Oral sex is associated with reduced incidence of recurrent miscarriage

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ABSTRACT

A possible way of immunomodulation of the maternal immune system before pregnancy would be exposure to paternal antigens via seminal fluid to oral mucosa. We hypothesized that women with recurrent miscarriage have had less oral sex compared to women with uneventful pregnancy.

In a matched case control study, 97 women with at least three unexplained consecutive miscarriages prior to the 20th week of gestation with the same partner were included. Cases were younger than 36 years at time of the third miscarriage. The control group included 137 matched women with an uneventful pregnancy. The association between oral sex and recurrent miscarriage was assessed with conditional logistic regression, odds ratios (ORs) were estimated. Missing data were imputed using Imputation by Chained Equations.

In the matched analysis, 41 out of 72 women with recurrent miscarriage had have oral sex, whereas 70 out of 96 matched controls answered positive to this question (56.9% vs. 72.9%, OR 0.50 95%CI $0.25-0.97$, $p = 0.04$). After imputation of missing exposure data (51.7%), the association became weaker (OR 0.67, 95%CI 0.36–1.24, $p = 0.21$).

In conclusion, this study suggests a possible protective role of oral sex in the occurrence of recurrent miscarriage in a proportion of the cases. Future studies in women with recurrent miscarriage explained by immune abnormalities should reveal whether oral exposure to seminal plasma indeed modifies the maternal immune system, resulting in more live births.

1. Introduction

About 1% of all couples trying to conceive, are confronted with recurrent miscarriage, which is often defined as three or more consecutive pregnancies losses prior to the 20th week of gestation (Coulam, 1991). Possible etiologic factors include uterine anomalies, endocrine disorders, maternal inherited and acquired thrombophilia, and parental chromosomal abnormalities (Branch et al., 2010; Larsen et al., 2013). However, in only about 25–50% of the couples an underlying cause for recurrent miscarriage can actually be identified (Rai and Regan, 2006; Branch et al., 2010).

Most research into the immunology of recurrent miscarriage focused on the maternal immune system, leaving paternal factors aside. However, males seems to be capable to affect the female immune system prior to conception (Robertson and Sharkey, 2001). Studies in mice have shown that during copulation, thus before implantation, fetus specific maternal tolerance toward paternal antigens is induced (Moldenhauer et al., 2009).

A well-known route to induce immune tolerance is via oral exposure, possibly because the gut has the most adequate absorption in the absence of an inflammatory environment (Sosroseno, 1995; Brandtzaeg, 1996). In transplantation models of rats, oral administration of MHC molecules diminishes the occurrence of allograft rejection (Hancock et al., 1993). In addition, Clark et al showed that direct seminal plasma antigen presentation to a mouse model of NK-cell mediated recurrent miscarriage may prevent the rejection of embryos (Clark et al., 2013).

Koelman et al hypothesized that a potent way of inducing tolerance towards paternal HLA antigens of the fetus in pregnancy would be exposure of these antigens to oral mucosa (Koelman et al., 2000). To

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support this theory, they showed that both oral sex and swallowing sperm reduced the incidence of preeclampsia (Koelman et al., 2000). Another study showed that the pattern of oral sex practice was similar in 66 women with two miscarriages and a control population (N = 110), but 44.5% women in the control group swallowed sperm compared to 24.2% of the women with recurrent miscarriage (Mattar et al., 2005). Here we describe the outcome of a matched case control study to assess the effect of oral sex on the occurrence of recurrent miscarriage in a well-characterized population.

2. Material and methods

2.1. Case group

From 433 women who visited the recurrent miscarriage clinic of the department of Obstetrics and Reproductive Medicine at the Leiden University Medical Centre (LUMC), a tertiary referral center in the Netherlands, between 2000 and 2014, 273 women were eligible and invited to participate in this study.

