ORIGINAL STUDY

Can Early hCG Levels be a Marker for Pregnancy Outcome in ART Cycles?

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Abstract

With advancing technology of assisted reproduction, physicians today have the ability to achieve conception in many couples who would have been totally incapable doing so only a few years ago. The anxiety and the uncertainty of pregnancy outcomes using ART procedures is widely accepted as one of the main psychological stresses the couples. The ability to predict outcome as soon as possible after assisted conception treatment is important for clinic staff and patients.

The aim of this observational study is to highlight the importance of hCG values in predicting the outcome of ART cycle and counseling the patients in case of adverse result. The ultimate aim is to improve the take home baby rate and initial hCG value can help us counsel our patients towards the ultimate outcome.

Embryo development in early pregnancy follows a preprogrammed-timing schedule and depends mainly on the embryonic age of the healthy, successfully implanted conceptus. The appearance of hCG in maternal serum is used to assess the time of clinically detectable implantation. bhCG has provided the best sensitivity and specificity for detection of normal and pathological pregnancies. After IVF, early pregnancy loss or multiple gestations may be predicted with high sensitivity and specificity by using cut-off values of serum hCG.

The median HCG concentration was 116 IU/l in viable pregnancies and 31 IU/l in nonviable pregnancies. The median hCG concentration in twin pregnancies was almost double that in singleton pregnancies (201 IU/l *vs* 116 IU/l). Thus we can reassure normally pregnant patients as well as filter and manage those with nonviable outcomes more efficiently.

Keywords: bhCG, ART, pregnancy prediction, pregnancy outcome.

INTRODUCTION

Infertility is not only a gynecological problem but also a social stigma. The price of motherhood is high and hapless couples seek to tide over this major crisis in their homes and society. Multiple studies have demonstrated the high incidence of psychological problems like low self-esteem; security and self-confidence among the childless couples. Women in particular suffer the deleterious consequences of infertility due to the common misconception that infertility is always the shortcoming of the female. This takes huge toll on the woman in terms of loss self-esteem, grief, and feelings of failure. As the technology of assisted reproduction continues to advance, physicians today have the ability to achieve conception in many couples who would have been totally incapable doing so only a few years ago. The ability to predict outcome as soon as possible after assisted conception treatment is important for clinic staff and patients. Early prediction of outcome is important in pregnancies following assisted reproduction treatment (ART).

The anxiety and the uncertainty of pregnancy outcomes using ART procedures is widely accepted as one of the main psychological stresses the couples. For the past 20 years investigators have sought to define tests that can help in predicting the results of pregnancy and decreasing this stress. Also, in ART pregnancies, the incidence of ectopic pregnancies (EP) varies from 2 to 5-fold more compared with that in spontaneous pregnancies, particularly in patients with tubal factor infertility. These patients therefore need special attention to avoid further impairment of fertility and hence an early diagnosis is imperative. The rate of multiple gestations is also high (20-25%) and early pregnancy loss is common, which causes anxiety in the couples involved.[1,2] Serum hCG has been found to be the most predictive of pregnancy outcome.

The aim of this study is to highlight the importance of hCG values in predicting the outcome of ART cycle and counseling the patients in case of adverse result. The ultimate aim is to improve the take home baby rate and initial hCG value can help us counsel our patients towards the ultimate outcome. The literature review is by the Medline and journal search, for the different options available for the same, and highlights the current modes of treatment.

A variety of hormones has been evaluated for their ability to predict outcome[3,4] but beta human chorionic gonadotropin (B-hCG) alone provided the best sensitivity and specificity for

detection of normal and pathological pregnancies.[5] Makers have been sought to distinguish between viable and nonviable pregnancies before verification of live intrauterine pregnancy by transvaginal sonography (TVS) is possible. A single determination of serum hCG concentration has been found to be predictive of pregnancy outcome,[6-8] even as early as 12 days after embryo transfer.[9,10] Other studies have shown that the daily increase in plasma hCG is significantly greater in viable pregnancies than in Ectopic or biochemical pregnancies.[10,11] Similarly hCG concentrations are significant higher in multiple compared with singleton pregnancies. This observation has been used to predict the probability of a multiple birth.[12] After IVF, early pregnancy loss or multiple gestations may be predicted with high sensitivity and specificity by using cut-off values of serum hCG. This sensitivity can be increased if we use the values derived from two measurements independently of the day of blood sampling.[13] For the purpose of this study we have used only one value on day.[12]

