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P-440 Impact of electrospun scaffold topology on the performance of *in-vitro* Folliculogenesis applied to preantral ovine follicles

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Study question: How to improve *in-vitro* Folliculogenesis (*iv*F) protocols to address the enlarged demand of fertility preservation?

Summary answer: Tissue engineering-based approach opens new frontiers for *iv*F improving 3D-technologies addressed to support immature-ovarian-follicle-growth to obtain an increased number of competent oocytes enrolled in Assisted-Reproductive-Technology.

What is known already: *iv*F is a promising Assisted-Reproductive-Technology (ART) for preserving and restoring fertility. This technology potentially reproduces the early stages of folliculogenesis and oogenesis *in-vitro* allowing to move a large amount of oocyte on individual basis towards the validated protocol of *in-vitro* maturation/*in-vitro* fertilization (IVM/IVF).

The current availability of biocompatible-supporting materials offers the challenging opportunity to mimic the native organ stroma in order to better reproduce the 3D environmental conditions leading to synergic follicles-oocyte development *in-vitro* with the aim to improve the performance of *iv*F in translational large sized mammal models.

Study design, size, duration: The present research aimed to compare preantral (PA) follicles culture on two different typologies of scaffolds fabricated using PCL(poly(epsilon caprolactone)), respectively made with patterned and randomly aligned fibers (PCL-Patterned/PCL-Randomic) with a standardized-single-follicle scaffold-free-method (3D-oil), widely validated on ovine model (Cecconi et al., 2004). The culture outcomes are compared analyzing follicle/oocyte growth, percentage of antrum differentiation and the incidence of meiotic competence, by exposing *iv*F growing oocytes to IVM protocol.

Participants/materials, setting, methods: PA follicles (mean size diameter: 250±4μm), mechanically isolated from slaughterhoused lamb ovaries, were individually cultured on electrospun PCL scaffolds (patterned *vs* randomic) or using the 3D-oil method. *iv*F were cultured alphaMEM-Fetal Bovine Serum free medium (5% Knockout Serum Replacement) supplemented with 4 IU/mL of equine Chorionic Gonadotropin (Di Berardino et al., 2021). At the end of *iv*F (14-days) the fully-grown oocytes isolated from early-antral follicles were tested on IVM.

Main results and the role of chance: PCL-Patterned electrospun scaffolds were able to strongly support a synergic oocyte and follicular growth. The 3D culture on Patterned electrospun scaffold supported the highest viability of follicles (87.5% *vs* 63% under 3D-oil conditions). On the contrary, the highest incidence of degenerated follicles was observed in cultures performed using PCL-Randomic materials (55 *vs* 37% *vs* 12.5% for PCL-Randomic *vs* 3D-oil *vs* PCL-Patterned, respectively; $p < 0.0004$). The greatest follicle diameter increment (74.7±1 *vs* 70±0.4 *vs* 60.9±2%, for PCL-Patterned *vs* 3D-oil *vs* PCL-Randomic, respectively $p < 0.0007$) and rate of antrum differentiation (87.5% *vs* 45% and *vs* 63%, for PCL-Patterned *vs* 3D-oil *vs* PCL-Randomic, for both $p < 0.0001$) were observed in PA ovine follicles cultured on PCL-Patterned scaffolds. Furthermore, PCL-Patterned electrospun scaffolds supported a complete functional development of the oocyte compartment. More in detail, the majority of fully grown oocytes isolated from early- antral follicles grown on PCL-Patterned materials reached the metaphase-II stage (MII 80%) at the end of IVM in comparison to the significant lower percentage in 3D-oil (MII 68%, $p = 0.04$) and PCL-Randomic (MII 18%, $p < 0.0001$) protocols, respectively.

Limitations, reasons for caution: -

Wider implications of the findings: Tissue engineering scaffold-based approach represents a valid strategy generating a multi-organ *in-vitro* system, where different compartments may cooperate generating the complexity of paracrine-mechanism controlling early-follicles outcomes. Scaffold topology is

essential to control early-follicles development. Indeed, exclusively PCL-Patterned can preserve long-term follicle 3D-microarchitecture supporting *in-vitro* oogenesis up to a complete meiotic-competence-acquisition.

Trial registration number: not applicable