The role of inhibin B in prediction of in vitro fertilization or intracytoplasmic sperm injection cycles' outcome

Ensieh S. Tehraninejad, MD, Elham Amirchaghmaghi, MD, Monir Owj, MD, Batol H. Rashidi, MD, Nasrin Jalilian, MD, Maria Sadeghi, BSc.

ABSTRACT

Objective: To investigate the usefulness of inhibin B concentrations obtained on the fifth day in predicting ovarian response and assisted reproductive technologies outcome.

Methods: In this prospective multi-center study, infertile women who were candidate for in vitro fertilization or intracytoplasmic sperm injection for the first time were enrolled. These patients were referred to the Royan Institute, Vali-Asr Hospital and Alvand Hospital, Tehran, Iran between 2003 and 2004. The inclusion criteria were female age (20-35 years), body mass index (BMI) of 20-28 Kg/m^2, duration of infertility >2 years, a normal menstrual cycle and a normal day 3 follicle stimulating hormone level of <8.5 IU/l. All patients underwent long standard gonadotrophin releasing hormone agonist protocol. Plasma level of inhibin B was checked on the fifth day of menstrual cycle. The diagnostic accuracy of inhibin B, were assessed by the area under the receiver operating characteristic (ROC) curve.

Results: In this study, 107 infertile patients were studied. Using the value of 283 pg/ml for inhibin B as the cut-off point, day 5 inhibin B had 77% sensitivity, 30% specificity, 31.2% positive predictive values (PPV) and 76.7% negative predictive values (NPV) for poor ovarian response. There were statistically significant correlation among day 5 inhibin B concentration and BMI, number of mature follicles, retrieved oocytes, developed and transferred embryos, chemical pregnancy, ovarian hyperstimulation syndrome (OHSS) and poor responder.

Conclusion: Although the chemical pregnancy, number of retrieved oocytes, developed and transferred embryos were higher in patients with higher day 5 inhibin B concentration but considering its sensitivity, specifity, PPV and NPV, it cannot be used as a strong test for prediction of cancellation, pregnancy, poor responses and OHSS.

Saudi Med J 2007; Vol. 28 (7): 1028-1033

Several factors have been used to predict ovarian reserve and assisted reproductive technologies (ART) outcome in response to gonadotrophin stimulation such as measurement of day 3 follicle stimulating hormone (FSH) in the preceding normal cycles,[1-3] follicular blood flow,[4,5] ovarian volume,[6,7] pre-treatment transvaginal ultrasound of the antral follicles,[8,9] basal estradiol concentration,[10] anti-Mullerian hormone (AMH)[11] and inhibin B.[12-14] Several studies reported different factors as the best predictors. Creus et al[15] reported that the stronger predictors of success in patients undergoing their first in vitro fertilization (IVF)/or intracytoplasmic sperm injection (ICSI) treatment cycle were age and basal FSH rather than inhibin B. Basal FSH concentration was a better predictor of cancellation rate than age, but age was a stronger predictor of pregnancy rate.[15] Penarrubia et al[14] showed that inhibin B concentrations obtained early in the follicular phase during ovarian stimulation under pituitary suppression for assisted reproductive treatment were highly predictive of ovarian response.

Inhibin A and inhibin B are dimeric polypeptides produced by granulose cells. Inhibin A secreted mostly in luteal phase, while inhibin B secreted predominantly in follicular phase[16] by early healthy antral follicles. High inhibin B concentration during the early follicular phase is responsible for the

decline in FSH serum levels closing the FSH window and assuring single dominant follicle selection in human. Early follicular phase inhibin B levels decrease over time, reflecting the recruitment of a diminished cohort of follicles with ovarian aging.[17] On the other hand, the decrease in inhibin B would be the earliest marker of the decline in follicle number across reproductive ageing.[18] Seifer et al[19] reported that women with declining ovarian responsiveness and clinical outcomes in assisted reproduction treatment consistent with declining ovarian reserve had decreased day 3 serum inhibin B concentrations despite having non-elevated day 3 serum FSH concentrations. Also, Fawzy et al[12] showed that normogonadotrophic normogonadal women with day 5 inhibin B <400 pg/ml in down-regulated cycles have a poor response to ovarian stimulation and are less likely to conceive compared with women with higher day 5 inhibin B.[12] However, several studies failed to find clinical value in measuring basal inhibin B with regard to IVF outcome.[20,21]

