Original Paper

DOI: 10.5455/jmood.20170824113610

Stress Lived Before Conception Alters the Maturation of the Offspring

Meriem Haloui[1], Amina Djouini[1], Sabri Benkermiche[1], Fatiha Bououza[2], Abdelkrim Tahraoui[1]

ABSTRACT:

Stress lived before conception alters the maturation of the offspring

To test the possibility that the pregestational chronic restraint stress can influence, indirectly, the development of the offspring, female albino wistar rats were divided into two experimental groups. The stress was applied 1 h daily for 4 days for 5 consecutive weeks, by placement into plexiglas tubes, so that the animal was unable to move. After parturition, the descent resulting from the control and stressed mothers was used for the behavioral study. Maternal glycemia was measured during stress. After birth, the neurobehavioral and physiological maturation of the offspring (somatic, physical, and reflexes) were studied from the 2^{end} to the 18th postnatal day (PND). Results showed hyperglycemia in the stressed mothers, while the little rats resulting from stressed group present difficulties in tests used for physical and behavio,ral development considered as consequences the nervous structure deterioration occurred during the development.

Keywords: pre-gestational stress, offspring development, behavioral maturation

Journal of Mood Disorders (JMOOD) 2017;7(4):191-8

[1]Laboratory of Applied Neuroendocrinology, Department of Biology, Faculty of sciences, University of Badji Mokhtar -Annaba-Algeria
[2]Laboratory of Animal Ecophysiology, Department of Biology, Faculty of Science, University of Badji Mokhtar -Annaba-Algeria

Corresponding Author:
Sabri Benkermiche,
Laboratory of Applied Neuroendocrinology, Department of Biology, Faculty of sciences, University of Badji Mokhtar -Annaba-Algeria

E-mail address:
sabri.benkermiche@univ-annaba.org

Date of received:
August 24, 2017

Date of acceptance:
November 12, 2017

Declaration of interest:
M.H., A.D., S.B., F.B., A.T.: The authors reported no conflicts of interest related to this article.

INTRODUCTION

Several studies have shown that environmental factors such emotional and stressful events (1,2) to which a mother is exposed during pregnancy in humans and animals (3-6) can influence behavioral and social development of the offspring (7,8). These changes could be mediated by in utero exposure of the developing brain to elevated levels of maternal glucocorticoids (GCs) secreted during HPA axis activities in mothers by stress. This can cross the placenta barrier and reach the developing fetal brain (9,10). Albeit with partial degradation by 11 ß h-hydroxysteroid dehydrogenase, it has been recognized that GCs access to receptors is determined by the presence of tissue-specific 11ß-hydroxysteroid dehydrogenases (11b-HSDs) that catalyse the interconversion of active corticosterone and inert 11-dehydrocorticosterone (11,12). They could affect the maturation of the fetal HPA axis and program the responsiveness of the hypothalamic–pituitary– adrenal (HPA) axis of the offspring (13,14). Prenatal GCS exposure "programs" permanently several central functions (12). Some studies report that the offspring of stressed mothers shows delay in the physical and reflex development of rat pups, deficits in learning and memory associated to depressive behavior in adulthood (15,16). Such behavioral developmental impairments are due to alterations in mother–infant relationships. It has been demonstrated that stressed dams during pregnancy have abnormal maternal behavior (17), which can affect mother–infant relationships immediately after birth and influence the development of the embryonic brain and permanently altering the offspring behavior (7,16).

This study was designed to explore the effects of chronic restraint stress applied before conception on both male and female pups on the parameters of early maturation/development; physiological maturation, and reflexes. In addition, we aimed to see whether pregestational chronic stress can affect the neuromotor and physiological development of the offspring.

Experimental Procedure

Animals

Albino rats (60 days old) coming from Pasteur Institute of Algiers were used during this study. The rats were acclimatized to the natural photoperiod standards conditions: an average temperature of 22±4°C and a relative humidity of 50-70%. After a three-week adaptation period, 28 females were selected according to the weight with an average of (140–170) grams (three months old at the beginning of the stress). Then, they were divided into 2 experimental batches each batch contains 14 rats. After parturition (with six days of mating), we randomly selected 15 male and 15 female pups from control rats and 15 male and 15 female pups from stressed rats for the behavioural studies.

