Comparison of the predictive value of progesterone-related indicators for pregnancy outcomes of women undergoing the short-acting GnRH agonist long protocol: a retrospective study

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Research

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Abstract

Background

There are many progesterone (P) elevation-related indicators for predicting pregnancy outcomes, including serum P, P-to-oestradiol ratio (P/E_2), P-to-follicle index (PFI), and P-to-mature oocyte index (PMOI); however, due to inconsistencies in study populations and controlled ovarian hyperstimulation (COH) protocols among studies, these indicators are controversial. Moreover, no researchers have included these four commonly used indicators in one study to compare their predictive efficacies. The objective of this study was to compare the predictive value of P-related indicators for pregnancy outcome of women undergoing the short-acting GnRH agonist long protocol.

Methods

A total of 612 infertile women undergoing IVF/ICSI were recruited for this study. Serum samples were obtained on the morning of HCG injection for serum P and E_2 measurements. Transvaginal ultrasound was performed to determine the follicle count (≥ 14 mm). The number of mature oocytes was observed in the embryo laboratory after oocyte retrieval.

Results

In cases of $P < 2.5$ ng/ml, there was no significant difference in the serum P level or P/E_2 between the pregnant group and the non-pregnant group. The PFI and PMOI of the pregnant group were significantly lower than those of the non-pregnant group. According to the stratified analysis of the ovarian response, only the PMI and PMOI of the pregnant women in the normal ovarian response group were lower than those of the non-pregnant women. In order to compare the predictive value of the PFI and PMOI in IVF/ICSI outcomes, patients were divided into four groups. The good-quality embryo rate and clinical pregnancy rate were highest in Group A (low PFI and low PMOI) and lowest in Group D (high PFI and high PMOI). In the two groups with discordant PFI and PMOI, namely, Group B (low PFI and high PMOI) and Group C (high PFI and low PMOI), the good-quality embryo rate and clinical pregnancy rate were not significantly different.

Conclusions

PFI and PMOI had equal value in predicting clinical pregnancy outcomes in the normal ovarian response group undergoing the short-acting GnRH agonist long protocol. Each clinical centre can choose one of the indicators according to their actual situation in clinical practice.

Background

During the process of controlled ovarian hyperstimulation (COH) using gonadotropins (Gn), serum progesterone (P) elevation is sometimes observed in the late follicular phase and on the day of human chorionic gonadotropin (HCG) administration. The frequency of serum P elevation varies according to the stimulation protocol. Studies have shown that the incidence of P elevation is 35% for the pituitary down-regulation protocol using gonadotropin-releasing hormone (GnRH) agonist and 38% for the GnRH antagonist protocol[1]. There are many possible mechanisms of P elevation, including the number and sizes of multiple follicles, the high dose of exogenous Gn, proliferation of granulosa cells and the increased activity of FSH-stimulated granulosa cells and LH-stimulated theca cells[2]. Excessive levels of progesterone may affect embryo quality and endometrial receptivity, reduce the embryo implantation rate, and thus reduce the clinical pregnancy rate. Some studies have reported a negative impact on pregnancy outcomes when serum P levels are increased, whereas others could not find any association between P levels and pregnancy rates[2]. There are many P elevation-related indicators for predicting pregnancy outcomes, including serum P, P-to-oestradiol ratio (P/E_2), P-to-follicle (≥ 14 mm) index (PFI), and P-to-mature oocyte index (PMOI);however, due to inconsistencies in study populations and COH protocols among studies, these indicators are controversial. Moreover, no researchers have included these four commonly used indicators in one study to compare their predictive efficacies. The objective of this study was to compare the predictive value of P-related indicators for pregnancy outcome of women undergoing the short-acting GnRH agonist long protocol.

Methods

Patients

In this retrospective study, we analyzed the clinical datas of 612 infertile women undergoing the short-acting GnRH agonist long protocol and fresh embryo transplantation from January 2018 to December 2018 at the Reproductive and Genetic Medical Center of Peking University First Hospital. Women with a history of endometrial lesions, endocrine diseases, including diabetes mellitus, thyroid disease, and hyperprolactinaemia were excluded from this study. This study was approved by the Clinical Research Institutional Review Board of Peking University First Hospital.

