ABSTRACT

It is now more than 35 years since the first world test-tube baby, Louise Brown, was born (1978) in England and it is estimated that since then more than 4 000 000 of children were produced by *in vitro fertilization* (IVF) worldwide.

The initial success of IVF was less than 20% in best clinics, but now it reaches about 40%. This is a consequence of introduction of new methods, standardization and exploitation of new manipulation and culture media, as well as the incorporation of research results. Nevertheless, the most important still remains the skill and experience of IVF clinics and IVF laboratories staff, especially their ability to critically evaluate the quality of biological material and to decide which cure and treatment are the best one. At least, some biological material (immature and low quality oocytes) can be used for training and also for some experiments aiming to explain some questions, which are not yet fully understood (for example aneuploidies in human oocytes and embryos). In addition, this training can facilitate the introduction of new progressive approaches and may also improve indirectly the quality of infertility treatments.

The first part of thesis is focused on the quality evaluation of oocytes collected by aspiration from follicles of stimulated patients. For labeling with specific antibodies against acetylated histones we used only those oocytes, that were not mature (metaphase II, MII). Our results showed that approximately 50% of oocytes with condensed chromosomes were positively labelled. This is much higher than in other species studied so far (approximately 10 times). Thus, this may explain why human oocytes are chromosomally abnormal with a very high frequency, when compared to the mouse and other species (5- 10%).

In the second part we have studied if the response of maturing human oocytes to butyrolactone 1 (BL1) is similar to that observed in the mouse, i.e. if they give off the polar body and form DNA replicating pseudo-pronuclei in the cytoplasm. These converted mouse oocytes cleaved and reached the blastocysts stage. From these embryos pluripotent ESC lines were then established. Our preliminary results show that human oocytes respond to BL 1 treatment similarly. Oocytes gave off the polar body (75-80%) and formed pseudo-pronuclei with several NPBs (nucleolus precursor bodies). In pseudo-pronuclei, DNA replication was detected in almost all cases. Some embryos cleaved further and we obtained abortive

blastocysts. Work is in progress with the aim to establish ESC lines from developing BL embryos.

The last part was focused on research on atypical nucleoli (nucleolus precursor bodies – NPBs), which are present in fully grown mammalian oocytes and early cleavage stage embryos. From human ART point of view, the evaluation (pattern) of a number and distribution of NPBs in pronuclei was used as a simple static indicator of successful embryo development (Hum. Reprod. 14, 1318-1323, 1999). The answer why this could be, was not known. It must be, however, noted here that some articles disagreed with this evaluation and did not confirm these observations. Our results support the rationale of this evaluation. NPBs material plays a crucial role in regulating major and minor satellite DNA sequences and chromosome dynamics and this is then absolutely essential for further embryonic development.

Conclusion: our results clearly document the usefulness of the exploitation of spare biological material from clinics of assisted reproduction for research. In our experiments, we were focused on epigenetic and structural aspects in oocytes that were not metaphase II staged after aspiration from follicles (immature and maturing oocytes). These oocytes are typically discarded. Extrapolation of results obtained in laboratory animals into a human ART indicates that it would be useful to reconsider some not commonly used evaluation approaches (pronuclear stage embryo evaluation) with their eventual introduction into human IVF clinical practice.