



The level of serum b-human chorionic gonadotropin hormone predicting clinical and continued pregnancy in IVF/ICSI cycles, in SPHMMC IVF center, Addis Ababa, Ethiopia, 2023

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Abbreviations and Acronyms

ART	Assisted Reproductive Technology
AUC	Area Under The Curve
CFRM	Center for Reproductive Medicine
ECL	Electrochemiluminescence
ET	Embryo Transfer
FMOH	Federal Ministry of Health
HCG	Human Chorionic Gonadotropin
ICS	Intracytoplasmic Sperm Injection
IVF	In vitro Fertilization
LR+	Likelihood ratio positive
LR-	Likelihood ratio negative
ROC	Receiver Operating Characteristics Curve
TVUS	Trans-vaginal Ultrasound

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Abstract

Background: After assisted reproductive technology (ART), most IVF centers in the world measure serum beta human chorionic gonadotropin level not only to diagnose pregnancy but also to predict pregnancy outcomes. However, the results were inconsistent from setup to setup in most IVF centers, due to the serum beta human chorionic gonadotropin (Hcg) hormone was determined at different dates ranging from day eight to sixteen and most studies recommend each IVF hospital laboratory is supposed to have its own beta hcg cut-off value to predict pregnancy outcomes. This study will help in counseling and avoid adverse psycho-social impacts on the couple life and unnecessary stress on the managing physician. It will also be baseline information for further studies.

Objective: The aim of this study was to determine the level of serum beta human chorionic gonadotropin hormone that can predict clinical and continued pregnancy in IVF/ICSI cycles in a recently opened public IVF center, Addis Ababa, Ethiopia, 2023.

Methods: Using secondary data from the SPHMMC's Department of Obstetrics and Gynecology, Reproductive Endocrinology and Infertility Unit database, a cross-sectional study design was used. Kobo toolbox was used to collect data from the electronic medical record of the center. After data completeness was checked, it was cleaned, coded and entered into computer software of STATA version 17 for analysis. Descriptive analysis was done for general characteristics of study participants. A receiver operating characteristic (ROC) curve was used to calculate the optimum β -hCG cutoff values to predict clinical pregnancy and continued pregnancy. The area under the curves and their 95% confidence intervals was calculated for each ROC curve.

Results: This study included 331 IVF/ICSI cycles with serum hCG hormone level of $>5\text{miu/ml}$. The cutoff value for beta human chorionic gonadotropin hormone to predict clinical pregnancy is 226miu/ml , with an AUC of 0.92, sensitivity of 90.24%, and specificity of 86.36%. The beta human chorionic gonadotropin hormone cutoff values to predict continued pregnancy was found to be at 448miu/ml with AUC of 0.74, sensitivity of 77.97%, and specificity of 60.78% on ROC analysis

Conclusion: The cutoff value of serum human chorionic gonadotropin hormone predicting clinical and continued pregnancy is relatively higher than the other studies.

1. Introduction

1.1 Background

One in every four couples in developing countries is affected by infertility (1). Infertility, as such, is one of the major public health problems identified in Ethiopia. It is estimated that infertility affects 15 to 20% of couples in Ethiopia(1, 2). From a cross-sectional study in three governmental hospitals in Addis Ababa, overall prevalence of infertility was 27.6%(3). Of these, 14.4% had primary infertility, and 13.2% had secondary infertility (3). Now a days, Assisted reproductive technology (ART) is the most preferred option of management for infertile couples to achieve pregnancy. However, Pregnancies achieved through in-vitro fertilization (IVF) are associated with adverse first trimester outcomes in comparison to spontaneously achieved pregnancies. In view of this, it is imperative to predict the success as well as prognosticate the pregnancy outcome of an IVF cycle. Different hormones have been used to predict pregnancy outcome after assisted reproductive treatments but Serum beta human chorionic gonadotropin (Hcg) hormone is a well-known strong biomarker used to diagnose pregnancy after assisted reproductive technology (ART)

Human chorionic gonadotropin (hCG) is a glycoprotein secreted during pregnancy by developing trophoblast cells of the placenta ((4). HCG is composed of two glycosylated distinguished subunits called alpha and beta (β) subunits.

Beta human chorionic gonadotropin (b-hCG) is a hormone secreted by placental syncytiotrophoblast and detectable in maternal serum as early as 8 days after ovulation (4, 5) The initial detection of b-hCG in urine or serum is the most common indicator of pregnancy onset(5)

The syncytiotrophoblast is the main source of hCG production whose 99% is secreted in maternal blood. hCG and its free beta subunit are detected in the maternal blood from the second week of pregnancy and their levels increase until reaching a peak at about 10 to 12 weeks of pregnancy and then decrease gradually, while its free alpha subunit increases progressively up to term(6).

