Ovarian volume may predict assisted reproductive outcomes better than follicle stimulating hormone concentration on day 3

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This study was undertaken to compare ovarian volume with other factors which are important for the success of assisted reproduction. The first treatment cycle for 261 patients meeting all entry criteria between September 1993 and June 1995 was considered. All cycles employed the same stimulation protocol and no interventions were based upon pre-treatment indicators. Pre-treatment ovarian volumes, cycle day 3 follicle stimulating hormone (FSH) and oestradiol concentrations, smoking status and age were compared to subsequent peak oestradiol concentrations, numbers of oocytes retrieved, cycle cancellation and occurrence of clinical pregnancy. Statistical evaluation was performed using simple and multiple logistic regression analysis to determine odds ratios. The resultant odds ratios suggest that age and small ovarian volume may predict retrieval of fewer mature oocytes, while the failure to achieve clinical pregnancy was predicted by current smoking and small ovarian volume. Day 3 FSH values failed to be a significant predictor when maternal age, smoking status and ovarian volume were known. It can be concluded that, like maternal age and smoking status, ovarian volume may be a clinically important predictor of reproductive success, being superior to cycle day 3 FSH or oestradiol concentrations as an assessment of ovarian reserve. Key words: assisted reproduction/ovarian reserve/ovarian volume/ovary

Introduction

Maternal age and smoking status provides the clinician with important information as to the likelihood of pregnancy after assisted reproductive treatment. This knowledge can be utilized in counselling patients prior to treatment. Advancing maternal age results in a decline in implantation rates and reproductive success as a manifestation of quantitative and qualitative reductions in ovarian and oocyte function (Padilla and Garcia, 1989; Tan et al., 1992; Navot et al., 1994). Cigarette smoking affects ovulation induction (Van Voorhis et al., 1992, 1996), the outcome of assisted reproduction (Van Voorhis et al., 1996) and ovarian reserve (Sharara et al., 1994). As an additional predictor of in-vitro fertilization (IVF) outcome, ovarian reserve is commonly assessed by basal or post-clomiphene citrate gonadotrophin testing (Scott et al., 1989, 1993; Toner et al., 1991; Scott and Hoffman, 1995; Sharara and Scott, 1997). Even so, ovarian reserve is a better indicator of ovarian hormone and oocyte responses to stimulation than of actual pregnancy rates following IVF (Toner et al., 1991; Sharara and Scott, 1997). Therefore, it is desirable to determine the best possible predictors of IVF pregnancy rates to counsel patients about their chances for success with IVF treatment.

Basal and post-clomiphene citrate gonadotrophin assessments of ovarian reserve have been described and reviewed by others (Scott and Hoffman, 1995; Sharara and Scott, 1997). Although less sensitive than provocative testing, the measurement of basal (cycle day 3) follicle stimulating hormone (FSH) concentrations is frequently utilized as a clinical assessment preceding assisted reproduction (Muasher et al., 1988; Scott and Hoffman, 1995; Sharara and Scott, 1997). While it is recognized that gonadotrophin measurements are subject to inter-cycle (Scott et al., 1990) and inter-institutional variability (Hershlag et al., 1992), elevated day 3 FSH concentrations portend poor gonadotrophin response and reduced clinical pregnancy rates (Scott et al., 1989; Toner et al., 1991; Scott and Hoffman, 1995). More recently, transvaginal ultrasound determinations of either the number of follicles of 2–5 mm diameter (Tomás et al., 1997) or the ovarian volume (Syrop et al., 1995; Lass et al., 1997) have been reported to define ovarian reserve and predict reproductive outcomes. Ovarian volume, separate from maternal age, predicts ovarian response to gonadotrophins as measured by the number of oocytes retrieved and cycle cancellation rates. Ovarian volume also predicts the occurrence of clinical pregnancy after IVF treatment (Syrop et al., 1995; Lass et al., 1997). The present retrospective observational study was undertaken to compare the relative importance of day 3 FSH values and ovarian volume as indicators of ovarian responsiveness and reproductive outcomes.