On the above evidence, this prospective multicentric study was undertaken to investigate the usefulness of inhibin B concentrations obtained on the fifth day of gonadotrophin therapy in predicting ovarian response and assisted reproductive treatment outcome in women undergoing ovarian stimulation under pituitary desensitization.

Methods. In this prospective multicenter study, infertile women who were candidate for IVF or ICSI for the first time were enrolled. These patients were referred to the Royan Institute, Vali-Asr Hospital and Alvand Hospital, Tehran, Iran between 2003 and 2004. The first IVF/ICSI cycles were chosen to avoid possible bias from experience with previous cycles regarding ovarian response to exogenous gonadotrophin stimulation. All patients signed the consent form. This study was approved by Royan Institute Ethics committee. The inclusion criteria were female age at time of research (20-35 years), BMI=20-28 Kg/m^2, duration of infertility more than 2 years, a normal menstrual cycle and a normal day 3 FSH level[2] (<8.5 IU/l) measured in a preceding cycle. Exclusion criteria were endocrine abnormalities including hyperprolactinemia and poly cystic ovary syndrome (PCOs), severe oligospermia or azospermia and previous ovary surgery. Following screening by vaginal ultrasound on day 1 of the menstrual cycle, patients underwent long standard gonadotrophin releasing hormone (GnRH) agonist protocol.[22] All cases received oral contraceptive pills-low dose (OCP-LD) from the 5th day of previous menstrual cycle then treated from the 21st day of cycle with the GnRH agonist (Suprefact, Hoechst, Germany) 500 µg/day, subcutaneously. When pituitary suppression was achieved (on second day of menstrual cycle progestrone ≤1 ng/ml, estradiol (E2) ≤50 pg/ml), busereline was reduced to 200 µg/day and human menopausal gonadotrophin (HMG) 150 IU/day (Menopur, Ferring, Holland) was started. The dose was changed according to follicular growth. Follicular development was monitored by transvaginal sonography. When more than 3 follicles ≥18 mm were seen, human chorionic gonadotrophin (HCG) 10000 IU (Pregnyle, Organon, Germany) were injected to induce final oocyte maturation and 36-40 hour later, ovum picked up was carried out. After 2-3 days if fertilization occurred, embryo transfer was performed. If more than 15 follicles were visited in each ovary or E2 >3000 pg/ml, the patient was considered as prone to ovarian hyperstimulation syndrome (OHSS)[23] and necessary management has been taken. Plasma level of inhibin B was checked on the fifth day of menstrual cycle (Fourth day of HMG administration) by enzyme-linked immunosorbent assay (ELISA) methods (Kit Inhibin B, ABL Company, Germany). For bias prevention, this measurement was performed in a single laboratory unit. Cycle would be cancelled if the ovarian response was poor with <3 follicles at day 8 of the cycle.[12] Maturity of oocytes was based on published data[24] and mature oocytes had a generous expansion of cumulus-corona and a distinct polar body. Chemical pregnancy was defined as increasing concentration of β HCG 2 weeks after embryo transfer. Presence of intrauterine gestational sac by transvaginal ultrasound 2 weeks after positive β HCG was considered as clinical pregnancy. Poor responder was defined as number of oocytes less than 4. For each patient a questionnaire was completed, which included: dose of required gonadotropin, duration of stimulation, number of mature follicles, number of retrieved and mature oocytes, number of transferred embryos and number of canceled cycles. The Statistical Package for Social Sciences (SPSS) version 11 was used for data analysis. For statistical analysis, the Mann Whitney test and the Spearman's test were used. The diagnostic accuracy of inhibin B, described as its ability to discriminate between patients with poor or normal ovarian response and non pregnant and pregnant patients were assessed by the area under the receiver operating characteristic (ROC) curve.[12,25]

