–2515, 2010
Advanced Access publication on August 4, 2010 doi:10.1093/humrep/deq189

human reproduction

ORIGINAL ARTICLE *Infertility*

Sexual violence, HSV-2 and HIV are important predictors for infertility in Rwanda

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Submitted on May 12, 2010; resubmitted on June 18, 2010; accepted on June 18, 2010

BACKGROUND: In order to formulate cost-effective health interventions aimed at preventing infertility it is necessary to identify modifiable risk factors for infertility in sub-Saharan Africa. This case–control study examined potential predictors and their population attributable fraction (PAF%) for various infertility types including lifestyle factors, sexual behaviour and reproductive tract infections (RTIs).

METHODS: Sexually active women aged 21–45 year presenting with infertility problems at the infertility clinic of the Kigali University Teaching Hospital ($n = 312$), and fertile controls who recently delivered ($n = 283$) were surveyed together with their male partners. Participants were interviewed about socio-demographic characteristics, sexual behaviours and lifestyle factors, and were tested for HIV and RTIs.

RESULTS: Variables significantly associated with tubal infertility were history of sexual violence [adjusted odds ratio (AOR) 2.41; 95% CI 1.36–4.25]; positive HIV (AOR 2.41; 95% CI 1.36–4.25), herpes simplex virus type 2 (HSV-2; AOR 1.67; 95% CI 1.03–2.71) and *Chlamydia trachomatis* serology (AOR 1.78; 95% CI 0.99–3.21), and current bacterial vaginosis by Amsel criteria (AOR 1.97; 95% CI 1.12–3.47). Among men, male factor infertility was associated with positive HIV (AOR 2.43; 95% CI 1.31–5.23) and HSV-2 serology (AOR 1.71; 95% CI 1.02–2.87) and current urologic abnormalities (AOR 2.38; 95% CI 1.01–5.31). Positive HSV-2 serostatus carried the greatest PAF% (26%) for tubal infertility, followed by positive HIV serostatus (20%) and history of sexual violence (17%).

CONCLUSIONS: Although temporal relationships are difficult to ascertain, history of sexual violence, HSV-2 infection and HIV infection are important predictors of infertility in Rwanda.

Key words: infertility / tubal factor / male factor / Africa / predictors

Introduction

Infertility is affecting a large number of couples in sub-Saharan Africa (SSA), with a prevalence of up to 29% in certain regions (Larsen, 2000; Boivin *et al.*, 2007). The consequences of infertility for the couples involved are often more severe in traditional societies, where motherhood is more important for the woman's status, than in Western societies (Gerrits, 1997; Sundby, 1997). Since modern infertility treatment is expensive, prevention of infertility should be the main focus of action in countries with limited resources.

A WHO multicentre investigation of 8500 infertile couples throughout the developed and developing world found that most cases of infertility in African couples are infection-induced and therefore possibly preventable (Cates *et al.*, 1985). Pelvic inflammatory disease (PID) can lead to damaged fallopian tubes in women and sexually transmitted infections (STIs) can lead to vas deferens blockage and decreased sperm quality in men (Pellati *et al.*, 2008). Investigations of risk factors for infertility have mostly been limited to secondary analyses of Demographic Health Surveys and World Fertility Surveys, which have indicated that a history of high-risk sexual behaviour, such as multiple marriages and unions and early age at first sexual intercourse, put women at substantial risk for infertility (Ericksen and Brunette, 1996; Larsen, 2003). Studies of risk factors for male infertility in SSA have been very limited. One case–control study in Nigeria found that infertility in men is associated with various proxies of STIs and poor healthcare-seeking behaviour for STIs

(Okonofua et al., 2005). Other elements of sexual experiences in women, such as sexual violence and transactional sex, and lifestyle factors in both men and women such as smoking, alcohol and obesity, have not been examined as risk factors for infertility in SSA so far. It also remains unclear which micro-organisms contribute to the pathogenesis of tubal infertility in SSA. *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are thought to be the major cause of PID and fallopian tube occlusion. *Neisseria gonorrhoeae* is difficult to examine as a risk factor for infertility because there are no good markers for past gonococcal infection. Other reproductive tract pathogens that have been associated with tubal disease are bacterial vaginosis (BV) related organisms, herpes simplex virus type 2 (HSV-2) and HIV (Gaudoin et al., 1999; Hettmann et al., 2008). The former two have not been examined in relation to infertility in an African population. A few studies in SSA have found a higher prevalence of HIV in infertile women and there is some evidence that HIV has a fertility-reducing effect (Favot et al., 1997; Gray et al., 1998).

