Supplementary Information (SI) for Lab on a Chip.

OoTrap: Enhancing Oocyte Collection and Maturation with a Field-Deployable Fluidic Device

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Supplementary File

Suppl. Table 1. Efficiency of oocyte maturation in microfluidic devices

Species	Device design	Linear flow (mm s^{-1})*	Maturation rate	Comments	Reference
Mouse	Channel: 7-mm L, 140-µm W, and 200-µm H Square microchambers: 120-µm W, 120-µm, 360-µm, 600-µm and 900-µm L, at a 250-µm interval distance	0.00027**	Perfusion: 83.17% 2D: 55.35%	Lower apoptosis and lipid peroxidation under perfusion than 2D Higher blastocyst formation under perfusion than 2D	38, 39
	Channel: 7-mm L, 140-µm W, and 200-µm H Square microchambers: 120-µm W, 120-µm, 360-µm, 600-µm and 900-µm L, at a 250-µm interval distance	0.00027**	Rates not indicated. But the maturation rate was similar to *in vivo* maturation.	Computational simulations show low shear stress levels on the oocyte. Denudation of oocytes occurred spontaneously by their addition into the microchannels without any external mechanical stimulation or enzymatic treatment.	10
Sheep	Commercial LB1 bioreactor	0.005 and 0.01	2D: 49% 50: 20% 100: 18%	Higher abnormal chromatin under perfusion than 2D	11
	LiveBox1bioreactor with COCs embedded in microbeads	0.005	No significant differences between 3D IVM and 3D-mIVM. Static microbead IVM: 57.89% 50: 53.53%	No difference in mitochondrial distribution pattern but oocyte mitochondrial membrane potential, intracellular ROS levels, and mitochondria/ROS colocalization increased after 3D-mIVM, compared with 3D-IVM controls	12
Bovine	Oocytes are trapped in a filter-like structure	NA***	No differences between 2D and perfusion (~90%)	Reduced fertilisation rate under perfusion compared to 2D	14
Porcine	NA	NA***	2D: 61% Microchannels: 71%	NA	40
	Well inserts, consisting of multiple microwells with connecting microchannels	Rocking	Well static: 61% PDMS well static: 56% Well rocking: 57% PDMS well rocking: 57%	Reduced blastocyst rates in rocking conditions.	13

*Linear flow values were calculated using the formula: Linear flow rate(cm h^{-1}): 60 x Volumetric flow rate(ml min^{-1}) / Column cross-sectional area (cm^2)
** Presented linear flow rate in the original study (0.5 mm s^{-1}) is different than the calculated value here
***Linear flow rate could not be calculated since dimensions of channels are not described in the studies

Chitubox slicing settings

The .stl files, generated using Autodesk Fusion 360, were imported into Chitubox software (v1.9.5) for slicing using the following parameters:

Layer Height:	0.050	mm	Bottom Lift Distance:	5.000	+	0.000	mm
Bottom Layer Count:	5		Lifting Distance:	5.000	+	0.000	mm
Exposure Time:	15.000	s	Bottom Retract Distance:	5.000	+	0.000	mm
Bottom Exposure Time:	24.000	s	Retract Distance:	5.000	+	0.000	mm
Waiting Mode During Printing:	Resti... ▼		Bottom Lift Speed:	50.000	&	0.000	mm/min
Rest Time Before Lift:	0.000	s	Lifting Speed:	505.00(	&	0.000	mm/min
Rest Time After Lift:	0.000	s	Bottom Retract Speed:	150.00(	&	0.000	mm/min
Rest Time After Retract:	0.000	s	Retract Speed:	150.00(	&	0.000	mm/min

Suppl. Table 2. Different post-printing protocols tested for mould post-processing

