Supervitri device validation in the mouse model. Part II (oocyte tests)

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Experimental protocol

Oocyte survival tests

Mouse oocytes were collected from F1 hybrid females (B6/CBA) and washed thoroughly. 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 liquid nitrogen (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) solution 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. After 1-2h, oocytes were evaluated as detailed below (see paragraphs 1, 2 and 3).

Control oocytes were evaluated following the same set-up and conditions, without exposure to vitrification/warming media nor LN2. 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.

1. **Morphological evaluation of vitrified oocytes at different time-points after warming**

   Oocyte survival and vacuolization rates were assessed 1-2h after warming under the inverted microscope.

2. **Meiotic spindle visualization after oocyte warming using a polarized light microscope**

   A glass bottom dish was prepared with microdroplets of manipulation medium covered with mineral oil. Between one and two hours after warming, metaphase II (MII) mouse oocytes were placed in the glass bottom dish maintained at 37.3°C. The birefringence signals of the meiotic spindles were analysed using polarAIDETM technology (Vitrolife).

3. **Oocyte fixation and processing for evaluation of the meiotic spindle structure and chromosome distribution by immunofluorescence**

   By 2h post-warming, oocytes were fixed and processed for the analysis of the meiotic spindle morphology and chromosome distribution. Triple-labelling protocol was used for the detection of microtubules, microfilaments, and chromatin. Labelled oocytes were examined using an epifluorescence microscope and digital images were acquired. Oocytes that were not subjected to vitrification were processed in parallel following the same protocol and used as controls. Meiotic spindles with a bipolar barrel shape and chromosomes aligned in the metaphase II plate were considered as morphologically normal. Meiotic spindles with an abnormal (elongated bipolar or multipolar) shape and dispersed chromosomes were considered abnormal.

Results

Oocyte survival tests

1. Morphological evaluation of vitrified oocytes at different time-points after warming

A total of 32 oocytes were vitrified/warmed following the previously described protocol. During the warming, 30/32 oocytes were retrieved (93.8%). The survival rate of oocytes vitrified with the Supervitri device was 96.7% (Table 1). After warming, major vacuolization did not occur and differences in minor vacuolization rates were not statistically significant (Fisher's exact test, p = 0.56) (Table 2). Examples of oocytes that lysed or presented minor vacuolization after the warming with the Supervitri are shown in Figure 1.

Survival			
	Processed oocytes n	**Retrieval n (%)**	**Survival n (%)**
Supervitri	32	30 (93.8)	29 (96.7)

Table 1. Retrieval and survival rates after warming with the Supervitri device.

Vacuolization			
	Processed oocytes n	**Major n (%)**	**Minor n (%)**
Control	10	0 (0)	0 (0)
Supervitri	29	0 (0)	4 (13.8)

Table 2. Oocyte vacuolization rates observed in the fresh control group and after the warming with the Supervitri device.

