Day 3 serum levels of Inhibin – B, FSH and Transvaginal Ultrasound as Predictors for Ovarian Reserve in IVF cycles

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Abstract: Background: Ovarianreserve is the remaining of resting and primary ovarian follicles and is used to define the quantity and quality of follicles present in the ovaries at a given time. Ultrasonographic markers of ovarian reserve is non invasive, however, they cannot predict future of the ovarian response to IVF cycle. inhibin B can be used in the same way as estradiol to monitor the follicular growth and correlates with the number of oocytes retrieved and fertilized. **The aim of is study is** to evaluate the role of cycle day 3 serum inhibin- B concentration, FSH and different ultrasound parameters in the prediction of ovarian reserve and fertility potential (pregnancy). **Patients and methods:** fifty women undergoing for their first IVF cycle in a pregnancy attempt were included. All patients underwent controlled ovarian hyperstimulation after baseline assessment of hormonal profile and ultrasound parameters, Transvaginal ultrasound monitor of ovarian response was conducted to assess ovarian response. **Results:** Day-3 antral follicle count and inhibin-B were more sensitive and specific than either day-3 ovarian volume or day-3 FSH in prediction of poor ovarian response (97% and 99% in antral follicle count and 95% and 99% in inhibin-B vs. 91% and 99% in ovarian volume and 40% and 35% in FSH, respectively). Also, day-3 antral follicle count and inhibin –B have higher predictivity for ovarian response than either day-3 OV or day-3 FSH (positive and negative predictive value was 98% and 99% in inhibin-B and AFC vs. 94% and 92% in ovarian volume and 47% and 50% in FSH, respectively). **Conclusions**: the predictive value of cycle day-3 inhibin-B and antral follicle countas regards assessment of ovarian reserve is higher compared to ovarian volume or cycle day-3 FSH. Basalinhibin-B and antral follicle counthave more or less similar value in predicting the ovarian reserve and the ovarian response to controlled ovarian stimulation in women undergoing infertility treatment with IVF.

[Amr A. Aziz khalifa ,Magdi A. Gawad Mohamed, Tagrid M. Mohamed. **Day 3 serum levels of Inhibin – B, FSH and Transvaginal Ultrasound as Predictors for Ovarian Reserve in IVF cycles.** *J Am Sci* 2014;10(1):199-206]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 31

Keywords: ovarian reserve, inhibin-B, FSH, ultrasound, ultrasonography, IVF, antral follicle count, ovarian volume, AFC, OV

1. Introduction:

The ovaries of the human female contain a number of immature, primordial follicles. These follicles each contain a similarly immature primary oocyte. After puberty and commencing with the first menstruation, a clutch of follicles begins folliculogenesis**. (1**) Folliculogenesis describes the progression of a number of small primordial follicles into large preovulatory follicles that enter the menstrual cycle, it ends when the remaining follicles in the ovaries are incapable of responding to the hormonal cues that previously recruited some follicles to mature. This depletion in follicle supply signals the beginning of menopause**.(2)**

As women age, double-strand breaks accumulate in their primordial follicle reserve. These follicles contain primary oocytes that are arrested in prophase of the first meiotic division. It was hypothesized that DNA double-strand break repair is vital for the maintenance of oocyte reserve, and that a decline in efficiency of repair with age plays a key role in the depletion of the ovarian reserve**.(3)**

Ovarian reserve is the remaining of resting and primary ovarian follicles and is used to define the quantity and quality of follicles present in the ovaries at a given time**.(4)** The decline in fecundity with female age, long before menopause occurs, is a well-known phenomenon**.(5)**

The timing of the menopause, caused by dysfunctional ovaries, is determined by the store of germ cells and the rate of depletion during life. The evaluation of ovarian reserve has been and still the focus of substantial clinical research**.(6,7)**

Measurement of ovarian reserve can only be approximated as precise tests**.(8)** The methods for assessing ovarian reserve are classified into two groups: Passive tests; age**(9,10)**,cycle day 3 serum follicle stimulating hormone(FSH) concentration **(11,12),** basal FSH/LH ratios**(12,13**), cycle day 3 serum estradiol concentration**(14-16),** cycle day 10 serum progesterone concentration**(17)**, cycle day 3 serum inhibin B concentration**(18-22),** serum Anti-müllerian hormone (AMH) level**(19,23-25**), ovarian volume (OV**) (26-28**), antralfollicle count (AFC) **(29-33)**, ovarian biopsies **(34-36),** ovarian stromal

Doppler **(37-40)**. Dynamic tests; Gonadotrophin agonist stimulation tes[t](9,41,42), clomiphene citrate challenge test**(42-44),** exogenous FSH ovarian reserve test **(45,46).**

