



Expression profile of H19 and Peg1 among diploid parthenogenetic, female sexed IVF and *in vivo* derived embryos during pre-implantation development in goat

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Parthenogenesis was induced in various species for the study of developmental ability of embryos and produced first parthenogenetic mice 'Kaguya' (Kono *et al.* 2004). This has opened up the hope of making parthenogenesis a successful method of cloning in domestic animals. Parthenogenesis does not occur in goats naturally, but artificially parthenogenetic embryo can be produced by various methods. The *in vitro* developmental potency of parthenogenetic embryos to different stages was done in goat (Pankaj *et al.* 2012, Ranjan *et al.* 2013a, Ranjan *et al.* 2013b). Further, the *in vivo* development potency of these embryos was also studied following transfer in goat and showed non return, up to 60–70 days (Ranjan *et al.* 2013c). The preceding information underlines the failure of parthenogenesis in goat to live birth. The major reasons are absence of paternal genes, which are required for normal embryonic development.

So, the present research work was proposed to study the comparative expression profile of paternally imprinted gene (H19) and maternally imprinted gene (Peg1) among diploid parthenogenetic (DIP), female sexed IVF and *in vivo* derived embryos in caprine.

Ethics: All the experiments were approved by ethics committee of the Indian Veterinary Research Institute, Izatnagar, India.

Embryos production and culture of embryonic cells isolated from early stage embryos: *In vitro* embryo production was done as per Ranjan *et al.* (2013a). For parthenogenetic diploid (DIP) embryo development, isolated oocytes were cultured in maturation media supplemented with 15µg/ml of CCB (Ranjan *et al.* 2013a). Surgical embryo collection was done from 10 goats as per Agrawal *et al.* (1982).

The DIP, IVF and *in vivo* derived embryos of 8–16 cells

and morula were zona lysed with proteinase-K (0.02%) and cultured in wells on mitomycin C inactivated goat fetal fibroblast monolayer. When the clumped blastomere of individual embryos were divided and made a colony, these cells were eluted by 0.25% trypsin EDTA solution.

Karyotyping of parthenogenetic embryo and sexing of embryonic cell colony: Karyotyping was done as per Ranjan *et al.* (2013a). The colonies of DIP embryos of cultures were used for gene expression studies. Colonies developed from individual IVF and *in vivo* derived embryos of 8–16 and morula stage embryos were sexed by *Sry* gene expression by real time PCR using genomic DNA (Ranjan *et al.* 2013b).

RNA extraction and reverse transcription: The total RNA was isolated from embryonic cell colony generated from 8–16 and morula stages of DIP (36 and 37), IVF (20 and 22) and *in vivo* (6 and 5) derived female sexed embryonic cell colonies by RNA kit cDNA was synthesized using

Table 1. Primers used for real-time PCR

Primer	Sequence (10pmol/µl)	Annealing temp (°C)	Product size (bp)
α-actin	F 5'TCATCACCATCGG CAATG3'	60	286
	R 5'CCAATCCACACGG AGTAC3'		
Gapdh	F 5'GGAGAA ACC TGC CAAGTATG3'	60	126
	R 5'TGAGTGTCTGCTGT TGAAGTC3'		
H19	F 5'AGCCAGGCATTCA TCCCGGT3'	60	134
	R 5'AGCGCTGTCTTCA CCTGC3'		
Peg1	F 5'CCGACCTTCTGAG AGTGAG3'	60	277
	R 5'CTGTGGATAGTGG CTAATGTG3'		
Sry	F 5'TGAACGAAGACGA AAGGTGGCTCT3'	60	342
	R 5'ATCAGGGACTGTG AGCGGCATAAT3'		

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iScript select cDNA synthesis kit.

Real time polymerase chain reaction: Relative quantification of different genes was performed using a real time PCR method by using Biorad CFX Software and Eva Green supermix as per standard protocol (Ranjan *et al.* 2014, Singh *et al.* 2014). The detail of primers is given in Table 1.

Statistical analysis: Relative expression of gene in fold changes was calculated by $2^{-\Delta\Delta C_t}$ method (Pfaffl *et al.* 2002) and analysed by one-way ANOVA using SPSS 16.

The expression of H19 gene was detected in all the samples tested in the present study (Figs 1–2). The expression of H19 gene was up regulated significantly ($P < 0.05$) in DIP and IVF derived 8–16 and morula as compared to *in vivo* (Figs 1–2). H19, a growth-related imprinted gene, was reported to be maternally expressed in mouse, human etc. (Bartolomei *et al.* 1993). In human, the H19 gene expression throughout embryonic development, is strictly controlled which suggested that regulation is essential for the growth of embryonic and extraembryonic tissues (Arima *et al.* 1997). In mouse, this gene showed monolallelic expression pattern in mouse pre and post implantation embryos (Jino *et al.* 2005). H19 expression in the parthenogenetic fetuses appeared to be somewhat higher than that in the control fetuses in sheep (Feil *et al.* 1998, Young *et al.* 2003). In our study, we also found higher expression of H19 in DIP compared to its IVF and *in vivo* counterpart (Figs 1–2).

