women). Even current versus lifetime estimates are problematic since lifetime estimates can be confounded when people sampled are still of reproductive age. Despite these problems we believe that a critical goal, and one that should be taken up by the World Health Organization, is to ascertain contemporary worldwide prevalence rates for infertility as the WHO cannot meet its Reproductive Health Strategy objectives regarding unmet reproductive health needs if it does not know the scope of the infertility problem, its nature (primary, secondary) or the interventions needed to address this global health problem.

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Reply: Influence of activating and inhibiting killer immunoglobulin-like receptors on predisposition to recurrent miscarriages

Sir,

We thank Moffett and Hiby for their interest in our paper entitled ‘Influence of activating and inhibiting killer immunoglobulin-like receptors on predisposition to recurrent miscarriages’. We share their concern regarding the association of KIRs in recurrent miscarriages in the light of existing confusions and conflicting reports. We undertook this study while being fully aware of the various reports published prior to this one concerning this issue and that there has not been a clear consensus established to date regarding the role of KIRs in the feto-maternal interactions and as predisposing factors to recurrent miscarriage (RM).

In the introduction as well as in the discussion of our paper, we have provided the details of other studies reported prior to our study which includes the following:

Witt et al. (2004) reported that the maternal KIR repertoire was not associated with RM, whereas Varla-Leftherioti et al. (2005) reported fewer appropriate inhibitory KIRs in RM women in a study of 26 patients. Likewise, Wang et al. (2007) and Hiby et al. (2008) reported an association between the maternal KIR repertoire and HLA-Cw alleles in 67 and 73 RM couples, respectively. To the best of our understanding, the possible reasons for this disparity could be:

(i) Relatively smaller sample sizes of recurrent miscarriage patients and different selection criteria opted by different workers. This warrants a methodical and well planned meta-analysis to be carried out on this subject.

(ii) The influence of different ethnic groups, while conducting such studies on the outcome of KIR frequencies, as KIR genotypes have a wide geographical distribution.

(iii) The methodology used for genotyping the KIRs is almost invariably the PCR–SSP technique in all the previous studies.

We planned our study keeping in mind the conflicting data available on the role of KIRs in feto-maternal interactions. The main objective was to evaluate these markers in the ethnically diverse population from the Indian subcontinent. We have considered all the above-mentioned points to decrease the discrepancy in our data i.e. sample size, exclusion–inclusion criterion for patient selection; selection of ethnically matched RM controls fulfilling the criteria of at least three live births and no history of miscarriages, pre-eclampsia, pre-term delivery and ectopic pregnancy; and selection of the reliable and most recent PCR–SSP protocol at the time of the commencement of this study.

We had followed the following strategy during the course of sample selection for this study: we initially collected samples from 526 RM patients and screened them for various known causes of miscarriages (as mentioned in the Methods section of the manuscript). After excluding those who do not fit into our inclusion criteria (including the secondary aborters), we were left with 205 cases (~39%) of varied ethnic distribution within the North Indian region. We then selected the control women from the renal transplant donors at our center. The healthy parous females who had at least three live
births and had no previous history of miscarriage, pre-eclampsia, ectopic pregnancy or pre-term delivery, we selected 224 controls subjects of the same ethnic distribution as that of RM subjects (Supplementary Table S1). The Supplementary Table (S1) reflects that there were no significant differences in terms of ethnicity among the patients and controls and that the differences seen in KIR frequencies are not due to non-ethnically matched controls as mentioned by Moffett and Hiby in their letter to the editor.

For the genotyping of KIR in our samples, we have largely followed the method described by Vilches et al. (2007), with some modifications suitings to our lab conditions. Vilches et al. (2007) primers amplify all the KIR alleles in the current Immuno-polymorphism Database (version 1.4.0, 4 June 2007). Individuals were determined to be negative for a KIR gene when a band of the expected size was absent in the presence of the control band. For easier size discrimination of KIR2DS4 full length and deletion variants, aliquots of PCR products of 2DS4 were run separately on the gel, and individuals were assigned positive when either or both the variants gave signals, whereas when both the variants were absent, the individual was labeled as 2DS4 negative. The data were verified and validated by Dr Raja Rajalingam from the University of California, Los Angeles (UCLA), CA, USA.

We used the working definition of assigning the KIR A and B haplotypes given by Rajalingam et al. (2008). Individuals who carried a fixed gene set of nine genes consisting of KIR3DL3–2DL3–2DL1–2DP1–3DP1–2DL4–3DL1–2DS4–3DL2, characteristic of Group A haplotypes, were considered as having two copies of Group A KIR haplotypes (AA genotypes). On the other hand, individuals lacking any of the four variable genes (KIR2DL1, 2DL3, 3DL1 and 2DS4) were regarded as carrying two copies of Group B haplotypes (BB genotypes). All the remaining combinations were regarded as heterozygotes carrying both the haplogroups, i.e. AB genotypes.

The RM control data reported by us varies from a previous report in North Indian population (Rajalingam et al., 2002) at several loci. To our understanding, this difference is because of the fact that our control data represent the healthy parous females of the eastern Uttar Pradesh Province of Northern India, and the absence of male individuals results in a bias as far as a representative population is concerned. Thus, we reckon our data cannot be considered as a representative of North Indian population because of the gender bias. Our data can very well be regarded as representative RM control data. Presently, we are working on the normal distribution of KIR genotypes among males and females from this part of the country which may provide a wider landscape of KIR distribution.

We agree with Moffett and Hiby that before publishing our control population data, we should confirm the accuracy and reliability of the KIR typing by using the DNA provided by the UCLA International KIR Exchange Program. However, the confusion about the role of KIR in RM is possible only through a multicentric study where samples of different ethnicities using the same selection criteria for the RM group are being used.

**References**


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**Re: Fertility preservation in adolescent males: experience over 22 years at Rouen University Hospital**

Sir,

Menon et al. (2009) report the high success rate of cryopreservation of seminal fluid from adolescents with a malignancy who are planned to undergo chemotherapy. They also, however, report that 40% of the young patients in their series were unable to benefit from the cryopreservation programme because of masturbation-related problems. A significant proportion of these patients (21%) had testicular cancer and had undergone orchidectomy.

We recently described how it is possible to recover spermatozoa using a testicular sperm extraction technique on the malignant testicle.