Reply: Testis development, beef consumption and study methods

Sir,

We appreciate the thoughtful letter by Drs. Amann and Seidel regarding our paper (Swan *et al.*, 2007) in which they correctly describe the goal of the study and their agreement with the importance we attribute to it. They did have several reservations, however, which we will try to address.

The first of these is a concern that the volume of the semen sample and total sperm count were not discussed. We chose not to analyse sample volume in relation to mother's beef because this parameter has seldom if ever been associated with exogenous exposures in prior studies, including ours (Swan *et al.*, 2003). However, in response to this letter we did so (Ejaculate volume was estimated by specimen weight, assuming a semen density of 1.0 g/ml). For mothers reporting $\leq$7 beef meals/week, the mean sample volume was 4.0 g [95% confidence interval (CI) 3.7, 4.3], compared with 4.1 g (95% CI 3.6, 4.7) for mothers who ate $>$7 beef meals/week. Similarly, sample volume was unrelated to number of beef meals reported by the mother when examined using the multivariate model: regression coefficient for number of beef meals was 0.003 (95% CI -0.05, 0.05). The variability of total sperm count reflects that of both concentration and volume, from which it is directly calculated. Therefore, in the absence of an association between our study variables and sample volume, total count would be expected to be somewhat less strongly associated than sperm concentration. Indeed, total sperm count was 19.3% lower in sons whose mothers ate $>$7 beef meals/week compared with those who ate $\leq$7 beef meals/week ($P = 0.087$) compared with a 24.3% reduction ($P = 0.014$) in sperm concentration. In fact, the role of total sperm count in epidemiological studies of semen quality is far from universally accepted. Furthermore, most clinical andrologists using sperm concentration to evaluate the fertility potential of a man, as can be seen by a quick PubMed search. For example, in a recent major meta-analysis of semen quality from 1549 healthy males in 30 studies worldwide, the study variable of interest was return (after male contraception) to a sperm concentration of 20×10^6/ml. There are no data provided on total sperm count (Liu *et al.*, 2006).

Amann and Seidel also suggest that we should have attempted to estimate daily sperm production in our study participants. In experimental studies, this parameter has indeed provided the 'gold standard'. However, while it would have been ideal to have a measure of daily sperm production, as can be done in nonhuman studies, we are not aware of this being done in large- scale observational studies of healthy males.

The third point discussed by Amann and Seidel is that our analysis was based on one semen sample per man, rather than two. We recently conducted an extensive analysis of semen parameters estimated on the basis of one versus two semen samples per man. In that analysis we demonstrated that, while multiple samples per man be necessary in a clinical evaluation of questionable fertility, this is not the case for population-based studies of semen quality (Stokes-Riner *et al.*, 2007). In fact, we showed, using data from the same population analysed to examine mother's beef consumption and semen quality, that among men who gave two samples, there were no significant differences between semen parameters based on the first or second samples, after covariate adjustment. Thus, as long as the model adjusts for important covariates, it makes little difference whether the analysis includes men who give one semen sample or two. Though we obtained two semen samples from a majority of men (and in both the count was determined using a disposable microcell chamber), only one was analysed by hemocytometer. Since, this is the most widely accepted method of determining sperm concentration, this is the method on which we reported.

Amann and Seidel also state; 'it is not clear if data were corrected for abstinence interval'. We are keenly aware of the need to adjust for this important variable, and have examined the functional relationship between sperm concentration and abstinence time extensively. In our methods section we described both our collection of information on abstinence time and our method for analysing this variable. Table 3 in Swan *et al.* (2007) includes regression coefficients for abstinence time, as well as for all covariates in the final multiple regression model.

As correctly noted by Amann and Seidel, there is considerable variability associated with estimates of all semen parameters, including concentration. In statistical terms, the data are 'noisy' and 'explanatory variables', singly or together, are not likely to explain the majority of this variation. In our data, the full model including number of beef meals consumed by the mother and all other influential variables 'explained' only 12% of the variability in (log) sperm concentration (adjusted $R^2 = 0.126$) and 88% remained unexplained. When we looked at the contribution of each covariate singly, abstinence time accounted for the greatest percent change in R^2 (5%) and each of the other covariates (including a history of sexually transmitted disease, age, alcohol and mother's beef meals) accounted for about 1%. We note that this is a more general phenomenon of studies of sperm parameters. For example, the large intra-individual variability of sperm concentration results in only a small percent explained by any single variable, and even abstinence time, which has long been recognized as one of the most important covariates of sperm concentration, can only account for a small fraction of the total variability. Therefore, it is particularly important to replicate any study that reports on a previously unrecognized predictor as ours did, as we stated in Swan *et al.* (2007).