We observed Peg1/Mest (paternally expressed gene-1/MEsoderm specific transcript) gene expression in both stages of DIP, *in vivo* and IVF derived embryonic cell colony (Figs 1–2). The expression was significantly ($P < 0.05$) down

regulated in both stages of DIP and *in vivo* embryos as compared to IVF derived ones (Figs 1–2). Interestingly, in both the stages, there was also no significant difference ($P < 0.05$) between DIP and *in vivo* derived embryos. Peg1/Mest, an alpha/beta hydrolase-encoding gene was reported to be expressed at high levels in mesodermal tissues (Sado *et al.* 1993). The mouse Peg1/Mest gene has recently been targeted by homologous recombination and this genetic study indicated that absence of this gene leads to a 20% reduction in fetal growth (Feil *et al.* 1998). In porcine, Peg1 expression was detected in oocytes and all the stages of preimplantation embryos (Park *et al.* 2011). In our study, we observed significantly ($P < 0.05$) low expression of Peg1/Mest in DIP compared to *in vitro* and *in vivo* derived embryos. In sheep, Peg1/Mest expression was detected in the control fetuses and their extra-embryonic membranes, but no expression was observed in the parthenogenetic fetuses and membranes (Feil *et al.* 1998). This result most likely indicated that in caprine also, Peg1/Mest is imprinted and expressed from the paternal chromosome.

Reports indicated that *in vitro* culture condition affects gene expression (Doherty *et al.* 2000, Khosla *et al.* 2001). Therefore, the changes of relative expression of these genes in DIP and IVF embryos in the present study might be due to the effect of *in vitro* culture condition. Platonov (2005) reported that death of parthenogenetic mammalian embryos is determined by the absence of expression of the genes of imprinted loci of the maternal or paternal genome, which leads to significant defects in development of tissues and organs. In our earlier experiment we observed early fetus death due to lack of formation of placenta or perturbed developmental gene expression (Ranjan *et al.* 2013c, Singh *et al.* 2013, Singh *et al.* 2014, Ranjan *et al.* 2014).

In conclusion, the present study demonstrated that in DIP embryos, the expression profile of both paternal and maternally imprinted genes were perturbed, which may be responsible for developmental failure of parthenogenetic embryos in this species.

SUMMARY

In the present study, we explored comparative expression profile of paternally imprinted gene (H19) and maternally imprinted gene (Peg1) among diploid parthenogenetic (DIP), female sexed IVF and *in vivo* derived embryos in caprine. Embryonic cell colonies were developed from 8–16 and morula stage DIP, IVF and *in vivo* derived embryos and these colonies were used for studying the paternal and maternal imprinted genes. The paternally and maternally imprinted gene was expressed in both the stages of DIP, IVF and *in vivo* derived embryos. It was further observed that the expression of H19 gene was up regulated significantly in DIP 8–16 and morula stage embryos as compared to their IVF and *in vivo* counterpart. The expressions of Peg1 gene was comparatively lower in DIP embryos than *in vivo* and IVF derived one. Interestingly, the expression of Peg1 gene was comparatively higher in IVF embryos than *in vivo* counterpart.

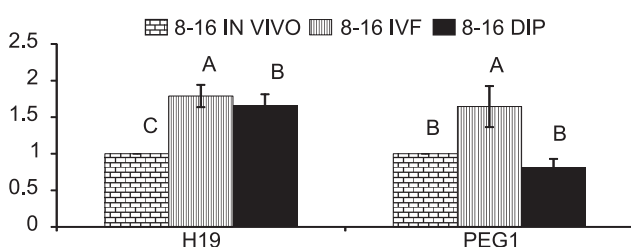


Fig. 1. Relative expression of H19 and Peg1 gene in embryonic cell colony derived from 8–16 cell stages of DIP, *in vivo* and IVF derived embryos in caprine (mean $\pm$ SE).

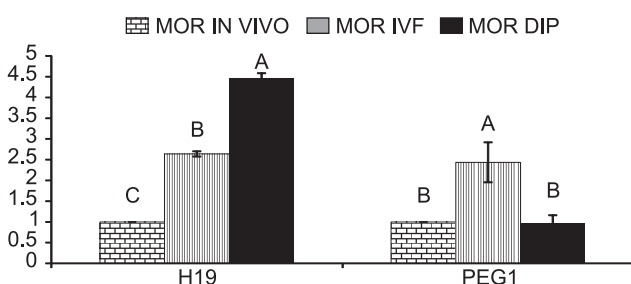


Fig. 2. Relative expression of H19 and Peg1 gene in embryonic cell colony derived from morula stages of DIP, *in vivo* and IVF derived embryos in caprine (mean $\pm$ SE).

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