Article

Effects of culture medium on HCG concentrations and their value in predicting successful IVF outcome

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Abstract

The hypothesis was tested that the medium used to culture embryos affects the concentration of human chorionic gonadotrophin (HCG) early in pregnancy. The value of these concentrations in predicting successful outcome was also assessed for each medium studied. Patients undergoing IVF between January 1998 and December 2004 and having a day 3 embryo transfer were stratified into one of four groups according to the medium in which their embryos were cultured (P1, IVF500, G1.2, and G1.3). Using receiver operating characteristic (ROC) curve analysis, cut-off values for serum HCG concentrations on day 15 after embryo transfer were calculated for optimal discrimination between cycles resulting in implantation failure and success for each medium. Cut-off points were chosen to maximize sensitivity and specificity. For viable singleton pregnancies, mean HCG concentrations were greater for G1.3 and lower for IVF500 compared with the other media. Discriminatory HCG cut-off concentrations for predicting implantation success were lowest for IVF500, intermediate for P1 and G1.2 and highest for G1.3. The data support the hypothesis that the medium used to culture embryos significantly affects the concentrations of HCG early in pregnancy. Furthermore, when using HCG cut-off concentrations to assess pregnancy outcome, medium type should be taken into consideration.

Keywords: *culture media, embryo transfer, HCG cut-off value, human chorionic gonadotrophin, ICSI, IVF*

Introduction

Early prediction of pregnancy outcome after assisted reproduction treatment may benefit assisted reproduction patients, since these treatment cycles are associated with an increased incidence of ectopic pregnancies (Dubuisson *et al.*, 1991; Sugantha *et al.*, 2000), multiple gestations (Bergh *et al.*, 1999) and spontaneous abortions (Ben-Rafael *et al.*, 1988). Therefore, a reliable and inexpensive diagnostic test that could identify those pregnancies at high risk of poor outcomes would aid clinicians when counselling these patients regarding their treatment alternatives. Not only might such a test decrease the costs associated with monitoring an abnormal pregnancy, but it might also reduce the emotional burden experienced by these patients.

Several studies have examined the effectiveness of hormones such as progesterone, oestradiol, relaxin, pregnancy-specific β1 glycoprotein, inhibin and CA-125 in predicting pregnancy outcome (Witt *et al.*, 1990; Carmona *et al.*, 2003; Anckaert *et al.*, 2005). Among the different proteins studied, serum human chorionic gonadotrophin (β-HCG) has provided the best sensitivity and specificity for detection of pathological pregnancies (Buyalos *et al.*, 1992). In fact, using receiver operating characteristic (ROC) analytical techniques, a single HCG determination on days 12–20 after embryo transfer has been shown to be highly predictive of pregnancy outcome (Glatstein *et al.*, 1995; Bjercke *et al.*, 1999; Papageorgiou *et al.*, 2001; Poikkeus *et al.*, 2002; Urbancsek *et al.*, 2002; Anckaert *et al.*, 2005), and therefore may be the best current method of predicting pregnancy outcome potential.

When considering the diagnostic efficacy of single serum HCG values, one must consider the many advances in assisted reproduction techniques and the possible effects that these advances may have on serum HCG concentrations. In this context, developments in human IVF culture media have resulted in a move away from utilization of simple media consisting of a basic salt solution supplemented with glutamine, lactate, pyruvate and glucose, to the use of more complex formulations with additional amino acids, vitamins and nucleic acid precursors (Quinn, 2004). These changes may impact the ability of the embryo to secrete HCG after implantation, and thus should be considered when using a single serum HCG value as a diagnostic tool. In fact, with the continued introduction of such advances in assisted reproduction technology, it is critical that there is constant surveillance of these relationships as they relate to clinical outcome.