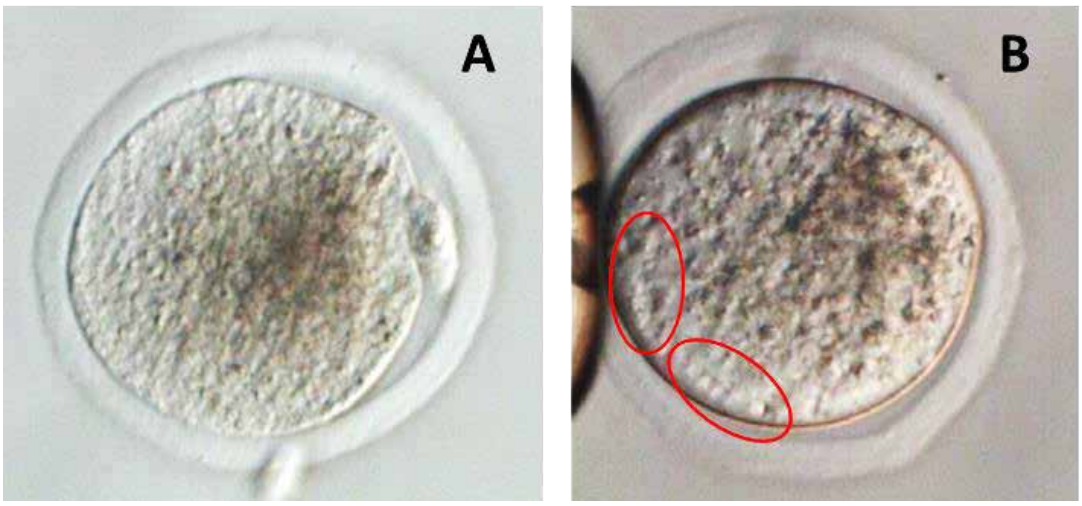

Figure 1. A) The only oocyte that did not survive after the warming with the Supervitri showing the lysed cytoplasm. B) Example of an oocyte showing minor vacuolization (indicated by red circles) after the warming with the Supervitri device.

Results

2. Meiotic spindle visualization after oocyte warming using a polarized light microscope

No differences in spindle birefringence signal were found between the control and Supervitri groups (Fisher's exact test, $p > 0.5$) (Table 3). Examples of oocytes with a positive spindle birefringence signal for both control and Supervitri groups are shown in Figure 2.

Spindle birefringence		
	Processed oocytes n	**Positive signal n (%)**
Control	10	10 (100)
Supervitri	29	28 (96.6)

Table 3. Analysis of the meiotic spindle birefringence in control oocytes and oocytes vitrified with the Supervitri device.

