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Growth factors and culture media dependent *in vitro* expansion and characteristics of enriched spermatogonial stem cells derived from adult caprine testis

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In vitro expansion of spermatogonial stem cells (SSCs) has gained significant attention, as it offers a promising alternative for preserving and utilizing these cells beyond their natural regenerative capacity. Present study aimed to investigate the effect of supplementation of growth factors in culture media and its comparison with commercially available media for *in vitro* expansion and maintenance of culture characteristics of adult caprine SSCs (cSSCs). In Trial-1, cSSCs were isolated from adult goats and an enriched population of cSSC was randomly divided into 5 groups i.e., group-1 (control; no growth factor), group-2 (GDNF; 40 ng/mL), group-3 (FGF2; 10 ng/mL), group-4 (EGF; 5 ng/mL) and group-5 (GDNF+FGF2+EGF). Following cultivation of cSSCs, morphological assessment, colony counting, and expression of alkaline phosphatase (ALP) and PGP9.5 were conducted and results were compared among the groups. Further, in Trial-2, the performance of optimized cSSCs culture media (in-house media) was compared with 5 commercial media viz, α-MEM, MesenPRO RS™, StemPRO®-34, Stemline®, and Ham's F-12 Nutrient Mix for improved growth and culture characteristics of cSSCs. The cluster-forming activity (CFA) assay, ALP staining, morphological evaluation of cSSCs colonies, and expression analyses of marker genes were performed. In Trial-1, the total number of colonies, size of colonies, and ALP expression were significantly (P<0.05) higher in group-5 compared with other groups. In Trial 2, the in-house media produced significantly (P<0.05) higher number and larger cSSC colonies among all the media tested. Similar results were observed in CFA and ALP staining. The results of expression analyses demonstrate upregulation of pluripotency (PGP9.5 and PLZF) and adhesion (E-cadherin) marker genes, and downregulation of apoptosis marker gene (BCL-6) in the cells when grown in in-house media. Overall, our results demonstrate that in-house media, with a combination of growth factors, provides a more favorable niche for proliferation, colony formation, and maintenance of functional characteristics of adult cSSCs in the *in vitro* culture systems. These results can be utilized for future studies and application that require optimum expansion of cSSCs or other stem cells.

Keywords: Spermatogonial stem cells, Testis, Growth factors, Culture media, Proliferation, Adult goat