Eligible cases were women who had three or more consecutive miscarriages prior to the 20th week of gestation with the same partner, and who were younger than 36 years at time of their third consecutive miscarriage. Women with known causes for miscarriage such as uterine anomalies, parental chromosomal abnormalities, and anti-phospholipid syndrome were not eligible. The clinical work-up and definition for known causes is previously described (Meuleman et al., 2017). Women with hereditary thrombophilia were not excluded because the evidence that hereditary thrombophilia is associated with recurrent miscarriage is only weak (Larsen et al., 2013; McNamee et al., 2012). Both women with primary recurrent miscarriage (no history of live birth) and secondary recurrent miscarriage (1 live birth followed by consecutive miscarriages) were eligible.

From the 273 eligible women, 100 eligible women were included (Fig. 1). Baseline characteristics from the 100 included women and 173 eligible, but not included women of which most women were non-responders, is depicted in Supplementary Table 1.

2.2. Control group

As it is postulated that the primary pathogenesis of various pregnancy complications is the same within individuals (Moffett et al., 2004), controls were women with no miscarriage and only uncomplicated pregnancy(ies), i.e. no history of pregnancy complications such as pregnancy-induced hypertension, preeclampsia, Hemolysis Elevated Liver enzymes and Low Platelets (HELLP) syndrome (all defined according to the criteria of the International Society for the study of Hypertension in Pregnancy (ISHHP)), preterm birth (24–37 weeks), fetal growth restriction (birth weight below the 2.3[th] percentile for gestational age and sex (Kloosterman, 1969)), and perinatal death (fetal loss after 20 weeks of gestation till 7 days after birth).

In the Netherlands it is common practice that community midwives are taking care of low-risk women (with no medical or obstetrical history) during pregnancy and child birth. The zip code of each woman with recurrent miscarriage was used to contact the nearest midwifery practice to control for the impact of socio-economic status (SES) and urbanity in the current analyses. Women with the same zip code, the same age (difference in birth date maximally 1 year), and of which the time of first delivery was close to the time of the third miscarriage of the matched exposed woman (maximum 6 months before or 6 months after) were asked to participate. We contacted at least 3 controls per case. Enrolment took place between 2012 and 2014. (Fig. 1)

2.3. Ethical approval

The protocol was approved by the Ethics committee of the LUMC (P12-099) and all participants gave informed consent. The study was registered with the Dutch trial registry NTR3402 and is part of the REMI (REcurrent MIscarriages) studies, which investigate causes and consequences of recurrent miscarriages.

2.4. Dutch reference group

In order to make the study more robust we obtained another control group, i.e, Dutch reference group in which participants were asked to fill in a digital questionnaire about relationships and sexual behaviour (de Graaf, 2012), for specific details about selection of participants: Wijsen and de Haas (Wijsen and de Haas, 2012). In total 14.892 persons, including men and women, were approached of which 4170 (28%) filled in the questionnaire completely. For our reference group we selected, from a total of 2075 women, 1259 women in the fertile age (between 16 and 50 years) with a heterosexual relationship.

2.5. Variables and definitions

All cases and controls were asked to participate by filling in a digital questionnaire or on paper in case women had no access to internet between 2012 and 2014. The questionnaire was made using ProMISe, an internet based, application for the design, maintenance, and use of data management projects. Data were entered and stored in a good clinical practice approved database (ProMISe Database, https://www.msbi.nl/promise/).

The questionnaire contained questions about personal characteristics, general disease history, intoxications (smoking, alcohol, drugs), use of medication at different time points, outcome and complications of all pregnancies, neonatal characteristics, family disease history, partner's characteristics, and questions about their recent sexual behaviour. Information about medical history, use of medication, intoxications, and pregnancy outcome was cross-checked in obstetrical records to overcome recall bias. The data of the obstetrical records were used in case of discrepancies between the questionnaire and obstetrical records. For the questions about sexual behaviour additional informed consent was requested. The sexual behaviour part entailed questions about recent (frequency of) oral sex, swallowing the ejaculate, length of the relationship, and monthly sexual frequency. To investigate whether vaginal exposure of sperm was different between cases and controls, recent contraception methods including use of condom were asked for. (Supplementary data, Appendix 1).