Higher β -hCG levels are associated with improved clinical outcomes, continued pregnancy and live birth while low level predicts adverse pregnancy outcomes.(7)

1.2 Statement of the problem

The first fourteen days post-embryo transfer after IVF/ICSI is the most stressful time for both couples and IVF specialist until pregnancy outcome is known. A reliable and inexpensive diagnostic test to predict early pregnancy outcome might reduce the psychological tension and anxiety present in many of these couples, and also reduce the cost and the stress to the managing physicians(8)

Following IVF treatment, most clinics offer routine β -human chorionic gonadotropin (β -HCG) testing around 9–14days after embryo transfer to verify whether the treatment has been successful (9). The results not only help to confirm whether pregnancy has occurred, but will also provide additional information regarding prognosis (9)

Early diagnosis of adverse pregnancy outcomes such as, miscarriage and ectopic pregnancy, allows effective action to prevent further complications to the woman. Fourteen days after embryo transfer serum beta hCG level will be determined and it will be documented as a positive pregnancy when it is ≥ 5 -10 mIU/ml. However only those with higher level of hCG will achieve a clinical pregnancy, continued pregnancy and live birth. On the other hand some other studies showed the possibility of live birth in patients with low serum beta hCG (10). Though there were tremendous studies regarding ability of beta hcg hormone in predicting pregnancy outcome, no ideal cut-off value was found to predict clinical pregnancy, live birth or early pregnancy loss.

From different previous studies the interpretation of hcg result was affected by multiple confounding factors. Among these, the grade and the number of embryos transferred, day of embryo transfer, and the type of fertilization were found to effects on the interpretations. On the other, some other studies also implicated the effect of these confounders is controversial. This is usually a challenge for the managing physicians whether to continue or stop the luteal phase support and regarding counseling of the couples about pregnancy outcome.

Several studies have been done on the cut-off value of serum beta human chorionic gonadotropin hormone (HCG) to predict pregnancy outcomes after IVF/ICSI cycles. However, the results are non-uniform and and timing of beta hCG determinations are different which could affect the interpretations (11-13). Therefore, this study aimed to determine the cut-off value of serum beta human chorionic gonadotropin hormone level that can predict clinical pregnancy.

1.3 Significance of the study

Though, there is an ample data regarding the level of serum beta hcg predicting pregnancy outcome still there is paucity of enough research in African population(14). Ideally, each hospital laboratory need to have its own optimistic value to diagnose pregnancy and predict IVF/ICSI outcome but we don't have any published studies about what level of serum beta human chorionic gonadotropin hormone level could predict clinical pregnancy and how low level can predict early pregnancy adverse outcomes. In addition we do not have a reference value of hcg for our population and the findings of this study will be a baseline data for Ethiopian population as this hospital is a public hospital serving for couples coming from different corners of the country.

Most importantly, this study will help to avoid adverse psycho-social impacts on the couples life and unnecessary stress on the managing physician during the post embryo transfer period especially the first two to four weeks after positive pregnancy result until ultrasound confirmation of pregnancy. It would also help us early detection of pregnancy complications and minimize the luteal phase support cost. Finally this research would be a baseline to establish our own counseling protocol that would be representative of our population than using the westerns population as a reference

1.4 Literature review

There were several studies performed worldwide in the last decade regarding the predictively of pregnancy outcome based the early serum beta hCG level fourteen days after embryo transfer of IVF/ICSI cycles. However, the results were inconsistent from one setup to the setup and in most IVF center, the serum beta human chorionic gonadotropin(Hcg) hormone was determined at different dates post- embryo transfer ranging from day 8 to 16(5, 9, 13, 15)

One retrospective cohort study, which included 772 vitrified-warmed single blastocyst transfer cycles. The initial maternal serum HCG concentrations measured on day 10 after blastocyst transfer were evaluated using receiver operating characteristic (ROC) curves to predict clinical pregnancy and live birth(12). Threshold values for predicting a clinical pregnancy were established in three different age groups: group A (21–29 years old, n = 360), group B (30–34 years old, n = 290) and group C (35–47 years old, n = 122). The areas under the ROC curves for

clinical pregnancy and live birth were 0.986 and 0.922, and the corresponding cut-off values were 113.28 and 146.37 mIU/ml, respectively.

The optimal threshold values for clinical pregnancy as indicated by Youden index values for the three age groups were 145.15, 126.25 and 94.44 mIU/ml, respectively (12).

Another retrospective cohort study showed rate of rise of β -hCG (β -hCG kinetics) was calculated in each cycle and its association with blastocyst quality and pregnancy clinical outcome was evaluated. Results: b-hCG kinetics was significantly different according to blastocyst expansion, but with considerable overlap between groups. b-hCG kinetics was also significantly different according to clinical outcome, with higher values in clinical pregnancies than in other groups. This remained true in subgroups' analysis according to blastocyst expansion and in top quality blastocysts. Conclusion(s): Early b-hCG kinetics after single-blastocyst transfer is different according to pregnancy outcome and is only slightly influenced by blastocyst quality. These results confirm the interest of b-hCG follow up in IVF pregnancies, and do not support the interest of building blastocyst-specific b-hCG ranges in IVF cycles (5)

