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 contries. 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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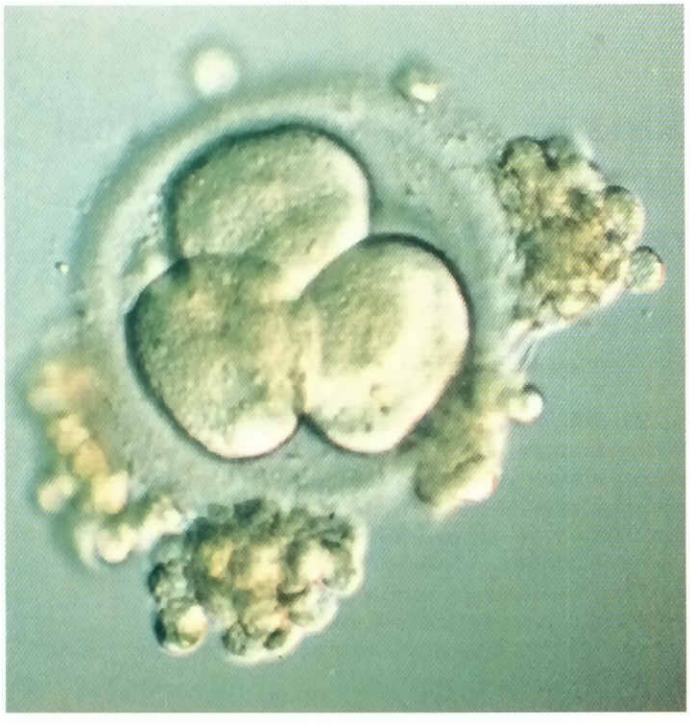