The sensitivity, specificity and PPV were calculated at the discriminating cut-off point for the prevalence of the study population. The PPV was calculated from the ROC analysis by plotting the percentage of true positives against the percentage of false positives at the best discriminating cut-off point. In summary, sensitivity was defined as true positive /true positive + false negative; specificity was defined as: true negative/ true negative + false positive. Positive and negative

predictive values (PPV and NPV) were calculated by these formulas:

PPV=true positive / false positive + true positive;
NPV=true negative / false negative + true negative.
p<0.05 was considered to be significant.

Results. In this study, 107 infertile patients referred to 3 infertility centers (Royan Institute, Vali-Asr Hospital and Alvand Hospital) were studied .The mean ± standard deviation (SD) of age and infertility duration were 29.08 ± 4.4 and 7.13 ± 4.3 years, respectively. The mean ± SD of BMI was 24.45 ± 2.4 Kg/m². The cause of infertility included: male factor (62.6%), tubal factor (19.6%), and unexplained infertility (17.8%). More than 50% studied cycles were ICSI (74.8%). The results of ART cycles were summarized in **Table 1 & 2**. Due to poor ovarian response, 12.1% of cycles were cancelled. In this study, no women were admitted to the hospital because of OHSS.

To analyze the diagnostic accuracy of inhibin B to discriminate between poor and normal ovarian response as indicated by the number of mature oocytes, area under curve$_{ROC}$ (AUC$_{ROC}$) values were determined by ROC analysis. Using the value of 283 pg/ml for inhibin B as the cut-off point, day 5 inhibin B had 77% sensitivity, 30% specificity, 31.2% PPV and 76.7% NPV for poor ovarian response in the study population (**Figure 1**). In the same cut-off point, inhibin B predicted cancellation rate with 85% sensitivity, 30% specificity, 14.3% PPV and 93.3% NPV. For OHSS, day 5 inhibin B gained 56% sensitivity, 77% specificity, 30% PPV and 90% NPV with AUC$_{ROC}$ = 0.69 (**Figure 2**).

Considering the value of 100 pg/ml for inhibin B as the cut-off point, day 5 inhibin B showed 57% sensitivity, 54% specificity, 29.6% positive predictive value and 77% negative predictive value for chemical pregnancy in the study population (**Figure 3**). There were statistically significant correlation among day 5

inhibin B concentration and BMI (*p*=0.03, correlation coefficient = -0.2), number of mature follicles (*p*=0.006, correlation coefficient = 0.26), retrieved oocytes (*p*=0.02, correlation coefficient = 0.21), developed embryos (*p*=0.03, correlation coefficient = 0.19), transferred embryos (*p*=0.02, correlation coefficient = 0.21), chemical pregnancy (*p*=0.04),OHSS (*p*=0.01) and poor responder (*p*=0.05). The mean of inhibin B concentration was 214.9 pg/ml in cancelled cycles and 255.7 pg/ml in non-cancelled cycles, which this difference was not statistically significant (*p* value >0.05). **Table 3** showed the mean of inhibin B in different groups of studied patients.

Discussion. One of the most doubtful issues in ART cycles is predicting the success rate. Pituitary FSH secretion increases with declining ovarian reserve. Day 3 serum FSH concentration is being routinely used in most assisted reproduction treatment programs.[3,26]

Theoretically, the direct products of granulosa cells might better reflect ovarian secretory capacity and follicle number. Inhibin is a heterodimeric glycoprotein produced by granulosa cells and consisting of α and β subunits.[15] With understanding the synthesis and secretion of the inhibins and their endocrine role in the regulation of FSH in human beings, attention has been focused on the possibility that these peptides may provide a more direct index of ovarian reserve and improved predictors of assisted reproduction treatment outcome. In females, the pattern of secretion of the inhibins suggests that early follicular phase inhibin B may reflect the number of follicles present at baseline, whereas inhibin α may indicate follicle maturity.[16] On this basis, recent reports have been focused based on day 5 inhibin B measurements in predicting successful outcome in assisted reproduction treatment.

