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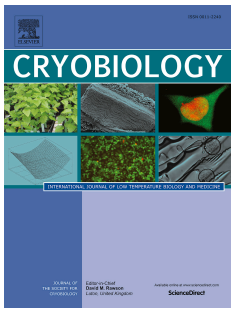

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Development of molecular markers for zebrafish (*Danio rerio*) ovarian follicle growth assessment following *in-vitro* culture in cryopreservation studies

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Abstract

Development of *in vitro* culture protocol for early stage ovarian follicles of zebrafish is important since cryopreserved early stage ovarian follicles would need to be matured *in vitro* following cryopreservation before they can be fertilised. Development of molecular markers for zebrafish (*Danio rerio*) ovarian follicle growth assessment following *in vitro* culture of early stage zebrafish ovarian follicles in ovarian tissue fragments is reported here for the first time although some work has been reported for *in vitro* culture of isolated early stage zebrafish ovarian follicles. The main aim of the present study was to develop molecular markers in an optimised *in vitro* culture protocol for stage I and stage II zebrafish ovarian follicles in ovarian tissue fragments. The effect of concentration of the hormones human chorionic gonadotropin and follicle stimulating hormones, and additives such as Foetal Bovine Serum and Bovine Serum Albumin were studied. The results showed that early stage zebrafish ovarian fragments containing stage I and stage II follicles which are cultured *in vitro* for 24 h in 20% FBS and 100mIU/ml FSH in 90% L-15 medium at 28°C can grow to the size of stage II and stage III ovarian follicles respectively. More importantly the follicle growth from stage I to stage II and from stage II to stage III were confirmed using molecular markers such as *cyp19a1a* (also known as *P450aromA*) and *vtg1* genes respectively. However, no follicle growth was observed following cryopreservation and *in vitro* culture.

Keywords: Zebrafish, ovarian follicle, *in-vitro* maturation, tissue fragments, molecular marker