MATERIAL AND METHODS

Subjects

From Jan 2005 to Dec 2006, a total of 198 embryo transfers were carried out at the Infertility Clinic. Records of the subjects who conceived following ART during that period were analyzed. Of these, 72 (Table 1) embryo transfer cycles fulfilled our inclusion criteria, which were, serum hCG had been assayed on day 12 post embryo transfer and its concentration was > 10 IU/l, (ii) the data on the outcome was available.

Table 1: Basic characteristics of patients included: n = 72

- Mean age at treatment was 32 years (range 21-41)
- Main cause of infertility was
  - Anovulation in 11 (15.2%) cycles,
  - Endometriosis in 7 (9.7%),
  - Male factor in 21 (29.1%),
  - Tubal factor in 12 (16.6%),
  - Unexplained in 10 (13.8%),
  - Combined in 11 (15.2%).

Treatment Protocols Followed in the ART Cycles

Briefly, in IVF or IVF with ICSI cycles, long luteal phase pituitary down-regulation with lupreolide acetate and followed by ovarian stimulation with either urinary or recombinant FSH. When two or more follicles reached a diameter of > 17 mm (in TVS), 10000 IU of hCG was administered. The half-life of the HCG used was 33 hours. Transvaginal follicle aspiration was performed 36 hours after hCG administration, and embryo transfer took place 48 hours later. Micronized vaginal progesterone at a daily dose of 800 mg was used for 2 weeks for luteal support.

In frozen embryo transfer cycles, hormone replacement with estradiol valerate given orally (4-6 mg daily) was used until endometrial thickness reached > 7 mm per two layers. Micronized vaginal progesterone (800 mg/day) was then started, followed by embryo transfer after 3 to 4 days. Estrogen and progesterone treatment was continued until the end of the first trimester in viable pregnancies.

RESULTS

The median hCG concentration was 116 IU/l in viable pregnancies and 31 IU/l in nonviable pregnancies. The median hCG concentration in twin pregnancies was almost double that in singleton pregnancies (201 IU/l *vs* 116 IU/l).

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). The hCG level of 76 IU/l is a suitable cut-off point for predicating viable pregnancies with 80% sensitivity and 82% specificity.

Table 2 shows the probabilities (%) of the different outcome within day 12 postembryo transfer hCG measurements. 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, and only one subject with EP had a level higher than this (144 IU/l).

In the subject with tubal factor infertility, the overall rate of EP was 5/72 (6.9%). Among viable singleton pregnancies, when male factor was present, the median hCG concentration was low compared with the remainder (100 IU/l), especially where ICSI was method of treatment. These results remained essentially the same when multiple pregnancies were included.

In nonviable pregnancies, no significant differences in hCG concentration were detected in the etiological subgroups. The outcome of pregnancies classified according to the treatment type is shown in Table 3. No correlation between age and hCG level was observed in viable pregnancies.

Table 4 shows the hCG value ranges based on the final outcome, which can be used when counseling patients regarding the final outcome based on the initial hCG values. Table 5 shows the details of all the 72 patients and their outcome based on hCG values.

Pregnancy Outcome

Viable pregnancy was defined as one resulting in delivery of at least one live fetus at $\geq$ 28 weeks gestation, which does not conform to the current World Health Organization's (WHO) definition. As per WHO definition, viable pregnancy is defined as any childbirth occurring after 24 completed weeks. With current neonatal facilities and a fair chance of survival, we have used 28 weeks as the cut off for viable, live birth. Nonviable pregnancies constituted biochemical pregnancies (a temporary rise of serum hCG without signs of intrauterine pregnancy in TVS), ectopic pregnancies (EP; diagnosed in TVS or laparoscopy), miscarriages (cessation of development of intrauterine pregnancy seen in TVS).