The design of cost-effective health interventions aimed at preventing infertility requires identification of modifiable risk factors for infertility in SSA. We conducted a case–control study in Kigali, Rwanda, to examine potential predictors and their population attributable fraction (PAF%) for infertility.

Methods

Subjects and settings

Between November 2007 and May 2009, an infertility research clinic was opened at the Kigali University Teaching Hospital in Rwanda, and infertile women were recruited mainly through word of mouth. To be eligible for participation in the study, women self-reported to be infertile needed to be between 21 and 45 years of age, residing in Kigali, willing to undergo HIV testing and having had sexual intercourse at least once in the last 2 weeks. Refusal to participate in the study did not influence access to further services. For this analysis, infertility was defined as having had regular unprotected intercourse for 1 year or more without conception with at least one regular partner, and included both primary and secondary infertility. Fertile controls were defined as non-pregnant women who recently (between 6 and 18 months ago) delivered. Controls were recruited at the level of the community, as no appropriate control group could be selected at the hospital. A list of all residential neighbourhoods (known as mudugudu) from where the infertile couples originated was compiled (120 umudugudus in total). Fourteen neighbourhoods were randomly selected from this list by blindly hand-picking numbers from a bowl. Community mobilizers in these neighbourhoods visited all families to identify potential eligible candidates and explained that they would receive free cervical cancer screening and treatment if indicated. This service was offered to both cases and controls.

Male partners of the enrolled participants were also asked to participate on a separate occasion, using a written invitation given to the woman at the end of her study visit. The study was approved by the National Ethics Committee of Rwanda, the Ethics Committee of the University Hospital Ghent and the Centre National de Lutte contre le Sida (CNLS) in Rwanda.

Study procedures

The purpose of the study was explained to the study participants in the local language (Kinyarwanda) and eligibility criteria were checked. All participants provided written informed consent before study initiation. A female nurse interviewer addressed the women and a male medical officer the male partners. Socio-demographic characteristics and information on past sexual behaviour and lifestyle factors were asked in face to face interviews using a structured questionnaire. All female participants received a clinical gynaecological examination by the same trained nurse, supervised by a gynaecologist. During the speculum examination vaginal swabs were collected for the preparation of a wet mount, potassium hydroxide testing and vaginal pH testing. Saline wet mounts were examined in the study clinic for the presence clue cells. A Gram stain slide was also prepared and read later for the presence of BV according to the morphological criteria of Nugent. Thereafter, the study nurse collected blood samples for serial rapid HIV testing on site as per national algorithm: determine Rapid HIV-1/2 Test (Abbott Laboratories, Abbott Park, IL, USA), Uni-Gold Rapid Test (Trinity Biotech Plc, Ireland) and the Capillus HIV-1/HIV-2 Rapid Test (Trinity Biotech Plc, Ireland). Participants were advised to return for test results and, if required, received treatment according to local guidelines. The serum sample was immediately processed in four aliquots and stored at $-80^{\circ}C$ until analysis. One aliquot was analysed for HSV-2 antibodies (Herpeselect 2 ELISA IgG, Focus Diagnostics) in the national reference lab of Kigali. Another aliquot was shipped, frozen on dry ice to the laboratory of the Ghent University Hospital for *C. trachomatis*-specific IgG antibodies by a synthetic-peptide-based EIA test (labsystems, Helsinki, Finland).

All participants were offered HIV counselling and same-day testing; those testing positive were enrolled in an HIV care and treatment clinic at the study site.

The women in an infertile relationship also received a tubal patency test using hysterosalpingography (HSG) in most and laparoscopy in some cases. Their male partners were offered semen analysis, which was performed by an experienced lab technician in a private lab. The following parameters were assessed: volume, pH, viscosity, concentration and motility. Assessment of morphology was not done due to lack of appropriate equipment.

