SUPERVITRI™ device validation in the mouse model.

COMPLETE REPORT

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SUPERVITRI™ device validation in the mouse model. Part I

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Measures

Measurements summary

SUPERVITRI™ device	Approximate measurements
Device length (capped)	13 cm
Device length (uncapped)	12,5 cm
Body length	9,8 cm
Body width	0,3 cm
Entire tip length	2.7 cm
Loading strip length	1.5 cm
Loading area length	0.8 cm
Loading strip width	1172.7 ± 0.7 µm

Evaluation of identification/labelling area

The labelling area is adequate and wide enough to write the sample identification with a marker. Similarly, the labelling area is also considered convenient to stick identification stickers (Figure 1).

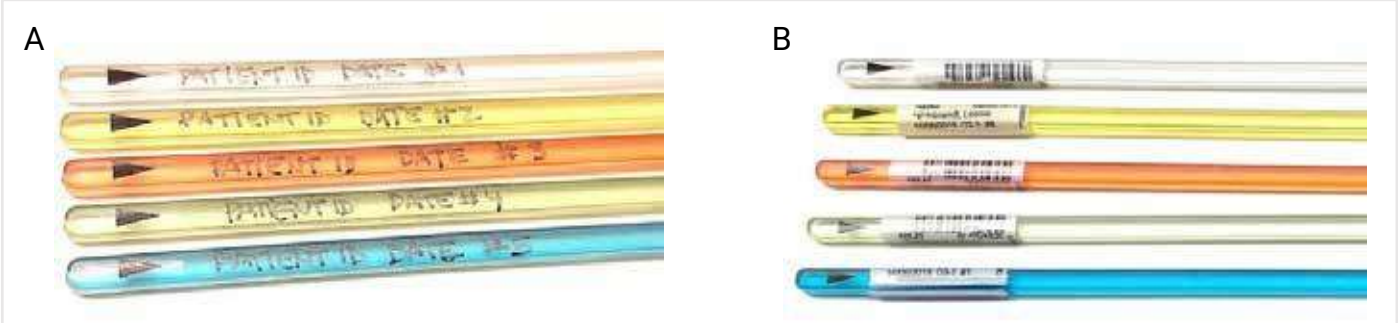

Figure 1. Identification or labelling area in the testing device. A) Pen marker labelling; B) Identification stickers.

Evaluation of minimum volumes needed

As a general characteristic of the surface type open vitrification systems, specimens can be loaded within a minimum volume (0.1 µl) of vitrification solution to ensure the best cooling and warming rates.

Resistance or tightness in liquid nitrogen

For the period of two weeks, SUPERVITRI™ devices were stored in LN2 and they showed to be resistant to LN2 storage and not propense to break or crack. Similarly, the cap of the device did not fall apart of the body of the device, break or crack and maintained properly sealed the tip, protecting the samples.

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Methods

Calculation of cooling and warming rates

Measurements were performed using a TC-08 data logger (Pico Technology) connected to a Type K thermocouple, which in turn was attached to the tip of the loading strip of the corresponding vitrification devices with the help of a copper wire. Temperature measurements were recorded in real-time every millisecond (ms) in a computer connected to the data logger until reaching a temperature plateau. The point of drastic slope regression line was calculated to determine the cooling and warming rates. Cooling and warming rates determined for the test device (SUPERVITRI™) were compared to those obtained in a similar surface open system already available in the market (control). For the determination of the cooling rates, the test and control devices were attached to a thermocouple at the tip of the loading strip and plunged directly in LN2. For the warming rates, the vitrification devices were quickly plunged in a warming solution previously warmed and stable at 37ºC. In this case, warming rates were determined in two different volumes of warming solution (4ml and 1ml), as both volumes are commonly used in the distinct vitrification protocols followed by human IVF laboratories (Figure 2).

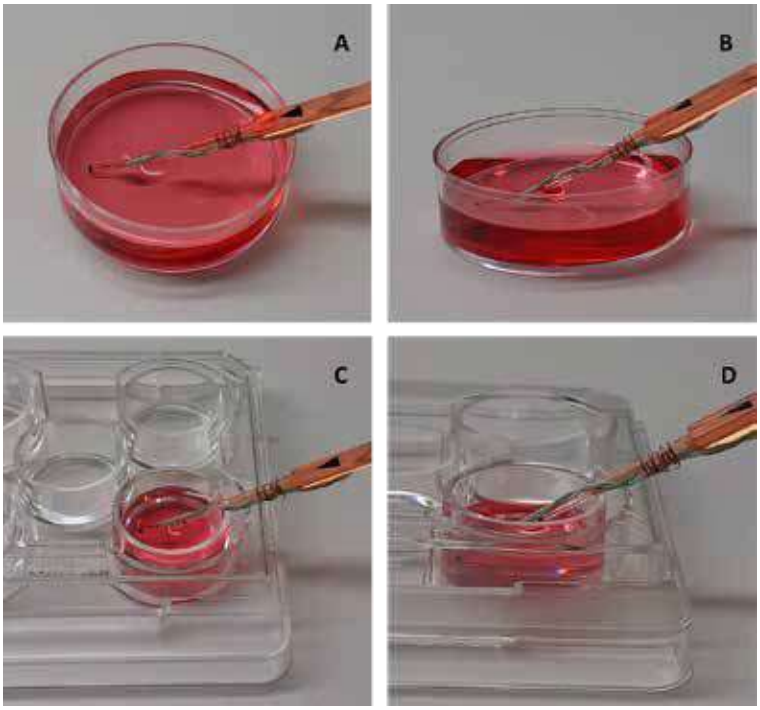

Figure 2. Warming set-up used to determine the warming rates in the SUPERVITRI™ device. A-B) 4ml of warming solution in a 35mm dish; C-D) 1ml of warming solution in a 5-well dish.

Three replicates were performed to assess the cooling rates and six replicas to determine the warming rates. The read values obtained in the different replicates were pooled and used to calculate the mean and standard deviation (SD). A t-test was then used to calculate statistical differences between the test and control devices. A p value < 0.05 was considered statistically significant.

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Results

Calculation of cooling and warming rates

Cooling rates

SUPERVITRI™ cooling rates were compared with a similar surface open system available on the market (Figures 3-4). The mean cooling rate obtained for the SUPERVITRI™ device was -30246ºC/min. Significant differences were not found between both groups (t-test > 0.05).

COOLING			
	Replicas	**Mean Cooling rate (ºC/min)**	**SD**
Control	3	-30670	730
SUPERVITRI™ device	3	-30246	2032

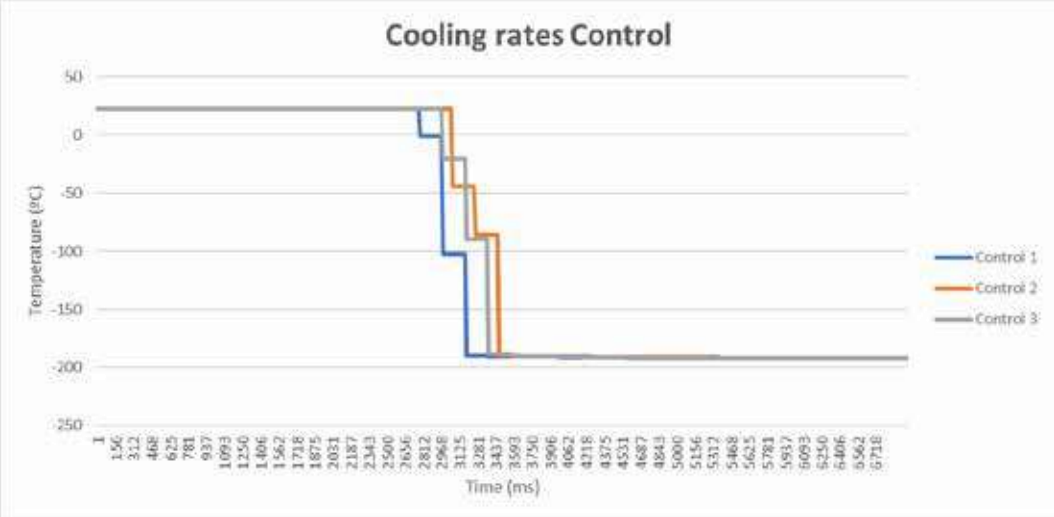

Figure 3. Graph with cooling ramps obtained in a surface open system used as a control. The results obtained in the three replicas are shown in different colours.

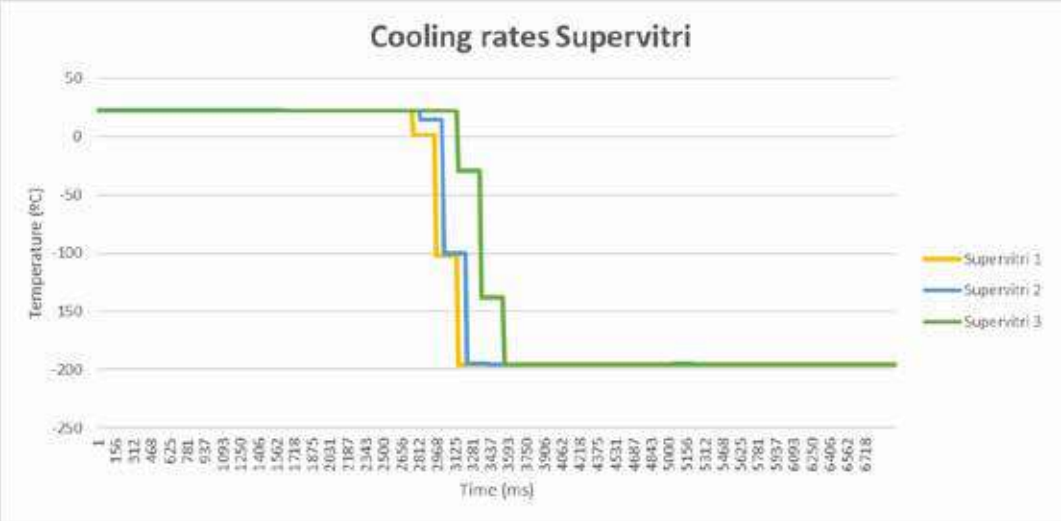

Figure 4. Graph with cooling ramps obtained with the SUPERVITRI™ device. The results obtained in the three replicas are shown in different colours.

