Ultrasound Examination (Antral Follicle Count and Ovarian Volume) Versus Serum FSH Measurement in Assessment of Ovarian Reserve

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Abstract

Aim of study: The current study was designed to compare antral follicle count (A.F.C), ovarian volume & day 3 FSH level, with respect to their ability to predict ovarian response of infertile women to ovulation induction agents (antiestrogens such as clomiphene citrate or tamoxifen, or exogenous gonadotrophins).

Material and Methods: Sixty-two patients between the ages of 19 - 43 years participated in this study when attending the infertility clinic of Babylon Hospital for Maternity and Children. All patients underwent a transvaginal sonography on day 3-5 of cycle to measure the ovarian volume and count of basal antral follicle, and basal FSH concentration was determined on day 2-4 of a spontaneous period.

Induction of ovulation in next cycle by using antiestrogens or gonadotroohins (pure FSH- gonal- F) for 3 successive cycles . Patients monitored by using TVS on other days, then on day 12 of cycle TVS was performed to assess the presence of dominant follicle.

Results: In this study the results showed that AFC has a higher predictability for successful fertility treatments by assessing ovarian reserve, i.e. when AFC < 10; the success rate was 13.3, while when AFC > 10; the success rate was 58.3.

The next predicting factor was ovarian volume, when < 3 ml; the chance of success was very poor (zero), while when volume is 3-9 ml; the success rate was 40.4% & when volume >9 ml; the success rate was 63.6%.

While day 3 FSH had the lowest predictability, when FSH < 10 m IU /ml; the success rate was 45.3%, while when FSH > 10 m IU / ml; the success rate was 38.8%

Conclusion: A.F.C. performs well as a test for ovarian response being superior to complex and time consuming endocrine tests. It is therefore likely to be the test for general practice.

الخلاصة

دراسة توقعية تضمنت (62) امرأة عند مراجعتهن لاستشارية علاج العقم في مستشفى بابل للولادة ولأطفال للفترة من (حزيران عام 2008 ولغاية كانون الثاني عام 2009), تم إجراء الفحوصات للتنبؤ باحتياط المبيض وقابلية استجابته للأدوية المنشطة:

1- إجراء فحص السونار المهبلي لكل النساء في الدراسة في اليوم 3-5 من الدورة الشهرية لقياس حجم المبيض وحساب العدد الكلي للبويضات الأولية الصغيرة .

2- قياس الهرمون المنشط للبويضة في الدم في اليوم 2-4 من الدورة الشهرية .

وبعد ذلك تم إعطاء المنشطات (كلوميفين ستريت او تاموكسيفين أو حقن الهرمون المنشط للبويضة) لمدة ثلاثة أشهر متتالية .

وقد أظهرت النتائج إن استخدام السونار المهبلي لحساب البويضات الأولية الصغيرة ويأتي بعده حساب حجم المبيض كان أكثر دقة للتنبؤ باحتياط المبيض وقابليته للاستجابة للمنشطات .

بينما كان قياس هرمون المنشط للبويضة الأقل حساسية للتنبؤ باحتياط المبيض .

Introduction

Fertility is reduced with increasing age of the female in both spontaneous conceptions and with the use of I. V. F. and other assisted reproductive methods[1].

The decrease in fertility with aging is probably due to a decreasing number of primordial follicles after birth [1].

Every woman is born with nearly 1 million

eggs, out of which only 300,000 to 400,000 survive up to puberty. Approximately 500-1000 eggs are lost every month through a degenerative process referred as Atresia.

Ovarian reserve refers to the number of healthy eggs present in a female's ovary at any given point of time. By "healthy" we mean eggs which respond to any biological stimulation efficiently.

A high ovarian reserve implies a greater number of eggs and thus, a higher possibility of fertility. A variety of factors can lead to a decline in ovarian reserve; the most significant of them is advancing age. Other factors which can prove detrimental to the ovarian reserve include any form of invasive ovarian surgery or some hereditary genetic disorder [(2)].