So far as is known, no previous reports have investigated the possibility that the culture medium used may affect embryo developmental potential as assessed by a single discriminatory HCG serum concentration. To fill this gap in knowledge, the hypothesis was tested that the medium used to culture embryos from days 1 to 3 affects the concentration of HCG early in pregnancy. The value of these concentrations in predicting successful pregnancy outcome was also assessed for each of the four medium formulations studied.

Materials and methods

The present study was approved by the Partners' Human Subjects Committee for medical record review.

Study population

All assisted reproduction cycles performed at Brigham and Women's Hospital between January 1998 and December 2003 (n = 8131) in patients <44 years old were screened to identify those having an embryo transfer on day 3 without use of a gestational carrier and/or egg donation. Gestational carrier and egg donor cycles were excluded, since their different hormone management for uterine receptivity might influence HCG concentrations. The resulting cycles (n = 6303) were further searched to identify those with an HCG determination on day 15 after transfer (day 0 being the day of transfer). These cycles (n = 1693) comprised the final dataset.

Patient stimulation regimens

All patients underwent ovarian stimulation according to the basic protocols as described by Racowsky *et al*. (2000). Briefly, ovarian stimulation was most commonly performed using luteal leuprolide acetate (Lupron; TAP Pharmaceuticals, Deerfield, IL, USA) down-regulation in conjunction with either highly purified FSH (Fertinex; Serono Laboratories, Norwell, MA, USA) or recombinant FSH (Follistim: Organon, West Orange, NJ, USA; Gonal-F: Serono). The standard daily gonadotrophin dosage was 3–4 ampoules (225–300 IU) administered as either a single or split dose. However, patients >40 years or those with a history of low gonadotrophin response were given up to a maximum of 8 ampoules daily (administered in divided doses), with or without human menopausal gonadotrophin (Repronex: Ferring, Tarrytown, NY; Pergonal: Serono). When at least two follicles reached a mean diameter of 18 mm and the oestradiol concentration was >500 pg/ml, 10,000 IU HCG (Profasi; Serono) was administered intramuscularly followed 36 h later by transvaginal oocyte retrieval. Luteal progesterone supplementation was initiated on the day after oocyte retrieval and was achieved by one of three regimens: (i) daily intramuscular progesterone (50 mg; Watson Laboratories Inc., Corona, CA, USA), (ii) daily vaginal gel (8% progesterone; Crinone; Wyeth-Ayerst, Philadelphia, PA, USA); or (iii) 3 times daily vaginal progesterone suppositories (200 mg tid; Serono Inc., Rockland, MA, USA) and continued until 10 weeks in patients who became pregnant.

Laboratory protocols

Oocytes were either inseminated in groups [3–5 oocytes in 1.0 ml HF-10 (Ham F10 nutrient mix with L-glutamine but without hypoxanthine; Sigma-Aldrich, St Louis, MO: stock # N3389) overlaid with 1.0 ml oil in Falcon 3037 dishes] using 100,000–400,000 motile spermatozoa (depending upon medium type) or underwent intracytoplasmic sperm injection (ICSI) 4–6 h after retrieval. Fertilization checks were performed 16–18 h after insemination or ICSI and zygotes were cultured in Falcon 1007 culture dishes (Becton Dickinson Labware, Franklin Lakes, NJ, USA) in 25 µl microdrops of medium [P1 (Irvine Scientific, Santa Ana, CA, USA); IVF500, G1.2, or G1.3 (Vitrolife, formerly known as Scandinavian IVF Science, Gothenburg, Sweden] overlaid with 8 ml oil purchased from one of three companies: Vitrolife, Irvine Scientific, Sage Biopharma, Bedminster, NJ, USA. The oil from each company was used at varying times during the study period, and all three oils were used with each of the four culture media. All cultures were maintained at 37°C in a humidified atmosphere with a controlled percentage of CO_2 in air in order to achieve the pH values as recommended by the manufacturer of the culture medium in use. This percentage ranged from 4.5 to 5.5%.

