

Pregnancy Outcomes of Patients with Low Serum β -hCG Level 14 Days After Fresh Embryo Transfer

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Research

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Abstract

Background: Although previous studies had successfully illustrated different pregnancy outcomes by different serum β -hCG levels after embryo transfer, prognosis of pregnancy outcomes remains elusive when the serum β -hCG level is extremely low (e.g., < 100 mIU/ml 14 days after embryo transfer). Therefore, the purpose of our study is to investigate the pregnancy outcomes of patients with low serum β -hCG level 14 days after day 3 embryo transfer.

Methods: A retrospective study was performed with 723 patients with a serum β -hCG level between 5 and 100 mIU/ml 14 days after day 3 fresh embryo transfer. Pregnancy outcomes (ongoing pregnancy, early miscarriage, biochemical pregnancy loss, and ectopic pregnancy) were analyzed according to the female patients' age. Receiver operating characteristic (ROC) curves were plotted to indicate the threshold for prediction of clinical pregnancy and ongoing pregnancy. Sensitivity and specificity were calculated according to the ROC curves as well.

Results: Of the 723 patients with serum β -hCG level < 100 mIU/mL 14 days after day 3 embryo transfer, 85.6% (619) had biochemical pregnancy, and only 14.4% (104) had clinical pregnancy (including 4.7% with ongoing pregnancy, 3.7% with ectopic pregnancy, and 5.9% with early miscarriage). The rate of ongoing pregnancy was significantly lower in ≥ 38 -year group compared with < 38 -year group (1.3% vs. 5.6%, $P = 0.029$). The serum β -hCG level to predict clinical pregnancy was 44.7 mIU/ml (sensitivity, 91.3%; specificity, 82.1%; area under the ROC curve (AUROC), 0.908). For ongoing pregnancy, the serum β -hCG level was 53.7 mIU/ml (sensitivity, 94.1%; specificity, 81.4%; AUROC, 0.902).

Conclusions: Initially low serum β -hCG level 14 days after day 3 embryo transfer indicated poor prognosis with minimal likelihood of ongoing pregnancy. **Keywords:** assisted reproductive technology; human chorionic gonadotropin; pregnancy; live birth; embryo transfer

Background

Human chorionic gonadotropin (hCG) is a hormone that is produced by differentiated syncytiotrophoblast cells from the time of implantation and increases as pregnancy progresses. It can also promote production of progesterone by ovarian corpus luteal cells through acting on the hCG/LH receptor. In addition, hCG has several other functions, such as promotion of angiogenesis of uterine vasculature, growth of uterus in line with fetal growth, immune-suppression, etc. [1]. In both the in vivo and in vitro fertilization (IVF) conceptions, β -hCG is used for diagnosis of pregnancy. Once the embryo implantation begins, hCG is secreted and can be detected in the maternal serum as early as 6 to 8 days after fertilization. In normal conceptions, hCG levels are doubled every 48 h, and thus, this increased pattern is often used to discriminate the normal pregnancy from pathological pregnancy [2].

Previous studies have focused on the prediction of clinical pregnancy by maternal serum β -hCG, which was tested 11-16 days following cleavage embryo transfer with the cut-off value of 42-80 mIU/ml [3-9]. A number of scholars investigated the relationship between pregnancy outcomes and the doubling times of

maternal serum β -hCG[10, 11] . Recently, some researches assessed the early prediction of pregnancy outcomes as early as 5-7 days after embryo transfer [12-14]. As the blastocyst transfer has become highly popular in recent years, several scholars studied the prediction of pregnancy by maternal serum β -hCG after blastocyst transfer, with the cut-off value of 152-527 mIU/ml 11-13 days after embryo transfer [2, 8, 15-18].