Table 2: Outcome chances based on hCG values

hCG value	*Nr*	*Outcome n = 72*					*Prediction of outcome*
		Biochemical	*G sac seen nonviable*	*Ectopic*	*Live birth*	*twins*	
10-25	1	1	0	0	0	0	100% accurate negative outcome
26-50	3	2	0	1	0	0	100% accurate negative outcome
51-75	7	2	2	2	1	0	85% accurate negative outcome. Ectopic suspected **Live birth not expected**
76-100	4	1	1	1	1	0	75% accurate negative outcome. **Live birth not expected**
101-125	5	0	1	0	4	0	80% accurate positive outcome. **Abortion not suspected**
126-150	27	0	1	1	25	1	88.8% accurate positive outcome. **Ectopic, abortion and twin not suspected**
151-175	12	0	0	0	12	2	83% accurate positive outcome. **Twins not suspected**
176-200	5	0	0	0	5	5	100% accurate
>200	8	0	0	0	8	6 + 1 triplet	87.5% accurate positive outcome. **Singleton not expected**
Total	72	6	5	5	56	15	

** Note – The negative prediction in these patients are marked in green in the table.

Table 3: Pregnancy outcome n = 198 cycles

	n	*%*
Pregnancy	72	36.3
Live birth	56	28.8
Ectopic	5	6.9
Biochemical	6	8.3
Spon abortion	5	6.9
Multiple pregnancy	15	20.83

Table 4: Median HCG Values for each outcome

Pregnancy outcome	*HCG value range*	*Median*
Viable pregnancy		76
• Fresh ET		• 116
• Frozen ET		• 115
• Multiple pregnancy		• 201
Miscarriage		58
Ectopic pregnancy		80
Biochemical pregnancy		31

DISCUSSION AND REVIEW OF LITERATURE ON OUTCOME OF ART PREGNANCIES

In our study, the median hCG concentration in viable pregnancies was about 3-fold compared with those in nonviable ones, which is in agreement with the results reported before (Qasim et al, 1996; Bjercke et al, 1999). Low maternal HCG is also associated with nonviable pregnancy later in the first trimester (Ong et al, 2000). The approximately double hCG level in twin pregnancies

Table 5: Details of all patients with cause of infertility and hCG values and the pregnancy outcomes

Sr. no	*Patient name*	*Cause of infertility*	*Day 12 hCG*	*Final outcome*
1	Dbc	Male factor	40	Abortion – 6 weeks
2	Nmk	Endometriosis	101	Live birth singleton
3	Nmm	Anovulation	34	Biochemical
4	Mkl	OAS + Tubal	87	Live birth singleton
5	Sjm	Male factor	89	Live birth singleton
6	Jrs	Tubal factor	54	Live birth singleton
7	Asd	Anovulation	63	Abortion – 8 weeks
8	Snn	Male factor	211	Live birth twins
9	Skm	Endometriosis	259	Live birth triplets – reduced to twins
10	Vkm	Unexplained	78	Live birth singleton
11	Ujk	Male factor	76	Live birth singleton
12	Nss	Tubal factor	57	Ectopic
13	Sss	Anovulation	89	Live birth singleton
14	Vmp	Male factor	103	Live birth singleton
15	Jap	Unexplained	189	Live birth twins
16	Rkp	Male factor	96	Live birth singleton
17	Msm	OAS + Anovulation	54	Abortion – 7 weeks
18	Amp	Endometriosis	94	Live birth singleton
19	Ass	Male factor	103	Live birth singleton
20	Map	Tubal factor	88	Ectopic
21	Rns	Unexplained	91	Live birth singleton
22	Gsm	Male factor	29	Biochemical
23	Abp	Male factor	108	Live birth singleton
24	Rab	Tubal factor	111	Live birth singleton
25	Pvs	Anovulation	190	Live birth twins

Contd...

