Quality of oocytes originating from in vitro grown ewe follicles

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Quality of oocytes originating from *in vitro* grown ewe follicles

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Ovarian follicles grow inside the ovaries until ovulation. During this growth period, which lasts several months in most mammals, the granulosa cells surrounding the oocyte proliferate and differentiate to form several layers, and an antral cavity forms later. Progressively, the oocyte grows and acquires meiotic competence, then developmental competence, i.e. the ability to later be fertilized and sustain embryo development, through accumulation of maternal factors, especially RNAs. Those events are coordinated due to an intricate communication system between somatic cells and the oocyte. It involves transzonal projections; they are cytoplasmic projections from granulosa cells passing through the oocyte's zona pellucida to reach its plasma membrane. Channels called gap junctions are formed where the projection and the oocyte membranes are in contact and they allow the exchange of small molecules.

Organotypic culture of ovine follicles can mimic folliculogenesis and is an invaluable tool to help delineate the mechanisms and factors involved in this process. Yet, *in vitro* cultured follicles also display differences when compared to their *in vivo* counterparts. For instance, our team previously showed that granulosa cell differentiation is accelerated in *in vitro* cultured follicles, and that Leukemia Inhibitory Factor (LIF) can modulate this phenomenon and has a beneficial effect on oocyte meiotic competence. Therefore, we hypothesize that LIF might enhance molecular communication between the gamete and surrounding somatic cells and, as a result, enable the oocyte to complete maternal RNA synthesis.

First, we have refined the follicle 3D-culture protocol. Preantral follicles 200-300 µm in diameter were dissected from pre-pubertal ewe ovaries and cultured individually for three weeks in enriched Minimum Essential Medium. Several culture dishes and surface treatment were tested. Follicle survival, 3D structure maintenance and antral cavity formation were evaluated. Growth was assessed on days 6, 13 and 20 using an in-house ImageJ script.

The objective is now to characterize the effect of follicle culture and LIF supplementation on oocyte quality. Oocytes from organotypic cultures with/without LIF will be compared to their *in vivo* counterparts on three main criteria:

1) Gene expression profile by transcriptomic and/or proteomic analysis
2) Formation and maintenance of transzonal projections and gap junctions; their abundance will be evaluated by automatic counting following immunofluorescent detection and confocal microscopic imaging
3) Embryonic development competence following parthenogenetic activation and/or *in vitro* fertilization.

Beyond improving knowledge of the mechanisms underlying folliculogenesis, our results will be useful for developing follicle culture in the context of human assisted reproductive technologies and for the preservation of endangered species.

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