ORIGINAL ARTICLE

Predicting successful live birth from single serum hCG measurement in assisted reproductive technology cycle

Rahilah Ahmad Shukri, MMed, Murizah Mohd Zain, MMed

Reproductive Unit, Department of Obstetrics and Gynaecology, Hospital Sultanah Bahiyah, Kedah, Malaysia

ABSTRACT

Introduction: Assisted reproductive technology may result in various outcomes, causing a significant stress both physically and emotionally to the patients. This study aims to determine the level of serum human chorionic gonadotrophin (hCG) following embryo transfer in predicting successful live births in in vitro fertilisation (IVF) cycles.

Materials and Methods: This is a retrospective analysis of 407 IVF pregnancies in Hospital Sultanah Bahiyah Kedah from 2014 to 2019. Serum hCG was withdrawn on either (i) day 16 post-oocyte retrieval for fresh IVF cycle or (ii) day 16 from the addition of progesterone in frozen embryo cycles. Outcomes of IVF pregnancies were analysed in relation to the level of serum hCG.

Results: The overall median hCG level in singleton live birth was 304.7 IU/L, 547.10 IU/L for multiple live births, and early pregnancy loss level was 77 IU/L. When the ROC graphs were plotted, serum hCG level of 152.85 IU/L predicted singleton livebirth with a sensitivity of 81.3%. Serum hCG of 322.40 IU/L predicted multiple live births with sensitivity of 78.6% and a specificity of 64.3%. In the subgroup analysis comparing prediction hCG level in singleton live birth; the cut-off point in frozen cycle was found to be higher as compared to fresh cycle, 277.05 IU/L vs 117.5 IU/L. Blastocyst pregnancies recorded overall higher predictor hCG level as compared to cleavage state in all the outcomes measured; singleton live birth (372.30 IU/L), early pregnancy loss (107.60 IU/L), and multiple pregnancies (711.40 IU/L).

Conclusion: A single reading of serum hCG taken at day 16 post-oocyte retrieval or day 16 from the addition of progesterone in a frozen cycle will help to determine the outcomes of IVF pregnancies and direct the physicians during counselling sessions and plan for further follow-up of the patients.

KEYWORDS:
Human chorionic gonadotrophin; assisted reproductive technology; livebirth; frozen embryo transfer

INTRODUCTION

Counselling is very important in assisted reproductive technology (ART) as patients endure high level of stress; both emotionally and physically.[1] In vitro fertilisation (IVF) is associated with multiple adverse pregnancy outcomes including biochemical pregnancies, failing pregnancies, miscarriages and ectopic pregnancies. In a good-quality blastocyst transfer study of 370 patients, the incidence of miscarriage was reported as 6.2%, biochemical pregnancies 8.1% and 1.1% ended with ectopic pregnancies.[2]

An easily available and reliable predictor of successful IVF pregnancies will help to reduce anxiety among patients awaiting the final results of their treatment. Many biomarkers have been evaluated to predict the outcomes of early pregnancies including serum oestradiol,[3] progesterone[4,5] and pregnancy-associated plasma protein A (PAPP-A).[6] One of the makers linked to a successful live birth in IVF pregnancies is the higher mean level of human chorionic gonadotrophin (hCG) on day 12 following embryo transfer.[7]

hCG is a hormone unique to pregnancies and has been used to confirm and monitor pregnancy outcomes. The hCG RNA expression occurred as early as eight cell stage embryo state[8] and is detectable in the serum 10 days after fertilisation.[9] Following blastocyst stage, hCG is mainly produced by the syncytiotrophoblast and serves as an important element in maintaining pregnancy. hCG then is detectable in the urine following 2–3 weeks of fertilisation; when the invasive trophoblastic activity is at maximum, and the levels continue to reach its peak at 10–11th week gestation.