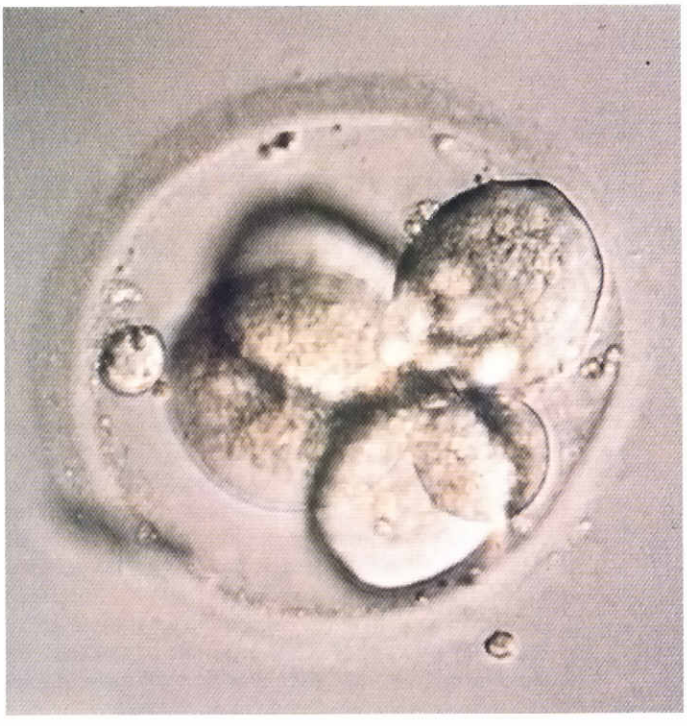

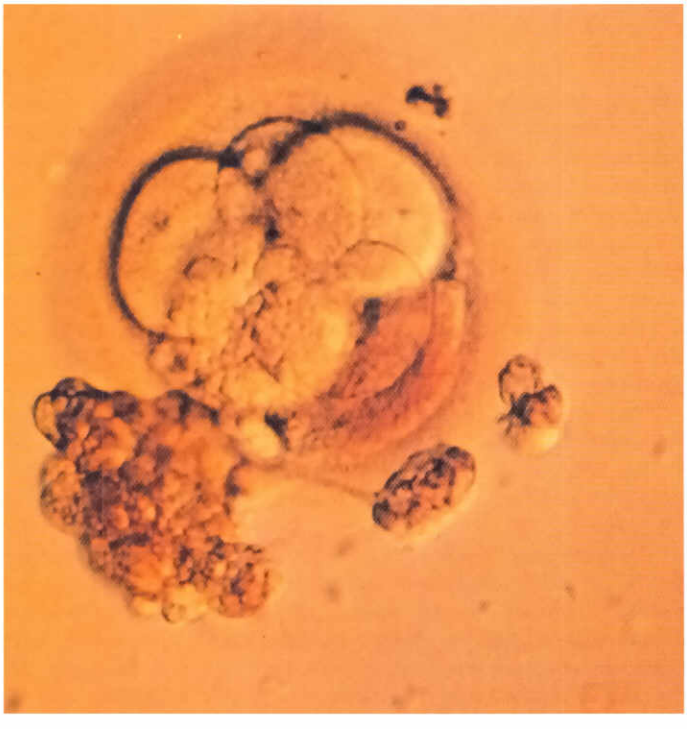

Fig. 1. High quality human embryo

Fig. 2. Poor quality human embryo

Fig. 3. Human embryo after cryopreservation and thawing loss of one blastomere

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.

