ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Effect of Prenatal Stress on Birth weight, Postnatal Weight gain and Developmental Milestones in Wistar Rats.

Saju Binu Cherian, K L Bairy and Muddanna S Rao.

Manuscript Info

Manuscript History:

Received: 15 July 2015
Final Accepted: 22 August 2015
Published Online: September 2015

Key words:
prenatal stress, restraint stress, low birth weight, developmental mile stones, birth weight, teeth eruption

Corresponding Author

Saju Binu Cherian.

Abstract

Introduction: The purpose of our study was to investigate the effects of prenatal stress on postnatal physical development parameters which include mortality, birth weight, body weight gain, pinna detachment, eruption of upper and lower incisors teeth and eye opening

Materials and Methods: Pregnant dams of Wistar incisors teeth and eye opening.

Results: We found that prenatal stress significantly affected the birth weight and body weight gain in the stressed group and also caused delay in pinna detachment whereas teeth eruption and eye opening were not affected.

Conclusion: This study reveals the pivotal significant adverse effect of stress during pregnancy on postnatal growth of the infant. Results of the study emphasize the importance of stress free pregnancy for the optimal physical growth of the child and its consequences on health in adult life.

Introduction:-

Prenatal or intrauterine development plays critical role in normal physical, mental and behavioural development of an individual. Maternal nutrition[1], exposure to environmental toxicants[2,3], and stressful disturbances[4,5] of the pregnant female are among the many variables that can affect *in utero* conditions and impair the maturational trajectory of the fetus. All sorts of early environmental influences can leave indelible imprints and influence the development of an offspring. In most of the cases, effects of such insults will be carried to the young age or even to the whole life span of the individual [6,7]**.** Though any system of the body is the target of flawed development, nervous system becomes the main target of faulty development.

Prenatal stress evokes a cascade of neurohumoral events which triggers HPA axis hyperactivity in response to stress throughout life[8].It promotes increased maternal hypothalamo-pituitary-adrenal gland (HPA) secretion of glucocorticoid (GC), leading to increased foetal and maternal GC receptor activity[9].Exposures during prenatal period have implications for pregnancy outcome as well as for morbidity and mortality. Considerable bodies of evidences have supported the view that prenatal stress causes reduction in birth weight[12]. D'mello et al have substantiated that maternal immobilization stress during weeks 1,2,3 had no effect on the gestational length, litter size or birth weight of the pups[13]. Thus inconsistencies between studies remain in literature. Since a wide variety of criticisms have been put forward regarding the effects of prenatal stress, the present study was undertaken to evaluate the effects of prenatal stress on postnatal physical development parameters which include mortality, birth weight, body weight gain, pinna detachment, eruption of upper and lower incisors teeth and eye opening.

Materials and methods:-

2.1. Experimental animals and housing conditions

In-house bred male and female Wistar strains of rats were used in the study. Animals were bred in Central Animal Reseach Facility of Manipal University, Manipal. Adult rats (3 months old) were housed in air conditioned animal rooms with constant light-dark cycle (12:12 h), controlled temperature (22±3ºC) and humidity (50±5%). Polypropylene cage with paddy husk as bedding materials was used for housing the rats. The animals had free

access to food (Gold Mohur; Lipton India Ltd.) and water *ad libitum*. Breeding and maintenance of animals were done according to the guidelines of Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA). Institutional Animal Ethical Committee (I.A.E.C) approval was obtained before the conduct of the study (IAEC/KMC/06/2005-2006) and care was taken to handle the rats in humane manner.

2.2 Timed pregnancy in rats

To get the pregnant rats of known gestational days, all female rats were subjected to vaginal smear test[14].The rats in the estrus cycle were mated with adult male rats overnight. Vaginal smear was examined within 12 hours after mating. The presence of sperms in the smear confirms the mating, and that day was taken as day zero of pregnancy for further counting the days. Pregnant females were assigned randomly into 'No stress' and 'stress groups' (n=20 in each group). The rats in 'No stress group' remained without any further procedures and allowed to deliver the pups. The rats in the 'Stress group' were subjected to restraint stress.

2.3 Prenatal stress protocol

Pregnant rats in the 'stressed group' were subjected to daily restraint stress from 11th gestational day, till they deliver the pups. The pregnant rats were restraint stressed by placing them individually in a wire mesh restrainer, 6 hours per day[15] . This type of restrain is known to induce stress in rats as indicated by increased serum cortisol level and adrenal gland weight in them[16]. The wire mesh restrainer has a wooden base and stainless steel wire mesh restrainer hinged to the base. A padlock and latch will help to secure the rat in the restrainer. The restrainers of two different dimensions were used. The restrainer with 11cm (Length) × 6cm (Breadth) × 6cm (Height) dimensions for restraining the pregnant rats from E11-E17, and restrainer with 11cm (Length) x 8cm (Breadth) x 8cm (Height) dimensions was used to stress the pregnant rats from E18 till delivery[16].This type of restrainer claimed to restricts the animal's movement without any pain, discomfort or suffocation. Control mothers were left undisturbed in the home cage throughout the duration of their pregnancies. The offsprings of both groups were raised by their biological mothers till weaning.