Results

Warming rates

SUPERVITRI™ warming rates were compared with a similar surface open system available on the market (Figures 5-6). The mean warming rate of the SUPERVITRI™ device was +55456ºC/min. Significant differences in the cooling rates were not found when compared to the control device (t-test > 0.05).

WARMING			
	Replicas	**Mean Warming rate (ºC/min)**	**SD**
Control	6	+57062	4927
SUPERVITRI™ device	6	+55456	5820

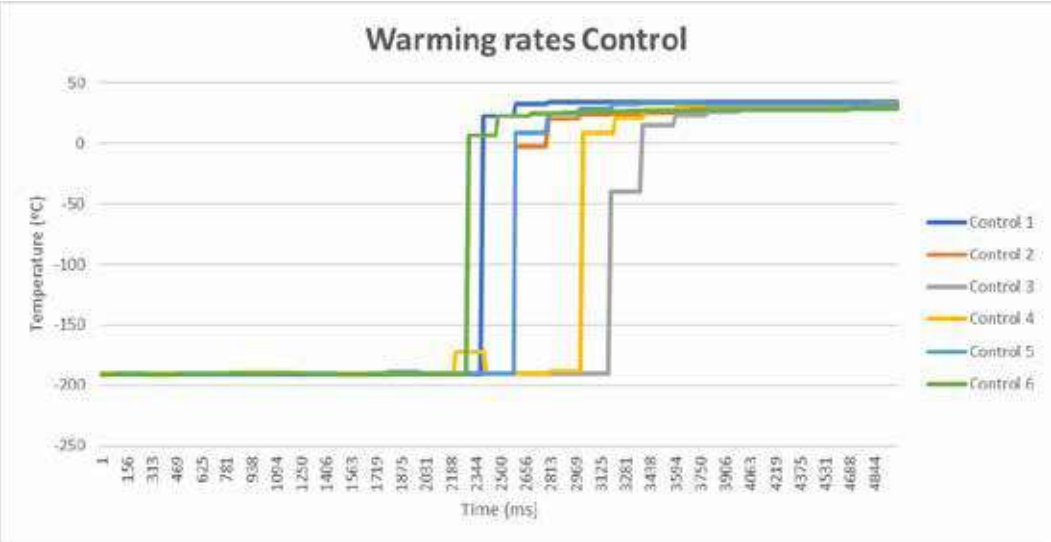

Figure 5. Graph with warming ramps obtained in a surface open system used as a control. The results obtained in the six replicas are shown in different colours.

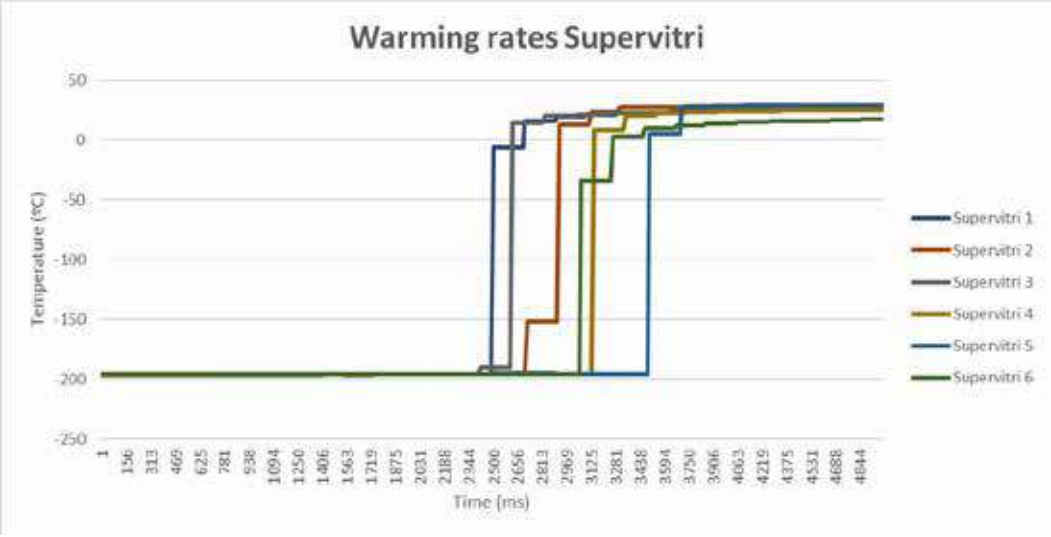

Figure 6. Graph with warming ramps obtained with the SUPERVITRI™ device. The results obtained in the six replicas are shown in different colours.

Results

Warming rates (1 vs. 4ml)

The warming rate for the SUPERVITRI™ device was assessed for two different set-ups. The SUPERVITRI™ was plunged into 4ml or 1ml of the first warming solution at 37ºC (Figures 7-8).A lower warming rate was observed when performing the warming procedure in 1ml of warming. Although the differences between both set-ups did not reach statistical significance (t-test, p= 0.17), a higher consistency in warming rates is achieved when higher volumes of medium are used in this step of the vitrification protocol (lower standard deviation).

WARMING (1 vs. 4ml)			
	Replicas	**Mean Warming rate (ºC/min)**	**SD**
SUPERVITRI™ device (1ml)	6	+49799	7581
SUPERVITRI™ device (4ml)	6	+55456	5820

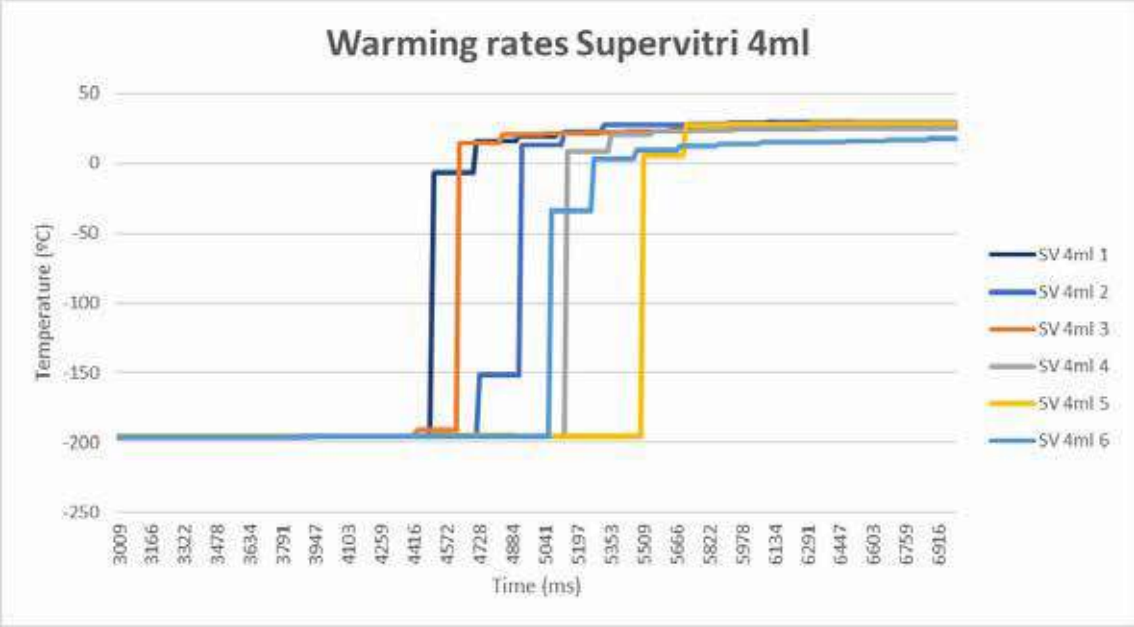

Figure 7. Graph with warming rates of the SUPERVITRI™ device plunged in 4ml. The results obtained in the six replicas are shown in different colours.