Induction of stress

Our model of stress is based on that of Bardin et al. (2009) (18), rats were restrained 1 h daily for 4 days in the morning for 5 consecutive weeks, by placement into plexiglas tubes, whose length was adjusted with a piston, so that the animal was unable to move. Stress was applied before putting the rats in gestation.

MATERNAL STUDY

Blood taking away

The taking away is done starting from the lachrymal vein at the end of the application of chronic restraint stress and before putting the rats in gestation. The blood samples were collected in the heparinized tubes, then centrifuged with 5000 rpm for 15 minutes. The plasma was put at the freezer for proportioning of the glycemia because it presents a close relationship to the variation of the body weight of the descent.

Proportioning of glycemia

The glycemia was determined by the method of trinder (19).

Offspring study

Pups were assessed after birth on the following measures of development:

Physiological maturation

Body weight: Weekly on postnatal day (PND) 2, 5, 9, 12, 15, 18 (20).

Tooth eruption: The date on which the inferior and superior teeth were first observed either visually or by touch. The verification was between PND 8 and PND 11 (20).

Eye opening: The first date on which a pup was observed to have opened its eyes. The verification was carried out between PND 12 and PND 16 (20).

Reflexes

Righting reflex (PND3): Also known as the labyrinthine righting reflex. It is considered to be a reflection of subcortical maturation (that corrects the orientation of the body when it is taken out of its normal upright position). In this test, the pups were placed on their backs on a plane surface and the time needed to recover their normal prone position (all four paws) was recorded. A maximum of 60 s per trial (3 trials) was allowed. Righting reflex was considered to be fully achieved when the pups turned 180 around their longitudinal axis, their four paws being in contact with the surface (20).

Grasping reflex (PND4): Young rats placed on a roasting tray must cling to not fall when the tray was set in rotation. The measured variable is the angle reached in relation with the horizontal when the young "clinches" and falls (20).

Negative geotaxis (PND9): This test is believed to test labyrinthine and cerebellar integration. We place pups on

an incline with their heads pointing down the slope and we measure the latency time for pup to perform a complete half turn on the inclined plane 25° (20).

Wire hanging maneuver (PND12): Test of neuromuscular and locomotor development. Pups were suspended with their forelimbs from a horizontal rod (2 mm thick, 70 cm long between poles 50 cm high). Suspension latencies (duration in seconds of hanging) were recorded. Animals were tested from PND12 (20).

Statistical Analysis

The results are presented as mean ± standard error of mean (SEM) and were analysed by using Student's t-test with the program Minitab (version13). They are regarded as significant $p<0.05$.

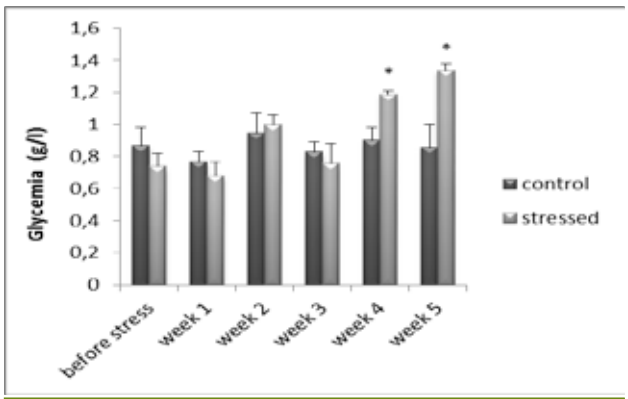

Figure 1: Variation of glycemia in stressed and control mothers (g/l). (*p<0.05; **p<0.01; ***p<0.001).

RESULTS

Maternal results

Glycemia

The figure 1 represents the effect of chronic restraint stress on glycemia. We note a significant increase in the fourth (1.19g/l±0.02) and the fifth week (1.34g/l±0.04) of chronic restraint stress of stressed mothers compared to the control o in the fourth (0.87g/l±0.08) and the fifth week (0.98g/l±0.04).

Offspring results

Physiological maturation

Body weight: After birth (in PND2), we recorded an increase in the body weight of females (Fig. 2.A) resulting from stressed mothers (PND2: **6.23g±0.49; PND5: ***8.46g±1.22; PND9: ***12.82g±0.79; PND18: **22.32g±2.67) compared to the females resulting from control mothers (PND2: 5.8g±0.46; PND5: 7.61g±0.42; PND9: 9.47g±1.41; PND18: 20.35g±2.13).