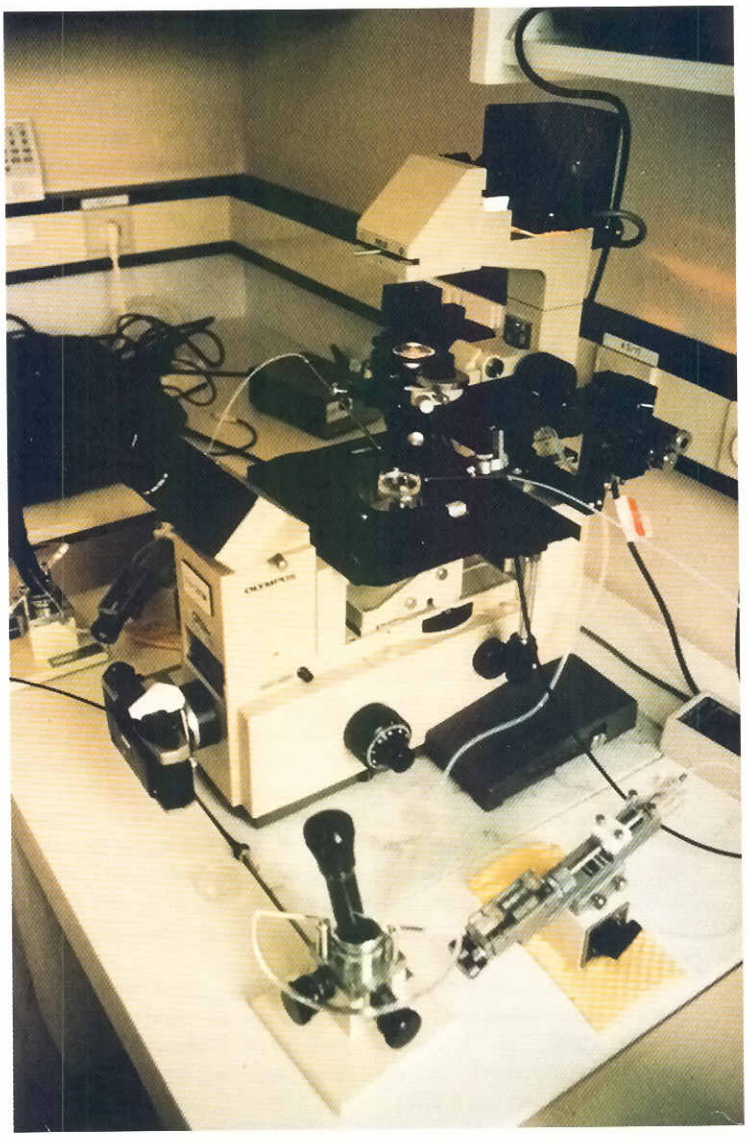

Fig. 4. Microscope for micromanipulation

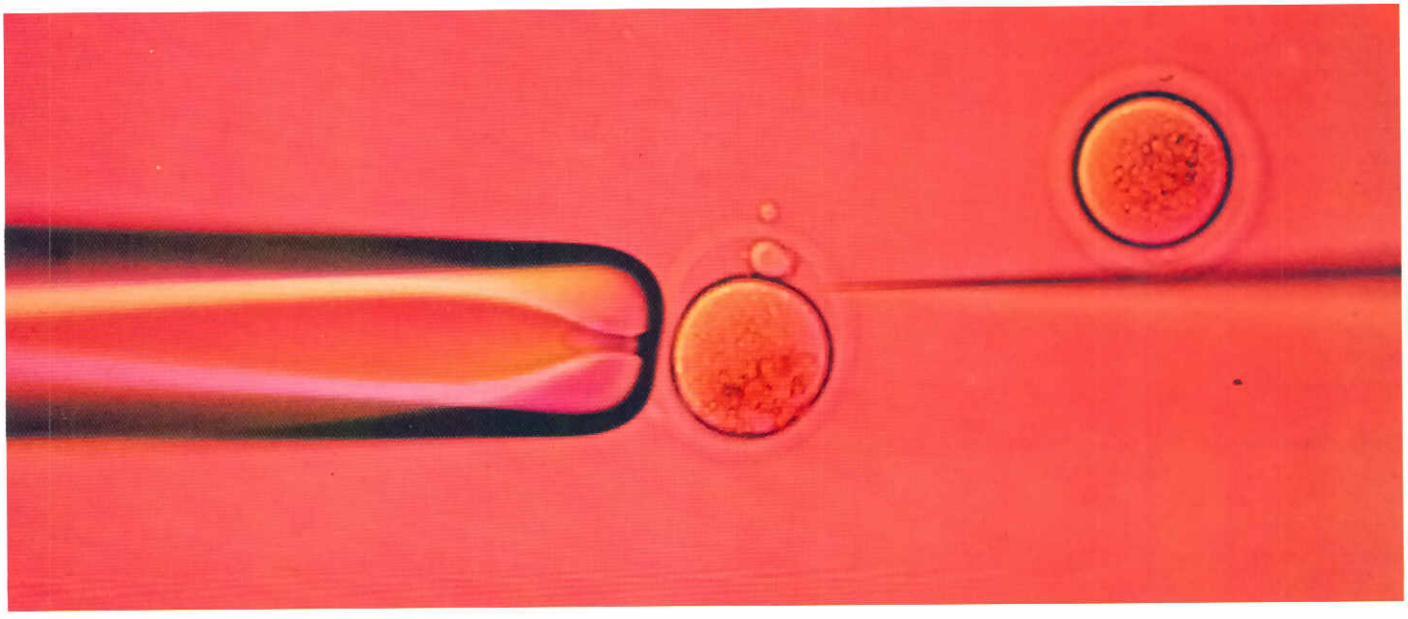

Fig. 5. Micromanipulation of mouse oocytes

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 (Dirnfeld *et al.*, 1985a). Since the detrimental effect of an endogenous LH rise was shown (Lejeune *et al.*, 1986b), various groups extended their studies to LHRH 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; Loumaye *et al.*, 1988a,b, 1989; Smitz *et al.*, 1990a), especially in patients with failed classical stimulations (Smitz *et al.*, 1987, 1988b; Loumaye *et al.*, 1988c).

The potential toxicity of this new drug was also evaluated by the assessment of its concentration in the follicular fluid (Loumaye *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 LHRH analog in IVF was published in 1990 (Loumaye, 1990a).

Poor ovarian response to stimulation is still a highly interesting topic in IVF. It is associated with tubal infertility and procures low prognostic value for patient's future IVF trials (Van Rysselberge *et al.*, 1989b). It may be predicted by a clomiphene citrate challenge test (Loumaye *et al.*, 1990b).

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 in the murine IVF model (Puissant *et al.*, 1986). This was not confirmed in human IVF practice (Hinting *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 (Puissant *et al.*, 1984).

Measuring hormones in follicular fluids collected during OPU taught us that follicular rupture induces a complete modification of peritoneal hormone levels (Loumaye *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 throughly 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), polyspermia (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; Hinting *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 azoospermia (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.*, 1991b; De Pijpere *et al.*, 1991; Palermo and Van Steirteghem, 1991; Van der Zwalmen *et al.*, 1992).

