Comparison Between Two hCG-to-Oocyte Aspiration Intervals on the Outcome of In Vitro Fertilization

SVERRE BJERCKE,^{1,2} TOM TANBO,¹ PER OLAV DALE,¹ and THOMAS ÅBYHOLM¹

Submitted: October 20, 1999

Accepted: February 26, 2000

Purpose: To determine whether there was any difference in the outcome of in vitro fertilization when retrieval of oocytes was done 34 hr (group A) or 38 hr (group B) after hCG injection.

Methods: A total of 170 patients with tubal failure were randomized into either group A (83 patients) or group B (87 patients). They underwent in vitro fertilization according to described protocols and were compared with regard to the frequency of spontaneous ovulation, number of oocytes retrieved, oocyte cumulus complex quality, embryo quality, and implantation and pregnancy rates.

Results: There was no significant difference for any of the parameters tested for in group A and group B.

Conclusions: HCG can be administered at any time within the time interval of 34 to 38 hr before retrieval of oocytes without affecting the results of in vitro fertilization.

KEY WORDS: In vitro fertilization; hCG; embryo quality; implantation and pregnancy rates.

INTRODUCTION

The timing of oocyte retrieval in women undergoing in vitro fertilization (IVF) requires knowledge of the exact time interval between the luteinization stimulus and ovulation. The time interval is important because a number of essential processes including the start of luteinization, expansion of the cumulus cells, and the resumption of the reduction division of the oocyte should be well established before aspiration. Steptoe and Edwards (1) reported that the preovulatory oocytes are in the predicted stages about 35 to 36 hr postinjection of human chorionic gonadotropin (hCG). After the introduction of combined therapy using human

menopausal gonadotropin (hMG) together with a gonadotropin-releasing hormone-agonist (GnRH-a), endogenous luteinizing hormone (LH) fluctuations are eliminated and result in low and stable LH environment throughout the follicular phase until the luteinization stimulus is given as a single hCG injection (2). The hCG is injected when a sufficient number of follicles have developed (typically more than three follicles > 18 mm in diameter). Although successful fertilization in vitro of oocytes retrieved 60 hr after hCG injection recently was reported (3), in most IVF units oocytes are aspirated after 34–38 hr to allow final maturation. It seems logical to assume that a long time interval will yield eggs in which all processes are initiated and therefore more likely to develop into 2PN zygotes and normally cleaving embryos. Significantly more cleaving embryos of high quality have been reported to be obtained when the interval between hCG injection and oocyte retrieval was 38 rather than 34 hr (4).

In this prospective randomized study on patients with tubal failure undergoing IVF, we have compared the number of spontaneous ovulation, number of oocytes retrieved, oocyte–cumulus complex quality, embryo quality, and implantation and pregnancy rates in two groups of women undergoing oocyte aspiration 34 and 38 h after hCG injection.

MATERIALS AND METHODS

Subjects

During January 1996 through December 1997, couples who proved to have infertility from tubal factor were randomly allocated into two groups: A (83 patients) and B (87 patients). All other causes of infertility were excluded. Randomization was performed by drawing of sealed envelopes by a nurse who was not an active participant in the study. All had undergone

¹ Department of Obstetrics and Gynecology, National Hospital, University of Oslo, 0027 Oslo, Norway.

² To whom correspondence should be addressed.

ultrasound and laparoscopy to exclude subdiagnoses like polycystic ovary, myoma uteri, malformations of the uterus, or endometriosis. The median age of the participants was 35 (25–41) years in group A and 33 (25–41) in group B. Embryos were characterized after morphological criteria described by Van den Abbeel *et al.* (5).