Embryos were evaluated for cell number, degree of fragmentation, and extent of symmetry 68–72 h post-insemination. Those with the highest cell number up to 8 cells, and having the lowest fragmentation score, and greatest symmetry in a given cohort were selected for transfer. Assisted hatching was performed in selected cases (i.e. patients ≥40 years, three previous failed IVF cycles, and/or with thick zonae) within 3 h of transfer using acid Tyrode's solution (Medicult, Jyllinge, Denmark). There were no differences in the distribution of patients with assisted hatching across the four media types. Embryo transfer was accomplished using Wallace catheters (Marlow/Cooper Surgical, Shelton, CT, USA) except in cases of difficult transfers, when a Marrs No. 4 or Marrs No. 5 embryo transfer catheter (Cook Ob/Gyn, Indianapolis, IN, USA) was typically used.

Determination of serum HCG concentrations

In the current programme, the target day for the first serum HCG determination is 15 days following day 3 transfer (day 0 being the day of transfer). Therefore, day 15 values were selected for study. These determinations were performed on site or at one of five satellite locations. Since several different serum HCG

assays were used among the sites (Bayer Immuno 1, Abbott Axsym, Roche Elecsys, Beckman Access 2, Dade Behring RXL), HCG values derived from satellite assays were corrected to the Bayer Advia Centaur assay that is used at Brigham and Women's Hospital (Bayer Corp., Diagnostics Division, Tarrytown, NY, USA). This correction was accomplished by using slopes of the least squares regression lines based on the 2003 College of American Pathologists surveys which demonstrated a coefficient of correlation better than 0.99 (for reference, please visit the website www.cap.org).

Analyses

In the first analysis, the effect of culture media on embryo viability was investigated by comparing day 15 HCG serum concentrations for gestations having only one implantation site on the first obstetrical ultrasound at 5 weeks post-embryo transfer, followed by a viable singleton pregnancy at 12 weeks. In the second analysis, ROC curves were derived to investigate the value of day 15 HCG serum concentrations in the prediction of implantation outcome following assisted reproduction. For the purpose of this study, implantation successes were defined by the presence of at least one viable fetus at or beyond 12 weeks post-embryo transfer. Conversely, implantation failures included all patients without viable fetuses at 12 weeks post-embryo transfer. This study group was expanded to include all gestational categories because no difference was found in the proportion of gestations that were vanishing or that comprised singletons, twins, triplets, or more than triplets among the four culture media types (data available from authors on request).

ROC analysis was performed using the Analyse-It statistical package (www.analyse-it.com) with Microsoft Excel (Microsoft Corporation, Seattle, WA, USA). ROC curves were generated for each culture medium that plotted the sensitivity of the day 15 post-embryo transfer HCG value to predict implantation outcome (true positive rate) versus the false positive rate (1–specificity) for multiple cut-off points. For each culture medium, the cut-off point with the highest sensitivity and specificity was identified, as determined by the software. Additionally, the positive predictive values, negative predictive values, areas under the curve (AUC), and 95% confidence intervals were generated for each medium. As previously described (Glatstein *et al.*, 1995), the following definitions were used: sensitivity (true positive rate) was the probability that a patient with an implantation failure would have a HCG concentration less than the cut-off value; specificity was the probability that a patient with an implantation success would have a HCG concentration greater than the cut-off value; positive predictive value was the probability that a patient with a HCG concentration less than the cut-off value would have an implantation failure; negative predictive value was the probability that a patient with a HCG concentration greater than the cut-off value would have an implantation success.

Statistical analyses

Comparisons of means were performed using the non-parametric Mann–Whitney and Kruskal–Wallis tests using either Analyse-it with Excel (www.analyse-it.com; Excel, Microsoft Corporation, Seattle, WA, USA) or StatView (SAS Institute Inc., Cary, NC, USA). Statistical significance was set at an alpha <0.05 (probability of a type I error) for all tests.

