

Letters to the Editor

Variability of ovarian reserve tests

Sir,

The article by Kwee *et al.* (2004) on the intercycle variability of ovarian reserve tests contains important methodological points that require further explanation and clarification before valid conclusions can be drawn.

(i) The authors mention that cycle day 2 or 3 serum FSH values were determined as basal values during clomiphene citrate challenge test (CCCT). It has been reported that there is considerable variation in serum FSH levels between days 2 and 3 (Brown *et al.*, 1995). The significant variation in that study (Kwee *et al.*, 2004) may partly be due to this intra-cycle variability. Therefore, we believe that the results on the intercycle variability of CCCT in that study should be cautiously interpreted.

(ii) In the relevant study, three ovarian reserve tests have been performed one to four times in subsequent cycles. Although it has not been mentioned whether subjects were on any recent treatment, i.e. ovulation induction, prior to enrollment, we assume that subjects did not have any ovulation induction before the first cycle. However, ovarian reserve tests in cycles 2, 3 and 4 were performed after ovulation induction. To our knowledge, the effect of clomiphene citrate on the ovarian reserve tests in the following cycle has not been discovered. It has been reported that significant plasma concentrations of clomiphene citrate could be detected up to one month after treatment with a single dose of 50 mg (Mikkelsen *et al.*, 1986).

Table III shows that the major variation was observed between cycles 1 and 2, i.e. between the cycle following a spontaneous cycle and that following an ovulation induction cycle. Any possible effect of clomiphene citrate may be responsible for that variability.

It may be more appropriate to analyse the intercycle variability between similar cycles, i.e. either between cycles with prior ovulation induction or between cycles without any prior treatment, and it may also be appropriate to exclude cycle 1 from the analysis in the relevant study.

(iii) To exclude the bias of pregnant subjects during the study, the authors mention that the CCCT and exogenous FSH ovarian reserve test (EFORT) groups were comparable. However, the basis of the study is the variability between cycles. It is obvious that subjects in each cycle are different due to pregnancies. It would also be inconclusive to compare characteristics of pregnant subjects with those of others to exclude any bias, due to the type II error. Due to the small number of pregnant subjects, it would not mean that pregnant subjects were comparable to others if the statistical analysis could not reveal any significance. The bias of pregnant subjects may have altered the results in the study.

(iv) The authors mention that there is significant intercycle variability in basal FSH and CCCT values based on the results, which were shown in Table III. Variance is: $\Sigma (\text{value} - \text{mean})^2 / (n - 1)$ and SD is the square root of the variance. To our understanding, variances in cycles 1 to 4 (per cycle variation) have been compared and this has been reported as the intercycle variability. However, since the populations in each cycle are different due to pregnancies, this comparison is not appropriate.

It may be more appropriate to calculate the variance per subject, i.e. variance could be calculated for the values of the same subject in subsequent cycles. That is how inter-assay variabilities are calculated: a constant serum sample is tested multiple times at different times and the variance of these values indicates the inter-assay variability. The intra-assay variation describes the variation between multiple assay wells on the same plate from the same sample. It is our understanding that variances of populations were compared in the relevant study, instead of variances of ovarian reserve tests in the same subject.

	Cycle 1	Cycle 2	Cycle 3
Subject 1	X_1	Y_1	Z_1
Subject 2	X_2	Y_2	Z_2
Subject 3	X_3	Y_3	Z_3

Variance of X_1 , Y_1 and Z_1 values are relevant for the intercycle variability. Variance of X_1 , X_2 and X_3 values indicate the population variances. Similar to the constant use of the same serum sample for analysis in the example of assay variability, subjects should be constant in each cycle. Otherwise, comparison of variances may mislead data, and any significant variability may be due to the inequality of the population.

References

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