ORIGINAL RESEARCH ARTICLE

Route of administration of exogenous progesterone for luteal support does not significantly affect the serum concentration in assisted reproductive technology

DOI: 10.29063/ajrh2021/v25i6.14

Yangyang Zhang, Yang Xu, Jing Shang, Fei Chen, Yanrong Kuai, Sheng Wang*

Department of Obstetrics & Gynecology, Peking University First Hospital, Beijing, China

***For Correspondence:** Email: *xuyangbdyy@163.com*; Phone: +86-13501356366

Abstract

The objective of this research is to study the guiding role of serum progesterone level on exogenous luteal support protocols. In the retrospective study, a total of 537 infertile women undergoing IVF/ICSI were recruited. Serum samples were obtained for serum progesterone measurements. The results demonstrated that the progesterone levels of all women gradually decreased over the course of 7 days after ET. The progesterone level of the pregnant women reached a nadir on day 7 after ET and subsequently began to rise, while the progesterone level of the non-pregnant women continued to decrease. Even with different routes of administration of exogenous progesterone, the progesterone levels followed the same patterns. The serum progesterone level does not represent the adequacy of exogenous progesterone supplementation. Therefore, there is no need to measure serum progesterone levels frequently after embryo transfer or adjust the dose according to serum progesterone levels. *(Afr J Reprod Health 2021; 25[6]: 134-142).*

Keywords: Progesterone level, luteal support, pregnancy outcome, assisted reproductive technology, infertility

Résumé

L'objectif de cette recherche est d'étudier le rôle directeur du taux de progestérone sérique sur les protocoles de soutien lutéal exogène. Dans l'étude rétrospective, un total de 537 femmes infertiles subissant une FIV/ICSI ont été recrutées. Des échantillons de sérum ont été obtenus pour les mesures de progestérone sérique. Les résultats ont démontré que les niveaux de progestérone de toutes les femmes diminuaient progressivement au cours des 7 jours suivant la TE. Le niveau de progestérone des femmes enceintes a atteint un nadir au jour 7 après la TE et a ensuite commencé à augmenter, tandis que le niveau de progestérone des femmes non enceintes a continué à diminuer. Même avec différentes voies d'administration de progestérone exogène, les niveaux de progestérone ont suivi les mêmes schémas. Le niveau de progestérone sérique ne représente pas l'adéquation de la supplémentation en progestérone exogène. Par conséquent, il n'est pas nécessaire de mesurer fréquemment les taux sériques de progestérone après le transfert d'embryons ou d'ajuster la dose en fonction des taux sériques de progestérone. *(Afr J Reprod Health 2021; 25[6]: 134-142).*

Mots-clés: Taux de progestérone, soutien lutéal, issue de la grossesse, techniques de procréation assistée, infertilité

Introduction

The corpus luteum (CL), derived from the ovulated follicle, is a temporary endocrine gland that is rich in blood vessels[1]. The main function of the CL is to produce steroid hormones, namely, progesterone and androgen, the latter of which is converted to oestrogen via the aromatase actions of the CL granule cells. If the oocyte is fertilized and implantation occurs, the CL continues to grow into corpus luteum graviditatis in the presence of human chorionic gonadotropin (HCG) from embryonic trophoblast cells. After 7 to 9 weeks of gestation, the main source of progesterone shifts from the CL to the placenta, which is known as the luteal-placental shift, and then, the CL gradually regresses. Studies demonstrate that the surgical removal of the CL before 7 weeks of gestation can lead to an abrupt decrease in serum progesterone levels followed by miscarriage; however, exogenous progesterone replacement after the removal of the CL maintains pregnancy[2]. The above study suggests that progesterone is an essential hormone for maintaining pregnancy. In early pregnancy, progesterone is responsible for preparing the endometrium for the implantation

process and maintaining the gestational sac in the uterus[3].

A growing number of women are seeking assisted reproductive technology (ART) to have a baby. Studies demonstrate that patients undergoing in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) suffer from luteal phase deficiency, which manifests as reduced serum progesterone levels, implantation rates and early pregnancy rates[4], due to the use of GnRH agonist (GnRH-a) and GnRH antagonist (GnRH-A) protocols in controlled ovarian hyperstimulation (COH). Therefore, exogenous luteal support has become a routine procedure in IVF/ICSI to improve CL function and the pregnancy outcome[5]. Data from a recent meta-analysis support the above conclusion[6]. There are many kinds of luteal support protocols, but there is no consensus on the optimal protocol regarding the types, doses and routes of drug administration[7,8]. The drugs for luteal support include progesterone, HCG, oestrogen and GnRH-a, among others. For luteal support, most IVF centres worldwide have mainly used progesterone[9]. Common routes of progesterone administration include intramuscular injection (IM), vaginal administration and oral administration.

