The Impact of Two Embryo Culture Media, Synthetic Oviduct Fluid and Commercial BO, on pre- and post-Implantation Development of Cloned SAANEN Goat Embryos

Mehdi Hajian, Ph.D.^{1*}, Farnoosh Jafarpour, Ph.D.¹, Sayed Morteza Aghamiri, Ph.D.², Shiva Rouhollahi Varnosfaderani, M.Sc.¹, Mohsen Rahimi Andani, M.Sc.¹, Mohammad Hossein Nasr-Esfahani, Ph.D.^{1*}

1. Department of Animal Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran

2. Department of Clinical Studies, School of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran

Gene	Relevant information	Gene ontology (GO)	Developmental signaling pathway	Phenotype -/-	Ref.
LIFR 1	LIFR also known as the leukemia inhibitory factor is a poly functional cytokine that affects the differentiation, survival, and proliferation of a wide va- riety of cells in the adult and the embryo and also has been shown to interact with Glycoprotein 130	Gene encodes a protein that belongs to the type I cytokine receptor family	JAK- STAT	Homozygotes for targeted null mutations die as neonates with reduced numbers of facial and spi- nal motor neurons, neurons of the nucleus ambiguus, and astrocytes. Mutants also show impaired pla- centation, severe osteopenia, and low hepatic glycogen stores.	(1)
BMPR1	Bone morphogenetic protein type I receptors are single pass, type I transmembrane proteins. They belong to a class of receptor serine/threonine kinases that bind members of the TGF beta superfamily of ligands-the BMPs. BMPR1A null mice died at embyonic day 8.0 without mesoderm specification, demonstrating its vital role in gastrula- tion, it has been shown that it plays a role in cell differentiation	Combining with a transforming growth factor beta (TGF β) and transmitting the signal from one side of the membrane to the other	TGF-β	Homozygous null mutants die by embryonic day 9.5, are smaller than normal, and form no mesoderm; a conditional knockout resulted in gross malformations of the limbs with complete agenesis of the hindlimb.	(2)
SMAD5	SMAD5 belongs to the SMAD, re- pressed the gene, decapentaplegic, in the embryo. SMAD proteins are signal transducers and transcriptional modu- lators that mediate multiple signaling pathways. This protein mediates the signals of the bone morphogenetic proteins (BMPs), which are involved in a range of biological activities including cell growth, apoptosis, morphogenesis, development and immune responses.	Transcription regula- tion	TGF-β	Homozygotes for targeted null mu- tations exhibit impaired allantois formation resulting in the lack of a placenta, and die around embry- onic day 9-10.	(3)
CTNNB	Catenin beta 1, also called beta-catenin (or β -catenin), is a dual function protein, regulating the coordination of cell–cell adhesion and gene transcription	Cell proliferation, cell fate specification	Wnt	Homozygous null embryos show anterior-posterior axis formation anomalies, but develop to E7	(4)
AKT	AKT is one of 3 closely related serine/ threonine-protein kinases (AKT1, AKT2 and AKT3) called the AKT kinase, and which regulate many processes including metabolism, proliferation, cell survival, growth and angiogenesis	Positive regulation of fat cell differentiation	FGF	Mutant homozygotes are smaller than sibs due to retarded prenatal and postnatal growth and exhibit increased apoptosis and decreased lifespan with genotoxic stress. Mice are fertile, but males have attenuated spermatogenesis and abnormal testes.	(5)

Table S1: Profile of expressed genes, main functions, GOs and null alleles in mouse embryos

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*Correspondence Address: P.O.Box: 8159358686, Department of Animal Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran

