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Does dexamethasone treatment change the expressions of proliferating cell nuclear antigen (PCNA), cyclin D3, p27, p57 in normal and dexamethasone-induced intrauterine growth restricted rat placentas?

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Intrauterine growth restriction (IUGR) described as only to those infants with birth weight and/or birth length below the 10th percentile for GA with a pathologic restriction of fetal growth [1]. It is a major clinical problem which causes perinatal morbidity and mortality and major etiological factor is abnormal placentation [2]. Despite the fact that placental development requires the coordinated action of trophoblast proliferation and differentiation, there are few studies on cell cycle regulators, which play the main roles in the coordination of these events and it is still not determined how mechanisms of coordination of proliferation and differentiation are influenced by dexamethasone-induced IUGR in the placenta.

Female rats were mated with male rats, presence of sperm in vaginal smear accepted day 0 of pregnancy. Rats were injected 100 µg/kg dexamethasone on day 13, 200 µg/kg dexamethasone on days 14-19 of pregnancy. Control animals were injected saline solution. Six rats each group were sacrified for each method. After Rattus norvegicus rats were sacrified on day 20 of pregnancy, blood samples were taken, placentas were formaline fixed-parafin embedded or snap-frozen. We applied Reverse Transcriptase Polymerase Chain Reaction (RT-PCR), immunohistochemistry, Western blotting, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling (TUNEL), glucocorticoid assay and did transmission electron microscopic observations.

Although there was a statistically significant ($p<0.001$) increase of glucocorticoid levels 60 minutes after dexamethasone injection, it was normal 180 minutes after the injection in IUGR group. Mean embryo and placenta weights of control rats were higher than IUGR group with statistically significant difference ($p<0.005$) (Figure 1) but dexamethasone didn't affect the number of embryos. According to RT-PCR, immunohistochemistry and Western blotting results, expression of PCNA was higher in control group than in IUGR group and it was statistically significant ($p=0,041$), expressions of cyclin D3, p27 and p57 were higher in IUGR group. TUNEL positive cell numbers in IUGR group placentas were higher than control group placentas ($p<0.001$). Electron microscopic observations were compatible with TUNEL results. Spongiotrophoblasts and labyrinth trophoblasts of IUGR placentas showed apoptotic cell characteristics (Figure 2).

Our data suggests that glucocorticoid-induced restriction of fetal-placental growth is mediated, in part, via inhibition of cell cycle proteins and increase in apoptosis. Previous studies showed that dexamethasone caused a decrease in growth-promoting genes [3]. Glucocorticoid metabolism during pregnancy is still debated. How dexamethasone acts in placental growth inhibition hasn't been determined. This study described decrease of proliferation, increase of apoptosis in dexamethasone injected IUGR rat placentas. Since dexamethasone is widely used to women having premature labor risk, reduces fetal growth and predisposes to increased risk of disease in later life, detailed studies should be done.

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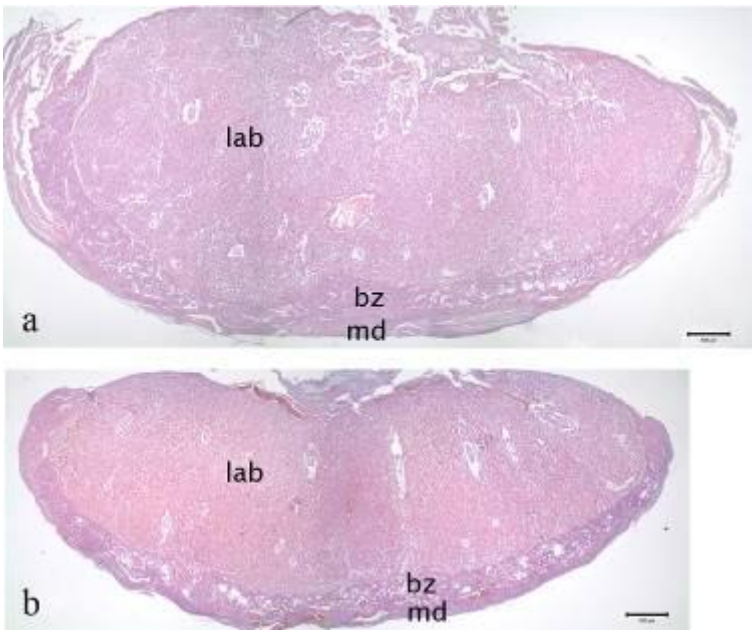

Figure 1. Light microscopy of control (a) and IUGR (b) placentas. The labyrinthine (lab) and junctional zones (bz) of control placenta are greater than the same zones of IUGR placenta. md: maternal decidua. Hematoxylin and Eosin. Bar: 500µm.

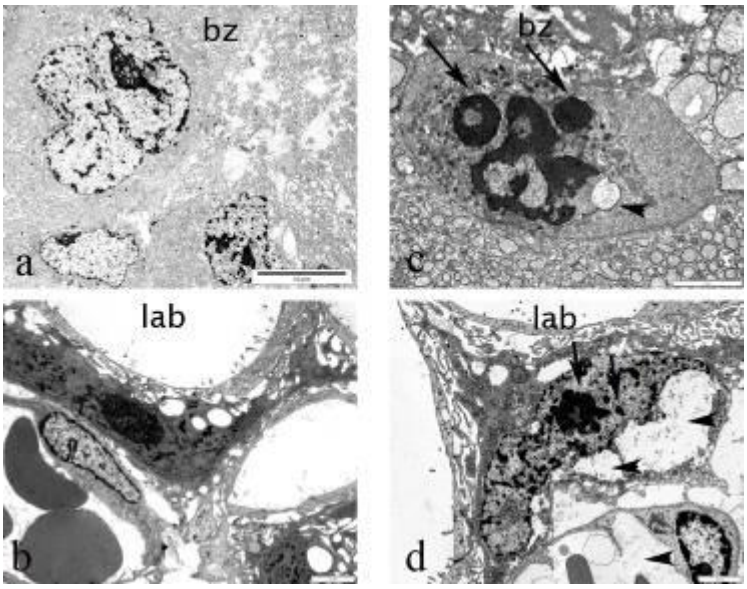

Figure 2. Electron micrographs of control (a, b) and IUGR (c, d) placentas. Normal spongiotrophoblast (a) and trophoblast (b) cells in control labyrinthine (lab) and junctional zones (bz) but a spongiotrophoblast with dense chromatin, fragmentation (arrow) (c) and degradation (arrow head) (c) was seen in junctional zone of IUGR placenta. A trophoblast with fragmentation (arrow) (d) and vacuoles (arrow head) (d) was seen in labyrinthine zone of IUGR placenta. Bars: a: 10µm, b, c, d: 2µm.