In Vitro Fertilization Protocol

The patients underwent IVF-ET according to described protocols (6, 7). Pituitary down-regulation with GnRH-a (Suprefact, Hoechst, Frankfurt am Main, Germany) was used in all patients. Ovarian hyperstimulation was performed using follicle-stimulating hormone (FSH) (Fertinorm HP, Serono, Aubonne, Switzerland). The initial dosage was 225 IE. After 8 or 9 days the dosages were individually adjusted. Transvaginal ultrasound (US) and serum estradiol (E_2) concentrations were used to monitor the IVF cycle. To induce ovulation, 10,000 IE units injected hCG (Profasi, Serono, Aubonne, Switzerland) were administered subcutaneously when three or more of the follicles had a diameter exceeding 18 mm. Oocyte aspiration was performed 34 or 38 hr after hCG was given in groups A and group B, respectively, using a transvaginal US-guided approach. On day 1 after aspiration the oocytes were examined for diploid fertilization and on day 2 scored according to the method described by Van Abbeel *et al.* (5). Embryo transfer was performed 4 days after oocyte aspiration and a maximum of two embryos transferred. All patients included in this series received progesterone support, either as vaginal suppositories (600 mg daily) (Progestan, Organon, Oss, The Netherlands) or intramuscular injections (25 mg daily) starting on the day prior to oocyte aspiration. The progesterone support was terminated if the patient had not become pregnant or extended until vaginal US at 7 weeks in the case of pregnancy. Serum hCG levels were examined on the 12th day after ET at the Department of Chemical Chemistry of our hospital. In order to potentially differentiate between the two groups the number of oocytes retrieved at ovum pick up (OPU) and the number of spontaneous ovulations were determined. Finally, implantation and pregnancy rates in the two groups were calculated.

Statistical Analysis

The Mann–Whitney U and χ^2 tests were used as appropriate to compare the two groups. Unless other-

wise stated, values are expressed as median with range in brackets.

In order to demonstrate an increase in top score (T_1) of day 2 embryos from 10 to 20% by increasing the time interval between hCG administration and oocyte pick up from 34 to 38 hr, a minimum of 155 couples had to be included in the study. This calculation was based on a maximum incidence of spontaneous ovulation of 5%, a median number of oocytes retrieved of 9, a 60% diploid fertilization rate, a power of 90% ($\beta = 0.1$), $\alpha = 0.05$, and a two-tailed test. To further strengthen the validity of the study an additional 10% more couples were included resulting in 170 couples being eligible for randomization.

RESULTS

The results are summarized in Table I. There was no difference in the number of oocytes retrieved. No incidence of spontaneous ovulation before OPU was detected in group A and only one in group B. Furthermore, no significant differences were found for the frequencies of oocytes with diploid fertilization on day

Table I. Results of IVF in Relation to Oocyte Retrieval 34 and 38 hr After hCG Administration

	Time interval between adm. of hCG and oocyte retrieval ^a		
	34 hr	38 hr	
Day of OPU			
Number of Spontaneous ovulations	0	1	
Oocytes retrieved	867	963	
Oocyte retr./OPU	9 (2–25)	10 (1–31)	NS ^b
Day 1 after OPU			
Diploid fertilization	554 (63.9)	598 (62.1)	NS
Day 2 after OPU			
Embryo score			
T_1	45 (11.6)	55 (13.5)	NS
T_2	199 (51.3)	238 (56.7)	NS
T_3	101 (26.0)	91 (21.7)	NS
T_4	43 (11.1)	36 (8.6)	NS
Day 4 after OPU (ET-day)			
No. of embryo	157	166	
Embryo score			
T_1	12 (7.6)	15 (9.0)	NS
T_2	98 (62.4)	110 (60.3)	NS
T_3	31 (19.8)	31 (18.7)	NS
T_4	7 (4.5)	5 (3.0)	NS
Morula	9 (5.7)	5 (3.0)	NS
Implantation rate	15.3%	12.0%	NS
Pregnancy rate	24%	20%	NS

^a Figures given as n (%)

^b NS, Not significant.

1 after OPU and embryo score on day 2 after OPU in the two groups.

On the day of ET (day 4 after OPU) the number of embryos that was decided to be replaced and their scores were calculated. In group A there were 157 embryos and in group B there were 166 embryos. Neither the number of embryos nor the embryo scores showed significant differences between group A and B. In group A the implantation rate was 15.3% and in group B 12.0%, while the figures for pregnancy rate in the two groups were 24% and 20%, respectively.

