

# Human Chorionic Gonadotrophine Level on Day, 7, 10, 14, Post-Embryo Transfer Following Day 3 Embryo Transfer can Predict Clinical Pregnancy in both fresh and frozen cycles

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## ABSTRACT

**Background:** human chorionic gonadotropin (HCG), a hormone produced by placental trophoblastic cells, and it is the earliest marker to detect and evaluate pregnancy outcome, it can be detected in maternal serum as early as 6-8 days post embryo transfer, several studies have demonstrated its important role in establishing and maintaining pregnancy, and early development of the embryo, and development of embryo brain other important role BHCG in protocols of IVF cycles.

**Aim:** To measure BHCG as early as possible to detect if pregnancy achieved or not after embryo transfer in (ICSI) cycle and can predict result of pregnancy as early as 7th day and 10th and 14th day post embryo transfer.

**Method:** Observation study was conducted in Baghdad in October 2019 till October 2020 in high institute for infertility diagnosis and ART, and KAMAL AL SAMARIA Hospital Baghdad, 50 lady were enrolled both fresh 72% and frozen cycle 28% had done (ICSI) cycle then measure (BHCG) in day 7, day 10, day 14 post embryo transfer in both fresh and frozen cycle, (BHCG) follow up to detect clinical pregnancy, follow up by vaginal ultrasound until 12 weeks of gestation

**Result:** BHCG titer in day 7 was significant P value 0.0001 comparing to day 10 and P value in day 7 was significant P value 0.0001 comparing to day 14, BHCG titer in day 10 was significant P value 0.0001 comparing to day 14, Pregnancy rate was 64%, NO ECTOPIC pregnancy. Miscarriage rate was 8% Pregnancy rate after fresh cycle was 58.3% & pregnancy rate after frozen cycle was 78.6%

- Cut of value in day 7 post embryo transfer is  $17.496 \pm 17.66$
- Cut of value for day 10 post embryo transfer was  $44,774 \pm 42.93$
- Cut of value for day 14 post embryo transfer was 454.27

**Conclusion:** Day 7 post embryo transfer BHCG titer was significant so it can be used to alleviate stress of the patient & can be used as early predictor of pregnancy. Day 10 post embryo transfer BHCG titer was significant it can be used as indicator for pregnancy. DAY 14 was significant post embryo transfer it can be used even before clinical pregnancy.

**Keywords:** Human chorionic gonadotropin, IVF cycles,

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## INTRODUCTION

According to a recent study, a lot of the couples of all ages in United Kingdom remained childless even after undergoing 6 complete IVF cycles<sup>1</sup>. That is, women aged  $\geq 35$  years would show a lower live birth rate in a single cycle than younger women. However, many patients discontinue treatment after 1 or 2 failed treatment cycles at the beginning of ART, although the final prognosis of achieving a live birth may remain promising. Furthermore, the number of embryos transferred (ET) in each cycle varies from 1 to 3, with 3 being the maximum. This reinforces that Clinical Live Birth Rate (CLBR) per cycle may not be a reliable index for providing informed advice to patients. The  $\beta$ -HCG in the urine or serum is used as a common indicator of pregnancy in most IVF centers. The serial measurement of  $\beta$ -HCG level is also used to differentiate normally progressing pregnancies from multiple gestations, ectopic pregnancies (EP), or spontaneous abortions<sup>2,3</sup>.

**Role of HCG in Implantation:** Many times, a rise in HCG (between the 1st and 2nd test) will be slow (failure to double every 48 hours). When this happens, a 3rd and sometimes even a 4th HCG test should be done at 2-day intervals. A failure to double on the 3rd and/or 4th test is a

poor sign. It usually indicates a failed or "dysfunctional" implantation but in some cases a progressively slow rising HCG level might point to a tubal (ectopic) pregnancy.<sup>(2)</sup> This sometimes suggests that there were initially more than one embryo implanting and that one of these subsequently succumbed and one survived to continue a healthy singleton pregnancy. In the event that serial blood quantitative beta HCG pregnancy tests indicate that one or more embryos are likely to be implanting, some IVF physicians advocate daily injections of progesterone or the use of vaginal hormone suppositories for several weeks to support the implanting embryo(s) physicians prefer to prescribe beta HCG injections three times a week for several weeks until the pregnancy can be defined by ultrasound. Some IVF centers do not prescribe any hormones at all, after the transfer<sup>4</sup>.