Resin	3D Printed mould treatment			Successful?*
	IPA Wash	***UV (h)***	***Oven***	
Elegoo black		8		No
			100°C ON	No
	RT 1 week			No
Anycubic ABS-like & Elegoo plant based black	RT 3x1 h			No
	RT 3x1 h	8		No
	RT 3x1 h		100°C ON	No
	RT 3x1 h	8	100°C ON	No
	RT 3x1 h	8	100°C ON	No
Elegoo water-based black	RT ON			No
NOVA3D transparent	RT 24h			No
Anycubic transparent	RT 24h			Partial
	RT 72h			Partial
	RT 15 min sonication			Partial
	RT 15 min sonication**		100°C 1h	Yes

*The success criteria refer to the reversal of PDMS curing inhibition. Unsuccessful treatments lead to the PDMS not completely curing and coming out of the mould considerably sticky and/or without preserving the shape. “Partial” success indicates that the PDMS construct kept the shape but was sticky.

**2 sets of sonications before and after oven treatment.
ON (overnight), RT (room temperature)

Suppl. Table 3. Step by step of follicular fluid (FF) loading in OoTrap

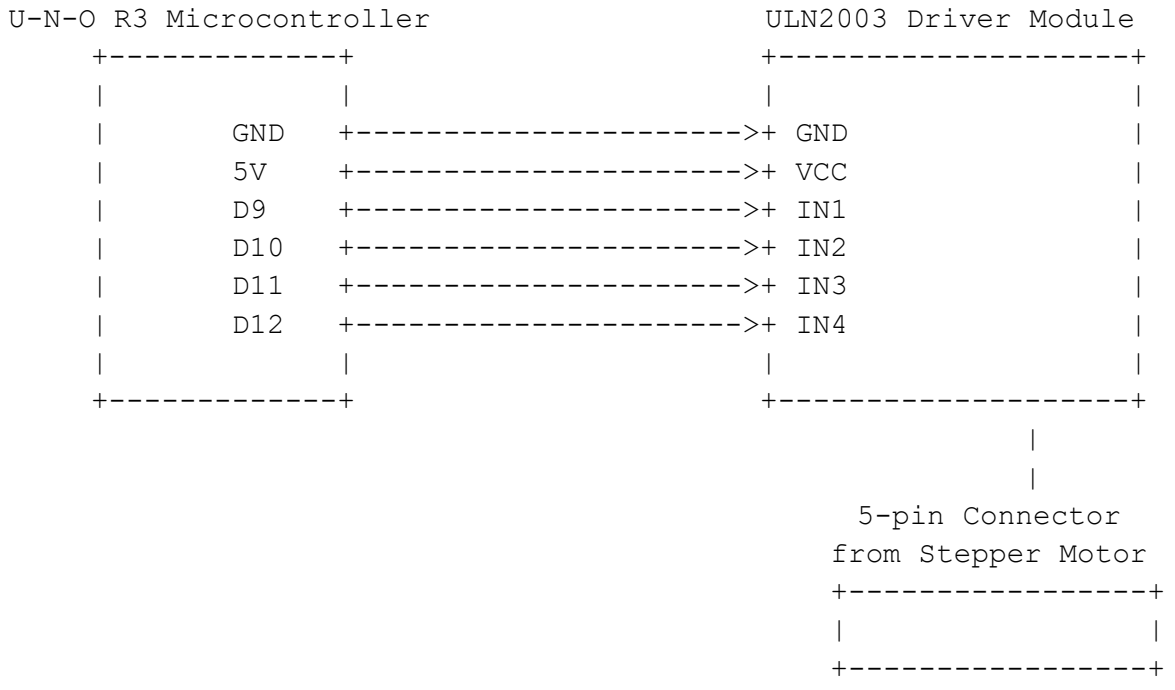
Step	Instructions
Adding FF + COCs into OoTrap	
1	Aspirate 5 ovaries in a tube and let COCs settle for 5 min.
2	Collect 2 mL of the pellet and transfer it to the reservoir.
3	Wait for 1 min to make sure most COCs are at the bottom of the reservoir.
4	Withdraw the FF by hand using the syringe: fast withdrawal of 500 µL and wait 30s. Repeat this until the FF is all inside the device.
5	Add 5 mL of wash media in the reservoir and slowly wash the device to remove cell debris. Usually, we wash twice.
Tip 1	If at any point, bubbles are introduced into the device, they can be removed by gently tilting the device so that the outlet is higher than the inlet. Since bubbles tend to travel upwards, by withdrawing the syringe in this position, the bubbles will be removed from the device into the tubing.
Tip 2	While withdrawing the wash media to remove cell debris, tilt the device to the left, right and bottom (reservoir up and outlet down) to accumulate bigger particles of cell debris to the sides to remove them easily. Repeat this every ~2 mL.
Tip 3	Do not withdraw too fast when the device is tilted, this may result in washing away some COCs but also do not withdraw too slowly as this will not efficiently remove the cell debris.
Tip 4	Whether some COCs are lost or not could be tested by collecting the excess wash media in the syringe to a culture dish, as described in our methods section.
6	Once all the washes are done, and most of the cell debris is washed away, place 5 mL IVM media in the reservoir and slowly fill the channel. For perfusion experiments, make sure to add enough volume of IVM media in the reservoir.
7	Keep the reservoir lid open and place the device in the incubator for the duration of IVM (22-24h).
12	Connect the device to the syringe pump with the desired flow rate and start perfusion using the withdrawal function.
Unloading COCs from OoTrap	
1	Add 5 mL of wash media in the reservoir and close the lid tightly.
2	Place the OoTrap on a straight surface and tap the top of the device forcefully from the middle (where the microwells are).
3	COCs and remaining cell debris will be floating within the device as you tap. Continue the tapping for 2 min.
4	Invert the device upside down to prevent COCs from going back into the microwells. Wait in this inverted position for ~30 sec.
Tip 1	At this stage, you could also position the device in such a way that the detached COCs float towards the outlet (reservoir higher than outlet with the reservoir facing against you).

