Human Reproduction vol.12 no.2 pp.220–223, 1997

Pretreatment transvaginal ultrasound examination predicts ovarian responsiveness to gonadotrophins in in-vitro fertilization

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The objective of this study was to determine the predictive value of the number of follicles seen by transvaginal ultrasound before gonadotrophin stimulation on the ovarian responsiveness of 166 infertile women undergoing in-vitro fertilization (IVF) treatment. The main variables were patient age, ovarian volume and number of ovarian follicles measuring 2–5 mm on transvaginal ultrasound before gonadotrophin stimulation. Based on the sum of ovarian follicles in both ovaries the patients were divided into three groups of inactive (<5 follicles), normal (5–15 follicles) or polycystic (PCO)-like ovaries (>15 follicles). The main outcome measure was the number of recovered oocytes. The number of follicles was correlated more strongly with the number of recovered oocytes (r^2 = 0.131; P = 0.0001) than age alone (r^2 = –0.053; P = 0.005). Fewer oocytes were recovered from patients with inactive ovaries (5.4 ± 2.5; P = 0.006) than with normal (7.5 ± 4.5) or PCO-like ovaries (10.5 ± 5.1). Ovarian volume was correlated with the number of follicles before stimulation (P = 0.0001), but not with the number of oocytes. The number of small follicles present before ovarian stimulation was a better predictor of the outcome than ovarian volume or age alone. Patients can be identified with inactive ovaries which will have a poor response to IVF treatment, a key factor for counselling couples and optimizing resources.

Key words: IVF outcome/low responder/ovarian reserve/predictive value/ultrasound

Introduction

In in-vitro fertilization (IVF) programmes, ovarian responsiveness to gonadotrophins is highly variable and difficult to predict. The number of follicles developing during ovarian stimulation influences the number of embryos available for transfer, selection and freezing, and the chance of pregnancy (Arnot *et al.*, 1995). A poor response to treatment occurs in ~10% of the cases (Pellicer *et al.*, 1987) and it is associated with a reduced number of recovered oocytes, increased cancellation rate and poor pregnancy rate.

The age of the patient alone is a weak predictor of the ovarian reserve and responsiveness to IVF stimulation (Check *et al.*, 1994). There are women aged >40 years who are able to conceive, and younger women who respond poorly to the stimulation regimens (Jacobs *et al.*, 1990; Toner, 1993).

In order to identify factors predictive of the ovarian response, hormonal markers like cycle day 3 serum basal follicle stimulating hormone (FSH), luteinizing hormone (LH), oestradiol concentrations, and challenge tests have been explored (Scott and Hofmann, 1995; Scott *et al.*, 1995; Smotrich *et al.*, 1995; Galtier-Dereure *et al.*, 1996). A cycle day 3 serum basal FSH concentration, as a single indicator, has a better predictive value than age alone (Toner, 1993). However, hormonal determinations may have inter-cycle variability (Scott *et al.*, 1990), are laborious, expensive, and have not achieved wide clinical acceptance.

Transvaginal ultrasound has contributed remarkably to the simplicity of modern IVF programmes (Wikland, 1992; Golan *et al.*, 1994; Nachtigall and Schwartz, 1996). With high resolution vaginal probes, the size and growth of the ovarian follicles can be monitored accurately (Tarlatzis *et al.*, 1984). A cohort of small follicles measuring 2–5 mm in diameter can be detected already at the end of the previous cycle.

In this study we evaluated whether the number of small follicles present at the beginning of the ovarian stimulation could predict ovarian responsiveness in an IVF programme.

Materials and methods

We studied 166 consecutive patients attending an IVF programme for the first time. All patients had both ovaries present. The main indications for treatment were tubal factor (n = 65), male factor (n = 40), endometriosis (n = 16), ovulatory dysfunction (n = 11), and unexplained infertility (n = 34).

The IVF protocol was similar for each patient. It included pituitary down-regulation with intra-nasal buserelin acetate 800 µg/day (Suprecur®; Hoechst AG, Frankfurt am Main, Germany) starting at day 23 of the previous cycle, followed by ovarian stimulation with human menopausal gonadotrophin (HMG, Pergonal®; Serono, Switzerland or Humegon®; Organon, Oss, The Netherlands). A step-up procedure with HMG was used when appropriate, based solely in the transvaginal ultrasound examinations. When at least two follicles measuring ⩾18 mm in diameter were detected, the gonadotrophin-releasing hormone agonist (GnRHa, buserelin) was discontinued and a dose of 5000–10 000 IU of human chorionic gonadotrophin (HCG, Pregnyl®; Organon) was given. Oocyte retrieval by transvaginal ultrasound-guided puncture was performed 36 h after HCG administration. No more than two embryos were transferred 48 h after ovum retrieval, followed by luteal support with HCG (Pregnyl) or natural progesterone (Lugesteron®; Leiras, Turku, Finland) for 2 weeks.

