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EFFECT OF VARYING CONCENTRATIONS OF RETINOIC ACID ON IN VITRO DIFFERENTIATION OF SHEEP SPERMATOGONIAL STEM CELLS

Being the precursor cells of spermatozoa, spermatogonial stem cells (SSC) are the only adult stem cell capable of transmitting genetic material to the next generation. They self-renew and differentiate into mature sperm through a process called spermatogenesis. The fate of SSC to self-renew or differentiate determine the foundation of normal spermatogenesis and fertility status of a male. One of the major differentiating signals for SSC arise from retinoic acid (RA), a vitamin A metabolite. Although it has been known that retinoids are essential for male fertility, the molecular and cellular events associated with RA remain largely undefined especially in livestock species. The objective of this study was to optimize the concentration of retinoic acid in inducing differentiation of sheep SSC in vitro. For this purpose, SSC were harvested from immature sheep testis (n=6) by two step enzymatic digestion followed by double enrichment in lectin and Geltrex (1%) coated plates. The purified SSC were cultured in differentiating media with 5% knockout serum replacement and varying concentrations of RA (0, 1, 5, 10, and 15µM). The same media without retinoic acid was taken as a control. After 24 hr and 48 hr of RA treatment, the cultured cells were trypsinized and subjected to toxicity assay, stemness (ALP) and metabolic activities (MTT) were compared among the groups. Gene expression studies were performed after 24 hr, 48 hr, 6th and 12th day. The viability percentage of the SSC in all the groups was comparable, with no toxicity for all RA doses. After 48hr of RA treatment there was significant ($p<0.05$) decrease in stemness activity (0.15±0.01 Vs 0.23±0.01 OD units) when treated with 15µM RA as compared to control group. Similarly, there was a significant increase in metabolic activity when treated with 15µM RA (1.3±0.04 Vs 1.1±0.02 OD units) as compared to control group. However, after 24 hrs, there were no significant difference either in stemness or metabolic activities among the groups. By the 12th day of RA treatment there was a significant ($p<0.05$) increase in CKIT expression (0.39±0.07 Vs 0.10±0.01 fold change). The study reveals that RA at 15µM concentration in SSC differentiating media improves metabolic activity and promote differentiation. These findings suggest 15µM RA can promote sheep SSC differentiation in vitro.

Author: A.T, Tomcy (DBT Category I Fellow (Ph.D Scholar))

Co-authors: Dr BALAKRISHNAN, Binsila (Senior Scientist); Dr B, Krishnappa; Dr N, Ramachandran; Ms E, Shiyamala; Mr M, Sadikh; Dr V, Aranganathan; Dr P KOLTE, Atul; Dr S, Selvaraju

Presenter: A.T, Tomcy (DBT Category I Fellow (Ph.D Scholar))

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