Results

No differences in HCG concentrations on day 15 post-embryo transfer were observed for cycles with or without assisted hatching or with or without ICSI for each of the four media tested (**Table 1**). Therefore, cycles involving either or both of these micromanipulation procedures were included in the subsequent analyses.

Table 2 shows the composition of each of the four culture media investigated. There were 1693 pregnancies for which day 15 HCG concentrations were analysed. Of these, the 686 viable singleton pregnancies at both 5 and 12 weeks were used to investigate the effect of culture media on embryo developmental potential in relation to day 15 post-embryo transfer HCG serum concentrations.

There were no significant differences across the four media types for infertility diagnosis (**Table 3**) or type of gonadotrophin used for ovarian stimulation (**Table 4**). Day 15 HCG concentrations were significantly impacted by media type, despite there being no difference among the four groups for maternal age, or number of embryos transferred ($P < 0.05$; **Table 5**). The concentrations of HCG significantly increased as each new generation of Vitrolife media was introduced ($P < 0.05$). In addition, while the mean HCG value associated with P1 was significantly higher ($P < 0.05$) than that for IVF500 (the first generation of Vitrolife media), it was not different from that for G1.2 (the second generation), and was significantly lower ($P < 0.05$) than that for G1.3 (the most recent generation).

Figure 1a–d show the ROC curves derived for each of the four culture media. Discriminatory HCG cut-off concentrations differed among the four media, with G1.3 having the highest cut-off value of 401 mIU/ml and IVF500 having the lowest value at 259 mIU/ml (**Table 6**). In fact, there was a 55% difference between the lowest and highest cut-off value. The sensitivity of the discriminatory cut-off value across the four media ranged from 75 to 84% and the specificities from 75 to 83%. Since the negative predictive values for all identified cut-off values were above 88%, any patient having an HCG concentration greater than the cut-off concentration had more than an 88% probability of having an implantation success. Among the four media, IVF500 had the greatest discriminatory power for predicting the probability for pregnancy outcome, with P1 having the lowest (AUC = 0.918 and 0.834 respectively). Additionally, compared with P1, there was increased sensitivity and specificity to predict pregnancy outcome for each of the Vitrolife media (**Table 6**).

Table 1. Human chorionic gonadotrophin concentrations on day 15 post-embryo transfer for cycles with or without assisted hatching (AH) or with or without intracytoplasmic sperm injection (ICSI).

Medium	*Cycle type*			
	ICSI	*ICSI/AH*	*IVF*	*IVF/AH*
P1	639.6 ± 639.7	591.9 ± 453.1	600.4 ± 494.4	564.5 ± 397.1
IVF500	356.6 ± 321.4	407.8 ± 224.3	457.2 ± 259.5	634.2 ± 619.4
G1.2	660.2 ± 515.3	594.8 ± 439.0	562.8 ± 423.08	577.5 ± 394.9
G1.3	695.7 ± 444.7	905.3 ± 520.5	658.9 ± 416.7	771.9 ± 558.4

Values are means ± SD.
There were no significant differences within rows between cycle types (Mann–Whitney test).

Table 2. Compositions of P1, IVF500, G1.2, and G1.3 culture media.

Component	*P1*	*IVF500*	*G1.2*	*G1.3*
NaCl (mmol/l)	101.6	+	+	+
KCl (mmol/l)	4.69	+	+	+
$MgSO_4$ (mmol/l)	0.20	+	+	+
$CaCl_2$ (mmol/l)	2.04	+	+	+
$NaHCO_3$ (mmol/l)	25	+	+	+
Na pyruvate (mmol/l)	0.33	+	+	+
Na lactate (mmol/l)	21.4	+	+	+
Na citrate (mg/l)	0.15			
Phenol red (g/l)	0.005			
Gentamycin (µg/ml)	10			
Penicillin G		+	+	+
K_2HPO_4		+	+	+
EDTA		+	+	+
Glucose		+	+	+
HSA		+	+	+
Alanine			+	+
Alanyl-glutamine			+	+
Asparagine			+	+
Aspartic acid			+	+
Glutamate			+	+
Glycine			+	+
Proline			+	+
Serine			+	+
Taurine (mmol/l)	0.05		+	+
Hyaluronan				+

HSA = human serum albumin. Concentrations are in mmol/l, unless otherwise stated. + = compound present, but concentrations are not available.

