

Review Article

The History of Embryologic Methods Implementation at the First Workplace of Assisted Reproduction in the Czech Republic

Žáková J*, Ventruba P and Crha I

Department of Gynecology and Obstetrics, Masaryk University, Czech Republic

*Corresponding author: Žáková Jana, Department of Gynecology and Obstetrics, Masaryk University and Faculty Hospital Brno, Obilní trh 11, 602 00 Brno, Czech Republic

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Abstract

The first test-tube baby in the Czech Republic and Central and Eastern Europe was born in our IVF centre in Brno (capital of Moravia) in 1982. It was the first Czech centre of infertility treatment. Since then, from the elementary *in vitro* fertilization and embryo transfer (IVF+ET) procedure, we gradually introduced new progressive methods into assisted reproduction practice in the Czech Republic. With sperm cryopreservation we started in 1991 and with embryo cryopreservation in 1995. Prolonged cultivation of embryos and micro manipulation techniques we practiced from 1994. Our centre built up the first Sperm bank (1995) and the Centre for fertility preservation of both male and female. All the time we were at the forefront of progress in the Czech Republic. We offer all available up-to-date methods and techniques of assisted reproduction.

Keywords: Oocyte fertilization; Embryo cultivation; Donor; Gamete cryopreservation; Micromanipulation

Abbreviations

IVF: *In Vitro* Fertilization; ET: Embryo Transfer; ICSI: Intracytoplasmic Sperm Injection; MESA: Mikro Epididymal Sperm Aspiration; PICS: Preselected Sperm for Intra Cytoplasmic Sperm Injection; TESE: Testicular Sperm Extraction

Introduction

The first test-tube baby in the Czech Republic and Central and Eastern Europe was born in our IVF centre in 1982, only four years after Luise Brown. At that time there were no equipment, no tools. Since then, the assisted reproduction methods have made a major progress and have become an integral part of the reproductive medicine [1]. More than thirty years of clinical and laboratory experiences based on our research activities are reflected in our treatment results, which are on par with those of the leading IVF centers worldwide. The aim of this contribution is to provide a short overview of the some important methods and techniques of clinical embryology, where the author was personally engaged with implementation, development and practical use in the embryological laboratory.

The Cultivation of Embryos

We developed the first method in Czech Republic how to extend length of the *in vitro* embryo cultivation beyond 48 hours. We were inspired by foreign literature [2]. Since 1993, we have extended this cultivation up to 120 hours, and for the first time, we have achieved the blastocyst stage embryo (1993), due to the co-culture with human oviductal cells. Mono layers of feeder cells were prepared from donor human oviductal epithelial cells collected from fallopian tubes which were obtained during hysterectomy in premenopausal viral screened women. From 1994 we started the usage one of the first synthetic medium (M3 Medium, Medi-Cult, Denmark) [3]. The use of medium

was safer and easier without potential risks. From 1995 extended cultivation in the synthetic media has become our daily practice and is currently performed in 98.5 % of IVF procedures.

Micromanipulation Techniques

Since 1994 we have provided a technique that helps an embryo leave the zona pellucida and its implantation – assisted hatching [4,5]. Assisted hatching is our daily routine. Intra Cytoplasmic Sperm Injection (ICSI) into the oocyte has become the most beneficial and the most widely used technique for oocyte fertilization since 1996 at our centre [6]. Currently, ICSI is carried out in more than 90% of the cycle search year. This technique is related to established methods for obtaining sperm from epididymal (MESA) and testicular tissue (TESE) and retrograde ejaculation. The possibility of sperm maturity evaluation, as a successive quality parameter, is the base of the PICS method (Pre selected sperm for Intra cytoplasmic Sperm Injection). Only pre-selected mature sperm are used for fertilization by PICS. We instituted this procedure in 2010, and patients choose this method in 71.5 % of oocyte insemination cases.

Cryopreservation of Gametes, Embryos and Tissues

We have cryopreserved sperm since 1991 and embryo freezing we introduced in 1995 [7]. In the beginning we performed slow freezing by Planer but now we prefer vitrification. Well-functioning cryopreservation was an essential prerequisite for a donation of gametes and embryos program. With in the center of assisted reproduction, we had the first and the largest sperm bank in the Czech Republic from 1995 (currently we have 100 donors) [8]. We have also built a Centre for fertility preservation of both male and female oncologic patients. Methods of preserving the fertility in young women

can be divided into three cryopreservative methods: embryo-, mature oocyte-, and ovarian tissue – cryopreservations. We have started as the first with ovarian tissue freezing and sperm freezing before gonadotoxic treatment in the Czech Republic [9-11]. From January 2006 to December 2013 we cryo preserved ovarian tissue of 20 women before gonadotoxic treatment (the most common were Hodgkin lymphoma -11x, non-Hodgkin's lymphoma -5x). During October 1995 to December 2012 we sperm of 1111 men – oncologic patients (557 – testicular cancer diagnosis). The testicular cancer survivors have a good chance to have a child by using sperm cryopreserved prior the oncology treatment, even when it contains only limited number of spermatozoa [12]. 41 patients who returned for infertility treatment under went 58 treatment cycles with cryopreserved sperm. Intrauterine insemination (IUI) was performed in 8 cases, *in vitro* fertilization in 4 cases and Intracytoplasmic Sperm Injection (ICSI) in 46 cycles. Totally 20 pregnancies were achieved (4 x IUI, 16 x ICSI), i.e. 34.5 % pregnancy rate. The implementation of all young oncological patient sperm cryo-preservation has an important place in our laboratory methods.

Conclusion

This offers clients an entire range of methods and as a part of a Masaryk University Complex, the center features complete clinical facilities; inter disciplinary collaboration, and an experienced team of experts.

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