EFFECT OF HEAT SHOCK PROTEIN A5 (HSPA5) ON MATURATION OOCYTE DURING BOVINE IN VITRO EMBRYO PRODUCTION

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INTRODUCTION AND OBJECTIVE

The oviduct plays a crucial role in the final oocyte maturation, gamete transportation, fertilization and, initial embryo development. Once in the oviduct, the ovulated cumulus- oocyte complexes (COCs) has direct contact with epithelial oviductal cell and oviductal fluid (OF). Proteins derived from OF have shown an active role in the reproductive physiological process and, the HSPA5 protein has been found in high abundance in bovine OF. ***Objective:*** Although the activity of HSPA5 in the oviduct is not clear, we aimed to investigate if the addition of HSPA5 in the last four hours of COCs in vitro maturation (IVM) could impact the further embryo produced.

MATERIAL AND METHODS

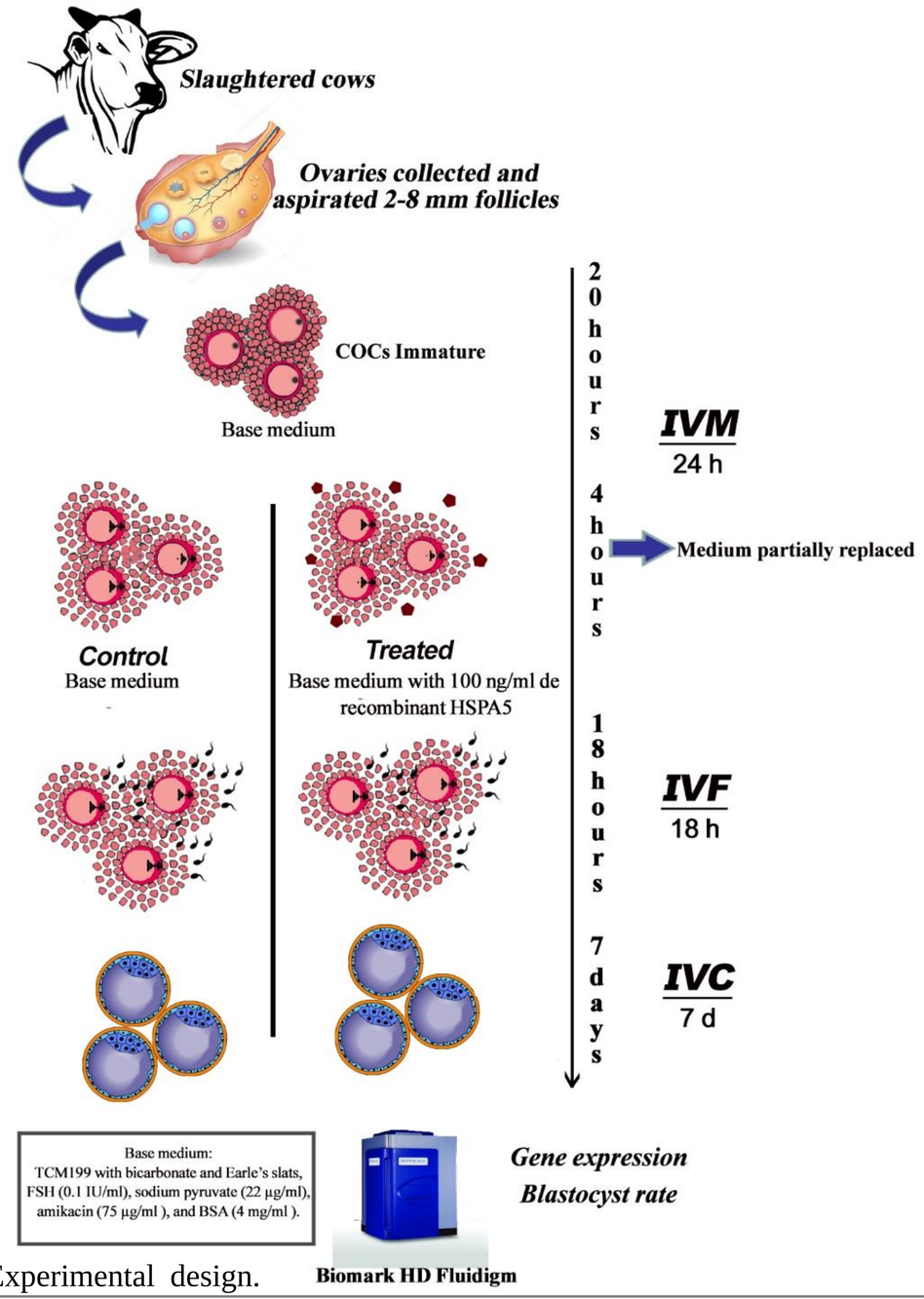

Figure 1. Experimental design.

