

its use should be based on the best evidence available. With our meta-analysis, no significant effect of treatment with IVIG was found, which was confirmed by a subsequent systematic review of IVIG for recurrent miscarriage (Ata *et al.*, 2011). In addition to IVIG, a systematic review of randomized trials found no significant beneficial effect of paternal or third party mononuclear cell immunization, and trophoblast membranes over placebo in improving the live birth rate in idiopathic recurrent miscarriage (Porter *et al.*, 2006). Presently, mononuclear cell immunization requires Food and Drug Administration approval under an investigational new drug application.

Although numerous immunologic tests have been proposed to identify an alloimmune etiology, there is a paucity of validated tests to assess the maternal immune response to pregnancy. Until miscarriages are routinely evaluated for numeric chromosome errors (trisomy, monosomy and polyploidy), validating immunologic tests will be fought with difficulties because of the admixture of 'explained' aneuploid/polyploid miscarriages with 'unexplained' euploid miscarriages.

Offering women with idiopathic recurrent miscarriage an evidence-based evaluation and management plan, to optimize their likelihood of having a subsequent successful pregnancy outcome, requires leaving entrenched personal biases behind and using results of well-designed RCTs. This will move the field forward.

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Women's age at menarche and offspring sex ratio

Sir,

Fukuda *et al.* (2011) recently suggested that women's age at menarche may be related to their offspring sex ratio. In their sample of 10 847 premenopausal Japanese women producing 21 208 live singleton births, women having the highest offspring sex ratio reached menarche at the age of 14 years. Women reaching menarche at younger or older age had lower offspring sex ratio (Fukuda *et al.*, 2011).

This is an interesting preliminary finding. Unfortunately, there are some methodological shortcomings that may have affected the results. First, as acknowledged by the authors themselves, offspring sex ratio in humans may potentially depend on various physiological, life-style and demographic factors (Lazarus, 2002). Therefore, it is rather surprising that the authors made no attempt to control for any of such confounding factors in their analyses, but relied on univariate association between menarche age and offspring sex ratio only. Second, the statistical procedure used in the study may not have been fully appropriate. This is because the analysis likely failed to account for the fact that since 10 847 women gave birth to 21 208 offspring, not all offspring could be regarded as independent data points. Independence of observations is a basic assumption of many statistical models and failure to account for it when needed likely produces underestimated variances and thus too narrow confidence intervals, making statistical inference unreliable (Littell *et al.*, 2006). Moreover, it may be argued that since the authors found no statistical support for their heterogeneity chi-square test, i.e. no overall association between women's age at menarche and offspring sex ratio, it was unwarranted to proceed to pairwise comparisons (Harrell, 2001).

Nonetheless, the findings of Fukuda *et al.* (2011), if real, are exciting and require replication. I examined the association between recalled age at menarche and lifetime offspring sex ratio using data on 241 postmenopausal Finnish women having a total of 494 singleton offspring (Helle and Lilley, 2008). In these women, mean age at menarche was 13.02 years (SD = 1.38, min = 10, max = 17) and mean offspring sex ratio, calculated as the proportion of males and not as the ratio of males to females (Wilson and Hardy, 2002), 0.531 (SD = 0.50). Due to small number of observations, the youngest and the oldest groups of age at menarche were added to the nearest age group. We applied logistic regression model with binomial errors and logit link function to model the likelihood of an offspring being a male in relation to her mothers' age at menarche (six categories). Because many mothers produced several offspring (i.e. pseudoreplication within a mother), we used generalized estimating equations (GEE) with exchangeable working correlation structure to accommodate such clustering (Lipsitz and Fitzmaurice, 2009). Statistical inference in this GEE model was based on Score test (Lipsitz and Fitzmaurice, 2009). Our model also controlled for several factors that have previously been suggested to relate to offspring sex: maternal age at birth (Lazarus, 2002), educational attainment (e.g. Almond and Edlund, 2007) and smoking (e.g. Fukuda *et al.*, 2002).

In these data, women's age at menarche was not associated with offspring sex ($\chi^2_5 = 6.84$, $P = 0.23$). Those women who reached menarche at the age of 12 years produced the highest proportion of sons [mean, 95% confidence intervals (CIs) = 0.55, 0.43–0.67]. Instead, the lowest proportion of sons (mean, 95% CIs = 0.38, 0.30–0.46) was produced by those women who experienced menarche at the age of 14 years. This conclusion remained unchanged when all the other predictors were omitted from the model ($\chi^2_5 = 6.88$, $P = 0.23$).

Although based on a smaller sample size, these results provide no evidence to suggest that women's age at menarche is associated with their lifetime offspring sex ratio. This result thus contrasts the findings of Fukuda *et al.* (2011). It would be interesting to see whether their results hold after correcting for those methodological shortcomings argued above. If the results remain and other studies provide similar observations, we may begin to evaluate what physiological, and perhaps even evolutionary, factors are responsible for the phenomenon.

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The sex ratio of offspring is associated with the mother's age at menarche

Sir,

We read with great interest the article by Fukuda *et al.* (2011) regarding the association between sex ratio of offspring and the mothers' age at menarche. The authors conclude that women entering menarche outside the normal range, especially those with earlier menarche, may have an increased chance of producing female offspring partially because of increased spontaneous abortions of male zygotes. While we agree that mothers' age at menarche may be related to offspring's sex, we have doubts that analyzing only mothers' age at menarche, offspring's gender and sex ratio is sufficient to draw this conclusion.

First, the methods for this study were not explained very clearly. The attending age of the participants at attendance was 37.5 ± 7.2 years, range 22–54, but the article did not specify the age at delivery, the gestational age, BMI, participants nutritional status or any other detailed information, such as delivery times. The authors state, that when the maternal age is less than 31 years old, sex ratio is 1.095 (1998:1824), and when the maternal age is more than 32 years old, the sex ratio is 0.931 (363:390). There were significant differences

($P = 0.0411$) between them. Therefore, the mother's age at pregnancy may be a confounding factor of sex ratio (Matsuo *et al.*, 2009). We consider that women over 35 and under 16 years old at delivery should have been excluded from the study, or that the authors should do stratified analysis. In short, factors affecting sex determination is not currently clear. We should consider other factors that may affect sex determination, rather than exclude other confounding factors.

Secondly, of the 10 847 women of this study, the largest proportion experienced menarche age between 11 and 14 years, 1350 had had menarche by 11 years old, 3241 women by age of 12, 2819 women by age of 13 and 2273 women by age of 14 group. The number of participants in the remaining groups was rather small meaning errors are more likely to occur; collectively only 254 experience menarche at the age of 9 or 10 and 910 in the age categories of 15–18 years. Thus in these groups, the true sex ratio might not have been calculated.

Thirdly, although previous studies (Emaus *et al.*, 2008) suggest that early age at menarche might lead to high levels of estradiol (E2), which can result in a higher sex ratio of newborn infants, the authors believe that spontaneous abortion of male zygotes might have led to lower sex ratio of the earlier menarche. However, they do not provide any information about spontaneous abortion rates, or serum E2 levels of these women and other indicators.

In our opinion the Fukuda *et al.* study is very interesting and bold. However, we feel that caution is required in drawing conclusions and further large-scale multi-center clinical studies are required.

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Reply: The sex ratio of offspring is associated with the mothers' age at menarche

Sir,

We thank Dr Helle for his interest in our work. He suggested that our work has some methodological shortcomings that may have affected the results. The data were collected from our clinical charts, which unfortunately did not include information on