Review of scientific contributions by the Belgian medical centers concerned with human in vitro fertilization and embryo transfer (IVF)

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ABSTRACT In vitro fertilization and embryo transfer (IVF) may be considered as a particular application of modern medical therapeutics linked to human reproduction. The treatment of human sterility therefore involves some fundamental human values such as life, love and death. The quality of this highly technological treatment with fast knowledge of outcome at the end of the patient’s menstrual cycle has been evaluated since the early 80s. It is a typically multidisciplinary team effort involving medical doctors, biologists, laboratory technicians, nurses and clerks that is representative of modern medical practice. IVF covers much more than just embryology, as this review will explain. IVF developed in close relation with clinical and experimental research protocols, which are the major topics of this paper. The newness of the techniques used led to the necessary interactions between clinicians and biologists working on animal experimental embryology.

KEY WORDS: in vitro fertilization and embryo transfer

Introduction
The short history of human IVF can be divided into two periods. The first one ended in the United Kingdom in 1978 with the birth of Louise Brown, the first test tube baby, and was characterized by a small number of publications by pioneers inspired by animal IVF. The second period, after 1978, is marked by a wide acceptance of IVF in most developed countries. The reason was a revolutionary approach combined with increasing success in a difficult area of traditional medical therapeutics.

Today scientific publications are very goal-oriented and IVF has become an important topic within the field of human reproduction. At the same time ethical debates have arisen within the scientific community, as well as among the public, in a very similar way to how abortion debates challenged established social values.

What is an IVF cycle?
The basic principle from which the names ‘test tube baby’ and ‘in vitro fertilization’ originated consists in collecting oocytes from the ovary, fertilizing them in vitro and placing them back in the maternal uterus. Originally developed in the natural cycle to help couples with fallopian tube defects, the technique resulted in a first success by R.G. Edwards and P. Steptoe with the birth in 1978 of Louise Brown in the U.K. (Steptoe and Edwards, 1978). It appeared very quickly that this kind of treatment could succeed in many other infertility problems. Ovarian stimulation resulting in a large number of collected oocytes increased significantly the chances of obtaining pregnancy. This approach was rapidly adopted world-wide and the actual basic IVF trial can be summarized as follows: during the first part of the menstrual cycle (follicular phase) ovarian stimulation is obtained by various protocols. By means of a close monitoring of the sexual hormones in the blood combined with an echographic control we can evaluate oocyte maturation. Ovulation, when not spontaneously triggered by the patient herself, is provoked by an intramuscular injection as soon as hormone levels and follicle size reveal oocyte ripeness.

The surgical part of the procedure takes place 36 hours after ovulation induction or just before spontaneous follicular rupture. During this time lapse oocytes progress in their meiosis from prophase 1 (before ovulation triggering) to metaphase 2 (at follicular rupture).

The most popular way of ovum pick-up (OPU) at present is carried out by ultrasound-guided transvaginal puncture; however the only available method for years was laparoscopic retrieval. Once collected, the oocytes are placed in culture and inseminated with capacitated sperm a few hours later. Fertilization is evaluated 18 hours post insemination by observation of two pronuclei.

Embryo cleavage and quality is assessed just before embryo replacement (embryo transfer) 48 hours after pick-up. The embryos are replaced by a simple injection in the uterus. Therefore a catheter is passed through the cervix during a vaginal examination.

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Again the measurement of the hormones allows us to monitor the luteal phase, and if no pregnancy is detected between days 12 and 14 after pick-up, menstruation returns.

Many different variations of this common schedule have been elaborated. This review will first mention them throughout the different steps of the IVF procedure: follicular phase, surgical procedures, laboratory techniques, oocyte-, embryo-, sperm evaluation and in particular male infertility. Secondly the review will deal with cryopreservation of embryos, genetics, oocyte and embryo donation, embryo transfer, luteal phase, complications of the treatment and the outcome of the pregnancies obtained. And finally ethical aspects will be summarized. The contribution of the Belgian teams to all the above-mentioned study objects will be pointed out each time in this review.
Fig. 4. Microscope for micromanipulation
Fig. 5. Micromanipulation of mouse oocytes
**Review of the scientific contributions of Belgian teams**

**From follicular phase to oocyte pick-up**

The endocrinology of the follicular phase in IVF has been widely studied due to the importance of hormonal monitoring of a controlled hyperstimulation (Van Steirteghem et al., 1988b) and the link with hyperthyroidism (Noppen et al., 1985) or decreased serum osteocalcin levels (Rozenberg et al., 1989).