Statistical analysis

Data collected during interviews and laboratory investigations were single entered, verified and cleaned using MS Access 2000 (Microsoft, Seattle, WA, USA). Intercooled Stata 9.2 (Stata Corporation, College Station, TX, USA) was used for statistical analysis. Socio-demographic characteristics of infertile and fertile women and their partners were compared using χ^2 tests or Mann–Whitney *U*-tests. The relationships between selected covariates and infertility were examined by comparing all infertile women (regardless of infertility type) and the subgroups of infertile women with and without tubal factor with fertile women and all infertile men and the subgroups of infertile men with and without male factor with fertile men. Within the infertile relationships, women with and without a tubal factor, and men with and without a male factor were also compared. Women were considered to have tubo-peritoneal infertility if HSG and/or laparoscopy had shown tubal obstruction and/or peritoneal adhesions. Men were considered to have a male factor if their sperm motility and concentration was abnormal according to the new WHO reference values (Cooper et al., 2010). Logistic regression was used to assess associations between each predictor of interest and infertility whereas controlling for age, marital status and educational level. In these initial models, seven measures of sexual behaviour for women and five measures for men were assessed. Engaging in sex before 15 years of age was considered a risk factor for women because PID at a young age is more damaging for the immature genital tract. The median age at first sexual intercourse in urban Rwanda is 20 years for men (Ministry of Health, 2006); we therefore chose the cut-off value of 20 years for men. Ever transactional sex was defined as ever having received money or gifts in exchange for sex. The cut-off values for alcohol use were based on distribution of the answers

and were at least one and nine units a week for women and men, respectively. All participants were weighed and measured at enrolment and obesity was defined as a body mass index (BMI) more than 25.

Predictors with a moderate association with infertility after controlling for age, education and marital status (*P*-value of <0.2) were fitted in a final logistic regression model to assess their independent relationship with tubal and male factor infertility. To reduce the number of covariates, composite variables were constructed for high-risk sexual behaviour. For women, high-risk sexual behaviour was defined as age at first sexual intercourse before 15 years and/or ever had a union dissolution and/or ever engaged in transactional sex; for men, it was defined as age at first sexual intercourse before 20 years and/or ever had a union dissolution. The number of lifetime sexual partners was not included, because we believe that a high number of lifetime partners may be a consequence of infertility as much as a risk factor. The same applies to lack of condom use. PAF% were calculated for selected risk factors using the following formula: pe[adjusted odds ratio (AOR) − 1]/AOR with pe the proportion of cases that is exposed to risk factor.

Results

Of a total of 339 potentially infertile women who presented to the clinic, 312 were confirmed eligible and enrolled. Of 407 fertile women identified in the community, 352 (86%) came to the clinic and 312 were confirmed eligible and enrolled. Of the 55 non-responders, half stated having no time to come to the clinic, one-third were afraid of the physical examinations and the remainder did not state a reason. Educational level and employment did not differ between responders and non-responders. Male partners of 81% of the infertile women (254 partners) and 61% of the fertile women (189 partners) agreed to participate. Twenty-nine women in the fertile group were excluded from the analysis because they tried to conceive for their last pregnancy for more than 12 months, for a final sample size of 283 women and 170 male partners in fertile relationships and 312 women and 254 male partners in infertile relationships.

Women in infertile relationships were more likely than women in fertile relationships to be married, to be older, and to have an occupation and an income (Table I). Infertile women without a tubal factor were better educated than fertile women and women with a tubal factor. All unmarried participants in both groups reported to have a steady sexual partner, and in most cases, this partner spend the night with them most of the time. Men in infertile relationships were older, better educated and had a higher income than men in fertile relationships.

All sexual behaviour covariates, except ever use of condoms, were associated with tubal infertility after controlling for age, marital status and educational level (Table II). Non-tubal factor infertility was associated with a history of sexual violence and more than three lifetime sexual partners but not with other sexual behaviour covariates. All measured reproductive tract infections (RTIs) were associated with tubal factor infertility (Table II). Apart from HIV, none of the RTIs was significantly associated with non-tubal infertility (Table II). Alcohol use and being overweight were not associated with infertility. Smoking was associated with tubal infertility but not with non-tubal infertility. Within the group of infertile women all measures of high-risk sexual behaviour, apart from transactional sex and all measured RTIs, with the exception of positive Chlamydia serostatus, were associated with tubal pathology (data not shown). Sexual violence (AOR 2.41; 95% CI 1.36–4.25), positive HIV serostatus (AOR 2.41; 95% CI 1.36–4.25), positive HSV-2 serostatus (AOR 1.67; 95% CI 1.03–2.71) and current BV by Amsel criteria (AOR 1.97; 95% CI 1.12–3.47) were significantly associated with tubal infertility in the final multivariable logistic regression model for women (Table IV).

Male factor infertility was associated with having had a union dissolution, never using condoms, positive HIV and HSV-2 serology, and current urological abnormalities after controlling for age, marital status and educational level (Table III). In the subset analysis of men with normal sperm analysis, infertility was associated with male circumcision and HIV. Lifestyle factors (smoking, alcohol and BMI) and reported STIs were not associated with infertility in men. Within the group of infertile men, no significant associations were found with abnormal semen analysis (data not shown). In the final logistic regression model, infertility was associated with positive HIV serostatus (AOR 2.43; 95% CI 1.31–5.23), positive HSV-2 serostatus (AOR 1.71; 95% CI 1.02–2.87) and urologic abnormalities (AOR 2.32; 95% CI 1.01–5.31) (Table IV).

