Human Chorionic Gonadotropin-to-Oocyte Collection Interval in a Superovulation IVF Program. A Prospective Study

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Purpose: *The aim of this study was to investigate whether the hCG–oocyte collection interval has any influence on the oocyte recovery rate, fertilization rate, and outcome of IVF-ET cycles.*
Methods: *Five hundred thirty-three consecutive patients undergoing their first IVF-ET treatment cycle at King's Assisted Conception Unit between 1993 and 1995 were included in this study.*
Results: *There was no significant difference in the oocyte recovery rates, fertilization rates, or outcome of IVF-ET treatment among the hCG–oocyte collection intervals examined (33–41 hr). None of the 533 women studied had ovulated before oocyte collection.*
Conclusions: *The results do not suggest a trend toward increased ovulation more than 36 hr after hCG administration.*

KEY WORDS: Human chorionic gonadotropin; interval; IVF; oocyte collection; pituitary downregulation.

INTRODUCTION

Most IVF programs currently use superovulation agents to obtain more oocyte and subsequently more embryos. Since the use of gonadotropins in conjunction with GnRH agonists was first reported for superovulation IVF (1), it has gained widespread popularity. The use of GnRH analogues has medical and practical advantages (2). Timing of human chorionic gonadotropin (hCG) injection is of critical importance in superovulation cycles without the use of GnRH analogues. A previous study has shown that precise timing of hCG administration has no significant advantages in cycles where a long protocol of GnRH analogue has been used (2). Most IVF programs time oocyte collections 33 to 36 hr after hCG administration. To the best of our knowledge, no study has addressed a prolonged hCG-to-oocyte collection interval in pituitary downregulated cycles and its influence on the outcome of IVF-ET cycles. We have conducted a prospective study to investigate the influence of widely varying intervals (33 to 41 hr) on oocyte recovery, fertilization, and cleavage rates and outcome of superovulation IVF-ET cycles where a long-protocol GnRH analogue is used. Patients with tubal damage, unexplained infertility, and male-factor problems were studied separately.

MATERIALS AND METHODS

Subjects

Five hundred thirty-three consecutive patients with all indications undergoing their first superovulation IVF-ET treatment cycle at the assisted conception unit of King's College Hospital between 1993 and 1995 were included in the study after ethical approval was obtained by the local research ethics committee. Patients were randomly allocated times for oocyte collection. All patients received hCG injections at 12 midnight. Time slots for egg collection were randomly allocated 36 hr after hCG administration.

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IVF Treatment

All patients were administered the GnRH analogue buserelin acetate (Suprefact; Hoechst, Hunslow, U.K.) starting on the first day of their menstrual cycle. The GnRH analogue was given either subcutaneously at a dose of 1000 μg per day or intranasally at a dose of 200 μg five times in 24 hr. Pituitary downregulation was considered to be achieved if the ovaries were inactive and the endometrium measured 3 mm or less as seen on transvaginal ultrasound after 14 days. If pituitary downregulation had not been achieved, buserelin was continued until downregulation was confirmed, before the administration of human menopausal gonadotrophin (hMG) was commenced. The dose of hMG was adjusted according to the patient's age (2 ampoules/day at <35 years, 4 ampoules at 35–37 years, 6 ampoules at ≥38 years), menstrual follicle stimulating hormone level, and previous ovarian response antiestrogens as FSH for ovulation induction therapy. The woman's response was assessed using ovarian and endometrial ultrasonography from day 8 of hMG therapy. The dose of hMG was increased if the follicular response was poor. When the mean diameter of the largest follicle reached 18 mm, 10,000 IU hCG was administered and the GnRH(a) stopped. All women received hCG injections at 12 midnight. Transvaginal ultrasound-directed oocyte recovery was performed 33–41 hr later (i.e., between 9 AM and 5 PM). The time of ovarian puncture was recorded. Embryo transfer was carried out approximately 48 hr after oocyte recovery. Progesterone pessaries (Cyclogest; Hoechst) were given at a dose of 200 mg/day for luteal support for 14 days starting on the day of oocyte recovery. A urine pregnancy test was done 2 weeks after ET if the patient had not bled. Clinical pregnancy was defined as the presence of a viable fetus as seen on ultrasound within the uterine cavity at 6 weeks of gestation.

Outcome Measures

For each woman the numbers of follicles aspirated, oocytes retrieved, oocytes fertilized, and embryos transferred were recorded, as well as evidence of clinical pregnancy at 6 weeks.

