Inclusion of tissue type plasminogen activator (t-PA) in IVF medium induces alterations in gene expression and affects blastocyst formation rate in bovine IVP

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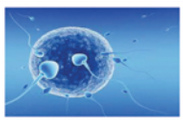
Introduction

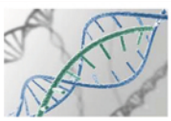
The proteolytic plasminogen activators/ plasmin system (PAA/PAI/PL) is involved in physiological processes such as fibrin degradation, matrix recomposition and cell invasion. The system is regulated by plasminogen activators (u-PA and t-PA) and inhibitors.

The presence or activity, of the PAA/PAI/PL has been detected in various cell types of the reproductive system, while its involvement into oocyte maturation, fertilization, embryo development and implantation has been shown in many studies. However, these factors are practically absent from the standard culture media used in IVF labs.

Here, through a modification of IVF medium, we investigated the effects of tissue type plasminogen activator (t-PA) on fertilizing capacity and subsequent embryo development.



Materials and Methods



- Bovine cumulus oocytes complexes (COCs) were collected from abattoir material and matured for 24 hours into TCM199 enriched with EGF and FCS at 39°C under 5% CO_2 in air and max humidity
- Matured oocytes were inseminated with frozen- thawed swim-up separated bull sperm and gametes were co-incubated in:
  - ➢standard IVF medium,
  - ➢modified medium containing 50 IU/ 0.1 ml t-PA, and
  - ➢medium containing t-PA and its inhibitor (ε-aminocaproic acid, final concentration 10 mM)
- Presumptive zygotes were cultured in SOF culture medium enriched with 5% FCS at 39°C under an atmosphere of 5% CO_2, 5% O_2 in air, max humidity
- Blastocyst formation rate (day-7 & day-8) was assessed in eight replicates
- For gene expression analysis, total RNA was extracted from 10 pooled day-8 blastocysts (three experimental replicates), followed by DNAse treatment to avoid DNA contamination, and reverse transcribed into cDNA
- To evaluate the quality of produced embryos, Real-Time PCR was carried out for nine gene transcripts (*PLAC8, AKR1B1, BIRC5, BBC3, PGHS2, BCL2L1, SLC2A5, MnSOD, PLG*) controlling apoptosis, metabolism, implantation and oxidation pathways. *18srRNA, H2a.z* and *GAPDH* housekeeping genes were used for normalization of gene expression data

Table 1: Details of primers used for RT-PCR and function of indicative marker genes analyzed for embryo quality