One study done in India From Jan 2005 to Dec 2006, a total of 198 embryo transfers, Day 12 post embryo transfer The hCG level of 76 IU/l was a suitable cut-off point for predicating viable pregnancies with 80% sensitivity and 82% specificity(16). All biochemical pregnancies were found at hCG levels < 100 IU/l. The probabilities of biochemical pregnancy, miscarriage and normal pregnancy were similar, about 31% each. Ectopic pregnancy (EP) was suspected with hCG ranges up to 80 IU/l(16). When calculating the results for the fresh vs frozen embryo cycles resulting in a viable singleton pregnancy, no difference in the median hCG values was observed (116 vs 115 IU/l respectively

One retrospective study conducted in China to determine the Independent value of serum b-human chorionic gonadotropin in predicting early pregnancy loss risks in IVF/ICSI cycles showed the optimal serum b-hCG cut-off value for predicting EPL was 542.45 IU/L. and results suggest that the serum b-hCG level has a strong independent predictive value for EPL occurrence in fresh IVF/ICSI cycles (13). B-hCG level was considerably lower in women who had EPL than in those who were ongoing pregnancy (564.03 ± 838.16 vs 1139.04 ± 1048.72 IU/ L, $p < 0.001$). More importantly, the b-hCG level could independently predict the occurrence of EPL and was comparable to the model that combined other risk factors.

However, another retrospective study in Jordan showed an HCG value of 145 IU/l emerged as the most suitable cutoff point to predict viable pregnancy and concluded that HCG cutoff values determined by a ROC curve analysis are useful but not absolute for discriminating between viable and non-viable pregnancy outcomes on day 14 after embryo transfer(11). Pregnancies were classified as viable (live fetus at 24 weeks gestation) or non-viable (biochemical pregnancy, miscarriage, ectopic pregnancy). Receiver operating characteristic (ROC) curve analysis was used to evaluate the cutoff value of HCG with maximal sensitivity and specificity to differentiate between viable and non-viable pregnancies. The median HCG concentration was 264 IU/l in viable pregnancies and 120 IU/l in non-viable pregnancies ($p < 0.001$). The median HCG concentration was 222 IU/l in singleton pregnancies, 389 IU/l in twin pregnancies and 809 IU/l in triplet pregnancies ($p < 0.001$). So, they recommended continuation of luteal phase support and monitoring even in those with low serum beta hCG level on day 14 after embryo transfer.

Another study in Canada with the objective of identifying the pregnancy outcomes based on day-16 β -hCG level assessed with modern assays, There was no difference on outcomes based on cleavage or blastocyst transfer. At a serum β -hCG level of 103 ± 13 (range 74–135), 50% had a biochemical loss. Biochemical pregnancy losses remained 21% at serum β -hCG range (136–197). It was only once serum β -hCG level reached 199–252 that the probability of a biochemical pregnancy loss was 12%. Interestingly, if a clinical pregnancy is present even at low day-16 serum β -hCG levels, the likelihood of live birth is approximately 50%. This maximizes to 75% when the serum β -hCG level was at least 253 IU/L. The relationship between serum day16 β -hCG levels and clinical pregnancy or live birth is quite strong with correlation coefficients above 0.8 which accounted for more than 75% of the variability in outcomes in both cases. Receiver operator curves determined that the cut-off for a clinical pregnancy was 190 and for live birth, it was 213 IU/L. An increase in the serum β -hCG levels at which to expect a reassuring outcome is required based on modern assays, as compared with the old cut-off levels(4)

Another retrospective study done in Seoul, Korea showed that Serum hCG- β levels of postovulatory day 12 and 14 with the sequential application of hCG- β fold change significantly increased predictability of pregnancy outcome after IVF-ET Cycle(15). The mean serum hCG- β level on POD 12 in the live birth group was 65.2 ± 32.4 mIU/mL in fresh cycles, which was significantly higher than those of biochemical pregnancy (20.0 ± 17.2 mIU/mL) and early

pregnancy loss groups (47.9 ± 30.3 mIU/mL) ($P < 0.001$). In frozen cycles, the mean serum hCG- β levels on POD 12 in live birth group was 73.0 ± 42.1 mIU/mL, which was significantly higher than those of biochemical pregnancy (29.3 ± 29.1 mIU/mL) and early pregnancy loss groups (51.5 ± 39.4 mIU/mL).

The mean serum hCG- β levels checked on POD 14 in the live birth group of fresh and frozen cycles were 197.2 ± 98.1 mIU/mL and 222.6 ± 131.6 mIU/mL each. These were significantly higher than those of biochemical pregnancy (fresh cycle, 38.6 ± 42.1 mIU/mL; frozen cycle, 60.2 ± 71.0 mIU/mL, $P < 0.001$) and early pregnancy loss groups (137.5 ± 87.1 mIU/mL, 148.9 ± 122.1 mIU/mL). The mean hCG- β levels on POD 12 and 14 after frozen cycles were significantly higher than those after fresh cycles regardless of pregnancy outcomes. They focused on the fold increase of hcg from day 12 to day 14: In fresh cycles, the increase in biochemical pregnancy, early pregnancy loss, and live birth groups were 2.0 ± 1.3 , 3.0 ± 1.0 , and 3.1 ± 0.8 , respectively. In frozen cycles, the increase were 1.9 ± 0.9 , 3.0 ± 1.0 , and 3.1 ± 0.9 in biochemical pregnancy, early pregnancy loss, and live birth groups, respectively. However, this seems impractical and not cost effective for resource limited setups like ours.