Materials and methods

From September 1993 until June 1995, serum cycle day 3 FSH and oestradiol concentrations were determined in all patients preceding a cycle of assisted reproduction. FSH (SI conversion = 1.00) was determined utilizing the Abbott IMX® assay (Abbott Labs, Abbott Park, IL, USA), calibrated to the WHO Second International Reference Preparation for human FSH (78/549). The FSH assay coefficients of variation were 4.4% (intra-assay) and 8.2% (inter-assay). Serum oestradiol (pg/ml, SI conversion = 3.671) was measured by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA,
USA) with intra- and inter-assay coefficients of variation of 8.9 and 10.0% respectively.

Transvaginal ultrasounds were performed utilizing a General Electric 3600® (5 MHz probe) or an Acuson XP128® (EV7 probe). Ovarian volumes were calculated as the volume of an ellipsoid \( \text{volume} = \frac{4}{3} \pi a b c \) from ultrasounds performed at initiation of leuprolide acetate down-regulation (7–9 days post-ovulation based upon a biphasic basal body temperature shift, urine luteinizing hormone surge or serum progesterone >5 ng/ml) in cyclic patients. In the absence of ovulation, this cycle was not used; however, another cycle was monitored for ovulation and subsequent treatment. Anovulatory patients were not included in this study but were treated by initiation of leuprolide acetate following 21 days administration of oral contraceptives. Intra- and interobserver variations in calculated ovarian volumes were previously reported at 6.9 and 5.2% respectively (Syrop et al., 1995). The smallest ovarian volume was defined as that of the lesser of the right and left ovaries. The ovarian stimulation protocol has been previously reported (Van Voorhis et al., 1996).

The doses of gonadotrophins utilized were not altered based upon ovarian volumes, basal FSH values or maternal age. Criteria for cancellation of treatment preceding retrieval were either (i) an absence of oestradiol response, or (ii) fewer than two follicles with diameter >18 mm or with <3 follicles in total, or (iii) a peak oestradiol of <500 pg/ml on the day of human chorionic gonadotrophin (HCG) administration.

Treatment cycle data and pretreatment ultrasound measurements were analysed for 261 patients who met all of the following criteria: (i) complete data was available from the first treatment cycle following determination of day 3 FSH/oestradiol and ovarian volume, (ii) ovarian volume was determined by one of two physicians (C.H.S or B.J.V), (iii) both ovaries were sonographically visualized and (iv) FSH and oestradiol determinations were performed by the same laboratory utilizing the aforementioned assay techniques. Pre-stimulation variables of smallest ovarian volume, day 3 FSH, day 3 oestradiol, recorded smoking status (never, ever, current) and maternal age were compared to subsequent outcome measures of peak oestradiol (pg/ml), peak oestradiol/ampoule (pg/ml per 75 IU human menopausal gonadotrophin (HMG)), number of mature oocytes retrieved, cycle cancellation and cycle results (clinical pregnancy). To retain independence of data, each analysed cycle represents an individual patient in the first cycle of treatment.

**Statistical methods**

Results are expressed as mean ± SD. Two of the study outcomes (peak oestradiol and peak oestradiol/ampoule) were measured on a continuous scale, one of the outcomes (number of mature oocytes) represented discrete counts, and the remaining two outcomes (cycle cancellation and clinical pregnancy) were dichotomous (yes versus no) in nature. In order to make the analyses consistent for all five main outcomes, the three outcomes that were continuous or discrete were dichotomized. Specifically, indicators were created as to whether peak oestradiol was <1000 pg/ml, whether peak oestradiol per ampoule was <26 pg/ml/75 IU HMG, and whether the number of mature oocytes was less than eight. These thresholds were chosen near the 25th percentile of the observed distribution of these variables in an attempt to delineate poor responders.

With all five of the dichotomized and dichotomous outcomes, simple logistic regression was applied to see whether age, smoking status (never, ever, or current), smallest ovarian volume, day 3 oestradiol and/or day 3 FSH predicted the outcomes in a univariate analysis. To determine which risk factors were significant when controlling for the others, all risk factors were entered into a multiple logistic regression model. Based on the results from the univariate and multiple logistic regression models, odds ratios (OR) and 95% confidence intervals (CIs) were calculated, with OR > 1.0 corresponding to positive associations and OR < 1.0 corresponding to negative associations. Confidence intervals which did not include the value of 1.0 correspond to \( P \) values < 0.05 and, hence, were considered statistically significant.