Although there are recommendations about the drug types and doses of progesterone in CL support and a progesterone supplement consensus, the protocols are not the same between different reproductive centres. All of the luteal support protocols are designed so that the drug dose will be sufficient and so that there will be no need to measure serum progesterone levels frequently after embryo transfer (ET); however, there is no relevant study to support these outcomes. Therefore, clinicians in some reproductive centres have repeatedly increased the progesterone dose in pursuit of higher clinical pregnancy rates and have even adjusted the dose according to serum progesterone levels after ET. Repeated blood draws increase the psychological and physical burden to patients. Furthermore, administration of a high dose of progesterone increases the economic burden on patients and increases the occurrence of adverse events, such as liver dysfunction, venous thrombosis and local sterile abscesses[10]. The objective of this research is to study the guiding role of serum progesterone level on exogenous luteal support protocols and the predictive value of serum progesterone levels at different times in pregnant outcome by analyzing the variation curve of serum mean P level after ET.

Methods

Patients

In the retrospective study, a total of 537 infertile women undergoing IVF /ICSI were involved in this study from January to December 2012 at the Reproductive and Genetic Medical Center of Peking University First Hospital. Women with a history of ovarian surgery, polycystic ovarian syndrome (PCOS) or other endocrine diseases (e.g., diabetes mellitus, thyroid disease) were excluded from this study.

COH protocols and luteal support protocols

During the study period, each patient was subjected to an individualized COH protocol according to specific characteristics, such as age, hormone level, and antral follicle count (AFC), and the different protocols included the long GnRH-a protocol (443 women), ultralong GnRH-a protocol (64 women), GnRH-A protocol (22 women) and microstimulation protocol (8 women). Gonadotropin therapy was performed, and follicles were monitored by transvaginal ultrasound regularly. Recombinant HCG (Ovitrelle, 250 μg, Merck Serono, Germany) was administered subcutaneously when the leading follicle was 18-20 mm in diameter. Oocytes were retrieved by transvaginal ultrasound-guided follicular aspiration within approximately 36 hours after HCG administration. Luteal support was started on the day of oocyte retrieval. According to personal preference, a total of 159 women received vaginal progesterone (vaginal P_4) preparations (Crinone, 90 mg per day, Merck Serono, Germany), and 378 women received IM-P_4 (Progesterone Injection, 40 mg per day, Xianju Pharma, China) plus oral dydrogesterone (OR-P_4) (Duphaston, 10 mg twice per day, Abbott Biologicals, Netherlands). Oocytes were fertilized by conventional IVF/ICSI, and embryos were transferred under abdominal ultrasound guidance on day 3 after oocyte retrieval. HCG tests were performed on day 14 after ET, and

if the result was positive, luteal support was continued as before until 10 weeks of gestation; otherwise, luteal support was discontinued. Clinical pregnancy was defined as the presence of an intrauterine gestational sac 4 weeks after ET.

Measurement of the serum progesterone level

Serum was sampled on the day of oocyte retrieval and on day 0, day 4, day 7 and day 14 after ET. Serum progesterone levels were measured by chemiluminescent immunoassay (Access Progesterone assay, Unicel DxI800 Access Immunoassay System, Beckman Coulter, U.S.A.). The analytical sensitivity of the assay was 0.1 ng/ml. The analytical performance characteristics had a coefficient of variation that was 8% and 10% for intra-and interassay precision, respectively.

Statistical analysis

All analyses were performed with SPSS 10.0 for windows. All data are expressed as the mean ± SD. Comparisons between two groups were performed with the independent sample T-test. $P<0.05$ was considered to be statistically significant. A receiver operating characteristic (ROC) curve analysis was performed to determine the most efficient predictive values of the progesterone level at different times that could be used as good predictors of successful implantation and clinical pregnancy. The highest value of the area under the curve (AUC) was determined.

Results

Patient characteristics

A total of 537 patients were enrolled in the study, and in this study, 292 women (54.38%) had a clinical pregnancy. The patients and cycle characteristics are shown in Table 1.

Variation curves of the serum mean P levels

(1) Variation curve of the serum mean progesterone level (all women)

The serum progesterone levels of all women were found to rise during the first few days after oocyte retrieval (peak level on day 3 after oocyte retrieval, that is, on the day of ET). Progesterone levels gradually decreased over the course of 7 days, reached a nadir on day 7 after ET, and subsequently began to rise in pregnant women while continuing to decrease in non-pregnant women. The results are shown in Figure 1. The serum progesterone levels of all women gradually decreased over the course of 7 days after ET. The progesterone level of the pregnant women reached a nadir on day 7 after ET and subsequently began to rise, while the progesterone level of the non-pregnant women continued to decrease.

