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No sign of actin polymerization or Hippo pathway inhibition in fragmented human ovarian tissue

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RESEARCH ARTICLE

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Hippo signaling, actin polymerization, and follicle activation in fragmented human ovarian cortex

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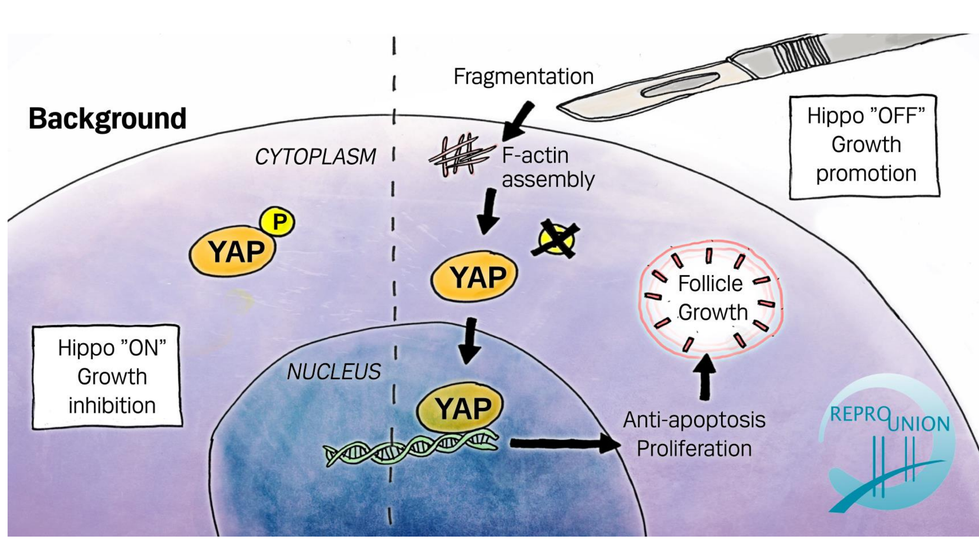
Does tissue fragmentation of human ovarian cortex result in activation of follicle growth through Hippo pathway inhibition?

- The Hippo pathway has been associated with regulation of early ovarian follicle growth in mammals including humans.
- Studies of murine ovaries suggest that changes in the actin cytoskeleton, caused by fragmentation, result in inhibition of the Hippo pathway
- In humans, *in vitro* and *in vivo* studies of fragmented ovarian tissue demonstrated upregulation of growth factors and growth of preantral follicles.
- However, the connections between fragmentation, the actin cytoskeleton and follicle activation in humans are yet to be confirmed.



Materials and methods

- Donated frozen ovarian cortex from six women (34-37 years) were thawed
- From each woman one cortex piece of 5x4x1mm was fragmented into 20 cubes of 1x1x1mm and one piece of the same size served as control tissue (**Figure 1**).
- Both exposed and control tissue were incubated for either 0, 10, 30, 60, 120 or 240 minutes prior to examination.
- Actin polymerization was examined by assessment of western blot of the ratio of F-actin to G-actin in exposed and control ovarian tissue.
- Inhibition of the Hippo pathway was examined by assessment of western blot of the ratio of phosphorylated YAP (pYAP/YAP)
- Quantification of gene expression of downstream growth factors CCN2, CCN3 and CCN5 in exposed and control ovarian tissue.
- Fragmented and control tissue were xenografted for six weeks before histological assessment of follicle growth (**Figure 2**)