**Results**

The ages of the patients included in this study ranged from 23–46 years, with a median of 34 years and a mean of 34 ± 4.4 years (90th percentile, 40 years). The median volume of the smallest ovary was 6.4 ml and mean was 7.2 ± 3.6 ml (10th percentile, 3.2 ml). Day 3 FSH values had a median of 8.5 mIU/ml with a mean of 9.5 ± 4.0 mIU/ml (90th percentile, 15 mIU/ml). The mean day 3 oestradiol was 39.6 ± 22.0 pg/ml (90th percentile, 63 pg/ml).

Linear regression revealed that day 3 FSH values were not significantly related to age, day 3 oestradiol or ovarian volume. Ovarian volume was unrelated to infertility aetiology, although subjects with unexplained infertility had a tendency towards smaller ovarian volumes but this was not statistically significant (\( P = 0.053 \)). No significant differences in ovarian volume or FSH were found according to type of embryo transfer [transcervical versus intratubal for zygote intra-Fallopian transfer (ZIFT) procedures]. A total of 202 subjects, had never smoked, while 44 were past and 15 were current smokers respectively.

Utilizing logistic regression, Table I provides a univariate analysis of identified clinical predictors and resultant low responses or clinical outcomes. Thus the importance of each factor is considered separately. Clearly, increasing maternal age produced significant reductions in peak oestradiol concentrations, peak oestradiol per 75 IU HMG and numbers of mature oocytes. Smoking within a month of oocyte retrieval significantly reduced the occurrence of clinical pregnancy. With decreasing volume of the smallest ovary, ovarian response (peak oestradiol/75 IU HMG), oocyte numbers, and the chances of pregnancy were reduced. Incremental increases in basal FSH or oestradiol values accounted only for decreases in number of mature oocytes (FSH) or increases in the odds of cycle cancellation (day 3 oestradiol) respectively.

Finally, multiple logistic regression models were applied in which all six predictor variables were considered simultaneously (Table II). Again, increasing age remained a significant risk factor for reduced ovarian response and oocyte numbers. Decreased volume of the smallest ovary posed a significant risk for reductions in ovarian hormonal and oocyte response and the occurrence of pregnancy. For example, the data suggested that a 40 year old non-smoker who has a smallest ovarian volume at the 10th percentile had only a 24% chance of a clinical pregnancy, whereas her chances would increase to 39% if her volume were at the 90th percentile. Elevations in day 3 oestradiol concentrations were a risk factor for cycle cancellation but not pregnancy. When considered with age, smoking status and ovarian volume, day 3 FSH values were not significant predictors of any outcomes analysed.
Table I. Simple logistic regression analyses of risk factors as predictors of reproductive outcomes. Odds ratios (95% CI) for predicting outcomes

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Peak oestradiol/ amp &lt;26 pg/ml/75 IU HMG</th>
<th>Mature oocytes &lt;8</th>
<th>Cycle cancellation</th>
<th>Clinical pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (5 years)</td>
<td>3.40 (2.30, 5.03)</td>
<td>3.41 (2.29, 5.10)</td>
<td>3.09 (2.10, 4.53)</td>
<td>3.23 (1.79, 5.82)</td>
</tr>
<tr>
<td>Former or current smoker</td>
<td>1.43 (0.77, 2.67)</td>
<td>0.77 (0.39, 1.53)</td>
<td>0.93 (0.48, 1.80)</td>
<td>2.27 (0.89, 5.77)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>1.30 (0.43, 3.95)</td>
<td>0.43 (0.09, 1.94)</td>
<td>0.68 (0.19, 2.48)</td>
<td>0.80 (0.10, 6.43)</td>
</tr>
<tr>
<td>Smallest ovarian volume (5 ml)</td>
<td>0.71 (0.48, 1.07)</td>
<td>0.54 (0.34, 0.84)</td>
<td>0.41 (0.25, 0.67)</td>
<td>0.66 (0.32, 1.33)</td>
</tr>
<tr>
<td>Day 3 oestradiol (10 pg/ml)</td>
<td>1.04 (0.94, 1.14)</td>
<td>1.03 (0.93, 1.14)</td>
<td>1.06 (0.96, 1.17)</td>
<td>1.14 (1.01, 1.28)</td>
</tr>
<tr>
<td>Day 3 FSH (1 mlU/ml)</td>
<td>1.05 (0.99, 1.12)</td>
<td>1.06 (0.99, 1.13)</td>
<td>1.09 (1.02, 1.17)</td>
<td>1.07 (0.97, 1.19)</td>
</tr>
</tbody>
</table>

