IN VITRO FERTILIZATION AND EMBRYO TRANSFER

Editors

Markku Seppälä R. G. Edwards

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Preface

MARKKU SEPPÄLÄ

Department I of Obstetrics and Gynecology
University Central Hospital
00290 Helsinki 29, Finland

ROBERT G. EDWARDS

Physiological Laboratory
University of Cambridge
Cambridge CB2 3EG, England

In vitro fertilization (IVF) has become an effective and widely used method of helping an infertile couple to conceive a child. In addition to offering new hope, this technique has brought about a vast increase in knowledge of early reproductive phenomena. Indeed, human *in vitro* fertilization has already opened the door to a reproduction revolution and, by doing so, has stimulated wild speculation and public arousal about the consequences that may follow. The Helsinki Congress was third in the sequence begun in Kiel (West Germany) in 1980 and followed, by the Second Congress, in Annecy (France) in 1982. Scientific meetings on *in vitro* fertilization have grown progressively larger each time. In Helsinki, more than 500 scientists working on embryology, developmental biology, endocrinology, and andrology were brought together with clinicians to exchange information and to present 235 scientific papers on various aspects of *in vitro* fertilization. Other disciplines, such as those represented by church and laypeople, were also represented. It was reassuring to note that *in vitro* fertilization is safe, and that the early fears of its harmful effects on progeny have turned out to have no basis in reality. According to a collaborative study reported to the Congress by 65 active teams worldwide, *in vitro* fertilization does not produce any more faults than nature itself does, nor does it distort the sex ratio of the offspring. By the end of January 1984, a total of 600 births were reported, and almost another 600 were expected from ongoing pregnancies brought about by *in vitro* fertilization. We believe that human *in vitro* fertilization is here to stay, despite the ethical, moral, and legal debate still attached to it. These issues were widely discussed at the Congress, and the Helsinki Statement on Human *in Vitro* Fertilization was prepared and signed by leading workers in the field to focus attention on key issues that need resolution. This *Annal* contains these reports and other selected papers presented at the III World Congress of *in Vitro* Fertilization and Embryo Transfer held in Helsinki in May 1984.

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IN VITRO FERTILIZATION AND EMBRYO TRANSFER[a]

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MARKKU SEPPÄLÄ AND R.G. EDWARDS

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In Vitro Fertilization and Embryo Replacement: Opening Lecture

R. G. EDWARDS

Physiological Laboratory
University of Cambridge
Cambridge CB2 3EG, England

Bourn Hall Clinic
Bourn Hall, Bourn
Cambridge CB3 7TR, England

It is a pleasure to present the opening lecture at this symposium, a convocation that represents another step in the progress of the development of *in vitro* fertilization and its application to medicine. The field of *in vitro* fertilization is moving forward very quickly. We are still dominated by the need to improve success rates, still too low in many clinics, but standards are being set by some clinics which show how successful the procedure can be. Obviously the method is here to stay, at least for the treatment of infertility, and there seems little doubt that it may soon be applied to treat other problems such as inherited disorders.

Let me give pride of place in this paper to the ethical issues that confront us. I imagine that we all agree that many aspects of the procedure are now fully accepted by society as ethical. One point that is hardly ever questioned today, for example, is the importance of *in vitro* fertilization in alleviating infertility. This is very different from the situation 15 years ago, when virtually everything about the procedure was questioned. We were accused of overpopulating the world, immorally collecting eggs and spermatozoa, opening a Pandora's box of biological tricks, and jeopardizing ethical standards. The infertile couple had no supporters. Those debates are now well behind us, however, and the alleviation of infertility is increasingly accepted as an urgent social and clinical need.

Today, different issues are raised in ethical debates. The frozen storage of embryos is rapidly being introduced in some countries, where ethical questions have presumably been resolved. In my opinion, the problems of frozen storage can be solved fairly easily by legal or social regulation. Research on the spare embryos growing *in vitro* raises difficult ethical issues, which are still being hotly debated in my country. I have no doubt that we must do this research, to help improve our methods, and to introduce new concepts in stem cell biology, although obviously there must be limitations. Freezing might help delay decisions about such research, but many frozen embryos may never be replaced in their mothers, and will one day be used for research or discarded. Freezing thus merely buys some time, but ultimately the ethical dilemma of the "spare embryo" must be faced squarely. In some countries, all the embryos growing *in vitro* must be replaced in their mother to avert the neccessity of discarding spare embryos; this principle is even expressed in the law in some places. Such legislation is unacceptable, because ethical considerations of the spare embryo or any embryo growing *in vitro* must be minor compared with those of children born in massive multipregnancies.

