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Effect of pyometra on in vitro maturation of canine oocytes collected in different stages of the estrous cycle

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The current in vitro maturation protocol in the dog is not reliable as percentages of oocytes reaching the metaphase II stage range from 0 to 58\% [1]. Previous studies have indicated that stage of reproductive cycle and culture medium composition are key factors contributing to low meiotic competence observed in this species. To date, there have been no studies examining the influence of uterine health on oocyte developmental competence. Uterine bacterial infections or inflammation during follicle growth, oocyte maturation and early embryo development are associated with infertility. Thus, the aim of this study was to evaluate the influence of pyometra on meiotic competency of oocytes obtained during anestrus versus diestrus. Ovaries were collected from 14 healthy bitches and 14 individuals having pyometra after ovariohysterectomy, transported in phosphate buffer saline (PBS) at 4\(^\circ\)C and serially sliced to release the cumulus oocyte complexes (COCs). Only COCs grade I were selected and cultured in HEPES-buffered TCM 199 medium supplemented with 26mM sodium bicarbonate, 1.5mM sodium pyruvate, 0.6mM cysteine, 0.03UI/mL hCG, 0.5μg/mL FSH, 20mg/mL E\(_2\) and 10ng/mL EGF. COCs were cultured for 72h at 38,5\(^\circ\)C and 5% CO\(_2\) in air in a humidified atmosphere. After IVM, the oocytes were denuded, fixed and stained with Hoescht 33342 (10μg/mL) to evaluate nuclear status. Statistical analysis was carried out by ANOVA and Fisher's test at 5\% significance level. In anestrous bitches, there was no effect of pyometra on metaphase I stage (M-I) (29\% versus 21\%) (p>0.05). However, percentage (p<0.05) of MII (11\%) and germinal vesicle breakdown (GVBD) oocytes (44\%) were higher in healthy females than pyometra individuals (3\% and 13\%, respectively). Furthermore, greater percentage (p<0.05) of degenerate oocytes in bitches with pyometra were observed (57\% versus 11.5\%). During diestrus, there was no effect of pyometra on metaphase II stage (M-II) (24\% versus 21\%). However, we found increased percentage (p<0.05) of GVBD (30\%) and MI oocytes (41\%) in healthy bitches compared with those with pyometra (22\% and 18\% respectively). High percentages (p<0.05) of degenerate oocytes in bitches with pyometra were also observed (35\% versus 5\%). This study demonstrated that uterine health and the stage of the estrous cycle influence in the oocyte maturation. Furthermore, oocytes recovered from females with pyometra can achieve nuclear maturation in vitro.