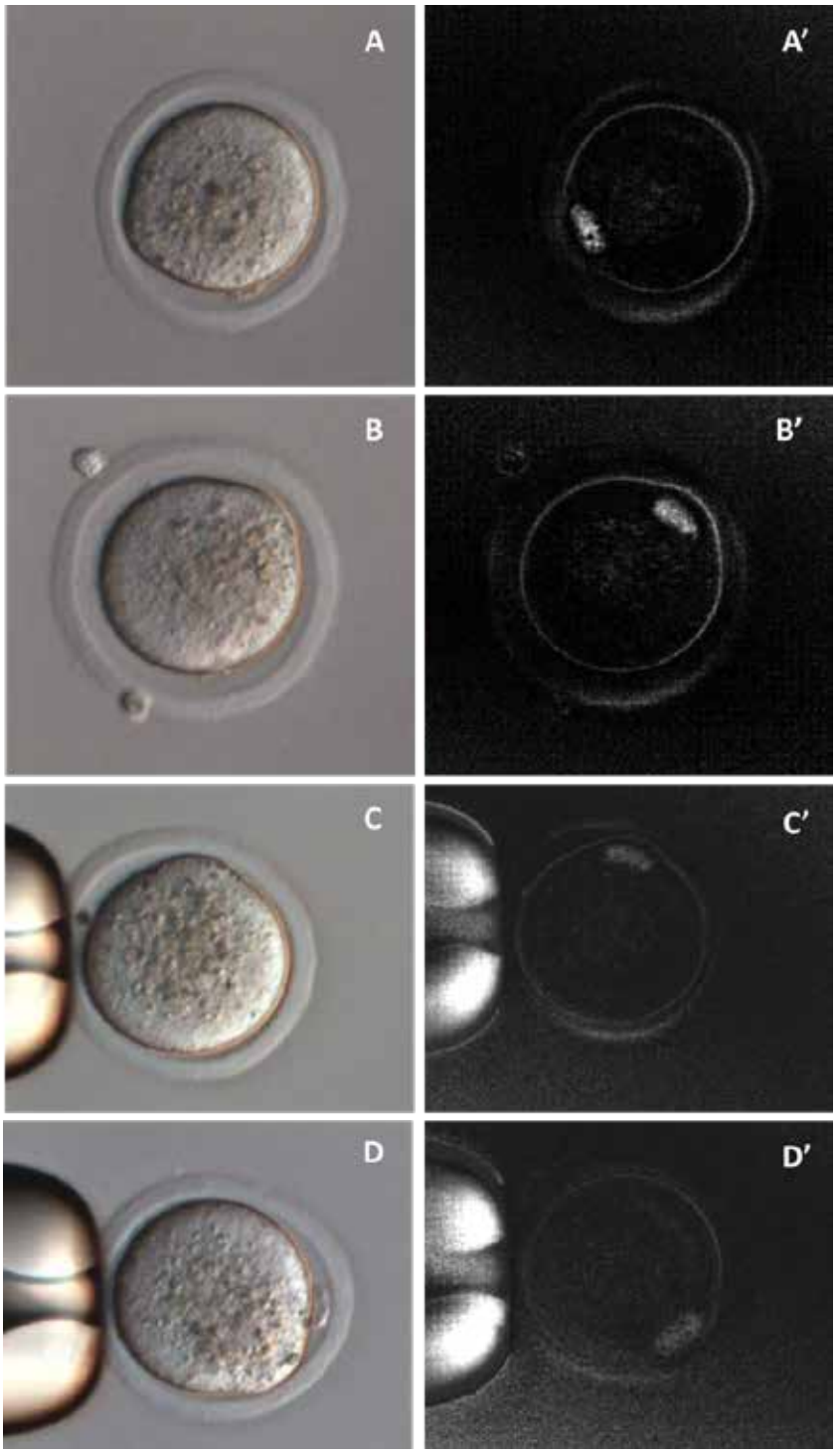

Figure 2. Spindle birefringence images of control oocytes (A-B) and Supervitri warmed oocytes (C-D).

Results

3. Oocyte fixation and processing for evaluation of the meiotic spindle structure and chromosome distribution by immunofluorescence

A 100% of normal meiotic spindle morphology and chromosome distribution was observed in both groups (Table 4). Examples of normal meiotic spindle morphology and chromosome distribution are shown in Figure 3.

Meiotic spindle morphology and chromosome distribution					
	Processed oocytes n	Meiotic spindle morphology		Chromosome distribution	
		Normal n (%)	Abnormal n (%)	Normal n (%)	Abnormal n (%)
Control	10	10 (100)	0 (0)	10 (100)	0 (0)
Supervitri	29	29 (100)	0 (0)	29 (100)	0 (0)

Table 4. Analysis of the meiotic spindle morphology and chromosome distribution in control oocytes and oocytes vitrified with the Supervitri device.

