168 INFLUENCE OF SEVERAL INSULIN PLASMA CONCENTRATIONS ON PROGESTERONE PRODUCTION AND HISTOLOGY OF CORPORA LUTEA IN SUPEROVULATED EWES

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Abstract

Nutritional status is essential for the determination of mammalian reproductive performance, and insulin is one of the main indicators for such condition. This hormone is able to interfere, directly or indirectly, with CL function, leading to alterations in steroidogenic capacity and cell development and, consequently, early embryonic development. The aim of this work was to identify CL histological alterations caused by different insulin plasma concentrations in superovulated ewes, as well as their interference in CL progesterone production. Santa Inês ewes (n = 24, 4 years old) were divided into 3 treatments: control (T1, n = 9); hypoinsulinemic (T2, n = 6), and hyperinsulinemic (T3, n = 9). In order to become hypoinsulinemic, ewes were treated with a single dose of alloxan monohydrate (50 mg kg^{-1} i.v; Sigma-Aldich, St. Louis, MO, USA) 48 h before the beginning of the superovulation protocol, whereas the hyperinsulinemic group was treated with administrations of 20 IU of NPH human insulin at every 12 h (Biolin-N; BIOBRAS, Montes ros, Brazil). All animals were fed corn silage and minerals ad libitum. Animals had their estrus synchronized with vaginal pessaries ntaining medroxyprogesterone acetate (60 mg) and were superovulated with porcine FSH (250 IU, PLUSET®, Calier, Spain) and eCG (250 IU, Novormon®, Schering-Plough, Kenilworth, NJ, USA), in a 13-day protocol. After removal of vaginal pessaries, blood samples used for quantification of progesterone and plasmatic insulin by radioimmunoassay were collected daily at 0700 h until the day before embryo recovery. Hysterectomy was performed in all groups after embryo recovery (T1: n = 3, T2: n = 6, and T3: n = 4), and ovaries were placed in buffered formalin saline. CL histological sections were evaluated by hematoxylin/eosin staining. Results were assessed by SAS statistical analysis software, using the MIXED procedure (SAS Institute Inc., Cary, NC, USA). Mean values were compared by Tukey's test. The mean CL number was lower in T2 (5.0 ± 2.7; $P < 0.05$) compared with T1 (10.2 ± 5.1) and T3 (11.3 ± 3.0) animals. No histological differences were observed between treatments T1 and T3. However, CL within the T2 group had a higher number of cells with picnotic nuclei and strongly contracted eosinophilic cytoplasm. Such alterations are suggestive of cellular apoptosis. The T2 group also differed for P4 production ($P < 0.01$) from the second (T2: 2.23 ± 0.71 ng mL^{-1}; T1: 5.42 ± 4.01 ng mL^{-1}; T3: 6.44 ± 2.76 ng mL^{-1}) to the sixth day post-estrus (T2: 7.58 ± 7.00; T1: 24.79 ± 8.40; T3: 32.07 ± 0.85 ng mL^{-1}). The mean insulin plasma concentration differed between treatments ($P < 0.01$); higher concentrations were obtained in the T3 group (20.05 ± 7.50 µIU mL^{-1}), whereas the T2 group had lower concentrations (10.18 ± 3.57 µIU mL^{-1}) compared to controls (T1: 14.52 ± 3.80 µIU mL^{-1}). In conclusion, low plasma concentration of insulin may restrict the response to superovulatory treatment and cause CL histological changes, suggesting a reduction in cell activity due to premature cellular senescence.

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