Although previous studies had successfully illustrated different pregnancy outcomes by different serum β -hCG levels after embryo transfer, prognosis of pregnancy outcomes remains elusive when the serum β -hCG level is extremely low (e.g., < 100 mIU/ml 14 days after embryo transfer). In this condition, the prognosis of the majority of pregnancies may be unfavorable, including biochemical pregnancy, ectopic pregnancy, and miscarriage. The management of different pregnancy outcomes is quite distinct: for biochemical pregnancy loss, only regular monitoring is required; for miscarriage, abortion with drugs or curettage is essential if it doesn't occur spontaneously; for ectopic pregnancy, an operation is required in the majority of the cases except for a number of special instances with appropriate prognosis. Therefore, it is highly important to discriminate clinical pregnancy from biochemical pregnancy when the serum β -hCG level is extremely low.

The aim of the present study was to assess the pregnancy outcomes when the initial maternal serum β -hCG level was < 100 mIU/ml 14 days after transfer of Day 3 fresh embryos. Meanwhile, it was attempted to identify the cut-off value to discriminate clinical pregnancy from biochemical pregnancy loss.

Methods

Populations

This retrospective study included patients who received in-vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) in Department of Reproductive Medicine of the Third Affiliated Hospital, Guangzhou Medical University (Guangzhou, China), between January 2012 and January 2019. All the patients transferred Day 3 fresh embryos and had a serum β -hCG level > 5 mIU/ml, while that was < 100 mIU/ml 14 days after transfer. This study was approved by the Ethics Committee of the Third Affiliated Hospital of Guangzhou Medical University.

Controlled ovarian hyperstimulation protocols

Both luteal phase pituitary downregulation protocol and gonadotropin-releasing hormone (GnRH) antagonist protocol were applied in the controlled ovarian stimulation (COS). For the luteal phase pituitary downregulation protocol, GnRHa (Triptorelin, Ipsen Pharma Biotech, Toulon France) 1.0–1.3 mg was administered in the luteal phase of the previous cycle.

Testing of serum levels of estradiol (E_2), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and ultrasound were performed after 14 days of downregulation. Ovarian stimulation was commenced with 150-300 IU rFSH (Gonal-f; Serono, Geneva, Switzerland) once the downregulation was satisfactory (i.e., serum E_2 level was < 50 pg/ml, follicular diameter was 4–6 mm, and endometrium thickness was < 5 mm). When at least three follicles were ≥ 17 mm in diameter, ovulation was triggered by administration of 250 ug rHCG (Serono, Geneva, Switzerland). Oocytes were retrieved after 34–36 h, and embryos were transferred 3 days after oocyte retrieval. Serum β -HCG level was tested 14 days after transfer, and ultrasound was carried out 28 days after transfer, if the β -HCG test was positive. Luteal function was supported by intravaginal progesterone (Crinone, 90 mg qd, Serone, Geneva, Switzerland, or Utrogestan, 0.2 g Bid, Besins Healthcare, Monaco) until the day of pregnancy test. Once pregnancy was confirmed, luteal support was continued until 10 weeks of gestation.

Hormone Measurement

Immunochemiluminometric assay was undertaken for testing of β -hCG (Architech i2000SR; Abbott Laboratories Inc., Chicago, IL, USA). The range of detection was between 1.2 and 225 000 mIU/mL. The sensitivity of the assay was 1.2 mIU/ml, and the intra-assay coefficient of variation was 7%. Our laboratory is annually checked for qualification by the External Quality Assessment of Clinical Laboratory Center (Ministry of Health of the People's Republic of China, Beijing, China).

Definitions of Pregnancy Outcomes

Clinical pregnancy was defined as an intrauterine/extrauterine gestational sac detected by ultrasound with positive serum β -hCG. Biochemical pregnancy loss was defined as serum β -Hcg level > 5 mIU/ml 14 days after transferring embryo, and declined to < 5 mIU/ml at the end without visible gestational sac by ultrasound. Early miscarriage was defined as fetal growth arrest or no cardiac activity detected in the gestational sac during the first 12 weeks of pregnancy. Ongoing pregnancy indicated pregnancy continued after 12 weeks of gestation with live fetus.