2.4 Experimental design:-

The male and female pups from 'No stress' dams were designated as normal control-male (NCM) and Normal control-female (NCF) groups. The male and female pups from 'Stressed' dams were designated as normal Stressed-male (STM) and Stressed-female (STF) groups.

2.4.1 Mortality:-

Total number of pups born and number of still born pups born to each control or stressed rat was counted at birth and percentage mortality was calculated.

2.4.2 Birth weight and body weight gain:-

Weight of pups born to control and stressed mothers were taken at birth (Birth weight). Body weight was measured on 7th, 14th and 21st postnatal days.

2.4.3 Pinna detachment (External ear development) :-

During early postnatal period, pups were observed daily to see the development of external ear (Pinna). The day on which external ear is 1mm in length and 0.5 mm in breadth was defined as the "day of pinna detachment".

2.4.4 Eruption of upper and lower incisors:-

Pups were observed daily during early postnatal period to witness the eruption of upper and lower incisor teeth. The day on which length of the erupted tooth is 0.5 mm, was considered as "day of teeth eruption".

2.4.5 Eye opening:-

A visible opening (2mm length) of palpebral fissure was defined as eyes are opened. The day on which eyes are opened was noted for each pup.

Statistical analysis:-

Data was expressed as mean±SEM. Data were compared with one way ANOVA test using Graph pad in stat software. If the ANOVA test is significant, Bonferroni's multiple comparision tests was applied to determine the significance between the groups.

Results:-

The present study was aimed to investigate the effect of prenatal stress on postnatal physical development parameters which include mortality, birth weight, body weight gain, pinna detachment, eruption of upper and lower incisors teeth and eye opening.

Study parameters at birth and neonatal period:-

4.1 Mortality:-

The number of pups delivered by each dam was statistically similar in stressed and control groups and physical malformation was not apparent in any of the groups. There were no still birth or dead neonates recorded in the prenatally stressed group.

4.2 Birth weight:-

Birth weight of pups born to stressed mothers was significantly affected by prenatal stress (Table 1). Stressed male rats (STM, $P<0.001$) and female rats (STF, $P<0.01$) showed significantly low body weight compared to respective control male (NCM) and control female (NCF) rats.

4.3 Body weight:-

Prenatal stress affected the body weight in the stressed group as weighed on postnatal 7th, 14th and 21st day (Table 2). Stressed males (STM) had lower body weight on 14th and 21st day of weighing compared to normal control males (NCM, $P<0.01$). Stressed females showed significant decrease in body weight only on 21st day compared to normal control females (NCF**,** $P<0.05$), but not on 7th and 14th day. Interestingly, body weight was significantly less ($P<0.01$) in normal control female (NCF) compared to normal male (NCM) on 14th day. This is suggestive of late onset of stress effects in stressed females and normal females have less body weight than normal males.

4.4 Pinna detachment:-

Both male and female offspring born to stressed rats showed a significant delay in pinna detachment when compared to respective control rats($P<0.001$ in both males and females; Table3).

4.5 Eruption of upper and lower incisor teeth:-

Stressed rats (both male and female) did not show any significant delay in eruption of upper and lower incisors when compared to respective control rats (control male and control female, Table3).

4.6 Eye opening:-

Prenatal stress did not have any significant effect on eye opening. Stressed rats [both male (STM) and female (STF)] did not show any significant delay in eye opening when compared to respective control rats [control male (NCM), and control female (NCF)] (Table4)

Birth weight (grams)

Male			Female			
Normal (NCM, n=6)	**Stressed (STM, n=6)**		**Normal (NCF, n=6)**	**Stressed (STF, n=6)**	**F value**	**ANOVA significance**
6.72± 0.19	**5.35 ± 0.07*** **		6.22± 0.16	**5.41 ±0.14** $^{\$\$}$	18.55	P<0.0001

Table 1: Birth weight in different groups. NCM-normal control male, NCF- normal control female, STM- stressed male, STF-stressed female. Note stressed rats (STM and STF) had significantly low birth weight compared to respective control rats (NCM and NCF). NCM vs STM: ***P<0.001; NCF vs STF: $^{\$\$}$ P<0.01, STM vs STF and NCM vs NCF: not significant (One way ANOVA, Bonferroni's test. Each data represents mean ± SEM).