STATISTICAL ANALYSIS:

Blastocyst rate was calculated as percentages and was arcsine-transformed. All data were tested for distribution normality by the Shapiro- Wilk test, and non-normal distribution was log-transformed. Follow, t-test or Wilcoxon-test (to non-parametric means) were performed to compared means. All data are presented as means ± SEM, and statistical significance was defined as $p \leq 0.05$. To analyses JMP software (SAS Institute, Cary, NC) was used.

RESULTS

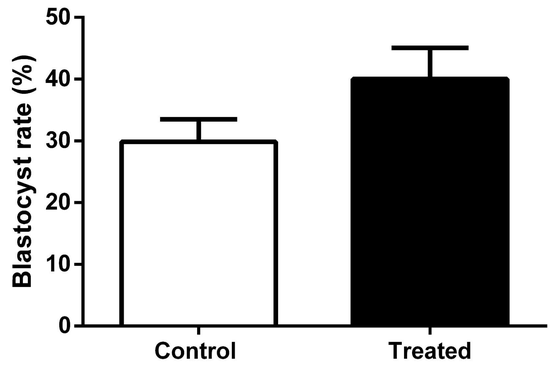

Figure 2. Blastocyst rates. The embryos was cultivated until 7 days. Data are means ± SEM of four biological replicates.

Tabel 1. Relative mRNA abundance of differently expressed genes on blastocyst ($p \leq 0.05$). Target genes were normalized with the reference genes (*HMBS*, *PPIA* and *RPL30*) geometric means using 2(-ΔCt) method. Data are means ± SEM of four biological replicates.

Gene symbol	Control	Treated	*p* Value	Function
ATP5L	2.30±0.08	2.56±0.10	0.04	Transmembrane transport
IGFBP2	1.97±0.12	2.79±0.28	0.02	Cell growth regulation
IMPDH1	0.04±0.004	0.06±0.005	0.04	Cell growth regulation
IMPDH2	0.23±0.01	0.31±0.03	0.03	Cell growth regulation
MAPK1	0.18±0.01	0.25±0.01	0.01	Cell differentiation
NANOG	0.04±0.01	0.10±0.01	0.006	Pluripotency
ATF4	0.29±0.06	0.47±0.02	0.02	ER stress
GFPT2	0.04±0.001	0.05±0.005	0.04	Cellular Stress
PRDX3	0.10±0.01	0.17±0.021	0.01	Oxidative Stress
XBP1	0.32±0.05	0.45±0.01	0.04	Oxidative Stress
SLC2A3	0.82±0.11	1.16±0.14	0.05	Energetic metabolism
SLC2A1	1.66±0.28	2.32±0.15	0.05	Energetic Metabolism
SREBF2	0.55±0.06	0.81±0.09	0.03	Lipid metabolism
DNMT3	0.63±0.09	0.87±0.06	0.04	Active DNA methilation
REST	0.37±0.04	0.61±0.08	0.02	Gene expression control
GLRX2	0.31±0.02	0.39±0.01	0.03	Oxidative Stress
HPRT1	0.26±0.02	0.37±0.04	0.04	Others
S100A10	0.72±0.10	1.12±0.11	0.02	Others

DISCUSSION

We did not find any difference in the blastocyst rates (P= 0.13); however, we figure out the upregulation of 18 genes involved in embryo quality, DNA methylation, cell growth, cellular development, and oxidative stress in the embryos produced from COCs treated with HSPA5 protein. Taken together, these results show that the addition of HSPA5 protein in the final stage of oocyte IVM might play a control role on embryo development modulating in low-scale the embryonic transcriptional profile.

CONCLUSION

- Our data shown that the COCs maturated with HSPA5 improves blastocyst development and modulates low-scale embryonic transcriptional profile.

FAPESP:#2013/11480-3
FAPESP: #2015/04505-5
CAPES: Finance Code 001