Riaz Shah et al., J Vet Sci Technol 2017, 8:4(Suppl)
DOI: 10.4172/2157-7579-C1-024

Production of pashmina (cashmere) goat through handmade cloning technique using continuous culture system

Riaz Shah, Abrar Malik, Maajid Bhat, Firdous Khan, Syed Hilal, Mujeeb Fazili, Mudasir Andrabi, Nadeem Shabir and Nazir Ganai
SKUAST Kashmir, India

Statement of the Problem: Pashmina (cashmere) goat is among the few species of livestock which can survive in their inhabitant harsh cold arid climate. This goat produces world's finest fibre. Due to high risk of genetic loss via inbreeding, reproductive technologies need to be implemented. Cloning is the fastest way to multiply the limited superior germplasm. Handmade cloning (HMC) is an alternative method of cloning which eliminates the use of costly sophisticated micromanipulator tools demanding greater degree of skill, in comparison to SCNT. The present study was designed to optimize *in vitro* continuous culture system for development of zona free handmade cloned pashmina goat blastocysts and they're *in-vivo* development after laparoscope aided intra uterine transfer into synchronized recipients.

Methodology & Theoretical Orientation: Skin derived fibroblasts at 5th to 9th passage was used as nucleus donor cells for HMC experiments. Cumulus–oocyte complexes (COCs) were *in vitro* matured and stripped of their cumulus investment and zona pellucida. Protrusion cone-guided bisection was performed for enucleation. Electro-fusion was carried out to generate triplets (two demicytoplasts and a donor cell). The reconstructed zygotes were then activated and cultured in different experimental groups wherein we compared different culture media and culture systems. The blastocysts were transferred into synchronized recipient goats by laparoscope aided transfer technique. Pregnancies were diagnosed through USG after 45 days of transfer. Identification and confirmation of the clone born was performed via microsatellite marker analysis.

Findings: The cleavage and blastocyst rates were determined at day 7 of embryo culture. G1. G2 medium gave the best cleavage percentage (86.84±2.26) while as RVCL (commercial medium from Cook™, Australia) gave the best blastocyst percentage (15.01±4.58). WID (well in drop) culture system was found to be most efficient with highest cleavage and blastocyst percentages i.e., 84.34±4.15 and 21.65±1.69 respectively. On day 45 post embryo transfer into 19 receipients, 3 pregnancies were detected out of which only one carried to term.

Conclusion & Significance: Using cost effective HMC technique, we successfully report the live birth of first handmade cloned cashmere goat. The birth weight of the cloned kid was 2.4 kg, like female kids from naturally bred Pashmina goats during the same period. No significant differences in growth rate between cloned goat and naturally bred goats (1.2 kg/month) were observed till it reached sexual maturity. Also, estrous cycle of the cloned goat was observed to be normal and bred normally. In our study, the embryos were cultured in a serum free media which could explain the normal birth weight of the cloned kid. Microsatellite analysis confirmed that the cloned kid was genetically identical to the fibroblast cell donor dam. In conclusion, this study elucidated the production of hand-made cloned blastocysts using a continuous culture system and birth of healthy cloned kid from Pashmina goat

Biography

Riaz Shah has completed his Graduation in Veterinary Sciences and Master's/PhD degree in Animal Biotechnology with specialization in Livestock Reproductive Biotechnology and Post-doctoral experience from AgResearch Ltd., Ruakura Research Centre, Hamilton, Newzealand in area of goat cloning. His current areas of research are the Application of Advanced Reproductive Technologies like IVF, SCNT and Stem Cell Production for augmenting production of Livestock. The recent successes in cloning of livestock (Buffalo and Pashmina goat) first time in India through Handmade Cloning technique during his research endeavors at National Dairy Research Institute, Haryana and SKUAST-K, Kashmir, India, has opened opportunities to achieve excellence in the application of such techniques for transgenic animal production and stem cell research. He is currently working as a Professor and Head, Division of Animal Biotechnology at Faculty of Veterinary Sciences in SK Agricultural University, Srinagar, J&K, India.

Notes:

drriazshah@gmail.com