Maternal age was defined as age at third consecutive miscarriage for cases or age at first pregnancy for controls. Socioeconomic status was categorized into high, middle or low by using mean household income levels of a neighborhood, which was determined with the first four digits of the zip code, using data from the Netherlands Institute for Social Research (SCP, 2006). Education was defined as whether or not university level (college and university education together). Ethnicity was based on country of birth of the woman and divided in 4 groups according to the rules of the Central Bureau of Statistics of the Netherlands. (CBS, 2013)

2.6. Sample size considerations

Sample size calculation was performed assuming that 40–50% of the cases and 60–80% of the controls would have oral sex (Koelman et al., 2000; Saftlas et al., 2014), leading to the following more precise assumptions adapted for the matched design:

Combination 1: (18%): Cases and controls both don't have oral sex
Combination 2: (15%): Cases have oral sex, controls don't have.
Combination 3: (35%): Cases don't have oral sex, controls have.
Combination 4 (32%): Cases and controls both have oral sex

This implies an odds-ratio on the event of 0.43 for oral sex vs. no oral sex. A sample size of 186 women (93 exposed, 93 non-exposed) was expected to provide sufficient power (two-sided alpha .05. power 80%), taking a 10% drop-out in consideration. We planned 1:1
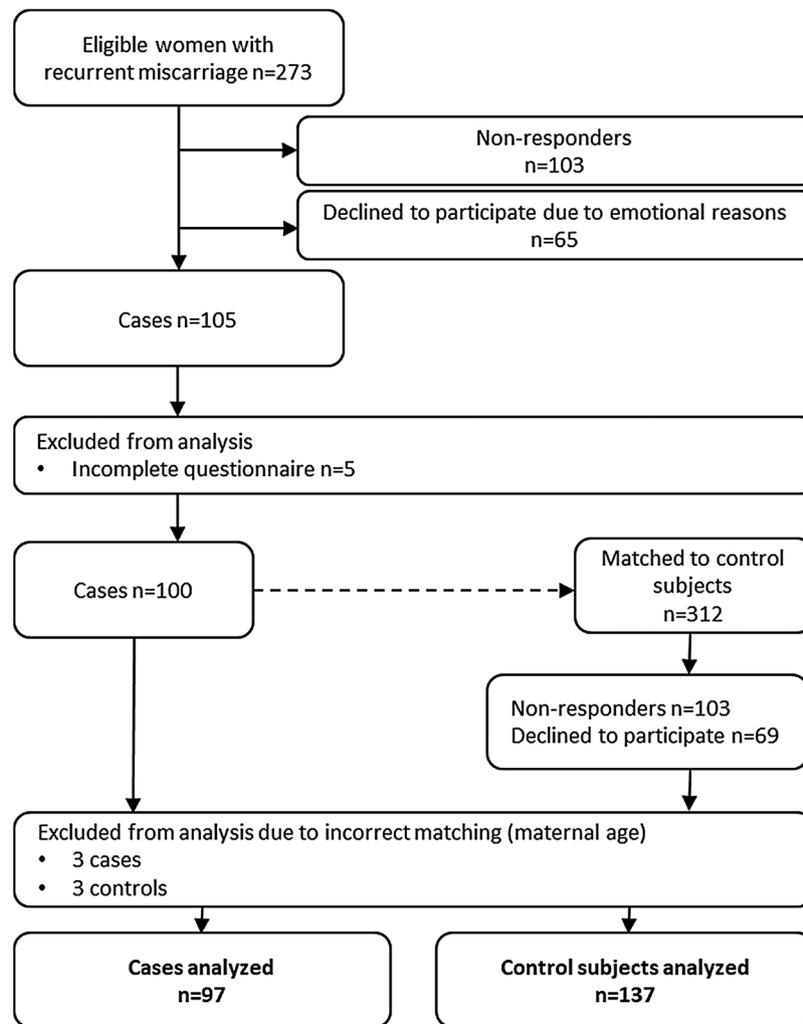
Fig. 1. Flowchart of subjects.

case:control ratio, that is, one woman who had recurrent miscarriage matched to one control. On forehand we expected a lot of non-responders and therefore we contacted at least 3 controls per case. PASS 2008, Power Analysis and Sample Size Software (Hintze J., NCSS Kaysville USA) was used for the sample size calculation.