It was also suggested that we examine the variability of the 387 data points used to calculate the regression line showing the relationship between log sperm concentration and beef servings per week. We did so and note that there are no unduly influential data points 'driving' this regression line (Figure available on request).

Amann and Seidel suggested that we should have presented data on alternative measures of morphology, and a detailed analysis of sperm defects in relation to mother's beef consumption. In fact, we obtained far more detailed morphological and motility data in this population than the two measures presented in Swan *et al* (2007). However, when we saw no evidence of any association between the percent of sperm that

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were moving at all (% motile) or the percent of men with normal morphology (WHO, 1999) we chose not to conduct further analyses on mother's beef consumption and the more detailed measures of motility and morphology. This could, of course, still be done, and might warrant a future publication.

Amann and Seidel wrote that, 'Swan *et al.* (2007) did not present data (apparently collected), on where the mothers resided while pregnant with the son studied'. In fact, the country in which mother resided is discussed quite extensively in our results section. We noted that 'most men (83.2%) were born in North America, 8.4% in South and Central America and that mothers of sons born in North America consumed an average of 1.3 more beef meals a week than other mothers ($P < 0.0001$)'. However, because only two mothers living outside North America at the time of their son's birth ate >7 beef meals/week, we concluded that this study is not able to provide information about the association between semen quality and high beef consumption outside North America.

Finally, we agree that it would have been of value to include information on cuts of beef or other details on the particular sources of beef consumed by the mother. We did not have this information. However, we did examine the association between mother's beef and son's sperm concentration within men recruited in a large urban center (Minneapolis, MN, USA) and a small agricultural area (Columbia, MO, USA) separately. These two areas might be expected to differ in the proportion of beef from cattle 'passing through a major feedlot' but the associations in these two centers, as stated in Swan *et al.* (2007), were very similar.

We appreciate Amann and Seidel's thoughtful comments and perhaps subsequent studies on this topic can address some of the points they have raised.

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Single embryo transfer in preimplantation genetic diagnosis cycles for women <36 years does not reduce delivery rate

Sir,

We read with interest the paper by Donoso *et al.* (2007), and feel it is important to make the following comments.

The authors have attempted to assess the effect of a single-embryo transfer (SET) policy on delivery rate in young preimplantation genetic diagnosis (PGD) patients compared with a double-embryo transfer (DET) policy in a matched group of patients. They have concluded that there was no significant difference in the delivery rate between the two groups and supported the implementation of a SET policy in young women undergoing PGD.

We believe that this conclusion is somewhat flawed as it cannot be substantiated by the data presented by the authors for the following reasons.

First, the sample size (62 versus 73 embryo transfers) may not allow for assessing, with confidence, the statistical significance of the difference between the study and the control groups as the likelihood of a type II (β) error is high. For example, in order to detect a 10% difference (between 30 and 20%) in the delivery rate with 80% power at an alpha of 5%, we need ~230 embryo transfers in each group.

It is interesting to note that when the authors reported the outcome of SET and DET in the IVF/ICSI cycles without PGD during the same period (which presumably involved much larger numbers), DET was associated with a significantly higher delivery rate (a difference of 11.1% in the delivery rate per embryo transfer, $P = 0.02$). However, when they restricted the analysis to embryo transfers in cases treated with PGD due to the presence of Robertsonian and reciprocal translocations, a similar difference of 10.6% in the delivery rate per embryo transfer between the DET and SET groups was described as non-significant ($P = 0.7$). An even larger difference of 15.3% in the delivery rate per embryo transfer between patients who had an 'elective' SET ($n = 27$) and those who had a 'non-elective' SET ($n = 46$) was also described as non-significant.

Second, the historical control group used to study the difference in delivery rate per embryo transfer makes the assessment subject to bias.

Finally, the study results do not justify the study's own conclusion to support the implementation of an SET policy in young women undergoing PGD, simply because the majority of patients in the SET group (63.1%) had the SET imposed on them by the lack of more than one embryo available for transfer, rather than by the SET policy implemented in July 2003.

We believe in the merits of SET for the appropriate patients, but this enthusiasm on SET should not justify relaxing the

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