As implantation is a complex process, a proper synchronisation of the blastocyst hormones, biomarkers and endometrial receptivity state is paramount in ensuring good pregnancy outcomes. hCG has been shown to be favouring endometrial tolerance towards the embryo and promoting angiogenesis to result in successful pregnancies.[10] It also plays a vital role in maintaining uterine quiescence by regulating gap junctions in myometrial smooth muscles and enhancing the expression of the progesterone receptors on these cells.[11] Immunomodulator properties of hCG have been well documented by mediating inhibitory properties to Th1 response leading to fetal survival.[12]

The objective of this study is to determine the level of hCG in predicting livebirth and early pregnancy loss in IVF pregnancies.

This article was accepted: 09 November 2022
Corresponding Author: Rahilah Ahmad Shukri
Email: rahilah2000@yahoo.com

MATERIALS AND METHODS

All patients who underwent ART in Hospital Sultanah Bahiyah Alor Setar from 2014 to 2019 were enrolled in this study. Patients' age, infertility factor, stimulation protocol and outcomes were analysed retrospectively. Cases with incomplete data were excluded from this analysis.

IVF Protocol in Fresh Cycle

In fresh IVF stimulation, the majority of the patients were stimulated with an antagonist cycle, except for cases of endometriosis where the ultra-long protocol was used. For antagonist protocol, stimulation typically began on day 1–2 menses, using recombinant follicle stimulation hormone (rFSH) alone or in combination with urinary or recombinant leutinizing hormone (LH), with an average dose of 150–350 iu daily. Trigger of ovulation in form of recombinant hCG was given once three or more leading follicles measured at least 17 mm from transvaginal scan. For patients who were at risk of ovarian hyperstimulation syndrome (OHSS), gonadotrophin releasing hormone (GnRH) agonist was used instead, followed by intensified luteal support (Humaidan protocol) after the oocyte retrieval (OR). In endometriosis patients, pituitary suppression was achieved by administrating long-acting GnRH agonist 3 months prior to IVF stimulation. A mixture of rFSH and urinary or recombinant LH was used, with a continuation of short-acting GnRH agonist for the rest of the stimulation protocols. Recombinant hCG 6500 iu (ovidrel) was used to trigger the oocytes, followed by oocyte retrieval after 36 hours. All patients had intracytoplasmic sperm injection (ICSI) procedures regardless of the cause of infertility.

Frozen Embryo Transfer Stimulation

In frozen embryo transfer, patients were randomly allocated to natural, mild stimulated or hormone replacement cycle. Patients were monitored transvaginally, and once the endometrial thickness reached 8 mm with trilaminar appearance, we started vaginal progesterone, followed by embryo transfer according to the age of embryo. Most of the patients were intended for blastocyst transfer, however in some cases we proceeded with cleavage state embryo transfer in avoidance of public holiday or weekends. Patients then continued their luteal support according to the protocol regime.

Embryo Transfer

All procedures were done as outpatient, using a Wallace or Emtrac embryo transfer catheter, both consisting inner and outer catheter. We transferred either 1–3 embryos at cleavage state or 1–2 blastocyst stage embryo based on the quality of the embryo and age of the patients.

Luteal Support

The luteal support consists of vaginal progesterone 90 mg daily, and 1,000 u hCG injection on days 0, 3, 6 following the oocyte retrieval for antagonist cycle. In patients who were stimulated with ultra-long protocol, oral oestrogen (estradiol valerate 2 mg BD) together with 1000u of hCG on days 0, 3, 6 and twice a day application of 90 mg vaginal progesterone was used for luteal support. For intensified luteal phase support, we followed Humaidan protocol whereby IM hCG injection of 1500u were given on the oocyte retrieval day, and day 5 post-OR along with vaginal progesterone 90mg BD and oestradiol valerate 2 mg TDS.

hCG Analysis

Serum hCG was taken on (i) day 16 post-oocyte retrieval or (ii) day 16 from the addition of progesterone in a frozen cycle.

