Proceedings of the 8th International Symposium on Canine and Feline Reproduction
ISCFR

June 22-25, 2016
Paris, France

In a joint meeting with the XIX EVSSAR Congress

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In vitro maturation of bitch oocytes with sequential addition of hormones

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The potential benefits of assisted reproduction techniques, such as in vitro maturation (IVM) and in vitro fertilization (IVF) in canids, are linked to the protection and saving of species threatened by extinction due to worldwide habitat destruction and pollution. Efficiency of in vitro oocyte maturation is very low in this species compared to that of other mammalian species. \cite{Luvoni2005} stated that canine oocyte is exposed to high concentration of progesterone in the follicular environment; it is ovulated in the dictyate state, and completes meiosis in the oviduct. Therefore, optimum conditions for in vitro maturation of dog oocytes may differ from other mammalian models in which estrogens is the dominant hormone and ovulate oocytes at the metaphase II stage of the first meiotic division. In this study a total of 134 ovaries were collected from bitches after routine ovariohysterectomy, from these ovaries 4848 COCs were retrieved. Out of the 4848 COCs, good qualities COCs were 2200. From these 1080 good quality COCs were randomly allotted to three groups which were cultured in TCM-199 medium supplemented with 10\% estrus bitch serum and incubated in 5\% CO\textsubscript{2} at 37°C for 96 h. In Group I (Control): without addition of any hormones. In Group II and III sequential hormone groups were designed in order to mimic the dog’s in vivo endocrine milieu at the time of oocyte culture. For this purpose, gonadotropins and steroids were added to the incubation medium of group I from 0 – 24 h were 2 \( \mu \)g/ml FSH + 20 \( \mu \)g/ml \( \beta \)-Estradiol and from 24-48 h: 2 \( \mu \)g/ml FSH + 20 \( \mu \)g/ml \( \beta \)-Estradiol + 1 \( \mu \)g/ml LH from 48-72 h: 10 \( \mu \)g/ml FSH + 5 \( \mu \)g/ml \( \beta \)-Estradiol +10 \( \mu \)g/ml LH+4 \( \mu \)g/ml Progesterone and from 72-96 h: 2 \( \mu \)g/ml FSH + 2 \( \mu \)g/ml Progesterone and in Group III same as in Group II up to 72 h and later from 72-96 h: 2 \( \mu \)g/ml FSH + 5 \( \mu \)g/ml LH + 4 \( \mu \)g/ml Progesterone were added to culture media. The nuclear maturation rates to GV, GVBD and MII stages in group I,II and III were 15.32, 19.81, and 0.9; 14.02, 32.71 and 3.74; and 13.27, 32.74, and 2.65 percent respectively in 5 COCs per droplet maturation medium, similarly in 10 COCs per droplet maturation medium were 15.98, 20.09 and 1.37; 13.51, 33.78, and 4.05; and, 14.41, 34.06 and 3.06 percent in group I, II and III respectively. Similar results were reported by \cite{Evecen2011} with the sequential hormone addition. In this study 10 COCs culture per droplet has given maturation results more than 5 COCs and sequential addition of hormones group has given better in vitro maturation of oocytes then the control group because the gradual changing of hormones mimicking the natural in vivo hormonal milieu even though the maturation results are lower than the other domestic species. It is due to the structural and functional variations in the cumulus cells and gap junction arrangements around the dog oocytes. So, further research has to be carried out to get the better in vitro maturation results in bitch oocytes by optimizing the FSH, LH, E2 and P4 concentrations in incubation medium.