Ovarian follicle growth was followed by transvaginal ultrasound

(General Electrics RT-X200, Milwaukee, USA), equipped with a 6.5 MHz vaginal probe. The first ultrasound examination was performed after ovarian suppression (oestradiol concentration <0.05 nmol/l) and just before the administration of the first dose of gonadotrophin. The maximum transverse (D1), antero-posterior (D2) and longitudinal (D3) diameters of both ovaries were measured, and their volume was estimated according to the approximate formula for the ellipse $(\pi/6)\times D1\times D2\times D3$. The number of all follicles measuring 2–5 mm was counted and, accordingly, the patients were divided into three groups: (i) those with inactive ovaries ($n = 17$) with <5 follicles in both ovaries; (ii) those patients with normal ovaries ($n = 125$) with 5–15 follicles; and (iii) those patients with polycystic (PCO)-like ovaries ($n = 24$) with >15 follicles.

The main variables were the age of the patients, the sum of the volume of both ovaries and the number of follicles measuring 2–5 mm in diameter presented before treatment, as described above. The main outcomes were the number of HMG ampoules (75 IU) administered and the number of retrieved oocytes.

Statistical analysis

A linear regression model was used to identify any correlation between two continuous variables. The Kruskal–Wallis test was used to compare variables between the three groups of patients with different types of ovaries. The box and whisker technique was used, in which the box represents the 25th, 50th (median) and 75th percentiles, while the lower and upper whiskers the 10th and 90th percentile respectively. The level of statistical significance was set at $P < 0.05$.

Results

There were 17 patients with inactive, 125 patients with normal and 24 patients with PCO-like ovaries. The number of ovarian follicles, seen by transvaginal ultrasound after down-regulation and before ovarian stimulation, was correlated linearly with the number of collected oocytes ($r^2 = 0.131$; $P = 0.0001$). As seen in Figure 1, the patients with PCO-like ovaries had more oocytes (mean ± SD) recovered (10.5 ± 5.1) than the patients with normal (7.5 ± 4.5) or inactive (5.4 ± 2.5) ovaries ($P = 0.006$). A similar trend was observed in the subgroup of patients aged <35 years (6.1 ± 2.7, 8.2 ± 4.7 and 11.1 ± 5.2 respectively; $P = 0.04$) and in patients aged ⩾35 years (4.6 ± 2.0, 6.7 ± 4.1 and 8.6 ± 4.8 respectively; not significant).

As judged by the sum of the volume of both ovaries, patients with PCO-like ovaries had larger ovaries (13.5 ± 6.8 ml; $P = 0.0008$) than patients with normal (9.7 ± 5.2 ml) or inactive ovaries (7.1 ± 4.9 ml). The ovarian volume was linearly correlated ($r^2 = 0.097$; $P = 0.0001$) with the number of follicles before ovarian stimulation (Figure 2), but not with the number of retrieved oocytes.

Patients in the PCO-like ovary group were younger (31 ± 4.9 years; $P = 0.003$) than those with inactive (35 ± 3.3 years) or normal ovaries (34 ± 3.7 years). Age was correlated inversely with the number of recovered oocytes ($r^2 = –0.053$; $P = 0.005$), but not with the number of follicles or the volume of the ovaries before stimulation.

There was no significant difference in the number of gonadotrophin ampoules used to achieve oocyte maturity between the three groups of patients with different ovarian types (25 ± 6, 23 ± 6 and 23 ± 8 ampoules for inactive, normal and PCO-like ovaries respectively).

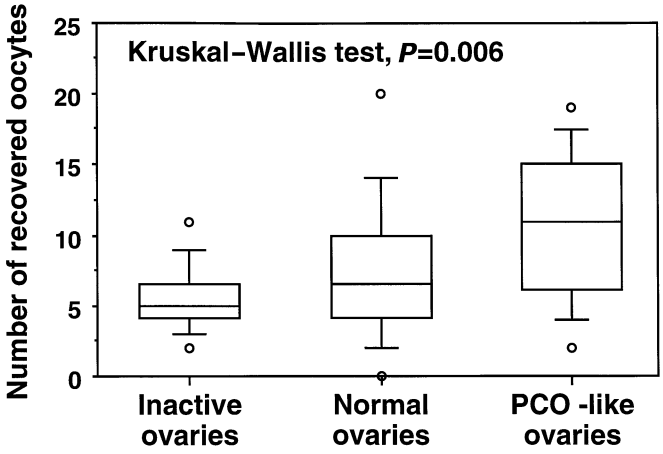

Figure 1. Box and whisker representation of the number of oocytes recovered after in-vitro fertilization (IVF) treatment in 166 patients with inactive (<5 follicles), normal (5–15 follicles) or polycystic (PCO)-like ovaries (>15 follicles).