Transvaginal ultrasonography (TVS) is suggested to be the preferred method for ovarian reserve determination rather than hormonal parameters, as TVS assessment of OV and the AFC confer a stronger correlation with chronological aging than Day 3 FSH level indices and aging**.(28)** The greatest advantage of ultrasonographic markers of ovarian reserve is their non invasiveness. The use of sonographic methods are somewhat limited, however, as they cannot predict future or the ovarian response to IVF treatment**.(47,48)**

Inhibins are glycoproteins produced by the granulosa and theca cells of the ovary and by the sertoli cells of the testis**.(49**) Inhibins are multifunctional molecules involved in the control of pituitary FSH secretion**.(12)** Both observational and experimental evidence in women suggests that inhibins are physiologically important regulators of FSH secretion**.(50)** At late reproductive years, regularly cycling women with elevated day 3 FSH levels have lower inhibin A and inhibin B levels compared to age-matched controls with normal FSH levels**.(51)** Apart from their essential role in the selective control of FSH secretion, inhibins are currently recognized as paracrine ovarian and testicular regulators and have multiple paracrine effects in the utero-placental unit, representing a promising marker for male and female infertility, gynecological and gestational diseases**.(52)**

During controlled ovarian stimulation for assisted reproduction treatment, inhibin B can be used in the same way as estradiol to monitor the follicular growth and correlates with the number of oocytes retrieved **(53)** and fertilized. **(54)**Inhibin B has also been evaluated as an additional marker to predict the response to ovulation induction in women whose main infertility factor was ovulatory dysfunction but, in such cases, it does not appear to be of clinical relevance. This is not surprising because anovulatory women who fail to respond to ovulation induction might have this resistance explained by a number of alternative mechanisms, apart from a diminished ovarian reserve**.(55,56)**

The relationship between increased female age, elevated basal FSH concentrations and diminished ovarian function with a lower chance of IVF success has been established**.(57-60)**The response to controlled ovarian hyperstimulation (COH) during assisted reproduction treatment is highly variable, even among women of similar ages**. (61)** This undoubtedly reflects the intersubject variation in ovarian reserve, which is primarily determined by the size of the primordial follicular pool at birth and the rate of its decline during reproductive life, both of which are genetically determined**.(62)**

2. Patients and methods

This study is prospective, single center study that was conducted at Ain Shams University Maternity Hospital in the period from May 2011 to May 2013. Fifty women who were referred to Assisted Reproduction center for their first IVF cycle in a pregnancy attempt were included in the study. Women with polycystic ovarian disease, endometriosis, hydrosalpinx, ovarian mass, fibroids, previous ovarian surgery, with endocrinal disorders (hyperthyroidism, hypothyroidism and hyperprolactinaemia) and with medical disorders (D.M, Hypertension) were excluded.

In day 3 of the normal menstrual cycle, all participants were subjected to blood sampling for measuring the serum inhibin-B and FSH as well as the ultrasound measurement of the AFC and OV.TVS was done using 7.5 H_z transvaginal probe of Madison Sonoco 8800 digital GAIA ultrasound machine with Doppler unit.

Ovarian Volume: the volume of each ovary was calculated by measuring the three perpendicular diameters and applying the formula for ovarian volume = D1 x D2 x D3 x 0.523 where D1, D2 and D3 are represent maximal longitudinal, antero-posterior and transverse diameter**.(63**) Mean ovarian volume is the reference value calculated in this study. Ovaries with cystic enlargements ≥ 15 mm were excluded from the analysis of the ovarian volume.

Antral follicle count: any round or oval structures in the ovaries were regarded as follicles. Follicles measuring smaller than 10 mm will be counted from lateral to medial margins of the ovary in order to determine the antral follicle count of each ovary. The total antral follicle count in both ovaries was recorded as reference value in the study**.(64)**

Serum E2, FSH and LH were measured in plasma specimens with an electrochemiluminescence immunoassay (ECLIA) on the Roche Elecsys 2010 immunoassay analyzer, using a commercial kit according to the manufacturer's sensitized assay protocol, the sensitivity of the assay is < 0.10mIU /mL. Serum inhibin B was determined using Enzyme Immunoassay (EIA). A commercial kit (RayBio) was used according to the manufacturer's sensitized assay protocol. The sensitivity of the assay is 34.6 pg. / mL.