Most studies paid attention only to the clinical results of ovarian hyperstimulation because of the well-demonstrated relation with the growth rate of estradiol secretion during the follicular phase (Dinfield et al., 1985a). Since the detrimental effect of an endogenous LH rise was shown (Lejeune et al., 1986b), various groups extended their studies to LH-RH analogs which were introduced in the stimulation regimens. One molecule (Busereline-Suprefact) was clinically explored for its gonadotrophin-inhibiting effect (Smitz et al., 1988c) and its clinical usefulness (Lejeune et al., 1988; Lournaye et al., 1988a,b, 1989; Smitz et al., 1990a), especially in patients with failed classical stimulations (Smitz et al., 1987, 1988b; Lournaye et al., 1988c).

The potential toxicity of this new drug was also evaluated by the assessment of its concentration in the follicular fluid (Lournaye et al., 1989a) and by reports of its accidental administration in early pregnancy (Smitz et al., 1991). A global review of the use of the LH-RH analog in IVF was published in 1990 (Lournaye, 1990a).

Poor ovarian response to stimulation is still a highly interesting topic in IVF. It is associated with tubal infertility and procures low progesterone and testosterone levels in follicular fluids (Verbessem et al., 1989a) and with perivaginal ultrasound-guided aspiration (Wisanto et al., 1988). Some of the technical problems associated with the transvaginal puncture were reported (Wisanto et al., 1989a). Laparoscopic follicle aspiration implies a CO_2 pneumoperitoneum involving pH drops (Verbessem et al., 1988) detrimental to the murine IVF model (Puisant et al., 1986). This was not confirmed in human IVF practice (Hunting et al., 1989a).

Nowadays oocyte pick-up is mainly performed by ultrasound-guided transvaginal puncture. This technique was compared with the previously used laparoscopic retrieval (Van Rysselberge et al., 1989a) and with perurethral ultrasound-guided aspiration (Wisanto et al., 1988). Some of the technical problems associated with the transvaginal puncture were reported (Wisanto et al., 1989a).

Laparoscopic follicle aspiration implies a CO_2 pneumoperitoneum involving pH drops (Verbessem et al., 1988) detrimental to the murine IVF model (Puisant et al., 1986). This was not confirmed in human IVF practice (Hunting et al., 1989a).

Moreover, laparoscopy usually means the use of general anaesthesia, which may interfere with the endocrine ovarian function (Heytens et al., 1987). The detrimental effect of ultrasound on the oocytes used in IVF was demonstrated in the murine model (Puisant et al., 1984).

Measuring hormones in follicular fluids collected during OPU taught us that follicular rupture induces a complete modification of the peritoneal hormone levels (Lournaye et al., 1985; Vanluchene et al., 1991). Progesterone was higher and testosterone lower in follicular fluids containing mature fertilizable oocytes (Vanluchene et al., 1990, 1991). Renin and inhibin concentrations were related with estradiol levels (Pampfer et al., 1989).

**From gamete collection to embryo transfer**

Laboratory procedures were thoroughly evaluated. Timing of the moment of insemination was determined (Khan et al., 1989b). Different sources of human serum for supplementation of fertilization and culture medium were compared (Psalti et al., 1989; Staessen et al., 1990). The prognostic value of the morphological quality of the cultured embryos was analyzed (Puissant et al., 1987; Puissant and Leroy, 1989).

Substances secreted by the in vitro cultured embryos have been studied as well (Punjabi et al., 1990). General problems occurring in the IVF laboratory were reviewed on a historical basis (Leroy et al., 1987b). Laboratory problems related to the spermatozoa were widely studied: sperm phagocytosis in vitro (Pijnenborg et al., 1985), polyspermy (Englert et al., 1986a), fertilization failures (Deschacht et al., 1988; Barlow et al., 1990), correlation between sperm characteristics and fertilization (Gerris et al., 1986; Englert et al., 1987b; Gerris and Khan, 1987; Hening et al., 1990b; Barlow et al., 1991; Berberoglugil et al., 1992) and the impact of semen preparation methods (Naaktgeboren et al., 1985b; Englert et al., 1992).

The importance of IVF as a therapy for patients with sperm antibodies (Naaktgeboren et al., 1985b; Devroey et al., 1986a; Palermo et al., 1989b), Hodgkin disease (Tournaye et al., 1992) or obstructive azospermia (Cognat et al., 1991) were published. The interest of IVF with donor semen after failure of artificial insemination was demonstrated (Vekemans et al., 1987) even when frozen sperm was used instead of fresh semen (Englert et al., 1989).