Contd...

Sr. no	*Patient name*	*Cause of infertility*	*Day 12 hCG*	*Final outcome*
26	Ssm	PCOS +OAS	217	Live birth twins
27	Tsw	Tubal factor	144	Ectopic
28	Jsn	Unexplained	88	Live birth singleton
29	Vmj	Anovul + OAS	99	Live birth singleton
30	Vsk	Male factor	158	Live birth twins
31	Rvs	Unexplained	100	Live birth
32	Ksr	Anovulation	30	Biochemical
33	Djd	Male factor	89	Live birth singleton
34	Ugd	Male factor	179	Live birth twins
35	App	Tubal factor	102	Live birth singleton
36	Tap	Anovul + OAS	123	Live birth singleton
37	Ppd	Tubal factor	76	Ectopic
38	Pns	Male factor	78	Live birth singleton
39	Nyp	Anovulation	94	Live birth singleton
40	Tdp	Unexplained	108	Live birth singleton
41	Saj	Male factor	149	Live birth twins
42	Rpj	Endometriosis	96	Live birth singleton
43	Bsu	Unexplained	66	Abortion – 7 weeks
44	Tsd	Tubal factor	89	Live birth singleton
45	Ssk	PCOS + Tubal	103	Live birth singleton
46	Kpm	Anovul + Tubal	159	Live birth twins
47	Udk	PCOS + OAS	111	Live birth singleton
48	Nss	Endometriosis	92	Live birth singleton
49	Sss	PCOS + Tubal	45	Biochemical
50	Trs	Male factor	121	Live birth singleton
51	Vvv	Male factor	105	Live birth singleton
52	Ssd	Anovulation	210	Live birth twins
53	Cam	Male factor	90	Live birth singleton
54	Vsm	Male factor	96	Live birth singleton
55	Agp	Tubal factor	67	Ectopic
56	Kam	Unexplained	109	Live birth singleton
57	Sps	Anovulation	79	Live birth singleton
58	Bpd	Tubal factor	289	Live birth twins
59	Fhr	Male factor	88	Live birth singleton
60	Yab	Anovulation	107	Live birth singleton
61	Ars	Endometriosis	102	Live birth singleton
62	Spp	Endomet + PCOS	126	Live birth singleton
63	Rse	Tubal factor	175	Live birth twins
64	Psk	Male factor	159	Live birth twins
65	Plp	Anovulation	76	Abortion – 6 weeks
66	Ppp	Unexplained	189	Live birth twins
67	Spm	Tubal factor	98	Live birth singleton
68	Srs		43	Biochemical
69	Sss	Male factor	245	Live birth triplets reduced to twins
70	Amp	Unexplained	56	Live birth singleton
71	Rmp	Anovulation	89	Live birth singleton
72	Mmp	Endometriosis	76	Live birth singleton

is in accordance with the results of previous studies. In viable pregnancies, the hCG concentrations in ICSI treatment cycles were a little lower than in other treatment groups. The same was true for the subgroup of male factor infertility. In with patients with a history of tubal infertility together with a low hCG calls for a second hCG and early localization of the pregnancy by transvaginal ultrasonography is necessary due to high incidence of ectopic pregnancy.

The aim of the study was to provide the probabilities for each type of outcomes for a given hCG level to help us counsel our patients and to plan the next step in her follow-up. Each hCG level has different promotion of pregnancy outcome. For example, equal proportion of biochemical pregnancies, miscarriages, and viable pregnancies were observed at an hCG level of 40 IU/l, which suggest that a second hCG reading a few days latter would be helpful. At an hCG level of 200 IU/l, two-third are live singletons, almost one third are twins, miscarriages are rare, and tubal pregnancies practically nonexistence.