Controlled Ovarian Stimulation Protocol: was performed according to a long GnRH agonist protocol starting in the midluteal phase. Seven days after ovulation, daily subcutaneous injections with triptoreline acetate (Decapeptyl 0.05 mg/day; Ferring

pharmaceuticals, Kiel, Germany) was started**.(65)** On day 3 of the next cycle, ovarian stimulation was started with daily IM injections of a dose of 150 – 225 I.U. HMG (Menogon 75 IU /ampoule; Ferring pharmaceuticals, Kiel, Germany). The starting dose of the gonadotropins was prescribed according to the age, body built of the subjects. Then the duration and daily doses were adjusted according to serum E2 levels and follicular number and size in an ultrasound scan of the ovary. Ovarian stimulation was continued until the largest follicle reach a diameter of ≥18mm. The maximum duration of HMG administration was not allowed to exceed 16 days. If these criteria was met, Menogon and Decapeptyl was discontinued and 10.000 IU of HCG (Pregnyl. 10.000 IU/ampoule: Organon, Oss, Netherlands) was administered.

After that oocyte retrieval and embryo transfer were scheduled followed by luteal phase support by natural progesterone supplements 100-200 mg/twice per day for at least two weeks.

Oocyte Preparation and fertilization: the oocytes were placed in culture medium and intra-cytoplasmic sperm injection was performed using Olympus CK40 inverted phase micro-manipulating equipment. The injected oocytes were incubated at 37ºc. Fertilization was diagnosed by the presence of two pronuclei in the injected oocyte.

Embryos Selection: embryo quality was assessed according to presence of nuclear fragments and size, shape, symmetry and cytoplasmic appearance of the blastomeres. Grading of day-2 embryos. [**66**]

Embryos Transfer: was done two days to five days after oocytes retrieval, up to four good quality embryos were transferred with a thin plastic cannula attached to a syringe (Cook embryo transfer catheter).

The study group was divided into two subgroups according to the number of oocytes retrieved. Patients with an oocyte count of five or more were considered good responders and patients with less than five were considered poor responders. Biochemical pregnancy was defined as a positive pregnancy test more than 3 days after the expected menses.

3. Results

Patients were grouped on the day of ovum pick up. Patients with an oocyte count of five or more were considered good responders (45 patients) and patients with less than five poor responders (5 patients). Between poor responders no female became pregnant while, thirty six patients of good responders had positive pregnancy tests with significant association.

Mean of basal levels inhibin-B in the studied women was 70.1±21, mean of OV and AFC was 5.5±1.2 and 9.8 ±2.0 respectively. Basal inhibin-B, OV and AFC had a statistically high significant decrease (P<0.001) between those with poor ovarian reserve compared with those with good ovarian reserve (table 1).

The patients' age ranged from 25 to 35 years with mean age being 29.8± 3.5 years.Mean of basal level of LH and FSH was 5.9±1.2 and 7.4±1.7 respectively and there was a statistically high significant increase (P<0.001) in those with poor ovarian reserve compared with those with good ovarian reserve (table 1).

Table (1);**hormonal and clinical parameters .**

	variable mean ± SD		ovarian reserve		p
			good	poor	
basal data	**age (years)**		29.3±3	34±1.3	<0.001 highly significant
	LH (mIU/mL)		5.7±1	7.6±1.1	
	FSH (mIU/mL)		7.2±1.5	9.9±1.2	
	Inhibin-B (pg/mL)		73.2±20	42.7±8	
	ultrasound	**OV**	5.7±2	3.2±1.3	
		AFC	10.2±1.6	5.4±0.5	
ovarian simulation outcomes	**Final E2**		3194±852	618±215	<0.001 highly significant
	HMG ampoules		37±6	56±2.8	
	Days of stimulation		12.3±0.9	13.8±0.4	
	Number of retrieved oocytes		11±2.7	3±0.5	
	Number of fertilized oocytes		9±2.5	2±0.8	
	Number of good embryos		7±2.3	2±0.9	

Day-3 AFC and inhibin –B were more sensitive and specific than either day-3 OV or day-3 FSH in prediction of poor ovarian response (97% and 99% in AFC and 95% and 99% in inhibin-B vs. 91% and 99% in OV and 40% and 35% in FSH, respectively). Also, day-3 AFC and inhibin –B have higher predictively for ovarian response than either day-3 OV or day-3 FSH (positive and negative

predictive value was 98% and 99% in inhibin-B and AFC vs. 94% and 92% in OV and 47% and 50% in FSH, respectively) (table 2, figure 1).

As regards clinical response to the controlled ovarian stimulation in the whole studied cases the average days of stimulation was 12.5±1.0, total 38.5±8.9 HMG ampoules and final E2 2937 on HCG day. Oocyte retrieval was performed with average retrieval of 10 oocytes. The good reserve group had higher final E2, shorter days of stimulation, lower number of HMG ampoules, higher number of retrieved oocytes and good embryos compared to the other group with significant difference between both groups (table 1).