Another recent study showed possibility of clinical pregnancy and live birth after ART even with low day fourteen serum beta human chorionic gonadotropin(10). The study included patients with serum β -hCG levels < 300 mIU/ml at 14 days after frozen blastocyst transfer, among which, 18.6% were live births, 47.4% were early miscarriages, 22.8% were biochemical pregnancies and 9.6% were ectopic pregnancies. ROC curve analysis showed that a predicted value of β -hCG for clinical pregnancy was 58.8 mIU/ml with an area under the ROC curve (AUC) of 0.752, a sensitivity of 95.0% and specificity of 53.5%

In conclusion, all the studies mentioned above have different timing of beta hCG determination and different cut off values suggesting that each laboratory of IVF setups need to have its own study to determine the cut off value of serum beta hCG at a specific date of post-embryo transfer to be applied as a protocol.

2. Objectives

2.1 General objective

To determine the level of serum b-human chorionic gonadotropin hormone predicting clinical and continued pregnancy in IVF/ICSI cycles, in SPHMMC IVF center, Addis Ababa, Ethiopia, 2023

2.2 Specific objectives

To determine the level of beta HCG hormone predicting clinical pregnancy

To determine the level of beta HCG hormone predicting continued pregnancy

3. Methodology

3.1 Study design

Cross-sectional study design was used.

3.2 Study Setting and Period

Review of record was conducted in the department of OBGYN reproductive endocrinology and infertility (REI) unit, center for fertility and reproductive medicine (CFRM), at SPHMMC from February, 2022G.C. to March, 2023 G.C. The center is governed by federal minster of health. It is the first and the only public IVF center for the country.

3.3 population

3.3.1 Source population

- The source population was all patients who have undergone embryo transfer and whose serum hcg level at day 14 was higher than 5miu/ml at Center for reproductive medicine

3.3.2 Study population

The study populations was all patients who have undergone embryo transfer having serum hcg level at day 14 was higher than 5miu/ml during the study period.

3.3.3 Sample population

Sample population was all study population who full fill the inclusion and exclusion criteria

3.4 Inclusion and exclusion criteria

3.4.1 Inclusion criteria

All patients who undergo IVF/ ICSI cycles and post-ET day 14 serum beta hcg level is >5miu/ml

3.4.2 Exclusion criteria

Patients who has early serum beta hCG determination but who are lost for ultrasound scan at 6 and 12 weeks. Patients whose serum beta hCG value is determined outside our laboratory.

Missing or incomplete documentation of patient profiles

3.5 Sample size and sampling techniques

3.5.1 Sample size calculation

The sample size was calculated with 95% confidence interval, 5% margin of error and 30% prevalence of clinical pregnancy rate from patients undergone IVF/ICSI in CFRM public ivf center, retrospective cross-sectional study done in Addis Ababa(2021, published)(1).

The required sample size was calculated using a single population proportion formula.

All consecutive patients who fulfill the study inclusion and exclusion criteria during the study period were reviewed until the desired sample size is reached.

$$n = \frac{(Z_{\alpha/2})^2 P (1-P)}{d^2}$$

$$n = \frac{(1.96)^2 \times (0.3(1-0.3))}{(0.05)^2} = 323$$

Where **n**= the minimum sample size

Z $\alpha/2$ = value of standard normal distribution (SND) corresponding to a significance level of alpha at 95 % (1.96) **P**=clinical pregnancy rate in Addis Ababa, Ethiopia, **d**= margin of error 5% (0.05). When a 5% missed or incomplete documentation is considered total sample size was 338.

3.6 Study variables

3.6.1 Dependent variable

Clinical and continued pregnancy

3.6.2 Independent variables

Patient age, type of service

Infertility duration, type and cause

History of pregnancy before ART (parity, abortion, ectopic pregnancy)

Method of sperm insemination (conventional, ICSI), method of sperm collection (ejaculate, TESE)

Ovarian reserve- (amh/AFC)

Type of ovarian stimulation protocol (Long, antagonist, minimal)

Embryology findings- number and grade of oocytes retrieved, number & grade of embryo transferred, day and type of embryo transfer

3.7 Operational definitions

Poor responders: study participants with AFC of <5 on trans-vaginal ultrasound scan.

Good responders: study participants with AFC of 5-15 on trans-vaginal ultrasound scan.

Hyper responders: study participants with AFC of >15 on trans-vaginal ultrasound scan.

Ovulatory dysfunction: the cause of infertility due to anovulation, diminished ovarian reserve or polycystic ovarian syndrome with or without oligo-menorrhea or amenorrhea.

