ABSTRACTS

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Introduction - Lack of developmental competence and degeneration of canine oocytes in vitro matured endorse the necessity of using more specific criteria to assess quality and integrity of gametes destined to in vitro maturation (IVM). Appropriate oocyte selection may in consequence enhance positively in vitro meiosis in the bitch. By far, morphological appearance of immature cumulus oocyte complexes (COCs), as determined by features such cumulus thickness, homogeneity of the ooplasm, and size [2] [3] has been routinely used to select dog oocytes subjected to IVM. In other species like bovine, swine, caprine, and more recently in the mice [5], the brilliant cresyl blue (BCB) test has been incorporated prior to culture to the selection methods as a non invasive technique for identifying fully-grown oocytes, with ability to mature to MII stage. The BCB test is based on the capability of glucose-6-phosphate dehydrogenase (G6PDH) to convert the dye from blue to colorless. Oocytes that have finished their growth show decreased G6PDH activity and will exhibit blue coloration (BCB+), whereas growing oocytes contain G6PDH and reduce the dye to a colourless solution (BCB-) [1].

Objective - The aim of the present study was to observe the features and levels of blue color impregnation in high quality (grade 1) immature canine oocytes stained with the BCB dye, as an indirect quality and integrity indicators of nuclear chromatin configuration in COCs selected to IVM. Moreover, because lack of developmental competence in oocytes has been attributed to follicular atresia, depending on ovarian donor reproductive status [4], we observed as well the influence of serum progesterone concentrations from ovary donors on BCB staining of immature oocytes.

Materials and methods - It was used the protocol proposed by El Shourbagy et al. [1] with slight modifications. Immediately after morphological selection, grade 1 oocytes were distributed in groups of maximum 5-7 and incubated in 80µl drops of 26µM BCB (Sigma, B-5388) diluted in modified PBS with 1.090mg/ml glucose, 35.2mg/ml pyruvate acid, 0.4% (w/v) BSA fraction V (Sigma A-9647) at +37 °C, in humidified atmosphere with 5% CO2 in air for 60 min. After the incubation time, the oocytes were examined under fluorescence microscopy and classified according to: (i) dark blue cytoplasmic staining (BCB+), (ii) fainty blue cytoplasmic staining (BCB±), and (iii) colourless cytoplasm (BCB–). Bitches were distributed in two groups according the serum progesterone levels as following: (i) bitches with serum progesterone varying from 0-2.5ng/ml (n = 5); (ii) bitches with serum progesterone varying from 2.6ng/ml to 16.7ng/ml(n = 4). Serum concentrations of progesterone were measured by chimoluminescence. The synchronization of BCB coloration between the ooplasm and cumulus cells was qualitatively assessed, but not included in the statistcal analysis.

Statistical analysis: Data were analyzed using Chi-square analysis with adjusted residual to compare the effect of oocyte’s morphology on BCB staining. ANOVA with repeated measures and two factors was performed to analyze the influence of serum progesterone concentrations of ovary donors on oocytes BCB staining, and ANOVA with repeated measures was used to to determine the differences on mean numbers (mean ± SD) of BCB
stained oocytes from 09 routines. The values were considered statistically significant when 
P<0.05.

**Results** - From 138 morphologically high quality COCs recovered from ovaries following 
sliding, the mean number of oocytes classified as the BCB+ was 9.9± 5.9, while the BCB± 
and the BCB- were 4.9± 2.9, and 0.6±1.7, respectively. Mean number of oocytes classified as 
grade 1 and stained BCB+ were statisically different from the BCB- (P= 0.010). Also, mean 
numbers of oocytes BCB± were different from those of BCB- (P= 0.014). In this experiment, 
the percentage of germinal vesicle (GV) in BCB+ stained immature oocytes was 67.4% 
(60/89) and much higher than the percentages observed in BCB± (52.2%) and BCB- (20%) 
stained oocytes. The rates of germinal vesicle break down (GVBD) stage also differ between 
the BCB groups with higher rates been observed at BCB± and BCB- stained oocytes (P= 
0.023). There was no effect of serum progesterone concentrations on the mean numbers 
of oocytes stained by the BCB dye (P= 0.680).

**Discussion and Conclusion** - In this experiment, the majority of BCB+ stained oocytes were 
observed at the germinal vesicle (GV) stage, demonstrating that in the dog this is the most 
probably feature to be expected in grade 1 oocytes previously selected by visual 
morphological appearence. Despite that, also we observed that resumption of meiosis, as 
identified by oocytes at the GVBD stage, was presented in 12.3% BCB+ oocytes, and this 
configuration may be vinculated to oocytes obtained from atretic follicles, as was previously 
proposed [2]. The GVBD pattern was found to be higher in BCB± and BCB- stained oocytes, 
and therefore the dye could possibly be used to separate oocytes from healthy follicles with 
developmental competence from those presumably derived from the atretic ones and therefore 
uncapable to achieve meiosis. One important factor was the inconsistency in BCB 
impregnation between the cumulus cells and the ooplasm in various from the observed 
oocytes. As reported by Wu et al. [5] for the mice, asynchrony in BCB coloration in COCs 
might suggest a disruption of metabolic coup ling between the oocyte and its cumulus cells. 
This criterion might be additionally useful in predicting canine oocyte competence in vitro. 
Therefore, the preliminary findings on BCB staining of dog oocytes seem worthy of further 
investigation.

**References**

competence and sperm penetration. Theriogenology 2000;54: 535-542
oestrus cycle and progesterone supplementation during culture on maturation of canine 