Table (2); Validity of inhibin-B, FSH , OV and AFC in prediction of ovarian reserve

	Inhibin-B	FSH	OV	AFC
Best cut off	44	7.5	4.6	6.5
AUC (area under the curve)	0.98	0.11	0.99	0.99
Sensitivity	95%	40%	91%	97%
Specificity	99%	35%	99%	99%
PPV	98%	47%	94%	98%
NPV	99%	50%	92%	99%

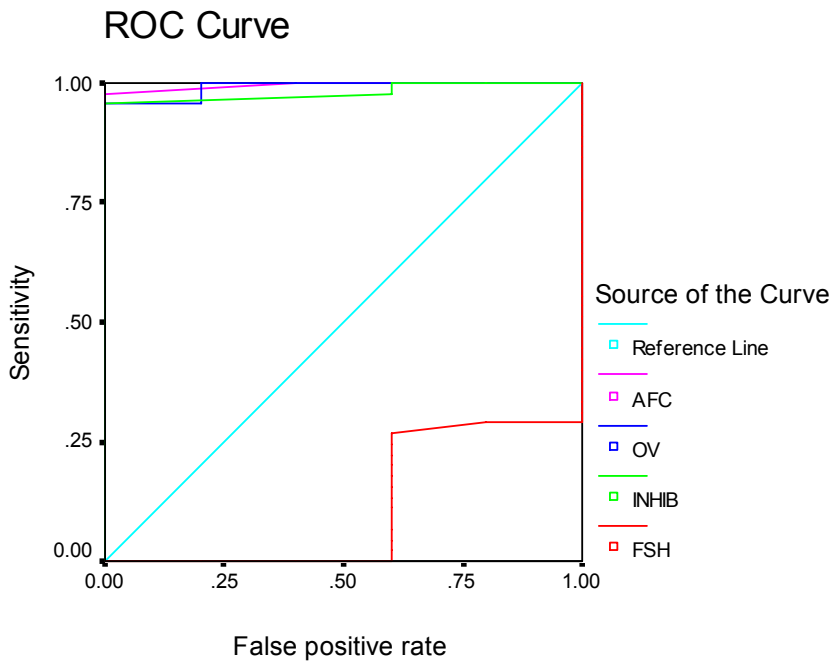

Figure (1): ROC curve for the parameters investigated in prediction of ovarian reserve in the studied women

4. Discussion

Pretreatment assessment of ovarian reserve allows appropriate counseling and modification of an individual's treatment protocol in an attempt to maximize their potential response**.(67)**

In this study 10% of the studied cases (n=50) had poor ovarian outcome and 90% had good ovarian outcome. Nine women with good ovarian outcome became non pregnant, so the occurrence of pregnancy was affected by multiple different factors involved in oocytes retrieval and embryos transfer that were out of our control.

AFC in early follicular phase of the menstrual cycle has highly significant increase (P<0.001) between those with good ovarian reserve compared with those with poor ovarian reserve. AFC is good predictor of poor ovarian response, as demonstrated by AUC (0.99). The sensitivity, specificity, positive and negative predictive values of AFC was 0.97%, 0.99%, 0.98% and 0.99% respectively.

Previous studies examining the ability of AFC to predict both the number of oocytes retrieved and the poor ovarian response found that AFC has at

least the same level of accuracy and clinical value for the prediction of poor response and non pregnancy as AMH**.(68,69)** However, three dimensions ultrasound was used in AFC provided more reliable and valid measurements**.(70)**

The mean OV has high significant increase (P<0.001) between those with good ovarian reserve compared with those with poor ovarian reserve. The sensitivity, specificity, positive and negative predictive values of OV were 0.91%, 0.99%, 0.94% and 0.92% respectively. Ovarian volume was predictive of both the number of oocytes retrieved and poor response on univariate analysis as reported in other studies**(71,72),** ovarian volume is an indirect indicator of the size of the follicle cohort and is not only influenced by the number of follicles but also by their size**.(44,73)**

The basal serum inhibin B level was statistically significantly lower in poor responders than normal responders. The sensitivity, specificity, positive and negative predictive values of inhibin-B was 0.95 %, 0.99 %, 0.98 % and 0.99 % respectively. In agreement with this study, Tan et al **(74)** found that both basal and stimulated serum inhibin B levels are lower in poor responders than in controls. Compared with AMH, basal and stimulated inhibin B are more accurate predictors of ovarian response in patients undergoing IVF. **(74)**Other studies confirmed that basal inhibin B concentrations are correlated directly with the parameters of ovarian response, ovum retrieval and fertilization outcome**.(75-77)**

Against our findings, 2 studies showed that inhibin B levels did not show any statistically significant differences between poor and good ovarian responders**. (69, 70)** It was reported that AFC and AMH are the most significant predictors of poor response to ovarian stimulation during ART compared to inhibin B**.(70)**

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1/12/2014