This study reveals that day 5 inhibin B concentration has significant correlation with number of mature follicles, retrieved oocytes, developed and transferred

Table 1 - The results of in vitro fertilization / intracytoplasmic sperm injection cycles in studied patients.

Variable	Mean	Standard Deviation
Duration of stimulation (Day)	10	2.1
Number of required HMG	26.5	9.4
Number of Mature follicles	9.5	6.2
Number of retrieved oocytes	7.2	5.3
Number of fertilized oocytes	4.6	4
Number of formed embryos	2.2	2.6
Number of transferred embryos	2.6	1.3

HMG - human menopausal gonadotrophin

Table 2 - The outcome of in vitro fertilization / intracytoplasmic sperm injection cycles in studied patients (n=107)

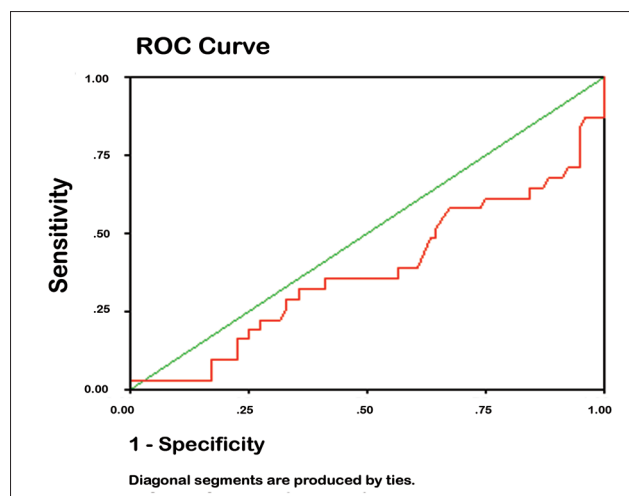| Variable	Frequency n (%)	
Chemical pregnancy	28	(26.2)
Clinical pregnancy	24	(22.4)
Cancellation rate	13	(12.1)
Ovarian hyperstimulation syndrome	16	(15)
Poor responder	31	(29)

Figure 1 - Receiver operating characteristic (ROC) curve for prediction of poor responder by day 5 inhibin B (AUC$_{ROC}$=0.3).
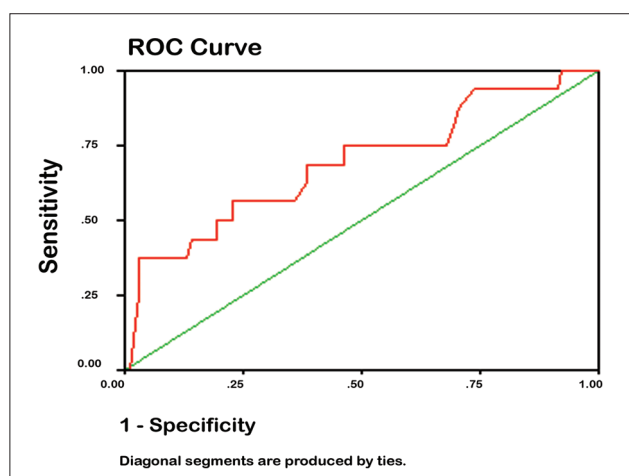

Figure 2 - Receiver operating characteristic (ROC) curve for prediction of OHSS by day 5 inhibin B (AUC$_{ROC}$ = 0.69).
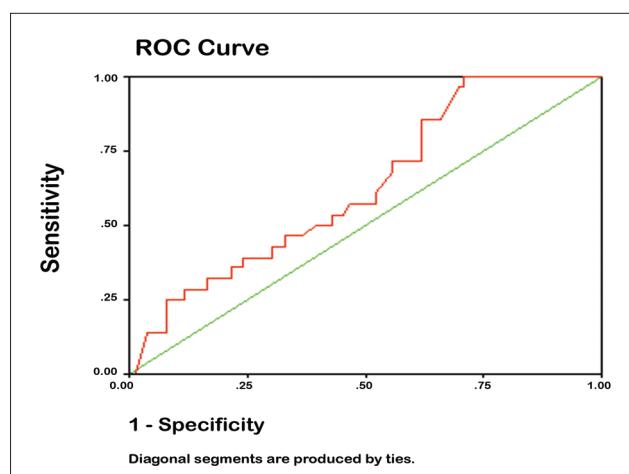