Males resulting from stressed mothers (Fig. 2.B) have shown an increase in the body weight (PND2: *6.33g±0.62; PND5: **8.59g±1.1; PND9: ***13.25g±0.69) compared to the males resulting from control mothers (PND2: 5.9g±0.48g; PND5: 7.64g±0.65; PND9: 10.09g±1.85).

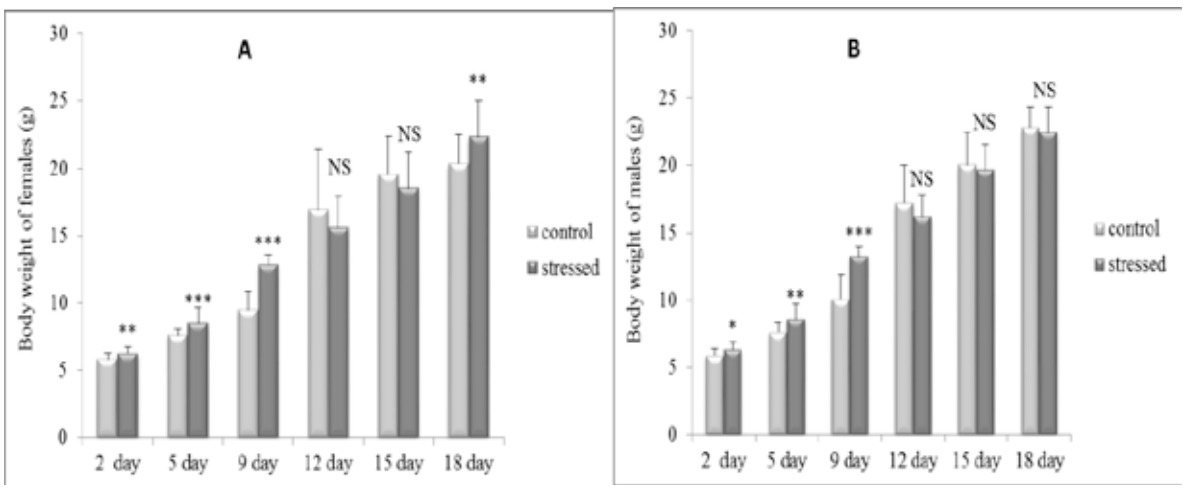

Figure 2: Body weight of the females (A) and males (B) pups between PND 2 and PND18. (*p<0.05;**p<0.01; ***p<0.001).

Tooth eruption and eye opening: There is no significant difference in the tooth eruption age of males (10.81±0.4) and females (10.64±0.49) compared to the control males (10.63±0.49) and control females (10.44±0.5) (Fig. 3.A and B).

In addition, no significant difference in eye opening between males resulting from stressed mothers (15.66±0.48) compared to the control males (15.37±0.51) and no difference between females resulting from stressed mothers (15.39±0.49) and females resulting from the control (15.38±0.5) (Fig. 3) has been noticed.

Reflexes (Behavior)

Righting reflex (PND3): A very significant difference between (stressed males: ***20.42s±5.31 vs control: 12.56s±1.33) and (stressed females: ***48.66s±9.95 vs control: 19.5s ± 3.44) has been noticed in the time needed to recover their normal prone position (180° around their longitudinal axis). The control take less time to stand on their four paws being in contact with the surface (Fig. 4).

Grasping reflex (PND4): A very significant difference was recorded between (stressed males: ***91.3°±5.21 vs control: 98.33°±3.7). A highly significant decrease between (stressed females: **90.56°±4.9 vs control: 96.86°±5.95) has been noticed because stressed rats fell quickly (Fig. 5).

Negative geotaxis (PND9): In this test, we recorded a very significant difference between stressed and control pups of both groups (stressed males: ***22s±1.66 vs control ones: 13.77s±3.13) and (stressed females: ***25.36s±1.71 vs control ones: 13.55s±4.12) in the latency time to turn and face up the incline 25° (Fig. 6).