Table 3. Infertility diagnosis for which assisted reproduction treatment was recommended.

Medium	*Infertility diagnosis (%)*						
	Ovulatory disorders	*Endometriosis factors*	*Uterine factors*	*Tubal factors*	*Male factors*	*Unexplained*	*Other*
P1	8.5	11.4	0.7	16.4	30.6	22.4	10.0
IVF500	6.2	14.8	0.0	18.5	25.9	28.4	6.2
G1.2	6.4	12.9	1.2	17.5	26.9	26.9	8.2
G1.3	6.5	12.4	0.7	9.2	26.8	34.6	9.8

There were no significant differences within columns between media (chi-squared test).

Table 4. Effect of urinary FSH (u-FSH) versus recombinant FSH (r-FSH) on HCG concentrations on day 15 post-embryo transfer in pregnancies with one viable fetus at 12 weeks.

Medium	*u-FSH*		*r-FSH*	
	No. of cycles	*HCG (mIU/ml)*	*No. of cycles*	*HCG (mIU/ml)*
P1	17	623.8 ± 513.4	264	599.9 ± 510.2
IVF500	49	415.6 ± 269.8	32	509.9 ± 380.8
G1.2	13	575.0 ± 249.4	158	591.1 ± 447.1
G1.3	0	N/A	153	727.7 ± 475.3

Values are means ± SD; N/A = not applicable.
There were no significant differences within rows between FSH types (Mann–Whitney test).

Table 5. Effect of culture media on HCG concentrations on day 15 post-embryo transfer in pregnancies with one viable fetus at 12 weeks.

Medium	*No. of cycles*	*Age (years)*	*No. of embryos transferred*	*HCG (mIU/ml)*
P1	281	34.8 ± 4.2[a]	3.4 ± 1.5[a]	601.3 ± 509.5[a]
IVF 500	81	35.5 ± 3.7[a]	3.7 ± 1.2[a]	452.9 ± 319.4[b]
G1.2	171	35.3 ± 4.0[a]	3.5 ± 1.6[a]	589.8 ± 434.8[a]
G1.3	153	35.9 ± 3.8[a]	3.5 ± 1.7[a]	727.7 ± 475.3[c]

Values are means ± SD.
[abc]Groups within the same column having different superscripts are significantly different (Kruskall Wallis test, $P < 0.05$).