COH protocols

During the study period, all subjects were subjected to the short-acting GnRH agonist long protocol. Follicular development was regularly monitored by transvaginal ultrasound. Recombinant HCG was administered subcutaneously when the leading follicle was ≥ 18 mm in diameter. Serum samples were obtained on the morning of HCG injection for serum P and E_2 measurements. Transvaginal ultrasound was performed to determine the follicle count (≥ 14 mm). Oocytes were retrieved by transvaginal ultrasound-guided follicular aspiration within approximately 36 hours after HCG administration.The number of mature oocytes was observed in the embryo laboratory after oocyte retrieval. Luteal support was started on the day of oocyte retrieval. Oocytes were fertilized by conventional in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI), and embryos were transferred under abdominal ultrasound

guidance on day 3 after oocyte retrieval. The transplantation was cancelled for the following reasons: (1)to prevent the occurrence of ovarian hyperstimulation syndrome (OHSS); (2) when no transplantable embryos were obtained; or (3) to accumulate embryos; or (4) when P levels were > 2.5 ng/ml on HCG day. HCG tests were performed on day 14 after embryo transfer (ET), and if the result was positive, luteal support was continued. Transvaginal ultrasound was performed on day 28 after ET, and clinical pregnancy was defined as the presence of an intrauterine gestational sac or embryonic heartbeat.

The following criteria were used to define the ovarian response according to oocyte yield[3]: poor ovarian response (POR), oocyte yield < 4; normal ovarian response, oocyte yield ≥ 4 and ≤ 15; and high ovarian response, oocyte yield > 15.

The P-related indicators were P (ng/ml), P/E_2 [P (ng/ml) × 1000/E_2 (pg/ml)], the PFI, and the PMOI.

Statistical analysis

All analyses were performed using the Software Package for Social Sciences (SPSS) version 13.0 for Windows. All normally distributed measurement data are expressed as the means ± standard deviation (SD). Comparisons between two groups were analysed using independent sample t-tests; comparisons among multiple samples were analysed using variance analysis; and intergroup multiple comparisons were analysed with the Bonferroni correction. $P < 0.05$ was considered statistically significant. A ROC curve analysis was performed to compare the predictive values of the progesterone-related indicators for pregnancy outcome. The highest value of the AUC was determined.

Results

A total of 612 patients were enrolled in the study, and 274 women (44.77%) had a clinical pregnancy. As shown in Table 1, there was no significant difference in age, infertility period or basal FSH between the pregnant and non-pregnant groups. The antral follicle count(AFC) of the pregnant group was significantly higher than that of the non-pregnant group.

Among the 612 infertility patients included in the study, there was no significant difference in serum P level or P/E_2 between the pregnant and non-pregnant groups. The PFI and PMOI of the pregnant group were significantly lower than those of the non-pregnant group (P = 0.002, 0.002). The results are shown in Table 1.

Table 1
Comparison of clinical data of patients in different groups

Parameters	Pregnant group (n = 274)	Non-pregnant group (n = 338)	P value
Age (years)	32.22 ± 4.03	32.61 ± 4.66	0.266
Infertility period(years)	3.33 ± 2.53	3.22 ± 2.32	0.570
Basal FSH (mIU/mL)	7.34 ± 1.82	7.63 ± 2.21	0.085
No. of AFCs (n)	12.74 ± 5.73	11.48 ± 5.41	0.006*
P on HCG day (ng/ml)	1.06 ± 0.45	1.02 ± 0.47	0.683
P/E_2	0.40 ± 0.26	0.43 ± 0.25	0.141
PFI	0.13 ± 0.08	0.15 ± 0.10	0.002*
PMOI	0.17 ± 0.12	0.21 ± 0.18	0.002*
* Indicates a significant difference ($P < 0.05$).			