Results

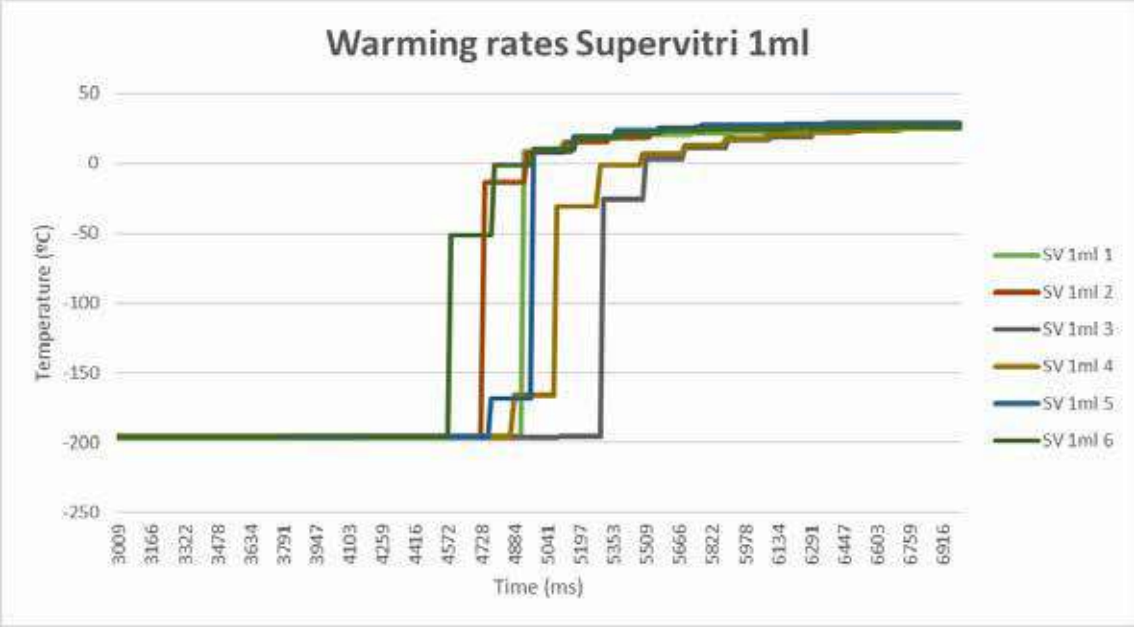

Figure 8. Graph with warming rates of the SUPERVITRI™ device plunged in 1ml. The results obtained in the six replicas are shown in different colours.

CONCLUSIONS

- Macroscopic and microscopic features are evaluated positively for the SUPERVITRI™ device. However, it is recommended to add a black mark on the caps of the devices to make it easier to visualise and use them under the LN2. In addition, if the mark is aligned with the black mark of the device body it will facilitate the insertion of the cap in the right position so that it fits tightly and seals the tip of the device properly.

- The labelling area is wide enough to write the sample identification with both markers and stickers.

- It is recommended to load the specimens for vitrification with a minimum volume (approximately 0.1 µl) to ensure the best cooling and warming rates possible, taking care not to leave the samples completely dry before plunging them in LN2.

- SUPERVITRI™ cooling and warming rates were -30246ºC/min and +55456ºC/min, respectively. The results are comparable to those obtained with a similar surface open system available on the market.

- It is recommended to use big volumes when performing the first step of the warming protocol as there is a tendency to achieve better warming rates when using 4 ml than when 1 ml is used. Similarly, a higher consistency can be achieved when using 4 ml compared to when 1 ml is used.

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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 a 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.

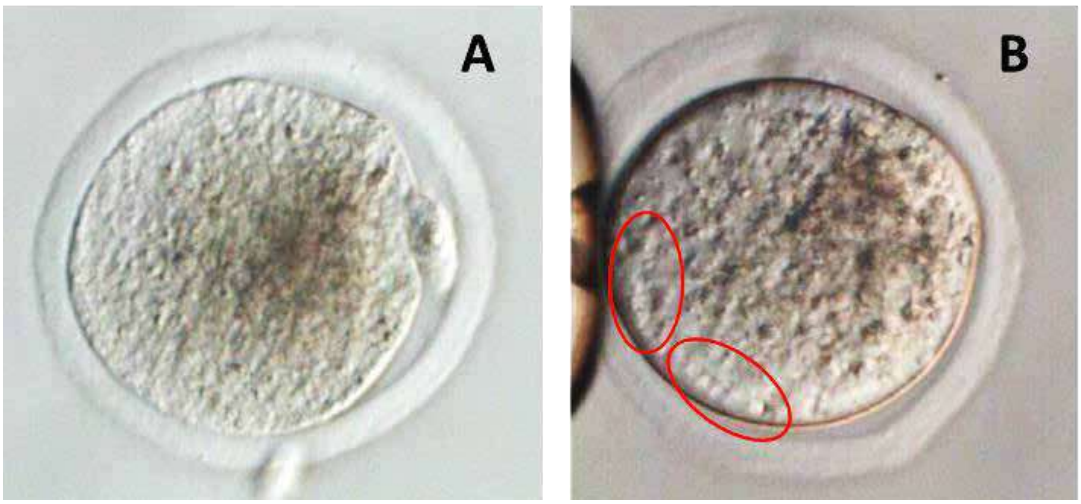

Figure 1. A) The only oocyte that did not survive after the warming with the SUPERVITRI™ showing the lysed cytoplasm. B) Example of an oocyte showing minor vacuolization (indicated by red circles) after the warming with the SUPERVITRI™ device.

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.

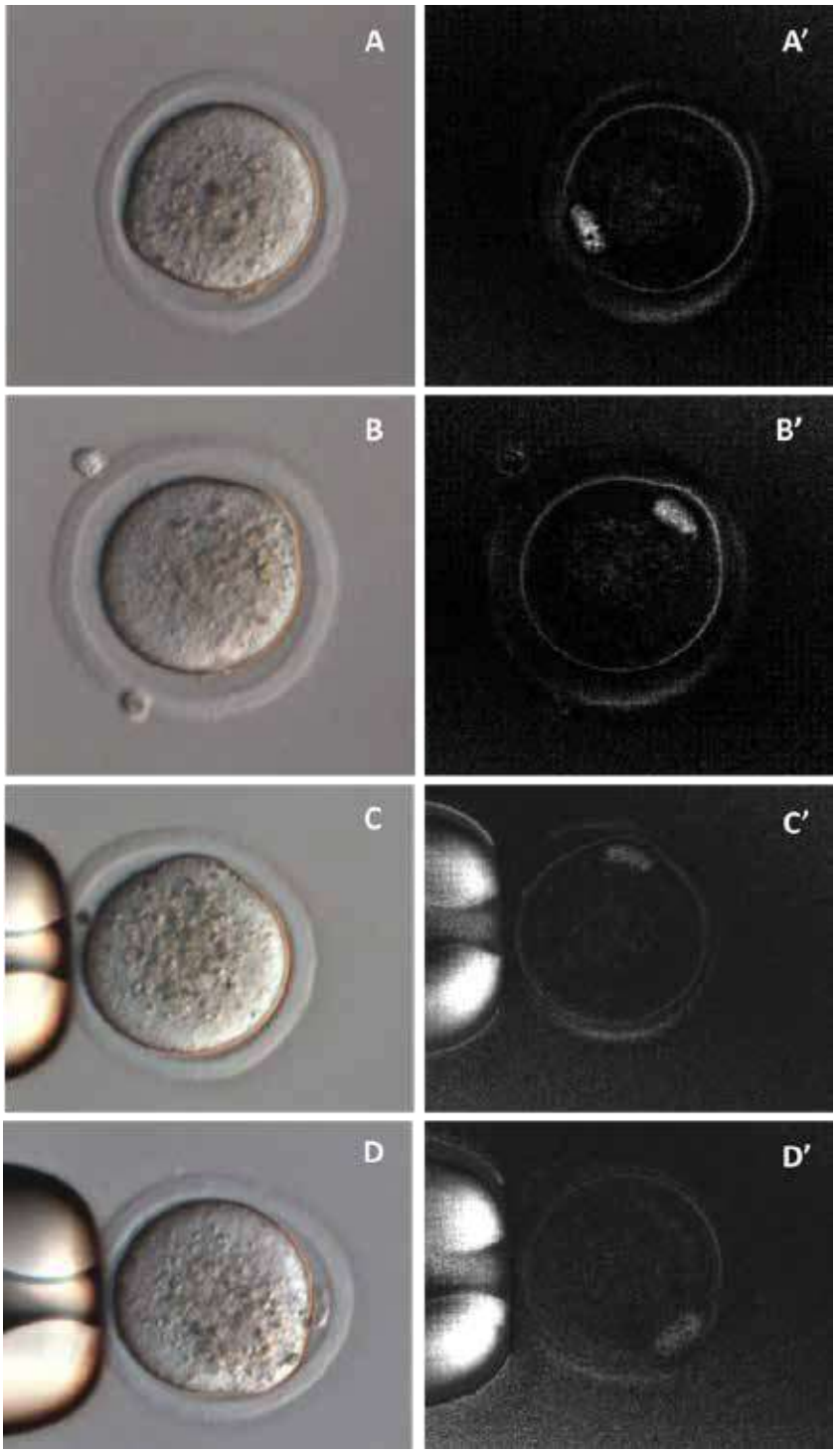

Figure 2. Spindle birefringence images of control oocytes (A-B) and SUPERVITRI™ warmed oocytes (C-D).

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.