INCIDENCE OF BIOCHEMICAL PREGNANCIES

Biochemical pregnancies occur commonly during ART procedures, but are more common when the initial day 14 hCG concentrations are low and are followed by an equally low day 21 values. When the initial hCG is low and though it rises and seem then to progress more normally, but never exceeds 200 IU/l by day 21, the possibility of biochemical pregnancies should be kept in mind. It is possible that some of these pregnancies were not inherently abnormal, but were simply delayed or late implantations relative to a normally evolving pregnancy. These implantations were probably hopelessly compromised from the start. This observation probably reflects the mixed etiology of those pregnancies which are lost prior to detection of a fetal heart or where no fetal heart could be demonstrated even with frequent scanning (anembryonic pregnancies). A small number of biochemical losses (approximately 6%) will occur despite completely normal hCG profiles. These are unexpected and cannot be predicted but could theoretically have resulted from some sort of implantation catastrophe occurring between day 21 and day 28. A falling hCG concentration therefore offers some reassurance against the possibility of an ectopic pregnancy.[14-16]

PREDICTING A CLINICAL ABORTION

If we know the probabilities for each type of outcome for a given hCG level, it would help us to counsel the patients and to plan the next step in their follow-up. Each hCG level has different proportions of pregnancy outcomes. Low maternal B-hCG is also associated with nonviable pregnancy later in the first trimester.[17] However, equal proportions of biochemical pregnancies, miscarriages, and viable pregnancies were observed at an hCG level of 40 IU/l on day 14 , which suggests that a second hCG level of >200 IU/l is more significance in the predictive values. The pattern of hCG in the pregnancies destined to end as FH^+ abortion is more variable. Approximately 73% of these pregnancies initially present with day 12 hCG levels > 50 IU/l, but subsequently hCG concentrations rise more slowly in FH^+ loss group compared with majority of equivalent pregnancies destined to end in a birth. It is difficult to quantify precisely FH^+ pregnancy losses because loss of one fetus in a

multiple implantation does not necessarily result in loss of the pregnancy.

Those pregnancies where a single fetal heart was demonstrated but had a low day 21-hCG (<1000 IU/l) is about twice as predictive of failure to progress to birth. It is likely that many of these losses were of chromosomally incompetent embryos. It was suggested[18] that there were differences in the distribution of chromosomal abnormalities in fetuses that aborted before the detection of a fetal heart and those that did so afterwards although they did not attempt to describe hCG profiles. However, a significant number of singleton pregnancies will continue and do so and hence the prediction becomes more difficult. Cut-off levels for predicting a viable pregnancy are different in different studies undertaken by different workers.[8-10]

PREDICTING A VIABLE PREGNANCY

Cut-off level for predicting a viable pregnancy is slightly higher than those reported with 80% sensitivity: 42 mIU/ml [9] 55 mIU/l[10] and 50 mIU/l.[8] Usually the hCG concentrations in singleton pregnancies remain similar regardless of the number of embryos transferred.

Male factor infertility was associated with viable pregnancy more often than the other causes of infertility, probably reflecting normal female fertility combined with suboptimal male fertility. In viable pregnancies, the hCG concentrations in ICSI treatment cycles were a little lower than in other treatment groups. This is probably as the early embryo cleavage is delayed in ICSI-derived embryos and the fragmentation of embryos is increased. However, the implantation potential is comparable with IVF-derived embryos.[19-21] The presence of both factors together resulted in the lowest hCG concentrations among viable pregnancies. Gold et al[22] in their study also reported lower hCG concentrations 16 days after embryo transfer in ICSI compared with IVF in the subgroup of unexplained infertility. The explanation for this was not clear. These data support the hypothesis that hCG levels greater than 200 mIU/ml on 14 days post-ET are more likely to have ongoing pregnancies; hCG levels greater than 600 have a high likelihood of a multiple gestation pregnancy.[23,24]