Statistical Analysis

Continuous, skewed variables were reported as medians and compared between groups using the Kruskal–Wallis analysis of variance. Categorical variables were compared using the chi-square test. The possible confounding effect of age was investigated by analysis of co-variance. A P value of less than 5% was taken to indicate statistical significance throughout.

RESULTS

Five hundred thirty-three consecutive women were included in this prospective study. Their indications for fertility, mean age, median time between hCG injection and oocyte collection, and various treatment outcomes are described in Table I. As expected, tubal damage was the largest single indication for IVF, and outcomes for those with an indication of male factor (at a time when intracytoplasmic sperm injection was not available) were noticeably worse.

For the purposes of comparison, the time between hCG injection and oocyte collection was collapsed

Table I. Descriptive Results, Overall and by Indication for Infertility

	Overall	Tubal damage	Unexplained indication	Male factor
No. of patients	533	258 (48.4%)	188 (35.3%)	87 (16.3%)
Median time since hCG (hr)	36.0	36.0	36.0	35.5
Mean age (yr)	33.4	33.1	33.3	34.1
No. of patients who had *no* oocytes collected	6 (1.1%)	1 (0.4%)	1 (0.5%)	4 (4.6%)
No. of patients who had embryos transferred	395 (74.1%)	222 (86.0%)	148 (78.7%)	25 (28.7%)
No. of pregnancies	68 (12.8%)	34 (13.2%)	32 (17.0%)	2 (2.3%)
Median nos. of				
Follicles aspirated	15	14	15	14
Oocytes retrieved	9	8	10	7
Fertilized oocytes (2PN)	4	5	5	0
Embryos	3	4	3.5	0
Median rates of				
Oocytes/follicles	63.2	63.5	66.7	55.6
2PN/oocytes	58.3	66.7	60.8	0.0
Embryos/2PN	75.0	83.3	75.6	0.0

Table II. Comparison of Outcomes by Time Since Administration of hCG

	Time since hCG administration (hr)			
	33 to <36	36 to <38	38 to <41	*P* value
Overall	$n = 258$	$n = 164$	$n = 111$	
Median rates of				
Oocytes/follicles	63.8	60.9	62.5	0.74
2PN/oocytes	54.2	55.9	66.7	0.23
Embryos/2PN	71.4	76.6	77.8	0.33
Pregnancies: no. (%)	27 (10.5)	21 (12.8)	20 (18.0)	0.14
Indication of tubal damage	$n = 126$	$n = 79$	$n = 53$	
Median rates of				
Oocytes/follicles	63.6	60.6	63.9	0.51
2PN/oocytes	66.7	66.7	70.6	0.65
Embryos/2PN	80.0	90.0	87.0	0.46
Pregnancies: No. (%)	12 (9.5)	12 (15.2)	10 (18.9)	0.20
Unexplained indication	$n = 88$	$n = 61$	$n = 39$	
Median rates of				
Oocytes/follicles	66.7	65.0	62.5	0.99
2PN/oocytes	57.1	55.0	66.7	0.042
Embryos/2PN	69.6	80.0	81.2	0.60
Pregnancies: No. (%)	14 (15.9)	8 (13.1)	10 (25.6)	0.25

into three groups as follows: less than 36 hr, 36 to less than 38 hr, and 38 hr or more. There were no statistically significant differences among the three time groups for any of the outcomes investigated, namely, percentage of clinical pregnancies and median rates for oocytes per follicle, two pronuclei (PN) per oocyte, or embryos per 2PN (Table II). Since an indication of male factor introduces as an additional variable influencing the likelihood of a successful treatment, the other two types of indications—i.e., tubal damage and an unexplained indication—were investigated separately. Only one statistically significant difference was obtained, between the median rates for 2PN per oocyte for the unexplained indication subgroup, the highest rate being observed in the "38+-hr" group.

The main factor known to influence the outcome of IVF treatment is maternal age. The mean ages of the three groups of women, for increasing time intervals, were 33.7, 32.9, and 33.2 years, respectively. An analysis of covariance was carried out to compare pregnancy outcome between groups while allowing for age. Neither time group ($P = 0.18$) nor age ($P = 0.15$) was statistically significant.

In the absence of statistically significant results, it is nevertheless wise to check for any apparent clinically important differences. No clear trends emerge from the data in Table II, except a possible suggestion that the percentage of women achieving clinical pregnancies is increased for the 38+-hr group. Table III shows the outcomes for all patients for eight separate hourly time intervals, and these also suggest a general upward trend in the rate of clinical pregnancies as the time interval increases.

