THE ROLE OF AUTOPHAGY ON EMBRYONIC DEVELOPMENT AT BLASTOCYST STAGE

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Study question:

- Does autophagy genes and proteins expression is deifferent between preimplantation embryos that developed to blastocyst and those which are arrested on day 5?

Summary answer:

- From all autophagy genes and proteins, Beclin 1 and LC3 expression is important for embryonic development to blastocyst on day 5.

What is known already:

- Autophagy is one of the molecular mechanisms that is important for the early embryonic development.
- It is a massive cytoplasmic degradation pathway mediated by the lysosome.
- Autophagy provides a"recycling system", As it engulfs unnecessary macromolecules proteins and organelles, degraded them to amino acids afree fatty acids which are important for the neo-synthesis of another proteins important for embryo development, It was known that autophagy is highly activated immediately after fertilization, and also found that fertilized embryos lack autophagy can not implant.

Study design, size, duration:

- Our study is a prospective study, 60 embryos were derived from oocytes aspirated from twelve patients undergoing ICSI between September 2019 to November 2019 at Madina fertility center.
- Embryos were devided on day 5 in to two groups according to its developmental stage and its ability to form blastocyst.

Participants/materials, setting, methods:

- Oocytes aspirated from twelve patients were injected with sperm by conventional ICSI, the injected oocytes were incubated till day 5.
- Embryos on day 5 were scored and devided in to two groups according to its developmental stage and its ability to form blastocyst:

Group A → Developed embryos (blastocyst formation)

Group B → Arrested embryos (No blastocyst formation) then biopsied to evaluate expression of autophagic genes (ELISA) and proteins (RT-PCR): LC3, PI3K, E2F, mTOR

Main results and the role of chance:

- Developed embryos to blastocyst stage on day 5 (Group A) shows significantly higher LC3 relative gene expression (1.12±0.51), Beclin 1 relative gene expression (1.43±0.33) and Beclin protein expression (3.8±0.028) than their expression in embryos that failed to form blastocyst on day 5 (Group B) (0.72±0.17,P=0.03), (0.35±0.12,P=0.0001),and (3.14±0.05, P=0.0001), respectively.
- While mTOR and PIK3C3 proteins expression were significantly higher in Group B (arrested embryos) than their expression within developed embryos (Group A) , P=0.007 and P= 0.0001 ,respectively. As well as the expression of E2F gene which is significantly lower within group A embryos (0.32±0.07) and remarkedly higher within group B embryos (4.38±1.16), P=0.0001.

	Developed (Group A)	Arrested (group B)	P
mTOR (ng)	1.13±0.053	1.23±0.09**	0.007
Beclin (ng)	3.8±0.028	3.14±0.05***	0.0001
PIK3C3 (ng)	18.6±1.4	24.5±1.5***	0.0001
LC3 relative gene expression fold	1.12±0.51	0.72±0.17*	0.03
Beclin 1 relative gene expression fold	1.43±0.33	0.35±0.12***	0.0001
E2F relative gene expression fold	0.32±0.07	4.38±1.16***	0.0001

Limitations, reasons for caution:

- Larger sample size is needed to support our results.

Wider implications of the findings:

- Investigation of autophagy function (specially evaluation of expression of Beclin 1 and LC3 genes and proteins) in embryos at late developmental stage introduce a new diagnostic tools for embryos with impaired development and those with impaired implantation potential.

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- I have no potential conflict of interest to disclose