The results were analysed in our biochemistry laboratory using the standard immunoassay method, and the level of 10 IU/L or more had been used to define biochemical pregnancy. For low level of HCG 10–20 IU/L, repeated samples were performed to determine the outcome of pregnancy. All pregnancies that ended with miscarriage, ectopic or failing biochemical pregnancy were recorded. Once the serum hCG was reported as biochemical pregnancy, we continued the luteal support and transvaginal ultrasounds were performed 3–4 weeks following the positive result to confirm the location and viability of pregnancy. Clinical pregnancies were confirmed with the presence of fetal heart activity from the scan and ongoing pregnancy was defined as pregnancy that lasted at least 12 weeks of gestation.

Statistical analysis

Statistical analysis was performed with SPSS version 25. The data were analysed separately, broadly divided into IVF outcomes – singleton livebirth and early pregnancy loss (miscarriage, ectopic pregnancy, and failed biochemical pregnancy). A separate analysis was performed for multiple pregnancy, knowing that the result would affect the overall level of hCG. Median values of hCG were computed and compared with non-parametric test (Mann–Whitney U test). Non-parametric receiver operating characteristics (ROC) curve was drawn to determine the cut-off value of hCG for predicting live birth in fresh and frozen cycle, multiple pregnancies with adequate sensitivity and specificity. For all the statistical test, the level of < 0.05 was taken as significant.

RESULTS

A total of 407 IVF pregnancies were analysed; 297 were fresh IVF cycles stimulation with 110 cases of frozen embryo transfers. Most of the embryo transfers were performed at cleavage state (D2-D3 embryo), comprised of 299 cases while the rest of 108 patients had blastocysts transfer.

Majority of the patients were diagnosed with male infertility; 122 patients (30%), followed by 81 cases of endometriosis (20%), 70 patients with PCOS and unexplained infertility (17% each group) and lastly tubal factors; 64 patients (16%). The mean age of the patients who attended our clinic was 32 years old. 13 patients (3%) were 40 years old and above; and 38% of them had early pregnancy loss despite of having good quality embryo transferred. Almost 70% of the IVF pregnancies ended with live birth, with the incidence of multiple pregnancies at 24%. Out of 129 patients who had early pregnancy loss, 18% had failing biochemical pregnancies, 8% had ectopic pregnancies and majority of the patients had miscarriages.

Table I: Median hCG level in all IVF outcomes (n=?)

	All pregnancies (cleavage and blastocyst) (n=407)	Cleavage embryo pregnancies (n= 299)	Blastocyst embryo pregnancies (n= 108)
	hCG level IU/L Median (QR)	hCG level IU/L Median (QR)	hCG level IU/L Median (QR)
Overall singleton livebirth (fresh and frozen cycles)	304.70 (267.40)	219.70 (257.00)	372.70 (424.78)
Fresh cycle singleton livebirth	223.00 (233.53)	208.00 (189.03)	312.95 (320.77)
Frozen cycle singleton livebirth	415.40 (398.70)	300.00 (393.90)	450.15(563.73)
Multiple live births	547.10 (584.20)	524.00 (349.90)	711.40 (1182.40)
Early pregnancy loss	77.00 (146.00)	53.55 (115.27)	107.60 (268.50)

hCG Prediction Level of IVF Outcomes
HG: human chorionic gonadotrophin, IUL: International Units Per Liter (IU/L)

Table II: hCG predictor level in of IVF outcomes

	All pregnancies (cleavage and blastocyst) (n=407)			Cleavage embryo pregnancies (n=299)			Blastocyst embryo pregnancies (n=108)		
	hCG level IU/L	Sen %	Spe %	hCG level IU/L	Sen %	Spe %	hCG level IU/L	Sen %	Spe %
Overall singleton live birth (fresh and frozen)	152.85	81.3	71.9	198.00	54.8	60.7	291.35	61.5	60.7
Fresh cycle singleton live birth	111.75	71.9	29.6	202.20	51.5	51.2	304.50	53.61	53.8
Frozen cycle singleton live birth	277.05	66.0	64.6	245.55	60.0	63.3	361.85	62.5	61.9
Multiple live births	322.4	78.6	64.3	303.75	78.0	77.9	553.30	72.7	76.3
Early pregnancy loss	174.50	61.5	60.7	153.55	77.6	77.6	265.95	71.1	71.4

Sen = sensitivity; Spe=specificity.