Positive HSV-2 serostatus carried the greatest PAF% (26%) for tubal infertility, followed by positive HIV serostatus (20%), a history of sexual violence (17%), BV by Amsel criteria (12%), high-risk sexual behaviour (9%), positive Chlamydia serostatus (9%) and lifestyle (9%) (Table V). Positive HSV-2 and HIV serology accounted for the largest PAF% for male factor infertility in men (22 and 13%, respectively).

Discussion

We found that a history of sexual violence, HIV infection and HSV-2 infection contribute most to infertility in Rwandan women, especially to tubal factor infertility. In men, HIV and HSV-2 infection were the most important risk factors for male factor infertility. Lifestyle factors could not predict infertility in our population.

In our study, a history of sexual violence contributed more to infertility than other measures of high-risk sexual behaviour. It is the first time that a history of sexual violence is found to be associated with infertility. Women in an infertile relationship had experienced three times more sexual violence during their lifetime than fertile women. Although we did not specifically ask, it is possible that many of these women were raped during the genocide of 1994 in Rwanda. Although the association between a history of sexual violence and tubal factor infertility can easily be explained, the association with non-tubal infertility is somewhat puzzling. This may be due to the fact that tubal pathology was diagnosed using HSG as opposed to the gold standard method laparoscopy; HSG is not a reliable test for tubal patency with only 65% sensitivity and 83% specificity (Crosignani and Rubin, 2000). Therefore, the non-tubal infertility group could have included an unknown number of cases of undiagnosed tubal infertility. In addition, psychological trauma resulting from sexual violence could have led to infertility through anovulation or sexual dysfunction. However, we did not identify an association between past sexual violence and current menstrual irregularities.

The association of HSV-2 with tubal factor infertility but not with non-tubal factor infertility, and its association with tubal pathology within the group of infertile women, suggest that HSV-2 plays a role in tubal pathogenesis. Prior studies in Western countries have

Table I Baseline characteristics of enrolled women and their male partners in fertile and infertile relationships and by infertility diagnosis in Kigali, Rwanda.

Variable	Fertile relationship (n = 283), n (%)	Infertile relationship (n = 312), n (%)	P-value[a]	Tubo-peritoneal factor (n = 189), n (%)	P-value[a]	No tubo-peritoneal factor (n = 90), n (%)	P-value[a]
Females							
Age, median (IQR)	27 (24–31)	30 (27–35)	<0.001	31 (27–36)	<0.001	30 (26–35)	<0.001
Education level							
Up to primary school	213 (75)	225(72)	0.38	145 (77)	0.72	58 (64)	0.045
Post-primary school	70 (25)	87 (28)		44 (23)		32 (36)	
Marital status							
Single	170 (60)	135 (43)	<0.001	88 (47)	0.015	32 (36)	<0.001
Married	112 (40)	176 (56)		100 (53)		58 (64)	
Separated, divorced or widowed	1 (0)	1 (0)		1 (0)		0 (0)	
Occupation							
Not employed	155 (55)	92 (29)	<0.001	55 (29)	<0.001	28 (31)	<0.001
Employed	128 (45)	220 (70)		134 (71)		62 (69)	
Income ($/day)[†]							
0 USD	159 (56)	98 (33)	<0.001	57 (31)	<0.001	32 (38)	0.008
<1 USD	26 (9)	79 (27)		58 (32)		13 (15)	
1–4 USD	88 (31)	85 (29)		44 (24)		31 (37)	
5 USD or more	10 (4)	34 (11)		23 (13)		8 (10)	
Variable	**Fertile relationship (n = 170), n (%)**	**Infertile relationship (n = 254), n (%)**	**P-value**	**Male factor (n = 144), n (%)**	**P-value**	**No male factor (n = 82), n (%)**	**P-value**
Males							
Age, median (IQR)	31.5 (27–38)	34 (30–40)	0.001	35 (30–41)	<0.001	34 (30–38)	0.07
Education level							
Up to primary school	132 (78)	151 (59)	<0.001	84 (58)	<0.001	52 (63)	0.02
Post-primary school	38 (22)	103 (41)		60 (42)		30 (37)	
Marital status							
Single	85 (50)	118 (46)	0.57	68 (47)	0.5	39 (48)	0.72
Married	85 (50)	135 (53)		75 (52)		43 (52)	
Separated, divorced or widowed	0 (0)	1 (0)		1 (1)		0 (0)	
Income ($/day)[†]							
0 USD	2 (1)	1 (0)	0.03	1 (1)	0.14	0 (0)	0.17
<1 USD	12 (7)	31 (13)		19 (14)		9 (12)	
1–4 USD	120 (71)	138 (58)		83 (60)		45 (58)	
5 USD or more	36 (21)	68 (29)		35 (25)		23 (30)	