5	Invert the device back to its original position. Quickly open the lid of the reservoir and withdraw the wash media with COCs by hand using the syringe.
6	Repeat these steps until all/most microwells are empty. This usually requires ~10 mL wash media, a lot of tapping and flipping the device from side to side.
Tip 2	If there are still some COCs stuck in the microwells (they will look darker compared to empty microwells), inverting the device completely and tapping from the bottom of the microwells, could help empty all microwells.
7	Place the wash media from the syringe containing the COCs in a dish and collect them once the device has been emptied.
Tip 3	Bubbles will be introduced during this process. When big bubbles are present or more than 30% of the device is empty, remove bubbles by quickly positioning the outlet end of the device upwards and withdrawing with the syringe and tapping the device.
Tip 4	When the device is inverted to remove COCs, keep an eye on the outlet connector: if a lot of force is applied to remove COCs from microwells, it detaches from the device and might lead to leaking of media with COCs inside.

3D-printed Syringe pump wiring and set up

The components used in this experiment include a U-N-O R3 microcontroller, a ULN2003 stepper motor driver module, a 28BYJ-48 5V stepper motor, connecting wires, and a power supply provided via the U-N-O R3 microcontroller's USB connection. To begin with, the ULN2003 driver module was connected to the U-N-O R3 microcontroller. The ULN2003 driver module serves as an interface between the microcontroller and the stepper motor, allowing the low-current control signals from the microcontroller to drive the higher current required by the stepper motor. The GND pin of the ULN2003 module was connected to one of the GND pins on the U-N-O R3 microcontroller. The VCC pin of the ULN2003 module was connected to the 5V pin on the U-N-O R3 microcontroller. For the control connections, IN1 on the ULN2003 module was connected to digital pin 9 on the U-N-O R3 microcontroller, IN2 was connected to digital pin 10, IN3 was connected to digital pin 11, and IN4 was connected to digital pin 12.

