

***In vitro* culture of goat preantral follicles**

José Ricardo de Figueiredo, Ana Paula Ribeiro Rodrigues & Valdevane Rocha Araújo

ABSTRACT

Background: The majority of thousands of oocytes in the ovaries are small, non-growing and reside in preantral follicles (PFs). The development of a culture system for preantral follicle may be very useful for understanding the complex mechanism in folliculogenesis at early stages of development as well as could offer a significant way for the propagation of livestock, including goats. This paper describes the results of a number of studies aimed to evaluate the effects of several substances on *in vitro* culture of caprine preantral follicles highlighting the many advances, limitations and prospects.

Review: Caprine PFs are usually cultured either in ovarian cortical slices or after isolation. Although IVC (*in vitro* culture) of PFs enclosed in cortical slices is practical, non-time-consuming, maintains three dimensional follicle architecture and preserves interactions between follicles and surrounding stroma cells, the cortical tissue may act as a barrier to IVC medium perfusion. Conversely, IVC of isolated PFs allows monitoring of individual follicles throughout the growing period, but is time-consuming, may be affected by the isolation procedure, demands a more sophisticated IVC system and is often applied to secondary and not to primordial and primary follicles. In general, several studies with farm animals and primates have successfully shown the activation and transition of primordial follicles to primary stages during *in vitro* culture of ovarian cortical slices. However, using these mammalian models primary follicles do not grow to secondary stages. Despite that, we succeeded to overcome this problem using an appropriate concentration of growth differentiation factor-9 (GDF-9). Indeed, Martins *et al.* [2] demonstrated that GDF-9 (200 ng/mL) maintained the survival of PF and promoted activation of primordial follicles. Furthermore, GDF-9 stimulated the transition from primary to secondary follicles, maintaining ultrastructural integrity of the follicles. Despite a small number of publications on IVC of isolated caprine PFs, promising results such as maintenance of follicular survival, follicle and oocyte growth, antrum formation, meiosis resumption and development of embryos after IVC of oocytes enclosed in caprine PFs were achieved [3]. The main results obtained by our research group (for review see [1]) after 18-day *in vitro* culture of isolated goat preantral follicles are as follows: follicular survival (89.74%), antrum formation (100%), Growth rate (25.52 $\mu\text{m}/\text{day}$), recovery rate of oocyte = 110 μm (77.10%), meiosis resumption (78.13%), nuclear maturation-MII (29.41%) and embryo production (n = 2).

Conclusions: The basic culture system for the *in vitro* culture of caprine PF which maintains follicular survival is well established. Primordial follicle activation and their further growth up to secondary stage *in vitro* were achieved. Isolated primary follicles can grow up to antral stage although this rate is still low. Antrum formation and fully grown oocytes were obtained from *in vitro* culture of large secondary follicles even yielding a few mature oocytes and embryos. At present, the key challenge for researchers is to increase the rates of maturation and *in vitro* production of embryos from goat oocytes enclosed in PF grown *in vitro*, specially from very early stages, in order to produce, in the future, large number of offsprings.

Keywords: preantral follicles, culture, goat.

REFERENCES

- 1 Figueiredo J.R., Rodrigues A.P.R., Silva J.R.V. & Santos R.R. 2010. Cryopreservation and *in vitro* culture of caprine preantral follicles. *Reproduction, Fertility and Development*. 23(1): 40-47.
- 2 Martins F.S., Celestino J.J., Saraiva M.V., Matos M.H.T., Bruno J.B., Rocha-Junior C.M., Lima-Verde I.B., Lucci C.M., Bão S.N., & Figueiredo J.R. 2008. Growth and differentiation factor-9 stimulates activation of goat primordial follicles *in vitro* and their progression to secondary follicles. *Reproduction, Fertility and Development*. 20(8): 916-924.
- 3 Saraiva M.V., Rossetto R., Brito I.R., Celestino J.J.H., Silva C.M., Faustino L.R., Almeida A.P., Bruno J.B., Magalhães D.M., Matos M.H., Campello C.C. & Figueiredo J.R. 2010. Dynamic medium containing FSH, LH and EGF produces caprine embryo from preantral follicles grown *in vitro*. *Reproductive Sciences*. 17(12): 1135-1143.