Pregnancies in Humans by Fertilization in vitro and Embryo Transfer in the Controlled Ovulatory Cycle

Abstract. *Normal pregnancies have been established in four women with tubal infertility by fertilization in vitro, embryo culture, and embryo transfer after stimulation of follicular growth with clomiphene citrate. In three of these women the time of oocyte maturation was controlled by human chorionic gonadotropin. This procedure for the control of ovulatory response has many advantages when compared with the previously successful method of using the natural ovulatory cycle.*

The fertilization and early preimplantation development of the human ovum in vitro has been proposed as a treatment of infertility caused by occluded or damaged Fallopian tubes where it is not possible for the gametes to associate for fertilization (*1*). It has also been proposed as a diagnostic test for infertility of unknown cause (*2*). However, in the two documented cases of successful establishment of pregnancy and birth by techniques of fertilization in vitro (*3*), the procedures were carried out in women with natural or spontaneous ovulatory cycles; no drugs or hormones were used to control the ovarian response or time of oocyte recovery. Previous attempts to induce multiple follicular development with exogenous gonadotropins or clomiphene citrate and to control the final maturation of oocytes with human chorionic gonadotropin (hCG) for fertilization in vitro did not result in the establishment of normal pregnancies (*4, 5*). Consequently, it was generally considered that successful fertilization in vitro would be limited to the spontaneous ovulatory cycle and that stimulation of follicular growth and the hormonal control of ovulation or oocyte maturation is contraindicated.

In the study described here, 50 patients whose long-term infertility was considered primarily tubal were allocated to three treatment groups on a random basis. All these patients had blocked or abnormal Fallopian tubes and had varying degrees of tubal disease or endometriosis, or they had undergone tubal sterilization and only tubal remnants remained.

The patients in group 1 (N = 20) remained on their natural ovulatory cycles. They were not given any drugs or hormones for ovarian stimulation or for control of the time of ovulation. From day 8 or day 9 after the onset of menstruation, cervical mucus was examined daily for preovulatory changes, and follicular size was estimated by ultrasound (*6*) on day 11 or 12. The patients were admitted to hospital when cervical mucus changes and estimated follicle size (> 1.7 cm in diameter) indicated that ovulation would occur within a few days. Urine samples were then obtained every 3 hours and the rate of luteinizing hormone (LH) excretion in the urine was calculated with the Hi-Gonavis kit (Mochida Pharmaceuticals, Japan) or by radioimmunoassay (RIA) (*7*). When three consecutive urine samples showed a sustained increase in LH excretion rate, laparoscopy for oocyte collection was timed for 25½ to 27½ hours after the midpoint of the first sample showing an increased LH excretion rate (greater than twice the sample standard deviation of the previous samples).

The patients in group 2 (N = 10) were given 150 mg of clomiphene citrate (Clomid; S. Merrell, Australia) daily on days 5 to 9 after the onset of menstruation (day 1). Ovulation was allowed to occur naturally. On day 11 or 12, follicle sizes were determined by ultrasonic measurement and the patient was admitted to hospital when it was estimated that the largest follicle would be in excess of 1.7 cm in diameter. When the size of the largest follicle was smaller than 1.2 cm in diameter, another ultrasonic examination was performed 2 days later. Urine samples were obtained after the patients were admitted to hospital and the rate of LH excretion and time of laparoscopy were determined as described for the patients in group 1.

The patients in group 3 (N = 20) were given clomiphene citrate, and follicle size was determined by ultrasound as described for group 2. On day 12, 13, or 14, depending on follicle growth rate and the availability of the operating room, 4000 IU of hCG (Pregnyl; Organon, Australia) were injected intramuscularly 35 to 36 hours before the predetermined time of laparoscopy.

Mature oocytes were recovered from the large (> 1.5 cm in diameter) follicles present at the time of laparoscopy (*8*). The husband's sperm was washed and added to the oocytes at a concentration of 2×10^5 to 15×10^5 sperm per milliliter, and the oocyte and sperm mixture was incubated in 1 ml of Ham's F10 medium (Flow Laboratories) under a humidified gas phase of 5 percent CO_2, 5 percent O_2, and 90 percent N_2 in 5-ml tissue culture tubes as described (*2*).

Embryos that developed to the four-cell or eight-cell stage after 45 to 76 hours in vitro were transferred to the uterine cavity of the patient using a fine bore open-ended Teflon catheter. Either one or two embryos were drawn up in 25 to 50 μl of culture medium and the catheter was introduced gently through the patient's cervix. The embryos were expelled from the catheter with an additional 50 to 100 μl of air and the catheter was removed and checked for the absence of embryos. Some patients were given 5 mg of diazepam (Valium) and 250 mg of mefenamic acid (Ponstan). A variety of positions for the patients was used for embryo transfer and all transfers were carried out in a hospital operating room under sterile conditions. The patients were advised to rest quietly for 24 hours after transfer. Some of the patients were given 200 mg of 17α-hydroxyprogesterone hexanoate (Proluton Depot) every 5 to 7 days starting the day after embryo transfer.

Blood samples were obtained at intervals beginning 7 days after laparoscopy and assayed for plasma progesterone and βhCG concentrations (RIA-Quant; Mallinckrodt). Normal fetal growth and development were determined by ultrasound.