[a]For all three columns of P-values: P-values are derived from comparison with women in fertile relationship.
[†]Sixteen missing values for infertile women and men.

Table II Predictors for tubal factor, non-tubal factor and infertility of any cause in Rwandan women.

Variable	All fertile women (n = 283), n (%)	All infertile women (n = 312), n (%)	Age, marital status and education adjusted OR (95% CI)	Infertile with tubal factor (n = 189),[g] n (%)	Age, marital status and education adjusted OR (95% CI)	Infertile without tubal factor (n = 90),[g] n (%)	Age, marital status and education adjusted OR (95% CI)
Sexual history							
Lifetime sex partners 3 or more	45 (16)	140 (45)	6.01 (3.87–9.31)	98 (52)	7.30 (4.50–11.88)	31 (34)	4.06 (2.22–7.42)
Ever had a union dissolution[a]	31 (11)	61 (20)	1.62 (0.97–2.73)	49 (26)	2.05 (1.18–3.55)	7 (8)	0.82 (0.33–2.08)
Ever transactional sex	16 (6)	26 (8)	2.75 (1.36–5.57)	18 (9)	2.81 (1.29–6.12)	5 (6)	2.58 (0.84–7.94)
Age at first intercourse < 15	22 (8)	32 (10)	1.81 (0.99–3.31)	24 (13)	2.13 (1.10–4.11)	3 (3)	0.43 (0.12–1.55)
Age at first intercourse > 30	3 (1)	11 (4)	1.00 (0.26–3.86)	4 (2)	0.54 (0.11–2.68)	4 (4)	2.02 (0.40–10.24)
Never used condoms	94 (33)	161 (52)	1.67 (0.64–1.41)	84 (44)	1.21 (0.80–1.83)	56 (62)	2.66 (0.73–1.86)
Ever exposed to sexual violence[b]	28 (10)	79 (25)	3.02 (1.85–4.91)	54 (29)	3.39 (2.00–5.74)	17 (19)	2.01 (1.01–4.00)
Past high-risk sexual behaviour[c]	57 (20)	100 (32)	2.17 (1.43–3.28)	74 (39)	2.50 (1.43–3.28)	15 (17)	1.06 (0.55–2.06)
Measured RTIs							
HIV serology positive[d]	39 (14)	98 (32)	3.61 (2.30–5.68)	65 (35)	4.01 (2.43–6.61)	20 (22)	2.63 (1.36–5.10)
HSV-2 serology positive[d]	115 (41)	180 (59)	2.15 (1.50–3.09)	122 (66)	2.71 (1.77–4.15)	40 (45)	1.27 (0.76–2.13)
Chlamydia serology positive[d]	44 (16)	57 (19)	1.80 (1.12–2.88)	38 (20)	2.11 (1.24–3.59)	14 (16)	1.41 (0.70–2.82)
BV by Amsel criteria	39 (14)	72 (23)	2.45 (1.54–3.90)	48 (25)	2.61 (1.56–4.38)	13 (14)	1.45 (0.71–2.80)
BV by Nugent criteria[d]	127 (48)	158 (52)	1.44 (1.01–2.06)	103 (56)	1.68 (1.11–2.54)	35 (40)	0.92 (0.55–1.55)
Lifestyle factors							
BMI > 25[d]	66 (24)	87 (31)	1.34 (0.90–1.99)	54 (32)	1.45 (0.92–2.28)	24 (30)	1.18 (0.66–2.10)
Alcohol (any)[e]	80 (28)	72 (23)	0.82 (0.55–1.21)	54 (29)	1.06 (0.68–1.63)	12 (13)	0.42 (0.21–0.83)
Smoker (past and/or now)	24 (8)	54 (17)	2.12 (1.22–3.70)	39 (21)	2.39 (1.32–4.35)	11 (12)	1.65 (0.73–3.73)
Lifestyle[f]	136 (48)	166 (53)	1.12 (0.80–1.58)	111 (59)	1.38 (0.93–2.05)	42 (47)	0.91 (0.55–1.49)