Wire hanging maneuver (PND12): Test of neuromuscular and locomotor development. Males resulting from stressed

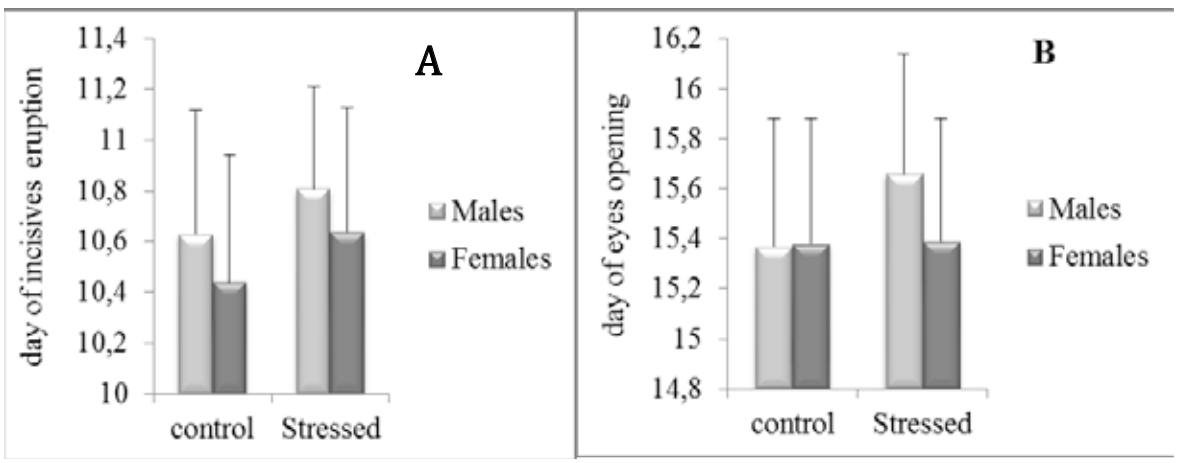

Figure 3: Tooth eruption (A) and Eye opening (B) of males and females pups. (*p<0.05;**p<0.01; ***p<0.001).

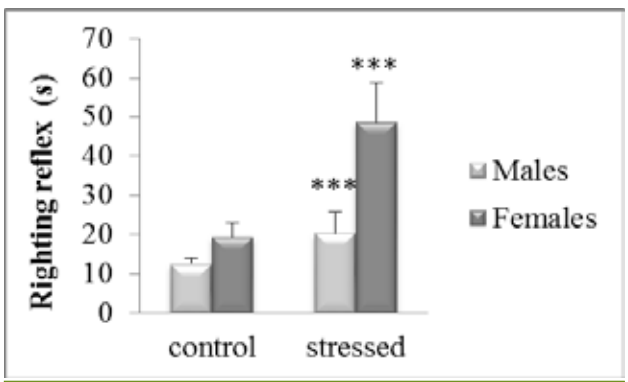

Figure 4: Effects of stress on the righting reflex in males and females pups. (*p<0.05; **p<0.01; ***p<0.001).

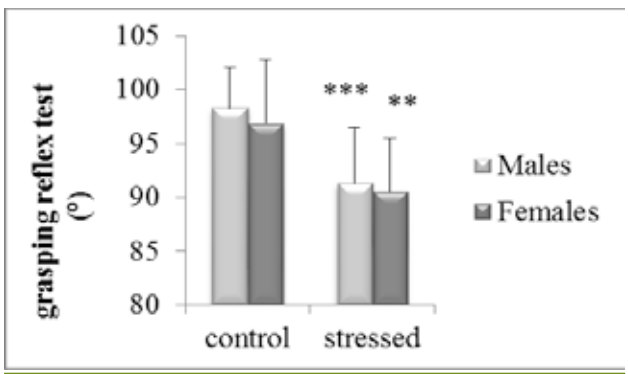

Figure 5: Effects of stress on the grasping reflex in males and females pups. (*p<0.05; **p<0.01; ***p<0.001).