Oocyte or embryo donation has also been practiced in some clinics and is likely to increase in frequency, raising new ethical issues. More questions concern the surrogate mother, yet even here an increasing number of articles in newspapers and in the media report successful surrogation, both from the point of view of the surrogate mother herself and from that of the natural parents. At present, virtually all commentators object to surrogate mothers, but I wonder how long this will last. Other issues are bound to arise or have arisen as the work progresses—the avoidance of excess multipregnancy, typing cleaving embryos for inherited defects, and one day in the future even using tissues from spare embryos for research or clinical practice.

I would now like to turn to the science and medicine of *in vitro* fertilization. Many aspects of the procedure are well established and accepted. These include the aspiration of oocytes, now reaching high proportions in most clinics, and perhaps the laboratory conditions for fertilization *in vitro*. Many clinics now attain good rates of fertilization, and the differences between the established and newer clinics are diminishing. There is no question either about the application of *in vitro* fertilization to various forms of infertility, not only in the wife, but also in the husband. There are new

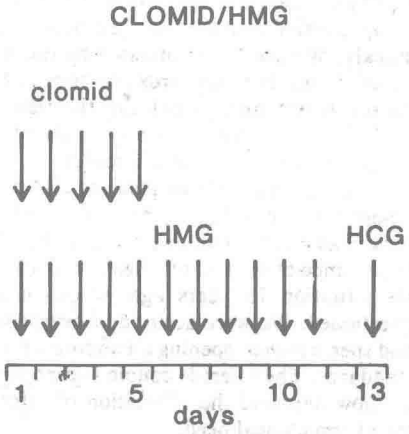

FIGURE 1. Schematic diagram of treatment with clomiphene, hMG, and hCG. This illustration shows that clomiphene and hMG were given from day 1, but this condition is seldom met and treatments usually start with clomiphene from day 2. hMG can be given at various times, for example, beginning on day 2, 5, or 7; our preliminary results suggest that day 5 might be preferable. hCG is given when urinary estrogens rise above 150–200 μg for 24 hr, and follicles exceed 2 cm in diameter.

methods, such as the various forms of ovarian stimulation, including the use of LH-RH agonists and antagonists, and the use of ultrasound alone for assessing follicle growth without the need for any endocrinologic procedures; ultrasound is also being used for the aspiration of follicles, replacing laparoscopy in this respect.

ENDOCRINOLOGY OF THE FOLLICULAR PHASE

Follicular Stimulation

Let me turn to the main substance of this paper: an assessment of experience at Bourn Hall and a comparison with the work of other clinics. I shall refer mostly to our own studies; extensive references to the work of other groups have been given elsewhere.[1] As do many others, we use clomiphene and hMG in combination for the stimulation of follicle growth (FIGURE 1). We were using this method in 1977, just

TABLE 1. Interval between Laparoscopy for Oocyte Recovery and the Subsequent Return to Menstruation in Patients Failing to Become Pregnant

Treatment	Days after Laparoscopy		
	9–11	12–13	14–15
Natural cycle	9 (11.1%)	17	55 (67.9%)
Clomiphene/LH surge	2 (3.8%)	22	47 (66.2%)
Clomiphene/hCG	4 (6.0%)	28	35 (52.2%)
Clomiphene/hMG/LH surge	17 (27.0%)	26	20 (31.7%)
Clomiphene/hMG/hCG	43 (42.2%)	33	26 (25.5%)

before Louise Brown was born, and it obviously has advantages. In my opinion, clomiphene has two major roles in this treatment: to stimulate follicular recruitment and to act an antiestrogen, preventing the short luteal phase caused by hMG. Clomiphene helps, but is not fully effective in this respect. This is seen clearly in the interval between laparoscopy for oocyte recovery and the return to menstruation (TABLE 1). With clomiphene alone, the interval in most patients is 14 days, as in the natural cycle. With clomiphene and hMG combined, the interval is shortened, and many patients have a very short luteal phase. Nevertheless, many pregnancies can be established without the need for any luteal support. With hMG alone, this period can be as short as 9 days, as shown in the data in FIGURE 2, published some years ago. The short luteal phase is presumably caused by the rising levels of estrogen in the follicular phase, which drives up prolactin, thus raising the levels of two luteolytic agents in the reproductive cycle.

