Abstract

Embryo cryopreservation with slow freezing or vitrification decrease rabbit (*Oryctolagus cuniculus*) embryo survival rate between 20-50%. This percentage depends on the genetic stock and the procedure followed. Higher mortality has been observed after implantation in vitrified rabbit embryos, suggesting that the procedure has negative delayed effects on foetal development. The aim of this thesis was to study the pre-implantatory transcriptome (at day 6), the embryonic and foetal development of rabbit embryos previously cryopreserved with the two common procedures for this species.

In particular, the aim of chapter I was to evaluate the effects of slow freezing on rabbit embryo development and gene expression. For this purpose, we first evaluated the distribution of embryo losses throughout gestation. We analysed the late blastocyst development, implantation and delivery rates. Then, we compared the transcriptomic profile of 6 day old embryos, previously frozen and transferred at morula stage, with their in vivo controls (day 6 post-insemination). We performed a two colour microarray analysis employing a generic platform specific for rabbit. Compared to controls, late blastocyst development, implantation and delivery rates were lower for frozen embryos. Likewise, differences were also observed in gene expression profile and viable frozen embryos showed 70 differentially expressed genes. This was the first approximation that provided us a list of candidate genes which could entail failures in maternal-embryo cross talk, implantation and formation of the placenta.

Chapter II aimed to evaluate the distribution of embryo losses throughout gestation due to the vitrification. However, in this case to evaluate the effects of vitrification on late blastocyst mRNA expression we performed a quantitative PCR (qPCR) with 10 candidate genes. These genes were selected because of their differential expression in rabbit frozen embryos (*SCGB1A1*, *EMP1*, *C1QTNF1*, *ANXA3*, *EGFLAM* and *TNFAIP6*) or for their important role in embryo development and implantation (*OCT4*, *VEGF*, *HBA* and *LAMA 4*). Moreover, we introduced ultrasonography data on foetal sack, foetus, foetal and maternal placenta at 10, 12 and 14 days of gestation. Results reported two major peaks of gestational losses: one before and the other after implantation. We also detected a reduction in development of foetus and maternal placenta in vitrified embryos between day 10 and 14 of gestation. Finally, we observed that the relative expressions of mRNA transcripts from *SCGB1A1*, *EMP1*, *C1QTNF1*, *ANXA3*, *EGFLAM* and *TNFAIP6* genes were significantly altered in vitrified embryos. These alterations were similar to the pattern

previously observed in frozen embryos.

In chapter III we directly compared the transcriptome of 6 day old embryos previously slow frozen or vitrified. We evaluated the distribution of embryo losses throughout gestation and we analysed the late blastocyst development, implantation and delivery rates. We also performed a two colour microarray analysis which directly compares the transcriptomic profile at day 6 of development of frozen and vitrified embryos. As in chapter I, we used the generic microarray platform specific for rabbit. We reported that slow freezing and vitrification have similar effects on embryo development till day 6, but the distribution of losses changed before and after implantation. The implantation and delivery rates were higher for vitrified embryos. The similarities at day 6 of development were also reflected in gene expression patterns, as no transcriptomic differences were found between both embryo types.

In chapter IV we compared the transcriptome of vitrified 6 day old embryos and 14 day old foetal placenta with the transcriptome of embryos and placentas that were transferred without cryopreservation. So only analysing the effect of vitrification. As previously described in chapter II, we observed that embryos that were able to reach late blastocyst stage were also able to implant but not all implanted embryos had the ability to continue with their gestation. Taking into account the ultrasonography results of this previous work, we weighted the foetus, foetal and maternal placenta at day 14. We also collected data on weight at day of birth. We detected a decrease in foetus and maternal placenta weights and an increase of weight at day of birth. For these reasons, we performed a transcriptomic analysis of 6-day-old embryos and 14 day-old-foetal placentas. For the gene expression analysis we introduced modifications to the experimental design. We used a microarray platform specially designed for the rabbit embryo and labelled the samples only with one colour. In case of the 6-day-old embryos we found that there were no differences in gene expression, but in the case of foetal placentas we identified 60 upregulated genes. Then, we performed a 2D-DIGE analysis of foetal placentas to find differences at proteomic level. We found 89 different expressed proteins in 14-day-old foetal placentas derived from vitrified embryos.

Due to our previous results on alterations a few days after implantation in transcriptome and proteome of foetal placenta, we focused chapter V on the study of proteomic alterations in foetal placentas at day 24 of development. We aimed to compare the protein profile of foetal placentas of vitrified and control embryos which were in the end of gestation and were about to be born. We performed a 2D-DIGE analysis and found that there were 32 different expressed proteins between experimental groups. These results demonstrate that vitrification induced a substantial alteration of placental protein expression at the end of gestation. So, apart from short-term effects on embryos, cryopreservation could

entail long-term consequences in those foetus that are going to be born.

The results of this thesis enabled us to propose that embryo cryopreservation in rabbit, whether by slow freezing or vitrification, may not be neutral. Moreover, for the first time it has been observed that there are transcriptomic and proteomic modifications in vitrified implanted embryos. Based on these findings, our work leaves the question open whether the effects of vitrification during foetal development could give rise to physiological or metabolic alteration in adulthood.