According to the stratified analysis of the ovarian response, amongthe poor ovarian response and high ovarian response groups, there was no significant difference in any P-related indicator between the pregnant and non-pregnant groups. In the normal ovarian response group, there was no significant difference in serum P level or P/E_2 between the pregnant and non-pregnant groups. The PFI and PMOI of the pregnant group (0.13 ± 0.07, 0.17 ± 0.11) were significantly lower than those of the non-pregnant group (0.14 ± 0.09, 0.20 ± 0.15) (P = 0.013, 0.010). The results are shown in Fig. 1.

In the normal ovarian response group, according to the receiver operating characteristic (ROC) analysis, the PFI and PMOI had some value in predicting clinical pregnancy outcomes, but the predictive efficacy was poor. The area under the curve (AUCs) of the PFI and PMOI were 0.554 (95% confidence interval [CI]: 0.504–0.603)and 0.560 (95% CI: 0.511–0.609). The optimal cut-off value for the PFI and PMOI were 0.17 and 0.18. The results are shown in Fig. 2.

To compare the predictive value of the PFI and PMOI in IVF/ICSI outcomes, we studied cases with PFI < 0.17 and PMOI < 0.18 (Group A), cases with PFI < 0.17 and PMOI ≥ 0.18 (Group B), cases with PFI ≥ 0.17 and PMOI < 0.18 (Group C) and cases with PFI ≥ 0.17 and PMOI ≥ 0.18 (Group D). As shown in Table 2, the good-quality embryo rate and clinical pregnancy rate were highest in Group A and lowest in Group D. In the two groups with discordant PFI and PMOI, namely, Group B (low PFI and high PMOI) and Group C (high PFIand low PMOI), the good-quality embryo rate and clinical pregnancy rate were not significantly different.

Table 2
Pregnancy outcomes of patients according to the PFI and PMOI levels

Parameters	Group A PFI < 0.17 and PMOI < 0.18 (n = 273)	Group B PFI < 0.17 and PMOI ≥ 0.18 (n = 98)	Group C PFI ≥ 0.17 and PMOI < 0.18 (n = 42)	Group D PFI ≥ 0.17 and PMOI ≥ 0.18 (n = 110)	p value
Good-quality embryo rate (%)	43.55% (885/2032)	34.77%▲ (169/486)	36.48%▲ (116/318)	34.02% (180/529	< 0.001
Clinical pregnancy rate (%)	52.38% (143/273)	40.82%▲(40/98)	50.00%▲ (21/42)	31.82% (35/110)	0.002

▲ There were no statistically significant differences between Group B and Group C(p = 0.651;0.355).

Discussion

During COH for treatment by assisted reproductive technology, oestrogen levels are far above normal physiological levels due to the development of multiple follicles. Some patients may also have elevated serum progesterone levels in the late follicular phase. Excessive progesterone can affect the embryo implantation rate and clinical pregnancy rate[4, 5]. This is mainly because excessive progesterone causes asynchronization between the endometrium and embryo development[6]. Therefore, if the progesterone level is too high in the late follicular phase, cancellation of the fresh embryo transplantation cycle and freezing of the embryos are recommended[7]. Compared with that of the fresh embryo cycle, the pregnancy rate of the frozen embryo transfer cycle is higher[8]. Recent studies have shown that excessive progesterone can also affect embryo quality. Vannie et al. studied 986 GnRH antagonist IVF/ICSI cycles, and the results demonstrated that a high-quality blastocyst rate was negatively correlated with high progesterone levels[9]. In cases of high progesterone levels, the “freeze-all" strategy is not the best way to solve the problem because the embryo utilization rate is significantly reduced[10]. However, a previous meta-analysis showed that elevated progesterone levels on the HCG day were not associated with pregnancy rates[11].

There are many progesterone elevation-related indicators for predicting pregnancy outcomes. The simplest indicator is serum P level on the day of HCG injection. Li Rong et al. proposed that serum P levels on the day of HCG injection can predict IVF pregnancy outcomes, but the predictive efficacy is poor (AUC = 0.599). For patients with P levels exceeding 6.0 nmol/L, cancellation of fresh cycle transplantation and freezing ofembryos for frozen embryo transfer with natural cycles are recommended[12]. It is well known that serum P levels during COH are associated with the ovarian response and increase in follicle count and oestradiol levels[13]. Therefore, P/E_2 may be more effective for predicting pregnancy outcome than serum P alone[14, 15], but its predictive efficacy is still poor[14, 16]. Shufaro et al. studied women with a normal ovarian response undergoing GnRH agonist and GnRH antagonist protocols, and the

