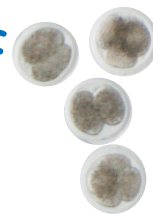


IN VIVO TRANSCRIPTOMIC RESPONSE OF BOVINE OVIDUCT EPITHELIAL CELLS TO THE EARLY EMBRYO



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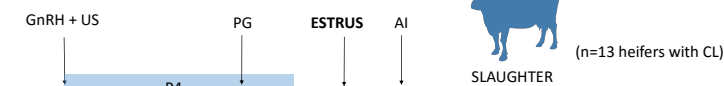


INTRODUCTION

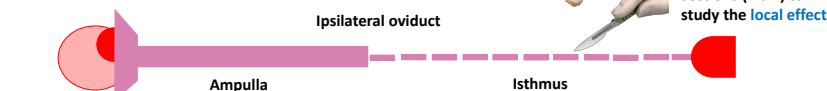
Based on previous data (Maillo *et al. Biol Reprod. 2015. 92: 144*) the presence of a single 8-cell embryo does not alter the transcriptome of the cells of the oviductal isthmus, although this apparent lack of effect might be due to a local effect at the precise position of the embryo which is missed if the whole oviduct is studied. Thus, we aimed to study the local embryo effect on the transcriptomic response of the epithelial cells of the oviduct *in vivo*.

MATERIALS AND METHODS

SYNCHRONIZATION OF THE HEIFERS



SAMPLES COLLECTION



I. Oviductal sections flushing

200 uL PBS
2cm oviduct

II. Embryo searching

(n=4 oviducts with an embryo)

III. Sections longitudinally opened + BOEC scratching

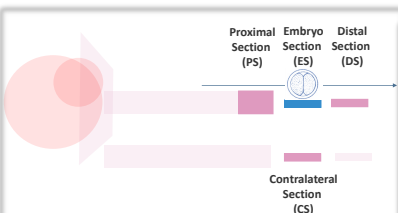
IV. BOEC storage (-80°C)

Fluid + cells centrifuged 14.000 rpm 1 min

BOEC

GENE EXPRESSION ANALYSIS in the BOECs (by RT-qPCR)

Oviductal sections selected for the analysis (experimental groups):



Genes selected: top 10 differentially expressed genes between the isthmus of pregnant and cyclic heifers (Maillo *et al. Biol Reprod. 2015. 92: 144*)

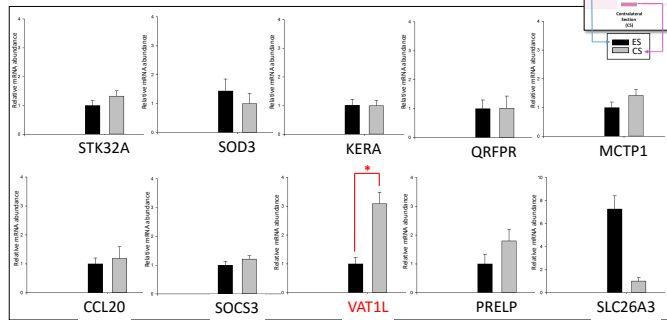
STK32A (6.22)	MMRN1 (4.21)	MUC12 (-11.54)	CXCL2 (-4.97)
SLC26A3 (6.22)	SLC24A4 (4.04)	RPS3A (-9.6)	VAT1L (-4.95)
KERA (5.88)	USP44 (3.93)	CASA (-8.75)	PRELP (-4.35)
QRFPR (4.85)	CBRN41 (3.83)	CCL20 (-6.04)	RYR1 (-3.73)
MCTP1 (4.22)	PIP (3.79)	SOD3 (-5.44)	ECSOD (-3.58)



* GnRH: Gonadotropin Releasing Hormone, US: Ultrasound Scan, P4: Progesterone, PG: Prostaglandin, AI: Artificial Insemination, CL: Corpus Luteum, BOEC: Bovine Oviduct Epithelial Cells, RT-qPCR: real time quantitative PCR.

RESULTS

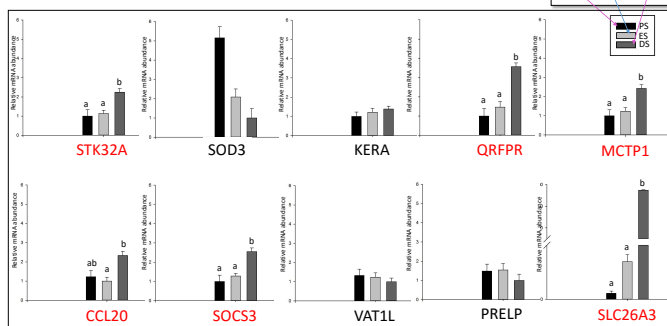
THE EMBRYO HAS AN EFFECT ON THE BOEC GENE EXPRESSION



* indicate significant differences based on the t-Test P<0.05

Comparison between ES and the CS revealed one significantly altered gene (VAT1L). This is in agreement with our *in vivo* results in which VAT1L was also down-regulated in the presence of embryos.

THIS EFFECT CAN BE SEEN ALONG THE IPSILATERAL OVIDUCT



^{a,b} letters indicate significant differences based on One Way analysis of variance (ANOVA, P<0.05)

Comparison within the ipsilateral oviduct of ES and PS samples revealed STK32A, SLC26A3, QRFPR, MCTP1 and SOCS3 transcripts significantly downregulated compared to DS samples, while the expression for CCL20 was different between ES and DS but similar to the PS.

CONCLUSIONS

- ❖ The fact that 6 out of 10 transcripts were different between the segment where embryo was collected and other locations in the oviduct suggests the presence of embryo site specific signal.
- ❖ Comparison between the ipsilateral embryo site with the contralateral site revealed one transcript different.
- ❖ Similarities in the ipsilateral oviduct between embryo and proximal site may be due to the passage of the embryo.



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