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EFFECT OF HEAT STRESS DURING MATURATION OF OOCYTES AND IN VITRO PRODUCTION OF EMBRYOS IN GOATS AND SHEEP

Santos Jr., E.R.[1]; Freitas Neto, L.M.[1]; Santos, M.H.B.[2]; Aguiar Filho, C.R.[1]; Almeida-Irmão, J.M.[1]; Bezerra, F.Q.G.[1]; Chaves, R.M.[1]; Lima, P.F.[1]; Oliveira, M.A.L.[1]

[1]Laboratório de Biotécnicas Aplicadas à Reprodução do Departamento de Medicina Veterinária da Universidade Federal Rural de Pernambuco, Recife, PE; [2]Bolsita (BFP) da Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE), Recife, PE – Brasil

E-mail: eduardorazza@gmail.com

In the Northeast region of Brazil, the environmental conditions compromise the reproductive efficiency of domestic animals with economic interest, resulting in economic losses in the region. The oocyte and embryo are the most affected t with the negative effects induced by thermal stress. This study aimed to determine the effect of heat stress during maturation of oocytes and in vitro production of embryos in goats and sheep. Ovaries were collected in a slaughterhouse and transported to the Laboratory of Biotechnical Reproduction of UFRPE. The cumulus-oocyte-complexes (COCs) were collected by the technique of "slicing" of the follicles from 2 to 6 mm in diameter and selected based on morphological classification and placed on the basic stages. In 10 replicates the COCs were submitted to the caloric heat stress at 41°C for 0 (the thermoneutral 39°C), 3, 6, 12, 18 and 24 hours of maturation in vitro. The percentage of oocytes was determined in the maturation, fertilization, cleaved (D-3), stage of 8-16 cells (D-4), morale (D-5), blastocyst (D-8) after fertilization and blastocyst positive for apoptosis by TUNEL assay. The statistical analysis was performed to compare variances and F test meant for variances with significance level of 5% ($P < 0.05$). Then, the t test was performed to compare means at significance level 5%. Significant difference ($P < 0.05$) was observed at all periods of maturation with thermal stress in both species. In goats, there was significant difference ($P < 0.05$) in fertilization 0h (42%), 3h (25.4%), 6 (17.7%), 12h (11.9%), 18h (7.9 %) and 24 (3.4%). On D-3 was different at 0h (28.1%) and 3h (23.6%), 6h (14.1%), 12h (6.8%), 18h (4.7%) and 24 (1.8%). On D-4 0h (26.9%), 3h (22.5%), 6h (12.1%), 12h (4.8%), 18h (2.6%) and 24 (1.5%) as the D-5 0h (20.3%), 3h (15.9%), 6h (8.8%), 12h (3.0%), 18h (1.6%) and 24 (1.0%) significant differences were observed between the different exposure times. The D-8 was not significantly different ($P > 0.05$) between the periods of 3 vs 6 to 18 vs 24 h and the blastocyst TUNEL positive at 0 vs 3, 3 vs 6, 12 vs 18 and 18 vs 24 h of heat stress. In sheeps, significant difference ($P < 0.05$) was observed between the different exposure times in maturation, 0h (75.8%), 3h (50.0%), 6h (35.4%), 12h (12.4%), 18h (9.9%) and 24h (4.6%). Significant difference ($P < 0.05$) was not observed in fertilization, D-3, D-4, D-5 and D-8 between the periods of 18 vs 24h and the blastocysts TUNEL positive at 0 vs 3, 3 vs 6, 12 vs 18 and 18 vs 24 h of heat stress. Under the conditions observed in this study, the results allows to conclude that a minimum of 3 hours exposure to heat stress at 41°C during in vitro maturation is sufficient to cause significant deleterious effects on embryonic development and in their level of apoptotic cells.

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LUTEAL VOLUME AND PROGESTERONE SERUM CONCENTRATION IN SANTA INES SHEEP SINCHRONIZED WITH GnRH IN A LONG OR A SHORT PROTOCOL

Teixeira, H.C.A.[1,2]; Silva[1], B.D.M.[1]; Silva, T.A.S.N.[1]; Paiva Neto, M.A.[2]; Neves, J.P.[1]; Ramos, A.F.[2]

[1]Faculdade de Agronomia e Medicina Veterinária, FAV/UnB, Brasília-DF; [2]Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF - Brasil

E-mail: heitortx@gmail.com; aleframos@cenargen.embrapa.br

This study had the objective to evaluate the number of CLs, the volume of the luteal mass and progesterone serum concentration in different protocols for estrus synchronization in Santa Ines sheep. Forty one sheep were subjected to four protocols for estrus synchronization and induction of ovulation: Long+GnRH (n = 11) where the vaginal sponge containing 60 mg of medroxyprogesterone (MAP) were maintained for 12 days, administration of 300 IU of eCG on day 12 and 25 ìg of GnRH (gonadorelin acetate) 27 hours after sponge removal; Long (n = 10) stayed with the sponge for 12 days and 300 IU of eCG was administered in its removal; Short+GnRH (n = 10) had sponge staying for 7 days, associated with administration of 37.5 ìg D-cloprostenol on the fifth day, 300 IU of eCG on the seventh day and 25 ìg of GnRH 27 hours after sponge removal; Short (n = 10) the sponge stayed for seven days, on the fifth day was administered 37.5 ìg of D-cloprostenol and at sponge removal was administered 300 IU of eCG. Twelve days after sponge removal blood was collected, and the serum was frozen at -20°C for later determination of progesterone concentration by radioimmunoassay. In the same day laparoscopic and ultrasonographic evaluation was performed to count CLs and for measuring the volume of the CLs. The volume of the luteal mass was calculated by summing the volume of each CL. For statistical analysis were used ANOVA and Duncan's test with statistical program SAEG. The estrus synchronization protocol Long+GnRH (2.3±1.1) showed a larger number of corpus luteum ($P<0.05$) when compared with the Long and Short+GnRH protocols (1.5±0.5 and 1.4±0.7 respectively) and was similar to Short protocol (1.7±0.5). The volume of the luteal mass (0.8±0.6 cm^3, 0.6±0.2 cm^3, 0.6±0.3 cm^3, 0.7±0.4 cm^3 in Long+GnRH, Long, Short+GnRH and Short protocols respectively) and serum progesterone concentrations (5.2±5.7 ng/ml, 5.3±2.2 ng/ml, 4.1±2.9 ng/ml, 5.7±2.4 ng/ml in Long+GnRH, Long, Short+GnRH and Short protocols respectively) showed no differences between treatments ($P> 0.05$). Despite the Long+GnRH protocol had a higher number of CLs, it showed no higher volume of the luteal mass and no higher progesterone concentration. By presenting the luteal volume and progesterone levels similar to other treatments, what is important to achieve a good pregnancy rate, the Long+GnRH protocol showed up as an alternative to increase ovulation rates in sheep TAI programs.