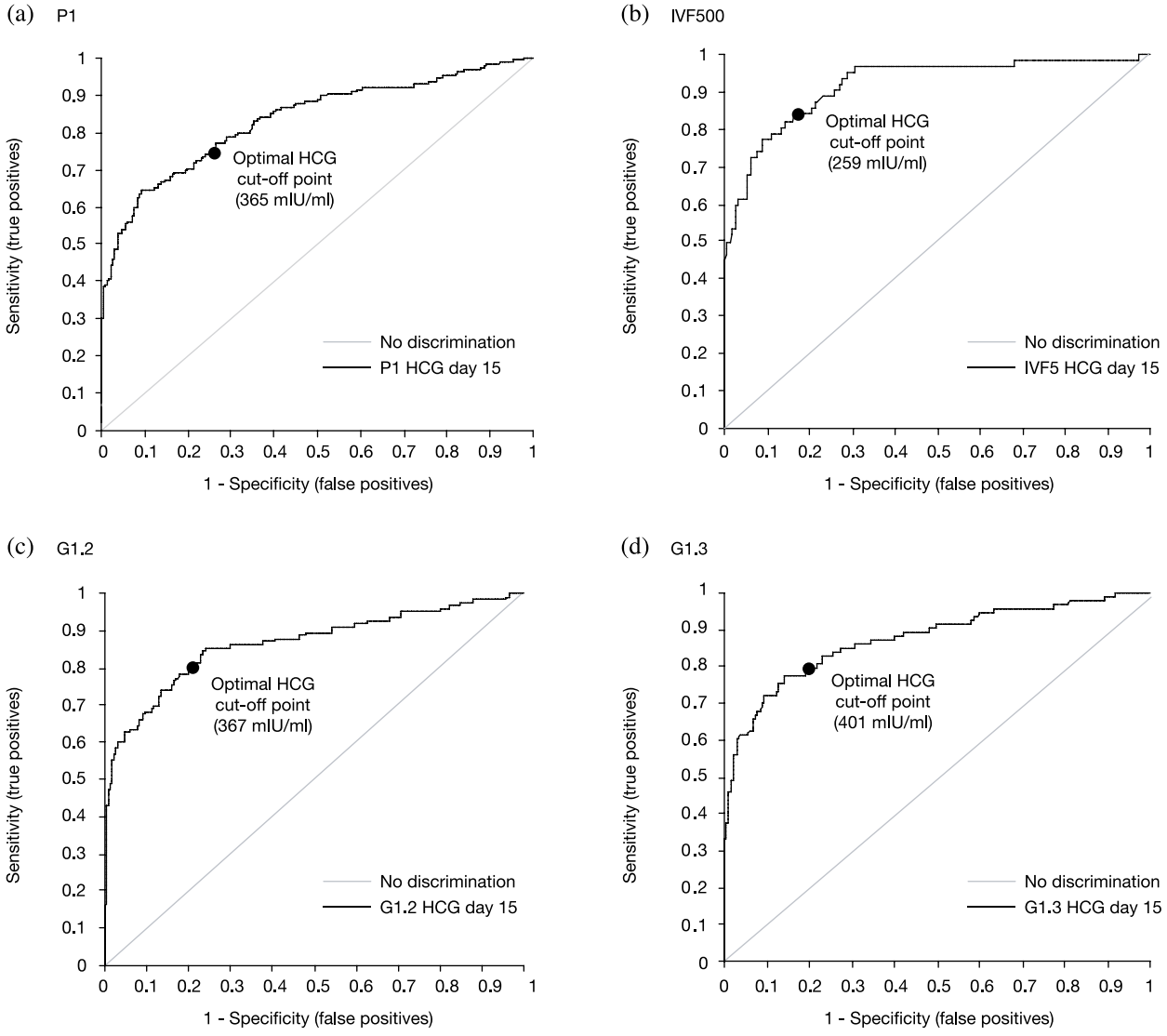

Figure 1. Receiver operating characteristic curves derived for P1, IVF500, G1.2 and G1.3.

Table 6. Prediction of implantation outcome by HCG serum concentrations on day 15 post-embryo transfer.

Medium	*No. of cycles*	*Cut-off value (mIU/ml)*	*Sensitivity (%)*	*Specificity (%)*	*PPV %*	*NPV %*	*AUC*
P1	683	365	75	75	53	89	0.834
IVF500	218	259	84	83	67	93	0.918
G1.2	446	367	80	80	60	91	0.865
G1.3	346	401	80	81	61	91	0.876

PPV = positive predictive value. NPV = negative predictive value. AUC = area under the curve.