Median hCG Level in IVF Outcomes
Table I shows the median hCG level measured in this study. The overall median of hCG level in singleton livebirth was 304.70 IU/L with 267.40 IU/L of interquartile range. When subgroup analysis was performed, the serum hCG level was significantly higher in frozen cycles, as compared to fresh cycles; 415.40 IU/L vs 223.00 IU/L (p 0.002). Patients with multiple live births showed a higher median hCG level of 547.10 I IU/L, and the median hCG level in early pregnancy loss was 77.00 IU/L.

Analysis was then further divided according to the state of embryos; cleavage vs blastocyst. Blastocysts pregnancies recorded overall higher median hCG level as compared to cleavage state in all the outcomes measured.

Singleton livebirth
Figure 1 shows ROC curve plotted the predicted value of hCG level in total singleton livebirth (fresh and frozen cycle) as 152.85 IU/L, with the area under the ROC curve (AUC) of 0.822, a sensitivity of 81.3% and a specificity of 71.9%.

Blastocysts embryos recorded a higher level of hCG; 291.35 IU/L (sensitivity 61.5% specificity 60.7%) as compared to the cleavage embryos. (Table II).

Fresh vs frozen cycle singleton livebirth
The optimal hCG prediction level in singleton livebirth for the frozen group was higher; 277.05 IU/l (Figure 2) with area under the ROC curve (AUC) of 0.647 (sensitivity 66.0% specificity 64.6%) vs 111.75 IU/l in fresh cycle transfer (sensitivity of 71.9% and a specificity 29.6%)

Blastocysts pregnancies recorded a higher singleton livebirth predicted level in both fresh and frozen cycles. The prediction level of in frozen was 361.85 IU/L (sensitivity 62.5%) as compared to 304.50 IU/L in fresh cycle with a sensitivity of 53.61% (Table II).

Multiple live births
The ROC curve analysis showed the predicted value of hCG level in multiple pregnancies as 322.40 IU/l with area under the ROC curve (AUC) of 0.758, a sensitivity of 78.6% and a specificity of 64.3% (Figure 3). The cut-off point in cleavage embryo is lower as compared to blastocysts multiple livebirth; 303.75 IU/L (sensitivity 78% specificity 77.9%) vs 533.30 IU/L (sensitivity 72.7% specificity 76.3%), as seen in Table II.

Pregnancy loss
The overall pregnancy loss predicted hCG level was 174.50 IU/L with a sensitivity of 61.5% and specificity of 60.7%. Higher level was observed in blastocyst transfer at 265.95 IU/L as opposed to cleavage embryo transfer at 153.55 IU/L (Table II)

DISCUSSION

Multiple approach of measuring hCG post-embryo transfer has been proposed in predicting the IVF outcomes; including single measurement during peri-implantation period (day 5

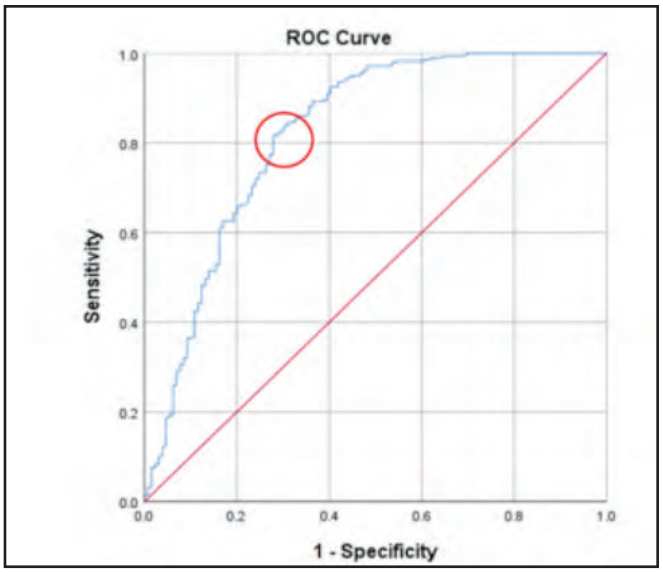

Fig. 1: ROC curve of hCG values predicting singleton livebirth in both frozen and fresh cycle