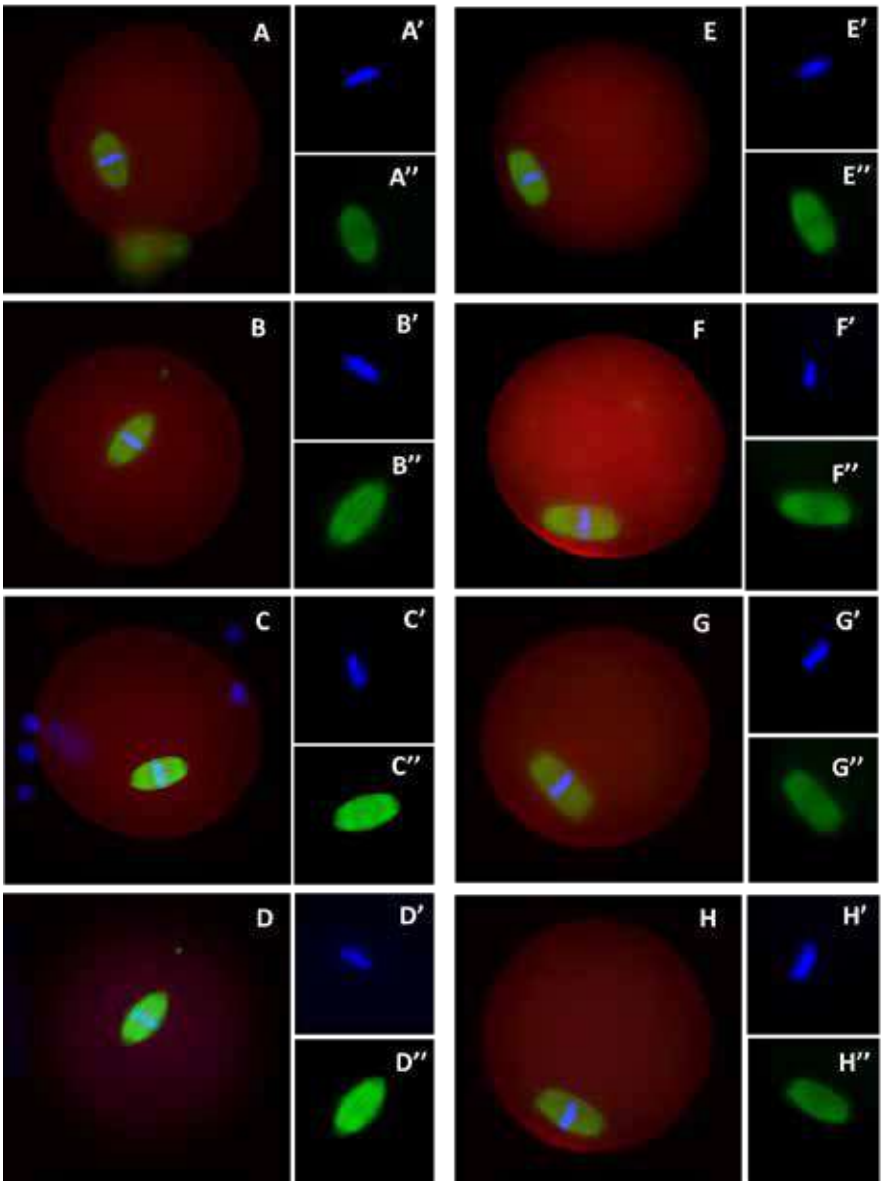

Figure 3. Immunofluorescence analysis of the morphology of the meiotic spindle and the chromosome distribution in metaphase II mouse oocytes. Control non-vitrified oocytes with a normal barrel-shaped spindle and the chromosomes aligned in the MII-plate (A-D). Oocytes were vitrified with the SUPERVITRI™ 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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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.

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)*.

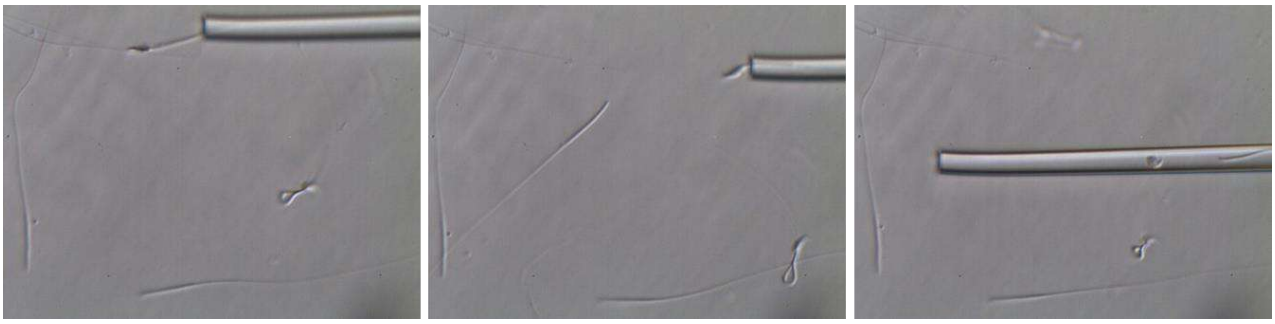

Figure 1. Decapitation of the mouse sperm. A) The sperm is aspirated into the piezo ICSI microcapillary with the tail first. B) The sperm is positioned to the tip end so that the head of the sperm just looks out. C) A piezo impulse is applied causing the separation of the head from the tail.

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.

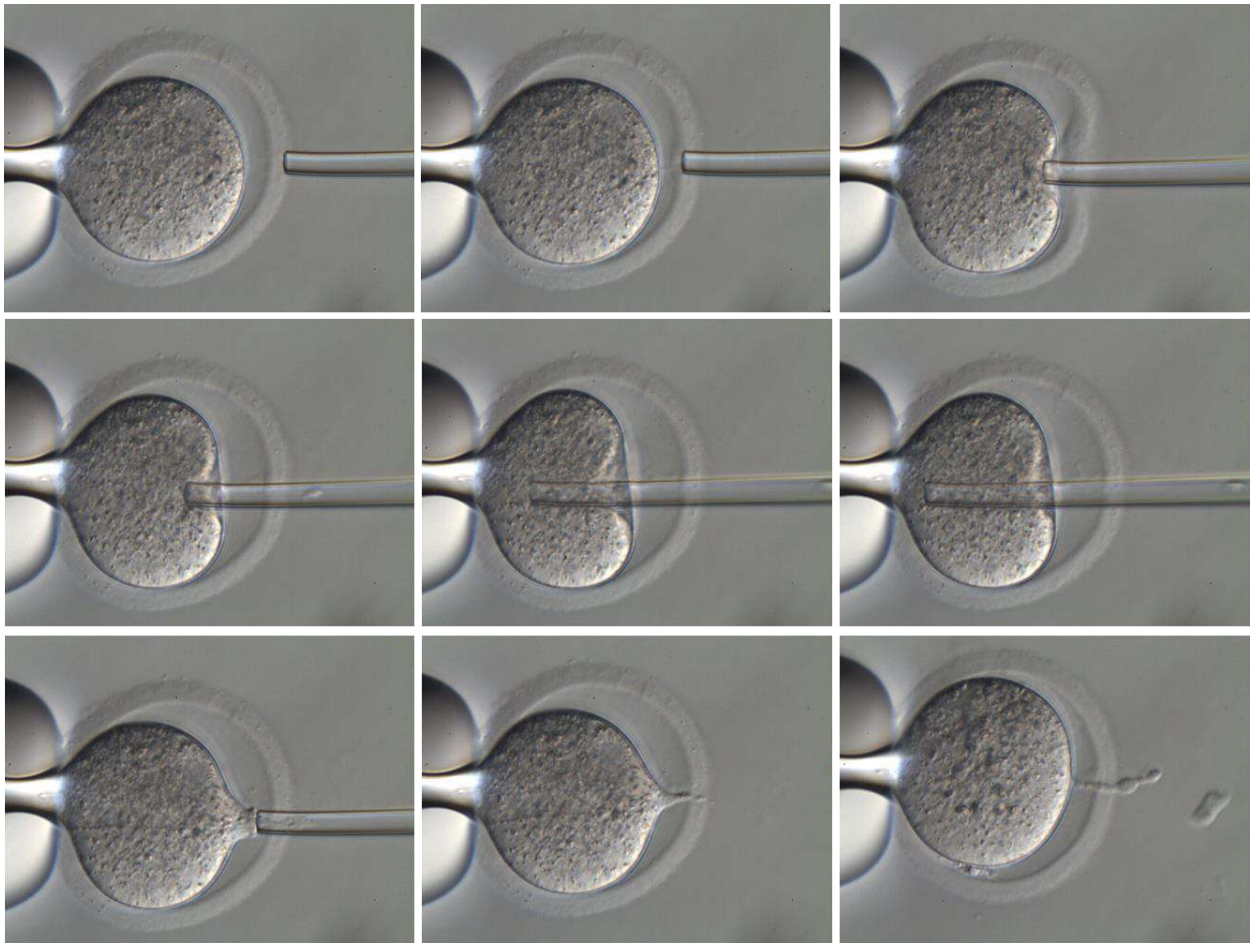

Figure 2. Mouse ICSI. (A–C) The equatorial plane of the oocyte and the ICSI capillary are aligned in microscopic focus. A piezo impulse is applied to ICSI capillary to penetrate the zona pellucida. (D–F) The ICSI capillary, loaded with the sperm head, is then moved deep into the oocyte. Minimal suction 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.

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. 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.

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Note. See Annex 1 (page 21) to see the embryo development photos.