The 28BYJ-48 stepper motor has a 5-pin connector that directly interfaces with the ULN2003 driver module. This connector was inserted into the corresponding socket on the ULN2003 module, ensuring proper alignment and secure connection, as seen below:



The U-N-O R3 microcontroller was programmed using the Arduino Integrated Development Environment (IDE). The following code was uploaded to the microcontroller to control the stepper motor:

```
#define STEPPER_PIN_1 9
#define STEPPER_PIN_2 10
#define STEPPER_PIN_3 11
#define STEPPER_PIN_4 12
  int step_number = 0;
     void setup() {
pinMode(STEPPER_PIN_1, OUTPUT);
pinMode(STEPPER_PIN_2, OUTPUT);
pinMode(STEPPER_PIN_3, OUTPUT);
pinMode(STEPPER_PIN_4, OUTPUT);

                }

          void loop() {
          OneStep(false);
           delay(29696);

                }


     void OneStep(bool dir){
               if(dir){
       switch(step_number){
               case 0:
digitalWrite(STEPPER_PIN_1, HIGH);
digitalWrite(STEPPER_PIN_2, LOW);
digitalWrite(STEPPER_PIN_3, LOW);
digitalWrite(STEPPER_PIN_4, LOW);
               break;
               case 1:
digitalWrite(STEPPER_PIN_1, LOW);
digitalWrite(STEPPER_PIN_2, HIGH);
digitalWrite(STEPPER_PIN_3, LOW);
digitalWrite(STEPPER_PIN_4, LOW);
               break;
               case 2:
digitalWrite(STEPPER_PIN_1, LOW);
digitalWrite(STEPPER_PIN_2, LOW);
digitalWrite(STEPPER_PIN_3, HIGH);
digitalWrite(STEPPER_PIN_4, LOW);
               break;
               case 3:
digitalWrite(STEPPER_PIN_1, LOW);
digitalWrite(STEPPER_PIN_2, LOW);
```

```
digitalWrite(STEPPER_PIN_3, LOW);
digitalWrite(STEPPER_PIN_4, HIGH);
break;
}
}else{
switch(step_number){
case 0:
digitalWrite(STEPPER_PIN_1, LOW);
digitalWrite(STEPPER_PIN_2, LOW);
digitalWrite(STEPPER_PIN_3, LOW);
digitalWrite(STEPPER_PIN_4, HIGH);
break;
case 1:
digitalWrite(STEPPER_PIN_1, LOW);
digitalWrite(STEPPER_PIN_2, LOW);
digitalWrite(STEPPER_PIN_3, HIGH);
digitalWrite(STEPPER_PIN_4, LOW);
break;
case 2:
digitalWrite(STEPPER_PIN_1, LOW);
digitalWrite(STEPPER_PIN_2, HIGH);
digitalWrite(STEPPER_PIN_3, LOW);
digitalWrite(STEPPER_PIN_4, LOW);
break;
case 3:
digitalWrite(STEPPER_PIN_1, HIGH);
digitalWrite(STEPPER_PIN_2, LOW);
digitalWrite(STEPPER_PIN_3, LOW);
digitalWrite(STEPPER_PIN_4, LOW);
}
}
step_number++;
if(step_number > 3){
step_number = 0;
}
}
```

After completing the assembly and programming, the circuit was tested to verify correct operation. The U-N-O R3 microcontroller was connected to a computer via USB to provide power and allow for code uploading. Once powered on, the stepper motor performed a single step every 29.696 seconds as per the delay specified in the code.

Determining the necessary delay between motor steps

First, gear reduction ratios were calculated based on the spikes on each component, which directly impacts the mechanical advantage achieved through the system. The reduction ratio from the motor wheel to the transmitter wheel (Ratio 1) was derived from the ratio of the number of spikes on the transmitter wheel to the motor wheel:

$$Ratio\ 1\ = Number\ of\ Spikes\ on\ Transmitter\ Wheel \div\ Number\ of\ Spikes\ on\ Motor\ Wheel$$

The reduction ratio between the transmitter and the second wheel (Ratio 2) was calculated as follows:

$$Ratio\ 2\ = Number\ of\ Spikes\ on\ Second\ Wheel \div\ Number\ of\ Spikes\ on\ Transmitter\ Wheel$$