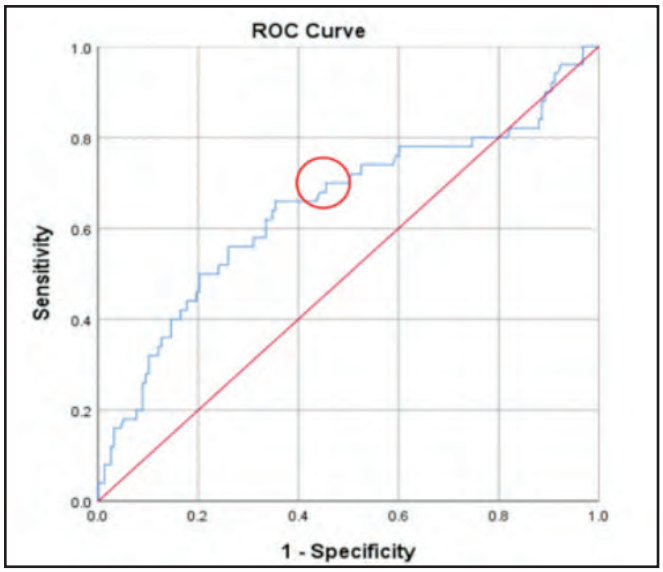

Fig. 2: ROC curve of hCG values predicting singleton livebirth in frozen cycle

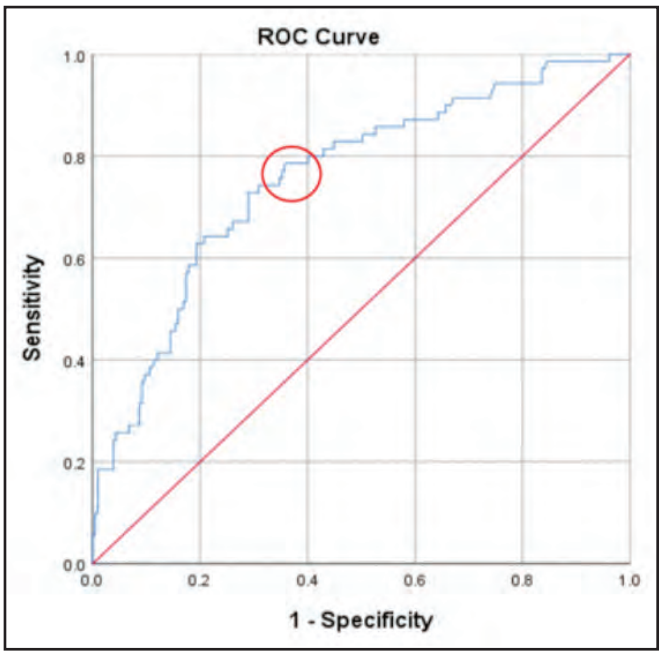

Fig. 3: ROC curve of hCG values predicting multiple pregnancies

post-transfer), day 12–14 post-embryo transfer and serial hCG measurement following embryo transfer. Saphiro et al[13] found that peri-implantation bHCG level of > 5IU/L (at day 5 following blastocyst transfer), resulted in 83% of ongoing pregnancy.