Table II. Multiple logistic regression analyses of risk factors as predictors of reproductive outcomes. Odds ratios (95% CI) for predicting outcomes

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Peak oestradiol/ amp &lt;26 pg/ml/75 IU HMG</th>
<th>Mature oocytes &lt;8</th>
<th>Cycle cancellation</th>
<th>Clinical pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (5 years)</td>
<td>3.53 (2.25, 5.32)</td>
<td>3.44 (2.27, 5.22)</td>
<td>3.17 (2.10, 4.78)</td>
<td>3.44 (1.80, 6.59)</td>
</tr>
<tr>
<td>Former or current smoker</td>
<td>1.25 (0.59, 2.67)</td>
<td>0.73 (0.33, 1.66)</td>
<td>0.84 (0.37, 1.88)</td>
<td>3.03 (1.04, 8.81)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>2.21 (0.58, 8.46)</td>
<td>1.07 (0.19, 5.94)</td>
<td>1.52 (0.33, 6.99)</td>
<td>0.84 (0.09, 8.09)</td>
</tr>
<tr>
<td>Smallest ovarian volume (5 ml)</td>
<td>0.71 (0.46, 1.11)</td>
<td>0.54 (0.33, 0.87)</td>
<td>0.42 (0.25, 0.70)</td>
<td>0.74 (0.36, 1.51)</td>
</tr>
<tr>
<td>Day 3 oestradiol (10 pg/ml)</td>
<td>1.02 (0.91, 1.14)</td>
<td>1.05 (0.89, 1.13)</td>
<td>1.15 (0.94, 1.18)</td>
<td>1.00 (1.01, 1.30)</td>
</tr>
<tr>
<td>Day 3 FSH (1 mlU/ml)</td>
<td>1.03 (0.96, 1.11)</td>
<td>1.03 (0.95, 1.11)</td>
<td>1.07 (0.99, 1.16)</td>
<td>1.06 (0.95, 1.18)</td>
</tr>
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</table>

Discussion

The present study was undertaken to consider simultaneously the variables of age, ovarian volume, basal FSH and oestradiol concentrations and smoking. It confirmed that ovarian volume (Syrop et al., 1995; Lass et al., 1997) may predict reproductive outcomes. Whether measured as peak oestradiol, peak oestradiol/ampoule of administered gonadotrophins, or number of mature oocytes, reduced ovarian response was predicted statistically by increasing age and decreasing smallest ovarian volume. Logistic regression was used to determine the importance of each variable in predicting the outcome of interest and can be judged by the odds ratios. Thus, the results reported in Table I and Table II demonstrated the importance of increasing age, smoking status and declining ovarian volume as risk factors for reduced ovarian response, oocyte numbers and pregnancy. Surprisingly, when ovarian volume and smoking status were included in the analysis, basal (day 3) FSH values failed to be a significant predictor of ovarian response, numbers of mature oocytes or the occurrence of clinical pregnancy.

Smoking affects reproductive response and ovarian reserve. The dose dependent influence of smoking on measures of ovarian response (past and current smoking) and pregnancy rates (current smoking) has been demonstrated (Van Voorhis et al., 1996). Using multivariate analysis, the current study suggests that past smoking increases the risk of cycle cancellation and that current smoking is a significant risk for failing to achieve a clinical pregnancy. Clomiphene citrate challenge testing of current smokers and those who have never smoked (ages 35–39) showed that smoking produced an accelerated decline in ovarian reserve but no differences in basal FSH (Sharara et al., 1994). Further, smokers have a 22% reduction in ovarian volume compared to non-smokers (Syrop et al., 1995). Thus, clinicians must counsel patients regarding the influence of smoking on reproductive response and ovarian reserve. Furthermore, the potential influence of smoking status must be considered during study design or analysis of assisted reproduction results.