Genes	GenBank Accession No.	Primer sequence 5'→3'	Amplicon (bp)	Function
18srRNA	NR_036642	5'-CCCCTCGATGCTCTTAGCTGAGTGT-3' 5'-CGCCGATCCAAGAATTTCACCTCT -3'	222	Component of the small eukaryotic ribosomal subunit (40S). Important for protein synthesis ***Housekeeping gene***
H2a.z	NM_174809	5'-AGGACGACTAGCCATGGACGTGTG-3' 5'-CCACCACCAGCAATTGTAGCCTTG-3'	208	Transcription regulation, DNA repair, DNA replication and chromosomal stability. ***Housekeeping gene***
GAPDH	NM_001034034	5'-CAAGTTCAACGGCACAGTCAAGG-3' 5'-ACATACTCAGCACCAGCATCACC-3'	123	Playing role in glycolysis and nuclear functions. Participate in transcription activation and initiation of apoptosis ***Housekeeping gene***
PLAC8	NM_001076987	5'-AATGAATGCTGTCTGTGCGGAAC-3' 5'-AGTGCGATTGGCTCTCCTTCTG-3'	167	Invasion specific gene. Placenta development and fetus maternal interface
AKR1B1	NM_001012519	5'-AGGAGAAAGTGGTGAAGCGTGAG-3' 5'-ATGAGGTAGAGGTCCAGGTAGTCC-3'	138	Carbohydrate metabolism. Metabolize Progesterone (implantation) and synthesizing PGF2a (pregnancy termination)
BIRC5 or survivine	NM_001001855	5'-GCCGTCAACCGCTGGATTTG-3' 5'-CGTTCTCAGTGGGACAGTGGATG-3'	198	Inhibits apoptosis by binding caspases - Regulates cell division
BBC3 or PUMA	XM_001251007	5'-CATGAAGAGCAAATGAGCCAAACG-3' 5'-GCAGAGCACAGGATTCACAGTC-3'	193	p53 upregulated modulator of apoptosis (pro-apoptotic protein)
PGHS2-COX2	NM_174445	5'-TCTGGTGCCTGGTCTGATGATG-3' 5'-GGATTAGCCTGCTTGTCTGGAAC-3'	127	Oxidoreductase activity. Mediates the formation of prostaglandins from arachidonate
BCL2L1	NM_001077486	5'-TGACTGTGGCTGGTGTGGTTC-3' 5'-CAATGGTGGCTGGACGGAGAG-3'	123	Act as anti- or pro-apoptotic regulator
SLC2A5 or GLUT-5	NM_001101042	5'-ATAGCTGCCTTTGGGTCGTC-3' 5'-CAGCAAGGTTCCTTTTCTGCC-3'	243	Fructose transporter, essential for nucleotide's synthesis
MnSOD	NM_201527	5'-GCACCACAGCAAGCACCAC-3' 5'-GGGCTCAGATTTGTCCAGAAGATG-3'	156	Destroys superoxide anion radicals which are normally produced within the cells and which are toxic to biological systems
PLG	NM_173951	5'-GGCGGTGGTGTTCCTACTTCTC-3' 5'-GTCTGTCTCCTCCTCACACTTGG-3'	163	Plasmin's zymogen. Plasmin dissolves the fibrin of blood clots and acts as a proteolytic factor in a variety of processes

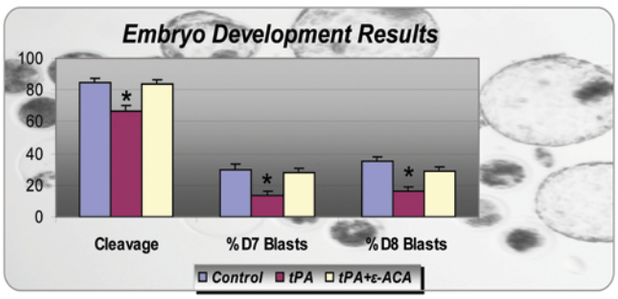
STATISTICAL ANALYSIS:

Differences in embryo development and mRNA expression were analyzed by one-way repeated measures ANOVA followed by multiple pairwise comparisons using both Tukey's and Dunkan's test. Results that are considered as significant have P value less than 0.05 (P< 0.05)

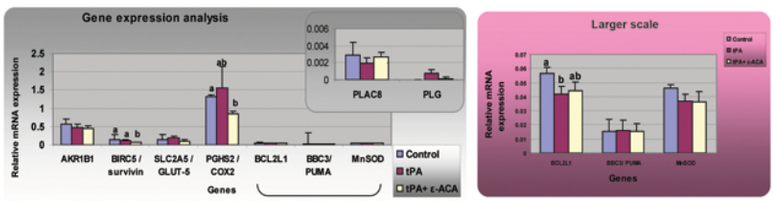
Results

Group	Total Zygotes	Cleavage %	Blastocyst Day 7 %	Blastocyst Day 8 %
Control	488	85	30	35
t-PA	583	66 ★	13 ★	16 ★
t-PA+ ε-ACA	259	84.3	28	30

Table 2: Effects of modification of IVF medium with the addition of t-PA and / or t-PA+ε-ACA. Within rows asterisks denote significant differences.



* Asterisks indicate statistically significant differences between three groups.



Upper panel: Bars with different supercripts within each gene transcript differ significantly (a:b P< 0.05).

Conclusion

- ➢ Under our lab's culture conditions t-PA addition in fertilization medium seriously affects embryonic development and reduces blastocyst yield that may be a sequel of downregulation of antiapoptotic genes.
- ➢ Further research is warranted to elucidate possible roles of this enzymatic system in the compromised fertility of dairy cows.