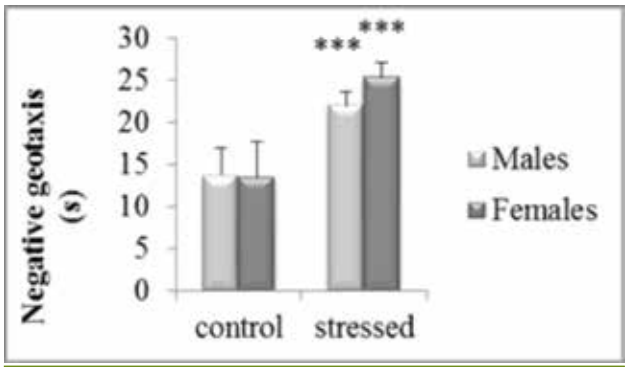

Figure 6: Effects of stress on the negative geotaxis in male and female pups. (*p<0.05; **p<0.01; ***p<0.001).

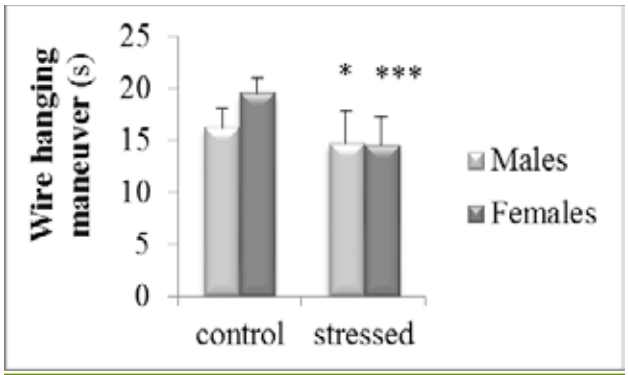

Figure 7: Effects of stress on the wire hanging maneuver in male and female pups. (*p<0.05; **p<0.01; ***p<0.001).

mothers have shown a significant difference (*14.72s±3.1) compared to males resulting from control mothers (16.19s±1.95). A very significant difference between females resulting from stressed mothers (***14.57s±2.77) and from the control ones (19.56s±1.42) (Fig. 7).

DISCUSSION

The direct neural connection between Corticotropin-releasing hormone CRH and gonadotophin-releasing hormone GnRH can induce perturbation in reproductive functions since receptors for CRH are identified in most of the female reproductive tissues including the ovary, uterus, and placental trophoblast (2,21). Which can affect the developments parametres of the offspring with perturbation in the energetic metabolism. Moreover, there is abundant evidence that the gonads affect the way the hypothalamic-pituitary-adrenal axis HPA responds to stress (22) have evaluated the presence of estrogen receptors in sheep adrenal glands. Ovarian steroids have been found to increase HPA-axis activity, enhance the HPA-axis response to psychological stress, and sensitize the HPG-axis to stress-induced inhibition in rhesus monkey (23).

Previously reported data by another group have shown that that diabetic pregnancy in rats yielded to impaired glucose metabolism in offspring (8,24). This can be related to chronic alterations in cortisol secretion in offspring which could affect body composition and be causative factors of early-onset obesity, metabolic syndrome, insulin resistance, and type 2 diabetes (25-28). Exposure to maternal glucocorticoids induces a structural change in the pancreas (reduced pancreatic β-cell number) (25) and functional alterations in hepatic tissue (29) which leads to the appearance of the metabolic syndrome.

The elevated levels of maternal glucocorticoids (GCs) secreted during HPA axis activities in mother by stress can cross the placenta barrier and reach the developing fetal brain (9, 10). Albeit with partial degradation by 11 ß-hydroxysteroid dehydrogenase of glucocorticoids to inactive products (30), The 11β-HSD1 enzyme plays a key role in regulating intracellular glucocorticoid concentrations, and obesity is associated with increased activity of 11β-HSD1 in adipose tissue in humans (31, 32) and animals (30,33). However, repeated prenatal stress exposure leads to a decrease in placental 11 β -HSD activity and there by an increase in maternal corticosterone reaching the fetus (34,35) because this hormone is released during the stress by the adrenal gland that acts on the metabolism. The onset of obesity and insulin resistance is sometimes associated with hormonal imbalances. In addition, the secretion of different hormones including cortisol, insulin, estrogens, dihydrotestosterone and growth hormone (36) regulates fat distribution in visceral and subcutaneous depots.

