Keywords: Chronic stress; Apoptosis; Cell proliferation; Placenta

Introduction

e placenta is an extraembryonic tissue located between the maternal and fetal compartments [1]. is structure provides highly specialized functions during gestation that are critical for the normal embryo/fetus development [2]. is structure performs the functions of most of fetal organs throughout gestation due to the immaturity of the embryo [3].

Rodents possess two placental structures: the choriovitelline placenta, which develops rst (until the day 11 of pregnancy) and the chorioallantoic placenta, which develops in the second half of gestation, showing considerable developmental changes with advancing gestation. Two prominent regions are formed within the chorioallantoic placenta: 1) Junctional Zone (JZ) and 2) Labyrinth Zone (LZ) [4]. Four di erentiated trophoblast cell phenotypes comprising the rat chorioallantoic placenta can be readily identi ed: 1) trophoblast

Stressing situations simultaneously activate both the hypothalamic-pituitary-adrenocortical axis and the sympathetic-medullary-adrenal axis, but it has been postulated that both axis respond di erently to stressful stimuli [9-13]. e Corticotropin-Releasing Hormone (CRH), secreted by the hypothalamus stimulates the Adrenocorticotropin-Releasing Hormone (ACTH) secretion by the pituitary [14]. In turn, ACTH stimulates the secretion of glucocorticoids by the adrenal cortex. In the course of pregnancy, CRH is also produced by the placenta and has the same biological activity as hypothalamic CRH [15-17]. e fetal sympathetic-medullary-adrenal axis, releases nally adrenaline and noradrenaline to restore homeostasis [18].

Materials and Methods

Animal's

Young female primipar Wistar-albino rats of 200 to 300 g were used. Four pregnant rats per cage were housed and, allowed *ad-libitum* access to food and water and maintained on a constant 12:12 light/dark cycle at constant room temperature (22°C ± 2°C) and humidity (60%) (Laboratory installations were adequate to disposition 6344/96 of the Administración Nacional de Medicamentos, Alimentos y Tecnología Médica, and Argentina. e Conclusions and Recommendation on the Reduction, Re nement and Replacement of Laboratory Animals Procedure of Declaration of Bologna were followed for animal experimentation. All experiments were conducted according to the principles and procedures of the NIH Guide for the Care and Use of Laboratory Animals (NIH publication n°85-23, revised 1985. Females were mated during the proestrous with a same strain male and the day on which spermatozoa were present in a vaginal smear was designated