Table 1: Demographics and baseline characteristics (entire sample)

Parameter	Parameter
Age (years)	32.12±4.82
Infertility period (years)	3.92±1.43
No. of AFCs	10.04±3.82
Basal FSH (mIU/mL)	7.74±2.15
Dose of Gn (IU)	2421.75±822.75
No. of retrieved oocytes	10.49±4.79

Abbreviation: AFC, antral follicle count; FSH, follicular stimulation hormone.

(2) Variation curve of the serum mean progesterone levels among women under different COH protocols

The serum progesterone levels of all women under different COH protocols followed the same pattern. progesterone levels gradually decreased over the course of 7 days, reached a nadir on day 7 after ET, and subsequently began to rise in pregnant women (34.10±5.37 ng/ml (the long GnRH-a protocol), 35.23±4.72 ng/ml (the ultra-long GnRH-a protocol), 38.41±5.94 ng/ml (the GnRH-A protocol), 39.94±0.25 ng/ml (the micro-stimulation protocol)), while continuing to decrease in non-pregnant women (12.12±3.50 ng/ml (the long GnRH-a protocol), 12.54±2.85 ng/ml (the ultra-long GnRH-a protocol), 14.04±1.88 ng/ml (the GnRH-A protocol), 10.36±2.00 ng/ml (the micro-stimulation protocol)). The results are shown in Figure 2. With the different COH protocols, the progesterone level followed the same pattern. The progesterone levels of all women gradually decreased over the course of 7 days after ET. The progesterone level of pregnant women reached a nadir on day 7 after ET and subsequently began to rise, while the progesterone level of the non-pregnant women continued to decrease.

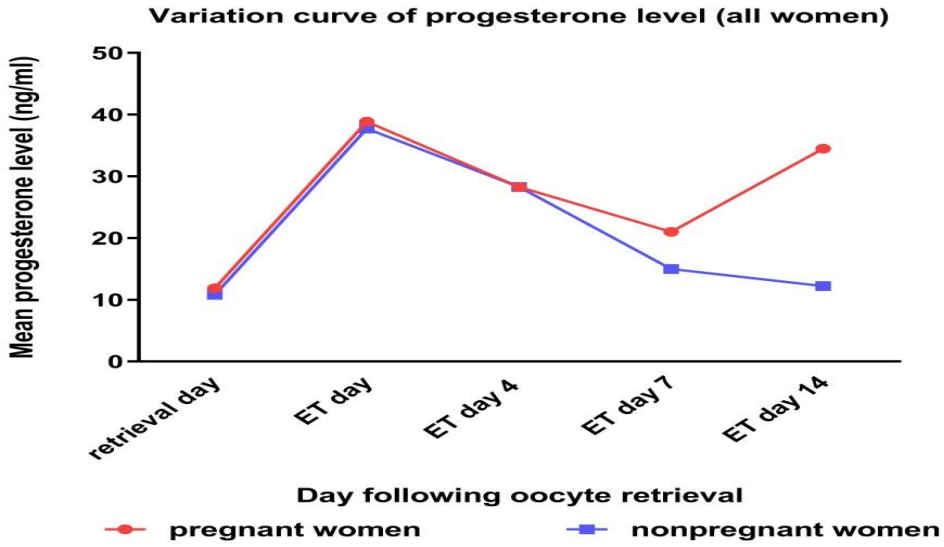

Figure 1: Variation curve of the serum mean progesterone levels (all women). The serum progesterone levels of all women gradually decreased over the course of 7 days after ET. The progesterone level of the pregnant women reached a nadir on day 7 after ET and subsequently began to rise, while the progesterone level of the non-pregnant women continued to decrease.