Few other researchers suggested comparing two levels of bhCG as a better predictor of IVF outcomes. For example, hCG index (day 12 hCG / day 8 hCG) of > 3.5 has been associated with 72.3% sensitivity and 100% specificity in predicting ongoing pregnancy.[2] Another paper by Hongbin et al suggested a hCG ratio on day 21 and day 14 post embryo transfer (hCG 21/hCG 14) as a predictor of viable pregnancy with level of >15 to be statistically significant.[14]

Considering cost-effectiveness and practicality point of view, majority of the practitioners still resort into single bHCG measurement at day 12–14 following embryo transfer. Even though the earliest serum bHCG is traceable in the serum at day 8–10 after fertilisation, it should be present and detectable from day 12 onwards. Based on the doubling level, measuring serum hCG on day 14–16 post-oocyte retrieval (fertilisation) represents the strength of implantation.[24]

In our centre, serum hCG is taken on day 16 post-oocyte retrieval (day 11–14 post-embryo transfer depending on the embryo age) not only for the reason mentioned above, but also for the fact that hCG injection was also used as part of the luteal support, and it takes 48–72 hours to be excreted from the system.[25] Meanwhile, for frozen embryo transfer, the day of progesterone commencement was referred to as the day of fertilisation.

In the present study, it is predicted that the overall median hCG level tends to be higher (304.70 IU/L) as the outcomes measured were singleton live birth. In a study by Poikkeus et al, a mean concentration hCG of 126 IU/L on day 12 post-embryo transfer was found to be associated with viable pregnancy; with a mean level of 115 IU/L in singleton and 201 IU/L in multiple pregnancies.[15] Kumbak et al3 further discussed a significant different levels of hCG in 2035 cycles for cleavage state embryo as compared to blastocyst transfer; with higher level found in D5 embryos.[3] Our result matched the previously published data with higher median hCG level recorded in blastocyst transfer as opposed to the cleavage state embryo; despite the small sample size.

Another interesting finding in this study is the mean hCG level of frozen embryo transfer was significantly higher than the fresh cycle. Even though the positive predictive values were low, the higher level of negative predictive values

indicates better accuracy of negative results. On the contrary, analysis in Sun Yat-Sen university hospital in Guangzhou reported a higher level of hCG predictor for live birth in both frozen (410.8 mIu/L) and fresh (222.86 mIU/L) cycle. However, their study only included blastocyst transfer which would eventually reveal a higher level.[16] Our blastocyst predictor hCG level was slightly lower; 361.85 IU/L for frozen cycle, and 304.50 IU/L for fresh cycle, most likely due to a smaller sample size.

Kalra et al[17] stated that the hCG rise level was noted to be higher in frozen embryo transfer even after adjustment of multiple pregnancies. They postulated the possibility of the best embryo surviving the whole thawing process together with physiologic endocrine environment during implantation might have influenced the outcomes and suggested further studies.[17] Previous studies also explained on the possibility of optimum endometrial receptivity in frozen cycle which result in better overall implantation rate, hence resulting in a higher hCG level.[18] The negative effect of the supra-physiological concentration of oestrogen and progesterone on the endometrium in fresh cycle may slow down the implantation process and release of hCG into the circulation. Another possible explanation is the rate of blastocyst growth during the transfer process, whereby some of the embryologist gave extra time for early blastocyst to grow in the thawing media and transfer a more mature embryo, therefore affecting the hCG result.[19]

Frozen embryo are also associated with higher birth and placental weight,[20] and previous data revealed that the level of hCG is also associated with fetal weight. A study by Barjaktrovic et al[21] found low level of bHCG in late first trimester has been associated with small gestational age in female fetus. On the other hand, Xiong et al[22] revealed a higher level hCG day 11 post blastocyst transfer in pregnant women with a male fetus in both fresh and frozen cycle. In our study, we did not look into the fetal weight and gender of the fetus to dispute or support this hypothesis.

In multiple live births, majority (90%) of our patients had twin pregnancies, followed by 9% of triplets and 1 % had quadruplets. Blastocysts pregnancies had a higher hCG level predictor as compared to cleavage embryos : 533.3 IU/L (sensitivity 72.7% specificity 76.3%) vs 303.75 IU/L (sensitivity 78.0% specificity 77.9%). A higher cut-off point was found in the study by Neeta Singh[23] with a level of 808 IU/L (sensitivity of 70% and specificity of 72%); however, the proportions of the higher order multiple gestations were not stated, which will affect the overall level of hCG.

