

**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**

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**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 µm 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-vitro* 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-vitro* 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-vitro* grown blastocysts becomes softer, probably due to the presence of zona lysins.

**P-056. 'Normal spermatozoa': IVF or ICSI?**

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**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.

	Fertilization (n = 33)	No fertilization (n = 41)
Duration of infertility	5.11	9.14
Family history of male infertility	0%	36%
Sperm morphology	0%	30%
Abnormal > 40% Kruger criteria		
Antisperm antibody positive	3%	37%

**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**

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**Introduction:** The management of potentially contaminated samples with HIV, HCV, and HBV in an IVF laboratory raises the question of the viral risk for the other samples of patients treated at the same time, the children conceived, and the embryologists. In France, the laboratory practice is supported by different texts, including a good practice guide for analysis laboratories, standard precautions to be observed when manipulating biological samples and virus classification, sug-