Email: mehdihajian2002@gmail.com, mh.nasr-esfahani@royaninstitute.org



Table S1: Continued

Gene	Relevant information	Gene ontology (GO)	Developmental signaling pathway	Phenotype -/-	Ref.
OCT4	Transcription factor that binds to the oc- tamer motif (5'-ATTTGCAT-3'). Forms a trimeric complex with SOX2 on DNA and controls the expression of a number of genes involved in embryonic develop- ment. Critical for early embryogenesis and for embryonic stem cell pluripotency	Blastocyst develop- ment., cell fate com- mitment involved in formation of primary germ layer	Pluripotent	Homozygosity for a targeted null mutation results in peri-implan- tation lethality prior to the egg cylinder stage	(6,7)
FZD	receptors in the Wnt signaling pathway and other signaling pathways. When activated, Frizzled leads to activation of Dishevelled in the cytosol. play key roles in governing cell polarity, embry- onic development, formation of neural synapses, cell proliferation, and many other processes in developing and adult organisms.	Canonical Wnt sign- aling pathway	Wnt	Mice homozygous for disruption of this gene die as embryos. Extra embryonic vascular development is abnormal.	(8)
SOX2	SRY (sex determining region Y)-box 2, also known as SOX2, is a transcription factor that is essential for maintain- ing self-renewal, or pluripotency, of undifferentiated embryonic stem cells. and have been shown to play key roles in many stages of mammalian develop- ment.	Cell cycle arrest, chromatin organiza- tion	Pluripotent	Homozygotes for targeted null mu- tations implant but fail to develop an egg cylinder or epiblast, and die shortly thereafter. Other mutations that affect only regulatory ele- ments show circling behavior and deafness, inner ear defects, and a yellow coat color.	(9)
FGFR4	tyrosine-protein kinase that acts as cell- surface receptor for fibroblast growth factors and plays a role in the regulation of cell proliferation, differentiation and migration, and in regulation of lipid metabolism, bile acid biosynthesis, glu- cose uptake, vitamin D metabolism and phosphate homeostasis	Cell migration, posi- tive regulation of cell proliferation	FGF	Homozygotes for a targeted muta- tion are viable, healthy and overtly normal, except for a 10% weight reduction at weaning. Mice doubly homozygous for disruptions of Fgfr3 and Fgfr4 show novel phe- notypes not seen in either single mutant, including dwarfism and defective respiratory alveogenesis.	(10)
ERK1	Mitogen-activated protein kinase 3(MAPK3) or ERK1 act in a signaling cascade that regulates various cellular processes such as proliferation, dif- ferentiation, and cell cycle progression in response to a variety of extracellular signals.	DNA damage induced protein phosphorylation, cell cycle, organ morpho- genesis	FGF	Mice homozygous for a targeted null mutation are hyperactive with impaired T cell maturation and proliferation. Mice homozy- gous for a knock-out allele on a CD-1 background exhibit normal Mendelian ratios, growth, and no obvious abnormalities.	(11)
CDX2	Involved in TE differentiation. Also involved in the transcriptional regula- tion of multiple genes expressed in the intestinal epithelium. Important in broad range of functions from early differen- tiation to maintenance of the intestinal epithelial lining of both the small and large intestine	Anterior/posterior pattern specification, positive regulation of cell differentiation, positive regulation of cell proliferation	Trophectodermal	Homozygotes for targeted null mutations die prior to gastrula- tion. Heterozygotes exhibit tail abnormalities, stunted growth, defects of the vertebrae and ribs, and multiple intestinal adenoma- tous polyps.	(1)
CDC25	regulation of CDK activity regulation of cyclin-dependent protein kinase activity Any process that modulates the frequen- cy, rate or extent of cyclin-dependent protein serine/threonine kinase activity	Biological_process	Cell cycle and proliferation	Mice homozygous for a targeted null mutation exhibit no discern- able phenotype; mice are viable and fertile with normal T and B lymphocyte development and proliferative responses.	(12)
GCN5	Catalysis of the transfer of an N-acetyl- glucosaminyl residue from UDP-N- acetyl-glucosamine to a sugar. positive regulation of AKT, PKB signalling cascade. up regulation of protein kinase B signaling cascade	Molecular_function biological_process	Histone acetyl- transferase	Homozygotes for targeted null mutations exhibit poorly devel- oped yolk sac blood vessels, retarded growth, absence of dorsal mesoderm lineages, failure to form somites, and lethality between embryonic days 9.5-11.5.	(13)

Table	S1:	Continued
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Gene	Relevant information	Gene ontology (GO)	Developmental signaling pathway	Phenotype -/-	Ref.
PCAF	cellular response to insulin stimulus negative regulation of cell proliferation regulation of protein ADP-ribosylation actomyosin histone deacetylase binding protein kinase binding negative regula- tion of cyclin-dependent protein serine/ threonine kinase activity transcription coactivator activity positive regulation of gluconeogenesis N-terminal peptidyl-ly- sine acetylation protein complex binding lysine N-acetyltransferase activity, acting on acetyl phosphate as donor positive regulation of transcription from RNA polymerase II promoter cyclin-dependent protein serine/threonine kinase inhibitor activity kinetochore Ada2/Gcn5/Ada3 transcription activator complex A band histone acetyltransferase activity histone H3-K9 acetylation I band	falz-related bromo- domain-containing proteins pthr22880	Histone acetyl- transferase	Mice homozygous for a null allele exhibit no abrnomal phenotype.	(13)
FoxD3	The initial formation of a blastocyst from a solid ball of cells known as a morula	Biological_process	Pluripotent	Mice homozygous for null alleles dsiplay embryonic lethality with failure of primitive streak forma- tion and gastrulation and failure to derive cultures of embryonic or trophoblast stem cells.	(14)
IFNT	Functions to control the survival, growth, differentiation and effector func- tion of tissues and cell	Molecular_function autocrine activity paracrine activity	Trophectodermal	Mice homozygous for a knock-out allele exhibit increased suscep- tibility to HSV-2 and Chlamydia infection.	(15)

Table S2: Retrospective and prospective results of in vivo developmental competence of SCNT goat embryos developed in SOF and BO culture media

Group	Transferred embryo	Recipient	Established preg- nancy (%)	Loss of pregnancy (%)	Full term pregnancy (%)	Live birth	SCNT efficiency (%)
SOF	96	24	10 (41.66)	5 (50)	5/10 (50)	6	6/96 (6.25)
BO	100	25	11 (44)	4 (36.36)	7/11 (63.63)	8	8/100 (8)

SCNT; Somatic cell nuclear transfer, SOF; Synthetic oviductal fluid, BO; Bracket-Oliphant IVC medium, and IVC; In vitro culture

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