### Clinical Applications of HCG

1. HCG Measurements in Normal and Abnormal Pregnancies. Level of HCG above 25 IU/L considered pregnant in viable pregnancies, a median HCG concentration of 126 IU/L is observed 12 days after embryo transfer, while levels below 76 IU/L are associated with early pregnancy loss. Approximately

- 20–30% of all pregnancies fail within days after implantation<sup>13</sup>.
- HCG is important marker for pre-eclampsia. usually elevated due to abnormal placentation. Abundant evidence indicates that the underlying pathology of preeclampsia occurs in the first trimester (14).
  - HCGβ levels in first trimester maternal serum proved to be extremely useful in screening for Down's syndrome. Pregnancies complicated by Down's syndrome are associated with elevated serum HCG and HCGβ concentrations.
  - HCG is crucial in the Diagnosis of Gestational Trophoblastic Disease<sup>6</sup>. Trophoblast tumors also produce high HCGβ levels, but usually at lower concentrations than HCG. Compared to other types of GTDs, placental site trophoblastic tumors frequently produce small amounts of HCG with 25% reported to be HCG negative<sup>18</sup>.
  - HCG Use in Assisted Reproductive Technology The mid-cycle LH surge is essential to achieve normal oocyte maturation and ovulation<sup>6</sup>.

Partially-purified urinary HCG (surrogate for LH) preparations are administered to achieve final oocyte maturation and ovulation during controlled ovarian hyperstimulation, and to facilitate correct timing of oocyte retrieval<sup>19</sup>.

HCG use in assisted reproduction may result in different responses than LH with respect to mature oocyte collection, embryo quality, implantation and pregnancy rate<sup>20,21</sup>. Moreover, Tesarik et al<sup>22</sup> showed that HCG administration to recipients increased endometrial thickness on the day of embryo transfer and improved endometrial receptivity. Recently, recombinant-HCG (r-HCG) became commercially available and subcutaneous administration of 250 µg of r-HCG was found to be equivalent to, or at least as effective as, intramuscular 10,000 IU of urinary HCG in initiating final oocyte maturation<sup>23,24</sup>.

## PATIENTS AND METHODS

Observational study was conducted between October 2019 to October 2020 for 50 patients at (ICSI) cycle in high institute for infertility, diagnosis and assisted reproductive technology and Kamal al Samarai Hospital, all of these lady were agreed to enrolled in our study conducted ethical committee in Arab board, history, examination, & investigation to all patient were evaluated, protocol was decided to be continued, all cases are subjected to antagonist flexible protocol ovulation induction by FSH & HMG gonadotropin started in day 2 then cetrorelix 0.25 g after that when follicle reach to 18 millimeter TRIGGER was done HCG injection followed by ultrasound, ova pickup after that BHCG titer was measured in day 7, 10, 14 post embryo transfer in both fresh and frozen cycles

- Fresh cycle was 32 case, 64% of embryo
- Frozen cycle was 18, 36% of embryo
- For different cause of freezing followed by frozen embryo transfer
- For all these cases (fresh & frozen)
- Luteal support was given by hydroxyprogesterone caproate injection 250 mg every 3 days, and vaginal suppository every day, there is no luteal support by BHCG
- THEN ASSESS
- Day 7 the (BHCG) titer in the luteal phase was measured
- Day 10 (BHCG) titer was measured
- Day 14 (BHCG) TITER Measured

Patient notify to come again and ultrasound was done to prove clinical pregnancy, gestational sac was seen intrauterine, follow up by vaginal ultrasound until 12 week of gestation

### Exclusion criteria

- Patient who disagree to enroll in our study
- Loss of follow up
- Any patient that use HCG in the luteal support

Table 1: Number and percentage of age of the patient, body mass index BMI, duration of infertility, cause of infertility, type of infertility, embryo transfer type whether fresh or frozen