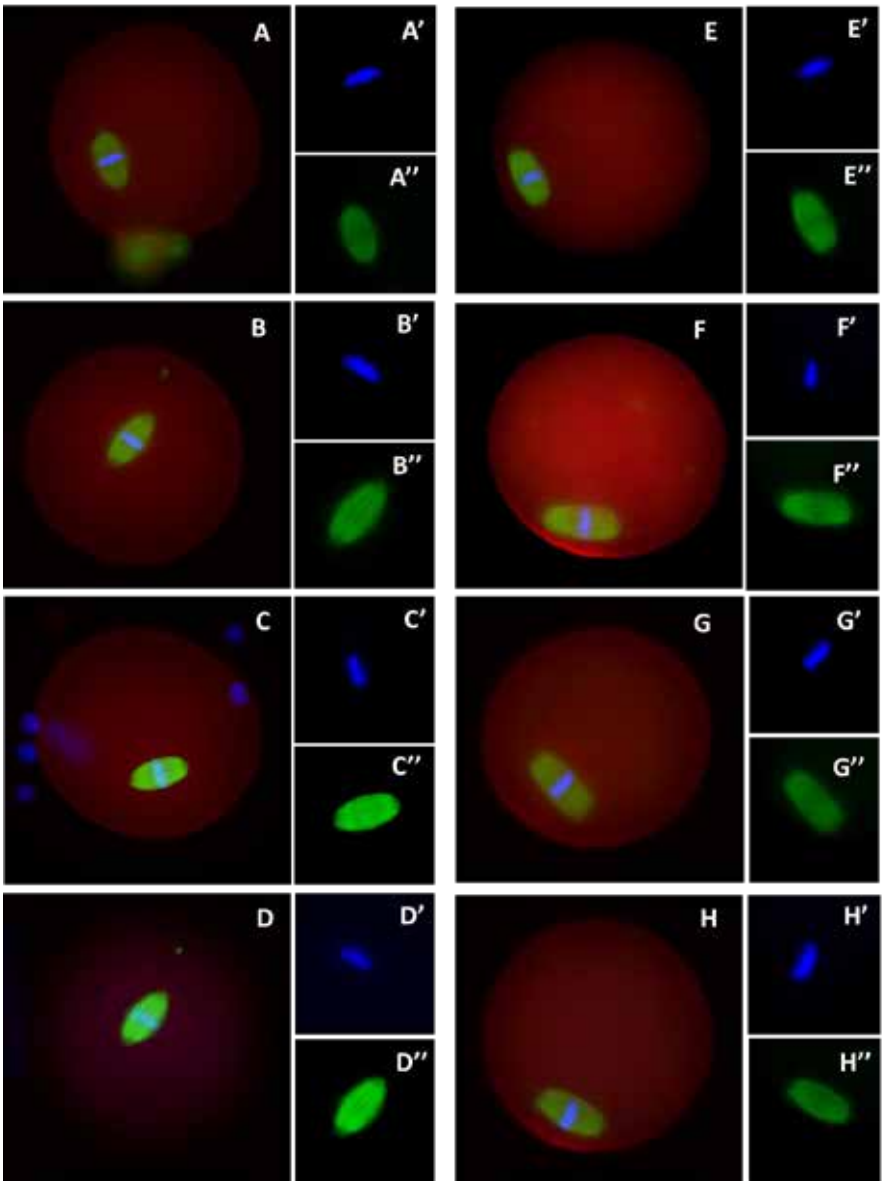

Figure 3. Immunofluorescence analysis of the morphology of the meiotic spindle and the chromosome distribution in metaphase II mouse oocytes. Control non-vitrified oocytes with a normal barrel shape spindle and the chromosomes aligned in the MII-plate (A-D). Oocytes vitrified with the Supervitri device with a morphologically normal spindle and the chromosomes correctly aligned (E-H). In the merged images of oocytes, microfilaments, microtubules and chromosomes are displayed in red, green and blue, respectively. The raw images of the chromosomes (A'-H') and meiotic spindle (A"-H") are displayed on the right side of its complementary image.

Conclusions

Conclusions:

- Oocytes vitrified with the Supervitri device showed very high survival rates.
- Oocyte spindle morphology and chromosome distribution was shown to be normal after vitrification with the Supervitri device.

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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.

50 YEARS EXPERIENCE

1968 — MEDICAL BUSINESS BEGINNING:
The founder, Carlo Bellini Sr, started his business in the medical field, founding some of the most important companies of our medical district

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.

2012 — THE EARTHQUAKE:
The company was damaged by an heavy earthquake: the family worked hard to keep the production open. After only 2 weeks from the EQ was back in business.

2014 — SPINE SURGERY:
Development and launch of Spine product line.

2015 — EXPORT EXPANSION:
Export success: 41 foreign countries served (35%TNR).

2016 — NEW SITE:
The intensive growth of the last few years required a new and bigger production site.

2018 — A.R.T. LINE:
Development and launch of Assisted Reproduction product line.

2019 — EXPORT ORIENTED:
70 country served (70% TNR).

2019 — REGENERATIVE & ORTHO-BIOLOGICS:
Development and launch of Regenerative Medicine & Ortho-Biologics product line.

2020 — SURGICAL MASKS:
New production line of Surgical Masks in response to COVID-19 emergency.

Our company

BPB MEDICA's™ philosophy is to grow alongside the needs of patients, doctors and hospital staff in general. Backed by the experience acquired by the company's specialized technical personnel and thanks to newly-adopted technologies, BPB MEDICA™ has quickly managed to make a name for itself on the domestic and international markets.

70

COUNTRIES SERVED

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.

BPB MEDICA™ operates with high qualitative standards aimed at increasing customer satisfaction through a continuous improvement regime. We conduct multiple quality tests during the entire production process: this allows to reduce the production waste and grant high quality products.

Thanks to the **internal R&D Department** BPB MEDICA™ conducts constant research in the reference pathologies with an aim to ever better qualifying and improving its production standards and aiding the development of new products.

OUR PRODUCT LINES

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Contact us for further information:

BIOPSYBELL S.R.L.
Via Aldo Manuzio 24
41037 Mirandola – MO – ITALY
T. +39 0535 27850 – F. +39 0535 33526
C.F./P.Iva 02615000367

BPB MEDICA

www.biopsybell.it

international1@biopsybell.it

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