Figure 3 - Receiver operating characteristic (ROC) curve for prediction of chemical pregnancy by day 5 inhibin B (AUC$_{ROC}$ = 0.63).

Table 3 - The mean of inhibin B in different groups of studied patients

Groups	Mean of inhibin B (pg/ml)	*P* value
Cancelled cycles vs. Non-cancelled cycles	214.9 vs. 255.7	0.1
Poor responder vs. normal responder	195.8 vs. 273.3	0.05*
Ovarian hyperstimulation vs. normal cycles	471.1 vs. 213.2	0.01*
Positive vs. negative chemical Pregnancy	340.9 vs. 220.2	0.04*
Positive vs. negative clinical Pregnancy	297.9 vs. 238.4	0.2

*Significant difference, vs. - versus

embryos, chemical pregnancy, OHSS and poor responder. Ocul et al[27] demonstrated that elevated fluid follicle inhibin α and β associated with good ovarian response and high pregnancy rate in IVF cycles. Seifer et al[19] reported significantly higher cancellation rates and lower pregnancy rate in woman with low (<45pg/ml) day 3 serum inhibin concentration.

With considering limitations of recent study such as non randomized sample selection, although recent study has shown the predictive value of inhibin B for ovarian response, its prediction value was lower than Fawzy's study.[12] Fawzy et al[12] showed that the serum day 5 inhibin B had the highest predictive power to discriminate between poor and normal ovarian response (in cut off point inhibin B = 400 pg/ml). However, the cause of this variation between these studies can be the difference in number of patients (54 versus 107), stimulation protocol (we used only HMG, while they used 4 products) and ART cycles (they studied only IVF cycles while in our study more than 50% of cycles were ICSI).

In recent study, the mean of inhibin B in poor responding group (195.8 pg/ml) was significantly lower than normal responder (273.3 pg/ml). This finding was similar to Fawzy et al[12] study. Also, present study showed that a cut off point of day 5 inhibin B of 283 pg/ml for poor ovarian response gave sensitivity, specifity, PPV and NPV of 77%, 30%, 31.2% and 76.7%, respectively. Tharnprisarn et al[28] studied 60 infertile women undergoing IVF. They found the mean inhibin B level of 113.18 ± 57.96 pg/ml in the poor responder group (n=20) and 94.05 ± 61.81 pg/ml in normal responder (n=40), which was not statistically significant. In comparison with the recent study, the number of patient were lower (60 patients in Tharnprisarn et al[28]

study versus 107 patients in present study). Present study shows inhibin B concentration was lower in cancelled cycles, but this difference was not statistically significant. Penrrubia et al[14] studied 80 women and reported that a cut off point of day 5 inhibin B of 141 pg/ml gave sensitivity of 94% and specifity of 81% for cancellation cycles. In comparison with their study, a sensitivity of 85% and specifity of 30% for cancellation rate in cut off point of 283 pg/ml were calculated in present study. The probable reason for this difference between these studies can be their high cancellation rate (25% in comparison with 12.1% in present study). The present study showed that the mean of day 5 inhibin B was statistically higher in patients prone to OHSS. In agreement with this finding, Enskog et al[29] reported that the elevation of serum inhibin B 3 days prior to oocyte retrieval was greater in patients who later developed OHSS.[29] Also, Fawzy et al[12] suggested day 5 inhibin B measurements may be a useful tool in predicting and monitoring OHSS.[12]

In conclusion, although in present study, the chemical pregnancy, number of retrieved oocytes, developed and transferred embryos were higher in patients with higher day 5 inhibin B concentration but considering its sensitivity, specifity, PPV and NPV, it cannot be used as a strong test for prediction of cancellation, pregnancy, poor responses and OHSS.