The results of the study are shown in Tables 1 and 2. Ovulation had already occurred in five patients at the time of laparoscopy (Table 1), but these patients were in groups 1 and 2 in which the time of laparoscopy was determined by the endogenous increase in LH. Invariably, oocytes are not recovered from ruptured follicles (*8*), so that in patients with single follicles (group 1), ovulation before laparoscopy results in failure of the procedure for that cycle. In the case of clomiphene-treated patients, multiple preovulatory follicles are frequently present, some of which may not have ovulated at laparoscopy. In such cases a mature oocyte may be recovered and fertilized. The complete absence of a large follicle or a visible recent ovulation occurred in two patients despite their apparently normal increase in LH (Table 1).

At least one mature oocyte was obtained from all patients given clomiphene and hCG. In this group (group 3), 27 of 31 oocytes (87 percent) developed to embryos of at least four cells. From one patient we obtained an oocyte on two separate collections, but fertilization failed to occur. This patient's husband produced semen that was within the normal quality standards of fertile men, and we are investigating the possibility of undetected abnormalities of the oocyte. In the other two groups there was a very

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Table 1. The recovery of oocytes from patients with tubal infertility and the development of embryos to at least the four-cell stage after fertilization in vitro.

Group and treatment	Ovarian response at laparoscopy			Patients from whom mature oocytes were obtained	Embryo development	
	Total number of patients	Patients who had ovulated	Patients with no large follicle		Number of mature oocytes	Number of embryos developed
1. Natural cycle	20	4	1	13	13	12
2. Clomiphene and natural ovulation	10	1	1	7	11	10
3. Clomiphene and hCG	20	0	0	20	31	27
Total	50	5	2	40	55	49

Table 2. The results of embryo transfer following in vitro fertilization.

Group and treatment	Number of			
	Patients with transferred embryos	Embryos transferred to each patient	Patients with increased elevated βhCG	Normal pregnancies
1. Natural cycle	11	1	1	0
2. Clomiphene and natural ovulation	5	1	0	0
	2	2	1	1*
3. Clomiphene and hCG	7	1	3	2
	9	2	3	1
Total	34		8	4

*Twin fetuses present.

high rate of embryo development from inseminations of mature oocytes.

Embryos were transferred to 11 of the original 20 patients (55 percent) in group 1 (natural cycle), but we found evidence of increased βhCG in only one of these patients. The one blood sample obtained from this patient, 14 days after laparoscopy, showed 9.2 mIU of βhCG per milliliter (the sensitivity of this assay is 4.2 mIU of βhCG (*9*). No exogenous hormones were given to this patient.

Embryos were transferred to seven of the original ten patients (70 percent) in group 2. To one of these patients we transferred two four-cell embryos, and ultrasonic examination showed normal twin fetuses. No exogenous hormones were given to this patient after laparoscopy.

Embryos were transferred to 16 of the original 20 patients (80 percent) in group 3. In three of these patients, one to four blood samples taken between days 7 and 16 after laparoscopy showed increases in βhCG from 6.6 to 9.4 mIU/ml. In another three patients, single fetuses were established that are normal by ultrasonic examination. To two of these patients we transferred one embryo; to the other we transferred two embryos. Two of these pregnancies have progressed normally to at least the 7th month of gestation. Two of the patients received 200 mg of hydroxyprogesterone hexanoate every 7 days for the first 12 weeks of gestation and the other patient received no hormonal treatment after laparoscopy.

These results demonstrate unequivocally that it is feasible to use hormones to control the ovulatory cycle of the human for fertilization in vitro. Thus the use of the natural ovulatory cycle for successful fertilization in vitro is not mandatory. A number of difficulties are associated with the natural ovulatory cycle. These difficulties include the necessity for frequent sampling of the blood or urine to assess the onset of LH release; the uncertainty of establishing the start of the LH surge in some patients when LH concentrations are low or variable; the increased chance of ovulation prior to laparoscopy; and the need for staff and facilities to be available 24 hours a day. An additional problem in some patients is that the surge in LH is occasionally not detected by Hi-Gonavis or RIA of the urine, and ovulation occurs undetected. These difficulties are largely overcome by the use of clomiphene and hCG. Patients may be allocated to surgical lists for oocyte recovery on a predetermined basis at least 3 to 14 days before laparoscopy. We have found that patient anxiety is reduced considerably if the time of laparoscopy is known well in advance. For the natural ovulatory cycle at least two assays must be done each day and the time of laparoscopy can only be determined 6 to 14 hours beforehand by the sustained increase in LH excretion.

Induction of the growth of multiple follicles by clomiphene does not induce the type of endocrine and follicular abnormalities reported by Edwards *et al.* (*5*) who used human menopausal gonadotropin (hMG) or hMG and clomiphene. In the patients that failed to become pregnant in our study, the luteal phase was not reduced in length even when there were three or more preovulatory follicles present in laparoscopy. Progesterone levels during the luteal phase were very variable as would be expected with multiple corpora lutea.

Although the success rate for establishment of pregnancy in this study was encouraging, for clinical purposes we consider the rate unsatisfactory. Our efforts were concentrated on maximizing oocyte recovery rates (*8*) and the rate of fertilization and embryonic development (*10*). The relatively low rate of pregnancy after transfer (four pregnancies in 34 patients receiving embryos) indicates that either the embryos have reduced viability or that the transfer procedure and subsequent treatment of the patient could be improved.

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References and Notes

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9. This patient reported heavy blood loss and clots at her period 20 days after laparoscopy.
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