Reproductive Seasonality Influences Follicle Dynamics and the Ovarian Extracellular Matrix Structural Properties in Ewes.

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Successful ovarian function, which includes follicular development, ovulation, and corpus luteum formation and regression, requires extensive cyclical tissue remodeling provided by its extracellular matrix (ECM). The ovarian ECM was long considered to be a passive structure that provides anchoring and mechanical support to the cells, but is now increasingly recognized as a pivotal element in the multi-directional communication that occurs between components of the ECM, stroma, theca, granulosa cells, and the oocyte. As new roles for the ECM emerge, in-depth examination of its changes across suspension and resumption of ovarian activity during seasonal breeding may reveal key aspects for improving oocyte yield and embryo production for agricultural purposes or restoring ovarian function in women. The aim of the present study was to investigate the influence of reproductive seasonality on follicle dynamics and the ovarian ECM properties.

Ovaries from ewes were obtained from the local abattoir, with eight of them collected during the breeding season (November/December) and eight during anoestrus (April/May). Ovaries were weighed and measured, and ovarian morphometric parameters as well as ECM-related proteins expression (collagen, fibronectin, laminin) were quantified using histological and immunohistological analysis.

Ovarian weight and volume were higher during the breeding season compared to the non-breeding season ($P < 0.001$). No difference was observed in the proportion of pre-antral follicles and earlier stages of development, but the percentage of antral follicles was significantly increased during anoestrus compared to oestrus ($P = 0.028$) while 6 out of the 8 ovaries (75%) collected during oestrus contained a corpus luteum, compared to none in those retrieved during anoestrus ($P = 0.010$). In addition, the tunica albuginea, the connective tissue layer located beneath the surface epithelium, thickened during the anoestrus season, rising from 110.3 µm ± 14.9 µm during the breeding season to 121.3 µm ± 18.8 µm during anoestrus ($P = 0.215$). Stromal collagen, fibronectin and laminin content were also quantified. Both collagen and fibronectin peaked during anoestrus compared to oestrus (collagen: 87.0% ± 3.3% positive area versus 72.4% ± 5.4%, respectively [mean ± SD], $P < 0.0001$; fibronectin: 33.5% ± 4.3% positive area versus 25.4% ± 6.0%, $P = 0.007$), while laminin slightly dropped (12.4% ± 2.8% positive area versus 16.1% ± 3.4%, $P = 0.032$). Collagen fibres thickness and packing density were further assessed and showed a significant increase of both thick and mid-sized fibres during anoestrus compared to the breeding season ($P = 0.003$ and $P < 0.001$, respectively), while the proportion of thin fibres dropped ($P < 0.001$). Given that collagen confers tissues with rigidity, these results suggest a stiffer microenvironment during anoestrus, corroborated with a thicker tunica albuginea. Similar features have been reported in patients with polycystic ovary syndrome and are likely to prevent ovulation. Ongoing

experiments are assessing the mechanical properties of the ECM at each reproductive phase to clarify the consequences of such structural changes.

These results offer a first look at the dynamic remodeling that occurs across the reproductive season, modulating the microenvironment to potentially regulate follicle growth and ovulation. They also reinforce the use of the ovine model, that share physiological characteristics with women, as a model to provide comparative insights into ovarian biology.

This study was supported by a grant from the Medical Research Council (MR/T025654/1 to E.E.T.).