

The effects of rFSH and Testosterone on in vitro maturation of mouse round spermatid in co-culture with Vero cells

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Abstract

Co-culture systems have important roles in maintenance of spermatogenic cells and process of spermatogenesis. These systems are used for in vitro maturation of germ cells and overcome on differentiation arrest of spermatogenic cells. FSH and Testosterone hormones are important in initiation and maintenance of spermatogenesis. Lack of these hormones causes spermatogenesis deficiency in vivo. In this study, the effects of both co-culture system with Vero cell and co-culture supplemented with rFSH and Testosterone were determined on maturation of round spermatids. Cell suspension was isolated from testis of NMRI male mice (8-12 weeks old) and divided into three parts. Suspensions in three groups include: control (culture on DMEM with 10% FBS), experimental 1 (culture on monolayer of Vero cell) and experimental 2 (culture on monolayer of Vero cell supplemented with rFSH and Testosterone), were cultured for 96-hours. The numbers of round, elongating and elongated spermatids, before and after of culture were recorded for 96-hr using light microscope. Survival rates of all kind of spermatids were evaluated using trypan blue test and the results of each group were compared statistically by repeated measure ANOVA test. The results of this study showed that in co-culture system on the first 24-hr, the number of round spermatid cells were reduced but elongating spermatid cells increased significantly ($P < 0.0001$). In co-culture system supplemented with rFSH and Testosterone, the numbers of elongated and elongating spermatid cells increased significantly after 24 and 48 hours respectively ($P < 0.001$) but after that the number of all kind of spermatid cells were reduced. Viability rates of all kinds of spermatid cells reduced during 96-hours culture and there were no significant differences. In conclusion, the results of this study confirms that round spermatid cells by using of co-culture system with and without hormones can progress into elongating over a short period (maximum 48 hours). Vero cell culture supplemented with rFSH and Testosterone can support in vitro maturation and viability of spermatogenic cells better than Vero cells without hormones.

Key Words: Infertility, Spermatogenesis arrest, Spermatid, Co-culture, In-vitro maturation, rFSH, and Testosterone.

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