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Morphological expression of angiogenesis in the mammalian ovary as seen by SEM of corrosion casts

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Summary

In the mammalian ovary, follicular and corpus luteum cycle is associated with intensive microvascular remodelling. The complex angiogenic dynamics are finely tuned by numerous regulatory factors acting as activators (up-regulators) or inhibitors (down-regulators) of angiogenesis. Alterations of such a tight modulation are involved in several pathologies, including infertility, polycystic ovarian syndrome, ovarian hyperstimulation syndrome and ovarian cancer.
We have demonstrated in several experimental models that ovarian function is critically and specifically dependent on angiogenesis for follicular development, ovulation, and corpus luteum growth.
The aim of this review is to summarize the results we have obtained on the morphodynamic remodelling of ovarian microvascularization, in polyovulatory (rat, rabbit and pig) and monovulatory species (cow), using scanning electron microscopy of vascular corrosion casts.
The knowledge of the morphological expression of the up- and down-regulation of angiogenesis occurring in mono and polyovulatory animals might provide useful information to preserve fertility and to increase of the effectiveness of reproductive management in species of domestic interest.

Key words

Angiogenesis, electron microscopy, ovary, mammals

Introduction

Ovarian vessels are rapidly remodelled during the reproductive lifespan in developing and atretic follicles or growing and regressive corpora lutea (CL) (Reynolds et al., 2002; Plendl, 2000; Robinson et al, 2009). The acquisition of an adequate vascular network, consequent to an up-regulation of angiogenesis, may be a priority step not only in the selection and maturation of ovulatory follicle(s), but also in the phases of CL maturation. These morphological changes are essential to ensure the adequate metabolic support to the follicular-luteal complex (FLC) (Motta et al., 2003) and, consequently, to fertility (Nottola et al., 1997; Plendl, 2000; Macchiarelli et al., 2006; Jiang et al., 2008). Nevertheless several aspects need to be clarified. For example: *i)* the dynamics of blood vessels growth and regression in FLC; *ii)* the morphological patterns of angiogenesis which drive vascular remodelling during the FLC develop-

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ment; *iii)* the patterns of secretion/modulation of ovarian angiogenic factors and *iv)* the activation and proliferation of perivascular cells. Indeed, consistent data are still missing concerning the comparison of the morphological expression of follicular and luteal angiogenesis between mono and polyovulatory species.

This review aims at summarizing results obtained by our group using scanning electron microscopy (SEM) on vascular corrosion casts (VCC) and correlated techniques in mono and polyovulatory animals, to provide a detailed morphological view of the angiogenic and angioregressive processes in follicles and CL, especially in terms of a further applicability of these data to studies on fertility control, treatment of diseases linked to abnormal angiogenesis and reproductive management of species of zootechnical interest (Tomanek and Schatteman, 2000; Ferrara and Kerbel, 2005).

Materials and Methods

Ovarian samples from female adult rats, rabbits, pigs, and cows were prepared according to previous experimental designs (Macchiarelli et al., 1991, 1993, 1998, 2006; Nottola et al., 1997; Jiang et al., 2002, 2003, 2008; Martelli et al., 2009). Immature infertile, hypothyroid *rdw* rats, normal *Wistar-Imamichi* rats and adult female *New Zealand* white rabbits were used to obtain antral, periovulatory and atretic follicles, growing and regressing pseudopregnancy CL, pregnancy CL. Ovaries collected at abattoir from mature gilts and *Japanese black* cattle were used to obtain developing follicles. Prepubertal *Large White* gilts, who underwent estrous synchronization (with e[equine]CG+hCG), allowed to collect preovulatory, early and late periovulatory follicles. The ovaries were processed for VCC as previously described to obtain almost three-dimensional images at relatively high resolution (Murakami, 1971; Macchiarelli, 2000) and to highlight differences in the vascularization of the FLC, both in terms of angiogenesis and angioregression, depending on the stage of ovarian cycle.

The animal studies reported in this article were approved by the local Ethics Committees of the Italian and Japanese Institutions involved in the projects and were in agreement with the related European Community regulations.