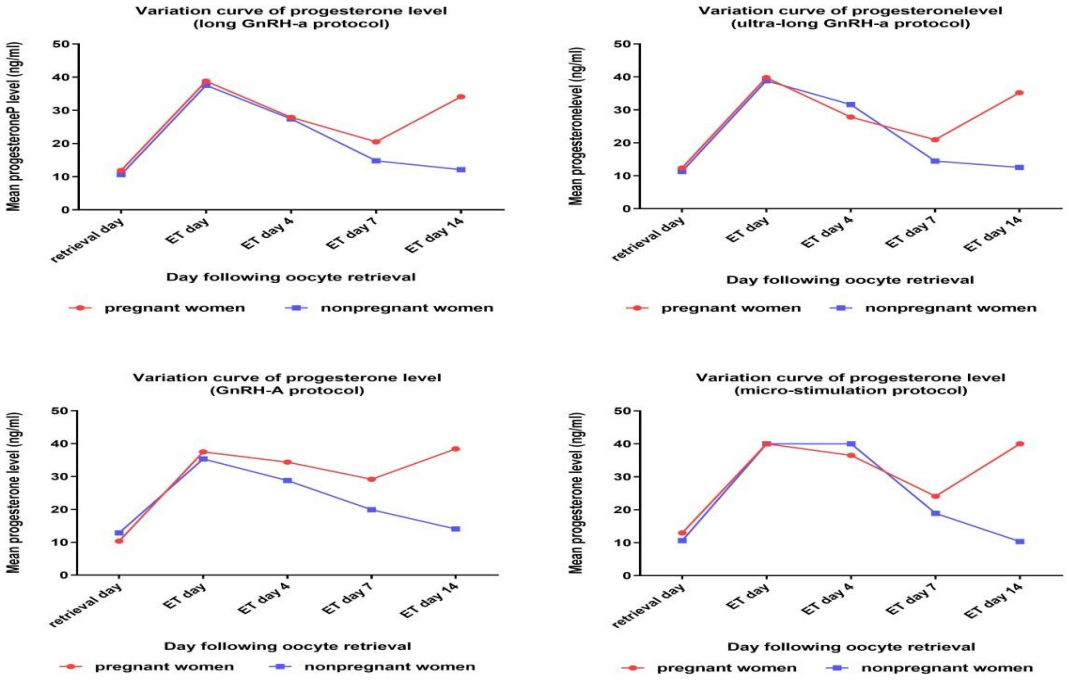

Figure 2: Variation curve of the serum mean progesterone levels among women under different COH protocols. With the different COH protocols, the progesterone level followed the same pattern. The progesterone levels of all women gradually decreased over the course of 7 days after ET. The progesterone level of pregnant women reached a nadir on day 7 after ET and subsequently began to rise, while the progesterone level of the non-pregnant women continued to decrease.

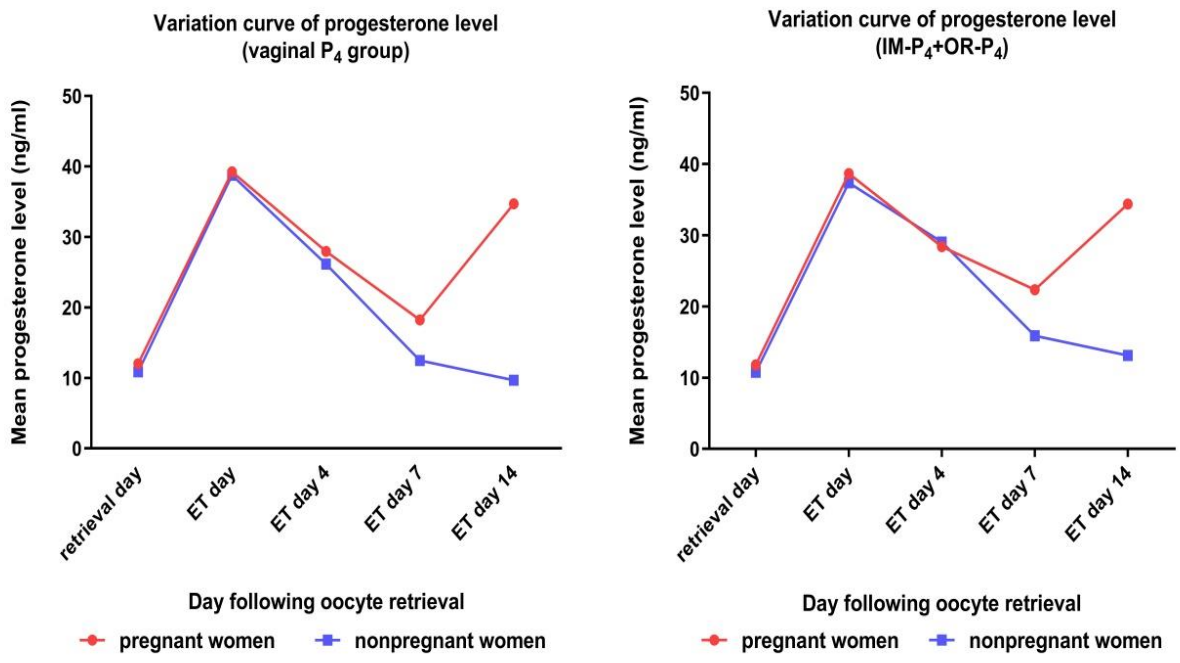

Figure 3: Variation curve of the serum mean progesterone levels among women under different luteal support protocols. With the different support protocols, the progesterone level followed the same pattern. The progesterone level of all women gradually decreased over the course of 7 days after ET. The progesterone level of the pregnant women reached a nadir on day 7 after ET and subsequently began to rise, while the progesterone level of non-pregnant women continued to decrease.