[a]Union dissolutions include all separations from previously cohabiting partners and excludes all union dissolutions which were reported to be a consequence of infertility.
[b]Includes sexual coercion by a stranger and/or a family member but not sexual coercion by an intimate partner.
[c]Composite variable for women: age first intercourse before 15 and/or marital dissolutions and/or ever engaged in transactional sex.
[d]HIV: 6 missing values infertiles; HSV-2: 2 missing values fertiles, 8 missing values infertiles; Chlamydia: 7 missing values infertiles; BV nugent: 19 missing values fertiles, 7 missing values infertiles; BMI: 3 missing values fertiles, 32 missing values infertiles.
[e]Cut-off value based on distribution of the answers.
[f]Composite variable combining using alcohol more than 1 unit a day and/or having BMI >25 and/or ever smoked.
[g]Tubal assessment was completed in 279 of 312 women in infertile relationships with 189 showing tubal pathology.
BV, bacterial vaginosis; BMI, body mass index.

Table III Predictors of male factor, non-male factor and infertility of any cause in Rwandan men.

Variable	Fertile relationship (n = 170), n (%)	Infertile relationship (n = 254), n (%)	Age, marital status and education adjusted OR (95% CI)	Male factor (n = 144),[g] n (%)	Age, marital status and education adjusted OR (95% CI)	No male factor (n = 82),[g] n (%)	Age, marital status and education adjusted OR (95% CI)
Sexual history							
Lifetime sex partners 4 or more	72 (42)	133 (52)	1.31 (0.87–2.00)	73 (51)	1.17 (0.72–1.88)	45 (55)	1.54 (0.89–2.68)
Ever had an union dissolution[a]	16 (9)	55 (22)	2.20 (1.15–4.18)	34 (24)	2.17 (1.08–4.34)	15 (18)	2.16 (0.90–5.20)
Age at first intercourse < 20	70 (41)	118 (46)	1.32 (0.87–1.98)	62 (43)	1.20 (0.75–1.93)	42 (51)	1.53 (0.88–2.63)
Circumcised	40 (23)	87 (34)	1.37 (0.86–2,18)	44 (31)	1.14 (0.67–1.94)	33 (40)	1.96 (1.08–3.54)
Never used condoms	45 (26)	87 (34)	1.51 (0.96–2.36)	55 (38)	1.77 (1.07–2.94)	22 (27)	0.97 (0.52–1.79)
Past high-risk sexual behaviour[b]	79 (47)	145 (57)	1.48 (0.99–2.22)	78 (54)	1.32 (0.83–2.11)	49 (60)	1.67 (0.97–2.87)
Reported STIs							
Ever had urethral discharge	45 (26)	69 (27)	0.97 (0.61–1.55)	45 (31)	1.15 (0.67–1.95)	18 (22)	0.78 (0.41–1.48)
Ever had a genital ulcer	41 (24)	43 (17)	0.66 (0.40–1.08)	27 (19)	0.73 (0.41–1.29)	12 (15)	0.59 (0.29–1.20)
Measured STIs							
HIV serology positive[c]	13 (8)	55 (22)	3.37 (1.73–6.56)	33 (23)	2.98 (1.43–6.18)	13 (16)	2.56 (1.08–6.07)
HSV-2 serology positive[c]	59 (35)	129 (51)	2.04 (1.33–3.12)	76 (53)	2.07 (1.27–3.38)	36 (44)	1.53 (0.87–2.70)
Lifestyle factors							
BMI[c]	23 (14)	42 (19)	1.26 (0.70–2.26)	23 (18)	1.21 (0.62–2.37)	14 (19)	1.38 (0.65–2.95)
Alcohol (>9 units/week)[d]	40 (23)	67 (23)	1.20 (0.75–1.92)	41(28)	1.30 (0.76–2.22)	20 (24)	1.11 (0.58–2.10)
Smoker (past and/or now)	85 (50)	112 (44)	0.82 (0.54–1.25)	65 (45)	0.81 (0.49–1.32)	37 (45)	0.86 (0.49–1.52)
Lifestyle[e]	109 (64)	160 (63)	0.89 (0.58–1.37)	91 (63)	0.87 (0.53–1.43)	52 (63)	0.92 (0.52–1.65)
Genital pathology							
Urological abnormality[f]	10 (6)	30 (12)	2.00 (0.93–4.28)	21 (15)	2.38 (1.05–5.40)	6 (7)	1.30 (0.45–3.79)