results demonstrated that the PFI can better predict the clinical pregnancy rate than serum P[17]. Matheus et al. performed a stratified analysis of patient age during treatment with the GnRH antagonist protocol and found that PFI with a cut-off value of 0.075 is a good predictor for IVF outcome among patients of all ages[18]. Cynthia et al. found that the PMOI was negatively correlated with the clinical pregnancy rate, live birth rate, and implantation rate. The PMOI could predict the live birth rate, while serum P had no predictive value[19]. The researchers also believe that the PFI is affected by the ultrasound equipment and the experience of the examiner and that the PMOI is more reproducible. In addition, studies have indicated that for patients with a PMOI exceeding 0.32, fresh cycle transplantation is recommended[20].

Although there are many studies on the predictive value of P-related indicators for IVF/ICSI pregnancy outcomes, no researchers have included these four commonly used indicators into one study to compare their predictive efficacies. The results of this study showed that there was no significant difference in serum P or P/E_2 between the pregnant group and the non-pregnant group in cases where P was < 2.5 ng/ml. The PFI and PMOI of the pregnant group were significantly lower than those of the non-pregnant group. According to the stratified analysis of the ovarian response, the results showed that only in the normal ovarian response group were the PMI and PMOI of the pregnant group lower than those of the non-pregnant group, and the differences were statistically significant. This may be because factors affecting pregnancy outcomes are more complicated among patients with a poor ovarian response or high ovarian response, including the number of retrieved oocytes and higher levels of oestrogen such that the effect of elevated P levels on pregnancy outcomes is relatively small. In this study, ROC analysis was performed to determine the predictive value of these indicators. The results showed that in cases of progesterone < 2.5 ng/ml, the PFI and PMOI had some value in predicting clinical pregnancy outcomes in the normal ovarian response group, but their predictive efficacies were poor. Compared with the PFI and PMOI, the prediction efficiency was approximately equal. Therefore, in the process of clinical practice, each clinical centre can choose one of the indicators according to their actual situation. For example, some centres do not always know the number of follicles (≥ 14 mm) on HCG day; in these cases, the PMOI can be used.

We speculate that there is a threshold for P levels and that below this threshold, the P level does not affect embryo quality, endometrial receptivity, or the clinical pregnancy rate. Different studies have different definitions of thresholds for excessive P levels[11], and our reproductive centre recommends cancelling fresh cycle transplantation when P exceeds 2.5 ng/ml[12]. Therefore, this study was conducted mainly to compare the predictive value of P-related indicators for pregnancy outcome of women undergoing the short-acting GnRH agonist long protocol when P is < 2.5 ng/ml.

The limitation of this study is that we cannot explore the predictive value of P-related indicators for pregnancy outcome when P exceeds 2.5 ng/ml due to ethical issues. Instead, we can use animal models to further explore the highest threshold for P that does not affect pregnancy outcomes.

Conclusions

In conclusion, in cases of P < 2.5 ng/ml,the PFI and PMOI had equal value in predicting clinical pregnancy outcomes in the normal ovarian response groupundergoing the short-acting GnRH agonist long protocol. Each clinical centre can choose one of the indicators according to their actual situation in clinical practice.

Conclusion

AFC, antral follicle count

AUC, area under the curve

COH, controlled ovarian hyperstimulation

Gn, gonadotropins

GnRH, gonadotropin-releasing hormone

HCG, human chorionic gonadotropin

OHSS, ovarian hyperstimulation syndrome

P, progesterone

P/E_2, P-to-oestradiol ratio

PFI, P-to-follicle index

PMOI, P-to-mature oocyte index

ROC, receiver operating characteristic

POR, poor ovarian response

SD, standard deviation

SPSS, Software Package for Social Sciences

Declarations

Ethics approval and consent to participate

This study was approved by the Clinical Research Institutional Review Board of Peking University First Hospital.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable.