Combined cause of infertility: more than one cause of infertility identified

Clinical pregnancy: the identification of intrauterine pregnancy with gestational sac with or without yolk sac, embryonic pole or fetal cardiac activity at 6-7 weeks of gestation by TVUS

Ectopic pregnancy- Ectopic pregnancy was defined as clinical pregnancy that did not occur inside the uterine cavity

Continued pregnancy- clinical pregnancy with detection of Fetal heart beat beyond 12 weeks

Number of gestations; no of gestational sacs detected by transvaginal ultrasound at 6-7 weeks

Ovarian stimulation;

In this fertility center we use three major stimulation protocols including conventional long protocol, antagonist protocol, and minimal stimulation protocols. **In the first long protocol**, it is usually applicable for patients younger than 35 with good ovarian reserve (AFC more than 5) and if no contraindication to start stimulation, financial issue is not the main concern. The patient is appointed day 21 of menses to give depot Goserelin (Zoladex) injection 3.6 mg subcutaneous stat after checking for cyst or dominant follicles, and then reappointed for stimulation either on second day of menses or 14 days from Zoladex injection whichever comes first

Antagonist protocol

It is used more recently for those who have no financial challenge and usually after failed minimal stimulation or for expected poor response.

Other rare protocols are estrogen primed or OCP primed antagonist protocols

Triggering and OPU

Trigger for all three protocols made with urinary HCG 10,000 IU or 5,000 IU SC, decided if 3 or more leading follicles reached 18mm or more in size or greater or equal to 5 follicles reach/exceed 16mm in size. Patients booked for ovum pick up procedure done with single lumen aspiration needle under transvaginal guidance after 36 hour from HCG injection. Husband give semen sample in the same day of ovum pick up and the cycles either will be for IVF or ICSI. After OPU prophylactic antibiotic and luteal support started with injectable together with PO progesterone with estradiol valarate.

Rarely decapeptyl used for trigger for those expected hyper-responders.

Fertilization and embryo transfer

Once the oocyte cumulus complex identified in the standard media it is kept in the incubator containing 8.8 % co₂ for 2 hours and decided for IVF or ICSI based on the sperm analysis finding and number oocytes collected and the embryo will be transferred back with embryo culture media to the incubator.

Patients called for embryo transfer by embryologist either on day 3 or day 5 after OPU based on grade and number of embryo available, if the grade of embryo is grade 1 and more than 2 embryos available extended culture to day 5 without changing embryo culture media otherwise day 3 embryos will be the final choice. Regarding to the number of embryos transfer, for blastocyst stage not more than 2 while for day 3 up to three embryos could be transferred. If transfer is successful luteal support will continue for 14 days.

SERUM BETA HCG TEST

Patients will be appointed fourteen days after embryo transfer to check for serum pregnancy test. The test will be considered as positive if serum beta hcg is above 5 mIU/ml and will be repeated

after 2 to 7 days if it is below 100miu/ml to see the doubling of hcg level. We use the cobase411 modern hormone analyzer which is a fully automated analyzer that uses ElectroChemiLuminescence (ECL) technology for immunoassay analysis with a regular control and calibration standardization tests. It has a minimum and maximum detection ability of serum Hcg of 0.01miu/ml and 10,000miu/ml with sensitivity of 97% and specificity of 99.9%. For those who have positive pregnancy test, luteal phase support will be continued and appointed at 6 weeks of gestation to be evaluated using TVUS to check for the presence of clinical pregnancy, location of pregnancy and number of gestational sacs.

3.8 Ethical considerations

Ethical approval was obtained from the institutional review board of SPHMMC.

The data extracted from the electronic medical record of Center for reproductive medicine of SPHMMC was kept confidentially.

3.9 Data collection procedures

Data was collected by the interviewer administered structured questionnaires using kobotoolbox. The questionnaires used in this study was developed from different literatures.

Data was collected by two BSC. Nurses and three general practitioners and one supervisor after they were trained for two days on data collection tools, study objectives and ethical issues.

3.10. Data quality Assurance

Daily checking of the collected data was made by the supervisor and questionnaires were checked for completeness and accurateness to determine validity. Consecutive sampling technique was used to collect data from patient charts and records.

3.11 Data Processing and Analysis

After the data collection was completed data was coded, entered, and edited into computer software of STATA version 17 for analysis. Descriptive analysis of general characteristics of the study participants was done. The data was presented in the form of text, tables, and figures.

A receiver operating characteristic (ROC) curve was used to calculate the optimum β -hCG cutoff values that can predict different reproductive outcomes (clinical pregnancy and continued pregnancy)

The area under the curves (AUC) and their 95% confidence intervals was calculated for each ROC curve. The sensitivity, specificity, positive likelihood ratio (LR+), and the negative likelihood ratio(LR-) was calculated for each cutoff value

4. Results of the study

This study included 331 IVF/ICSI cycles with serum hCG hormone level of >5miu/ml.

