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BRIEF HISTORY OF IN-VITRO FERTILIZATION IN EUROPE: INSIDER VIEW

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ABSTRACT
The first birth after in-vitro fertilization (IVF) in humans was achieved in Great Britain in 1978. Soon thereafter this technique was successfully applied in different European countries. The author of this paper has been involved in the development of different clinical and laboratory procedures used in IVF from the very beginning until now, in nine different countries, mainly in Europe (Spain, France, Italy, Czechoslovakia, Russia) and occasionally outside Europe (Brasil, Egypt, Saudi Arabia, United Arab Emirates). Therefore, rather than an observational review by someone located outside, this paper presents the story as viewed by an insider. Although this approach leads to a relatively high degree of autocitations, it does not lack objectivity, since all important contributions by other authors are also included. The paper also outlines ideas about the possible future techniques, still in the way of development and testing as to their safety and efficiency.

KEYWORDS: In-vitro fertilization, ovarian stimulation, sperm and oocyte treatment, intracytoplasmic sperm injection, use of immature male germ cells, gametes made from somatic cells.

INTRODUCTION AND BACKGROUND

Many human couples suffer from infertilily due to different factors, both of the male and the female origin. At present, in-vitro fertilization (IVF) can help, with more or less success. in most, though not all, of these cases. However, it was not so at the beginnings,[1] and the therapeutical indications of IVF have been continuously expanding over years.[2] Still while a medical student in the ancient Czechoslovakia, where I was born, I was sent to France by the main promoter of IVF in that country, Professor Milan Dvorak, in order to refine our protocols of ovarian stimulation and gamete handling. As a result of this new experience, we managed to obtain mature human oocytes and early preimplantation embryos for electron microscopic analysis before going ahead with the clinical application.[3,4] Subsequently we proceeded to the clinical application, resulting in a normal childbirth, in 1982. At the same period we developed a new technique whereby spermatozoa and oocytes were transferred into the oviduct during laparotomic microsurgical repair.[5] This was the first demonstration that in vitro prepared spermatozoa and oocyte can form normal embryos when transferred into the oviduct. Whereas the first birth of an IVF baby was achieved in a couple with apparently normal gametes, in whom infertility was caused by bilateral tubal obstruction,[1] subsequent evolution of IVF showed that it can also be adapted for cases of both sperm and oocyte abnormalities.[2] This evolution will be resumed in further text below.

Sperm abnormalities

The currently known sperm abnormalities include problems of sperm count, motility, morphology and DNA integrity. After leaving Czechoslovakia, I continued my carrier in France, mainly at the American Hospital of Paris, alternating with the University of Granada in Spain. During this period, my team discovered a number of new sperm abnormalities, not related to those previously described. To begin with, it became still more evident that sperm preparation for fertilization (capacitation and acrosome reaction) are finely tuned events whose disturbances can seriously compromise fertilization. Sperm capacitation depends on the removal of various factors, of seminal plasma origin, from the sperm surface. It normally occurs during sperm passage through the oviduct. However, in IVF it must be effected in vitro by sperm washing in culture medium.

Capacitated spermatozoa acquire the ability to develop a specific kind of movement, called hyperactivated, which facilitates their penetration through the outermost oocyte envelope – the cumulus oophorus, consisting of modified granulosa cells and a viscous itercellular material. At the same time, they become capable of binding to the surface of the following oocyte envelope, the zona pellucida (ZP). A specific ZP protein, ZP3, acts on a

complementary receptor, located on the sperm plasma membrane and, upon its activation, it triggers a chain of intracellular signaling events leading to the acrosome reaction (AR). AR means exocytosis of spem acrosome, a modified lysosome located in the anterior portion of the sperm head. Hydrolytic enzymes from the acrosome enable the penetration of the spermatozoon through the ZP into the oocyte. However in order for this to occur, AR must be synchronized with sperm passage.[6] Consequently, we defined two opposite, but equally harmful, AR pathologies: AR insufficiency and AR prematurity. The former condition means that spermatozoa lack functional receptors to induce AR in response to ZP3, whereas the latter refers to the condition in which AR occurs prior to sperm contact with ZP. Because AR implies a loss of the plasma membrane from the anterior sperm head region, this latter condition prevents any further sperm-oocyte interaction. Treatments for both of these conditions were suggested,[7] and led to success of IVF in many cases.