The use of clomiphene and hMG also has another major advantage. It results in the

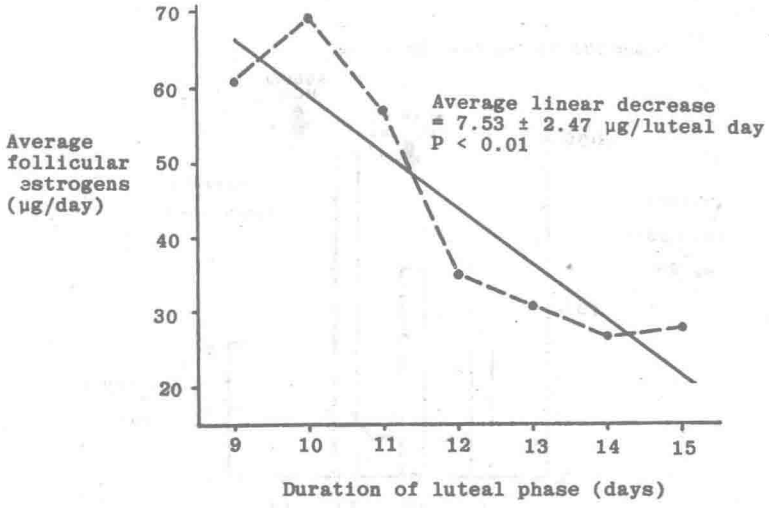

FIGURE 2. Duration of the luteal phase in relation to the average secretion of follicular estrogens in patients given hMG and hCG. There is a close relationship between follicular estrogens and the return to menstruation, although the luteal phase is greatly shortened in patients according to the amount of estrogen they secrete. (Reprinted by courtesy of the *British Journal of Obstetrics and Gynaecology*.)

TABLE 2. Numbers of Embryos Replaced in Patients after Various Follicular Treatments

Treatment	Embryos Replaced		
	1	2	3
Clomiphene/LH surge	380	222	44 (6.8%)
Clomiphene/hCG	136	157	68 (18.8%)
Clomiphene/hMG/LH surge	62	47	44 (28.8%)
Clomiphene/hMG/hCG	28	49	132 (63.2%)

continued development of several recruited follicles so that three or more preovulatory oocytes can be obtained from the majority of patients, and three embryos can be replaced in many of them. The distribution of patients with one or more embryos differs after treatment with clomiphene alone or with clomiphene and hMG combined, for the majority of patients have three embryos with the combined treatment (TABLE 2). Those patients with an endogenous LH surge have fewer embryos than those given hCG, hence most embryos are obtained with clomiphene and hMG followed by hCG. This evidence indicates the value of suppressing the endogenous LH surge, hence LH-RH antagonists could be invaluable by suppressing the propensity of a dominant follicle to induce an LH surge at an optimal time for itself and to inhibit the growth of its neighboring follicles. We have not yet used such agonists or antagonists.

Nevertheless, the best combination of clomiphene and hMG has not been reliably assessed, especially the optimal timing of injections of hMG. We are measuring the responses of patients to these different treatments (FIG. 3). All are given clomiphene daily from days 2 to 6, but hMG is given from day 2, day 5, or day 7, and continued daily until a satisfactory estrogen response is obtained. This work is still incomplete, but already it seems that the administration of hMG on day 2 is less successful than on

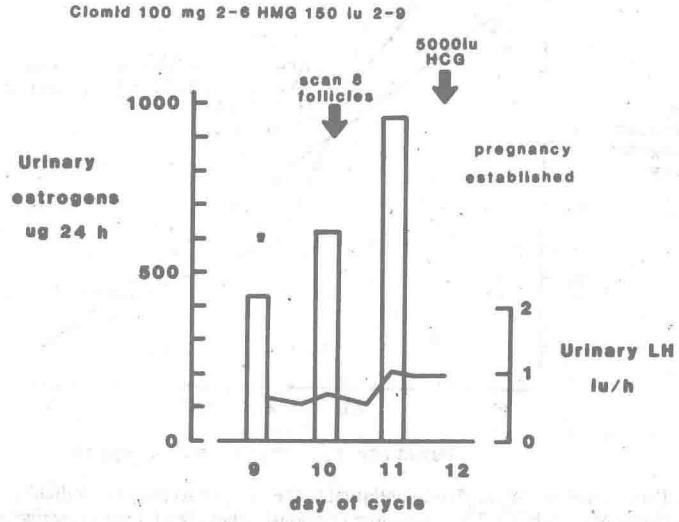

FIGURE 3. High levels of estrogens in a patient given clomiphene, hMG and hCG. No LH surge occurred. Pregnancy was established in this patient.

day 5 with respect to increasing the incidence of pregnancy and to reducing the chances of abortion.