[a]Union dissolutions include all separations from previously cohabiting partners and excludes all union dissolutions which were reported to be a consequence of infertility.
[b]Composite variable: had a marital dissolution and/or age first intercourse before 20 years.
[c]HIV: 1 missing value infertiles; HSV-2: 3 missing values infertiles; BMI (body mass index): 35 missing values infertiles.
[d]Cut-off value based on distribution of the answers.
[e]Composite variable: using alcohol more than 1 unit a day and/or having BMI >25 and/or ever smoked.
[f]Abnormalities found during male genital exam including atrophic or non-palpable testes, cystic epididymus, non-palpable vas deferens, hydrocoele and varicocoele.
[g]Semen analysis was performed in 226 of 254 men in infertile relationships with 144 men diagnosed as having abnormal sperm parameters.
BMI, body mass index.

shown a possible association between HSV-2 and PID and Fallopian tube obstruction, but its direct effects on PID pathogenesis remain unclear (Lehtinen *et al*., 1985; Paavonen *et al*., 1985; Heinonen and Miettinen, 1994; Cherpes *et al*., 2006; Hettmann *et al*., 2008). Further research is needed to clarify whether HSV-2 infection facilitates movement of bacteria from the lower to the upper genital tract, and whether HSV-2-related genital immune activation and inflammation facilitates tubal scarring (Cherpes *et al*., 2006). In our study, HSV-2 was also associated with male factor infertility but not with non-male factor infertility, suggesting a causal role of this pathogen in male factor infertility as well. Within the infertile men, positive HSV-2 serostatus was only weakly associated with abnormal semen analysis, but this may have been due to insufficient statistical power.

In our study, HIV was consistently associated with all types of infertility in both men and women. The association between infertility and HIV has predominantly been documented in women but much less in men (Favot *et al*., 1997; Ikechebelu *et al*., 2002; Adesiyun *et al*., 2008). Both tubal and non-tubal infertility were significantly associated with HIV in our study, but the association with tubal infertility was stronger. Within the infertile women, HIV infection was also significantly associated with tubal pathology. Positive HIV and HSV-2 serostatus could be markers for other past STIs known to be tubal pathogens such as *C. trachomatis* and *G. neisseria*. We could not measure past

Table IV Multivariate logistic regression analysis of variables associated with tubal factor infertility (women) and male factor infertility (men).

Variable	Women with tubal factor AOR (95% CI)[a]	Men with abnormal semen analysis AOR (95% CI)[a]
Past high-risk sexual behaviour[b]	1.43 (0.86–2.39)	NI
Ever exposure to sexual violence	2.41 (1.36–4.25)	NI
HIV serology	2.41 (1.36–4.25)	2.43 (1.31–5.23)
HSV-2 serology	1.67 (1.03–2.71)	1.71 (1.02–2.87)
Chlamydia serology	1.78 (0.99–3.21)	NI
BV (Amsel)	1.97 (1.12–3.47)	NI
Lifestyle[c]	1.18 (0.76–1.82)	NI
Urologic abormaltities	NI	2.32 (1.01–5.31)

[a]AOR, adjusted odds ratio, model includes in addition of all variables listed age, marital status and education.
[b]Composite variable combining for women: age first intercourse before 15 and/or union dissolutions and/or ever engaged in transactional sex; for men: ever had a marital dissolution and/or age first intercourse before 20 years.
[c]Composite variable combining using alcohol more than 1 unit a day and/or having BMI >25 and/or ever smoked.
BV, bacterial vaginosis.

Table V PAFs of selected risk factors for tubal and male factor infertility.

Variable	Tubal factor		Male factor	
	Pe (%)	PAF%	Pe (%)	PAF%
Past high-risk sexual behaviour[a]	39	12		
Ever exposure to sexual violence	29	17		
HIV serology	35	20	23	13
HSV-2 serology	66	26	53	22
Chlamydia serology	20	12		
BV (Amsel)	25	9		
Lifestyle[b]	59	9		
Urologic abnormalities			15	8

PAF = population attributable fractions were calculated using the following formula: pe(AOR − 1)/AOR, pe is proportion of cases that is exposed to risk factor, AOR = adjusted odds ratio.
[a]Composite variable combining for women: age first intercourse before 15 and/or marital dissolutions and/or ever engaged in transactional sex; for men: ever had a marital dissolution and/or age first intercourse before 20 years.
[b]Composite variable combining using alcohol more than 1 unit a day and/or having BMI >25 and/or ever smoked.