Nearly half of the individuals in the study were under 30 years old, and 292 (88.2%) were nulliparous. Two hundred forty three of the study participants had primary infertility and 97 (29.5%) of them had tubal cause of infertility. Half of the study participants were good responders for controlled ovarian stimulation. The mean of oocytes retrieved was 12.45 ± 9.466 . ICSI fertilization was done for 239 (69.5%) of the study participants. 197 (59.5%) of the study participants had a day three embryo transfer and more than three fourth had fresh embryo transfer. (**Table 1**)

Table 1: general characteristics of study participants at SPHMMC, Department of Obstetrics and Gynecology, Reproductive Endocrinology and Infertility Unit from February, 2022 G.C. to March, 2023 G.C

Variables	Category	N	%
Age of the patient	<30 years	165	50.2
	30-35 years	111	33.7
	36-40 years	48	14.6
	>=41 years	5	1.5
Type of service	Government	141	42.6
	Private	190	57.4
History of abortion	Yes	50	15.1
	No	281	84.9
Parity	Nulliparous	292	88.2
	Primiparous	27	8.2
	Multiparous	12	3.6
Type of infertility	Primary	243	73.4
	Secondary	88	26.6
Causes of infertility	Tubal	97	29.5
	Male factor	76	23.1
	Ovulatory dysfunction	30	9.1

	Unexplained	44	13.4
	Combined	73	22.2
	Other	9	2.7
AFC count	Poor responders	62	18.8
	Good responders	165	50.2
	Hyper responders	102	31.0
Type of protocol used	Antagonist	145	44.2
	Minimal stimulation	93	28.4
	Long protocol	90	27.4
Sperm collection method	Ejaculate	258	77.9
	TESE	73	22.1
Type of fertilization	Conventional	101	30.5
	ICSI	230	69.5
Day of embryo transfer	Day 3	197	59.5
	Day 5	134	40.5
Embryo transfer type	Fresh	263	79.5
	Frozen	68	20.5
number of embryo transferred	One embryo	35	10.6
	Two embryos	280	84.6
	Three embryos	16	4.8

Among the 331 IVF/ICSI cycles, 287 (86.7%) had clinical pregnancy and two hundred thirty three of them achieved continued pregnancy.

Table 2: pregnancy outcome characteristics of study participants at SPHMMC, Department of Obstetrics and Gynecology, Reproductive Endocrinology and Infertility Unit from February, 2022 G.C. to March, 2023 G.C

Variables	Categories	Number(N)	Percent (%)
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U/S detection of gestational sac at 6 week	No clinical pregnancy	44	13.3
	Clinical pregnancy	287	86.7
Fetal heart was detected at 6 week	Not detected	31	10.9
	Detected	254	89.1
Ectopic pregnancy	No	287	99.0
	Yes	3	1.0
US detection of FHB beyond 12 weeks	Continued pregnancy	233	81.2
	No continued pregnancy	54	18.8
No of gestations	Singleton	217	75.6
	Twin	66	23.0
	High order pregnancy	4	1.4

Only 14.3 % of the individuals in the study achieved clinical pregnancy when hcg level was below 100miu/ml and 98.3% of them achieved clinical pregnancy when hcg level was 601-1000miu/ml.

Table 3: Clinical pregnancy with beta hcg level (descriptive)

Variables	Categories	Ultrasound detection of gestational sac at six weeks (N=331)	
		No clinical pregnancy (n(%))	Clinical pregnancy (n(%))
Serum beta Hcg level(miu/ml)	≤100	36(85.7%)	6(14.3%)
	101-200	-	18(100%)
	201-600	4(4.3%)	88(95.7%)
	601-1000	1(1.7%)	58(98.3)
	1001-2000	2(2.8%)	70(97.2%)
	>2000	1(2.1%)	47(97.9%)
Total		44(13.3%)	287(86.7%)

Only seven or 36.8% achieved continued pregnancy when hcg level was 101-200 miu/ml and 67(94.4%) achieved continued pregnancy when hcg level was 1001-2000 miu/ml.

Table 4: frequencies of continued pregnancy with beta hCG level

Variables	Categories	Ultrasound detection of fetal heart beat beyond 12 weeks (N=287)	
		No continued pregnancy (n (%))	Continued pregnancy (n (%))
Serum beta Hcg level(miu/ml)	≤100	5(62.5%)	3(37.5%)
	101-200	12(63.2%)	7(36.8%)
	201-600	21(25.6%)	61(74.4%)
	601-1000	7(12.1%)	51(87.9%)
	1001-2000	4(5.6%)	67(94.4%)
	>2000	5(10.2%)	44(89.8%)
Total		54(18.8%)	233(81.2%)

The cutoff value to predict clinical and continued pregnancy was 226 and 448.6 respectively.

Table 5: Comparison of the optimal cutoff value of serum hcg hormone and ROC curve characteristics to predict clinical and continued pregnancy.