Statistics

Statistical analysis was performed by using SPSS 21.0 software (IBM, Armonk, NY, USA). One-way analysis of variance (ANOVA) was used for comparing mean values, and causes of infertility were analyzed by Chi-squared test. Receiver operating characteristic (ROC) curve was plotted to explore threshold of the serum β -hCG level for prediction of clinical pregnancy. P -value < 0.05 was considered statistically significant.

Results

Pregnancy outcomes of patients with low β -hCG level

Of the 723 patients with serum β -hCG level < 100 mIU/mL 14 days after day 3 of embryo transfer, 619 (85.6%) had biochemical pregnancy, while only 104 (14.4%) had clinical pregnancy (34 (4.7%) with ongoing pregnancy, 27 (3.7%) with ectopic pregnancy

Table 1

Pregnancy outcomes of patients with serum β -hCG level < 100 mIU/ml 14 days after day 3 of embryo transfer

| Pregnancy outcomes | < 38 years | > 38 years | Total | P |
|----------------------------|------------|-------------|-------------|-------|
| | %(n) | %(n) | %(n) | |
| Biochemical pregnancy loss | 85.3(489) | 86.7(130) | 85.6(619) | 0.680 |
| Ongoing pregnancy | 5.6(32) | 1.3(2) | 4.7(34) | 0.029 |
| Ectopic pregnancy | 3.8(22) | 3.3(5) | 3.7 (27) | 0.771 |
| Early Miscarriage | 5.2(30) | 8.7(13) | 5.9 (43) | 0.114 |
| Total | 100.0(573) | 100.0 (150) | 100.0 (723) | |

and 43 (5.9%) with early miscarriage) (Table 1). For 32 ongoing pregnancies, 26 were live birth, 4 are currently beyond 12 weeks of gestation, and the other 2 were late miscarriages. The lowest level of β -hCG for clinical pregnancy was 9.32 mIU/ml, which was an ectopic pregnancy. The minimal value of β -hCG for live birth was 36.14 mIU/ml. For patients aged < 38 years old, the rate of biochemical pregnancy loss was 85.3%, which was not significantly different from that of patients' age > 38 years old (P = 0.68), when the maternal serum β -hCG level was < 100 mIU/ml 14 days after day 3 of embryo transfer. However, the rate of ongoing pregnancy was remarkably lower in \geq 38-year group, compared with < 38-year group (1.3% vs. 5.6%, P = 0.029).

Of the 104 patients with clinical pregnancy, ongoing pregnancy accounted for 38.1% (32/84) in < 38-year group, which was significantly higher than that in \geq 38-year group (10.0%). Rate of early miscarriage was notably lower in < 38-year group

Table 2

Outcomes of patients with clinical pregnancy when the serum β -hCG level was less than 100 mIU/ml 14 days after day 3 embryo transfer

| Pregnancy outcomes | < 38 years %(n) | \geq 38 years %(n) | Total %(n) | P |
|---|--------------------|-------------------------|---------------|-------|
| Ongoing pregnancy | 38.1(32) | 10.0(2) | 32.7(34) | 0.016 |
| Ectopic pregnancy | 26.2(22) | 25.0(5) | 26.0(27) | 0.913 |
| Early Miscarriage | 35.7(30) | 65.0(13) | 41.3(43) | 0.017 |
| Total | 100.0 (84) | 100.0 (20) | 100.0(104) | |
| compared with that in \geq 38-year group (35.7% vs. 65.0%, P = 0.017). No significant difference was found when the rate of ectopic pregnancy was compared between the two groups(Table 2). | | | | |
| Baseline of clinical pregnancy vs. biochemical pregnancy | | | | |
| Patients' mean age in the group of clinical pregnancy was 33.3 ± 4.4 years old and that was 32.9 ± 4.6 years old in the group of biochemical pregnancy (P > 0.05). Causes of infertility in both groups were mainly tubal/peritoneal factors (40.9% vs. 45.5%, P > 0.05). The levels of E ₂ ,P, and endometrial thickness on the day of HCG administration were similar in the two groups (P > 0.05). No significant differences were noted in the number of of embryos transferred in both groups (1.93 ± 0.55 vs. 1.98 ± 0.51 , P > 0.05). The serum β -hCG level 14 days after transferring embryo was markedly different (clinical pregnancy: 71.9 ± 18.5 mIU/mL; biochemical pregnancy: 23.9 ± 18.5 , P = 0.00) (Table 3). | | | | |
| The ROC curve showed a significant predictive value of serum β -hCG level 14 days after embryo transfer for clinical pregnancy and live birth. Area under the ROC curve | | | | |