Ovarian reserve testing is performed predominately in women 35 years of age and older. It is quite unusual for women under the age of 35 years to have reduced ovarian reserve. This low prevalence leads to more false positive results in younger women [(3)].

Various methods of ovarian reserve testing have been established with the goal of predicting a patient's response to ovulation induction agents and her prognosis for both pregnancy and miscarriage [(4, 5)].

Basal or day 3 follicle stimulating hormone (F.S.H.) has been extensively used as a screening method to estimate ovarian reserve. Day 3 F.S.H. levels have been shown be predictive of cycle outcome and overall pregnancy rate. Elevated levels have been associated with poor response during assisted reproductive technique cycles and decreasing pregnancy rates, indicative of an age – related decline in fertility. There are differing opinions as to what cutoff value should preclude starting an assisted reproductive technique cycle [(6, 7)].

F.S.H. levels do not seem to vary significantly between days 2, 3, 4 of the menstrual cycle in normal women. It is therefore reasonable to use a level on any of these days as an assessment of ovarian reserve. Day 3 F.S.H. levels do seem to trend slightly higher than either day 2 or day 4 levels [(8)].

Antral follicles are small follicles about 2-8 mm in diameter, present very early in the follicular phase of the cycle [(9)]. These follicles are in an antral phase, and are easily detected by transvaginal ultra-sound, as they contain a small amount of antral fluid. The number of small antral follicles is clearly related to age and could well reflect the size of the remaining primordial pool in women with proven fertility [(10, 11)].

A high value of A.F.C. implies a higher ovarian reserve and thus, a higher chance of getting pregnant. A.F.C. mainly declines with increasing age, but some other hereditary or genetic factors might also bring about a decline in its value.

A.F.C. is typically done during the 2^{nd}-4^{th} days of menstrual flow, though it can probably be as accurately done during other times of the menstrual cycle [(12)].

The number of antral follicles counted on each ovary can be used to complement F.S.H. levels in ovarian response assessment [(13)].

A.F.C. may be a useful tool for predicting pregnancy loss in I.V.F. pregnancies. Women with a pregnancy loss had a lower A.F.C. than those with healthy deliveries [(14)].

There is intercycle variation of A.F.C. and ovarian volume. More variation was observed in the A.F.C. of young infertile patients [(15)].

Real time two – dimentional (2D) pelvic ultrasonography is a relatively accurate and reliable method of determining ovarian volume and morphology [(16)]. Interobserver and intra-observer measurements have been shown to be very low when using T.V.S [(17, 18)].

The mean ovarian volume increases from 0.7 ml at 10 years to 5.8 ml at 17 years of age. It has been suggested that there are no major changes in ovarian volume during reproductive years until the premenopausal period. In women > 40

years old, there is a dramatic drop in ovarian volume, which is not related to parity [19, 20].

Several studies demonstrate that ovarian volume, as determined by T.V.S., is a predictor of ovarian reserve and clinical pregnancy rate. Lass et al. confirmed that decrease in ovarian volume is an early sign of depletion of the follicles and its measurement is likely to be clinically useful [21, 22, 23, and 24].

Navot et al. (1987) first described the clomiphene citrate challenge test (CCCT) [25].

This test consists of day 3 FSH value and oestradiol obtained via a blood draw. The woman then takes clomiphene citrate, 100 mg from day 5- 9 of her menstrual cycle. On day 10 a repeat FSH value is drawn. If either value is greater than 12 m I.U/ml, this suggests reduced ovarian reserve. This test can provide earlier evidence of declining ovarian function. It is more sensitive than day 3 FSH test alone. It can discover some cases of poor ovarian reserve that are still showing a normal day 3 FSH.

Abnormal CCCT has been shown to be predictive of poor ovarian reserve, cycle cancellation and reduced pregnancy rate [26, 27, and 28].