	Clinical pregnancy	Continued pregnancy
Beta hcg cut off	226	448.6
AUC	0.9189	0.7337
95%CI of AUC	0.85497-0.98293	0.65013-0.81723
Sensitivity %	90.24	77.97
Specificity %	86.36	60.78
Accuracy	89.73	74.82
LR+	6.6179	1.9883
LR-	0.1130	0.3624

Receiver operating characteristics (ROC) analysis

ROC analysis was carried out to identify the predictive ability of 14th day beta HCG on the clinical pregnancy. The ROC analysis indicates that the AUC is 0.92 which indicates that the 14th day beta HCG is predictive of clinical pregnancy

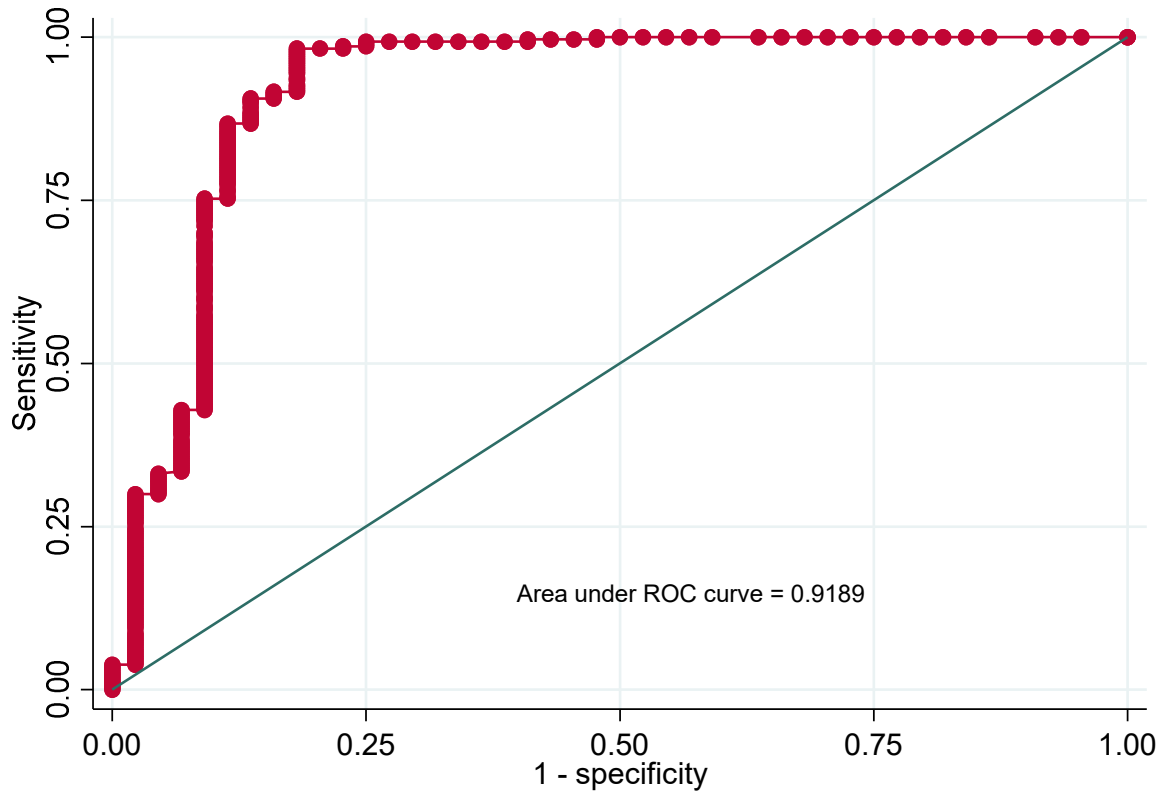


Figure 1; ROC curve showing the predictive ability of serum HCG on clinical pregnancy of study participants at SPHMMC, Department of Obstetrics and Gynecology, Reproductive Endocrinology and Infertility Unit from February, 2022 G.C. to March, 2023 G.C.