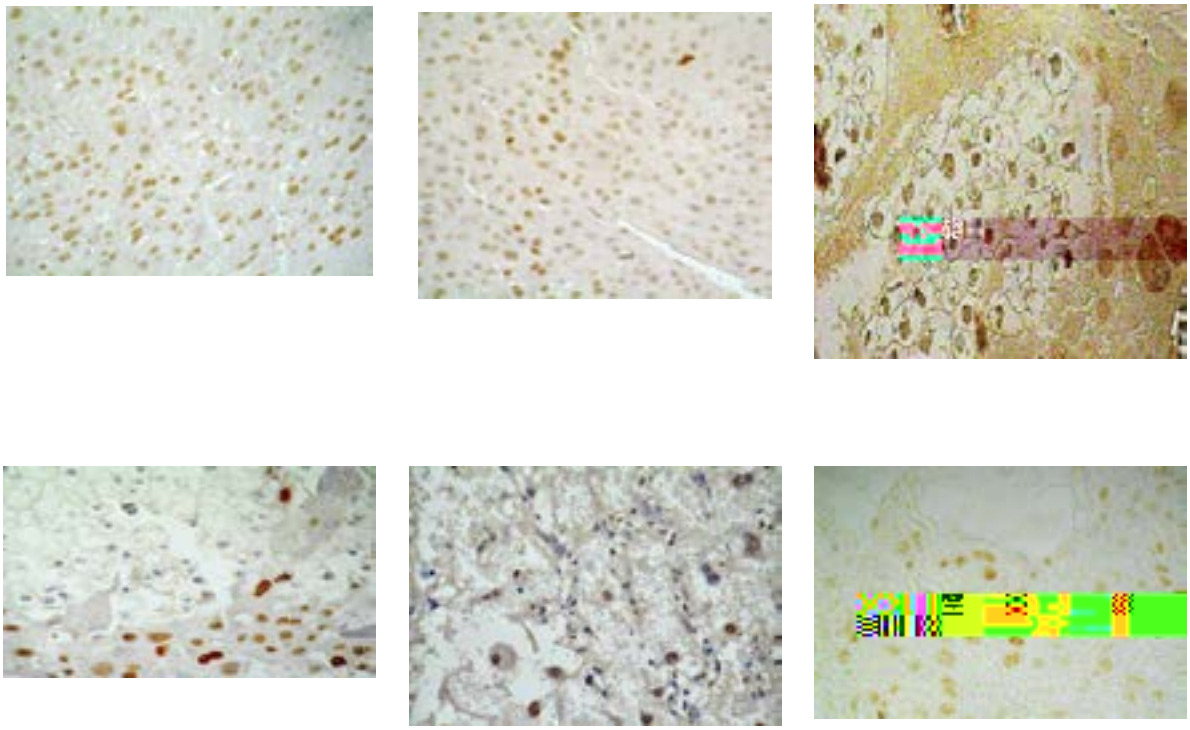

Figure 1: Histological sections of the placental zones from control (A, C, E) and stressed rats (B, D, F) at days 12 (A, B), 17 (C, D) and 21 (E, F) of

days, AI increase in the S group compared to C group. Data are shown in Figure 2.

BrdU detection

Di erent zones of the placenta with proliferating and normal nuclei are shown in Figure 3.

Proliferating nuclei were present in both JZ and LZ. Most proliferative nuclei were observed in the S group, at all gestational stages.

BrdU marked nuclei were more abundant on the 17th day of pregnancy and less frequent on days 12 and 21 of pregnancy. is feature occurred in both groups.

ANOVA test revealed no signi cant interaction between gestational stages and groups ($p=0.9$). However, signi cant di erences between groups ($p<0.0001$) and among gestational stages ($p=0.002$) were observed.

A post hoc test was performed to determine existing di erences among gestation days. Results revealed signi cant di erences between days 12 and 17 ($p=0.0044$) and between 17 and 21 ($p=0.01$). No signi cant di erences were observed between days 12 and 21 ($p=0.91$). ese results were observed in C and S groups.

Cell proliferation is higher in S group than in C group at all gestational stages. Data are shown in Figure 4.

Discussion

Apoptosis has been present in normal placenta throughout pregnancy. Its concurrent appearance with cell proliferation re ects the growth and remodeling of the placenta. e two processes together maintain tissue homeostasis [21]. In line with other studies [26,29,30] our study has shown that both processes were observed in normal placental tissue development (C group).

Apoptosis and cell proliferation were observed in both placental zones. In LZ, these processes were observed mainly in spongiotrophoblast cells, whereas in JZ, they were observed in

