

## Supplementary Information

### Investigating the mechanical properties of zona pellucida of whole human oocytes by atomic force spectroscopy

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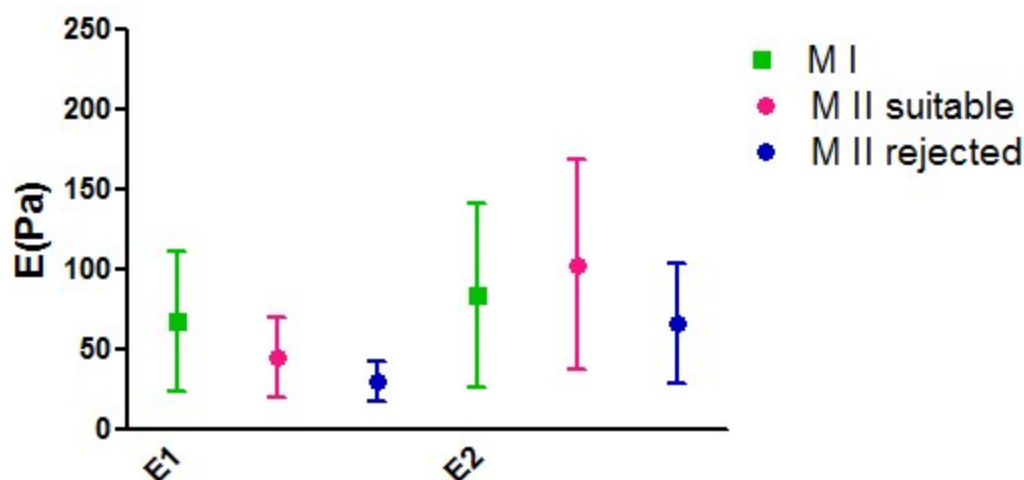
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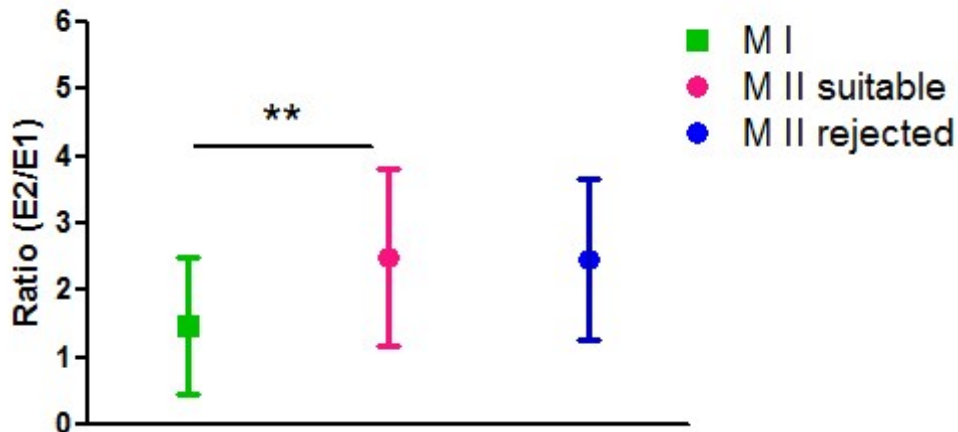
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#### 1S. Analysis of E1 and E2 values for oocyte group



**Figure 1S:** The E1 and E2 mean values per oocyte are obtained and then averaged for each group and reported in the plot: mean of the means values (symbol)  $\pm$  standard deviation (bar).



**Fig 2S:** The mean E1 and E2 values for each oocyte are used to evaluate the E2/E1 ratio per oocyte, which is then averaged for each group and reported in the plot: mean of the means (symbol)  $\pm$  standard deviation (bar). A statistical analysis between the groups was performed by the non-parametric statistical analysis of the Mann–Whitney test (two-tailed distribution) with GraphPad Prism 5.0. A p value  $< 0.05$  was considered to be statistically significant. A significant difference among MI and MII suitable was observed ( $p=0,0096$ ); while no difference can be observed between suitable and rejected MII.

## 2S. Clinical details about patients, their oocytes and outcome of ICSI procedure

In Tab.IS are reported the total number of the oocytes retrieved after hormonal stimulation of the patients involved in this work, together with details about fertilization steps and outcome. Oocytes degenerated are those that degenerated during manipulation immediately after retrieval. Generally the number of the embryos transferred to a woman is  $\leq 3$  to avoid multiple pregnancy, which can cause pregnancy complications. The rest can be frozen according to the patient requirements.

In Tab. IIS are reported only oocytes donated for our experiments (a small part of those reported in Tab.IS) together with their maturation stage, quality, relation to infertility and pregnancy. These oocytes were not inseminated.