Subjects and Methods

Patients aged 19 – 43 years attending the infertility clinic at Babylon Hospital for Maternity and Children between June 2008 and January 2009, participated in this prospective study.

Total number of patients was 75; 13 patients lost to follow up, and only 62 patients continue to follow up in the study.

Duration of their infertility ranged from 2-12 years. In 14 of patients the cause of infertility was combined male & female factors, and in the remaining 48 patients the cause of infertility was only female factor (anovulation) .There was no cases of unexplained infertility in our study.

Poor visualization of ovaries because of abdominal position, presence of an ovarian cyst >20 mm in diameter, cases of hyperprolactinemia, and the presence of polycystic ovaries on scanning were excluded from the study. Every woman gave a written informed consent prior to participating in the study.

A basal cycle day 2-4 FSH was measured in the blood in all patients. AFC and ovarian volume were determined on days 3-5 of the cycle using transvaginal ultrasound by experienced sonologist in the ultrasound department in the same hospital. AFC was determined during transvaginal scanning using a 6.5 MHZ vaginal probe. The follicles visualized and counted by TVS are 2-10mm in diameter. To determine the diameter of the follicle, the mean of measurements in two perpendicular directions was taken.

The volume of each ovary was calculated by measuring in three perpendicular directions and applying the formula for an ellipsoid: D1x D2 x D3 x π /6.

Serum FSH concentration was measured using commercially available kits by a two – site sandwich immunoassay.

FSH assay is standardized against the World Health Organization 2nd International Standard reference material.

During the follow up in the first cycle, there was no mature follicle on day 12 ultrasound in all women in the study.

In the next cycle induction of ovulation by clomiphene citrate 100 mg for 5 days (from day 2-6 of cycle) used by 56 women for three successive cycles .

While in the remaining 6 women; pure FSH (recombinant) ampules 150 IU/ day for eight days, were used for 3 successive cycles, because of failure of induction of ovulation on clomiphene or tamoxifene.

In the same cycle ultrasound was done on day 12 to assess the presence of mature follicles.

Results

Table 1. Study group characteristics

Age		
< 35 years	**39**	**62.9%**
> 35 years	**23**	**37.1%**
Type of infertility		
Primary	**56**	**90.3%**
secondary	**6**	**9.7%**
Duration of infertility		
< 5 years	**22**	**35.5%**
> 5 years	**40**	**64.5%**

Table 2. Relationship between AFC and success rate of induction of ovulation per cycle

AFC	NO. of subjects	%	Success rates of induction of ovulation per cycle No. of cycles	%
<4	2	3.2%	Zero 1/ 6 (cycles)*	Zero% to clomiphene 16.6% to high dose FSH
5-10	20	32.3%	8/ 60 (cycles)*	13.3% (to clomiphene only)
>10	40	64.5%	70/ 120 (cycle)*	58.3%

*: for each subject 3 treatment cycles are tried.
P < o.oo1 (highly significant)

Table 3. Relationship between day 2-4 serum FSH level and success rates of induction of ovulation per cycle.

FSH (m IU / ml)	NO. of subjects	%	Success rates of induction of ovulation per cycle No. of cycles	%
< 10	**50**	**80.6%**	**68/ 150 (cycle)***	**45.3%**
>10	**12**	**19.4%**	**14/ 36 (cycle)***	**38.8%**

*: for each subject 3 treatment cycles are tried.
P > 0.05 (no significant difference)

Table 4. Relationship between mean ovarian volume (ml) and success rates of induction of ovulation per cycle.

Ovarian volume (ml)	NO. of subjects	%	Success rates of induction of ovulation No. of cycles	%
<3	**2**	**3.2%**	**Zero/ 6 (cycle)***	**Zero% even to high dose of FSH**
3-9	**38**	**61.3%**	**46/ 114 (cycle)***	**40.4%**
> 9	**22**	**35.5%**	**42/ 66 (cycle)***	**63.6%**

*: for each subject 3 treatment cycles are tried.
P < 0.01 (significant)

Discussion

The significance of AFC has been evaluated extensively in infertile patients undergoing ART treatment.