The results of the present study shows also that restraint stress (RS) was responsible for the appearance of neuromotor and behavioral disorders at the descent, resulting from the stressed mothers before gestation after their evaluation in the behavioral tests. These disorders may be the result of the dysfunction in the hypothalamo-pituitary-adrenal axis (HPA) of mothers and in the rise of the plasma concentration of glucocorticoids and glycemia. Aiming at facing the stressing situation (37,38). The hyperactivation of the HPA axis of stressed mothers activates HPA axis of their offspring, which affects mother–infant relationships immediately after birth, influencing

the development of the embryonic brain and permanently altering the behaviour of the offspring (14,39). The behavioral development of the offspring is likely mediated by functional and/ or anatomical abnormalities of the central nervous system (CNS). Our results have shown that repeated pregestational stress can affect normal cerebral functions such as coordination control, righting reflex, negative geotaxis and the body weight of the offspring male and female. Because glucocorticoids can act at the central nervous system on the hippocampus, amygdala, the prefrontal cortex and the hypothalamus, which are the centers of integration of emotional and physical information. These structures are rich in receptors of glucocorticoids (GR) and mineralocorticoids (MR). The latter increases the response to stress in mother, which result in high levels of glucocorticoids exposure to the fetus. Leading to down-regulation of GR in hippocampus and attenuation of negative feedback of the HPA axis and enhancement of the HPA axis activity; resulting alteration of behavior, emotion (40). The prolonged activation of HPA axis leads to structural modifications in the central nervous system (41). Pregestational chronic stress can delay some neurobehavioral and psychological developments and cause neuropsychiatric disorders in later life of behavior and neurophysiological parameters.

Environmental signals can be transmitted from the mother to the fetus, affecting specific vulnerable tissues in their sensitive developmental stage, modulating normal development trajectory, remodelling their structure and function and reprogramming the resiliency or susceptibility to disease in postnatal life. The programming effects of HPA function by prenatal stress and the difference in results could be allotted to the chronic duration of stress, and the experimental procedures (e.g., day-night, the phase of the application of the stress), time and genetic factors what can affect the behavior (42,43).

CONCLUSION

The intrauterine environment is critical to fetal development; perturbations of this environment can have significant short and long-term consequences on the offspring. Pregestational chronic restraint stress causes modifications in neurobehavioral and emotional activities of the offspring.

References:

1. Dudley KJ, Li X, Kobor MS, Kippin TE, Bredy TW. Epigenetic mechanisms mediating vulnerability and resilience to psychiatric disorders. Neurosci Biobehav Rev. 2011;35(7):1544-51. [CrossRef]
2. Chrousos GP, Gold PW. The concepts of stress and stress system disorders: overview of physical and behavioral homeostasis. JAMA. 1992;267(9):1244-52. [CrossRef]
3. Huizink AC, Mulder EJ, Buitelaar JK. Prenatal stress and risk for psychopathology: specific effects or induction of general susceptibility? Psychol Bull. 2004;130(1):115-42. [CrossRef]
4. Kofman O. The role of prenatal stress in the etiology of developmental behavioural disorders. Neurosci Biobehav Rev. 2002;26(4):457-70. [CrossRef]
5. Jarvis S, Moinard C, Robson SK, Baxter E, Ormandy E, Douglas AJ, et al. Programming the offspring of the pig by prenatal social stress: neuroendocrine activity and behaviour. Horm Behav. 2006;49(1):68-80. [CrossRef]
6. Neigh GN, Ritschel LA, Kilpela LS, Harrell CS, Bourke CH. Translational reciprocity: Bridging the gap between preclinical studies and clinical treatment of stress effects on the adolescent brain. Neuroscience. 2013;249:139-53. [CrossRef]
7. Mychasiuk R, Gibb R, Kolb B. Prenatal bystander stress induces neuroanatomical changes in the prefrontal cortex and hippocampus of developing rat offspring. Brain Res. 2011;1412:55-62. [CrossRef]
8. Kiss AC, Woodside B, Felício LF, Anselmo-Franci J, Damasceno DC. Impact of maternal mild hyperglycemia on maternal care and offspring development and behavior of Wistar rats. Physiol Behav. 2012;107(3):292-300. [CrossRef]
9. Zarrow MX, Philpott JE, Denenberg VH. Passage of 14C-4-corticosterone from the rat mother to the foetus and neonate. Nature. 1970;226(5250):1058-9. [CrossRef]
10. Weinstock M. Alterations induced by gestational stress in brain morphology and behaviour of the offspring. Prog Neurobiol. 2001;65(5):427-51. [CrossRef]
11. Otten W, Kanitz E, Tuchscherer M, Schneider F, Brüssow KP. Effects of adrenocorticotropin stimulation on cortisol dynamics of pregnant gilts and their fetuses: implications for prenatal stress studies. Theriogenology. 2004;61(9):1649-59. [CrossRef]
12. Diaz R, Brown RW, Seckl JR. Distinct ontogeny of glucocorticoid and mineralocorticoid receptor and 11βhydroxysteroid dehydrogenase types I and II mRNAs in the fetal rat brain suggest a complex control of glucocorticoid actions. J Neurosci. 1998;18(7):2570-80.
13. Andrews MH, Matthews SG. Programming of the hypothalamo–pituitary–adrenal axis: serotonergic involvement. Stress. 2004;7(1):15-27. [CrossRef]