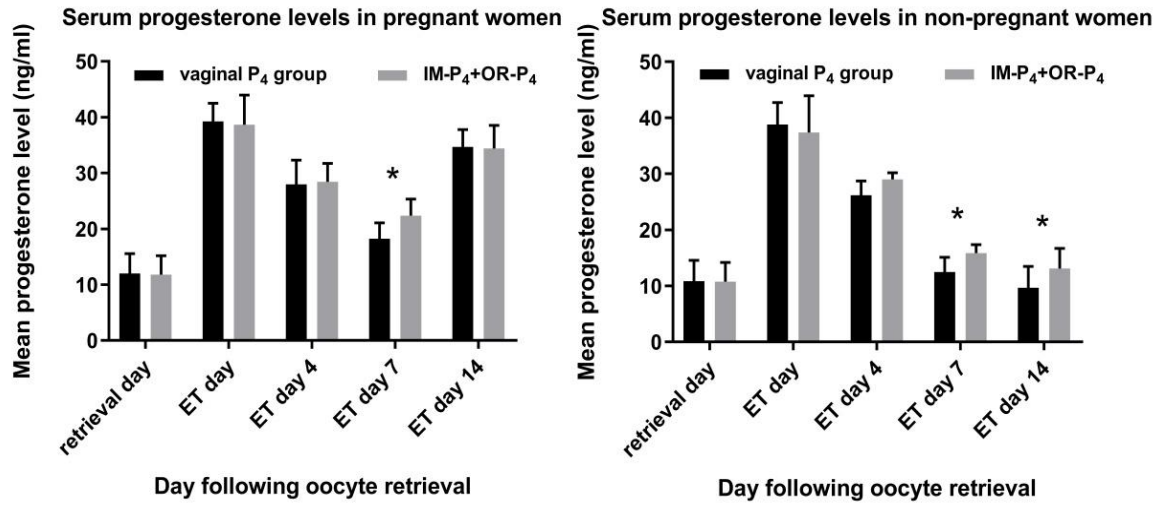

Figure 4: Serum progesterone levels for different routes. In pregnant women, none of the serum progesterone levels were significantly different between the vaginal P4 group and the IM-P4+OR-P4 group, except on day 7 after ET. In non-pregnant women, there were significant differences between the two groups on day 7 and day 14 after ET.

