

## Quantity and quality of oocytes recovered from donor bitches of different ages

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### Abstract

In vitro studies that use isolated oocytes benefit from the ability to harvest oocytes of excellent morphological quality in sufficient numbers to allow the replicability of techniques and experiments. The objective of the present study was to verify the effect of the age of the donor bitch on the quantity and quality of oocytes recovered from isolated ovaries, using the slicing technique. Ten bitches (45 days to 13 years) were ovariectomized, and the ovaries were placed in phosphate buffered saline (PBS), supplemented with bovine fetal serum (5%), and oocyte-cumulus-complexes (OCCs) were obtained by slicing the ovarian tissue. The OCSs were classified morphologically as Degree I (DI, best), Degree II (DII) and degenerated. A total of 427 oocytes were acquired, including 81, 109 and 237 that were graded as DI, DII and degenerated, respectively. Slicing yielded no OCS from animals <2 months of age. In senile (>9 years) bitches, bitches, there were more oocytes per bitch, compared to adult (2–6.5 years) bitches, but fewer DI oocytes, and more DII and degenerate oocytes. We inferred that using donors that were post-pubertal but not senile, would assure the recovery of high-quality oocytes by the slicing method. Additional studies are required to assess the quality of oocytes collected from pre-pubertal versus post-pubertal bitches <2 years of age.

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**Keywords:** Oocytes; Bitch; Age

### 1. Introduction

The search for methods to preserve threatened or endangered canid species has stimulated research on oocyte physiology, morphology, development and maturation and methods to increase rates of oocyte in vitro maturation (IVM) and in vitro fertilization (IVF) in canid species, including the domestic dog and fox [1–4]. In studies involving canine oocyte IVM and IVF,

one of the main concerns is the quantity and quality of oocytes recovered. The classification of oocyte quality is typically done by analysis of the cytoplasmic appearance and the presence of multiple layers of cumulus cells. Large variations in oocyte quality are described in the literature. Oocyte degeneration indices can range from 20 to 68% [2,4]; it is noteworthy that suboptimal quality oocytes do not yield good results when used for IVM or IVF [3]. The reproductive state or age of the donor (or both) may also influence oocyte quality, although study of age effect has been limited. Young bitches have a better oocyte index than older bitches [2]. The present study aimed to further assess the effect of the age of donor bitches on the quantity and quality of oocytes recovered from their isolated ovaries.

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Table 1

Numbers of oocytes in each oocyte-quality class obtained from individual bitches in the three age groups

Oocyte class	Young (<60 days)					Adult (2–6.5 years)					Senile (>9.5 years)		
	1	2	3	4	Average	1	2	3	4	Average	1	2	Average
All classes combined (total no. recovered)	0	0	0	0	0	62	68	45	54	57.25	89	109	99
DI	0	0	0	0	0	20	19	9	13	15.25	8	12	10
DII	0	0	0	0	0	19	21	12	11	15.75	20	26	23
Degenerated	0	0	0	0	0	23	28	24	30	26.25	61	71	66

## 2. Materials and methods

### 2.1. Dogs

Ten bitches between 45 days and 13.5 years of age were ovariohysterectomized at the Zoonosis Control Center of Campos dos Goytacazes, Brazil. The dogs were divided into three age groups: young donors (45–60 days;  $n = 4$ ), adult donors (2–6.5 years,  $n = 4$ ) and senile donors (9.5 and 13 years;  $n = 2$ ).

### 2.2. Ovaries and oocyte collection (sampling)

After surgery, the ovaries were transported to our laboratory (in 0.9% NaCl solution at room temperature). The ovaries were placed in a 5% bovine fetal serum solution in phosphate buffered saline (PBS) and sliced to recover oocyte-cumulus-complexes (OCCs).

### 2.3. Oocyte assessment

Oocyte morphology was assessed via stereomicroscopy and classified as Degree I (DI), Degree II (DII), and degenerated, according to Hewitt and England (1998) [3]. In brief, the classification involved the following. The DI oocytes were considered as having the best quality, characterized by a strongly dark cytoplasm and completely surrounded by layers of cumulus cells. The DII oocytes had light cytoplasmic pigmentation and incomplete layers of cumulus cells, whereas the degenerated oocytes had pale cytoplasm lacked cumulus cells. The morphological classification and scoring of OCCs was done separately for each donor.

### 2.4. Statistical analysis

Averages were determined by summing the totals of every bitch in the age group for each oocyte category, divided by the number of females in the group (Table 1). The average number of Degree I, II and deg oocytes in

each age group compared with ANOVA, and differences located with the Tukey-HSD test. Probability values  $< 0.05$  were considered significant.

## 3. Results and discussion

A total of 427 oocytes were recovered, including 81 GI, 109 GII and 237 degenerated (Table 1). No oocytes were obtained from young bitches, in contrast to the observations reported by Hay (1996) [2]. However, Durrant et al. [1] reported that ovaries of young donors (<6 months of age) had fewer follicles than the ovaries of middle-age donors (7–48 months of age), therefore young bitches yield a small number of oocytes. The females used in the present study were very young (1.5–2 months old) and no follicles were grossly apparent on the ovarian surface. Furthermore, the absence of oocyte recovery in these donors may have been related to increased difficulty to release oocytes from pre-antral follicles by the slicing technique used. For adult bitches, the total number of oocytes per bitch varied between 45 and 68. For the group of senile bitches, total oocytes per bitch varied from 89 to 109, greater ( $P < 0.05$ ) than in the adult bitches (Table 2). The senile bitches yielded on average more total oocytes, significantly fewer DI oocytes, and more degenerate oocytes than adults (Table 2). These results corroborated our preliminary

Table 2

Total numbers of oocytes and oocytes classified according to quality obtained from bitches in each age group

Oocytes	Young	Adult	Senile
Total	0	57.2 <sup>a</sup>	99.0 <sup>b</sup>
DI	0	15.2 <sup>a</sup>	10.0 <sup>b</sup>
DII	0	15.8	23.0
Degenerated	0	26.2 <sup>a</sup>	66.0 <sup>b</sup>

Different letters within the same oocyte group indicate a difference between adults and senile animals ( $P < 0.05$ ). Data for young bitches were considered null and they were excluded from the statistical analysis. DI: Degree I (best) oocytes; DII: Degree II (intermediate quality) oocytes.

observations [5] and were in agreement with an earlier observation that senile bitches have decreased oocyte quality [3].

The present results, albeit on a limited number of bitches, suggested for the domestic dog that adult bitches <7 years old are likely to yield more oocytes of higher quality for in vitro study than very young or very old animals. However, the pre- or post-pubertal ages at which similar or better oocyte yields might be obtained, and the age (from 5 to 9 years) at which oocyte quality declines, have not been studied in detail and merit further investigation.

### Acknowledgement

The authors would like to thank the CCZ for the surgeries and ovary donation for the experiments.

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