		No	%
Age (years)	20--24	6	12.0
	25--29	15	30.0
	30--34	19	38.0
	=>35y	10	20.0
	Mean±SD (Range)	30.4±5.2 (20-42)	
BMI (Kg/m2)	Normal (18.5-24.9)	16	32.0
	Overweight (25-29.9)	23	46.0
	Obese (=>30)	11	22.0
	Mean±SD (Range)	26.8±3.4 (21-35)	
Duration of infertility (years)	<5 years	10	20.0
	5--9	26	52.0
	=>10 years	14	28.0
	Mean±SD (Range)	7.2±3.1 (3-15)	
Cause of infertility	Male cause	19	38.0
	Female cause	21	42.0
	Both	10	20.0
Type of infertility	Primary	34	68.0
	Secondary	16	32.0
Embryo transfer type	Fresh	36	72.0
	Frozen	14	28.0
Outcome	Pregnant	32	64.0
	Failure	18	36.0

Table 2: Mean and standard deviation and standard error of mean and range of day 7 and day 10 and day 14 result of BHCG titer after embryo transfer

BHCG level (nUI/L)	7th Day	10th Day	14th Day
Mean	17.496±17.66	44.774±42.93	292.665±342.99
Standard Deviation	17.66	42.925	342.992
Standard Error of Mean	2.498	6.070	48.506
Range	1.0-103.0	0.8-189.0	0.4-1100.0
Percentile05 <sup>th</sup>	2.0	1.90	1.0
25 <sup>th</sup>	6.0	8.0	9.0
50 <sup>th</sup> Median	13.0	38.0	169.035
75 <sup>th</sup>	23.0	70.0	471.0
95 <sup>th</sup>	34.0	124.0	1030.0
99 <sup>th</sup>	103.0	189.0	1100.0
Compared to 7 <sup>th</sup> Day	-	0.0001*	0.0001*
Compared to 10 <sup>th</sup> Day	-	-	0.0001*

\*Significant difference between two dependent means using Paired-t-test at 0.05 level.

Table 3: p value of day 7,10,14 ‘

	Outcome				P value
	Pregnant		Failure		
	No	%	No	%	
BHCG level (nUI/L) at 7th day	24.24±18.85(7.0-103)		5.50±3.60(1.0-13.0)		0.0001*
HCG level (nUI/L) at 10th day	67.16±38.29(12.0-189)		4.98±4.31(0.8-17.0)		0.0001*
HCG level (nUI/L) at 14th day	454.27±333.25(25.7-1100)		5.37±5.02(0.4-15.0)		0.0001*
P value comparing 7 <sup>th</sup> x 10 <sup>th</sup> Day	0.0001^		0.309		
P value comparing 7 <sup>th</sup> x 14 <sup>th</sup> Day	0.0001^		0.850		
P value comparing 10 <sup>th</sup> x 14 <sup>th</sup> Day	0.0001^		0.461		

\*Significant difference between two independent means using Students-t-test at 0.05 level.  
 ^Significant difference between two dependent means using Paired-t-test at 0.05 level.

Table 4: Comparing embryo transfer type to outcome of ICSE cycle (pregnant, failure)

Outcome		Embryo transfer type			
		Fresh		Frozen	
		Count	Column N %	Count	Column N %
Pregnant		21	58.3%	11	78.6%
	Failure	15	41.7%	3	21.4%

P=0.181

Table 5:Result variables of day 7,10,14 respectively & Confidence Intreval

Test Result Variable(s)	Area Under the Curve (AUC)	Std. Error	P value	95% Confidence Interval	
				Lower Bound	Upper Bound
BHCG level (nUI/L) at 7th day	0.959	0.025	0.0001*	0.909	1.000
HCG level (nUI/L) at 10th day	0.998	0.003	0.0001*	0.993	1.000
HCG level (nUI/L) at 14th day	1.000	0.001	0.0001*	1.000	1.000

**DISCUSSION**

In our studytable 1the mean age of our patient was 30.4 and the standard deviation is 5.2 and the range of age of the patient is between 20 and 42 years old 38% of patient are between 30 and 35 years old, while Gillian human and Susan Brown found thatthe mean age of their study was 32.8 years and the rang (19.9-50.9) Body Mass Index BMI (kg/m<sup>2</sup>) the mean 26.8 and the standard deviation 3.4 the range is (21-35) most of our patient(BMI) 46% of our patient was overweight.