Discussion

The number of small ovarian follicles (measuring 2–5 mm) detected by transvaginal ultrasound after pituitary down-regulation and before ovarian stimulation, predicted the number of oocytes collected in an IVF programme.

The process of folliculogenesis starts months before the actual cycle, with follicular recruitment occurring in the late luteal phase of the previous cycle (Nachtigall and Schwartz, 1996). A cohort of follicles measuring 2–5 mm are present very early in the follicular phase of the cycle (Gougeon and Lefèvre, 1983; Taymor, 1996). These follicles are in an early antral phase, and are easily detected by transvaginal ultrasound, as they present a small amount of antral fluid (Taymor, 1996).

The number of small follicles at the beginning of the cycle may well represent the actual functional ovarian reserve. During spontaneous cycles, the selection of one of the follicles and atresia of the others is the rule, while during stimulated cycles, a set of follicles has the chance to develop. If the amount of FSH administered surpasses a threshold concentration, those antral follicles will grow up until they reach a mature state. The number of developing follicles is known to be correlated with the number of recovered oocytes and the number of embryos available for transfer and cryopreservation (Arnot *et al.*, 1995). Our study confirms that the number of follicles seen already at the beginning of the treatment is correlated with the number of recovered oocytes. In this study, the number of recovered oocytes was seen as the main outcome, as it is directly related to the ovarian characteristics studied (ovarian volume and number of follicles). The pregnancy rate and other IVF outcomes were not in focus in this study, because they are affected by many other factors.

The known effect of age on the number of recovered oocytes (Tan *et al.*, 1992; Check *et al.*, 1994) was also observed in this study. The inverse correlation with the number of recovered oocytes, however, was weaker than the correlation of the

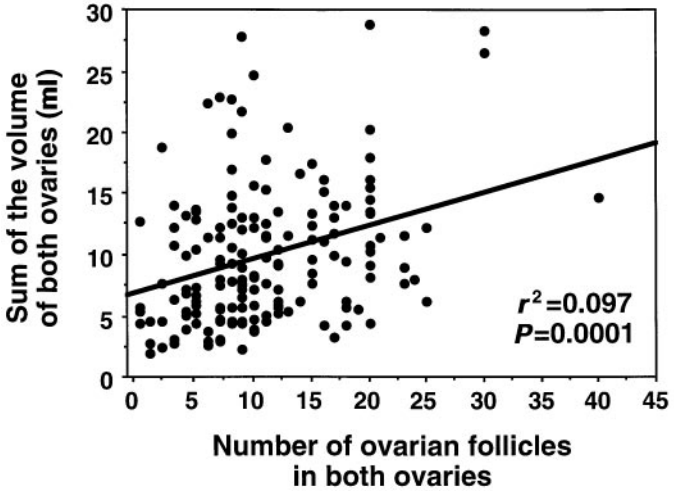

Figure 2. Linear correlation between ovarian volume and the number of ovarian follicles before ovarian stimulation and after pituitary down-regulation, in 166 patients enrolled in an in-vitro fertilization (IVF) treatment.

number of follicles with the recovered oocytes. This indicates that the number of follicles alone is a better indicator than age alone in predicting ovarian responsiveness. More important, transvaginal ultrasound at the beginning of an IVF trial makes it possible to detect a group of patients with inactive ovaries that will have a poor response. This occurs independently of their age, although high doses of gonadotrophins are used. This confirms the observation that some older patients may show a normal response, while some younger patients have a poor response (Jacobs *et al.*, 1990; Toner, 1993). In the present study, the differences in recovered oocytes between the patients with inactive, normal and PCO-like ovaries reach statistical significance in the patients aged <35 years, but not in the older patients. There was, however, a clearly similar trend (4.6 $\pm$ 2, 6.7 $\pm$ 4.1 and 8.6 $\pm$ 4.1 oocytes respectively) in this group, which is of obvious clinical importance. The lack of statistical significance may be related to the small number of patients in this subgroup. The possible concomitant effect of age and the number of small follicles will have to be addressed in further, larger studies.