A similar pattern was observed in pregnancy loss level, with a higher hCG level noted in blastocyst transfer; suggesting a need for closer monitoring with hCG level of 265.95IU/L or lower, as the sensitivity for miscarriage and ectopic pregnancy was 71%.

LIMITATIONS

This study has a few limitations. Firstly, it involves a small number of participants. Secondly, outcomes of the study may be less accurate as it involves both cleavage and blastocysts stage embryo transfer, therefore generating a slightly lower level of overall hCG. And lastly, a single study centre also serves as another limitation to the study, as it may increase the bias in result interpretation.

It is well known that there are many factors affecting the outcomes of IVF pregnancies; including the embryo qualities, endometrium, uterine factors and maternal age. However, for this study purpose, we focused on patients who were confirmed pregnant, based on single reading of hCG to further guide in monitoring and counselling.

We proposed a larger data collection involving only blastocyst transfer to capture the real value of predictive hCG level with consideration of other parameters such as the gender and birth weight of the babies which may affect the overall hCG level.

CONCLUSION

Single measurement of hCG on day 16 post oocyte retrieval (or day 16 from the addition of progesterone in frozen cycle) remains a practical and reliable approach to predict IVF outcomes. Not only it helps patients to understand the possibility of different IVF results at the end of treatment, it also serves as a guide for the practitioners in planning for further follow-up and subsequent management for the patients.

REFERENCES

1. Kee BS, Jung BJ, Lee SH. A study on psychological strain in IVF patients. J Assist Reprod Genet 2000; 17(8): 445-8.
2. De Neubourg D, Gerris J, Mangelschots K, Van Royen E, Vercruyssen M, Elseviers M. Single top quality embryo transfer as a model for prediction of early pregnancy outcome. Hum Reprod 2004; 19(6): 1476-9.
3. Kumbak B, Oral E, Karlikaya G, Lacin S, Kahraman S. Serum oestradiol and β-HCG measurements after day 3 or 5 embryo transfers in interpreting pregnancy outcome. Reprod Biomed Online 2006; 13(4): 459-64.
4. Martínez F, Coroleu B, Clua E, Tur R, Buxaderas R, Parera N, et al. Serum progesterone concentrations on the day of HCG administration cannot predict pregnancy in assisted reproduction cycles. Reprod Biomed Online 2004; 8(2): 183-90.
5. Ioannidis G, Sacks G, Reddy N, Seyani L, Margara R, Lavery S, et al. Day 14 maternal serum progesterone levels predict pregnancy outcome in IVF/ICSI treatment cycles: A prospective study. Hum Reprod 2005; 20(3): 741-6.
6. Yaron Y, Heifetz S, Ochshorn Y, Lehavi O, Orr-Urtreger A. Decreased first trimester PAPP-A is a predictor of adverse pregnancy outcome. Prenat Diagn 2002; 22(9): 778-82.
7. Bjercke S, Tanbo T, Dale PO, Mørkrid L, Abyholm T. Human chorionic gonadotrophin concentrations in early pregnancy after in-vitro fertilization. Hum Reprod 1999; 14(6): 1642-6.
8. Bondueue M-L, Dodd R, Liebaers I, Steirteghem A Van, Williamson R, Akhurst R. Chorionic gonadotrophin-/3 mRNA, a trophoblast marker, is expressed in human 8-cell embryos derived from tripronucleate zygotes. Human Reprod 1988; 3(7): 909-14
9. Makrigiannakis A, Vrekoussis T, Zoumakis E, Kalantaridou SN, Jeschke U. The role of HCG in implantation: A mini-review of molecular and clinical evidence Int J Mol Sci 2017; 18(6): 1305.
10. Tsampalas M, Gridelet V, Berndt S, Foidart JM, Geenen V, d'Hauterive SP. Human chorionic gonadotropin: A hormone with immunological and angiogenic properties. J Reprod Immunol 2010; 85(1): 93-8.