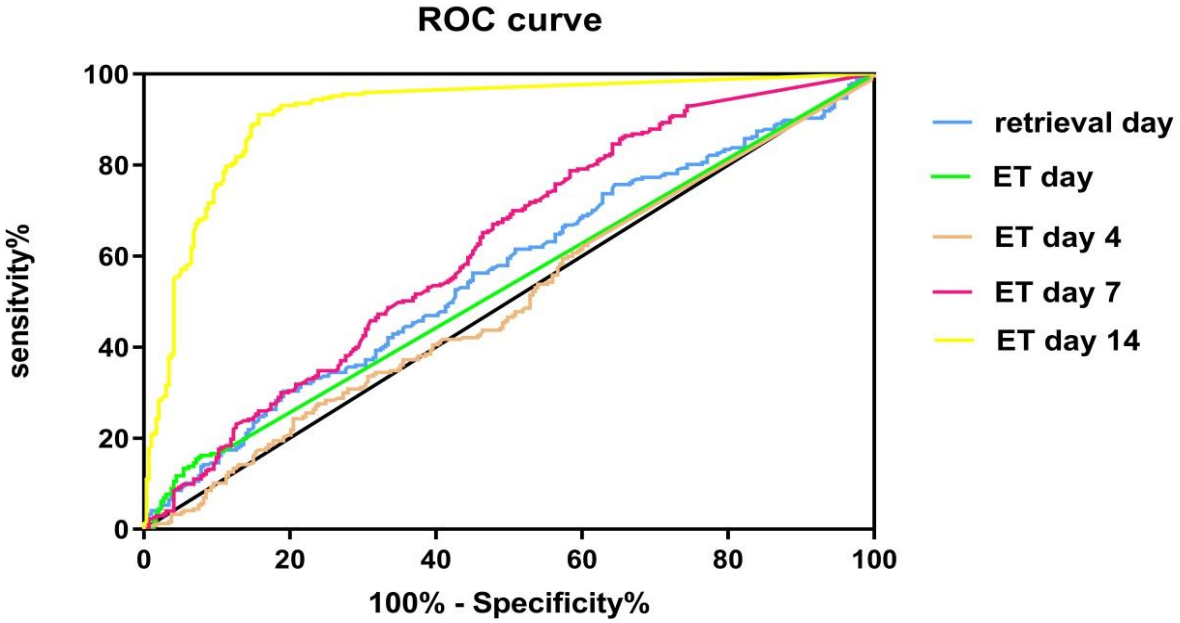

Figure 5: Predictive values of the serum mean progesterone level at different times after ET in terms of pregnancy outcome. The progesterone levels at different times were able to predict the pregnancy outcome, but only the progesterone level on day 14 after ET had a more significant predictive value on pregnancy prognosis.

(3) Variation curve of the serum mean progesterone levels among women with different luteal support protocols

The serum progesterone levels of all women with different luteal support protocols followed the same pattern. Progesterone levels gradually decreased over the course of 7 days, reached a nadir on day 7 after ET, and subsequently began to rise in pregnant women (34.70±3.10 ng/ml (the vaginal P_4 group), 34.39±4.14 ng/ml (the IM-P_4+OR-P_4 group)), while continuing to decrease in non-pregnant women (9.67±3.81 ng/ml (the vaginal P_4 group), 13.10±3.58 ng/ml (the IM-P_4+OR-P_4 group)). The results are shown in Figure 3. With the different support protocols, the progesterone level followed the same pattern. The progesterone level of all women gradually decreased over the course of 7 days after ET. The progesterone level of the pregnant women reached a nadir on day 7 after ET and subsequently began to rise, while the progesterone level of non-pregnant women continued to decrease.

When the pregnant women were included in the analysis, none of the serum progesterone values were significantly different from those of the vaginal P_4 group or the IM-P_4+OR-P_4 group, except on day 7 after ET. In the non-pregnant women, there were significant differences between the two groups on day 7 and day 14 after ET. The results are shown in Figure 4. In pregnant women, none of the serum progesterone levels were significantly different between the vaginal P_4 group and the IM-P_4+OR-P_4 group, except on day 7 after ET. In non-pregnant women, there were significant differences between the two groups on day 7 and day 14 after ET.

(4) Predictive values of the serum mean progesterone levels at different times after ET in terms of pregnancy outcome

ROC analysis was performed to determine the most efficient predictive value of the progesterone level to predict pregnancy outcome. The AUCs for predicting clinical pregnancy were 0.560, 0.534, 0.504, 0.632, and 0.917 on day 0, day 4, day 7 and day 14, respectively, after ET. The progesterone levels at different times were able to predict the pregnancy outcome, but only the progesterone level on day 14 after ET had a more significant predictive

value on pregnancy prognosis. The results are shown in Figure 5. The progesterone levels at different times were able to predict the pregnancy outcome, but only the progesterone level on day 14 after ET had a more significant predictive value on pregnancy prognosis.

Discussion

Studies demonstrate that almost 100% of women undergoing COH for IVF/ICSI will suffer from luteal phase deficiency[4]. Exogenous luteal support has become a routine procedure during COH in the vast majority of reproductive centres to improve luteal function, increase the implantation rate, increase the early pregnancy rate, and reduce the miscarriage rate. There are many kinds of luteal support protocols, but there is no consensus on the optimal route of progesterone administration. In a recent Cochrane review, vander Linden *et al*. reported that the route of P administration was not associated with an improvement in pregnancy outcomes[11]. Yanushpolsky *et al*. conducted a prospective randomized controlled study of 468 patients undergoing IVF, 407 of who completed the entire study. The results showed that the vaginal gel and IM-P_4 were equally effective for luteal phase support in IVF[12]. Herman *et al*. carried out an RCT on the effectiveness of oral dydrogesterone and micronized vaginal progesterone (MVP) for luteal support in IVF, and the study demonstrated that there was no difference in the rate of ongoing pregnancy[13]. In a recent survey, Vaisbuch *et al*. found that in more than 90% of reported cycles, a vaginal progesterone product was used, either alone or in combination with IM-P_4 due to convenience and fewer adverse reactions, which became the preferred treatment for CL support in ART[14]. Progesterone administered vaginally is expected to be associated with a higher level of uterine progesterone concentrations and lower systemic absorption than IM-P_4[15]. However, our study found that there were no significant differences in the progesterone levels between the vaginal P_4 group and the IM-P_4+OR-P_4 group, except on day 7 after ET in pregnant women and on day 7 and day 14 after ET in non-pregnant women. The differences on day 7 after ET (i.e., day 10 after oocyte retrieval) might be due to the fact that the corpus luteum began to degenerate and the endogenous secretion of progesterone levels were low. The differences on day 14 after ET might be due to the fact that the corpus luteum of the non-pregnant women had completely degraded. Therefore, exogenous progesterone supplementation by different routes showed differences in systemic absorption, that is, the progesterone levels of the vaginal P_4 group were lower than those of the IM-P_4+OR-P_4 group.