A comparison of the E1 and E2 distributions of oocytes from a single patient, who donated both suitable MII and MI or both suitable and rejected MII is performed and shown in Fig. 3S. Although the number of oocytes donated by a single patient is very limited, this analysis seems to reflect the trend observed in the data pool analysis. In patient 4 and 7 we noticed that the outer ZP of MI is significantly stiffer than that of suitable MII.

In the case of those patients who donated both suitable and rejected MII, we observed some variability: in patient 11 and 13 the outer ZP of rejected MII is softer than that of suitable MII although the data are not significant statistically, while the rest of the ZP-oocyte is significantly

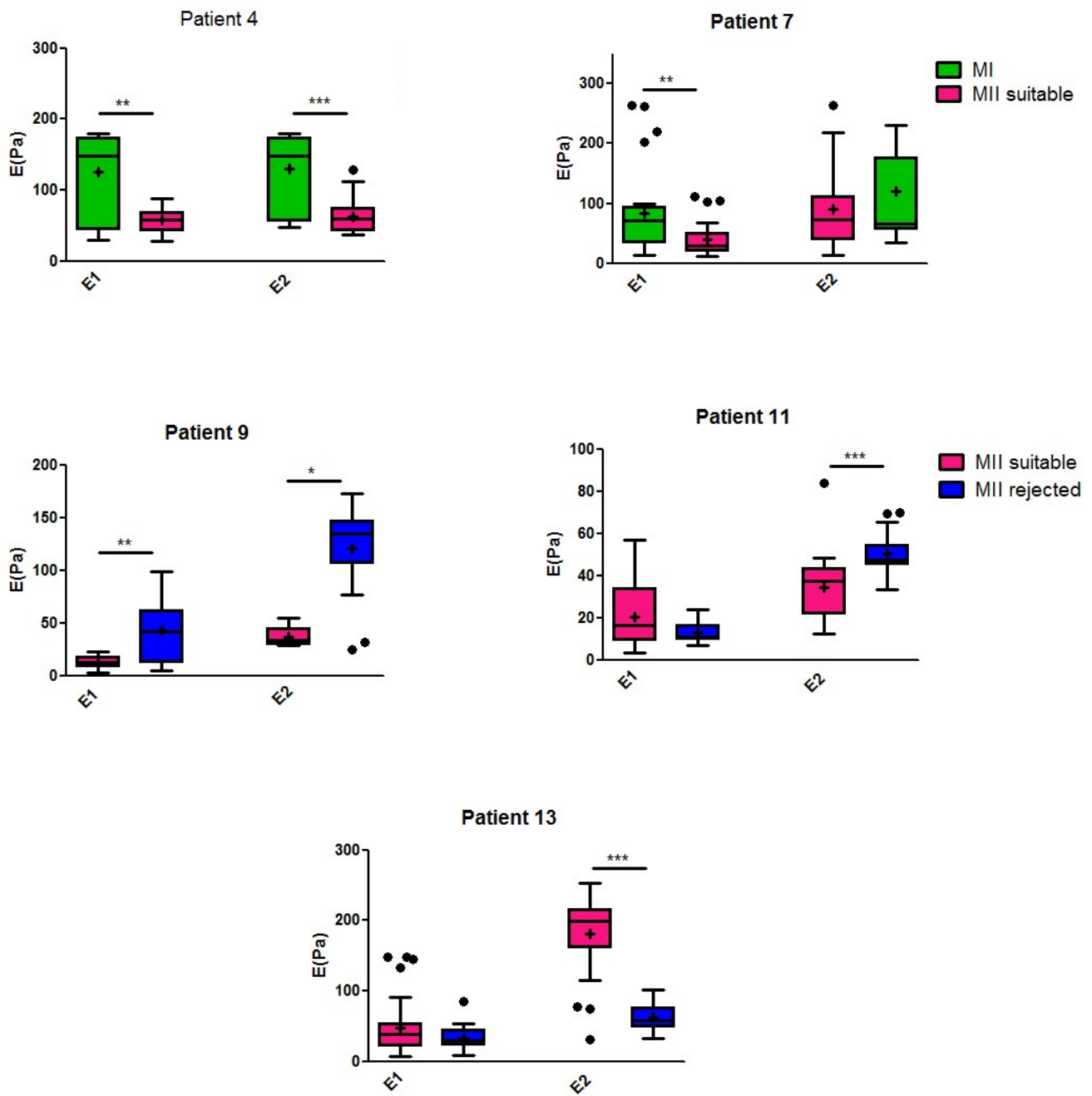
stiffer in patient 11 and softer in patient 13 than the suitable MII. Whereas for patient 9 the entire MII rejected oocyte (both outer ZP and inner ZP-oocyte) is significantly stiffer than suitable MII.