Authors' contributions

YZ and YX conceived and coordinated the study, designed and analysed the experiments, and wrote the paper. QX, JS, XY and YW carried out the data collection and data analysis and revised the paper. All authors read and approved the final manuscript.

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Figures

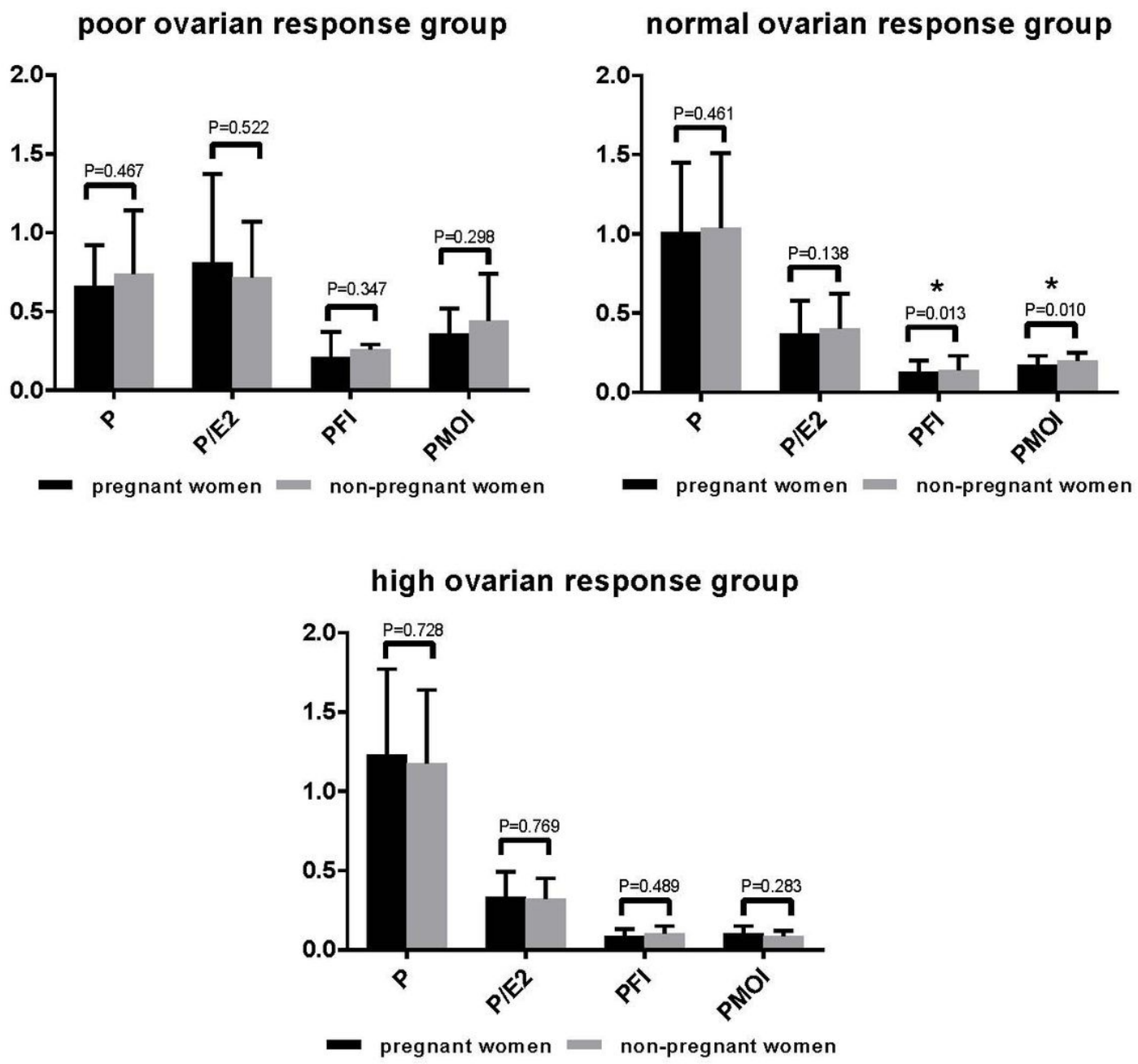

Figure 1

Progesterone-related indicators and pregnancy outcomes of women with different ovarian responses. Serum P levels increased with the number of oocytes retrieved, was lowest among women with a poor ovarian response and was highest among women with a high ovarian response. In the normal ovarian response group, there was no significant difference in serum P or P/E2 between the pregnant and non-pregnant groups. The PFI and PMOI of the pregnant group were significantly lower than those of the non-pregnant group.