Results

Microvasculature architecture of follicle and CL

Thecal capillaries from small to ovulatory follicles changed their distribution from a single-layer to a more complex structure, in **all species**. Further differences in the richness of vascularization were found among species and between dominant and subordinate follicles (Fig 1a). In all the species, the casted microvasculature of developing follicles was organized in a basket-like vascular plexus (VP) surrounding an empty central antral cavity. Species-specific differences included plexus and vessel diameter, the number of vascular layers as well as the presence and localization of angiogenic figures. In periovulatory follicles, the VPs appeared very large, of ovoid shape, multilayered, with an avascular area located at the apical pole. The basal pole presented an enriched vascularisation. The VPs of subordinate follicles resulted poor-

ly capillarized (Fig 1a). Atretic follicles presented irregular VPs with the central cavity partially occupied by newly formed vessels, as a consequence of the inflammatory process. Growing CL showed spherical VPs. Newly formed small arterioles and venules supplied luteal VPs and originated a dense capillary network penetrating the plexus. The blood supply of pregnant CL was ensured by large plexuses of ovoid shape.

Angiogenesis.

In **rats**, angiogenic figures found in follicles and CL mainly consisted of budding, sprouting and dilation of activated capillaries (Fig. 1b), often accompanied by proliferation of pericytes as seen by transmission electron microscopy (Fig. 1c). In **rabbits**, sprouting angiogenesis was found during all the phases of developing follicles and CL. Degenerative and atretic follicles showed signs of sprouting capillary growth within the antrum, associated with wide phenomena of angioregression (Fig. 1d). Periovulatory follicles showed massive capillary changes such as dilation of capillaries, budding, sprouting and non-sprouting (intussusceptive; Fig. 1d) angiogenesis. During CL formation, several capillary sprouts protruded into the plexus. In **gilts** and **cows**, capillary buds and sprouting occurred in developing follicles. Proliferative budding, sprouting and non sprouting angiogenic figures were detected in the inner layer of largest VPs. Atretic follicles showed thin regressing capillaries. In cows differences in the number of angiogenic sprouts between apical, equatorial and basal regions of the inner network were detected.

Discussion

Capillary remodelling was demonstrated during follicle and CL development in all species. The species-specific differences were related to follicular size and to the mechanisms of follicle selection (Mihm and Evans, 2008). In the rat, rabbit and pig, angiogenesis was mainly found in the inner thecal capillaries. In larger follicles of domestic animals, the vascular remodelling extended to all thecal layers. SEM of VCC showed: 1) sprouting and non-sprouting angiogenesis; 2) gradual formation of a dense sinusoidal thecal network in dominant follicles; 3) rapid formation of a dense capillary plexus in growing CL; 4) intensive remodelling of cortical venules and arterioles during CLF cycle, especially after ovulation; 5) regressive vascularization in atretic or subordinated follicles as well as degenerating CL (Douglas et al., 2005).

Two types of ovarian angiogenesis were recognized: sprouting and non-sprouting (Fig. 1b). In sprouting angiogenesis, the new capillaries generally form by outward growth of endothelial cells from the pre-existing vessels. Sprouting is the most represented process of vascular growth in the adult. Non-sprouting angiogenesis is an alternative method of vessel formation from pre-existing vessels (Burri et al., 2004). It consists in splitting of capillaries inward from their vessels of origin (intussusception i.e. "growth within itself") (Fig. 1b). Non-sprouting angiogenesis occurs with low endothelial cell proliferation, is achieved at low vascular permeability levels, and requires only 4-5 h for completion and is the optimal physiological solution to sustain a rapid angiogenesis during the development of ovarian endocrine functions (Burri et

al., 2004). In the ovay, the initial rapid proliferation of granulosa and thecal cells asks for the cooptation of interstitial vessel and a quick subsequent sprouting angiogenesis for providing nutrients and extraovarian endocrine stimuli. As soon as maturing follicles differentiate towards endocrine function, by expressing the proper steroidogenic activity, intussusceptive angiogenesis is required in order to create a sinusoidal network that is able to sustain both nutritional and functional microcirculation. This process is not only ''structural'', but also functional. In fact, FLC microcirculation shows hemodynamic control activation at capillary and postcapillary level: capillaries activate, enlarge and develop into sinusoids; pericytes proliferate and activate; venular sphincters activate (Jiang et al., 2003 Macchiarelli et al., 2006).

The angiogenic process is sustained in dominants follicles; all other cyclically recruited follicles, the subordinate ones, are destined to atresia (Motta et al., 2003). Angiogenesis is halted in these follicles and regressing CL as well. Active angiogenesis may be still present contemporary to capillary degeneration, this is likely related to the time of onset of atresia and to the residual endocrine functions of atretic follicles until they degenerate (Macchiarelli et al., 2006).

