Endocrinol Metab Syndr 2015, 4:4
http://dx.doi.org/10.4172/2161-1017.C1.009

OMICS International
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3rd International Conference on
Endocrinology
November 02-04, 2015 Atlanta, USA

# Embryo production from *in vitro* grown caprine preantral follicles using three dimensional follicle culture matrix

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*In vitro* culture of preantral follicles provides a valuable tool to study early folliculogenesis including the critical and complex interactions regulating follicle and oocyte development and may also have implications for fertility preservation. Goats are considered suitable models for human. In fact, follicular development in the adult goat (i.e., 10 weeks) was the closest to that observed in adult women. The aim of this study was to establish a culture system that improves the *in vitro* development of isolated caprine preantral follicles. In the first experiment we determined the optimal concentration of alginate (ALG). In a subsequent experiment we investigated the effects of the multiple follicle culture and the type of hydrogel (ALG, fibrin-alginate [FA] and hyaluronate [HA]) on the *in vitro* follicular development. For this, secondary follicles (200 µm) were encapsulated or not (control) in ALG (0.25%, 0.5% or 1%) and cultured for 18 days and the recovered oocytes were destined for *in vitro* maturation. Estradiol and progesterone were measured and the mRNA levels of CYP19A1 and 3βHSD were quantified. Yet, follicles were cultured individually or in group (n=5), in multiple ALG beads or in the same bead, for 18 days. Next, follicles were encapsulated or not (control) in ALG, FA or HA and cultured for 18 days. Estradiol and progesterone were measured and the mRNA levels of CYP19A1, 3βHSD, Cx43, Cx37, MMP-9 and TIMP-2 were quantified. Finally, groups of 5 follicles were encapsulated in FA and cultured for 30 days. The *in vitro* matured oocytes were parthenogenetically activated. ALG 0.25% improved the antrum formation, growth, hormone production, gene expression of CYP19A1 and 3βHSD and the meiotic resumption compared to the others ALG concentrations ($P<0.05$). The culture of 5 follicles in the same ALG bead significantly enhanced follicular diameter compared to the individual culture, and antrum formation and meiotic resumption in relation to the culture in group in individual ALG beads. Follicles cultured in FA grew progressively until day 18 and generated mature oocytes. The hormone secretion was higher in follicles encapsulated in hydrogels ($P<0.05$). mRNA levels for all genes evaluated, except for CYP19A1, were similar between the FA group and uncultured antral follicles. 8-cell embryos were obtained after parthenogenetic activation of oocytes from follicles cultured in FA for 30 days. In conclusion, ALG 0.25% improved the *in vitro* development of goat preantral follicles. The encapsulation of 5 follicles in the same FA bead improved oocyte maturation which, after parthenogenetic activation, resulted in 8-cell embryos.

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