P-055. Hardness of the zona pellucida at different stages of mouse embryonic development in vitro and in vivo as measured indirectly by a 1.48 μm diode laser

Montag M.1, Rink K.2, Delacretaz G.3 and van der Ven H.1
1Department of Endocrinology and Reproductive Medicine, Sigmund-Freud-Str. 25, 53105 Bonn, Germany,
2MTM Medical Technologies Montreux SA, 1815 Clarens, Montreux and 3Institut d’Optique Appliquée, Ecole Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland

Introduction: During fertilization and early embryonic development, the zona pellucida plays an important role and serves as an embryonic coat, in vitro and in vivo. It has been reported that in-vitro culture conditions might alter the properties of the zona pellucida and lead to zona hardening, being a possible cause for improper hatching and failed implantation. However, an easy means to measure zona hardening has not been established by now. Here we report our experience with a 1.48 μm diode laser which allows indirect examination of zona hardness.

Materials and methods: Mouse oocytes and embryos up to the blastocyst stage were cultured in vitro. In-vivo grown embryos were isolated by flushing the oviduct or the uterus at various stages of embryonic development. For indirect measurement of zona hardness, we used a non-contact, 1.48 urn diode laser system (Fertilase®; MTM Medical Technologies Montreux SA, Switzerland) coupled to an inverted microscope. Laser-drilling of the zona pellucida was performed on each day of development. We always applied the same, constant laser energy (0.6 mJ) in a single laser pulse by using standardized conditions. We then determined the diameter of the drilled openings which served as an indirect measure of zona hardness for that embryonic stage.

Results: The size of a laser-drilled opening in freshly isolated oocytes was 17 μm. In zygotes and up to the morula stage the diameter was 14 μm in in-vitro cultured embryos and 13 μm in in-vivo grown and flushed embryos. With the onset of blastocyst formation and especially during expansion, the diameter of the drilled opening in the zona of in-vitro cultured embryos was 4 μm for fully expanded blastocysts. The thickness of the zona decreased as well, due to blastocyst expansion. In contrast, in-vitro grown and flushed blastocysts showed a diameter of the drilled opening in the zona up to 17 μm while the thickness of the zona remained the same as in earlier embryonic stages (6-7 μm).

Conclusions: Our data show that the size of a laser-drilled opening in the zona pellucida of early embryos may serve as an indirect measure of the zona hardness, where a smaller opening may indicate a harder zona. We conclude that up to the time of blastocyst formation, in-vitro and in-vivo grown embryos show only slightly differences in zona hardness. However, in vitro the formation of the blastocyst leads to structural changes in the zona, which becomes harder mainly due to blastocyst expansion. In contrast, the zona of in-vivo grown blastocysts becomes softer, probably due to the presence of zona lysins.

P-056. ‘Normal spermatozoa’: IVF or ICSI?

Aboujaoude I.1, Boulos J.1, Hachem F.1, Hamze M.1 and Attieh E.2
1Center for Reproductive Medicine and Genetics, Aboujaoude Hospital, Beirut; 2Hotel Dieu de France, Beirut, Lebanon

Introduction: Ten per cent of the patients undergoing IVF show no fertilization with normal spermatozoa. The objective of this study is to determine parameters necessary for the choice between IVF or ICSI in case of ‘normal spermatozoa’.

Materials and methods: Seventy-four couples underwent IVF and ICSI simultaneously and were classified into two groups depending on fertilization or no fertilization after conventional IVF.

Results: Forty-one couples showed a fertilization failure after conventional IVF, while the remaining 33 showed normal fertilization. The two groups were comparable in mean age of female patients, number of ampoules taken, day of HCG injection, number of eggs retrieved, number of embryos transferred, number of spermatozoa, sperm motility, and pregnancy rate. Four parameters were found statistically different in the two groups.

Conclusion: These four parameters (duration of infertility, family history of male infertility, sperm morphology, and presence of antisperm antibodies) allow us to choose for each couple the more appropriate technique: IVF or ICSI.

P-057. The viral risk in IVF laboratories dealing with carrier patients: implications for current practice

Selva J.1, Merlet F.2, Letur H.3, Aggouné M.4, Poirot C.5, Platé C.1 and Pibarot M.L.4
1Reproductive Biology Laboratory, Hôpital A Béclère, 157, rue de la porte de Trivaux, 92140 Clamart; 2Reproductive Biology Laboratory, Hôpital de Poissy St Germain en Laye, 78303 Poissy; 3CECOS, Hôpital Necker-Enfants Malades, 75007 Paris; 4Service vigilance hygiène prévention AP-HP, 75001 Paris; 5Reproductive Biology Laboratory, Hôpital Cochin, 75014 Paris

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