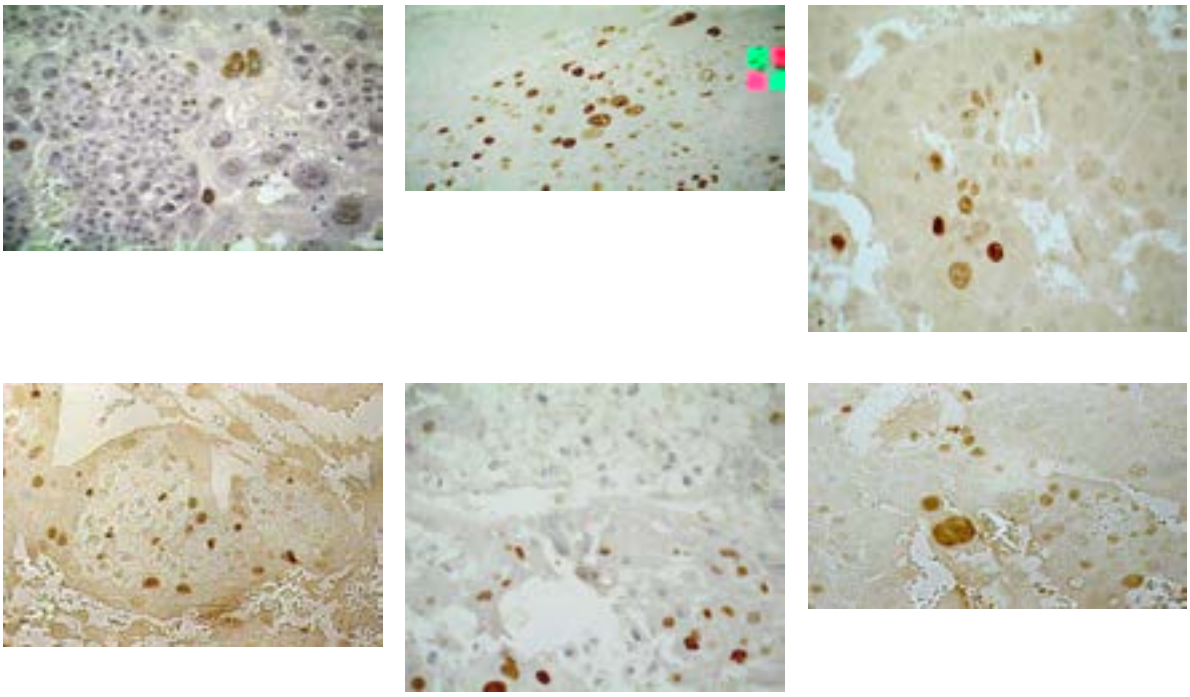

Figure 3: Histological sections of the placental zones from control (A, C, E) and stressed rats (B, D, F) at days 12 (A, B), 17 (C, D) and 21 (E, F) of pregnancy stained by using the BrdU proliferative assay. Arrows indicate positive BrdU nuclei. Proliferating nuclei were staining in the junctional zone in trophoblast giant cells (TGC) and in the spongioblast cells in all stages of pregnancy, control and stress groups.

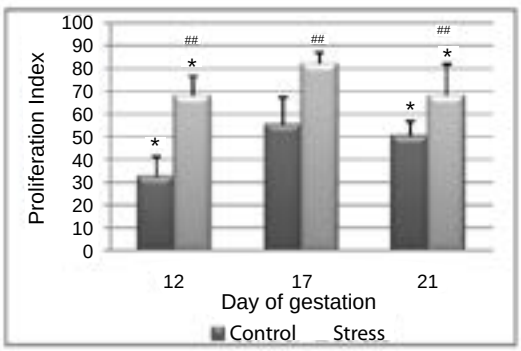

Figure 4: Total proliferation index in the placenta on days 12, 17 and 21 of pregnancy. Control rats (C) vs Stress rats (S). A signifcant increase in the proliferation index was detected between day 12 and 17 and day 17 and 21 for both groups C and S. (*p<0, 05). Signifcant differences between C and S groups in all gestational stages were observed (##p<0, 0001).

trophoblast giant cells. Our results are similar to those reported by Waddell et al. [30], who rst proposed that apoptosis was clearly evident in both placental zones, and it was particularly prevalent in the JZ near term. ese authors also demonstrated that apoptosis in JZ was stimulated by increased glucocorticoid exposure.

According with [27], apoptosis increases at the end of pregnancy. Agreeing with these authors, we have demonstrated a signi cant decrease in apoptosis on the 17th day of pregnancy, followed by an increase toward the end of gestation, in both C and S groups, whereas by contrast, cell proliferation showed an increase on the 17th day and a decrease on the 21st day of pregnancy.

Signi cant di erences in AI and in PI were observed among gestational stages analyzed. ese results were due to the fact that the placental tissue undergoes changes as pregnancy progresses. As a consequence, apoptosis increased over midterm and term in normal pregnancies. Our Immunohistochemical results showed that positive TUNEL nuclei gradually decreased from day 12 to 17 and gradually increased over day 21. For S group, the immunostaning pattern was very similar to that of C group. However, the number of apoptotic nuclei in placentas of S group was higher than in C group on days 12 and 21, while it was higher in C group on day 17. e opposite situation occurs in terms of cell proliferation. In accordance with Unek et al. [1] in placentas with Intrauterine Growth Restriction, we observed that BrdU expression was very strong during the early days of pregnancy but gradually decreased over midterm and term in normal rats. Our Immunohistochemical results showed that the number of BrdU immunolabeled cells gradually increased from day 12 to 17, being the highest on day 17 and gradually decreasing on day 21, for placental removal during delivery. For S group, the immunostaning pattern was very similar to that of C group. However, the number of BrdU immunolabeled cells in placentas of the S group was higher than in C group placentas on all days, which are in agreement with the results of Acar et al. [19].

On the other hand, statistical analysis about studied groups revealed signi cant di erences between groups in which cell proliferation was studied, but no signi cant di erences were observed between groups in which apoptosis was studied. However, during exposure to a stressor when the hypothalamus-pituitary-adrenal and the sympatho-adrenal axis are activated, individuals may respond di erently to an identical stressful stimulus [15]. Normally, placental tissue works as a bu er organ to the e ects produced by the stress applied and it has the ability to adapt to di erent environmental conditions. erefore,

no signi cant di erences in apoptosis between groups were observed. However, the cell proliferation process was a ected by stressful stimulus, probably due to the e ects of increased glucocorticoids during abnormal pregnancies [14].

Since the action of glucocorticoids on homeostasis is widespread, a ecting most of the tissues, as well as the placenta, one can speculate that the chronic stress applied to mothers can generate a deleterious environment for the fetus development.

e molecular mechanisms, by which glucocorticoids alter cell proliferation in stressed rat placentas, are not yet known [36-38].

In conclusion, chronic stress by immobilization did not produce e ects on apoptotic process. However it produces an increase in