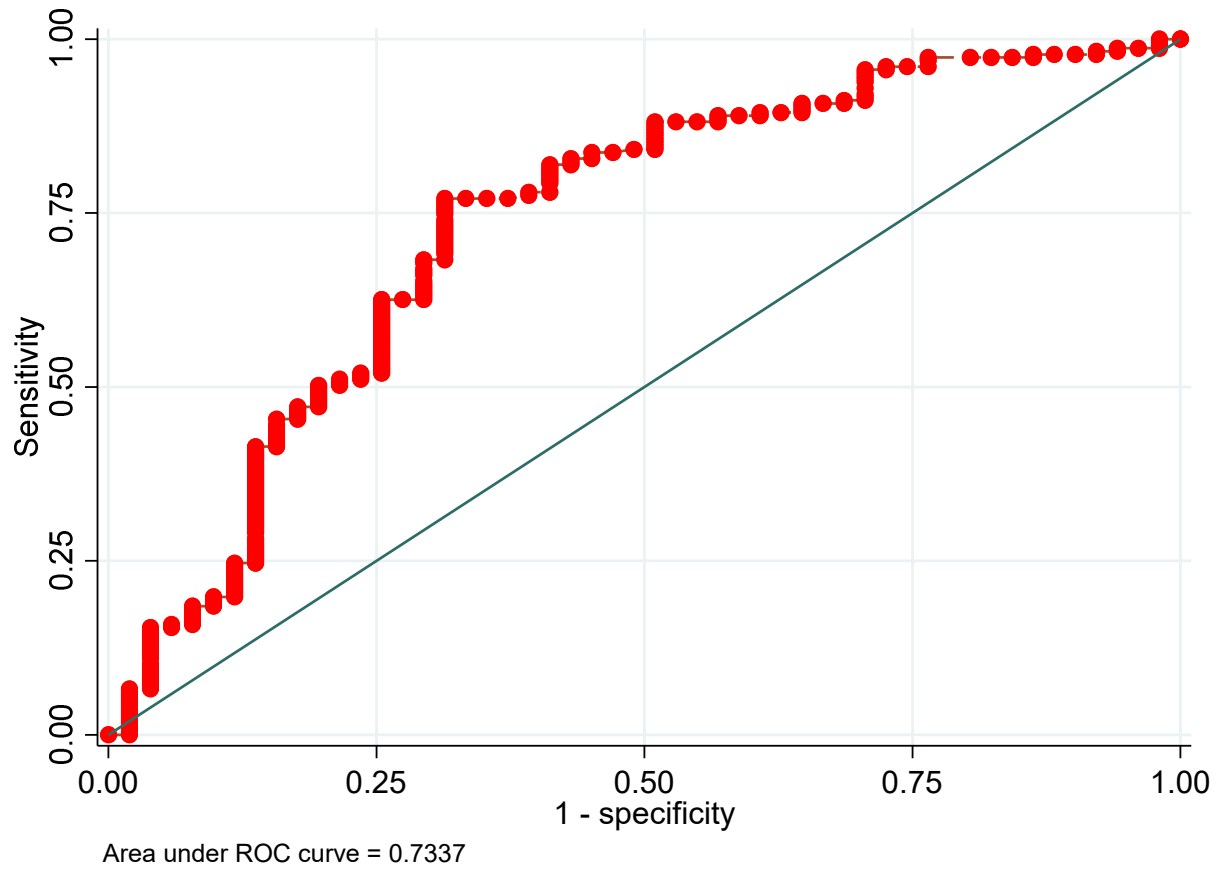


Figure 2; ROC curve showing the predictive ability of day 14th serum HCG level on the continued pregnancy

5. Discussion

In affluent nations, a lot of research has been done on the level of serum human chorionic gonadotropin hormone in predicting the outcomes of early pregnancy. Each laboratory should identify its own optimistic β -hCG levels to predict the early pregnancy outcomes.

According to ROC analysis, the cutoff value for beta human chorionic gonadotropin hormone to predict clinical pregnancy is 226 mIU/ml, with an AUC of 0.92, sensitivity of 90.24%, and specificity of 86.36%. This finding is in line with the studies reported by Zhang Y and his colleagues conducted on diagnostic value of a single β -hCG test in predicting reproductive outcomes in women undergoing cleavage embryo transfer(7). Another study conducted in Uganda on Prognostic value of a serum β -hCG after embryo transfer was consistent with this finding(14). This consistency might be due to the number of embryos transferred and population correspondence.

On the other hand, this finding's cut off value of serum beta human chorionic gonadotropin hormone to predict clinical pregnancy is higher than studies conducted in China, USA, and Canada (4, 10, 17). This discrepancy can be the result of participant population variances in the study. In addition; this might be explained by the differences in the type, number, and quality of embryos transferred.

The beta human chorionic gonadotropin hormone cutoff values to predict continued pregnancy was found to be at 448 mIU/ml with AUC of 0.74, sensitivity of 77.97%, and specificity of 60.78% on ROC analysis. This finding is higher than a study done by Khadra and his colleagues on Human chorionic gonadotropin cutoff value to predict pregnancy outcomes (11). A study conducted in USA also showed the beta hCG cut off value to predict continued pregnancy was lower than this study(18). This potential discrepancy could be caused by racial population variances, the day of embryo transfer (day 3 vs. day 5), or laboratory hCG assay techniques.

6. Strength and limitations

The strength of this study is it is the first study conducted in the country and will be a baseline data. The limitations of this study are; it was a cross-sectional study, it did not include other pregnancy outcomes (miscarriage, ectopic pregnancy and live birth). The study has not addressed the possible determinant factors that could affect the level of serum beta hcg predicting clinical pregnancy

7. Recommendation

For IVF specialists and fertility clinics

- ✓ It would be better to use these cut off values to predict early pregnancy outcomes.
- ✓ It is also good to consider the cut off value for couples counseling on its probabilities of continuation.

For policy makers

- ✓ Policy makers better to use this cut off values to be incorporated as a baseline finding to develop population specific serum hcg cut off value to develop guideline for IVF/ICSI services.

For researchers

- ✓ Researchers better to use this finding as a baseline finding to explore possible factors that could affect the level of beta HCG for early pregnancy outcomes in IVF/ICSI cycles.
- ✓ It is better to conduct further study on level of beta hcg that can predict live births in IVF/ICSI cycles.
- ✓ Better to investigate on population differences on the level of serum beta hcg to predict pregnancy outcomes.

8. Conclusion

The level of serum beta human chorionic gonadotropin hormone on day fourteen after embryo transfer are helpful to predict clinical and continued pregnancy. The cutoff value of serum human chorionic gonadotropin hormone predicting clinical and continued pregnancy is relatively higher than the other studies. This finding can be used as a reference for counseling purpose on the possible outcomes of early pregnancy. However, routine monitoring of ART and luteal phase support should be continued until ultrasound confirmation of clinical pregnancy.

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