References

1. Cahill DJ, Prosser CJ, Wardle PG, Ford WC, Hull MG. Relative influence of serum follicle stimulating hormone, age and other factors on ovarian response to gonadotrophin stimulation. *Br J Obstet Gynaecol* 1994; 101: 999–1002.
2. Fawzy M, Harrison RF, Barrett T, Mallon E. Stratification improves the prognostic capability of normal range day 3 FSH for IVF. *Adv Contracept Deliv Syst* 1997; 13: 278–289.
3. Scott RT, Hofmann GE. Prognostic assessment of ovarian reserve. *Fertil Steril* 1995; 63: 1–11.
4. Nargund G, Bourne T, Doyle P, Parsons J, Cheng W, Campbell S, et al. Associations between ultrasound indices of follicular blood flow, oocyte recovery and preimplantation embryo quality. *Hum Reprod* 1996; 11: 109–113.
5. Van Blerkom J, Antczak M, Schrader R. The developmental potential of the human oocyte is related to the dissolved oxygen content of follicular fluid: association with vascular endothelial growth factor levels and perifollicular blood flow characteristics. *Hum Reprod* 1997; 12: 1047–1055.
6. Syrop CH, Dawson JD, Husman KJ, Sparks AE, Van Voorhis BJ. Ovarian volume may predict assisted reproductive outcomes better than follicle stimulating hormone concentration on day 3. *Hum Reprod* 1999; 14: 1752–1756.
7. Lass A, Skull J, McVeigh E, Margara R, Winston RM. Measurement of ovarian volume by transvaginal sonography before ovulation induction with human menopausal gonadotrophin for in-vitro fertilization can predict poor response. *Hum Reprod* 1997; 12: 294–297.
8. Tomás C, Nuojua-Huttunen S, Martikainen H. Pretreatment transvaginal ultrasound examination predicts ovarian responsiveness to gonadotrophins in in-vitro fertilization. *Hum Reprod* 1997; 12: 220–223.
9. Chang MY, Chiang CH, Hsieh TT, Hsieh TT, Soong YK, Hsu KH. Use of antral follicle count to predict the outcome of assisted reproductive technologies. *Fertil Steril* 1998; 69: 505–510.
10. Licciardi FL, Liu HC, Rosenwaks Z. Day 3 oestradiol serum concentration as prognostic data of ovarian stimulation response and pregnancy outcome in patients undergoing in vitro fertilization. *Fertil Steril* 1995; 64: 991–994.
11. Muttukrishna S, Suharjono H, McGarrigle H, Sathanandan M. Inhibin B and anti-Mullerian hormone: markers of ovarian response in IVF/ICSI patients? *BJOG* 2004; 111: 1248-1253.
12. Fawzy M, Lambert A, Harrison RF, Knight PG, Groome N, Hennely B, et al. Day 5 inhibin B levels in a treatment cycle are predictive of IVF outcome. *Hum Reprod* 2002; 17: 1535-1543.
13. Ravhon A, Lavery S, Michael S, Donaldson M, Margara R, Trew G, et al. Dynamic assays of inhibin B and oestradiol following buserelin acetate administration as predictors of ovarian response in IVF. *Hum Reprod* 2000; 15: 2297–2301.
14. Peñarrubia J, Balasch J, Fábregues F, Carmona F, Casamitjana R, Moreno V, et al. Day 5 inhibin B serum concentrations as predictors of assisted reproductive technology outcome in cycles stimulated with gonadotrophin-releasing hormone agonist–gonadotrophin treatment. *Hum Reprod* 2000; 15: 1499-1504.
15. Creus M, Peñarrubia J, Fábregues F, Vidal E, Carmona F, Casamitjana R, et al. Day 3 serum inhibin B and FSH and age as predictors of assisted reproduction treatment outcome. *Hum Reprod* 2000; 15: 2341–2346.
16. Groome NP, Illingworth PJ, O'Brien M, Pai R, Rodger FE, Mather JP, et al. Measurement of dimeric inhibin B throughout the human menstrual cycle. *J Clin Endocrinol Metab* 1996; 81: 1401–1405.
17. Laven JS, Fauser BC. Inhibins and adult ovarian function. *Mol Cell Endocrinol* 2004; 225: 37-44.
18. Welt CK, McNicholl DJ, Taylor AE, Hall JE. Female reproductive aging is marked by decreased secretion of dimeric inhibin. *J Clin Endocrinol Metab* 1999; 84: 105–111.
19. Seifer DB, Scott RT Jr, Bergh PA, Abrogast LK, Friedman CI, Mack CK, et al. Women with declining ovarian reserve may demonstrate a decrease in day 3 serum inhibin B before a rise in day 3 follicle-stimulating hormone. *Fertil Steril* 1999; 72: 63–65.
20. Corson SL, Gutmann J, Batzer FR, Wallace H, Klein N, Soules MR. Inhibin-B as a test of ovarian reserve for infertile women. *Hum Reprod* 1999; 14: 2818–2821.
21. Hall JE, Welland CK, Cramer DW. Inhibin A and inhibin B reflect ovarian function in assisted reproduction but are less useful at predicting outcome. *Hum Reprod* 1999; 14: 409–415.
22. Kondaveeti-Gordon U, Harrison RF, Barry-Kinsella C, Gordon AC, Drudy L, Cottell E. A randomised prospective study of early follicular or midluteal initiation of long protocol gonadotropin-releasing hormone in an in vitro fertilisation program. *Fertil Steril* 1996; 66: 582–586.