G. neisseria infection. However, the association between HIV and tubal infertility remained significant after adjusting for positive Chlamydial antibodies and high-risk sexual behaviour, which could be a marker for gonococcus infection. This supports the current hypothesis that HIV plays a role in tubal pathogenesis (Kamenga *et al.*, 1995; Cohen *et al.*, 1998).

Also of interest is our finding of higher rates of BV, both by Amsel and Nugent criteria, in women with tubal infertility compared with fertile women and compared with infertile women with normal tubes. This association has been described by other groups in Western countries but has never been examined in an African context (Gaudoin *et al.*, 1999; Wilson *et al.*, 2002).

The limited role of *C. trachomatis* in infertility in Rwanda is in contrast with findings from Ghana, Gabon, Gambia and Nigeria where Chlamydia antibodies were significantly more often found among infertile women compared with fertile women (Mabey *et al.*, 1985; Reniers *et al.*, 1989; Omo-Aghoja *et al.*, 2007; Siemer *et al.*, 2008). All these studies use different diagnostic assays for identification of Chlamydia antibodies, rendering the comparison of results difficult.

The association between male circumcision and infertility in men is surprising since circumcision is known to protect men from HIV infection and possibly other STIs (Moses *et al.*, 1998; Morris, 2007). However, no association was found with abnormal semen analysis within the group of infertile men. Further research is needed to explore the association between circumcision and infertility.

The cross-sectional design of our study limits our ability to ascertain temporal relationships between HIV, HSV-2 and infertility. The association between infertility and high-risk sexual behaviour is thought to be bidirectional. Anthropological research in SSA has shown that couples with fertility problems are more likely to face extramarital relationships, polygamous unions and divorce, all of which are known risk factors for HIV and HSV-2 infection. In our study population current high-risk sexual behaviour (such as concurrent partners) was also associated with infertility and data on this are described elsewhere (Dhont, unpublished data).

To further elucidate temporal relationships between infertility and high-risk sexual behaviour, STIs and HIV, longitudinal studies of the reproductive and sexual behaviour and incidence of infections in newly married or cohabiting couples are needed.

Another limitation of this study is the possibility of selection bias. The infertile study population is a selection of couples who are willing to undergo infertility investigations; they do not necessarily represent all infertile couples in Kigali. This may explain the difference in marital status between the two groups and the differences in education between the men, educational level of women was similar for cases and controls. Married infertile couples might be more motivated to participate in the study than non-married infertile couples since marriage often represents a stronger commitment and a higher expectation to produce children than non-legalized cohabitation. It is not clear how this could have influenced the differences in HIV, HSV-2 and high-risk sexual behaviour in the past between infertile and fertile women. The relationship between socio-economic status and HIV is complex and contradictory results are reported in literature (Wojcicki, 2005; Msisha *et al.*, 2008). The high response rate of fertile women (86%) and the fact that the educational level is the same for responders and non-responders indicates that the selection bias for fertile women is likely to be small. We do not know the response rate of infertile women since they were recruited from the community through

word of mouth from other participants and it is not known how many women heard about the study.

Despite these limitations, our study has several implications. In addition to the promotion of safer sex, provision of STI treatment to rape victims in special gender-based violence clinics has the potential to prevent tubal factor infertility in this group of women. Prevention of HIV and HSV-2 infection are likely to decrease the incidence of male and tubal factor infertility in SSA. Whether long-term antiviral suppression therapy of HIV and HSV-2 can also contribute to the prevention of infertility is not known.

Authors' roles

The study was conceived and designed by N.D., M.T. and J.W.; N.D., J.V. and C.M. executed the study. N.D. analysed the study data and drafted the article. S.L. and J.W. assisted with data analysis and interpretation of data. S.L., C.M., J.V., J.W. and M.T. revised the article critically.

Acknowledgements

We thank Rosette Busasa, Ammiel Gasarabwe and the Community Mobilizers for their dedication during data collection, Dr Gilles Ndabisaya for programming of the database, and other members of the Projet Ubuzima team and the EDCTP microbicides project team for their contributions and support.

Funding

This study was funded by a PhD grant from the Flemish Interuniversity Council (VLIR-UOS) and by the European and Developing Countries Clinical Trials Partnership (EDCTP) through a project entitled: 'Preparing for Phase III vaginal microbicide trials in Rwanda and Kenya: Preparedness studies, capacity building and strengthening of medical referral systems'. EDCTP cannot accept any responsibility for information or views expressed herein. The project also received funding from the 'Walking Egg npo', Genk 2000.

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