According to our observations we may conclude: *i)* that angiogenesis in mammalian ovary occurrs in sequential steps: budding, sprouting (and elongation), dilation, infolding (intussusception), duplication (splitting and elongation), sinusoidalization and finally capillary thinning and re-absorption (Fig. 1d); *ii)* that the selection of the dominant follicle is greatly related to up and down regulation of angiogenesis; and therefore *iii)* that angiogenesis is a significant regulative factor of mammalian CLF cycle, in both mono and polyovulatory animals.

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References

Burri P.H, Hlushchuk R., Djonov V. (2004) Intussusceptive angiogenesis: its emergence, its characteristics, and its significance. Dev. Dyn. 231:474-488.

Douglas N. C., Nakhuda G. S., Sauer M. V., Zimmermann R. C. (2005) Angiogenesis and ovarian function. J. Fertil. Reprod. 15: 7-14.

Ferrara N., Kerbel R.S. (2005) Angiogenesis as a therapeutic target. Nature 438: 967-974.

Jiang J.Y., Macchiarelli G., Miyabayashi K., Sato E. (2002) Follicular microvasculature in the porcine ovary. Cell Tissue Res. 310: 93-101.

Jiang J.Y., Macchiarelli G., Tsang B.K., Sato E. (2003) Capillary angiogenesis and degeneration in bovine ovarian antral follicles. Reproduction 125: 211-223.

Jiang J.Y., Miyabayashi K., Nottola S.A., Umezu M., Cecconi S., Sato E., Macchiarelli G. (2008) Thyroxine treatment stimulated ovarian follicular angiogenesis in immature hypothyroid rats. Histol. Histopathol. 23: 1387-1398.

Macchiarelli G. (2000) The microvasculature of the ovary. A review by SEM of vascular corrosion casts. J. Reprod. Dev. 46: 207-255.

Macchiarelli G., Nottola S.A., Vizza E., Kikuta A., Murakami T., Motta P.M. (1991) Ovarian microvasculature in normal and hCG stimulated rabbits. A study of vascular corrosion casts with particular regard to the interstitium. J. Submicrosc. Cytol. Pathol. 23: 391-395.

Macchiarelli G., Nottola S.A., Vizza E., Familiari G., Kikuta A., Murakami T., Motta P.M. (1993) Microvasculature of growing and atretic follicles in the rabbit ovary: a SEM study of corrosion casts. Arch. Histol. Cytol. 56: 1-12.

Macchiarelli G., Nottola S.A., Picucci K., Stallone T., Motta P.M. (1998) The microvasculature of the corpus luteum in pregnant rabbit. A scanning electron microscopy study of corrosion casts. Ital. J. Anat. Embryol. 103 (Suppl 1): 191-202.

Macchiarelli G., Jiang J.Y., Nottola S.A., Sato E. (2006) Morphological patterns of angiogenesis in ovarian follicle capillary networks. A scanning electron microscopy study of corrosion cast. Microsc. Res. Tech. 69: 459-468.

Martelli A., Palmerini M.G., Russo V., Rinaldi C., Bernabò N., Di Giacinto O., Berardinelli P., Nottola S.A., Macchiarelli G., Barboni B. (2009) Blood vessel remodeling in pig ovarian follicles during the periovulatory period: an immunohistochemistry and SEM-corrosion casting study. Reprod. Biol. Endocrinol. 7: 72.

Mihm M., Evans A.C. (2008) Mechanisms for dominant follicle selection in monovulatory species: a comparison of morphological, endocrine and intraovarian events in cows, mares and women. Reprod. Domest. Anim. 43 (Suppl 2): 48-56.

Motta P.M., Nottola S.A., Familiari G., Makabe S., Stallone T., Macchiarelli G. (2003) Morphodynamics of the follicular-luteal complex during early ovarian development and reproductive life. Int. Rev. Cytol. 223: 177-288.

Murakami T. (1971) Application of the scanning electron microscope to the study of fine distribution of the blood vessels. Arch. Histol. Jpn.. 32: 445-454.

Nottola S.A., Macchiarelli G., Motta P.M. (1997) The angioarchitecture of estrous, pseudopregnant and pregnant rabbit ovary as seen by scanning electron microscopy of vascular corrosion casts. Cell Tissue Res. 288: 353-363.