Cryopreservation methods for embryos issued from IVF were tested with animal models (Leroy *et al.*, 1984, 1987a; Massip *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 (Nijs 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 laparascopy and were studied (Braeckmans *et al.*, 1987; Khan *et al.*, 1988; 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). The

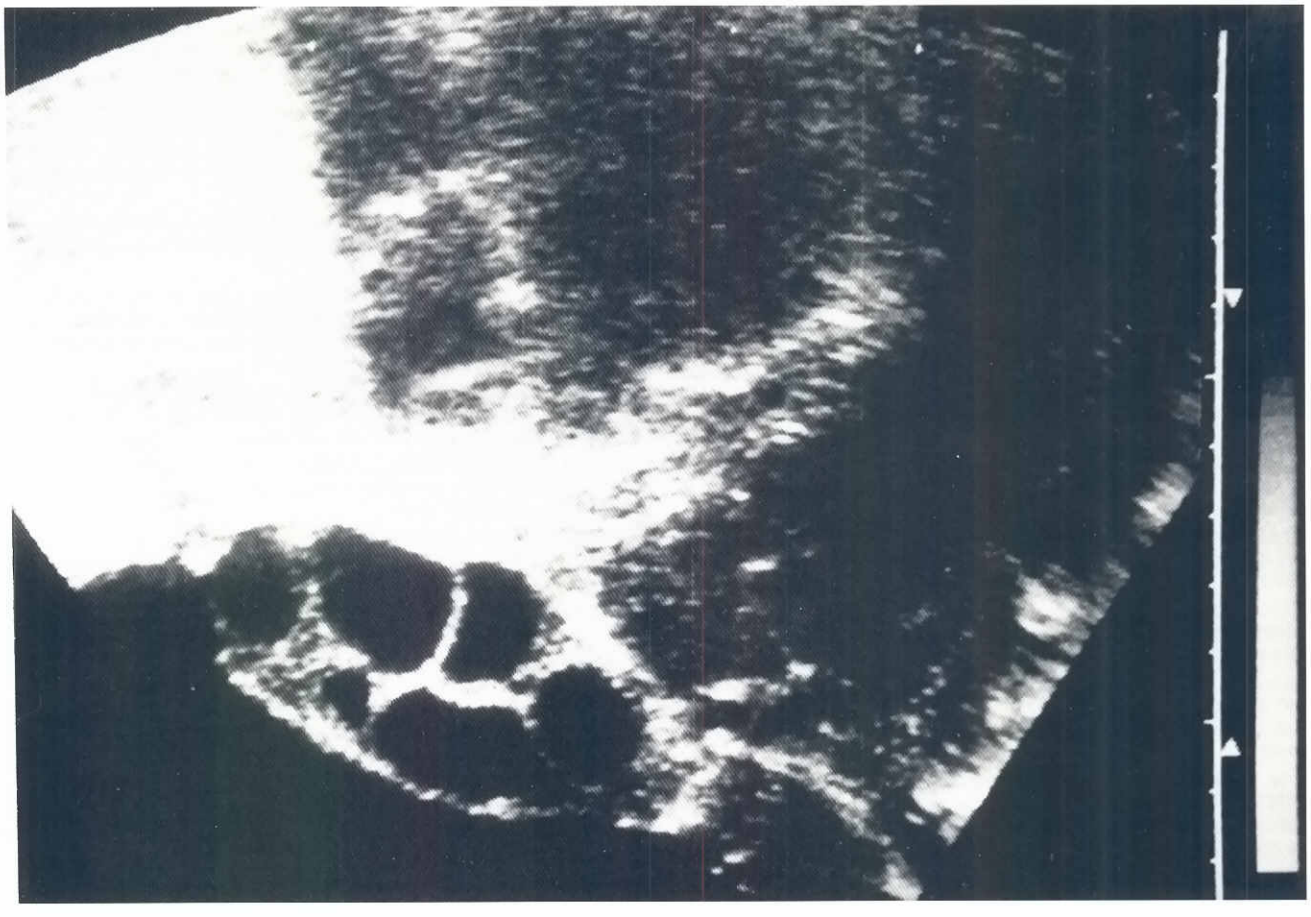

Fig. 6. Vaginal echography of a hyperstimulated ovary

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

publications, but it would be easy to add a large number of national publications. From the 1989 world survey on IVF we learned that Belgium is doing better than the mean: 741 clinical pregnancies out of 3750 oocyte pick-ups (19.8 %) vs 12,480 clinical pregnancies out of 76,030 pick-ups (16.4 %).

Belgium may not be so small after all.

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