23. Golan A, Ron-El R, Herman A, Soffer Y, Weinraub Z, Caspi E. Ovarian hyperstimulation syndrome: an update review. ***Obstet Gynecol Surv*** 1989; 44: 430–440.

24. Veeck LL, Wortham JWE Jr, Witmyer J, Sandow BA, Acosta AA, Garcia JE, et al. Maturation and fertilization of morphologically immature human oocytes in a program of in vitro fertilization. ***Fertil Steril*** 1983; 39: 594–602.

25. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. ***Clin Chem*** 1993; 39: 561–577.

26. Ashrafi M, Madani T, Seirafi Tehranian A, Malekzadeh F. Follicle stimulating hormone as a predictor of ovarian response in women undergoing controlled ovarian hyperstimulation for IVF. ***Int J Gynaecol Obstet*** 2005; 91: 53-57.

27. Ocal P, Aydin S, Cepni I, Idil S, Idil M, Uzun H, et al. Follicular fluid concentrations of vascular endothelial growth factor, inhibin A and inhibin B in IVF cycles: are they markers for ovarian response and pregnancy outcome? ***Eur J Obstet Gynecol Reprod Biol*** 2004; 115: 194-199.

28. Tharnprisarn W, Leepipatpaiboon S, Boonkasemsanti W, Virutamasen P. Day 3 serum inhibin B level of poor and good ovarian responders in the IVF-program. ***J Med Assoc Thai*** 2002; 85: 612-616.

29. Enskog A, Nilsson L, Brannström M. Peripheral blood concentrations of inhibin B are elevated during gonadotrophin stimulation in patients who later develop ovarian OHSS and inhibin A concentrations are elevated after OHSS onset. ***Hum Reprod*** 2000; 15: 532–538.

Related topics

Alsunaidi M. Incidence of ectopic pregnancy after assisted reproduction treatment. ***Saudi Med J*** 2005; 26: 1107-1111.

Moini A, Zafarani F, Haddadian S, Ahmadi J, Honar H, Riazi K. Effect of low-dose aspirin therapy on implantation rate in women undergoing in-vitro fertilization cycles. ***Saudi Med J*** 2007; 28: 732-736.

Gafar AH, Abotalib ZM. Coasting. A viable option for patients at risk of ovarian hyperstimulation syndrome. ***Saudi Med J*** 2002; 23: 1398-1401.