Table 3
Characteristics of clinical pregnancy vs. biochemical pregnancy

| Characteristics | Clinical pregnancy | Biochemical pregnancy | P |
|--|--------------------|-----------------------|-------|
| N | 44 | 218 | |
| Female patients' age(years) | 33.3 ± 4.4 | 32.9 ± 4.6 | NS |
| Male patients' age (years) | 35.7 ± 6.0 | 34.9 ± 5.5 | NS |
| Duration of Infertility (years) | 4.3 ± 3.2 | 4.8 ± 3.4 | NS |
| Causes of Infertility n (%) | | | |
| Tubal/ Pelvic | 18(40.9) | 99(45.4) | NS |
| Ovulatory dysfunction | 3(6.8) | 12(5.5) | NS |
| Male factor | 7(15.9) | 46(21.1) | NS |
| Others | 16(36.4) | 61(28.0) | NS |
| No. of cycles | 1.4 ± 0.9 | 1.3 ± 0.6 | NS |
| BMI (kg/m ²) | 23.1 ± 4.5 | 22.0 ± 3.5 | NS |
| AMH (ng/ml) | 3.83 ± 3.40 | 4.04 ± 3.51 | NS |
| Days of Gn | 12.1 ± 2.4 | 12.6 ± 2.5 | NS |
| Total dose of Gn (IU) | 3118 ± 1176 | 2983 ± 11.8 | NS |
| E ₂ on hCG day (pmol/L) | 9952.6 ± 3778.1 | 9875.4 ± 4177.0 | NS |
| P on HCG day (nmol/L) | 2.25 ± 1.00 | 2.35 ± 0.90 | NS |
| EMT on hCG day (mm) | 10.3 ± 2.0 | 10.4 ± 1.9 | NS |
| No. of oocytes retrieved | 9.5 ± 4.1 | 9.8 ± 4.5 | NS |
| No. of usable embryos | 4.2 ± 2.4 | 4.3 ± 3.1 | NS |
| No. of embryos transferred | 1.93 ± 0.55 | 1.98 ± 0.51 | NS |
| Serum β-hCG (mIU/mL) | 71.9 ± 18.5 | 23.9 ± 18.5 | 0.000 |
| <p>(AUROC) for clinical pregnancy was 0.908 (95% confidence interval (CI): 0.882–0.935), and the AUROC for ongoing pregnancy was 0.902 (95% CI: 0.872–0.932) (Figs .1 and 2). The threshold for predicting a clinical pregnancy was 44.7 mIU/ml with the sensitivity of 91.3% and specificity of 82.1%. The cut-off value for predicting an ongoing pregnancy was 53.7 mIU/ml, with sensitivity of 94.1% and specificity of 81.4%. For patients with serum β-hCG level < 44.7 mIU/ml, 98.3% had biochemical pregnancy loss (negative predictive value (NPV): 98.3%). For patients with serum β-hCG level ranged between</p> | | | |

Table 4

Thresholds of serum β -hCG level for prediction of clinical pregnancy and ongoing pregnancy.

| Pregnancy outcomes | Threshold of β -hCG level(mIU/mL) | AUROC | 95% CI of AUROC | Sensitivity % | Specificity% | PPV % | NPV % |
|--|---|-------|-----------------|---------------|--------------|-------|-------|
| Clinical pregnancy | 44.7 | 0.908 | 0.882–0.935 | 91.3 | 82.1 | 46.1 | 98.3 |
| Ongoing pregnancy | 53.7 | 0.902 | 0.872–0.932 | 94.1 | 81.4 | 20.0 | 99.6 |
| PPV = positive predictive value; NPV = negative predictive value | | | | | | | |

44.7 and 100 mIU/ml, only 46.1% had clinical pregnancy (positive predictive value (PPV): 46.1%). For patients with serum β -hCG level < 53.7 mIU/ml, 99.6% had non-ongoing pregnancy (NPV: 99.6%). For patients with serum β -hCG level ranged between 53.7 and 100 mIU/ml, only 20.0% had ongoing pregnancy (PPV: 20.0%) (Table 4).