14. Gheorghe CP, Goyal R, Mittal A, Longo LD. Gene expression in the placenta: maternal stress and epigenetic responses. Int J Dev Biol. 2010;54(2-3):507-523. [CrossRef]

15. Emack J, Kostaki A, Walker CD, Matthews SG. Chronic maternal stress affects growth, behaviour and hypothalamo–pituitary–adrenal function in juvenile offspring. Horm Behav. 2008;54(4):514-20. [CrossRef]

16. Trickey D, Siddaway AP, Meiser-Stedman R, Serpell L, Field AP. A meta-analysis of risk factors for post-traumatic stress disorder in children and adolescents. Clin Psychol Rev. 2012;32(2):122-38. [CrossRef]

17. Patin V, Lordi B, Vincent A, Thoumas JL, Vaudry H, Caston J. Effects of prenatal stress on maternal behavior in the rat. Brain Res Dev Brain Res. 2002;139(1):1-8. [CrossRef]

18. Bardin L, Malfetes N, Newman-Tancredi A, Depoortere R. Chronic restraint stress induces mechanical and cold allodynia, and enhances inflammatory pain in rat: relevance to human stress-associated painful pathologies. Behav Brain Res. 2009:205(2);360-6. [CrossRef]

19. Trinder P. Determination of blood glucose using 4-amino phenazone as oxygen acceptor. J Clin Pathol. 1969;22(2):246. [CrossRef]

20. ETAP-Applied Ethology. Study report. Ingredia. No. 18/1100 / ING 911. Behavioral Toxicology. 2001.

21. Maeda K, Tsukamura H. The impact of stress on reproduction: are glucocorticoids inhibitory or protective to gonadotropin secretion? Endocrinology. 2006;147(3):1085-1086. [CrossRef]

22. Van Lier E, Pérez-Clariget R, Forsberg M. Sex differences in cortisol secretion after administration of an ACTH analogue in sheep during the breeding and non-breeding season. Anim Reprod Sci. 2003;79(1):81-92. [CrossRef]

23. Roy BN, Reid RL, Van Vugt DA. The effects of estrogen and progesterone on corticotropin-releasing hormone and arginine vasopressin messenger ribonucleic acid levels in the paraventricular nucleus and supraoptic nucleus of the rhesus monkey. Endocrinology. 1999;140(5):2191-2198. [CrossRef]

24. Fujisawa Y, Nakagawa Y, Li RS, Liu YJ, Ohzeki T. Diabetic pregnancy in rats leads to impaired glucose metabolism in offspring involving tissue-specific dysregulation of 11beta-hydroxysteroid dehydrogenase type 1 expression. Life Sci. 2007;81(9):724-731. [CrossRef]

25. De Vries A, Holmes MC, Heijnis A, Seier JV, Heerden, J, Lou, J, et al. Prenatal dexamethasone exposure induces changes in nonhuman primate offspring cardiometabolic and hypothalamic-pituitary-adrenal axis function. J Clin Invest. 2007;117(4):1058-1067. [CrossRef]

26. Pervanidou P, Chrousos GP. Metabolic consequences of stress during childhood and adolescence. Metabolism. 2012;61(5):611-619. [CrossRef]

27. Paternain L, Batlle MA, De la Garza AL, Milagro FI, Martinez JA, Campion J. Transcriptomic and epigenetic changes in the hypothalamus are involved in an increased susceptibility to a high-fat-sucrose diet in prenatally stressed female rats. Neuroendocrinology. 2012;96(3):249-260. [CrossRef]

28. Haloui M, Tahraoui A, Bououza F, Bairi A, Boukhris N, Boulaakoud MS, et al. L. Effects of chronic restraint stress on energetic metabolism and the evolution of depression, evaluated in the open field test in female wistar rat. Ann Biol Res. 2014;5(2):1-7.

