Young cancerous women who underwent chemo or radiotherapy often suffer from premature ovarian failure (POF). In order to preserve and restore fertility of these cancerous patients, ovarian cortical fragments can be frozen before treatment and then thawed and transplanted after remission. Twenty babies were born worldwide following this procedure. However this technique still needs to be optimised. follicles density and vascular network within cryopreserved ovarian tissue have a profound impact on the subsequent success of this transplantation. For ethical reasons, we choose to use sheep ovarian tissue to establish our model to evaluate fertility preservation after transplantation. Sheep ovaries indeed demonstrate similar size and morphology as compared to human ovaries. The first step of our study was to evaluate the distribution of follicles and vascular network within cortical pieces.

Four ovaries harvested from 2 young ewes (5 and 7 months old) were included in this study. About 20 fresh cortical fragments per ovary (2.5 x 2.5mm), randomly chosen, were entirely sectioned into 5µm thick serial sections. Every sixth slide was stained with hematoxylin and eosin and follicles were counted and classified according to their maturity. The vascular network was analyzed after an immunostaining for anti-van Willebrand Factor.

Our results show that follicle distribution and vascular network are extremely heterogeneous in sheep ovarian tissue, as it has already been demonstrated for human ovaries. Moreover, no direct correlation between follicular distribution and vascular network was evidenced. However, no method can allow the enumeration of follicles and vessel density within the cortical pieces ready to be transplanted. Statistical analysis established that at least 10 fragments from the same ovary and 20 sections of each fragments have to be examined in order to detect an improvement of follicle preservation independently of fragment heterogeneity.

Altogether our data emphasize that the experimental design needs to take into account the heterogeneity of follicular and vascular distributions. Consequently, the number of different ovarian pieces will depend on the level of confidence we intend to reach.