2.7. Statistical analysis

The association between oral sex and recurrent miscarriage was studied with conditional logistic regression using a stratified Cox regression and odds ratios (ORs) were estimated. Statistics were performed using SPSS (Version 24.0, Inc., Chicago, IL, USA). A p-value < 0.05 was considered statistically significant.

Of the 97 cases and 137 controls, 51.7% did not complete all the questions about sexual behaviour, including questions about oral sex. We compared the cases and controls who did complete questionnaires to cases and controls who did not using chi-square tests or Fisher's exact tests or Mann Whitney U tests, whichever were appropriate. We repeated the analyses with missing exposure data imputed using Imputation by Chained Equations. In the imputation models the case/controls status oral sex, swallowing the ejaculate, relationship duration at time of index pregnancy, sexual frequency, and condom use as contraception, and the variables used for matching cases and controls (SES, urbanity, maternal age at time of index pregnancy) were included. In addition, variables that were significantly different between cases who completed all questions on sexual behaviour and cases who

did not complete these questions were also included in the imputation model. Ten imputed datasets were created.

3. Results

3.1. Baseline characteristics

In total, 97 women with recurrent miscarriage were included and 137 matched controls (Fig. 1). Table 1 shows the baseline characteristics of the study population.

In the case group, 63 women (64.9%) had primary recurrent miscarriage and 34 (35.1%) secondary recurrent miscarriage. A total of 65 women had 4 or more consecutive miscarriages (67.0%), and 39 women (40.2%) had 5 or more miscarriages. A total of 6 (6.2%) cases had hereditary thrombophilia, i.e, factor V Leiden (n = 4), prothrombin gene mutation (n = 3), or antithrombin deficiency (n = 1). None had protein C or S deficiencies. Out of 97 cases, 70 had at least one live birth after the consecutive miscarriages (72.2%).

3.2. Sexual behaviour

Of the 97 cases, 46 cases (47.4%) and of the 137 controls, 75 controls (55.9%) did not complete all the questions about sexual behaviour. In Table 2 characteristics are shown of women with completed and women with not-completed questionnaire. Cases who did not complete the questions about sexual behaviour were significantly more often

Table 1
Baseline characteristics.

	Cases with primary recurrent miscarriage (N = 63)	Cases with secondary recurrent miscarriage (N = 34)	Controls without miscarriage (N = 137)
Maternal age at index pregnancy (years;median[IQR])	29.0 (27.0-32.0)	31.0 (27.7-33.0)	30.0 (27.0-32.0)
Maternal age at time of questionnaire (years;median[IQR])	35.0 (31.0-38.0)	35.5 (33.0-38.2)	36.0 (33.0-39.0)
BMI (median[IQR])[a]	23.2 (21.6-27.4)	23.4 (20.9-25.7)	23.1 (21.0-25.8)
Smoking at time of questionnaire	10 (15.9)	4 (12.1)	18 (13.2)
Use of alcohol at time of questionnaire	34 (54.0)	17 (50.0)	87 (64.4)
Ethnic origin			
Native/Caucasian	59 (93.7)	27 (79.4)	130 (94.9)
Turkish/Moroccan	2 (3.2)	1 (2.9)	2 (1.5)
Antillean/Surinamese	1 (1.6)	1 (2.9)	1 (0.7)
Other non-Caucasian immigrants	1 (1.6)	5 (14.6)	4 (2.9)
University level education	35 (55.6)	12 (35.3)	82 (59.9)
Urbanity			
Few	9 (14.3)	5 (14.7)	15 (10.9)
Strong to moderate	37 (58.7)	16 (47.1)	78 (56.9)
Very strong	17 (27.0)	13 (38.2)	44 (32.1)
SES			
Lowest 25% (< 25%)	4 (6.3)	6 (17.6)	19 (13.9)
Median 50% (25-75%)	33 (52.4)	18 (52.9)	64 (46.7)
Highest 25% (> 75%)	26 (41.3)	10 (29.4)	54 (39.4)
Gravidity (median[IQR])	5 (5-8)	7 (6-8)	2 (1-2)
Parity (median[IQR])	1 (0-2)	2 (1-2)	2 (1-2)