11. Horiuchi A, Nikaido T, Yoshizawa T, Itoh K, Kobayashi Y, Toki T, et al. HCG promotes proliferation of uterine leiomyomal cells more strongly than that of myometrial smooth muscle cells in vitro. Mol Hum Reprod 2000; 6(6): 523-8
12. Schumacher A, Costa SD, Zenclussen AC. Endocrine factors modulating immune responses in pregnancy. Front Immunol 2014; 5: 196
13. Shapiro BS, Daneshmand ST, Restrepo H, Garner FC. Serum HCG measured in the peri-implantation period predicts IVF cycle outcomes. Reprod Biomed Online 2012; 25(3): 248-53.
14. Chi H, Qiao J, Li H, Liu P, Ma C. Double measurements of serum HCG concentration and its ratio may predict IVF outcome. Reprod Biomed Online 2010; 20(4): 504–9.
15. Poikkeus P, Hiilesmaa V, Tiitinen A. Serum HCG 12 days after embryo transfer in predicting pregnancy outcome. Hum Reprod 2002; 17(7): 1901-5
16. Zhao W, Li Y, Ou J, Sun P, Chen W, Liang X. Predictive value of initial serum human chorionic gonadotropin levels for pregnancies after single fresh and frozen blastocyst transfer. J Huazhong Univ Sci Technol - Med Sci 2017; 37(3): 395-400.
17. Kansal Kalra S, Molinaro TA, Sammel MD. Viable pregnancies following fresh versus frozen embryo transfer: is there a difference in the rate of serum human chorionic gonadotropin (hCG) rise? Fertil Steril 2008; 90: S205.
18. Evans J, Hannan NJ, Edgell TA, Vollenhoven BJ, Lutjen PJ, Osianlis T, et al. Fresh versus frozen embryo transfer: Backing clinical decisions with scientific and clinical evidence. Hum Reprod Update 2014; 20(6): 808-21.
19. Zhu W, Yeung Q, Chan D, Chi L, Huang J, Wang Q, et al. Maternal β-HCG concentrations in early IVF pregnancy: association with the embryo development stage of blastocysts. Reprod Biomed Online 2019; 38(5): 683–90.
20. Pereira N, Baergen RN, Kelly AG, Pryor KP, Elias R, Rosenwaks Z. Birth weight differences of term singletons after frozen or fresh embryo transfer: what does placental histology reveal? Fertil Steril 2017; 108(3): e82.
21. Barjaktarovic M, Korevaar TIM, Jaddoe VWV, de Rijke YB, Visser TJ, Peeters RP, et al. Human chorionic gonadotropin (hCG) concentrations during the late first trimester are associated with fetal growth in a fetal sex-specific manner. Eur J Epidemiol 2017; 32(2): 135-44.
22. Xiong F, Sun Q, Li GG, Chen PL, Yao ZH, Wan CY, et al. Initial serum HCG levels are higher in pregnant women with a male fetus after fresh or frozen single blastocyst transfer: A retrospective cohort study. Taiwan J Obstet Gynecol 2019; 58(6): 833–9.
23. Singh N, Begum AA, Malhotra N, Bahadur A, Vanamail P. Role of early serum beta human chorionic gonadotropin measurement in predicting multiple pregnancy and pregnancy wastage in an in vitro et fertilization cycle. J Hum Reprod Sci 2013; 6(3): 213-8.
24. Lambers MJ, van Weering HGI, van't Grunewold MS, Lambalk CB, Homburg R, Schats R, et al. Optimizing hCG cut-off values: A single determination on day 14 or 15 is sufficient for a reliable prediction of pregnancy outcome. Eur J Obstetrics Gynecol Reprod Biol 2006; 127(1): 94-8.
25. Bjercke S, Tanbo T, Dale PO, Mørkrid L, Abyholm T. Human chorionic gonadotrophin concentrations in early pregnancy after in-vitro fertilization. Human Reprod 1999; 14(6): 1642-6.