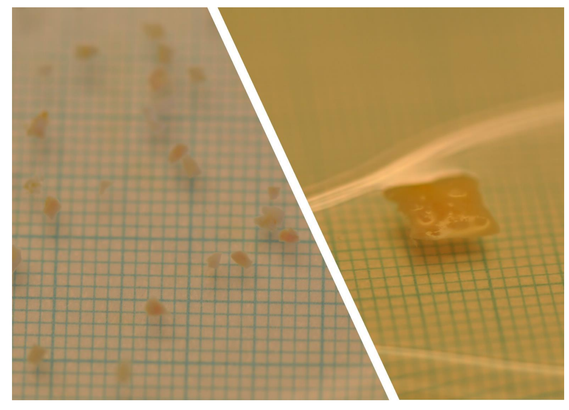


Figure 1: Fragmented and intact tissue pieces

Figure 2: Immundeficient mouse with transplanted tissue

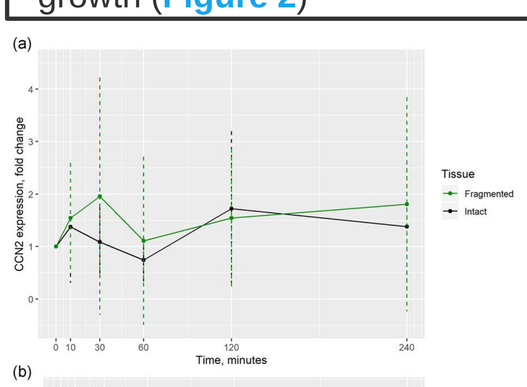


Figure 5: CCN expression

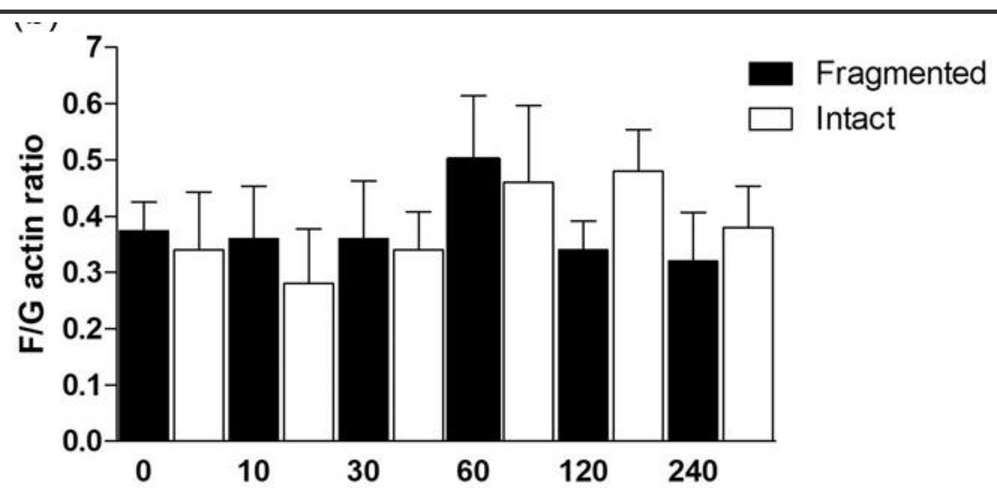


Figure 3: F/G actin ratio

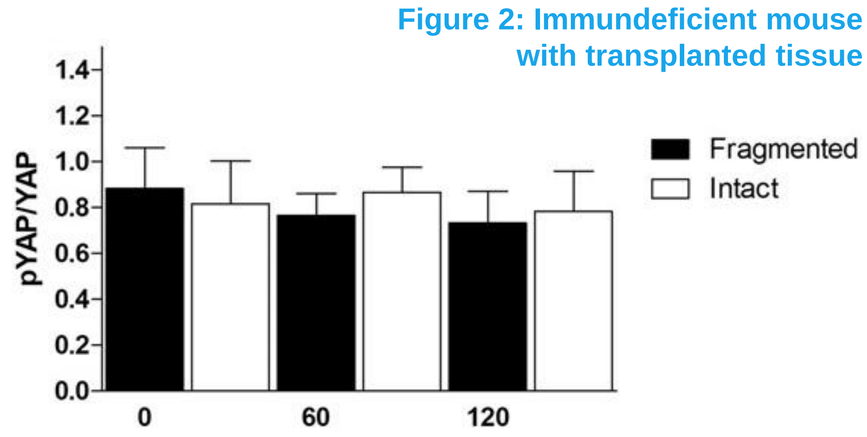


Figure 4: pYAP/YAP ratio

Table 1: Distribution of follicles from six women at different developmental stage in fragmented and intact ovarian cortical tissue after xenografting

	Number of follicles (%)									
	Fragmented ovarian tissue					Intact ovarian tissue				
Case	Primordial	Primary	Secondary	Tertiary	Total	Primordial	Primary	Secondary	Tertiary	Total
1	6 (75)	2 (25)	0 (0)	0 (0)	8	21 (60)	10 (28.6)	4 (11.4)	0 (0)	35
2	59 (68.6)	22 (25.6)	5 (5.8)	0 (0)	86	353 (76.1)	96 (20.7)	14 (3)	1 (0.2)	464
3	51 (86.4)	6 (10.2)	1 (1.7)	1 (1.7)	59	40 (50.6)	31 (39.2)	8 (10.1)	0 (0)	79
4	0 (0)	1 (100)	0 (0)	0 (0)	1	291 (74)	56 (14.2)	36 (9.2)	10 (2.5)	393
5	31 (44.3)	21 (30)	13 (18.6)	5 (7.1)	70	0 (0)	1 (100)	0 (0)	0 (0)	1
6	9 (60)	4 (26.7)	0 (0)	2 (13.3)	15	3 (100)	0 (0)	0 (0)	0 (0)	3
Total	156 (65.3)	56 (23.4)	19 (8)	8 (3.3)	239	708 (72.6)	194 (19.9)	62 (6.4)	11 (1.1)	975

Results

- Both F-actin and G-actin were detectable in all samples, but no significant difference was found in the ratio in exposed tissue compared with the control tissue for each timepoint (p=0.8). (**Figure 3**)
- Both YAP and phosphorylated YAP (pYAP) were expressed in all samples. At the three selected timepoints (0, 60 and 120 minutes) there were no difference in the ratio of YAP/pYAP in exposed and control tissue (p=0.6). (**Figure 4**)
- CCNs were expressed in all tissue samples. Fragmentation did not induce upregulation of gene expression (**Figure 5**)
- Histological assessment of the xenografted ovarian tissue revealed fewer follicles in the exposed tissue compared with the control tissue, 239 versus 975 preantral follicles, respectively. A logistic regression model accounting for paired samples showed that the proportions of growing follicles, defined as all non-primordial follicles, in the exposed tissue (34.7%) and the control tissue (27.4%) were not different (p = 0.56) (**Table 1**).