Data are n (%) unless otherwise indicated, BMI; Body mass index, SES; Socioeconomic status, IQR; interquartile range.
[a] 1.7% missing values (1 of 97 cases with secondary recurrent miscarriage and 3 of 137 controls).

smokers ($p = 0.02$). No other statistical differences were observed. Due to incomplete questionnaires on sexual behaviour matched analysis on oral sex was performed with 72 cases matched with 96 controls.

In the matched analysis, 41 out of 72 women with recurrent miscarriage reported to have oral sex, compared to 70 out of 96 matched controls (56.9% vs. 72.9%, OR 0.50 95%CI 0.25−0.97, $p = 0.04$) (Table 3). From the 41 women with recurrent miscarriage who indicated to have oral sex, 39 women filled in the question on swallowing the sperm and 9 indicated to swallow sperm (23.1%). In controls 68/70 matched controls who indicated to have oral sex filled in this question

Table 2
Baseline characteristics of cases and controls with complete and incomplete information on sexual behaviour.

	Cases (N = 97)			Controls (N = 137)		
	Incomplete questions about sexual behavior (n = 46)	Complete question about sexual behavior (n = 51)	P-value	Incomplete questions about sexual behavior (n = 75)	Complete question about sexual behavior (n = 62)	P-value
Maternal age at index pregnancy (years;median[IQR])	30 (27-32.2)	30 (27-32)	0.82[a]	30 (27-32)	30 (27.7-31)	0.77
Maternal age at time of questionnaire (years;median[IQR])	35 (33-39)	34 (31-37)	0.12[a]	36 (33-39)	35 (32-39)	0.35
BMI (median[IQR])[b]	23.0 (21.5-25.7)	24 (21.0-27.1)	0.31[a]	23.1 (20.9-26.0)	23.1 (21.0-25.8)	1.00
Smoking at time of questionnaire	11 (23.9)	3 (6.0)	0.02	11 (14.9)	7 (11.3)	0.54
Use of alcohol at time of questionnaire	25 (54.3)	26 (51.0)	0.74	51 (68.9)	36 (59.0)	0.23
Ethnic origin			0.19			0.73
Native/Caucasian	42 (91.3)	44 (86.3)		72 (96.0)	58 (93.5)	
Turkish/Moroccan	1 (2.2)	2 (3.9)		1 (1.3)	1 (1.6)	
Antillean/Surinamese	2 (4.3)	0 (0.0)		0 (0.0)	1 (1.6)	
Other non-Caucasian immigrants	1 (2.2)	5 (9.8)		2 (2.7)	2 (3.2)	
University level education	22 (47.8)	25 (49.0)	0.91	45 (60.0)	37 (59.7)	0.97
Urbanity			0.62			0.68
Few	8 (17.4)	6 (11.8)		7 (9.3)	8 (12.9)	
Strong to moderate	23 (50.0)	30 (58.8)		42 (56.0)	36 (58.1)	
Very strong	15 (32.6)	15 (29.4)		26 (34.7)	18 (29.0)	
SES			0.13			0.73
Lowest 25% (< 25%)	7 (15.2)	3 (5.9)		12 (16.0)	7 (11.3)	
Median 50% (25-75%)	26 (56.5)	25 (49.0)		34 (45.3)	30 (48.4)	
Highest 25% (> 75%)	13 (28.3)	23 (45.1)		29 (38.7)	25 (40.3)	
Gravidity (median[min-max])	6 (5-8.2)	7 (5-8)	0.79[a]	2 (1-2)	2 (1-2)	0.81
Parity (median[min-max])	1 (1-2)	2 (1-2)	0.36[a]	2 (1-2)	2 (1-2)	0.61