SUPERVITRI™ device validation in the mouse model. Part III

Annex I

Examination Report

Client/Company: **Embryotools**
Code: EXP.014.911.2020
Group: **SUPERVITRI™ R1** (ET-RP-25)
Date: 06/07/2020

Examination ID	Examiner	Lab	Date of Examination
7246	EM, D4	Embryotools	09/07/2020

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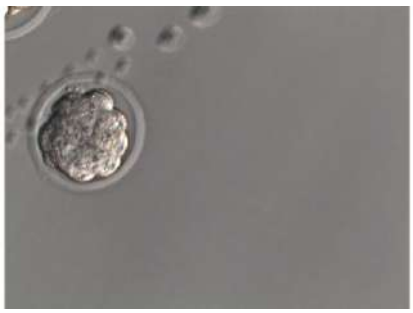

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Examination ID	Examiner	Lab	Date of Examination
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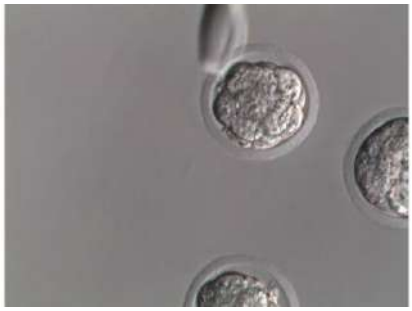

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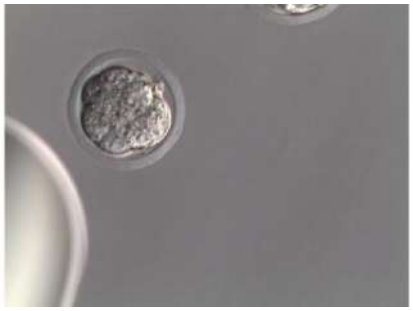

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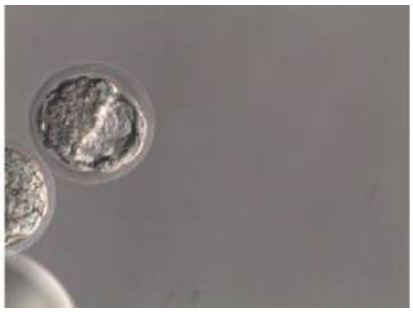


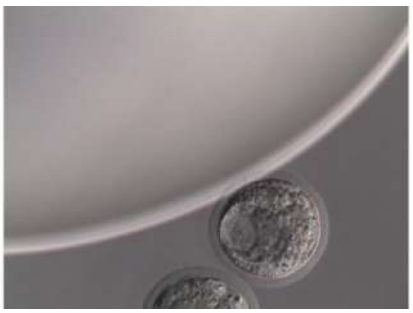
Examination Report

Client/Company: **Embryotools**
Code: EXP.014.980.2020 D4
Group: **SUPERVITRI™ R2** (ET-RP-25) 13/07/2020
Date:

Examination ID	Examiner	Lab	Date of Examination
7272	MG	Embryotools	16/07/2020

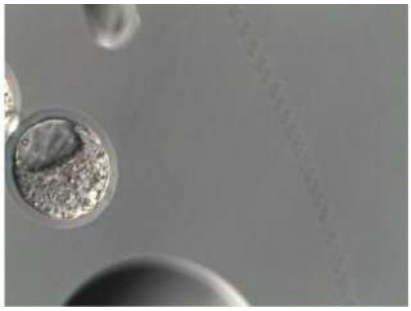
#1, 16/07/2020 14:12:35

#2, 16/07/2020 14:12:37



#3, 16/07/2020 14:12:47

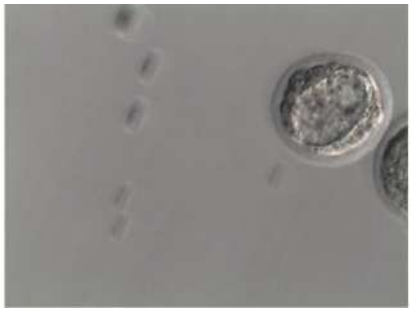
#4, 16/07/2020 14:12:49



#5, 16/07/2020 14:13:01

#6, 16/07/2020 14:13:03

#7, 16/07/2020 14:13:15

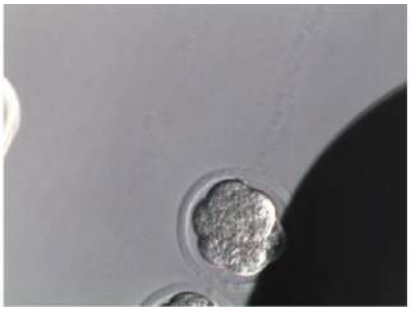


#8, 16/07/2020 14:13:16

#9, 16/07/2020 14:13:29

#10, 16/07/2020 14:13:30

#11, 16/07/2020 14:16:13



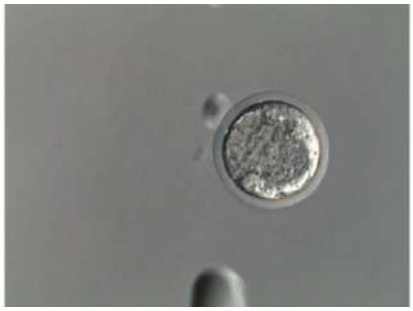
#12, 16/07/2020 14:16:13

Examination ID	Examiner	Lab	Date of Examination
7272	MG	Embryotools	16/07/2020

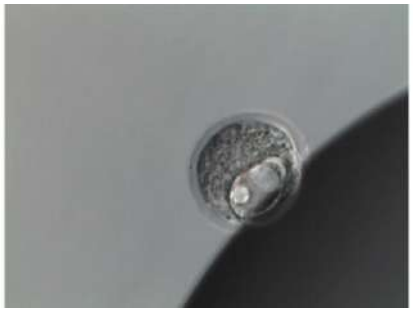
#13, 16/07/2020 14:16:26

#14, 16/07/2020 14:16:28

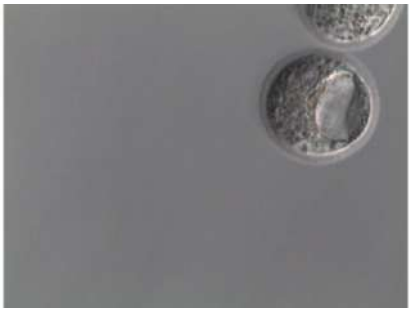
#15, 16/07/2020 14:16:38



#16, 16/07/2020 14:16:41



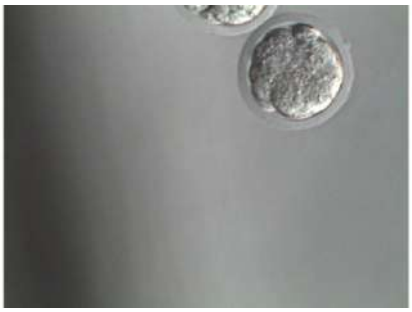
#17, 16/07/2020 14:16:52



#18, 16/07/2020 14:16:53

#19, 16/07/2020 14:17:05

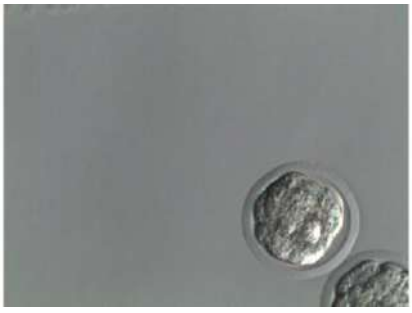
#20, 16/07/2020 14:17:06



#21, 16/07/2020 14:17:32

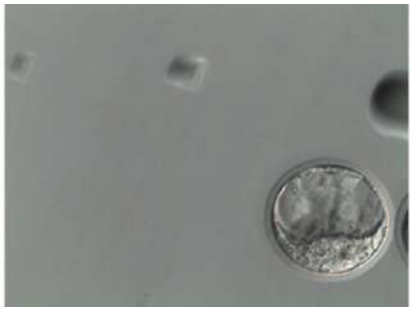
#22, 16/07/2020 14:17:40

#23, 16/07/2020 14:17:51

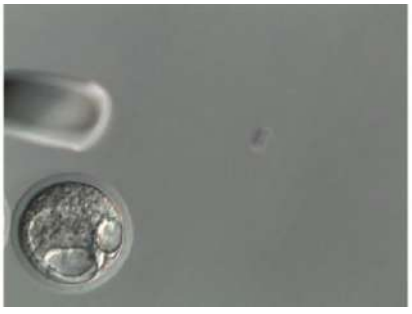


#24, 16/07/2020 14:17:52

#25, 16/07/2020 14:18:05



#26, 16/07/2020 14:18:06

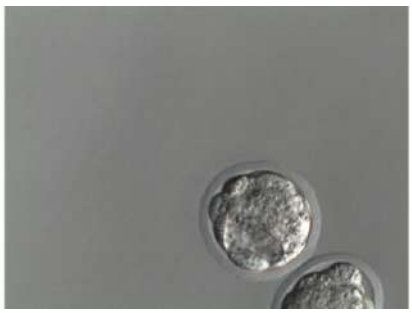


Examination Report

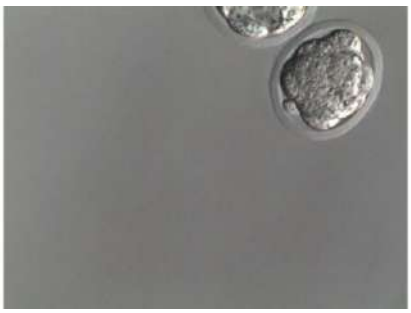
Client/Company: **Embryotools**
Code: EXP.014.1030.2020 D4
Group: **SUPERVITRI™ R3** (ET-RP-25) 21/07/2020
Date:

