7. Summary

Immunohistochemical, light and electron microscopic study on in vitro angiogenesis in cultured endothelial cells from the bovine ovary.

Angiogenesis implicates the formation of blood vessels by sprouting of capillaries from existing vessels. In angiogenesis research in vitro models are used, displaying the migration and proliferation of endothelial cells, the formation of tubular structures and the deposition of basement membrane-like material. One aim of the present study was to examine the steps of in vitro angiogenesis. For this purpose endothelial cells from the midstage and the regressing corpus luteum were isolated, cultured and examined by phase contrast microscopy and electron microscopy. The formation and localization of a basement membrane-like material was shown by immunohistochemical demonstration of laminin, a basement membrane component.

After passaging endothelial cells strongly proliferated and established contacts by long cellular protrusions. When reaching confluence, involution of these protrusions occured and the cells displayed a polygonal shape showing the so-called cobble-stone pattern.

The beginning of in vitro angiogenesis was marked by depositon of laminin, that formed short intercellular fibrils. Subsequently endothelial cells began to form elongated protrusions and arranged side-by-side. In the next phase the cells re-arranged to ring-like structures and finally they developed a network, that covered the entire culture. Between the cells of these ring-like structures laminin and fibrillary material was found, but mainly it was located at the external side of the rings. The unilateral deposition of basement mebrane-like material pointed towards a definite polarity. Larger meshes of the network could be devided by cellular sprouts, that displayed basement membrane-like material in their center. This process is called intussusception, the splitting of vessels by pillars, that has been observed in vivo in different tissues. Subsequently remodeling of the primary network led to the establishment of cellular cords whereas fine endothelial structures began to vanish. In the center of the solid cords laminin and fibrillary material could be seen,

thus determinating again the basal and luminal side of the cells. Lumen formation by intracellular vacuoles was observed. For the first time the spontaneous regression of previously formed endothelial structures could be observed in a long term culture that had been maintained for eight months.

The course of angiogenesis in vitro showed strong similarity with angiogenesis in vivo. The processes concerned were the formation of ring-like strucures and net-works in vitro and the building of a primary capillary mesh by anastomoses and loop formation in vivo. The formation of solid cords and the vanishing of the fine endothelial network are comparable to the remodeling of a primary vascular plexus into mature structures with larger and smaller vessels. The results of this work imply that angiogenesis in vitro is not only determined by a sequence of specific events, but also has to take place in a characteristic temporal pattern.

The second aim of this study was to demonstrate the expression of vascular endothelial growth factor (VEGF), its receptors VEGFR-1 and VEGFR-2 and the expression of the receptor for growth hormone by PCR. These proteins were expressed by all cells examined. No significant differences between the steps of angiogenesis were found. A remarkable result was the expression of VEGF by endothelial cells. The expression of the receptor for growth hormone was very strong suggesting that GH is involved in vessel formation, particularly influencing the proliferation of endothelial cells via the GHR.