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# ABSENCE OF GREEN FLUORESCENCE IN CUMULUS OOPHORUS CELLS, IN CUMULUS-OOCYTE-COMPLEXES WITH FLUORESCENT OOCYTES FROM C57/BL6/EGFP MOUSE

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Generation of animal models, as GFP transgenic mice, is an important tool to understand the morphology and physiology of cells, isolated or in association to other cells. The functional unity of the cumulus-oocyte-complex (COC) is of essential importance to oocyte growth, maturation and fertilization understanding. The fluorescence of cumulus oophorus cells could be explored (coupled with nonfluorescent oocytes) to studies of the influence of those cells on denuded oocytes. The aim of this report was describe the lack of complete green fluorescence of the COC from EGFP mice. Four C57BL/6-Tg (CAG-EGFP) C14-Y01-FM131 Osb females, weighting around 35g, were sacrificed to other purposes (i.e., embryo recovery). Ovaries were collected and manipulated in PBS with 0.4% BSA. They were manually fragmented (23G needle) in order to release COCs. After 3 to 4 washes, groups of COCs and eventually primordial follicles, were evaluated with bright and UV light under an inverted microscope (Eclipse Ti-S, Nikon, Japan). Control COCs from four ovaries (Swiss Webster strain) were used to standard absence of fluorescence. Images were captured (NIS-Elements Advanced Research, Nikon, Japan) with both light sources and merged. As expected, control COCs did not fluoresce with specific UV light to EGFP (488nm). However, oocytes from C57/BL/6 EGFP fluoresced with high intensity, independently if they came from primordial or antral follicles. Interestingly, cumulus oophorus cells did not fluoresce even with their counterpart (i.e., oocyte) strongly glowing. When put together control COC and C57/BL/6 EGFP COC, no difference was detected between cumulus oophorus cells from both COCs under UV light. Herein it was reported that the cumulus-oocyte-complex from C57/BL/6 EGFP did not follow the constitutive gene expression of EGFP, as related previously when the Tg mouse was generated ("Green mice" as a source of ubiquitous green cells; Okabe et al., FEBS Letters, 1997:407;313-9). Cumulus cells should be put together with hair and red blood cells as the only cells/structures that do not fluoresce on described EGFP mouse. The authors wish to acknowledge Dr. Masaru Okabe (Genome Information Research Center, Research Institute for Microbial Diseases, Osaka University, Japan) for C57BL/6-Tg (CAG-EGFP) C14-Y01-FM131 Osb mice and FAPESP (São Paulo, Brazil) for funding (06/06491-2ª) and fellowships (09/16254-6-ª, 09/15919-4<sup>b</sup>, 09/17605-7° e 07/07705-9ª).

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# OVULATORY DYNAMICS OF TOGGENBURG GOATS SUBMITTED TO ESTROUS INDUCTION WITH AUTOCLAVED REUTILIZED INTRAVAGINAL DEVICES

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Reutilization of progesterone intravaginal devices may cause health risks. The aim of the study was to evaluate the influence of the autoclaving process in intravaginal devices on the ovulation parameters in Toggenburg goats. It was conducted in October of 2008 (non-breeding season), Piau-MG, Brazil (latitude 21<sup>3</sup>, 35' and longitude 43<sup>1</sup>, 15'). Twenty-one goats were selected and distributed according to weight and body condition scores, respectively, into three treatments: animals received new devices containing 0.33 g progesterone (CN Eazi-Breed CIDR®, Pfizer Animal Health, São Paulo, Brazil, n = 6, 40.7 ± 8.5 kg,  $3.4 \pm 0.2$ ), autoclaved (121 ° C, 1 atm, 15 min) devices used previously for six days (C6, n = 8, 45.6 ± 9.4 kg,  $3.5 \pm 0.3$ ) or 12 days (C12, n = 7, 49.8 ± 15.6 kg,  $3.5 \pm 0.2$ ). On the day of its insertion, 5 mg dinoprost (Lutalyse®, Pfizer Animal Health) laterovulvar and 24 h before its removal 200 IU eCG (5000 Novormon®, Sintex Industries Biochemistry, Buenos Aires, Argentina) latero-vulvar were given. The devices were maintained for six days in all treatments. Estrous detection was performed twice daily with the use of bucks. Goats were examined by transrectal ultrasonography (Aloka SSD 500®, Tokyo, Japan) daily from device insertion to its removal (D0-D6) and every 12 h until ovulation or 96 h after device removal (D6-D10). Progesterone plasmatic concentration was determined by the use of solid phase radioimmuneassay technique seven days before device insertion. Statistical analysis was performed using all tests with a significant interval of 95%. Nonparametric variables were compared among treatments using the chi-square test. Parametric variables were submitted to ANOVA and SNK by the SAEG program. The results are described as mean ± SD. All goats showed subluteal (<1ng/mL) progesterone concentrations seven days before receiving the devices, reflecting the reproductive seasonality of this breed at that latitude. No difference was detected (P> 0.05) among treatments on the following parameters: females ovulating rate (%) for NC (100.0; 6/6), C6 (87.5, 7/8), C12 (100.0, 7/7); Number of ovulations for NC ( $1.5 \pm 0.5$ , 9/6), C6 ( $1.9 \pm 0.7$ , 13/7), C12 ( $1.7 \pm 0.8$ , 12/7); Interval from device removal to ovulation (h) for NC (72.0 ± 13.1), C6 (61.7 ± 4.5), C12 (72.0 ± 17,0); Interval from estrus to ovulation (h) for NC (40.0 ± 9.8), C6 (29.1 ± 13.6), C12 (41.1 ± 11.7); Diameter of dominant follicle (mm) for NC (7.6 ± 1.0), C6 (7.3 ± 0.4), C12 (7.4 ± 0.3) and Diameter of codominant follicle (mm) for NC (7,1 ± 1.1), C6 (6.9 ± 0.5), C12 (6.5 ± 0.2). It is concluded that reused autoclaved devices present similar efficiency regarding goats reproductive parameters associated to ovulation. Financial Support: CNPg, Embrapa Goats and Sheep Keywords: Caprine, CIDR, Follicular Dynamics, Reproductive Efficiency

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