Data are n (%) unless otherwise indicated, BMI; Body mass index, SES; Socioeconomic status, IQR; interquartile range.
All χ^2 tests or Fisher's exact tests except [a] Mann Whitney U test. [b]1.7% missing values (1 of 97 cases with incomplete questions and 3 of 137 controls with incomplete questions).

Table 3
Oral sex in women with recurrent miscarriage.

	Recurrent miscarriage (N = 97)	No miscarriage (N = 137)	OR (95% CI)	P	OR (95% CI)	P
			Missing values not imputed		With missing values imputed	
Oral sex[a]	41/72 (56.9)	70/96 (72.9)	0.50 (0.25-0.97)	0.04	0.67 (0.36-1.24)	0.21
Relationship duration at time of index pregnancy (median [IQR])[b]	7 (4-9)	7 (4-9.2)	0.94 (0.84-1.05)	0.30	0.97 (0.88-1.07)	0.56
Sex frequency (median [IQR])[c]	8 (4-8)	4 (4-8)	1.07 (0.96-1.19)	0.18	1.03 (0.95-1.12)	0.41
Condom use as contraception[d]	7/56 (12.5)	15/69 (21.7)	0.59 (0.23-1.49)	0.27	0.82 (0.39-1.70)	0.60

Data are n (%), OR; odds ratio, CI; confidence interval, P; p-value.
[a] 17.5% missing values (20 of 97 cases and 21 of 137 controls), 10.6% lost by matching (5 of 97 cases and 20 of 137 controls).
[b] 26.0% missing values (21 of 97 cases and 40 of 137 controls), 13.6% lost by matching (13 of 97 cases and 19 of 137 controls).
[c] 32.4% missing values (29 of 97 cases and 47 of 137 controls), 18.3% lost by matching (16 of 97 cases and 27 of 137 controls).
[d] 30.3% missing values (26 of 97 cases and 45 of 137 controls), 16.2% lost by matching (15 of 97 cases and 23 of 137 controls).

and 10 controls (14.7%) swallowed sperm. No significant differences were observed in the incidence of oral sex in women with primary recurrent miscarriage and secondary recurrent miscarriage (63.3% vs. 46.4%, p = 0.15).

Table 3 also shows results after missing values being imputed. The association became weaker with a crude OR of 0.67 (95%CI 0.36–1.24, p = 0.21).

Out of the 1259 women selected as Dutch reference group, 1206 women filled in the question on oral sex. From the 1206 women 1076 women stated to have oral sex (89.2%) compared to 44 out of 77 women with recurrent miscarriage who filled in this question (57.1%) (OR 0.16, 95%CI 0.09 − 0.26, p < 0.001).

4. Discussion

This matched case control study suggests that women with recurrent miscarriage had less oral sex compared to women with uneventful pregnancy. This is in line with the hypothesis that the gut has the most adequate absorption in the absence of an inflammatory environment (Sosroseno, 1995; Brandtzaeg, 1996), and seminal fluid contains soluble HLA antigens which can already induce maternal immune tolerance towards inherited paternal antigens of the fetus before implantation.

The strength of this study is that a large homogenous well-characterized case group of women with at least three consecutive unexplained recurrent miscarriages less than 20 weeks of gestation with the same partner was included. Furthermore, we compared our data in women with recurrent miscarriage to a representative group of Dutch women in the fertile age. In this reference group 89.2% of the women stated to have oral sex, this percentage is comparable to research on heterosexual behaviour in the USA, that showed that 83.5% of the women between age 35 years and 44 years ever had oral sex (Leichliter et al., 2007). In addition, in a recent study on oral and vaginal exposure to the father's seminal fluid in preeclampsia, 78.6% of controls subjects had oral sex (Saftlas et al., 2014). In contrast, in our study this was only 56.9% of the women with recurrent miscarriage, suggesting indeed that having less oral sex might be associated with pregnancy complications such as recurrent miscarriage.