Can Endocrinology of the Follicular Phase Be Abandoned?

Many clinics, perhaps sustained by the success obtained with clomiphene and hMG and with ultrasound for assessing follicle size, have abandoned any endocrinologic assessment of their patients. Ultrasound alone is used to determine follicle growth and to aspirate follicles. We remain to be convinced about the value of this procedure. In

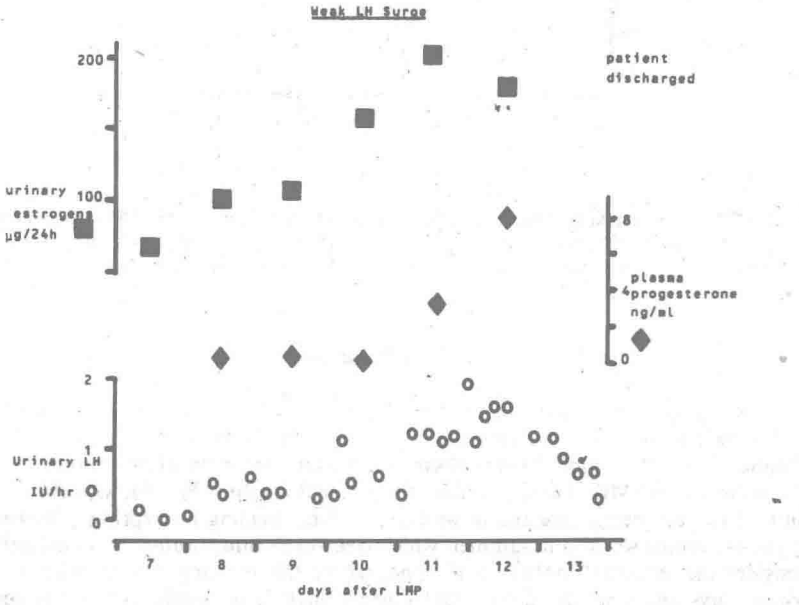

FIGURE 4. A weak LH surge in a patient led to increasing secretion of progesterone before laparoscopy could be attempted. Notice that the levels of urinary estrogens rose steadily and then declined on the day the LH surge occurred. The weak LH surge in this patient was detected, and the patient was discharged without laparoscopy for oocyte recovery.

some patients with many follicles, estrogens rise to very high levels indeed (FIG. 3), possibly leading to problems at ovulation or later in pregnancy. Many patients display an endogenous LH surge; and this must be monitored to avoid the loss of oocytes through spontaneous ovulation (FIG. 4). In other patients, the LH surge is very weak and can be very difficult to detect, sometimes being found, but occasionally overlooked. In these patients, hCG can be given in error after the LH surge has occurred so that follicles are luteinized at laparoscopy and both fertilization and embryonic growth are abnormal (FIG. 5), with dispermic eggs and fragmenting embryos. We believe that it is essential to monitor estrogen and LH to maintain the highest chances of success since many patients display an LH surge (TABLE 3).

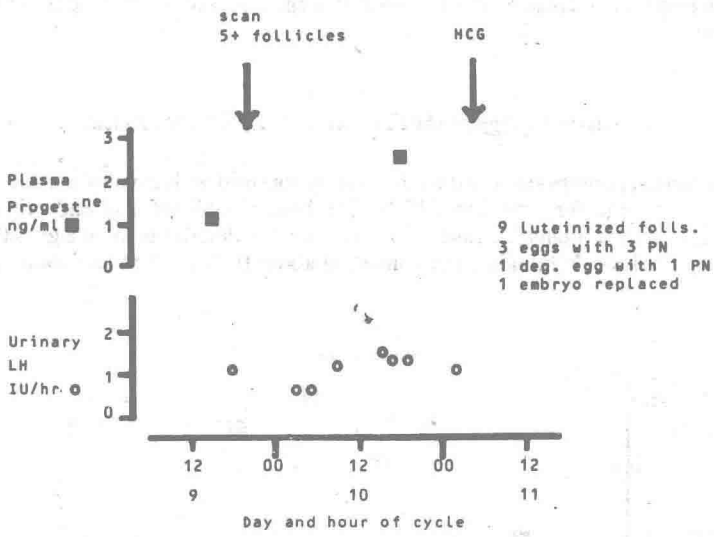

FIGURE 5. A second patient receiving clomiphene and hMG in whom the LH surge was barely detectable, and was therefore overlooked. hCG was given, but was obviously administered too late. At laporoscopy, follicles were luteinized and several fertilized eggs possessed three pronuclei.