PREDICTING A MULTIPLE IMPLANTATION

At the other end of the scale, the probability of a multiple pregnancy is negligible if day 12 hCG concentrations were less than 50 IU/l. However, the incidence is almost 50% if the day 12 hCG is >100 mIU/l. And this becomes highly likely when hCG concentrations are rising strongly and by day 21, concentrations are >1000 IU/l. While in the majority of singleton births show an initial day 12 hCG between 50 and 100 IU/l. A cut-off level of 50 IU/l predicts pregnancy outcome with a sensitivity of 75% and a specificity of 81%, while an hCG value >135 IU/l predicts a multiple ongoing pregnancy with a sensitivity of 80% and a specificity of 88%.[6,7,10,16] The approximately doubled hCG level in twin pregnancies is similar as other pregnancies. As expected the mean hCG is greatest in the multiple pregnancies and this same observation has been made by many workers.[6,7,10,16,24-26]

PREDICTING AN ECTOPIC IMPLANTATION

The probability of an ectopic is high if the day 12 hCG is less than 50 IU/l. Rates of ectopic pregnancy[27] as high as 11% have been reported and are clearly associated with low hCG values. They could not be distinguished from the much larger group of other pregnancies with similar levels of hCG. However, above 50 mIU/l the odds were more favorable for intrauterine gestation and only about 1-2% present as an ectopic implantation. Among the women with tubal factor infertility, EP is seen in 10-20% of patients with the hCG values 5-66 IU/l. Also most ectopic pregnancies typically presented with a low hCG on day 14 but with in a week concentrations may have risen dramatically and in some a good hCG concentration (>1000 IU/l) was observed by day 21. A low hCG concentration alone is therefore not the only marker for EP. Tubal factor infertility is associated with higher incidence of nonviable pregnancies, at least partly due to the increased incidence of EP. Thus a history of tubal infertility together with a low hCG calls for second hCG and early localization of the pregnancy by transvaginal ultrasonography.[28-31] Another important factor to be kept in mind is the high incidence of heterotrophic pregnancies after ART and finding a intrauterine sac with values not in synchrony or with patient with symptoms, one must make an effort to look for and rule out a simultaneous ectopic pregnancy.[30,31]

TIMING OF hCG TESTING

Embryo development in early pregnancy follows a preprogramed-timing schedule and depends mainly on the embryonic age of the healthy, successfully implanted conceptus. The appearance of hCG in maternal serum is used to assess the time of clinically detectable implantation. Furthermore, because hCG production is a marker of trophoblastic activity, its serum doubling time is used as an reliable indicator of embryo quality. The importance of the timing of the hCG sample is reinforced by multiple studies and it is clear that day 14 and day 21 readings should be taken into account before predicting pregnancies. It is also clear that day 21 measurement gives a better sensitivity and specificity.[32-35] B-hCG serum levels on day 16 postretrieval were highly predictive of pregnancy outcome after a blastocyst transfer.[22]

An hCG concentrations of <100 IU/l is shown to discriminate between viable and nonviable outcomes. The incidence of a live birth is only 6% if the hCG was <100 IU/l but 82%if it is >100 IU/l. However a similar study demonstrated that a single hCG measurement is too early to differentiate between biochemical pregnancies abortions and ongoing pregnancies. [32] When the day 21 hCG is < 200 IU/l, then, the prognosis is poor and no birth occurs irrespective of day 14 concentrations.

CONCLUSION

A single hCG reading on day 12 after embryo transfer helps to plan the subsequent follow-up. The importance of the timing of the hCG sample is reinforced by multiple studies and it is clear

that day 12 and day 21 readings should be taken into account before predicting pregnancies. It is also clear that day 21 measurement gives a better sensitivity and specificity. Male factor infertility and ICSI are associated with relatively low hCG values in viable pregnancies. B-hCG serum levels on day 16 postretrieval were highly predictive of pregnancy outcome after a blastocyst transfer. hCG concentrations did not differ overall in the conventional IVF pregnancies compared with those achieved by ICSI. It is possible to make reasonable predictions of outcome after two estimations of hCG taken on days 14 and 21 after the procedure (egg collection). A day 21 hCG > 200 IU/l is associated with a reasonable prognosis with around 73% of such patients achieving a birth. Taking account of the day 14 hCG concentration can further refine the probabilities. Thus we can reassure normally pregnant patients as well as filter and manage those with nonviable outcomes more efficiently.

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