29. Nyirenda MJ, Lindsay RS, Kenyon CJ, Burchell A, Seckl JR. Glucocorticoid exposure in late gestation permanently programs rat hepatic phosphoenolpyruvate carboxykinase and glucocorticoid receptor expression and causes glucose intolerance in adult offspring. J Clin Invest. 1998;101(10):2174-2181. [CrossRef]

30. Livingstone DE, Kenyon CJ, Walker BR. Mechanisms of dysregulation of 11 beta-hydroxysteroid dehydrogenase type 1 in obese Zucker rats. Journal of endocrinology, 2000;167(3):533-539. [CrossRef]

31. Rask E, Olsson T, Soderberg S, Andrew R, Livingstone DE, Johnson O, et al. Tissue-specific dysregulation of cortisol metabolism in human obesity. J Clin Endocrinol Metab. 2001;86(3):1418-1421. [CrossRef]

32. Rask E, Walker BR, Söderberg S, Livingstone DEW, Eliasson M, Johnson O, et al. Tissue-specific changes in peripheral cortisol metabolism in obese women: increased adipose 11βhydroxysteroid dehydrogenase type 1 activity. J Clin Endocrinol Metab. 2002;87(7):3330-3336. [CrossRef]

33. Livingstone DE, Grassick SL, Currie GL, Walker BR, Andrew R. Dysregulation of glucocorticoid metabolism in murine obesity: comparable effects of leptin resistance and deficiency. J Endocrinol. 2009;201(2):211-218. [CrossRef]

34. Mairesse J, Lesage J, Breton C, Bréant B, Hahn T, Darnaudéry M, et al. Maternal stress alters endocrine function of the feto-placental unit in rats. Am J Physiol Endocrinol Metab. 2007;292(6):E1526-E1533. [CrossRef]

35. Baibazarova E, van de Beek C, Cohen-Kettenis PT, Buitelaar J, Shelton KH, van Goozen SH. Influence of prenatal maternal stress, maternal plasma cortisol and cortisol in the amniotic fluid on birth outcomes and child temperament at 3 months. Psychoneuroendocrinology. 2013;38(6):907-915. [CrossRef]

36. Mattsson C, Olsson T. Estrogens and glucocorticoid hormones in adipose tissue metabolism. Curr Med Chem. 2007;14(27):2918-2924. [CrossRef]

37. Bourke CH, Owens MJ. Corticotropin-releasing factor. In: Stolerman IP, ed. Encyclopedia of Psychopharmacology. Springer, New York; 2010. p. 355-360.

38. Bourke CH, Raees MQ, Malviya S, Bradburn CA, Binder EB, Neigh GN. Glucocorticoid sensitizers Bag1 and Ppid are regulated by adolescent stress in a sex-dependent manner. Psychoneuroendocrinology. 2013;38(1):84-93. [CrossRef]

39. Brummelte S, Lieblich SE, Galea LA. Gestational and postpartum corticosterone exposure to the dam affects behavioral and endocrine outcome of the offspring in a sexually-dimorphic manner. Neuropharmacology. 2012;62(1):406-418. [CrossRef]

40. Fietta P, Fietta P, Delsante G. Central nervous system effects of natural and synthetic glucocorticoids. Psychiatry Clin Neurosci. 2009;63(5):613-622. [CrossRef]

41. Radley JJ, Rocher AB, Rodriguez A, Ehlenberger DB, Dammann M, McEwen BS, et al. Repeated stress alters dendritic spine morphology in the rat medial prefrontal cortex. J Comp Neurol. 2008;507(1):1141-1150. [CrossRef]

42. Harris, A, Seckl J. Glucocorticoids, prenatal stress and the programming of disease. Horm Behav. 2011;59(3):279-289. [CrossRef]

43. Haloui M, Tahraoui A. Effects of pregestational chronic restraint stress on the generation of psychological and neurobehavioural disorders in female wistar rats and their offspring. International Journal of Science and Research (IJSR). 2014;3(8).