Discussion

In the field of clinical assisted reproduction, use of chemically defined media for culturing embryos offers many advantages over the earlier undefined media, including less batch-to-batch variation, better quality control, greater reproducibility, and a reduction in the possibility of contamination from enzymes and growth factors (Karamalegos and Bolton, 1999; Summers and Biggers, 2003; Quinn, 2004). It is not surprising, therefore, that such media have widespread use in all domains of modern assisted reproduction (Mauri *et al*., 2001). Since the early 1980s when these media were first developed, their formulations have ranged from very simple, basic salt solutions such as B3 (Menezo *et al*., 1984) and human tubal fluid (HTF; Quinn *et al*., 1985) media to more complex media containing a variety of amino acids, vitamins, and nucleic acid precursors, such as KSOM^{AA} (Biggers *et al*., 2000; Biggers and Racowsky, 2001), recently modified and now known as Global Medium, and G1.2 and G1.3 (Gardner *et al*., 1998; Gardner *et al*., 2004).

The 'simplest' medium analysed in the present study was P1. This medium contains no glucose or phosphate, but does contain taurine and citrate. The second most complex medium was IVF500, containing glucose, phosphate, EDTA, human serum albumin, but neither taurine nor citrate. In contrast, the most complex culture media, G1.2 and G1.3, contain amino acids, lactate, pyruvate and glucose, with the sole difference between these two media being the addition of hyaluronan to G1.3.

Numerous studies have compared several commercially prepared media with respect to fertilization rate, embryo cleavage rate, embryo quality (as assessed by morphology), implantation rate, abortion rate and pregnancy rate (Staessen *et al*., 1994; Conaghan *et al*., 1998; Parinaud *et al*., 1998; Mauri *et al*., 2001; Van Langendonckt *et al*., 2001; Zollner *et al*., 2004). Although, taken together, the results of these studies seem to suggest that no single medium is superior when all these parameters are used as comparison standards, there are studies showing superiorities of some of these media when one or more of these outcomes were considered either separately or together (Ben-Yosef *et al*., 2004; Nedambale *et al*., 2004; Aoki *et al*., 2005). However, the possibility exists that a biochemical marker of embryo quality, such as HCG secretion, may provide a more sensitive assessment of embryonic developmental capacity. Indeed, several studies with mouse (Ho *et al*., 1994, 1995; Doherty *et al*., 2000), sheep (Young *et al*., 2001) and cow (Wrenzycki *et al*., 1996, 1999, 2001; Niemann *et al*., 2000) have demonstrated that culture media formulations affect embryonic genetic expression. In fact, altering the concentration of a single component in the medium can induce a change in such expression (Ho *et al*., 1994).

The results show that for viable singleton pregnancies, G1.3 medium (the most complex medium of the four media) was associated with higher HCG concentrations on day 15 post-embryo transfer compared with G1.2, P1 and IVF500 media. Of interest, however, the simplest medium, P1, was associated with HCG concentrations comparable with those of cycles in which the more complex G1.2 medium was used, and significantly higher ($P < 0.05$) than those using IVF500. It is unknown what underlying mechanisms might influence the differential effects on embryonic development that lead to these disparate HCG concentrations. Several possibilities exist, including alterations in genetic expression that lead to modulations in HCG secretion, and/or effects on the kinetics of embryo development that, in turn, either delay or accelerate implantation or influence the process of implantation itself. Culture media could have different influences on early developmental kinetics. This may be important because cleavage rate has been related to the potential of human embryos to form blastocysts *in vitro* (Muggleton-Harris *et al*., 1995; Alikani *et al*., 2000). Furthermore, embryos cleaving either too fast or too slowly have a compromised implantation potential (Racowsky *et al*., 2003) and, interestingly, cell number on day 3 has been associated with birth weight (Lieberman *et al*., 2006). In addition, many compounds are known to be key regulators of embryo metabolism and viability (e.g. amino acids and vitamins), and the culture of embryos in medium lacking these regulators could result in an inability of the embryo to control its metabolism (Lane and Gardner, 1998). Furthermore, in animal studies, the presence of globulin with human serum albumin has also been shown to increase the rate of embryo development and pregnancy rates beyond the concentrations achieved for human serum albumin alone (McKiernan and Bavister, 1994). It would seem, therefore, that media containing such metabolic regulators are required to support embryo development in the human, at least to the blastocyst stage. Clearly, further studies are necessary to explore these and other possible mechanisms that may underpin the associations between culture media type and HCG concentrations reported here.