In this study it is found that AFC is more predictive of ovarian reserve than ovarian volume & day 3 FSH, this is in agreement with other studies.

Tomas et al. (1997) first concluded that AFC before ovarian stimulation was a better predictor of the ovarian response than ovarian volume or age alone [(29)].

Other studies (Frattarelli et al. , 2000 ,

Hsieh et al. , 2001 , Nahum et al., 2001,Kupesic et al. 2002, Popovic-Todorovic et al. , 2003, also confirm the importance of AFC in the prediction of ovarian response [30].

Recently Hendriks et al. [31], published a meta-analysis on the AFC as a predictor for poor ovarian response and concluded that AFC is an adequate test for the prediction of poor ovarian response, compared to FSH.

The high intercycle stability of AFC and its potentially likely attractive cost features are likely to make this test rather attractive for routine practice.

A great advantage of AFC over any other test is its potential usefulness for its ability to concomitantly predict low and high responders.

A gradual decrease with advancing age in the number of sonographyically detectable antral follicles has been shown in many studies [32].

AFC do not seem to vary significantly from side to side within patients .This provides basic clinical usefulness when evaluating patients with one ovary that is not easily imaged.

In general women found to have fewer than five egg follicles will likely not respond well to fertility medications and will have too few mature eggs for retrieval thereby making them unsuitable candidates for IVF. A woman whose follicle count falls between (5 and 11) is still a fairly reduced number of follicles although IVF may be successful for some. If follicle count between 12 and 30, then IVF is likely to be successful. Women who have more than 30 follicles are considered to be good candidates for IVF. However, the high number of follicles could indicate the presence of polycystic ovaries as well as the possibility of over stimulating the ovaries.

Ovarian volume has also been evaluated to predict ovarian responses during ART treatment (Syrop et al. 1999; Lass et al. 1997) and was a better measure than basal FSH level (this also is in agreement with our study).

Inhibin –B levels fall in women with decreased ovarian reserve prior to the elevation in FSH level [33], prompting investigations into the potential of inhibin - B as a marker of ovarian reserve.

One study demonstrated that women with low day 3 serum inhibin - B concentrations (< 45 pg / ml) had a poorer response to ovulation induction and were less likely to conceive through ART relative to woman with a high day 3 inhibin - B [34]. Other studies, however, have failed to show inhibin –B to be predictive of pregnancy in women undergoing infertility or ART therapies [35, 36].

Moreover, there is no accepted international standard for the inhibin – B assay, making correlation between laboratories difficult.

A meta – analysis (Bancsi et al. 2003) showed that the performance for basal FSH concentration for predicting poor response was moderate and the performance for predicting no pregnancy was poor. Therefore, a challenge test such as CCCT will be able to identify more women with impaired ovarian reserve than basal FSH screening alone [37].

Although ovarian reserve tests should be performed in women above 35 years, but in our study 62.9 of women were < 35 years & only 37.1 were > 35 years; these is because the majority of women attending the infertility clinic in our hospital are young & have long history of infertility.

Future studies will have to be carried out to determine if other ovarian reserve tests such as the measurement of Anti – Mullerian Hormone (AMH) are better predictors for ovarian reserve [38, 39, and 40].

Low serum levels of AMH (< 2.7 ng / ml) suggest the presence of a depleted ovarian follicle pool.

AMH may have an especially useful role in identifying reduced ovarian follicle pool in certain types of patients, such as in cancer patients.

Recommendations

1. All infertile women over the age of 30 years should be screened because ovarian reserve begins to diminish approximately at that time.
2. Younger women with anovulation & long history of infertility (after exclusion of PCO, hyperprolactinemia) should be assessed for ovarian reserve.
3. A larger study using other ovarian reserve tests like AMH and inhibin–B comparing them with AFC.

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