DISCUSSION

This article gives the result of a prospective randomized investigation of the administration of hCG 34 or 38 hr before OPU in a group of 170 patients undergoing IVF. It is well known that with GnRH-a-controlled ovarian hypostimulation for IVF there is an implicit LH suppression and a longer in vivo maturation time than is possible without the use of GnRH. By producing a hypogonadotropic state it is possible to control the follicular development more readily. The optimal time interval between the administration of hCG and ovulation has been subject to controversy. Originally and before the days of GnRH agonist most centers carried out oocyte recovery between 32 and 36 hr after hCG administration because of data obtained from clomiphene and clomiphene + hMG treated cycles (8, 9) showing high biological variation in the incidence of preoperative ovulation in cycles where the interval between the luteinizing signal and oocyte recovery was greater than 35 hr. Edwards and co-workers (8, 10) declared that preoperative ovulation rarely occurred before 36 hr, so the delay of < 36 hr is a compromise between relative oocyte immaturity and avoidance of preoperative ovulation. This compromise results in a requirement for preinsemination oocyte maturation in vitro to reduce the incidence of polyspermi (9).

In women with normal menstrual cycles, the LH surge usually starts between days 12 and 16 and is between 1 and 2 days in duration (11, 12). Follicle rupture generally occurs about 36 hr after the detection of the onset of the surge (13). Some of the important changes initiated by LH surge or by an ovulation-inducing hCG injection (14) are changes in steroid hormone and prostaglandin biosynthesis, resumption of oocyte meiotic maturation, cumulus expansion, reduced cumulus cell-oocyte coupling, and the synthesis of preovulatory enzymes. The time between LH surge and ovulation varies among species, being only

about 12 to 15 hr in rodents, but as long as 42 hr in the pig (15). In the human, ovulation occurs between 40 and 63 hr after the E₂ peak in peripheral serum (16). This indicates that ovulation occurs between 16 and 39 hr after the LH surge. Peters and McNatty (17) have given a figure of 28 to 36 hr after LH surge for human. As a result of the hormonal changes taking place around the time of ovulation, changes also occur in the cellular investments surrounding the ovum.

The use of long-course GnRH-a combined therapy suppresses the LH concentrations, and probably reduces the variation in responses to hCG. It has been reported that no ovulation occurs within a delay of less than 39.5 hr, indicating that a longer in vivo period of maturation is possible in patients treated with long-course GnRH-a. The incidence of fully expanded cumulus cells was increased in the patients with longer delays (>36 hr). This apparent benefit was reflected in higher fertilization and cleavage rates (18).

The hCG is used as a surrogate LH surge to trigger the final follicular maturation before oocyte retrieval. Original observations described ovulation as a rare event before 36 hr after hCG administration (10). Of the oocytes retrieved 33–36 hr after hCG, 85% are in the metaphase II stage (19). Gudmundsson *et al.* (20) described 39 hr as the critical time to avoid spontaneous ovulation. They suggested that prolonged luteinization-to-oocyte-retrieval delay increased the production of oocytes with fully expanded cumuli, which may reflect improved oocyte maturation. They thought that the subsequent proportion of oocytes proceeding to fertilization and cleavage also increased, implying that a longer interval between hCG and oocyte retrieval may improve gamete quality by allowing more optimal in vivo maturation. Later, De Vits *et al.* (4) reported that significantly more cleaving embryos of high quality are obtained when the interval between hCG injection and oocyte retrieval was 38 hr rather than 36 hr. Since the time interval between the moment of hCG administration and oocyte retrieval probably determines the degree of cellular and cytogenetic maturation, the authors did find their results logical and assumed that the longest interval would yield eggs in which all processes are completed and therefore more likely to develop into 2PN zygotes and normally cleaving embryos. The number of investigations and the size of the studies so far reported are not sufficient to draw certain conclusions as to the optimal time for hCG administration.

We were prompted by the two latter reports to do a prospective, randomized study to look for evidence for differences in the results between administration

of hCG 34 hr (group A) or 38 hr (group B) before OPU. No significant differences could be found for the pregnancy rate, implantation rate, embryo score, or any of the other parameters examined. Intrafollicular changes in the preovulatory follicle triggered by ovulation-inducing hCG injection therefore seems to be sufficiently developed 34 hr after administration compared to 38 hr.

CONCLUSIONS

Our results indicate no differences for any of the parameters tested for whether hCG was administered 34 or 38 hr before OPU in women undergoing IVF. In fact, we believe that hCG can be offered to the patients at any time within this 4-hr time interval. As a consequence, the administration of hCG injections may not have to be subjected to as strict rules as previously practiced by most IVF units.