Plendl J. (2000) Angiogenesis and vascular regression in the ovary. Anat. Histol. Embryol. 29: 257-266.

Reynolds L.P., Grazul-Bilska A.T., Redmer D.A. (2002) Angiogenesis in the female reproductive organs: Pathological implications. Int. J. Exp. Pathol. 83:151-163.

Robinson R.S., Woad K.J., Hammond A.J., Laird M., Hunter M.G., Mann G.E. (2009) Angiogenesis and vascular function in the ovary. Reproduction 138: 869-881.

Tomanek R.J., Schatteman G.C. (2000). Angiogenesis: new insights and therapeutic potential. Anat. Rec. 261: 126-135.

Figures

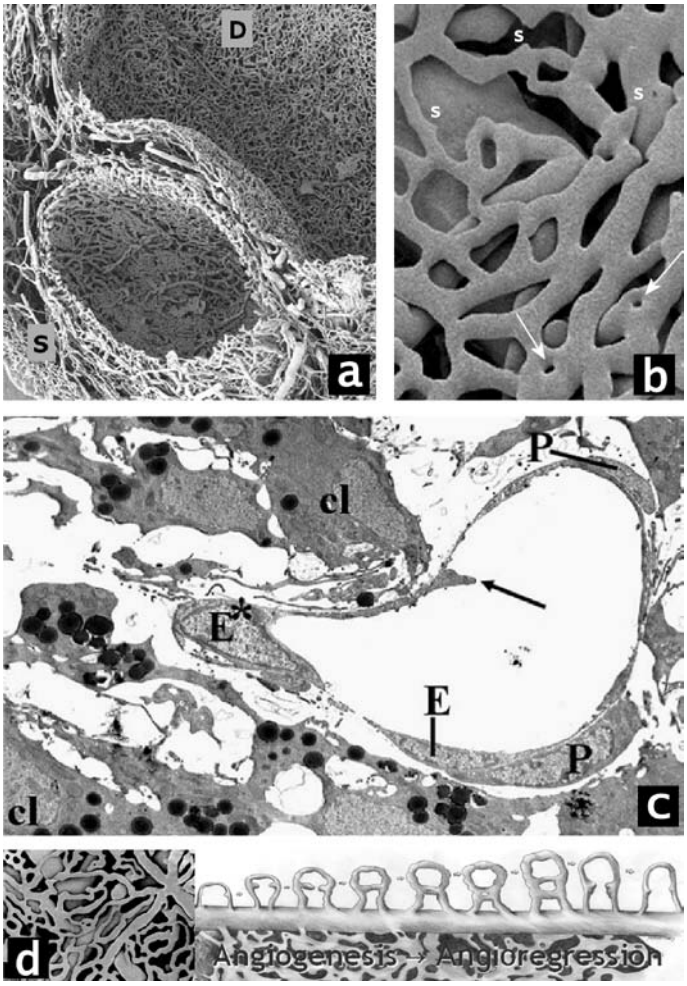

Figure 1a – SEM of a vascular corrosion cast (VCC) representing a dominant (D) bovine ovarian follicle, showing capillary growth, and a subordinate follicle (S) with evident signs of capillary regression (Modified from Jiang et al., 2003, with permission).
Figure 1b – SEM of a VCC of a rabbit preovulatory follicle showing sprouting (s) and non-sprouting (arrows) angiogenesis.
Figure 1c – An activated capillary of a large antral follicle of a T4/eCG-treated *rdw* rat is shown. Two pericytes (P) enveloping the endothelial cells (E) are evidenced. Arrow: short cytoplasmic protrusion from the leading cell (*). Original magnification: x 1,600. (Modified from Jiang et al., 2008, with permission).
Figure 1d – Left panel: representative picture of angiogenesis and angioregression as seen by SEM of VCC. Right panel: the art work shows the sequence of capillary differentiation cycle in folliculo-luteal complexes as seen by SEM of VCC. A thin capillary loop gives off small buds, which transform into sprouts that fuse to other sprouts to form a new capillary loop. Then new capillaries dilate, showing a wall infolding that enlarges and forms capillary splitting (duplication), followed by the formation of new sinusoidal loops. When angiogenesis is downregulated, the neocapillaries undergo thinning and rapid loss of newly formed loops.