Supervitri device validation in the mouse model. Part III

Oocyte fertilization and embryo development

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Experimental protocol

1. Study groups

In the present part of the study, two groups were processed in parallel:

- **Test group.** Oocytes were vitrified/warmed using the Supervitri device and inseminated by ICSI following the protocol described below.
- **Control group.** Control oocytes were prepared following the same set-up and conditions as the test group, without exposure to vitrification/warming media nor liquid nitrogen (LN2). Fresh oocytes were inseminated through ICSI and cultured in parallel with the test group.

2. Oocyte retrieval

B6CBAF1 female mice were induced to superovulate by intraperitoneal injection of 7.5 I.U. of pregnant mare serum gonadotropin (PMSG) followed 48 h later by 7.5 I.U. of human chorionic gonadotropin (hCG). Females were euthanized by cervical dislocation 15h post-hCG administration. Oocytes were then collected by tearing the walls of the oviductal ampulla under a stereoscopic microscope in hyaluronidase droplets at 37.3 °C, until cumulus cells dispersed. Oocytes were washed thoroughly and those showing the presence of the first polar body (MII) were cultured at 37.3°C and optimal %CO2 and %O2 in air until use.

Oocytes showing good morphology were randomly selected for each study group:

- Control group, n= 141
- Test group (Supervitri), n= 150

3. Oocyte vitrification with Supervitri

For the test group, oocytes were vitrified following a standardized protocol and using commercial vitrification solutions. Briefly, samples were exposed to buffer solution and equilibration solution gradually for 15 min and transferred to vitrification solution for 1 min.
Afterwards, oocytes were loaded onto the surface strip of the Supervitri device and directly plunged into LN2. Subsequently, the Supervitri device containing the loaded samples was capped under the LN2. A maximum of 5 oocytes were loaded at a time on each Supervitri device.

For the warming, the cap was first removed from the Supervitri under LN2, and then the Supervitri strip was transferred from the LN2 into the first warming solution (4ml, at 37oC) for 1 min. Then, oocytes were gradually moved to dilution solution for 3 min, to washing solution 1 for 5 min and finally to washing solution 2 for an additional 1 min (at room temperature). After warming, samples were extensively washed and kept in culture medium awaiting ICSI. Control oocytes were not exposed to vitrification/warming media nor LN2.

4. Intracytoplasmic Sperm Injection (ICSI)

After 1h post-warming, oocytes were inseminated with the ICSI technique using a PiezoXpert® (Eppendorf, Germany) device.

4.1. Sperm collection and sperm head isolation

Fresh mouse sperms were collected from cauda epididymis taken from an adult male in a microdroplet of culture medium and cultured for 15 min at 37°C and optimal %CO2 and %O2 concentration. After incubation, 3 µL of the sperm concentration were taken with a pipette and further diluted into a 150 µL droplet of culture medium. For the isolation of the sperm heads, sperms were transferred into a droplet of PVP 10% (v/v), previously prepared in the micromanipulation dish. Single, motile spermatozoa were selected, and the head was separated from the tail using a piezo pulse *(Fig. 1)*.

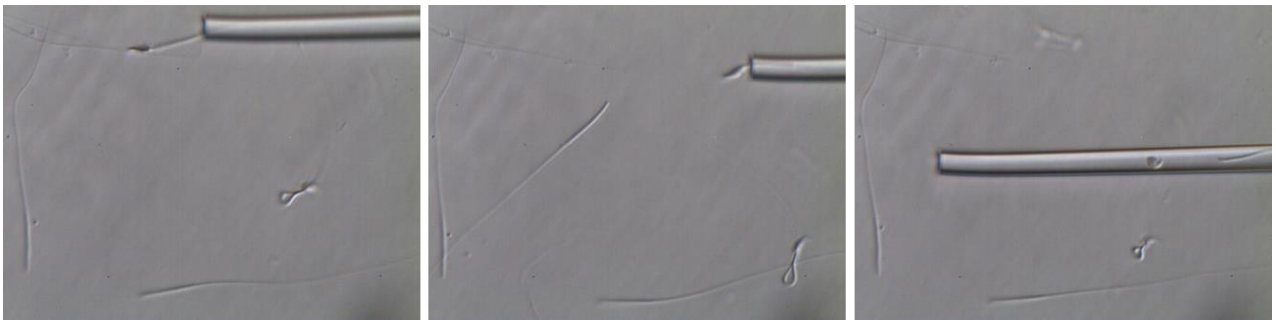

Figure 1. Decapitation of the mouse sperm. A) The sperm is aspirated into the piezo ICSI microcapillary with the tail first. B) The sperm is positioned to the tip end so that the head of the sperm just looks out. C) A piezo impulse is applied causing the separation of the head from the tail.