The total gear reduction combines these ratios to quantify the overall mechanical advantage:

$$Total\ Reduction\ =\ Ratio\ 1\ \times\ Ratio\ 2$$

To establish the steps required per mm of syringe plunger movement, we first calculate the linear movement achievable per revolution of the motor by considering the total gear reduction and the circumference of the second wheel. The circumference of the second wheel (Circumference Second Wheel) was computed as:

$$Circumference\ Second\ Wheel\ =\ \pi\ \times\ Diameter\ of\ Second\ Wheel$$

Therefore, the linear movement per revolution was:

$$Linear\ Movement\ Per\ Revolution\ =\ Circumference\ Second\ Wheel\ \times\ Total\ Reduction$$

The number of steps per mm of plunger movement (Steps Per mm) was derived by dividing the stepper motor's total steps per revolution by the linear movement per revolution:

$$Steps\ Per\ mm\ =\ Steps\ Per\ Revolution \div\ Linear\ Movement\ Per\ Revolution$$

To achieve a precise flow rate of 20 µL h^{-1}, we performed the following calculations, given the syringe diameter of 4.78 mm, the cross-sectional area (Area) was:

$$Area\ =\ \pi(Diameter \div\ 2)^{\ 2}$$

The movement per step was:

$$Movement\ Per\ Step\ =\ 1\ \div Steps$$

Therefore, the volume per step was:

$$Volume\ Per\ Step\ =\ Area\ \times\ Movement\ Per\ Step$$

The number of steps needed to achieve a flow rate of 20 μL h^{-1} was calculated as:

$$Steps\ Per\ Hour\ =\ 20\ \div\ Volume\ Per\ Step$$

Finally, the delay between each step was:

$$Delay\ (ms)\ =\ 3600\ seconds/hour\ \times\ 1000\ ms/second\ /\ Steps\ Per\ Hour$$

In our setup, the first gear reduction ratio was 2.0, the second gear reduction ratio was 0.375, and the total gear reduction was 0.75. The number of steps per mm of plunger movement was approximately 108.67 steps mm^{-1} (the stepper motor has 2,048 steps per revolution), and the volume per step was approximately 0.165 μL $step^{-1}$. To achieve a flow rate of 20 μL h^{-1}, approximately 121.21 steps per hour are needed, resulting in a delay of approximately 29,696 ms between steps.

Suppl. Table 4. Step by step of follicular fluid (FF) loading in the heated OoTrap

Step	Instructions
Adding FF + COCs into heated OoTrap	
1	Aspirate 5 ovaries in a tube and let COCs settle for 5 min.
2	Collect 500 µL of the pellet and transfer it to the reservoir.
3	Wait for 1 min to make sure most COCs are at the bottom of the reservoir.
4	Withdraw the FF by hand using the syringe: fast withdrawal of 100 µL and wait 30s. Repeat this until the FF is all inside the device.
5	Add wash media in the reservoir and slowly wash the device to remove cell debris. Usually we wash twice (with ~2.5 mL wash media in total).
Tip 1	If at any point, bubbles are introduced into the device, they can be removed by gently tilting the device so that the outlet is higher than the inlet. Since bubbles tend to travel upwards, by withdrawing the syringe in this position, the bubbles will be removed from the device into the tubing.
Tip 2	While withdrawing the wash media to remove cell debris, tilt the device to the left, right and bottom (reservoir up and outlet down) to accumulate bigger particles of cell debris to the sides to remove them easily. Repeat this every ~1 mL
Tip 3	Do not withdraw too fast when the device is tilted, this may result in washing away some COCs but also do not withdraw too slowly as this will not efficiently remove the cell debris.
Tip 4	Whether some COCs are lost or not could be tested by collecting the excess wash media in the syringe to a culture dish, as described in our methods section.
6	Once all the washes are done, and most of the cell debris is washed away, place 2.5 mL IVM media in the reservoir and slowly fill the channel. For perfusion experiments, make sure to add enough volume of IVM media in the reservoir.
7	Perform IVM (22-24h).
12	Connect the device to the syringe pump with the desired flow rate and start perfusion using the withdrawal function.
Unloading COCs from OoTrap	
1	Add 2.5 mL of wash media in the reservoir and close the lid tightly.
2	Place the OoTrap on a straight surface and tap the top of the device forcefully from the middle (where the microwells are).
3	COCs and remaining cell debris will be floating within the device as you tap. Continue the tapping for 1 min.
4	Invert the device upside down to prevent COCs from going back into the microwells. Wait in this inverted position for ~30 sec.
Tip 1	At this stage, you could also position the device in such a way that the detached COCs float towards the outlet (reservoir higher than outlet with the reservoir facing against you).
5	Invert the device back to its original position. Quickly open the lid of the reservoir and