More recently "assisted fertilization", a technique based on micromanipulation, was developed to help patients with previous fertilization failures or with an unacceptably low sperm count for classical IVF (Barlow et al., 1991; De Pijper et al., 1991; Palermo and Van Steirteghem, 1991; Van der Zwalm and et al., 1992).

Cryopreservation methods for embryos issued from IVF were tested with animal models (Leroy et al., 1984, 1987a; Maspio et al., 1984) and several clinical situations were analyzed (Van den Abbeel et al., 1988; Camus et al., 1989; Gordts et al., 1990; Noto et al., 1991). Some experimental work on oocyte freezing was conducted (Pensis et al., 1989).

Genetic aspects of infertility (Hens et al., 1988) and chromosome abnormalities in unfertilized oocytes were reported (Vercheval et al., 1988; De Sutter et al., 1991). Preliminary work on preimplantation diagnosis was done (Njis and Van Steirteghem, 1990; Sermon et al., 1991).

**From embryo replacement to birth**

Embryo transfer is usually performed by the vaginal way. Different experimental and clinical studies about this part of the IVF procedure were made: the risk of expulsion (Englert et al., 1985), the materials (Wisanto et al., 1989b) and media to be used (Khan et al., 1991) and the technique (Englert et al., 1986b) for embryo transfer were described. Two alternative techniques of replacement are: gamete intrafallopian transfer (GIFT) and zygote intrafallopian transfer (ZIFT). Both are based on transfer into the fallopian tube during laparoscopy and were studied (Braeckmans et al., 1987; Khan et al., 1986; Palermo et al., 1989a) and compared (Devroey et al., 1990a).

The endocrinology of the luteal phase (Lejeune et al., 1986a; Lejeune, 1988; Smitz et al., 1988a; Van Steirteghem et al., 1988a) and the implantation of the human embryo were extensively studied in relation to other animal species (Leroy and Lejeune, 1985; Lejeune et al., 1986b; Leroy, 1982).

Drug administration schemes (Devroey 1989a,b) for patients without functional ovaries were analyzed in oocyte donation programs. The effectiveness of these schemes was studied on the basis of endometrial morphology (Dehou et al., 1987; Bourgain et al., 1990) and clinical results (Devroey et al., 1988a,b, 1989).
hyperstimulation syndrome, a specific complication of controlled stimulation for multiple oocyte retrieval, was studied (Smitz et al., 1990b). The characteristics of these hyperstimulation cycles were analyzed to discover predictive criteria (Delvigne et al., 1991). Radioimmunoassays for the detection of IVF pregnancies were compared (Heip et al., 1986). Early pregnancies showed unusual patterns suggesting delayed implantation (Englert et al., 1984; Naaktgeboren et al., 1986, 1987).

Treatment results were analyzed as an entity (Staessen et al., 1989) or according to specific indications, such as endometriosis (Devroey et al., 1987) or male infertility (Englert et al., 1987b; Tournaye et al., 1991).

Success rates were compared in relation to the different causes of infertility (Naaktgeboren et al., 1985a) and mathematical models were developed attempting to predict the success rate after several trials (Bouckaert et al., 1989).

The evolution of the pregnancies characterized by a higher early pregnancy loss (Barlow et al., 1989) and more multiple pregnancies (El Khazen et al., 1986b; Bollen et al., 1991) was reported. A method for reducing the risk of multiple pregnancies was analyzed (Leroy et al., 1990). Certain placental abnormalities supposed to be linked to perturbations in the orientation of the blastocyst at the moment of implantation were studied (Englert et al., 1987a; Jauniaux et al., 1990).

**Ethical aspects**

IVF brought to the attention of the public ethical questions initially faced by the medical teams alone. Within society questions concerning these new technologies were raised. Most of the Belgian IVF teams participated from 1986 to May 1987 in a national reflection ending with a congress and a publication in which nearly all ethical aspects of gamete donation and the status of the embryo were discussed (Demeester and Demeyer, 1987). The large number of contributions in natural scientific publications, mass-audience books and newspapers illustrate the desire of the scientific and medical world to keep in closer contact with society than ever before.

**Conclusion**

Belgium's scientific contributions in the area of IVF are at a higher level than one would suppose for a country so small in size. The review has limited itself to international medical and scientific

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Fig. 6. Vaginal echography of a hyperstimulated ovary
Selected References


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