It has been proposed that the number of remaining ovarian follicles influences age of menopause and the reproductive age of the woman. Chronological age influences more the quality of the oocyte and its capacity for implantation and development (Faddy *et al.*, 1992; te Velde, 1993; Navot *et al.*, 1994; Faddy and Gosden, 1995).

Ovarian volume was correlated with the number of follicles, but not with the number of recovered oocytes. This partly agrees with the recent study of Syrop *et al.* (1995) indicating that ovarian volume had a prognostic value in ovarian responsiveness. We believe that the volume of the ovaries is an indirect indicator of the activity of the ovaries. In our study the number of follicles was more accurate than ovarian volume in this sense, and could be used as a first method for predicting the ovarian response to gonadotrophins. In cases in which obesity or a distant localization of the ovaries perturbs the image quality, ovarian volume may be used to predict ovarian responsiveness. Ovarian volume, however, is of little value in the case of patients who have had reductive ovarian surgery, or in the presence of endometriotic lesions.

As expected, patients with PCO-like ovaries had larger ovaries than the other patients, which is related to increased ovarian stroma in these patients. It is interesting to note that patients with PCO-like ovaries needed similar numbers of gonadotrophin ampoules as patients with inactive or normal ovaries. It is important to stress, however, that we were not dealing specifically with PCO patients. The decision for adjusting gonadotrophin dose was based only on the ultrasound follow-up and by using a step-up procedure as needed. This indicates that the number of ampoules used did not influence directly the number of recovered oocytes, which was more dependent on the intrinsic characteristics of the ovaries. It is also known that increasing doses in the middle of the stimulation does not obviate the poor responsiveness of the ovaries.

All patients in this study had ovarian suppression by GnRHa for 2 weeks. The effects of GnRHa on the number of small ovarian follicles have not been studied. We believe, however, that use of GnRHa does not influence the number of recruitable follicles as their growing starts much earlier.

Ovarian reserve and prediction of ovarian stimulation response have been, so far, evaluated by single hormonal measurements, obtained at the beginning of the cycle, or by dynamic hormonal challenge tests. Basal serum FSH concentration is, together with maternal age, the main factor influencing the outcome of ovarian stimulation (Toner *et al.*, 1991). Magarelli *et al.* (1996) evaluated a group of patients referred by other centres after unsuccessful infertility treatments, with ages >35 years. They found that a model including maternal age, a single FSH determination and the number of prior treatments is a good predictor of pregnancy (Magarelli *et al.*, 1996). Other authors, however, suggest that the combination of single or dynamic tests does not seem to improve the prediction of ovarian response over the basal serum FSH concentration alone (Galtier-Dereure *et al.*, 1996). There has also been some concern that serum FSH concentrations can vary between cycles (Scott *et al.*, 1990), although in a recent study of 19 non-infertile women, Hansen *et al. (*1996) did not find any significant FSH inter-cycle variability. Furthermore, they observed that FSH did not vary significantly during cycle days 2–5.

Obtaining blood samples is bothersome and expensive, and subject to discrepancies between different laboratories. Furthermore, blood samples must be obtained with a certain regularity as to follow-up the ovarian reserve at different stages. In the present study, we have not obtained serum FSH determinations. We share the opinion of Wikland (1992), that IVF treatment and its follow-up, needs to be simplified.

Transvaginal ultrasound has an important role in IVF protocols (Wikland, 1992; Golan *et al.*, 1994; Nachtigall and Schwartz, 1996). Its ease and availability permits the identification of a group of patients for which ovarian stimulation will not be effective, despite high doses of gonadotrophin being used at the beginning of, or during the treatment.

Patients presenting with apparently inactive ovaries will generate small numbers of mature follicles, even with the use

of increased amounts of gonadotrophins. They are also at risk of having their cycles cancelled, achieving a reduced number of embryos. An alternative for some of these patients can be oocyte donation, in which good pregnancy rates have been observed independently of age (Remohi *et al.*, 1993). The information obtained by ultrasonography is very useful in counselling the couples and planning their treatment, because it gives an estimation of the actual ovarian reserve. Furthermore, realistic expectations for success can be formulated before couples are enrolled in expensive IVF trials. It is possible that this information could already be obtained before starting ovarian suppression with GnRHa, and we have initiated studies to test this hypothesis. In a period when economic resources have to be optimized, this is important information for rationalizing the use of expensive drugs and treatments. Transvaginal ultrasound is an easy and reliable method to complement other predictor factors of ovarian responsiveness, including age or basal FSH determinations.

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Received on July 17, 1996; accepted on November 7, 1996