	withdraw the wash media with COCs by hand using the syringe.
6	Repeat these steps until all/most microwells are empty. This usually requires ~5 mL wash media, a lot of tapping and flipping the device from side to side.
Tip 2	If there are still some COCs stuck in the microwells (they will look darker compared to empty microwells), inverting the device completely and tapping from the bottom of the microwells, could help empty all microwells.
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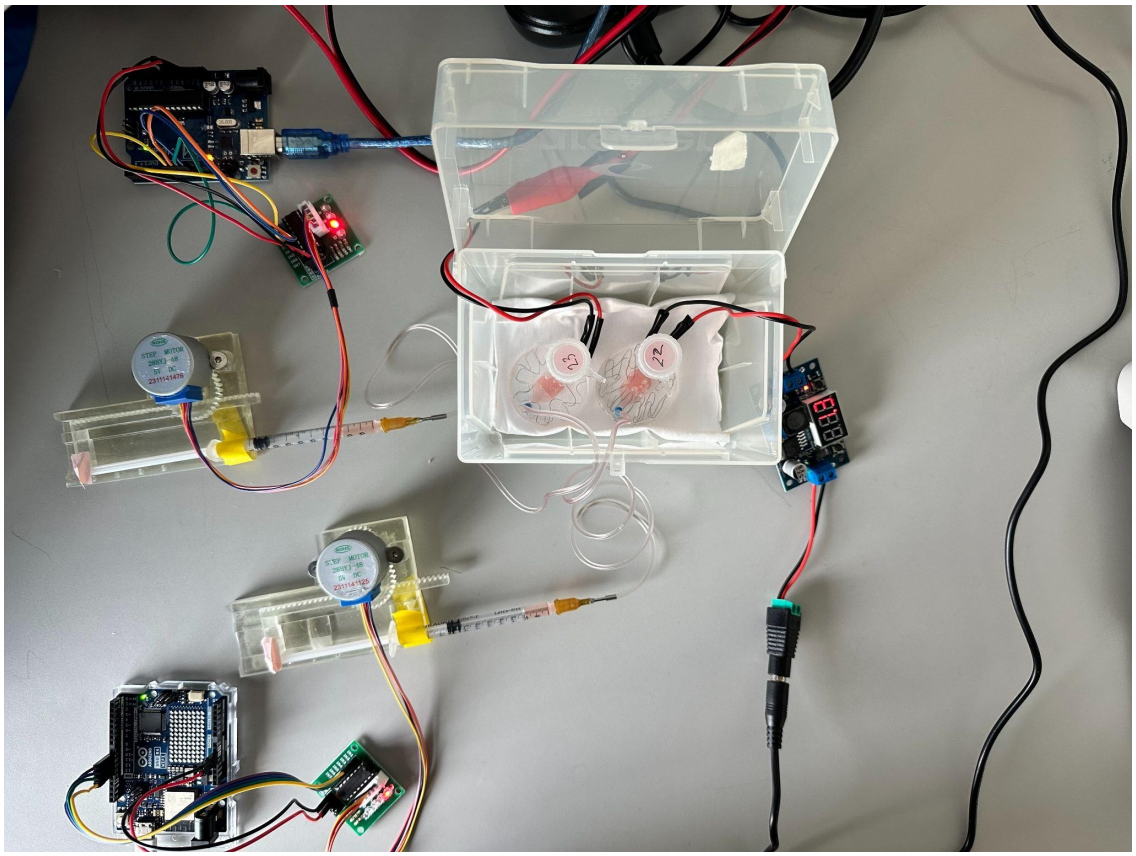
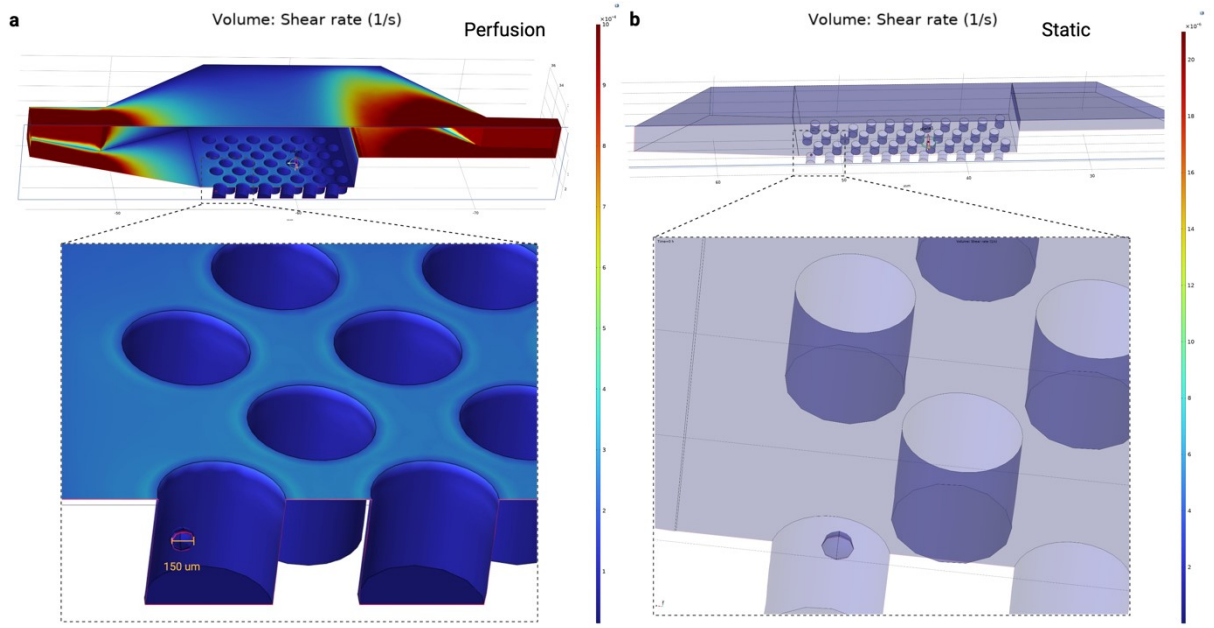
Tip 4	When the device is inverted to remove COCs, keep an eye on the outlet connector: if a lot of force is applied to remove COCs from microwells, it detaches from the device and might lead to leaking of media with COCs inside.