Discussion

Many studies investigated the relationship between serum β -hCG level and pregnancy outcomes. However, these studies mainly predicted the outcomes of all patients after embryo transfer. To date, a limited number of researches demonstrated the pregnancy outcomes when the patients' serum β -hCG level was below 100 mIU/ml 14 days after day 3 of embryo transfer. The present study indicated that the majority of the patients had unfavorable prognosis, including biochemical pregnancy loss, early miscarriage, and ectopic pregnancy. Hence, the management is quite distinct for different pregnancy outcomes. Consequently, prediction of pregnancy outcomes is highly significant especially when the serum β -hCG level is insignificant.

In the current research, the incidence of biochemical pregnancy loss was as high as 85.6% when the serum β -hCG level was < 100 mIU/ml 14 days after day 3 of embryo transfer. The rate of ongoing pregnancy was only 4.7%, which was consistent with a study conducted by Heiner et al. They illustrated that the rate of live birth was only 6% (2/31) [19]. However, their study included the transfer of embryos in both pronuclear and cleavage stage, which may affect the serum hCG level.

The ROC curve analysis revealed the cut-off value to distinguish biochemical pregnancy loss and clinical pregnancy was 44.7 mIU/ml, with the optimal sensitivity and specificity of 91.3% and 82.1%, respectively. The threshold to predict ongoing pregnancy was 53.7 mIU/ml, with the sensitivity and specificity of 94.1% and 81.4%, respectively. This may assist performing follow-up schedules for patients with low serum β -hCG level. For patients with serum β -hCG level < 44.7 mIU/ml, 98.3% experienced biochemical pregnancy loss. For patients with serum β -hCG level \geq 44.7 mIU/ml, 46.1% had clinical pregnancy. Nevertheless, the majority of the patients with clinical pregnancy had poor prognosis. For such patients,

intensive monitoring is required especially for ectopic pregnancy. If serum β -hCG level decreases in the following test, weekly testing of β -hCG is essential until it is negative. If the doubling time of β -hCG is greater than 2 days, weekly ultrasound is required, as well as monitoring of serum β -hCG level. If the doubling time of β -hCG is less than 2 days, ultrasound can be arranged two weeks later. Once an intrauterine pregnancy is confirmed, ultrasound can be scheduled every two weeks to follow up the growth of the fetus.

A number of previous researches concentrated on the association between pregnancy outcomes and serum hCG level 12 days after embryo transfer. Qasim et al. [3] also assessed the predictive value of β -hCG on pregnancy outcomes in a prospective study of 153 IVF patients. They demonstrated that the rate of normal pregnancy was 93.9% (PPV) when the serum β -hCG level was ≥ 42 mIU/ml 12 days post-ET, with the sensitivity of 79.3% and specificity of 83.8%. In a study of 774 cycles with serum β -hCG level ≥ 5 IU/L 12 days after embryo transfer, they reported serum β -hCG level of 76 IU/L to predict viable pregnancy. However, this study included both the fresh and frozen-thawed embryos transferred in IVF cycles, with embryos of day 2 to day4 [5]. Frishman et al. performed a retrospective cohort study to investigate the prognosis of IVF pregnancies with initially low serum hCG level. They compared the miscarriage rate of the 65 IVF pregnancies with serum hCG level ≤ 20 mIU/ml 17 days after administration of hCG into 130 pregnant cases with serum hCG level > 20 mIU/ml. They found that the miscarriage rate at 6 weeks of gestation was significantly higher in the group with low serum hCG level compared with that in the group with high serum hCG level (38.5% vs. 9.2%, odds ratio (OR) = 5.7, $P < 0.0001$). However, once the pregnancy progressed to 13 weeks, the rate of miscarriage between the two groups was comparable ($< 1\%$) [20].