The LH Surge

I will speak briefly about the LH surge. We still find that in the majority of patients LH is discharged in the morning at approximately 0700 hr in urine or at 0300 hr in plasma. This astonishing diurnal rhythm is maintained even after stimulation with clomiphene and hMG. There is a hint of a seasonal rhythm (FIG. 6), with the clearest diurnal rhythm being detected in winter, and freewheeling into spring, whereas the rhythms become weakest in summer, which freewheels into autumn. It is interesting to consider the seasonal rhythms in Finland, where this meeting is held, with its brief winter days and summer nights. This diurnal rhythm is closely tied to the cortisol rhythm. Patients coming to Bourn Hall from the Western Hemisphere keep their own "clock" time. This diurnal rhythm in LH must regulate the timing of ovulation because the interval between the plasma LH surge and follicle rupture is 37 hr. The majority of women must therefore ovulate in the afternoon (FIG. 7).

OOCYTE RECOVERY

I do not wish to speak in detail about the use of laparoscopy or ultrasound for aspirating oocytes, because this point will be discussed by other groups of investigators

TABLE 3. Proportion of Patients with Endogenous LH Surge

Treatment	Induction of Maturation	
	Endogenous LH Surge	Injection of HCG
Clomiphene	646 (58%)	361
Clomiphene/hMG	153 (42%)	209

elsewhere in this volume. High rates of success are clearly enjoyed by many clinics, especially with the use of laparoscopy, and some of our own data are shown in TABLE 4. I suspect that a combination of the two methods will prove best, and it will be of great interest to hear the points of view of workers using both methods.

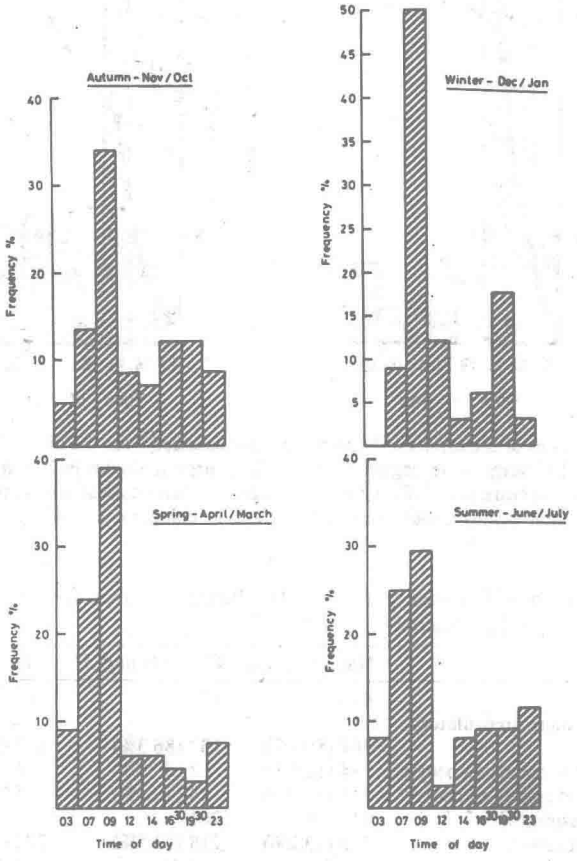

FIGURE 6. The beginning of the LH surge in patients in Bourn Hall throughout the year. The diurnal rhythm is most pronounced in winter and it freewheels into spring. It is weakest in summer and this pattern freewheels into autumn.

FERTILIZATION AND CLEAVAGE *IN VITRO*

Fertilization in Vitro

Let me briefly consider fertilization and embryo growth *in vitro*. Obviously, several media will sustain human fertilization, and we still use Earle's medium containing pyruvate and 8% serum. Our results remain good, with fertilization occurring in more than 80% of our patients. Several forms of infertility can be treated by this method (TABLE 5). In a sense fertilization is the major problem to overcome for some couples, especially in cases of male infertility caused by inflammation or antibodies. Even in