Cycle cancellation secondary to inadequate ovarian stimulation response was related to age, ever smoking status, and basal oestradiol but not to ovarian volume, or day 3 FSH. The possibility that basal oestradiol is a predictor of cycle...
cancellation confirms some reports (Smotrich et al., 1995), but not others citing basal FSH (Toner et al., 1991) or ovarian volume (Lass et al., 1997) as predictors of cycle cancellation. However, these latter studies either included multiple treatment cycles from some patients and/or ignored smoking status. The inclusion of multiple cycles from some patients introduces bias by loss of independence of data. Thus, the test values of poor responders and non-pregnant subjects are considered repetitively, each with the same weight as a single cycle of data from a good responder or pregnant patient. This leads to an over estimate of the importance of a single test and its predictive value.

The current study regarded numbers of mature oocytes and occurrence of clinical pregnancy as measures of the quantitative and qualitative gametic responses to ovarian stimulation. When age, ovarian volume and smoking status were factored, basal FSH was not predictive of the number of mature oocytes or the occurrence of clinical pregnancy. Prior studies defining basal gonadotrophins as predictors of oocyte numbers or pregnancy rates included more than one cycle per patient and did not account for ovarian volume or smoking. (Scott et al., 1989, 1990; Toner et al., 1991). The value of ovarian volume is independent of the identified demographic variables of age and smoking status. The importance of decreasing ovarian volume to predict reductions in retrievable mature oocytes and pregnancy is demonstrated in Table II. Thus, the 9 ml reduction in smallest ovarian volume of patients with a 12 ml volume compared to those with a 3 ml volume increased the odds of obtaining fewer than eight mature oocytes (25th percentile response) by 380% (odds ratio 4.8). This same decline in ovarian volume was associated with a 50% decrease in the odds of clinical pregnancy.

Three prior reports have investigated ovarian volume as a predictor of reproductive outcomes. The first (Syrop et al., 1995) studied the first cycle of treatment in 188 patients before initiation of down-regulation. Total ovarian volume and volume of the smallest ova were found to be predictors distinct from maternal age for predicting numbers of oocytes and embryos obtained. The volume of the smallest ova, but not total ovarian volume, predicted clinical pregnancy occurrence. Of note, ovarian volume correlated poorly with age. Two subsequent reports (Lass et al., 1997; Tomás et al., 1997) studied ovarian volumes following the initiation of gonadotrophin releasing hormone (GnRH) agonist down-regulation but neither considered smoking status. In one study (Tomás et al., 1997), the ovaries of 166 new patients were morphometrically characterized as ‘PCO-like’, ‘inactive’ or ‘normal’. The sum of the ovarian volumes was 13.5 ± 6.8 ml in PCO-like ovaries compared to 9.7 ± 5.2 ml for normal ovaries. The only outcome measures analysed were the numbers of HMG ampoules utilized and of oocytes retrieved. Ovarian volumes appeared to be correlated with age and numbers of follicles but not with numbers of oocytes. A second study (Lass et al., 1997) examined 140 subjects after excluding those ≥36 years with FSH >15 mIU/ml (reference standard unspecified) or with an ovarian cyst >10 mm. Gonadotrophin doses for ovarian stimulation were varied according to maternal age and past history of poor cycle responses. The mean ovarian volume following down-regulation was 6.3 ml and a volume of <3 ml was below one standard deviation from the mean. Those subjects with a reduced mean ovarian volume (more than one standard deviation below the mean) required a greater administration of gonadotrophins and obtained fewer oocytes. Unlike the current study, a reduced mean ovarian volume was also associated with a higher basal FSH and greater cancellation rate.

This study confirms that ovarian volume may have the ability to predict ovarian reserve. Ovarian volumes were determined prior to pituitary down-regulation. Like maternal age and smoking status, the measurement of ovarian volumes provides information to the clinician and patient before the effort and expense of down-regulation. Since gonadotrophin doses were not influenced by prior ovarian responses or age and subjects were not excluded by FSH or ovarian morphology, the current study emphasizes the importance of this information.

Ovarian volume can be determined before initiation of down-regulation and provides the clinician with a measurement of ovarian reserve that is determined readily, inexpensively, and with minimal invasiveness. Beyond maternal age and smoking status, ovarian volume is a better measure of ovarian reserve than basal FSH.

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References


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