**Tab. IS:** Total number of oocytes retrieved by the patients involved in this work and fertilization steps.

<b>Patient</b>	<b>MI</b>	<b>MII suitable</b>	<b>Oocytes degenerated</b>	<b>Inseminated</b>	<b>Total Embryos</b>	<b>Transferred Embryos</b>
1	2	19	0	10	6	0
2	1	6	0	6	4	2
3	0	18	0	10	9	1
4	2	5	0	4	2	2
5	1	3	0	3	3	2
6	3	3	0	3	1	1
7	3	8	1	8	2	2
8	1	6	0	4	2	2
9	1	15	0	6	5	0
10	1	12	0	9	8	1
11	0	11	0	6	1	0
12	0	8	0	5	1	0
13	1	13	0	7	5	2
14	4	12	0	6	5	0

**Tab. IIS:** Maturation stage and quality of the oocytes donated by the patients (small part of the total number of the oocytes reported in Tab. IS) in relation to infertility and pregnancy.

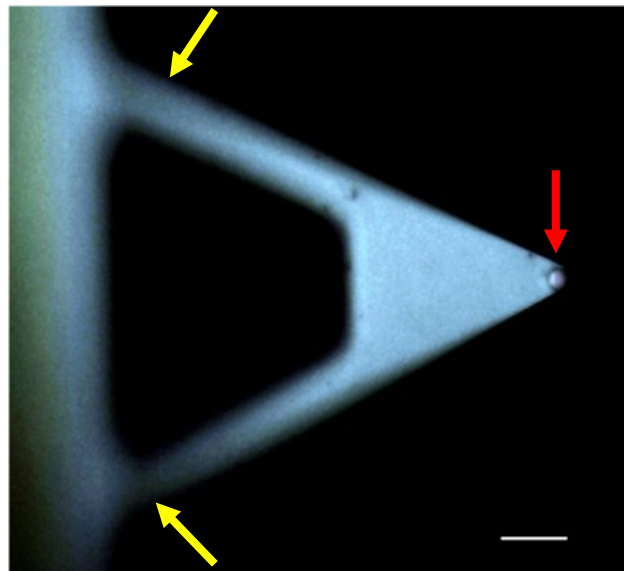
Patient	M I	M II suitable	M II rejected	♀ infertility	♂ infertility	Pregnancy
1		1			x	
2	1				x	
3		1		x	x	
4	1	3			x	x
5	1			idiopathy		
6	3				x	
7	2	1		x	x	x
8		1		x		x
9		1	1	x	x	
10	2				x	
11		3	1		x	
12		3		idiopathy		
13		2	2	x		x
14	1				x	



**Fig 3S:** All  $E1$  and  $E2$  values, as obtained by fitting procedure for all curves obtained for MI or suitable and rejected MII from a single patient are displayed as box-whisker plot.  $p$  value  $< 0.05$  is considered significant (Mann–Whitney test, two-tailed distribution). Regarding patient 4, both  $E1$  and  $E2$  of MI differ significantly from those of suitable MII ( $E1$   $p=0,0071$ ;  $E2$   $p=0,0009$ ), while for patient 7 only the  $E1$  distribution of MI differs significantly from MII ( $E1$   $p=0,0021$ ). For patient 9, the  $E1$  and  $E2$  distribution of rejected MII are significantly different from suitable MII ( $E1$   $p=0,0014$ ;  $E2$   $p=0,0118$ ), whereas for patient 11 and 13 only  $E2$  distribution of rejected MII is significantly different from that of suitable MII (patient 11 and 13  $E2$   $p<0,0001$ ).

### 3S. Calibration of cantilevers before and after attaching a microsphere

In Fig. 3S is shown an optical image of the bead mounted cantilever where the bead is generally positioned on the apex of the triangular cantilever with a negligible amount of glue (red arrow), while the mechanical response of the cantilever is mainly determined at the cantilever base (yellow arrows). To assess the influence of the bead gluing on the cantilever mechanical properties, the spring constant of two independent cantilevers was calibrated by thermal noise method before and after gluing the micrometer bead (see Tab. SIII). The measurements were reproducible within a variation of 5% , which is far below the mechanical differences observed on the oocytes.



**Fig. 4S:** Optical image of the bead glued to a tipless cantilever before measurements (bar 10  $\mu\text{m}$ ).

**Tab. IIS:** Spring contact values (K) for two cantilevers before and after gluing the micrometer bead.

	<b>K (N/m) (no bead)</b>	<b>K (N/m) (plus bead)</b>
<b>Cantilever 1</b>	0,0967	0,1034
<b>Cantilever 2</b>	0,0709	0,0667