REFERENCES

1. Steptoe PC, Edwards RG: Laparoscopic recovery of preovulatory human oocytes after priming of ovaries with gonadotropins. *Lancet* 1970;1:683–689
2. Flemming R., Coutts JR: Induction of multiple follicular growth in normally menstruating women with endogenous gonadotropin suppression. *Fertil Steril* 1986;45:226–230
3. Al-Mizzen ESS, Balet R, Lower AM, Wilson C, McClure A.F, Al-Shawaf T, Grundzinskas JG: Unexpected successful fertilization in vitro of oocytes retrieved 60 hours after human chorionic gonadotrophin injection. *Hum Reprod* 1998;4:1020–1021
4. De Vits A, Gerris J, Joostens M, Aytos A: Comparison between two hCG-to-oocyte aspiration intervals (36 versus 38) on the outcome of in-vitro fertilization. *Hum Reprod* 1994;9 (Suppl 4):12–
5. Van den Abbeel E, Van der Elst J, Van Waesberghe L, Camus M, Devroey P, Kahn I, Smits J, Staessen C, Wisanto A, Van Steirteghem A: Hyperstimulation: The need for cryopreservation of embryos. *Hum Reprod* 1988;3 (Suppl 2):53–57
6. Åbyholm T, Tanbo T, Dale PO: The first attempt at IVF treatment. Results and requirement for satisfactory success rate. *Eur J Obstet Gynecol Reprod Biol* 1990;38:125–132
7. Tanbo T, Omland A, Dale PO: In vitro fertilization/embryo transfer in unexplained infertility and minimal peritoneal endometriosis. *Acta Obstet Gynecol Scand* 1995;74:539–543
8. Edwards RG, Fishel SB, Cohen J: Factors influencing the success of in vitro fertilization for alleviating human infertility. *J In Vitro Fertil Embryo Transfer* 1984;1:3–23
9. Trounson AO, Mohr LR, Wood C, Leeton JF: Effect of delayed insemination on in vitro fertilization, culture and transfer. *J Reprod Fertil* 1982;64:285–194
10. Edwards RG, Steptoe P: Indication of follicular growth, ovulation and luteinization in the human ovary. *J Reprod Fertil* 1975;22:121–163
11. McIntosh JEA, Matthews CD, Crocker JM, Broom TJ, Cox LW: Predicting the luteinizing hormone surge: Relationship between the duration of the follicular and luteal phases and the length of the human menstrual cycle. *Fertil Steril* 1980;34:125–130
12. Hoff JD, Quigley ME, Yen SSC: Hormonal dynamics in mid-cycle: A reevaluation. *J Clin Endocrinol Metab* 1983;57:792–796
13. Testart J, Frydman R: Minimum time lapse between luteinizing hormone surge or human chorionic gonadotropin administration and follicle rupture. *Fertil Steril* 1982;37:50–53
14. Hillier SG, Wickings EJ. Cellular aspects of corpus luteum function. *In The Luteal Phase*, SL Jeffcoate (ed). Chichester, John Wiley, 1985, pp 1–23
15. McNatty KP, Moore-Smith D, Makris A, Osathanondh R, Ryan KJ: The microenvironment of the human antral follicle: Interrelationships among the steroid levels in antral fluid, the population of granulosa cells, and the status of the oocyte in vivo and in vitro. *J Clin Endocrinol Metab* 1979;49:851–860
16. Richards JS: Maturation of ovarian follicles: Actions and interactions of pituitary and ovarian hormones on follicular cell differentiation. *Physiol Rev* 1980;60:51–89
17. Peters H, McNatty KP: *The Ovary*. London, Granada Publishing, 1980
18. Flemming R, Coutts JRT: Induction of multiple follicular development for IVF. *In Assisted Human Conception*, Vol 46, RG Edwards (ed). pp 596–615
19. Van Steirteghem AC, Liu J, Joris H: Higher success rate by ICSI than by subzonal insemination. A report of a second series of 300 consecutive treatment cycles. *Hum Reprod* 1993;8:1055–1060
20. Gudmundsson J, Fleming R, Jamieson ME: Luteinization to oocyte retrieval delay in women in whom multiple follicular growth was induced as part of an in vitro fertilization/gamete intrafallopian transfer program. *J Fertil Steril* 1990;53:735–737