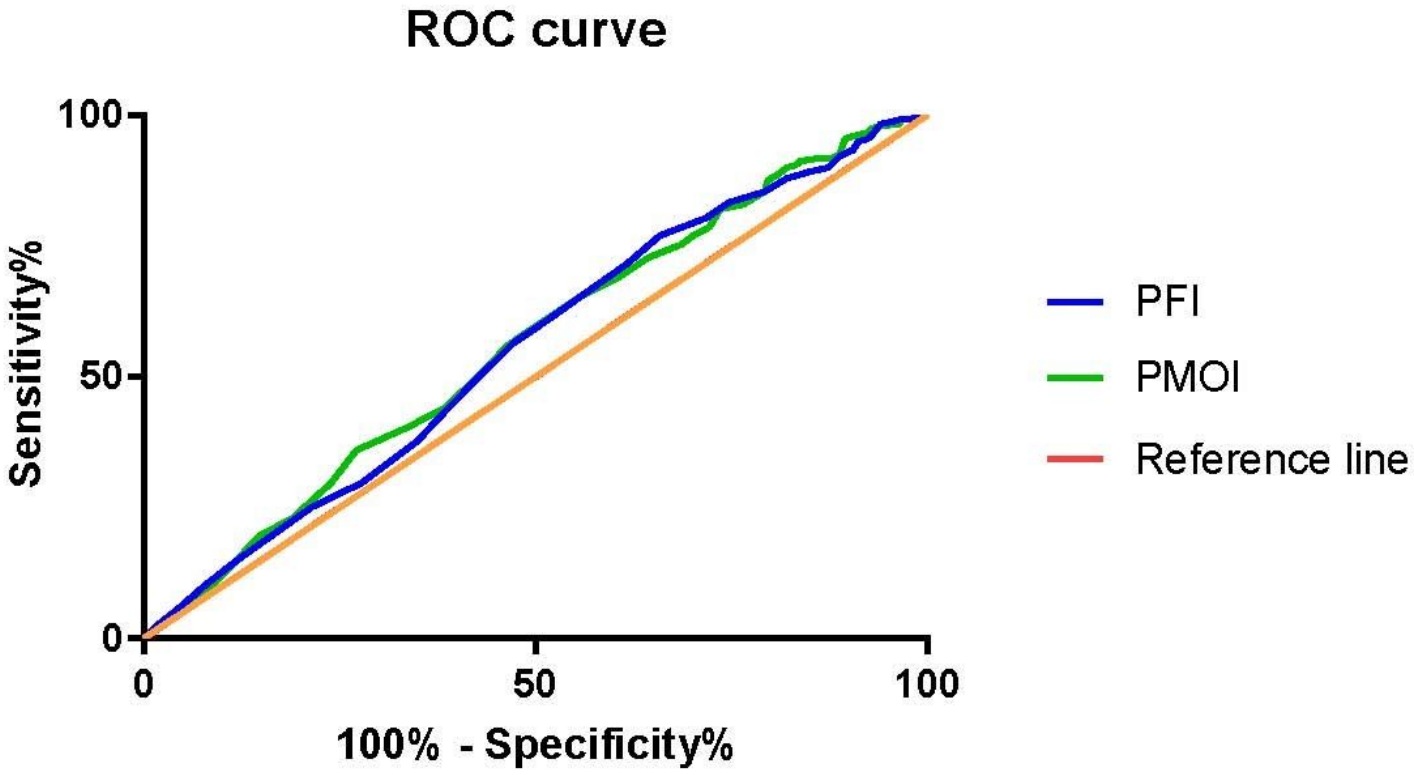

Figure 2

Predictive efficacies of progesterone-related indicators for pregnancy outcomes among women with normal ovarian response.