4.2. Oocyte injection

Oocytes were transferred to the micromanipulation dish in manipulation medium supplemented with cytochalasin B. Detailed instructions of the ICSI procedure in the mouse with the Eppendorf PiezoXpert® have been described by our group elsewhere [1] *(Fig. 2).* After the injection, oocytes were washed thoroughly and cultured under optimal conditions for 4 to 5 hours.

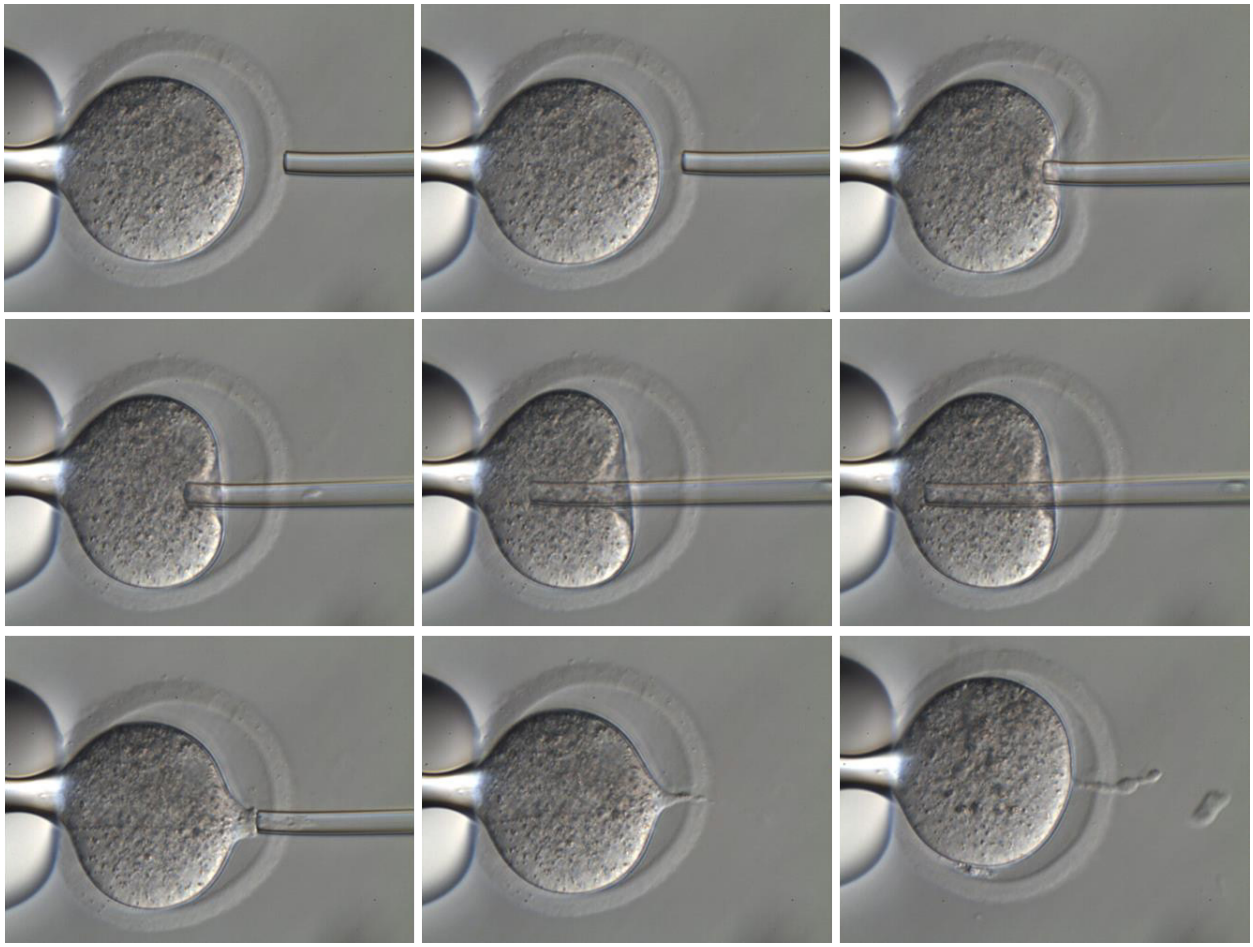

Figure 2. Mouse ICSI. (A–C) The equatorial plane of the oocyte and the ICSI capillary are aligned in microscopic focus. A piezo impulse is applied to ICSI capillary to penetrate the zona pellucida. (D–F) The ICSI capillary, loaded with the sperm head, is then moved deep into the oocyte. Minimal suction is applied into the ICSI capillary and with a single soft piezo impulse the oolemma is penetrated and the sperm is released into the cytoplasm. (G–I) the ICSI capillary is withdrawn out of the injected oocyte very carefully to allow the oolemma to close.