Examination ID	Examiner	Lab	Date of Examination
7305	MG	Embryotools	24/07/2020

#1, 24/07/2020 13:45:11



#2, 24/07/2020 13:45:13



#3, 24/07/2020 13:46:04

#4, 24/07/2020 13:46:05

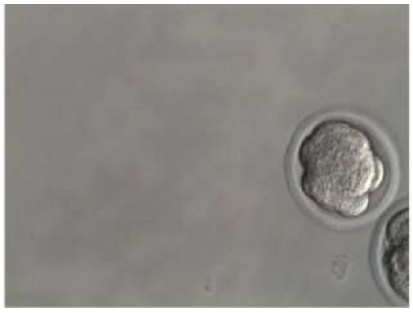
#5, 24/07/2020 13:46:16

#6, 24/07/2020 13:46:17

#7, 24/07/2020 13:46:27

#8, 24/07/2020 13:46:29

#9, 24/07/2020 13:46:49



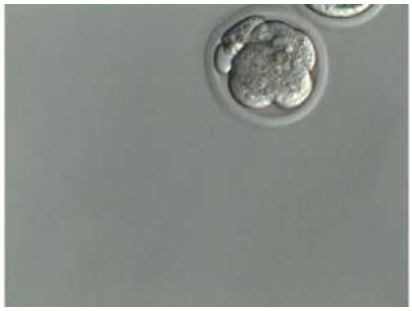
#10, 24/07/2020 13:46:51

#11, 24/07/2020 13:47:19

#12, 24/07/2020 13:47:20

Examination ID	Examiner	Lab	Date of Examination
7305	MG	Embryotools	24/07/2020

#13, 24/07/2020 13:47:31



#14, 24/07/2020 13:47:32

#15, 24/07/2020 13:47:43

#16, 24/07/2020 13:47:45

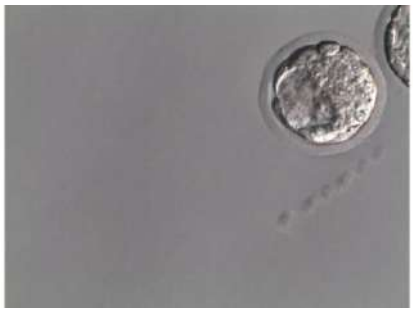
#17, 24/07/2020 13:47:54

#18, 24/07/2020 13:47:56

#19, 24/07/2020 13:48:13

#20, 24/07/2020 13:48:14

#21, 24/07/2020 13:48:34



#22, 24/07/2020 13:48:37

#23, 24/07/2020 13:48:38

Examination Report

Client/Company: **embryotools**
Code: EXP.015.037.2020 D4
Group: **SUPERVITRI™ R4** (ET-RP-25) 03/08/2020
Date:

Examination ID	Examiner	Lab	Date of Examination
7317	MG	embryotools	06/08/2020

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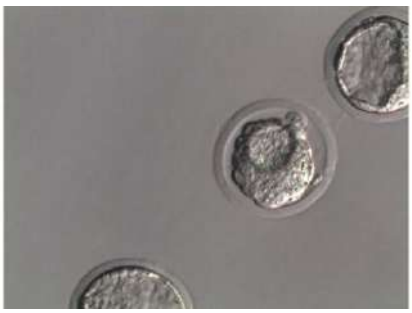
#2, 06/08/2020 13:07:27



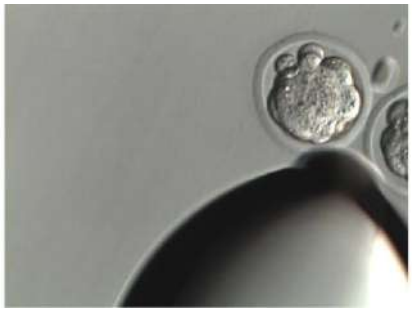
#3, 06/08/2020 13:07:29

#4, 06/08/2020 13:08:11

#5, 06/08/2020 13:08:16

#6, 06/08/2020 13:08:18

#7, 06/08/2020 13:08:32



#8, 06/08/2020 13:08:34

#9, 06/08/2020 13:08:46

#10, 06/08/2020 13:08:48

#11, 06/08/2020 13:08:56

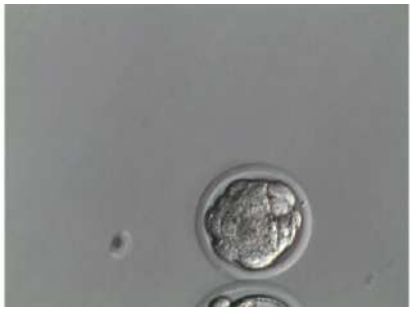
#12, 06/08/2020 13:08:56

Examination ID	Examiner	Lab	Date of Examination
7317	MG	embryotools	06/08/2020

#13, 06/08/2020 13:09:24

#14, 06/08/2020 13:09:26

#15, 06/08/2020 13:09:51



#16, 06/08/2020 13:09:52

#17, 06/08/2020 13:10:09

#18, 06/08/2020 13:10:10

#19, 06/08/2020 13:10:19

#20, 06/08/2020 13:10:21

#21, 06/08/2020 13:10:31

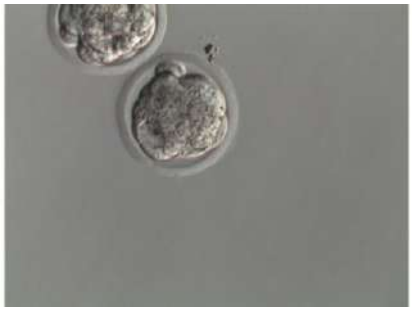
#22, 06/08/2020 13:10:32

#23, 06/08/2020 13:10:52

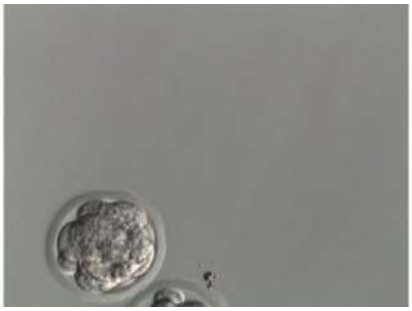
#24, 06/08/2020 13:10:54

Examination ID	Examiner	Lab	Date of Examination
7317	MG	embryotools	06/08/2020

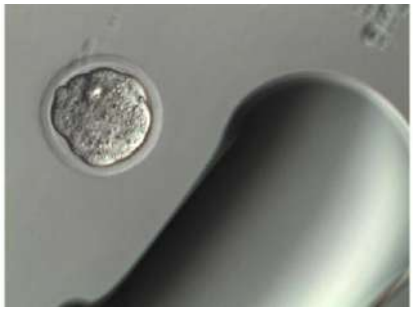
#25, 06/08/2020 13:11:15



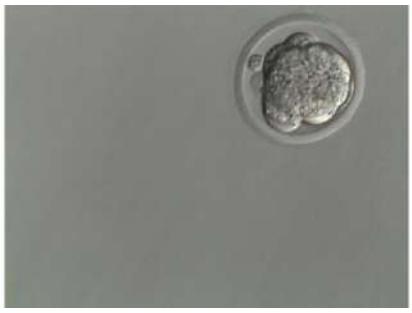
#26, 06/08/2020 13:11:15



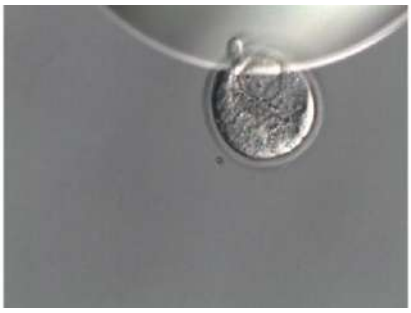
#27, 06/08/2020 13:11:27



#28, 06/08/2020 13:11:29



#29, 06/08/2020 13:11:37



#30, 06/08/2020 13:11:39

#31, 06/08/2020 13:11:45

#32, 06/08/2020 13:11:46

Examination Report

Name: **Embryotools, Control R1 (ET-RP-25)**
ID: 1526
Date of Birth: 06/07/2020
Code: EXP.014.912.2020

Examination ID	Examiner	Lab	Date of Examination
2220	MG	Embryotools	09/07/2020

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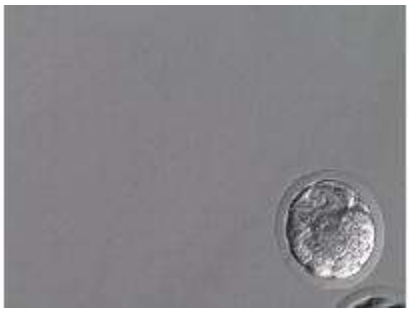