A number of scholars assessed predictive value of serum hCG level 16 days after oocyte retrieval on pregnancy outcomes. Lambers et al. found that the cut-off value of 223 IU/l for day 16 after oocyte retrieval or ovulation was able to discriminate ongoing pregnancy from non-ongoing pregnancy (sensitivity, 72.2%; specificity, 83.3%) [21]. For single cleavage stage embryo transfer, the threshold for prediction of clinical pregnancy was 79 IU/L 16 days after oocyte retrieval, with sensitivity and specificity of 90% and 74%, respectively [16]. Homan et al. analyzed the prognosis of low serum hCG level 16 days after ovulation, and found that the rate of ongoing pregnancy was $< 35\%$ when the serum hCG level was between 25 and 50 IU/L [22]. However, their study contained both assisted artificial technology (ART) cycles (IVF, ICSI, gamete intra fallopian transfer (GIFT), and intrauterine insemination (IUI)) and natural conception, which may increase the variability of the results. Tong et al. investigated the relationship between serum hCG level 16 days after oocyte retrieval and the rate of clinical miscarriage in 1054 IVF patients, who had been proven of clinical pregnancy by ultrasound with fetal cardiac activity. They declared that low serum hCG level (< 25 th: 5-124 mIU/ml) remarkably increased the risk of clinical miscarriage (16.7%) compared with the normal serum hCG levels (25-75th: 9.9%; > 75 th: 8.0%) [23].

With analysis of an association between pregnancy and serum hCG level measured 14 days after cleavage stage embryo transfer, the threshold to predict the outcomes varied greatly. Schmidt et al. [24] investigated a relationship between pregnancy outcomes and serum β -hCG level, which was achieved 14

days after cleavage stage embryo transfer or 16 days after gamete transfer. They found that the threshold of 100 mIU/ml was able to discriminate viable pregnancy from nonviable pregnancy with sensitivity and specificity of 91% and 71% (AUC, 0.83), respectively. When the serum β -hCG level was < 100 mIU/ml, the rate of nonviable pregnancy was 83%, which was lower than the rate mentioned in the present study (95.3%). However, their sample size with serum β -hCG level < 100 mIU/ml was extremely small to draw the conclusion. Zhang et al. studied an association between pregnancy outcomes and the serum hCG levels measured 17 days after oocyte retrieval in 6560 patients. They demonstrated that the rates of clinical pregnancy were only 23.5% and 40.1% when the serum hCG levels were 20–50 and 50–100 IU/L, respectively. In patients with clinical pregnancy, rates of live birth were only 16.4% (9/55) and 18.6% (18/97) in the above-mentioned two low-hCG groups. Their rate of clinical pregnancy was higher than that reported in the current study in patients with serum hCG level < 100 IU/L (63.6% vs.14.4%). The explanation for this is that they only included patients with serum hCG level \geq 20 IU/L, while we included patients with serum hCG level \geq 5 IU/L. However, once clinical pregnancy progressed, the rates of live birth between two studies were similar (35% vs.32.7%) in patients with serum hCG level < 100 IU/L. Nevertheless, their study included patients who transferred embryos 2–5 days after oocyte retrieval. Although testing of hCG was arranged on the same day after oocyte retrieval, a study had demonstrated that serum hCG level was different between cleavage-stage and blastocyst-stage embryo transfer [25].

Sugantha et al. analyzed the pregnancy outcomes of 429 patients after undergoing ART, in which serum hCG level was measured 14 and 21 days after oocyte collection or intrauterine insemination. The ROC curves showed that serum hCG level of 50 IU/L on day 14 and 200 IU/L on day 21 corresponded to the maximal sensitivity and specificity to predict non-viable birth. When serum hCG level was < 50 IU/L on day 14 and < 200 IU/L on day 21, the probability of live birth was 0%; when serum hCG level was < 50 IU/L on day 14, while > 200 IU/L on day 21, the probability of live birth was 44% [26].

