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In-vitro transfection efficiency of Sheep Spermatogonial Stem cells using Lipofectamine Reagents

Spermatogonial stem cells (SSCs) are the foundational cells of the male reproductive system, responsible for the lifelong production of sperm. As the progenitors of spermatozoa, SSCs hold significant potential for germline genetic modification, wherein any alterations introduced into these cells can be stably transmitted to the offspring. This makes SSCs a powerful tool for the direct incorporation of desirable traits such as enhanced growth, fertility, or disease resistance. To harness this potential effectively, it is essential to evaluate transfection efficiency in SSCs. The objective of the study is to optimize the enhanced green fluorescent protein (eGFP) gene transfection efficiency into sheep SSC using the liposomal carriers

SSCs were harvested from prepubertal sheep testis (n=6) by two-step enzymatic digestion and purified by double enrichment through differential plating. The purified SSC were proliferated and trypsinized for transfection with plasmid DNA (pPy-CAG-GFP-IRES-Pac). The transfection was done using Lipofectamine™ 3000 reagent.

Transfection efficiency was evaluated 72 hours post-transfection using varying concentrations (3.75 Vs 7.50 µl) of LipofectamineTM 3000. Additionally, the effect of media change at 24 and 48 hrs hours post-transfection was assessed. Following this initial optimization, different concentrations of puromycin (0.75,1.00,1.25 and 1.50 µg/ml) were tested to determine the optimal dose for effective selection of transfected cells. The identification of transfection was monitored by green coloured SSC colony through fluorescent microscopy, and eGFP quantification were carried out using flow cytometry. Lipofectamine at 7.5 µl for 48 hrs induces transfection efficiency of 21% in SSCs. Puromycin @ 1µg/ml enriched the eGFP positive SSCs to 29%.

Lipofectamine @ 7.5 µl for 48 hrs along with puromycin selection effectively induces 29% transfection in Spermatogonial stem cells

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