The Influence of the Culture Medium on *In Vitro* Meiotic Competence of Canine Oocytes

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Abstract

Although in carnivores modern breeding biotechnologies have developed particularly over the last 15 years, studies in the bitch oocytes are less than in other species due to the reduced availability of biological material. A major problem for *in vitro* embryo production in dogs is the particular ovulation physiology in this species. *In vitro* maturation of bitch oocytes is influenced by several factors: age of the donor female, estrous phase, hormonal stimulation, transport conditions of the samples, size and quality of the oocytes, the culture medium and atmosphere. To increase the rate of in vitro maturation of bitch oocytes, over time different media were used. The present approach focuses on summarizing the most frequently used mediums and their results for *in vitro* maturation of canine oocytes.

Keywords: canine, in vitro, maturation, medium, oocyte

In vitro maturation of bitch oocytes was first described in 1976 [1]. Unlike other mammalian species in witch oocyte is expelled from the preovulatory follicle in metaphase II stage of meiosis, the bitch oocyte is released in the immature stage of germinal vesicle, in prophase I, in a particular hormonal environment of preovulatory luteinisation [2]. The maturation occurs only after 48 – 72 hours spent in the oviduct. *In vitro* maturation rate is very low compared with that obtained in cattle, and maturation time required is higher (48 – 96 h) [3, 4].

Different culture media have been used, from simple media such as mKRB (modified Krebs Ringer Bicarbonate) or SOF (synthetic oviductal fluid) to more complex media such as TCM 199 (Tissue Culture Medium), with a more complex composition (inorganic salts, amino acids, vitamins).

Ovaries were collected from ovariectomies of healthy bitches in different stages of oestrus cycle.

Oocytes were collected by slicing for anoestrus ovaries, by follicular puncture, or by tubal flushing after ovulation.

TCM 199 is currently the most used medium, but the compounds responsible for its efficiency are not really known. The basic medium was supplemented with different compounds, trying to improve the rate of *in vitro* maturation.

Medium THY, 5% CO_2 and 95% air: approximately 32% of oocytes reached the metaphase II stage after 72 hours of culture in Krebs-Ringer modified solution (THY medium), containing 10% calf serum and gentamicin sulfate 30mg/kg [5].

Culture in isolated oviducts: the close physical interaction between the canine oocytes and the oviductal tract positively affects oocyte maturation, and meiosis is resumed within 30 hours of culture. Moreover, oocyte survival is better preserved within 30 hours in the ligated oviduct compared with the conventional culture in drop or to the culture in the open oviduct, but the ligated oviduct does not assure viability of the oocytes up to 48 h oh culture [6].

TCM 199 with fetal bovine serum and antibiotics: 99% of apparently healthy oocytes were in germinal vesicle stage at the time of collection,

and 25% of them reached metaphase I or II in 72 hours of culture [1].

Bovine serum albumin (BSA): high concentrations of BSA or FCS (fetal calf serum) appear to be optimal for *in vitro* maturation of bitch oocytes collected in the stage of germinal vesicle or germinal vesicle brake down, cultivated for 48 and 96 hours [4].

Epidermal growth factor (EGF) and ITS (insulin, transferrin, selenium): although the donor females were in anoestrus, resumption of meiosis occurred in a large proportion (on average 77.3%). ITS has a positive effect on nuclear progression to late stages (from metaphase I to metaphase II). Supplementation with EGF significantly reduced the percentage of oocytes with unidentifiable nuclear stage [7].

Progesterone: supplementation of culture medium with progesterone either during maturation or during meiotic arrest before maturation does not increase the rate of *in vitro* maturation of canine oocytes [8].

Proteins and heterologous hormones: the highest rates of meiotic resumption were achieved with the 0.4% BSA (bovine serum albumine) supplementation. A positive influence on the metaphase II acquisition rate was observed with hTS (human somatotropin) supplement. Oocytes cultured with 10% EBS (estrous bitch serum) did not develop to the MII stage. Protein and hormone supplements to TCM-199 culture medium did not promote the final steps of IVM of bitch oocytes [9].

Estradiol and hST: supports nuclear and cytoplasmic maturation of canine oocytes. Meiotic competence was verified by *in vitro* production and development of embryos up to the 8 cell stage [10].

Follicle-stimulating hormone (FSH) and luteinizing hormone (LH): in general, they play important roles in the regulation of cumulus cell expansion and oocyte maturation. A FSH supplement in IVM medium can increase cumulus cell expansion and nuclear maturation, while the nuclear maturation rate remained low [11].

Polyvinyl-pirolidone (PVP) and ECS (estrous cow serum): nuclear maturation of domestic dog oocytes can be achieved using TCM 199 supplemented with PVP, and this yields results similar to those obtained using media containing serum, gonadotropins, or estradiol [12].

Oocytes embedded in collagen gel: the percentage of the oocytes reaching the metaphase I and metaphase II was significantly higher in cumulus-oocytes complexes with collagen-gel embedding than in COCs without collagen-gel embedding. The percentage of oocytes that were arrested at the germinal vesicle stage was significant lower in oocytes cultured with gonadotrophins than in oocytes cultured without gonadotrophins. However, there were no significant differences in the percentages of groups, irrespective of the duration of exposure to gonadotrophins. These observations indicate that embedding of COCs by collagen enhances the meiotic competence of canine oocytes, but removal of hormone supplement from maturation medium does not improve the ability of the oocytes to reach MII stage [13].

To improve the rate of *in vitro* maturation of canine oocytes, further studies are needed, primarily aimed at a better understanding of the complexity of the maturation process of oocytes *in vivo*, so it may be adapted to culture conditions *in vitro*.

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