The rate of ongoing pregnancy was only 4.7% in our research, which indicated that low level of serum β -hCG 14 days after fresh embryo transfer indicates poor prognosis, with extremely high rate of early pregnancy loss (including ectopic pregnancy). There are two possible etiologies for this. First, the low level of β -hCG resulted from later implantation of embryos. According to the research of Wilcox etc., later implantation increased the risk of early pregnancy loss [27]. During the later implantation, the poor-quality cleavage embryos maybe develop more slowly, thus implant later than the normal embryos, which leads to the lesser and later production of hCG [28]. Second, 27% of the patients were ectopic pregnancy, in which all the embryos implanted in the fallopian tubes. The environment in the fallopian tubes is less nutritious, in contrast to that in the endometrium. Therefore, the ectopic embryos may poorly develop, resulting in the less production of hCG.

As age is closely associated with the pregnancy outcomes, the risk of spontaneous abortion is markedly increased in aged women. In the present study, the rate of early miscarriage in women who aged \geq 38 was nearly doubled (65.0% vs.35.7%, $P = 0.017$) in contrast to women who aged < 38 years with low serum hCG level. Therefore, for aged women with low serum hCG level, the prognosis could be even worse. The rate of ongoing pregnancy was only 1.3% with initial serum hCG level < 100 mIU/ml. We didn't

analyze the impact of paternal age here because our previous study proved that paternal age had little effect on pregnancy outcomes [29].

The present research possesses the following advantages. First, all the included patients transferred only at day 3 fresh embryos and had serum β -hCG level measured exactly the same day after embryo transfer, which would increase the accuracy of serum β -hCG level. Second, the serum β -hCG levels of all the samples were tested at the same laboratory with a single assay, which may minimize the inter- and intra-assay variability. Third, only progesterone, instead of hCG, was used for luteal support, which may eliminate the effect of exogenous hCG. Last but not least, we only analyzed patients with low serum hCG level instead of investigating all the patients. Therefore, the present research may assist to predict the prognosis of patients with low serum hCG level and provide guidelines for the clinical consultation.

However, the current study contains some disadvantages. First, the number of embryos transferred varied from one to three. This may cause vanishing twin syndrome, which may affect the initial serum hCG level. It has been reported that if two sacs are identified sonographically, loss of one twin can be expected in 27.1% of pregnancies achieved after ART [30]. In the present research, the rate of clinical pregnancy was only 14.4% with initial serum hCG level < 100 mIU/ml on day 14. Thus, the rate of vanishing twin syndrome would quite low with such low serum β -hCG level, and can be neglected. Second, we studied the patients with β -hCG tested 14 days after embryo transfer, which was later than most of the study. This may limit its wide application because serum β -hCG level was not measured in all the patients 14 days after embryo transfer.

Conclusions

Initially low serum β -hCG level 14 days after embryo transfer indicated poor prognosis with minimal likelihood of ongoing pregnancy. For patients with serum β -hCG level < 44.7 mIU/mL, luteal support is suggested to withdraw, and serial weekly measurement of serum β -hCG level is essential until measured level would be negative. For patients having a serum β -hCG level \geq 44.7 mIU/ml, intensive monitoring is required especially for ectopic pregnancy.

Declarations

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Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YXH designed research. YXW analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the ethics committee of the Third Affiliated Hospital of Guangzhou Medical University. Each patient has signed a informed consent on obtaining and analyzing their clinical data prior to the initiation of IVF/ICSI-ET treatment.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