#2, 09/07/2020 16:19:11

#3, 09/07/2020 16:19:14

#4, 09/07/2020 16:19:46

#5, 09/07/2020 16:19:52

#6, 09/07/2020 16:19:53

#7, 09/07/2020 16:20:06

#8, 09/07/2020 16:20:08

#9, 09/07/2020 16:20:09

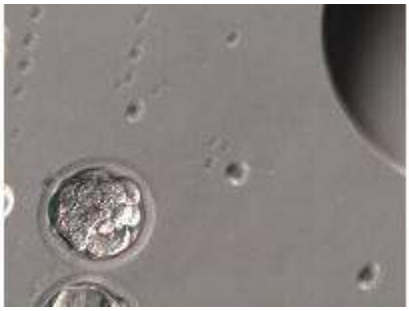
#10, 09/07/2020 16:20:22

#11, 09/07/2020 16:20:24

#12, 09/07/2020 16:20:26

Examination ID	Examiner	Lab	Date of Examination
2220	MG	Embryotools	09/07/2020

#13, 09/07/2020 16:20:43



#14, 09/07/2020 16:20:44

#15, 09/07/2020 16:21:48

#16, 09/07/2020 16:21:50

#17, 09/07/2020 16:22:55

#18, 09/07/2020 16:22:58

#19, 09/07/2020 16:23:09

#20, 09/07/2020 16:23:10

#21, 09/07/2020 16:23:25

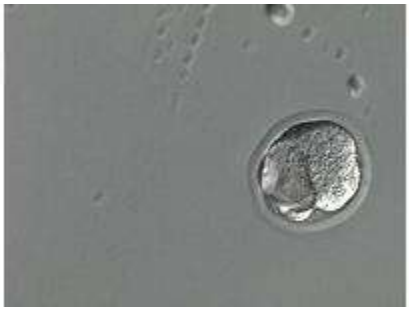
#22, 09/07/2020 16:23:26

#23, 09/07/2020 16:23:37

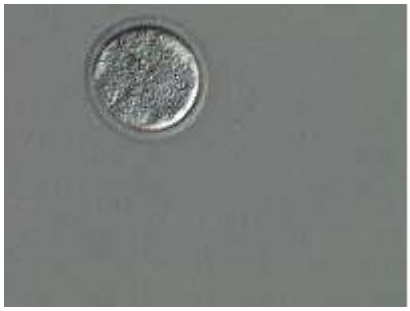
#24, 09/07/2020 16:23:38

Examination ID	Examiner	Lab	Date of Examination
2220	MG	Embryotools	09/07/2020

#25, 09/07/2020 16:24:20



#26, 09/07/2020 16:24:21



#27, 09/07/2020 16:24:27

#28, 09/07/2020 16:24:28

#29, 09/07/2020 16:24:53

#30, 09/07/2020 16:24:55

#31, 09/07/2020 16:25:06

#32, 09/07/2020 16:25:08

#33, 09/07/2020 16:25:18

#34, 09/07/2020 16:25:20

Examination Report

Client/Company: **Embryotools**
Code: EXP.014.979.2020 D4
Group: **Control R2** (ET-RP-25)
Date: 13/07/2020

Examination ID	Examiner	Lab	Date of Examination
7273	MG	Embryotools	16/07/2020

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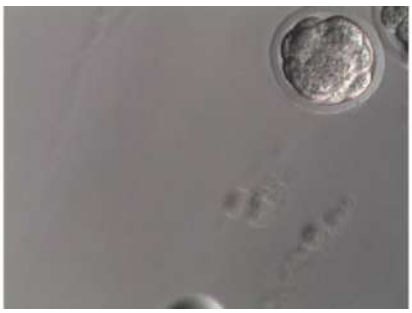
#2, 16/07/2020 14:21:26



#3, 16/07/2020 14:21:29

#4, 16/07/2020 14:21:44

#5, 16/07/2020 14:21:45

#6, 16/07/2020 14:21:56

#7, 16/07/2020 14:21:57

#8, 16/07/2020 14:22:07

#9, 16/07/2020 14:22:09

#10, 16/07/2020 14:22:18

#11, 16/07/2020 14:22:20

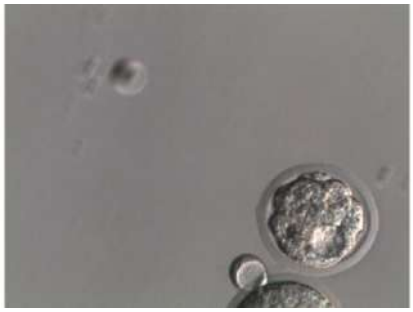
#12, 16/07/2020 14:22:42

Examination ID	Examiner	Lab	Date of Examination
7273	MG	Embryotools	16/07/2020

#13, 16/07/2020 14:22:44

#14, 16/07/2020 14:22:55

#15, 16/07/2020 14:22:56



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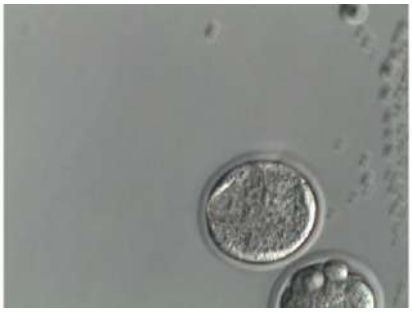
#17, 16/07/2020 14:23:09



#18, 16/07/2020 14:23:19

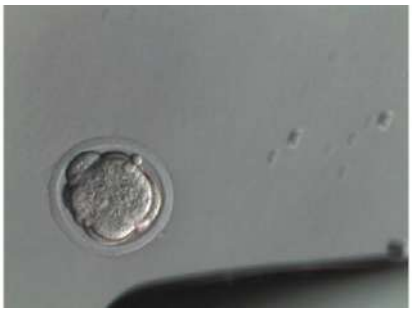
#19, 16/07/2020 14:23:20

#20, 16/07/2020 14:23:32

#21, 16/07/2020 14:23:33

#22, 16/07/2020 14:23:58

#23, 16/07/2020 14:23:59

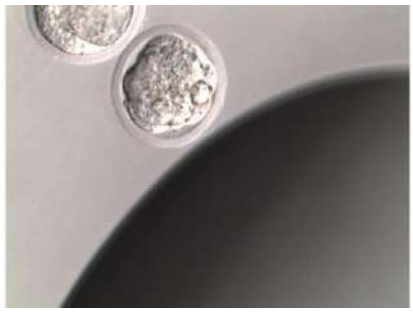


#24, 16/07/2020 14:24:11

#25, 16/07/2020 14:24:12

#26, 16/07/2020 14:24:22

#27, 16/07/2020 14:24:24



Examination Report

Client/Company: **Embryotools**
Code: EXP.014.1029.2020
Group: **Control R3** (ET-RP-25)
Date: 21/07/2020

Examination ID	Examiner	Lab	Date of Examination
7304	MG	Embryotools	24/07/2020

#1, 24/07/2020 13:36:14

#2, 24/07/2020 13:36:17

#3, 24/07/2020 13:36:29

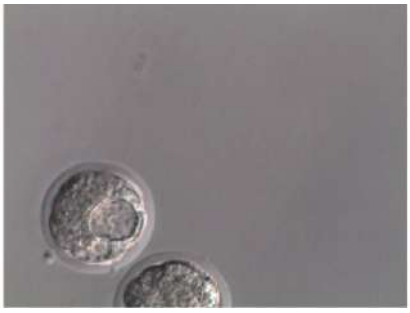
#4, 24/07/2020 13:36:31

#5, 24/07/2020 13:36:45

#6, 24/07/2020 13:36:46

#7, 24/07/2020 13:36:59

#8, 24/07/2020 13:37:00



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#10, 24/07/2020 13:37:57

#11, 24/07/2020 13:38:38

#12, 24/07/2020 13:38:39

Examination ID	Examiner	Lab	Date of Examination
7304	MG	Embryotools	24/07/2020

#13, 24/07/2020 13:38:52

#14, 24/07/2020 13:38:53

#15, 24/07/2020 13:39:04

#16, 24/07/2020 13:39:05

#17, 24/07/2020 13:39:17

#18, 24/07/2020 13:39:18

#19, 24/07/2020 13:39:28

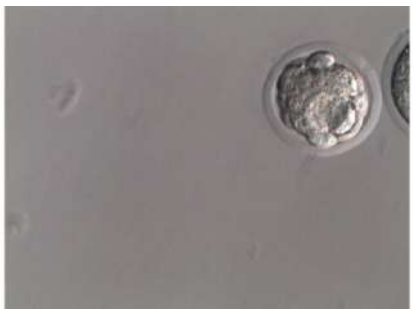
#20, 24/07/2020 13:39:29

#21, 24/07/2020 13:39:52

#22, 24/07/2020 13:39:55

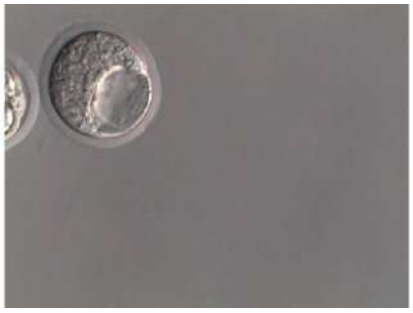
#23, 24/07/2020 13:39:56

#24, 24/07/2020 13:40:11



Examination ID	Examiner	Lab	Date of Examination
7304	MG	Embryotools	24/07/2020

#25, 24/07/2020 13:40:13



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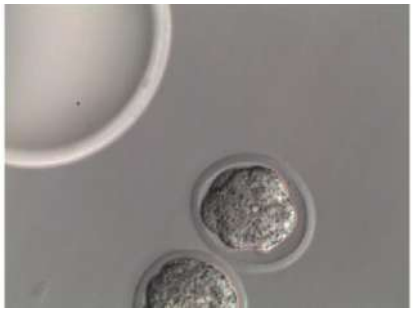
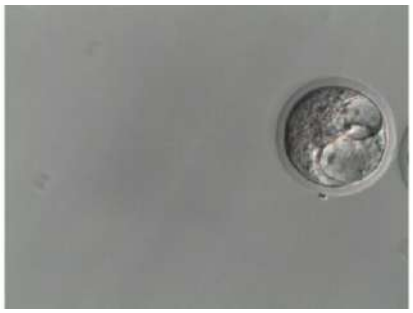
#27, 24/07/2020 13:40:26