Duration of infertility, mean is 7.2 yearsand standard deviation is 3.1 range is3-15 years , more than half of our patientis more than five years infertilitytype of infertility ,male infertility was 38%, female infertility was 42%, the rest is mixed,68%of our patientwas primary infertilitymost embryo transfer type was fresh was 72% while 28% of embryo was frozen. Outcome of ICSE cycle percentage of ladies that got pregnant is 64%, and percentageof ladies

those fail to get pregnant 36%. Urine and serum β-HCG concentration are dependent on the number of implanting/implanted embryos, and β-HCG levels in females with multiple pregnancies are higher than those of single embryo transfer

Table 2 showed the measurement of BHCGin day 7,10,14. The mean of day 7 was 17.496 reaching to44.774in day 10and it increase 2-3 time in day 10 and it reach as high as 292 in day 14

In this table P value issignificant in day 7 , alsoin day 10 it was significant and for day 14,whilea study Wang et al found that a serum BHCG The clinical pregnancy rate was 57.7 and 29.2% in women <35 years old, and women >35 yearsG cutoff level of 2.5 MIU/Lon the seventh dayafter ET can predicted pregnancy outcome and a serum BHCG cut off value of 10.8 can predict multiple pregnancy<sup>25</sup>.while cut off value for day 7 post ET was 24.24in our study andcutoff value for day 10 post ET was 67.16several scholars studied prediction of pregnancy with cut off value of 152\_

527 mIU/ml on day 11-13 after ET. Initially low serum  $\beta$ -hCG level 14 days after day 3 embryo transfer indicated poor prognosis with minimal likelihood of ongoing pregnancy<sup>26</sup>. In table 3 show the difference of BHCG level in day 7,10,14 divided into pregnant and non-pregnant group showed the P value of day 7, 10,14 post ET, there is significant difference between two independent means using Student-t-test at 0.05 level and showed significant difference between two dependent mean using Paired -t-test at 0.05 level.

Table 4 showed type of embryo transfer, pregnancy rate after fresh ET was 58.3% while pregnancy rate after frozen ET 78.6%, means that pregnancy rate with frozen embryo more than fresh embryo BUT pregnancy rates for fresh embryo transfer during in vitro fertilization were similar to the freeze-all strategy, and time to pregnancy was significantly longer with the freeze-all approach, according to a study published in BMJ. New randomized controlled trials have reported conflicting results in reproductive outcomes after the freeze-all strategy compared with fresh transfer in women who have regular menstrual cycles." Sacha Stormlund, MD, PhD, from the Fertility Clinic in the department of obstetrics and gynecology at Hvidovre University Hospital in Denmark<sup>27</sup>. In table 5 result of variable of day 7,10,14 respectively & Confidence Interval the area under the curve for day 7 is 0.959 & for day 10 is 0.998 & for day 14 is 1.000

The Confidence Interval lower bound for day 7 is 0.909 for day 10 is 0.993 and for day 14 is 1.000 respectively, While Lihua found that Serum  $\beta$ -hCG levels on the seventh day after FET were higher in the single OP group compared with the biochemical pregnancy group ( $p < 0.001$ ). Besides, the serum  $\beta$ -hCG cut-off level at 4.34 mIU/mL on the seventh day showed high predictive value (area under the curve (AUC)=0.852). the serum  $\beta$ -hCG cut-off level at 17.95 mIU/mL on the seventh day showed high predictive value (AUC=0.903)<sup>27</sup>.

## CONCLUSION

Human chorionic gonadotropin level on day 7,10,14 post embryo transfer following day 3 embryo transfer can predict clinical pregnancy in both fresh and frozen cycles result in BHCG in day 7 post ET was not significant while in day 10 post ET it was significant and can be used even before clinical pregnancy.

**Conflict of Interest:** Conflict of Interest the authors declare that there are no potential conflicts of interest related to the study.

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