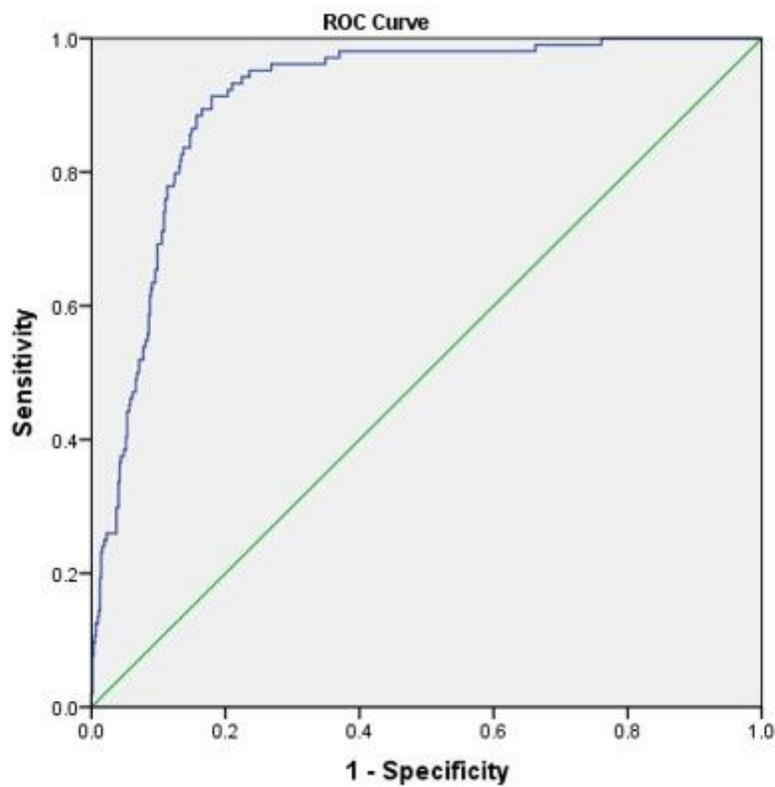
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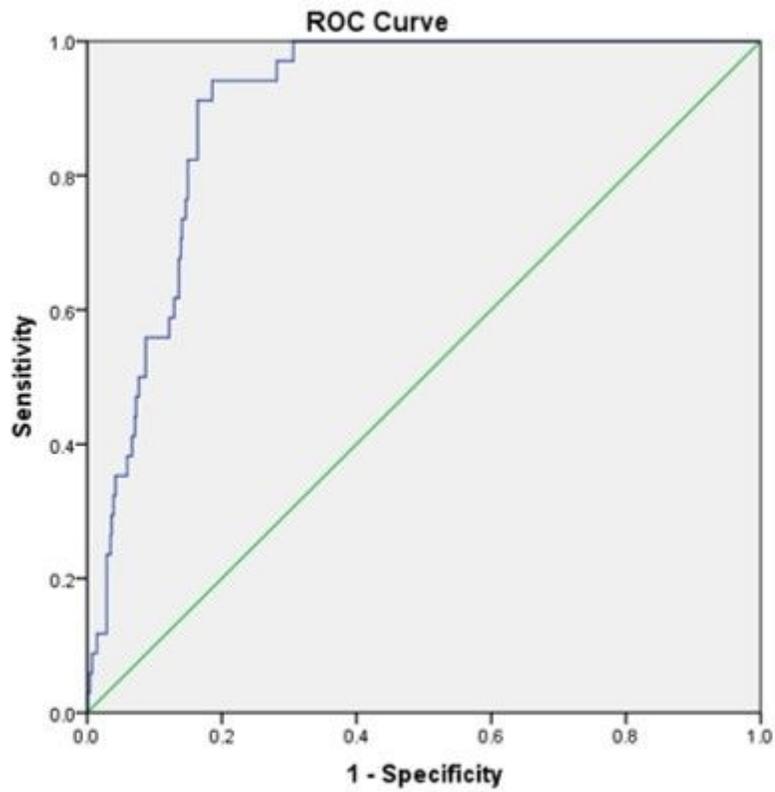
Figures



Threshold of β -hCG: 44.7mIU/mL; Sensitivity: 91.3%; Specificity: 82.1%

Figure 2

Receiving operating characteristics (ROC) curves for prediction of clinical pregnancy by serum β -hCG level 14 days after embryo transfer.



Threshold of β -hCG: 53.7mIU/mL; Sensitivity: 94.1%; Specificity: 81.4%

Figure 4

ROC curves for prediction of live birth by serum β -hCG level 14 days after embryo transfer.