

### INTRODUCTION

The success of assisted reproductive technology programs in patients with endometriosis cysts is still very low, this is strongly associated with impaired oocyte maturity. FFEC exposure is thought to be associated with impaired oocyte maturity and the addition of ATP is expected to improve oocyte maturity. The prevalence of endometriosis which manifests clinical infertility in Asia is 15.7% and 20-60% in several hospitals in Indonesia. This experimental and experimental research aims to prove that people with endometriosis cysts have low levels of Bcl-2 and high cytochrome C. Furthermore, the addition of ATP as an energy source is likely to play a major role in oocyte maturity through the process of maturation of the cytoplasm and its nucleus. This will be proven through indicators of cyclin B1 levels and the presence of PB I.

### METHODS

The study was experimental using experimental animals with a randomized post-test only control group design. In stage I, 6 replicates of mature mice oocyte tubes as treatment group 1, 6 as treatment group 2, and 6 as a control group. In stage II, treatment group 2 in stage I became the treatment group and treatment group 1 in stage I became a control group. The level of Bcl-2, Cytochrome-C, Cyclin-B1 was determined by ELISA and Polar Body-I by an inverted microscope Nikon Eclipse T1 Series. Data analysis using SPSS version 16.0 for Windows.

### RESULT

Between the treatment group and the control group using the one way ANOVA test on the age and weight variables of mice found no significant differences in the two groups. In stage I, there was a tendency for a decrease in Bcl-2 levels in the treatment group, but not significantly different. Bcl-2 levels in the treatment groups 1, 2 and controls were  $627.83 \pm 146.42$ ,  $634.50 \pm 140.62$ , and  $678.83 \pm 152.71$ , respectively ( $p = 0.838$ ) and Cytochrome-C levels in treatment group 1, 2 and controls were  $314.75 \pm 228.50$ ,  $3104.45 \pm 211.29$ , and  $2738.28 \pm 227.45$ , respectively ( $p = 0.021$ ). In stage II, the study was found that the addition of 1 mM ATP can cause cytoplasmic oocyte maturation of mice after exposure to CFKE. Cyclin-B1 levels in the treatment and control groups were  $238.96 \pm 12.84$  vs.  $173.79 \pm 21.24$ , respectively ( $p = 0.029$ ). Polar Body I in the treatment and control groups were  $30.33 \pm 3.33$  vs.  $24.50 \pm 4.37$ , respectively ( $p = 0.026$ ).

### CONCLUSION

The results of this study are the effect of FFEC exposure on decreased oocyte maturation is through mitochondrial apoptosis characterized by high levels of cytochrome C. The effect of the addition of ATP in vitro on oocytes exposed to CFKE has been shown to increase oocyte maturation, characterized by high levels of cyclin B1 and the presence of Polar Body I.

Table 1. Phase I Research: Levels of Bcl-2 and Cytochrome C

Group	n	Bcl-2 Levels	SB	F	p
Treatment 1	6	627,83	146,42	0,18	0,838
Treatment 2	6	634,50	140,62		
Controls	6	678,83	152,71		
Group	n	Cytochrome C Levels	SB	F	p
Treatment 1	6	3147,75	228,50	5,07	0,021
Treatment 2	6	3104,45	211,29		
Controls	6	2738,28	227,45		

Table 2. Phase I Research: Levels of Cyclin B1 and Polar Body I

Group	n	Cyclin level B1	SB	t	P
Treatment	6	238,96	12,84	2,55	0,029
Controls	6	173,79	21,24		
Group	n	Polar Body I Levels	SB	t	P
Treatment	6	30,33	3,33	2,60	0,026
Controls	6	24,50	4,37		

### REFERENCES

- Huniadi CA, Pop OL, Antal TA, Stamatian F. The effects of ulipristal on Bax/Bcl-2, cytochrome c, Ki-67 and cyclooxygenase-2 expression in a rat model with surgically induced endometriosis. *Eur J Obstet Gynecol Reprod Biol.* 2013 Jul;169(2):360-5. doi: 10.1016/j.ejogrb.2013.03.022. Epub 2013 Apr 22. PMID: 23619346.
- Banu SK, Lee J, Speights VO Jr, Starzinski-Powitz A, Arosh JA. Selective inhibition of prostaglandin E2 receptors EP2 and EP4 induces apoptosis of human endometrial cells through suppression of ERK1/2, AKT, NFkappaB, and beta-catenin pathways and activation of intrinsic apoptotic mechanisms. *Mol Endocrinol.* 2009 Aug;23(8):1291-305. doi: 10.1210/me.2009-0017. Epub 2009 Apr 30. PMID: 19407222; PMCID: PMC5419188.
- Soysal D, Kızıldağ S, Saatlı B, Posacı C, Soysal S, Koyuncuoğlu M, Doğan Ö. A novel angiogenesis inhibitor bevacizumab induces apoptosis in the rat endometriosis model. *Balkan J Med Genet.* 2015 Apr 10;17(2):73-80. doi: 10.2478/bjmg-2014-0077. PMID: 25937801; PMCID: PMC4413445.
- Banu SK, Stanley JA, Lee J, Stephen SD, Arosh JA, Hoyer PB, Burghardt RC. Hexavalent chromium-induced apoptosis of granulosa cells involves selective sub-cellular translocation of Bcl-2 members, ERK1/2 and p53. *Toxicol Appl Pharmacol.* 2011;251:253-266.
- Collins BJ, Stout MD, Levine KE, Kissling GE, Melnick RL, Fennell TR, Walden R, Abdo K, Pritchard JB, Fernando RA, Burka LT, Hooth MJ. Exposure to hexavalent chromium resulted in significantly higher tissue chromium burden compared with trivalent chromium following similar oral doses to male F344/N rats and female B6C3F1 mice. *Toxicol Sci.* 2010;118:368-379.
- Felici MD, Carlo AD, Pesce M, Iona S, Farrace MG, Piacentini M. Bcl-2 and Bax regulation of apoptosis in germ cells during prenatal oogenesis in the mouse embryo. *Cell Death Differ.* 1999;6:908-915.
- Cecconi S, Rossi G, Santilli A, Stefano LD, Hoshino Y, Sato E, Palmerini MG, Macchiarelli G. Akt expression in mouse oocytes matured in vivo and in vitro. *Reprod Biomed Online.* 2010;20:35-41.

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