The ROC analyses performed in this study revealed that the HCG cut-off values obtained were dependent upon the type of culture medium used (**Table 5**). Interestingly, despite the difference in composition between P1 and G1.2, the cut-off values were virtually identical (365 and 367 mIU/ml respectively). However, those for IVF500 and G1.3 differed by 55% (259 and 401 mIU/ml respectively).

A patient who had an HCG concentration higher than the cut-off value for her respective culture medium had a probability of implantation success equal to the negative predictive value for that culture medium (89, 93, 91, 91% for group P1, IVF500, G1.2 and G1.3 respectively). Conversely, a patient who had an HCG concentration lower than the cut-off value had a probability of an implantation failure equal to the positive predictive value for that culture medium (53, 67, 60, 61% for group P1, IVF500, G1.2 and G1.3 respectively). Among the four media, IVF500 had the greatest discriminatory power for predicting the probability for pregnancy outcome (AUC = 0.918) and P1 had the least discriminatory power (AUC = 0.834). Taken together, these observations indicate that it is important to know in what type of medium the embryo was cultured, in order to make a prognosis about pregnancy outcome based on day 15 post-embryo transfer HCG concentrations.

There are several limitations to a clinical retrospective analysis of this type. First of all, the study dataset comprised cycles spanning a period of 6 years. During this interval, the four media were used successively over time using the same culture system composed of microdrops of media under oil. Although there were some expected changes in personnel, it would seem unlikely that these impacted the present findings, since all practitioners were certified to the same standards and adhered to the clinical protocols in use at that time. However,

due to the changing technology of assisted reproduction, there were some inevitable modifications to some of the protocols during the study period. Perhaps the most significant of these modifications involved the types of gonadotrophins used for ovarian stimulation. While the gradual change from urinary FSH to recombinant FSH might have contributed to the media differences observed, a further subanalysis showed no gonadotrophin-related statistical differences on day 15 post-embryo transfer HCG concentrations in pregnancies with one viable fetus at 12 weeks among the culture media assessed (**Table 4**). Also, the average of 3.5 embryos transferred is unacceptably high. However the study spanned 6 years from 1998 to 2004. In 1998, patients were allowed to dictate the number of embryos that were transferred, which inevitably resulted in more than was desirable and a high multiple rate. From 1999 onwards, an algorithm based on internal data was used which stipulates the optimum number of embryos to transfer for each patient. Use of this algorithm, in addition to improved embryo selection, has resulted in an average of 2.4 embryos currently being transferred.

It is possible that the conclusions drawn from this study are skewed by early implantation sites which failed prior to detection at 5 weeks after embryo transfer. These possible additional implantations, theoretically, would have increased the HCG concentrations on day 15 post-embryo transfer. The present study design could not assess this possibility, since HCG titres were not monitored sequentially prior to day 15. A future prospective trial would be necessary to investigate this possibility.

In conclusion, so far as is known, this is the first study to investigate the relationship between culture medium type and day 15 HCG concentrations and the possible relevance of this relationship to the ability to predict discriminatory HCG serum concentrations for successful IVF outcomes. Within the constraints of this retrospective analysis, as discussed, the results reveal that day 15 HCG concentrations are significantly affected by the type of culture medium used. Furthermore, discriminatory HCG cut-off values have been determined which are specific for each medium. These findings will be of value when counselling pregnant patients. Furthermore, the data demonstrate the need for identification and constant surveillance of markers with which to assess the potential impact of any changes in laboratory procedure on clinical outcome from IVF. The present results contribute to a growing body of literature that highlights the importance of investigating the possible relationship between various media components and their effect on embryonic genome expression.

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