Suppl. Fig. 1. The setup of the heated OoTrap device with a 3D-printed syringe pump is shown. Each device incorporates a nichrome wire for heating and is connected to a 1 mL syringe placed on a 3D-printed syringe pump. The syringe pumps are controlled by a U-N-O R3 Microcontroller Board. The nichrome wires are connected to a DC-DC Step Down Converter Module, enabling Joule heating to maintain the devices at the required temperature. To provide humidity and prevent media evaporation, the devices are placed in a pipette tip box containing wet clean paper. This configuration allowed for the maturation of oocytes to be performed directly on the bench.

Suppl. Table 5. Supplies needed for Field-friendly heated OoTrap with costs

Component	Part name	Supplier	Cost (Euro)
Heating component	DC-DC Step Down Converter Modules Adjustable Buck Converter Module with LED Display 4-40V to 1.25-37V	Heelom	3.50
	Nichrome wire 0.25 mm		0.35
	28BYJ-48 ULN2003 Stepper Motor with Drive Module Board for Arduino Motor	Angeek	2.18
	USB C Male to 5 Pin Screw Terminal Female	XMSJSIY	2.49
Controller	U-N-O R3 Microcontroller Board Mega328 Board Motherboard, Mega328-R3 Microcontroller Board with USB Cable Compatible with Arduino	APKLVSR	7.05
Wiring	Jumper wire	ELEGOO	0.70
	Silicone Electrical Wire Cable - .32 mm² tinned copper wire, 22 AWG	Powergrace	0.20
Syringe pump	28BYJ-48 ULN2003 Stepper Motor with Drive Module Board for Arduino Motor	Angeek	2.18
	3D-printing Clear resin	Anycubic	0.93
Microfluidics device	PDMS	Dow Corning	0.70
	Tubing connector	Chipshop	0.74
	Reservoir	Chipshop	4.84
	Tubing	Tygon	0.45
	1 mL Syringe	Fisher	0.15
Total			**26.46**



Suppl. Fig. 2. Shear rate distribution in the heated OoTrap device as simulated in COMSOL Multiphysics 6.1. The design of the heated OoTrap was sliced through the middle, and a sphere with a diameter of 150 µm, representing an oocyte, was placed in the microwell as shown in the bottom image. The colour spectrum indicates the intensity of the shear rate within the device for perfusion (a) and static (b) conditions. As depicted, under perfusion, the shear rate is higher at the inlet and outlet, where the channels are narrower, and decreases significantly in the middle of the device where the microwells are located. No differences in shear rates were observed under static conditions.

Suppl. Table 6. Efficiency of oocyte maturation without CO_2 controlled environment

Species	Culture media description	Culture conditions	Maturation rate	Comments	Reference
Sheep	Medium199 with FBS, FSH, LH, HEPES (concentration not indicated), and pen/strep	Non-CO_2 controlled portable incubator at 39C. Media not equilibrated.	Control: 72.55% Portable device: 66.96%	No significant differences in terms of maturation, fertilisation, and developmental potential.	50
Bovine	Control: M199 with Earle's salts with10% FBS, 0.5 mM pyruvate, 0.1 mM cysteamine, 25 µg/mL gentamicin and 5 mU FSH at 38.5°C and 6% CO_2 in air Experimental: EqMM, Synthetic oviductal fluid medium (SOFaaci) with 3 mM glucose, 18 mM NaHCO3, 20 mM HEPES, 0.5 mM pyruvate, 0.1 mM cysteamine, 2 µM resveratrol, 25 µg/mL gentamicin, 5 mU FSH, 5% v/v FBS, 5% v/v serum substitute	Experimental group: Tube containing oocytes were placed in a portable incubator at 38.5°C and matured for 22 hours in atmospheric air. Control: Oocytes were placed in Nunc dishes with media and covered with sterile oil. Matured at 38.5°C and 6% CO_2	Not described	Similar cleavage and blastocyst rates, with embryos having similar lipid content and mitochondrial activity.	51
Equine		Experimental group: EqMM for 30 hours at 38.5 C in atmospheric air Control: Placed in Syngro holding medium for 18–20 hours at room temperature (22°C–25°C) in atmospheric air and then matured in control-M for 30 hours at 38.5°C and 6% CO_2 in air.	Control: 73% Portable device: 70%	Similar maturation, cleavage, blastocyst, and pregnancy rates.	
Porcine	TCM 199 supplemented with 0.1% PVA, 3.05 mM D-glu- cose, 0.91 mM sodium pyruvate, 0.57 mM cysteine, 0.5 µg/ml LH, 0.5 µg/ml FSH, 10 ng/ml epidermal growth factor, 75 µg/ml penicillin G, and 50 µg/ml streptomycin.	Culture in the dry incubator was tightly capped and sealed with parafilm.	Control: 87.9% Dry incubator: 91.4%	Oocytes can develop to the blastocyst stage after parthenogenetic activation.	52