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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 intracycle 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 (Mikkelson *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: Σ (value $-$ 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

Brown JR, Liu HC, Sewitch KF, Rosenwaks Z and Berkeley AS (1995) Variability of day 3 follicle-stimulating hormone levels in eumenorrheic women. J Reprod Med 40,620–624.

Kwee J, Schats R, McDonnell J, Lambalk CB and Schoemaker J (2004) Intercycle variability of ovarian reserve tests: results of a prospective randomized study. Hum Reprod 19,590–595.

Mikkelson TJ, Kroboth PD, Cameron WJ, Dittert LW, Chungi V and Manberg PJ (1986) Single-dose pharmacokinetics of clomiphene citrate in normal volunteers. Fertil Steril 46,392–396.

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Reply: Variability of ovarian reserve tests

Sir,

We thank Dr Elter *et al.* for their comments concerning our paper on intercyclical variability of ovarian reserve tests.

The authors raise the issue of possible differences between day 2 and day 3 FSH values which in our study may have contributed to the observed variation of FSH between cycles. In the first place, only in ~10% of the participants was FSH not measured on all third days of the cycle. The cited small subanalysis in 20 patients by Brown *et al.* (1995). indicated a possible within-cycle coefficient of variation for FSH of 14.8% It should be realized that such a variation also included the assay varation (4.8% intra-assay variation and 6.2% inter-assay variation) and that value indicates that the within (intra)-cycle variation of FSH measurement is probably only limited. And indeed Hansen *et al.* (1996), in the study that we cited, measured FSH on cycle days 2–5 in order to investigate the intra- and intercycle variability in a healthy population of 44 women with regular menstrual intervals in a total of 66 cycles on cycle days 2, 3, 4 and 5, and FSH concentrations were not different between the various cycle days.

The second point raised was about the possible carry-over effect of clomiphene from one cycle to the other. Indeed, it has been reported that significant plasma concentrations of clomiphene citrate could be detected up to 1 month after treatment with a single dose of 50 mg (Mikkelson *et al.*, 1986). But this is predominantly the so-called isomeric Zu variant of clomiphene. Glasier *et al.* (1989) investigated the effects on follicular development of clomiphene citrate and its two isomers En clomiphene and Zu clomiphene. It was concluded that the En isomer, which has largely the antiestrogenic properties, is the isomer active in inducing follicular development. The biologically active En clomiphene is elimated much more quickly than the biologically inactive Zu clomiphene. Moreover, Opsahl *et al.* (1996) showed that patterns of gonadotrophin response, follicular development, and endometrial growth and maturation remain consistent across consecutive cycles of clomiphene citrate treatment. This is why we believe that there is no carry-over effect of clomiphene citrate. Taking this altogether, we assumed that a biological carry-over effect of clomiphene in our study could be negligible.

The third point raised was whether the bias of pregnant subjects may have biased the results in the study. Indeed we do have a potential bias here in that women who became pregnant during the three test cycles did not reach the IVF cycle in which the ovarian reserve was evaluated. We admit the possibility of this bias but we could not think of a way to avoid it in an ethically acceptable manner. It turned out that the number of pregnant subjects was (relatively) small. Therefore this bias, if present, has only contributed to a limited extent.

Finally, it was suggested that we should have calculated the variance per subject, i.e. variance within-subject over cycles. This is in fact exactly what we have done: SD = square root of variance measured *within* each female patient.

References

Brown JR, Liu HC, Se Witch KF, Rosenwaks Z and Berkeley AS (1995) Variability of day 3 follicle-stimulating hormone levels in eumenorrheic women. J Reprod Med 40,620–624.

Glasier AF, Irvine DS, Wickings EJ, Hillier SG and Baird DT (1989) A comparison of the effects on follicular development between clomiphene citrate, its two separate isomers and spontaneous cycles. Hum Reprod 4,252–256.

Hansen LM, Batzer FR, Gutmann JN, Corson SL, Kelly MP and Gocial B (1996) Evaluating ovarian reserve: follicle stimulating hormone and oestradiol variability during cycle days 2-5. Hum Reprod 11,486–489.

Mikkelson TJ, Kroboth PD, Cameron WJ, Dittert LW, Chungi V and Manberg PJ (1986) Single-dose pharmacokinetics of clomiphene citrate in normal volunteers. Fertil Steril 46,392–396.

Opsahl MS, Robins ED, O'Connor DM, Scott RT and Fritz MA (1996) Characteristics of gonadotropin response, follicular development, and endometrial growth and maturation across consecutive cycles of clomiphene citrate treatment. Fertil Steril 66,533–539.

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Reciprocal translocation carriers in recurrent miscarriage parents may yield an unbalanced fetal chromosome pattern

Sir,

We read with interest the study by Goddijin *et al.* (2004) on clinical relevance of structural chromosome abnormalities in couples with repeated miscarriage. The authors concluded that karyotyping of 1324 couples ascertained for repeated miscarriage did not yield an unbalanced fetal chromosome pattern after the ascertainment of parental carrier status. We disagree with the conclusion because reciprocal translocation cases do exist for recurrent miscarriages who give birth to offspring with unbalanced fetal chromosomes.

Our recent analysis showed that one of 34 offspring of successful pregnancies of reciprocal translocation carriers examined for recurrent miscarriage had an unbalanced translocation (Sugiura-Ogasawara *et al.*, 2004). This 2.9% is not negligible and is equivalent to the frequency at which 43 year old women have a fetus with an abnormal chromosome karyotype ascertained by amniocentesis.

We have another patient with 46,XX, t(4;15)(q33; q26) who was found to be a carrier after examination for recurrent miscarriage in another hospital and who gave birth to two malformed children with 46,XX,-15,;der(15)t(4;15)(q33;q26)-mat and 46,XX,der(4)t(4;15)(q33;q26)mat after three miscarriages. Our previous study did not include this case because the patient came to our hospital for preimplantation genetic diagnosis.

Midro *et al.* (1991) also reported 10 families with reciprocal translocations and one woman with 46,XX, t(6;13)(q27;q12) who had two malformed children with three spontaneous