Recovery rate, morphological quality and nuclear maturity of canine cumulus-oocyte complexes collected from anestrous or diestrous bitches of different ages

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Abstract

Canine cumulus-oocyte complexes (COC) were recovered from ovaries of post-pubertal animals (1–3, 4–6 and 7–10 years old) at different ovarian estrous phases (anestrus and diestrus). The number of COCs, and the number and nuclear maturity of high-quality (grade-1) oocytes were assessed. For all animals, no significant differences were found between the two reproductive phases relatively to the total number of COCs and grade-1 oocytes recovered. However, significant higher numbers of COCs were recovered from young than from elderly animals, and the proportion of grade-1 oocytes was also significantly higher in the younger group than in the other two age-groups. Of 226 grade-1 oocytes, 73% were at the germinal vesicle stage (GV), 10% had resumed meiosis (9% at germinal vesicle breakdown; 1% at metaphase-I) and 17% were degenerated. A significant effect of the reproductive phase on oocyte nuclear maturity was found only for adult animals, with a higher number of GV oocytes being found at anestrous (79%) due to higher rates of meiosis resumption (34%) at diestrous. The high number of grade-1 oocytes with meiosis resumption and fragmented or unidentified nuclear contents, indicates that current criteria for the selection of viable canine COCs are not optimized and need a new definition.
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1. Introduction

In dogs, the most common method to obtain oocytes for in vitro maturation and fertilization is to slice ovaries obtained after routine ovariohysterectomy (OVH) to release the cumulus-oocyte complexes (COCs), and then select the best ones under stereomicroscopy based on morphological criteria [1–3]. Elective OVH is usually performed in anestrous or diestrous bitches of different ages and recovery rates of cumulus-oocyte complexes, as well as quality of the oocytes, could be affected by the ovarian estrous phase and by aging of the donor bitch.

Studies evaluating the effect of animal age on the total number of cumulus-oocyte complexes recovered and the proportion of high-quality (grade-1) oocytes, revealed contradictory results [4–6]. Some have shown that young and adult animals (1–6 years old) display a higher number of cumulus-oocyte complexes than older (>6 years old) animals [4,5], whereas others suggested that bitches greater than 9 years old have a higher

number of cumulus-oocyte complexes; although, the proportion of grade-1 oocytes was higher in the younger group [6]. Controversial data also exist in relation to the effect of the ovarian estrous phase on the recovery rate of cumulus-oocyte complexes and grade-1 oocytes [7]. Several studies found that ovarian reproductive phase had no effect on total number or on the quality of the recovered COCs [2,4,8], whereas others obtained a lower mean number of grade-1 oocytes from ovaries with functional luteal tissue, as compared to ovaries without functional luteal tissue [1]. Despite the fact that several studies confirmed that the large majority of the ovulated oocytes are arrested at prophase-I of meiosis [9–11], an experiment carried out after OVH of bitches in several phases of the reproductive cycle revealed that 7% of the recovered oocytes had resumed meiosis (germinal vesicle breakdown stage) and 15% had non-identifiable nuclear material [12]. Studies relating rates of oocyte nuclear maturity with the ovarian reproductive phase also revealed contradictory findings. While nuclear maturity was shown to be independent of the ovarian estrous phase in one study [12], another found a higher incidence (48%) of germinal vesicle breakdown at the anestrous stage [13].

Therefore, a heterogeneous population of COCs in terms of quality and nuclear maturity is retrieved from canine ovaries and the competence of the selected canine oocytes could be highly variable. This might be the reason for the low oocyte in vitro maturation efficiency in this species.

A selection of the donor bitches might be the key factor for optimizing the recovery of highly competent COCs. However, information on the characteristics of retrieved COCs from bitches of different ages and in anestrous or diestrous phase of the cycle are still needed and prompted this investigation.

For this purpose, the recovery rate of canine cumulus-oocyte complexes and of grade-1 oocytes, as well as the nuclear status of the recovered oocytes, were determined in relation to animal age (young, adult and elderly) and ovarian reproductive phase (anestrous and diestrous).

2. Materials and methods

Canine ovaries were collected after routine ovariohysterectomy (OVH) from 37 healthy, post-pubertal bitches, with ages ranging from 1 to 10 years that were either at the anestrous or diestrous phases of the ovarian estrous cycle. The reproductive status was established by gross macroscopical examination of the ovarian surface. Ovaries without visible follicles or a corpus luteum were considered to be from bitches in anestrous, whereas those with one or more pronounced corpora lutea were classified as being in diestrous [14]. A total of 37 animals were subdivided into 3 age-groups: 13 young (1–3 years), 18 adult (4–6 years) and 6 elderly (7–10 years old) dogs.

After OVH, ovaries were immediately transported to the laboratory at room temperature in 0.9% NaCl supplemented with 1% penicillin–streptomycin. They were then completely sliced in phosphate buffered saline (PBS) supplemented with 0.1% of bovine serum albumin (BSA) to release cumulus-oocyte complexes (COCs). The total number of the recovered COCs was then recorded. After two or three washings, COCs were examined under a stereomicroscope at a final magnification of $90\times$ to select those exhibiting high-quality (grade-1) oocytes, according to the following morphological criteria: oocytes exhibiting ≥ 1 cumulus-cell layers, completely surrounding the oocyte, and containing an oocyte $> 100~\mu m$ in diameter with a uniformly dark pigmented ooplasm [3,7,15]. Only grade-1 oocytes were used to evaluate the oocyte nuclear maturation stage. To assess the nuclear maturity status, cumulus-cells were removed by vortexing the oocytes in 1% sodium citrate buffer or by mechanical cell displacement using a small-diameter glass micropipette. The
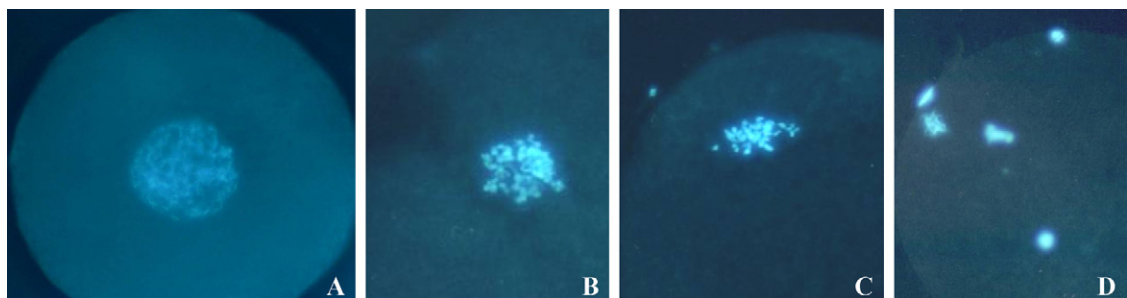

Fig. 1. Canine grade-1 oocyte chromatin configurations. Germinal vesicle (A), germinal vesicle breakdown (B), metaphase-I (C) and degenerated (D) ($400\times$).