#28, 24/07/2020 13:40:41



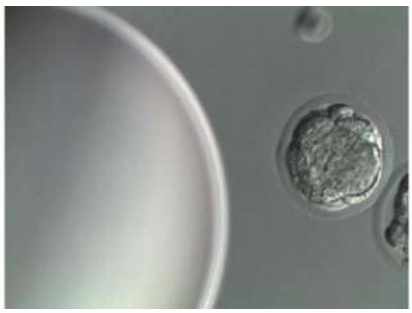
#29, 24/07/2020 13:40:43

Examination Report

Client/Company: **EMBRYOTOOLS**
Code: EXP.015.038.2020 D4
Group: **CONTROL R4** (ET-RP-25)
Date: 03/08/2020

Examination ID	Examiner	Lab	Date of Examination
7318	MG	EMBRYOTOOLS	06/08/2020

#1, 06/08/2020 13:15:39



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#3, 06/08/2020 13:15:44

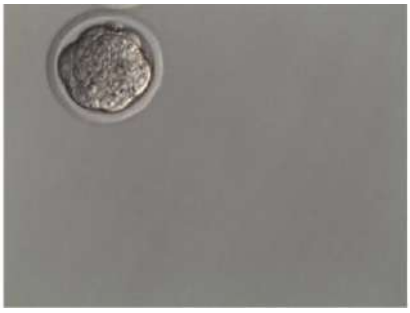
#4, 06/08/2020 13:15:55

#5, 06/08/2020 13:15:56

#6, 06/08/2020 13:16:12

#7, 06/08/2020 13:16:13

#8, 06/08/2020 13:16:24



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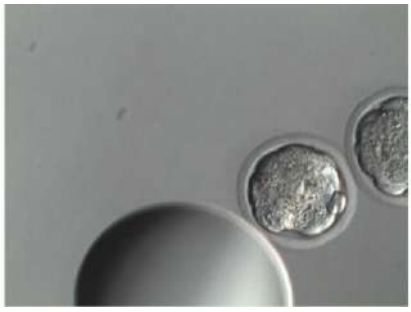

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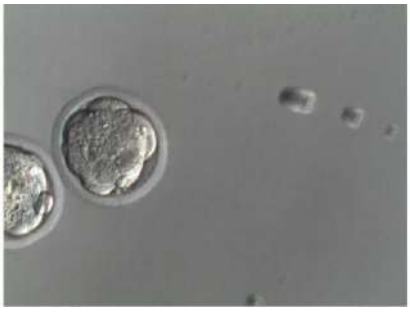

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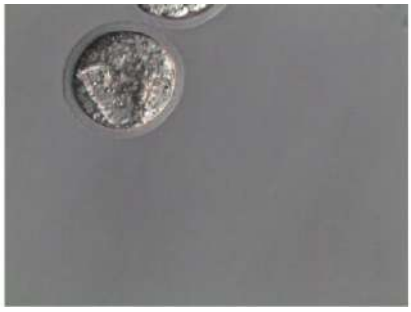


Examination ID	Examiner	Lab	Date of Examination
7318	MG	EMBRYOTOOLS	06/08/2020

#13, 06/08/2020 13:16:55

#14, 06/08/2020 13:17:07

#15, 06/08/2020 13:17:09

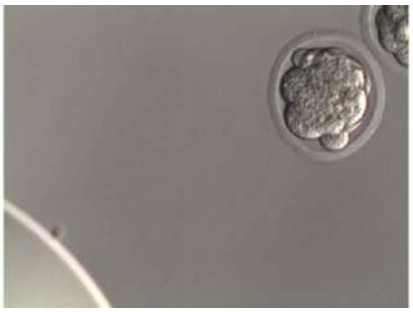
#16, 06/08/2020 13:17:18

#17, 06/08/2020 13:17:19

#18, 06/08/2020 13:17:36

#19, 06/08/2020 13:17:37

#20, 06/08/2020 13:17:48



#21, 06/08/2020 13:17:49

#22, 06/08/2020 13:18:08

#23, 06/08/2020 13:18:09

#24, 06/08/2020 13:18:18

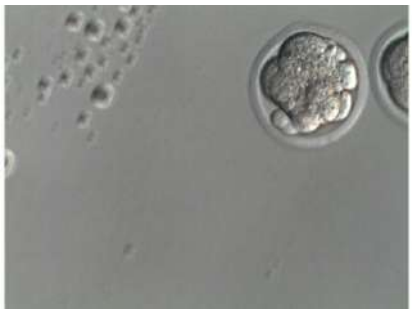
Examination ID	Examiner	Lab	Date of Examination
7318	MG	EMBRYOTOOLS	06/08/2020

#25, 06/08/2020 13:18:20

#26, 06/08/2020 13:18:56

#27, 06/08/2020 13:18:58

#28, 06/08/2020 13:19:07



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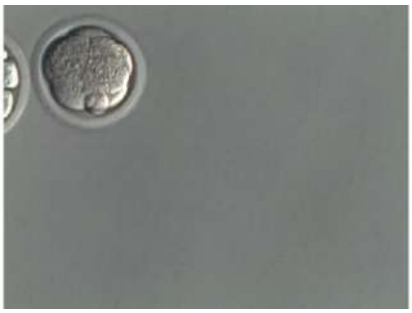

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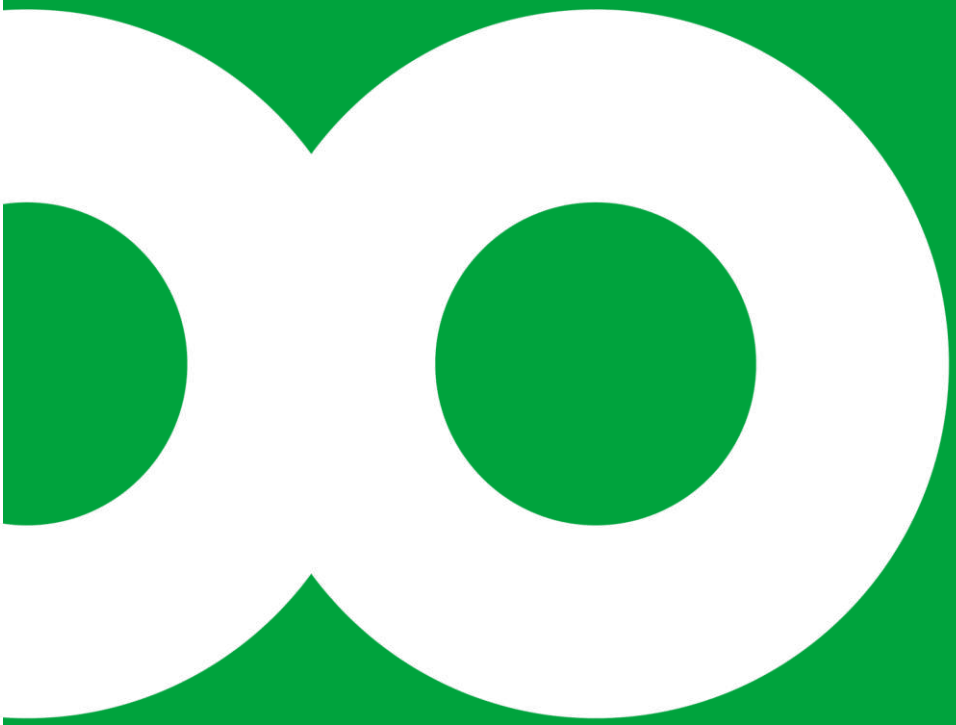
#31, 06/08/2020 13:19:17

Our Company



Our company

BPB MEDICA™ is an Italian manufacturing company specializing 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 an international level.

50 YEARS

OF EXPERIENCE

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

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

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

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

2014 — SPINE SURGERY: Development and launch of the 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 the Assisted Reproduction product line.

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

2020 — REGENERATIVE & ORTHO-BIOLOGICS: Development and launch of Regenerative Medicine & Ortho-Biologics product line.

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 conduct 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 reducing production waste and grants high-quality products.

Thanks to the internal R&D Department BPB MEDICA™ conducts constant research in the reference pathologies intending to ever better qualify and improve its production standards and aid the development of new products.

OUR PRODUCT LINES

- SPINE SURGERY
- ORTHO-BIOLOGICS
- ASSISTED REPRODUCTION
- BIOPSY
- INTENSIVE CARE
- AESTHETIC

METAL REFINISHING DEPARTMENT
Cutting, grinding, sharpening, cleaning, echogenic marking, sealing, reduction.

CLEAN ROOM
Manual & automatic assembly, packaging.

MOULDING DEPARTMENT
Vertical and horizontal moulding.

OUR SERVICES

PRODUCTION PROCESS CONTROL
Complete manufacturing process carried out internally, from design to final packaging.

RESEARCH & DEVELOPMENT
Constant research to increase production and quality standards while developing new products.

OEM & PRIVATE LABEL SERVICES
À la carte production, with the customer's brand name and custom colour.

MARKETING SUPPORT
Video tutorials, case reports, presence at the major medical congresses, organization of training and courses.

QUALITY & REGULATORY DEP.
Our primary aim is product quality and providing painstaking service. Our regulatory and quality teams conduct rigorous tests for this purpose.

FOUR WEEKS DELIVERY
Thanks to the optimization of the production process, we satisfy our customer's orders within 4 weeks.


medica

Contact us for further information:

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