4.3. Fertilization check

Four hours post-ICSI, fertilized zygotes (identified by the presence of two polar bodies and two pronuclei) were selected and transferred into a culture dish. Parthenogenetic (only one pronucleus), not fertilized (no pronuclei) or lysed oocytes were discarded.

4.4. Embryo development

Embryo development of control and test groups was followed every 24 h.

5. Statistical analysis

A Fisher's exact test was used to analyse statistical differences between the test and control oocytes. A p value < 0.05 was considered statistically significant.

Results

In the present study, a total of 150 oocytes were vitrified/warmed using the Supervitri device following the previously described protocol. During the warming, 143 oocytes were retrieved, all of which survived to the vitrification/warming procedure *(Table 1)*.

Table 1. Efficiency rates of vitrification/warming and fertilization

	Oocytes vitrified	Oocytes retrieved	Vitrification survival n (%)
Test group	150	143	143 (100)

ICSI was then performed to the test oocytes. The survival rates after ICSI were above 90% in the control and test group. No significant differences were found in the fertilization rates between the control and the test group *(Table 2)*.

Table 2. Efficiency rates of fertilization

	n	ICSI survival n (%)	Fertilized n (%)
Test group	129	117 (90.7)[a]	105 (89.7)
Control group	139	135 (97.1)[b]	121 (89.6)

a-b Values with different superscripts differ significantly within the same column (p = 0.04; Fisher's test).

Almost all embryos developed to the two-cell stage on the next morning *(Table 3)*. Significant differences were not found regarding the embryo development at 48 and 72h post-ICSI between groups *(Table 3)*.

Table 3. In vitro development for up 72h post-ICSI

	n cultured	Two-cell stage 24h post-ICSI n (%)	Cleavage stage 48h post-ICSI n (%)	Morula/early-blastocyst 72h post-ICSI n (%)
Test group	105	104 (99)	102 (97.1)	100 (95.2)
Control group	121	118 (97.5)	118 (97.5)	116 (95.9)

Conclusions

- Maximum oocyte vitrification survival rates were achieved using the Supervitri device.
- Mouse oocyte vitrification using the Supervitri did not affect the fertilization rate.
- Mouse oocyte vitrification using the Supervitri did not affect their developmental potential on days 2, 3 and 4 of in vitro culture.

Note. Click here to see the embryo development photos.

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Our company

BPB MEDICA™ is an Italian manufacturing company specialized in the design, production and marketing of high qualitative healthcare products for medical use and medical-surgery devices.

BPB MEDICA™ was founded in 1999 by the Bellini family, boasting thirty year's experience in the biomedical sector. The founder, Carlo Bellini Sr., started the business in 1968 and has passed down to his heirs ethics, integrity and spirit of sacrifice. Today BPB MEDICA™ has leveraged its 50 years experience to develop new innovative product lines, growing the company on international level.

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1999 — BIOPSYBELL S.R.L. FUNDATION:
Carlo Bellini Sr's daughter founded Biopsybell srl: "Biopsy" as the initial core business and "Bell" as Bellini family name.

2008 — THIRD GENERATION OF THE FAMILY:
Carlo Bellini, founder's nephew, joined the company and focused on export sales development.

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Development and launch of Spine product line.

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Export success: 41 foreign countries served (35%TNR).

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Our company

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70

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BPB MEDICA™ provides painstaking service to its clientele and its primary aim is product quality. The **internal Regulatory and Quality Departments** conducts rigorous tests, from the raw materials to the equipment and the finished product. This allowed the company to obtain CE, ISO 13485 and the establishment registration by FDA.

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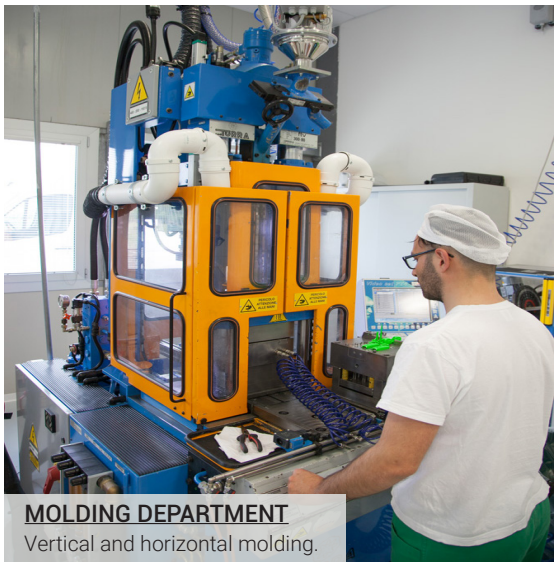
Manual & automatic assembly, packaging.



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