There is no uniform standard regarding the optimal dose of progesterone. A meta-analysis showed that the dose of IM-P_4, which varied between 25 and 100 mg per day, was not consistent with the clinical pregnancy rate[6]. In addition, the most recent Cochrane review demonstrated no difference in the live birth or ongoing pregnancy rate between standard (90 mg/day) or high (equal or more than 100 mg/day) doses of vaginal progesterone without heterogeneity among studies[11].

The present research results demonstrated that the serum progesterone levels of all women gradually decreased over the course of 7 days after ET. The progesterone level of the pregnant women reached a nadir on day 7 after ET and subsequently began to rise, while the progesterone level of the non-pregnant women continued to decrease. Even with different COH protocols, the P level followed the same pattern. In this study, exogenous luteal support was performed by means of different routes of administration. If the serum progesterone level was affected by absorbed progesterone from exogenous supplementation rather than by endogenous secretion from the CL, its level should be stable, with a steady continuous absorption of progesterone expected; however, the results indicated that this not the case. Therefore, one can speculate that the serum progesterone level is mainly derived from the endogenous secretions of the CL. In agreement with our study, Mitwally *et al*. analysed the serum progesterone levels of women who used vaginal P_4 and IM-P_4 for luteal support. The authors found that the P level began to decline after a brief rise during the first few days after oocyte retrieval, dropped to a nadir on days 9-10 and started to rise again in pregnant women while continuing to decrease in non-pregnant women[15]. In addition, progesterone administered vaginally is expected to be associated with a higher level of

uterine progesterone concentrations and lower systemic absorption than IM-P_4[15]. However, our study found that there were no significant difference in the progesterone levels of the vaginal P_4 group and the IM-P_4+OR-P_4 group on the day of oocyte retrieval, on day 0 and day 4 after ET among all women, or on day 14 after ET in non-pregnant women. Therefore, the results of the above studies fully suggest that serum progesterone is mainly derived from the endogenous secretions of the CL and that the progesterone level is either not affected by exogenous progesterone supplementation or is mildly affected after COH. In another words, the serum progesterone level does not represent the adequacy of exogenous progesterone supplementation. Therefore, the serum progesterone level cannot be used to guide the dose of exogenous progesterone supplementation.

Since progesterone is a necessary hormone to maintain early pregnancy, one can predict the pregnancy outcome by means of the serum progesterone levels after ET. Sonntag et al. studied the relationship between the progesterone levels and pregnancy outcome from the day of ET and throughout the luteal phase until ET +14. The results showed that the progesterone levels in pregnant patients were higher than those in non-pregnant patients[16]. Bakas *et al*. included 126 infertility patients who underwent artificial insemination, and the study showed that the AUC for predicting pregnancy outcomes was 83.9% at 6-10 days after artificial insemination[17]. A recent study showed that progesterone levels above 17 ng/ml on day 7 after ET can be a better predictor of cycle outcome[18]. In this study, ROC analysis was performed to determine the most efficient predictive value of the progesterone level to predict pregnancy. The results showed that the serum progesterone levels at different times can serve as a predictive factor for pregnancy outcomes but had a higher predictive value only on day 14 after ET. Therefore, there is no need to measure the serum progesterone levels frequently after ET, and the best measurement time can be delayed to 14 days after ET.

Conclusion

In conclusion, serum progesterone is mainly driven from the endogenous secretions of the CL, and its level is either not affected by exogenous progesterone supplementation or is mildly affected after COH. In another words, the serum progesterone level does not represent the adequacy of exogenous progesterone supplementation. Therefore, there is no need to measure serum progesterone levels frequently after ET or adjust the dose according to serum progesterone levels.

Acknowledgement

We thank American Journal Experts (AJE) for English language editing. This manuscript was edited for English language by AJE.