Although it is suggested that particularly the vaginal route of exposure to paternal antigens is critical to successful pregnancy (Saftlas et al., 2014), earlier findings suggest that oral exposure to paternal antigens reduced the incidence of preeclampsia (Koelman et al., 2000), which is in line with our findings in recurrent miscarriage. Seminal fluid contains all types of immunoregulatory factors such as cytokines, hormones and soluble HLA (sHLA) antigens (Politch et al., 2007) including sHLA-G. sHLA-G appears to have an important role in creating tolerance during pregnancy (Athanassakis et al., 1999; Pfeiffer et al., 2000; Zidi et al., 2016), and sHLA-G in seminal fluid may affect the maternal immune system before implantation of the embryo (Larsen et al., 2011). The gut has the most adequate absorption in the absence

of an inflammatory environment (Sosroseno, 1995; Brandtzaeg, 1996), and therefore having oral sex before implantation of the semi-allogeneic fetus could be a potent way of inducing immune tolerance to the paternal HLA antigens.

We were confronted with incomplete data from questionnaires, especially missing data on sexual behaviour, which was our exposure of interest. We tried to overcome this problem by imputation, a standard statistical approach to deal with missing data (Schafer, 1999). However, valid imputation assumes missing at random, meaning that other variables with complete information are completely accountable for the missing data. However this missingness at random is an untestable assumption, but may be valid in our study as comparing responders to non-responders showed no significant difference, except for smoking. However, missingness at random could still be dependent on variables not included in this study. The observed negative association between oral sex and recurrent miscarriage became smaller after imputation of the missing data, and the confidence interval included the null effect. When performing an unmatched analysis between cases and controls using only complete matched pairs, results were similar to the matched analysis, when performing an unmatched analysis using all cases and controls, results were similar to the results of the imputed analysis (data not shown). This shows that our results should be interpreted with caution. Potential information bias should also be taken into account, as misclassification may have occurred due to the use of questionnaires and self-reported data, which is impossible to overcome. Importantly, seminal fluid exposure is not commonly recognized as a potential factor that could influence the occurrence of recurrent miscarriage, this will likely not have influenced the way women filled in the questionnaire. For this reason, information bias is not likely explanation for the observed association.

Our study is limited by the fact that the questions about sexual behaviour and contraception did not concern the period before the index pregnancy. This might explain the discrepancy in frequency of sexual intercourse between our study and others showing that limited seminal exposure or the use of barrier methods before conception play a role in the occurrence of pregnancy complications such as preeclampsia (Klonoff-Cohen et al., 1989; Robillard and Hulsey, 1996; Kho et al., 2009). In our study the frequency of sexual intercourse was similar for women with recurrent miscarriage and controls. It is unknown how sexual behaviour changes during the years in individuals and therefore the questions about sexual behaviour might not reflect sexual behaviour before the index pregnancy especially in the women with recurrent miscarriage. By questioning sexual behaviour after the occurrence of recurrent miscarriage, the question remains whether having recurrent miscarriage affects sexual behaviour or sexual behaviour influence the occurrence of recurrent miscarriage.

Another possible limitation is that couples with recurrent miscarriage who did not participate in this study had overall significantly fewer children and fewer live births after they had recurrent miscarriages. However, this suggests that the observed effects are rather an

underestimation due to the fact that the group with worse outcome amongst the recurrent miscarriage cases did not participate.

Despite the limitations of this study and the issues addressed, orally exposure to seminal fluid seems to induce maternal tolerance to paternal antigens and therefore influence pregnancy outcome in a positive way. Our results suggest an association between less oral sex and the occurrence of recurrent miscarriage; this however needs confirmation given the limitations of the present study.

Acknowledgements

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jri.2019.03.005.

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