The main objection to lengthening the interval between hCG injection and oocyte collection is the increased risk of the woman having already ovulated. In our sample, the number of women for whom no

Table III. Outcomes by Time Since Administration of hCG: Hourly Groups

	Time since hCG administration (hr)							
	33–<34	34–<35	35–<36	36–<37	37–<38	38–<39	39–<40	40–<41
No. of patients	23	101	134	108	56	53	37	21
Median rates of								
Oocytes/follicles	50.0	63.6	66.7	62.5	54.6	58.8	62.5	66.7
2PN/oocytes	60.0	50.0	57.1	59.4	50.0	65.4	66.7	66.7
Embryos/2PN	66.7	70.0	75.0	76.0	77.4	76.2	82.4	77.8
Pregnancies: No. (%)	1 (4.3)	7 (6.9)	19 (14.2)	16 (14.8)	5 (8.9)	9 (17.0)	7 (18.9)	4 (19.0)
Patients who had *no* oocytes collected: No. (%)	1 (4.3)	2 (2.0)	1 (0.7)	0 (0.0)	0 (0.0)	1 (1.9)	0 (0.0)	1 (4.8)

oocytes were collected from intact follicles was one, two, one, zero, zero, one, zero, and one, respectively (Table III), in eight increasingly hourly time groups. None of the 533 women had ovulated 33 to 41 hr after hCG injection.

DISCUSSION

To our knowledge, this is the first study reporting a wide (33- to 41-hr) hCG–oocyte recovery interval in an IVF program and its influence on oocyte recovery, fertilization, and cleavage rates and outcome for treatment cycle. We found no evidence of increased prior ovulation in patients with longer hCG–oocyte recovery intervals, and if anything, the clinical pregnancy rates appeared higher in such patients, although these results were not statistically significant.

One previous study (3) which looked at the 35- to 37-hr interval in cycles with GnRH analogues reported that oocyte maturity is attained 36 hr after hCG injection, and therefore oocyte recovery should not be performed before 36 hr, with no risk of spontaneous ovulation in the 36- to 37-hr interval. No study has looked at the interval later than 37 hr. Nader *et al.* (4) studied the pharmacokinectics of hCG and its relation to ovulation and concluded that ovulation may occur earlier than 36 hr in some women. They advised aiming for a <35-hr hCG–oocyte recovery interval if ovulation is to be avoided. Our study shows that no woman ovulated up to 41 hr after hCG injection. The timing of oocyte maturation in vivo after an injection of hCG was first estimated by Jagiello *et al.* (7). They found that many oocytes were in metaphase 1, although some were in metaphase 2, by 28 hr after hCG administration. The differences between the timing of follicular rupture after the LH surge and hCG injection may be due to the time required for hCG to become available after intramuscular administration (5). Natural ovulation after the LH surge may occur between 30 and 36 hr, whereas ovulation after hCG injection may occur between 36 and 40 hr in superovulation cycles without the use of GnRH analogues (6). Despite the wide use of pituitary downregulated cycles, IVF programs have continued to time oocyte collections 34–36 hr after hCG administration. We hope that our preliminary findings will reassure those who fear ovulation and diminished quality of oocytes if the hCG–oocyte collection interval is prolonged.

This is a prospective, study involving a large number of couples undergoing their first IVF treatment cycle. Proper randomization was not possible in this clinical context, as patients' choices had to be taken into account such as work and travel arrangements. Administration of hCG to all women at 12 midnight and allocation of random time slots for egg collection were achievable. We do not believe that the random allocation of time for oocyte collection in this paper biased the results in any way. Clinical criteria were not taken into account for any random allocation, but purely patients' choices based on convenience.

However, the most likely confounding factor, namely, maternal age, was almost-identical for different time intervals, and when we adjusted for this variable within the statistical analysis, the interpretation of the results was unchanged. The number of patients who waited more than 38 hr between hCG injection and oocyte recovery was relatively small, and hence the data lacked some sensitivity to detect differences in rare outcomes such as prior ovulation. Larger samples are therefore desirable, and these may also establish as statistically significant the apparent trend which we observed toward pregnancy outcomes with lengthening intervals. Further studies, with more patients allocated a hCG-to-oocyte collection interval of 38 hr or more, are needed to confirm the results of this research.

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