Contribution of authors

YZ and YX conceived and designed the study, and wrote the paper. JS and FC carried out the data collection and verification. YK and SW performed data analysis and revised the paper. All authors read and approved the final manuscript.

References

1. Devoto L, Fuentes A, Kohen P, Cespedes P, Palomino A, Pommer R, Munoz A and Strauss JR. The human corpus luteum: life cycle and function in natural cycles. Fertil Steril, 2009; 92(3):1067-79.
2. Csapo A I, Pulkkinen M O, Ruttner B, Sauvage JP and Wiest WG. The significance of the human corpus luteum in pregnancy maintenance. I. Preliminary studies. Am J Obstet Gynecol, 1972; 112(8):1061-67.
3. Czyzyk A, Podfigurna A, Genazzani A R and Meczekalski B. The role of progesterone therapy in early pregnancy: from physiological role to therapeutic utility. Gynecol Endocrinol, 2017; 33(6):421-4.
4. Fatemi H M. The luteal phase after 3 decades of IVF: what do we know?. Reprod Biomed Online, 2009; 19 Suppl 4:4331.
5. Palomba S, Santagni S and La Sala G B. Progesterone administration for luteal phase deficiency in human reproduction: an old or new issue?. J Ovarian Res, 2015; 8:77.
6. Pritts E A and Atwood A K. Luteal phase support in infertility treatment: a meta-analysis of the randomized trials. Hum Reprod, 2002; 17(9):2287-99.
7. Conforti A, Strina I, Mollo A, Amoroso R, Marrone V, Alviggi C, Marci R and de Placido G. The efficacy of modified luteal phase support with intramuscular progesterone in IVF/ICSI cycles: a retrospective observational study. Eur Rev Med Pharmacol Sci, 2017; 21(4):657-61.
8. Connell M T, Szatkowski J M, Terry N, DeCherney AH, Propst AM and Hill MJ. Timing luteal support in

assisted reproductive technology: a systematic review. Fertil Steril, 2015; 103(4):939-46.
9. Yanushpolsky E H. Luteal phase support in in vitro fertilization. Semin Reprod Med, 2015; 33(2):118-27.
10. Propst A M, Hill J A, Ginsburg E S, Hurwitz S, Politch J and Yanushpolsky EH. A randomized study comparing Crinone 8% and intramuscular progesterone supplementation in in vitro fertilization-embryo transfer cycles. Fertil Steril, 2001; 76(6):1144-9.
11. van der Linden M, Buckingham K, Farquhar C, Kremer JA and Metwally M. Luteal phase support for assisted reproduction cycles. Cochrane Database Syst Rev, 2015; (7):D9154.
12. Yanushpolsky E, Hurwitz S, Greenberg L, Racowsky C and Hornstein M. Crinone vaginal gel is equally effective and better tolerated than intramuscular progesterone for luteal phase support in in vitro fertilization-embryo transfer cycles: a prospective randomized study. Fertil Steril, 2010; 94(7):2596-9.
13. Tournaye H, Sukhikh G T, Kahler E and Griesinger G. A Phase III randomized controlled trial comparing the efficacy, safety and tolerability of oral dydrogesterone versus micronized vaginal progesterone for luteal support in in vitro fertilization. Hum Reprod, 2017; 32(5):1019-27.
14. Vaisbuch E, de Ziegler D, Leong M, Weissman A and Shoham Z. Luteal-phase support in assisted reproduction treatment: real-life practices reported worldwide by an updated website-based survey. Reprod Biomed Online, 2014; 28(3):330-5.
15. Mitwally M F, Diamond M P and Abuzeid M. Vaginal micronized progesterone versus intramuscular progesterone for luteal support in women undergoing in vitro fertilization-embryo transfer. Fertil Steril, 2010; 93(2):554-69.
16. Sonntag B, Loebbecke K C, Nofer J R, Kiesel L and Greb RR. Serum estradiol and progesterone in the mid-luteal phase predict clinical pregnancy outcome in IVF/ICSI cycles. Gynecol Endocrinol, 2013; 29(7):700-3.
17. Bakas P, Simopoulou M, Giner M, Drakakis P, Panagopoulos P and Vlahos N. Predictive value of repeated measurements of luteal progesterone and estradiol levels in patients with intrauterine insemination and controlled ovarian stimulation. Gynecol Endocrinol, 2017:1-4.
18. Aslih N, Ellenbogen A, Shavit T, Michaeli M, Yakobi D and Shalom-Paz E. Can we alter pregnancy outcome by adjusting progesterone treatment at mid-luteal phase: a randomized controlled trial. Gynecol Endocrinol, 2017:1-5.