However, soon thereafter everything changed, owing to the introduction of intracytoplasmic sperm injection (ICSI) to assist IVF.[8] ICSI obviates the importance of AR timing, because each single spermatozoon is introduced into an oocyte by means of micromanipulation. Hence, evaluation of AR insufficiency and prematurity was relegated to the use as a diagnostic means to help understand the cause of infertility, and ICSI was recommended to all couples in this condition.[9]

Sperm DNA damage (fragmentation) is caused by oxidative factors that may result from unhealthy lifestyle (such as smoking) but can also appear without any apparent explanation. Sperm DNA is partly protected against oxidative damage by its tight package in association with proteins (protamins). However, some spermatozoa may have local disturbances of this chromatin package, showing intranuclear minivacuoles which can only be visualized in living spermatozoa by high-magnification light microscopy. This can explain how sperm selection at a high magnification can improve ICSI outcomes by avoiding the injection of spermatozoa showing this abnormality.[10] However, the most commonly used treatment in this condition is a direct antioxidative action using mixtures of antioxidants taken orally for 2-3 months.[11]

Finally, spermatogenesis can be arrested before the stage of mature spermatozoa in some men. Before the era of ICSI, this condition did not have any viable solution. Thanks to the pioneering animal experiments led by Professor Ryuzo Yanagimachi it became clear that normal offspring can be obtained by fertilization of mouse oocytes with round spermatids.[12] Encouraged by this achievement, we tried IVF with round spermatids in humans, but using round spermatid injection (ROSI), a technique derived from ICSI, instead of electrofusion used by Yanagimachi's group. The technique worked well and led to the birth of the world's first two healthy babies conceived with round spermatids.[13] These results were later confirmed by ourselves[2] and another independent study.[14] Moreover we also achieved pregnancies in cases of men with spermatogenesis arrest at the primary spermatocyte stage, by culturing segments of seminiferous tubules in hormone-enriched medium, leading to the evolution of spermatocytes into round spermatids that were subsequently injected into oocytes.[15]

Oocyte abnormalities

In Europe, most oocyte abnormalities detected in infertile couples nowadays are related to the increasing female reproductive age related to delaying reproduction for different reasons. This kind of abnormalities can be related to disturbances of oocytes nuclear and cytoplasmic maturation, resulting in meiotic errors and aneuploidy and/or to mitochondrial aging and the depletion of the stock of maternal mRNAs used during the early stages of postfertilization development. In fact, in all animal species the early embryo development is dependent on stored maternal mRNA species stored in the oocyte cytoplasm. The time of activation of embryonic genome transcription and expression varies among species, being relatively late in humans. We showed previously that the first signs of embryonic gene transcription (detected by autoradiography) begins between the 4-cell and 8-cell stage of development,[16] while the first ultrastructural signs of embryonic genome expression can only be observed from the 8-cell stage.[17] Consequently, paucity of stored maternal mRNA in human oocytes is a more serious problem as compared to species in which embryonic gene activation occurs earlier.

Interestingly, a randomized controlled trial showed that the inclusion of growth hormone in the ovarian stimulation protocol improved significantly delivery and live birth rates in women aged >40 years,[18] apparently corresponding to age-related decrease in the endogenous growth hormone secretion. Techniques enriching the cytoplasm of older oocytes with that from young oocyte donors[19,20] were also shown to be of help. On the other hand there are doubts about the usefulness of preimplantation genetic testing for aneuploidies because this technique can cause harm even to healthy oocytes.[21]

Uterine (endometrial) receptivity for embryos

Endometrial receptivity is critically dependent on progesterone secreted by the corpus luteum (CL) during the luteal phase and, later, after the luteoplacental shift, by the placenta. Both functions are often disturbed by ovarian stimulation protocols.[22] Hence, serum progesterone levels should be checked frequently (at least on the day of embryo transfer and then once a week) to prevent consequences of a possible CL deficiency and of a delayed luteoplacental shift and to administer exogenous progesterone when its endogenous production is too low with regard to the duration of

pregnancy.[22] In some, relatively rare, cases implantation failure and pregnancy loss can occur despite normal serum progesterone levels. In this condition, several experimental treatments, including endometrial scratching,[23] intentional injury to the endometrium using instruments, most frequently a Pipelle catheter inserted through the uterus, and subcutaneous injections of growth hormone,[24] were successful in some cases. However, the efficiency of these treatments remains to be corroborated by sufficiently powered randomized controlled trials.

CONCLUSIONS

Most sperm abnormalities were resolved by using ICSI and ROSI, and the uterine receptivity can also be controlled relatively easily On the other hand, oocyte abnormalities, mainly those related to advanced female age, still remain a problem open for future research. Creation of human artificial oocytes using patient's somatic cell nuclei is a possible approach to this problem,[25] but more research is needed to make us sure about the efficiency and safety of this technique.

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