Body weight gain (grams)

Table 2: Body weight gain in rats belonging to different groups as on 7th 14th and 21st postnatal day. NCM-normal control male, NCF- normal control female, STM- stressed male, STF-stressed female. Note that stressed males (STM) had lower weight gain on 14th and 21st day. Stressed females showed significant decrease in body weight gain on 21st day, though there was no significant difference on 7th and 14th days. NCM vs STM: **P<0.01; NCF vs STF: $ P<0.05; STM vs STF: not significant, NCM vs NCF: †† P<0.01; (One way ANOVA, Bonferroni's test. Each data represents Mean ±SEM).

Groups	Postnatal day		
	7th Day	**14th Day**	**21st Day**
NCM(n=6)	13.76 ± 1.13	24.73 ± 1.25	37.33 ± 3.68
STM(n=6)	11.82 ± 0.85	**17.25 ± 0.43****	**25.0 ± 0.44****
NCF(n=6)	11.71± 1.13	**18.30 ± 0.52**††	35.83 ± 2.72
STF(n=6)	9.58 ± 0.16	18.49 ± 1.95	**24.67 ± 0.42**$
F value	3.48	7.87	8.68
ANOVA significance	P< 0.05	P<0.01	P<0.001

Day of pinna detachment, upper and lower incisor teeth eruption

	Male			Female			
	Normal (NCM, n=6)	**Stressed (STM, n=6)**		**Normal (NCF, n=6)**	**Stressed (STF, n=6)**	**F value**	**ANOVA significance**
Pinna detachment	5.35± 0.07	**6.72 ± 0.07*****		5.41 ±0.14	**6.22±0.16** $$$	18.55	P<0.0001
Upper incisor teeth eruption	8.23± 0.06	8.38 ± 0.04		8.26± 0.04	8.41 ±0.03	3.45	P<0.05
Lower incisor teeth eruption	8.69± 0.04	8.5 ± 0.05		8.68± 0.04	8.68 ±0.04	3.45	P<0.05

Table 3: Day of pinna detachment, upper and lower incisor teeth eruption in rats belonging to different groups. NCM-normal control male, NCF- normal control female, STM- stressed male, STF-stressed female. Note the stressed rats (STM and STF) had significant delay in pinna detachment compared to respective control rats (NCM and NCF). There was no significant difference in day of upper or lower incisor teeth eruption between groups. NCM vs STM: ***P<0.001; NCF vs STF: $$$ P<0.001, STM vs STF and NCM vs NCF: not significant (One way ANOVA, Bonferroni's test. Each data represents mean ± SEM).

Day of eye opening

	Male			Female			
	Normal (NCM, =6)	**Stressed (STM, n=6)**		**Normal (NCF, n=6)**	**Stressed (STF, n=6)**	**F value**	**ANOVA significance**
Day of eye opening	8.69± 0.04	8.5 ± 0.05		8.68± 0.04	8.68 ±0.04	3.45	P<0.05

Table 4: Day of eye opening in different groups. NCM-normal control male (n=6), NCF- normal control female (n=6), STM- stressed male (n=6), STF-stressed female (n=6). Note there is no significant difference in the day of eye opening among the groups. (One way ANOVA, Bonferroni's test. Each data represents mean±SEM).

Discussion:-

In the present study, the effects of prenatal stress on postnatal growth parameters were studied. This study is novel and potentially important since only a few animal or human studies have utilized females as subjects.

5.1 Restraint stress method:-

The restraint method of prenatal stress used in the present study is one of the well-known methods of stress [17] .Stress by restrainer method used in the present study is convenient and animals will not suffocate, but at the same time stress them. Stress by this method is known to hyperactivate the hypothalamo-pituitary adrenal (HPA) axis, adrenal gland weight and glucocorticoid hormonal level[17].

5.2 Growth parameters:-

The results of the present study showed higher preweaning mortality in the stressed group which is comparable with the previous reports[11].The increased rate of mortality observed could be due to the low birth weight seen in the rat pups born to stressed mother rats.

Low birth weight is a broad marker of disturbed fetal development. In the present study, the stressed rats (both male and female) showed significant reduction in body weight when compared to respective control rats. Prenatal stress response is characterized by the activation of HPA axis. HPA axis activation involves a cascade of events that starts with the release of corticotropin-releasing hormone (CRH) from the hypothalamus. This leads to the release of adrenocorticotropic hormone (ACTH) by the pituitary, resulting in adrenal cortex release of glucocorticoids (cortisol) and adrenal medulla release of norepinephrine and epinephrine[18,19] . Maternal cortisol is also regulated by the placenta. The placenta secretes increasing amounts of CRH into the fetal and maternal bloodstreams. The increasing unbound circulating placental CRH levels during pregnancy stimulate ACTH production in the pituitary leading to increased adrenal function levels[20].10–20% of the mothers' cortisol crosses the placenta[21].Cortisol and norepinephrine are reported to elicit vascular constriction [22].It has also been shown to directly affect the intrauterine vasculature with norepinephrine reliably inducing uterine artery vasoconstriction[23] and cortisol increasing the density of uterine artery adrenoreceptors and thereby causing norepinephrine-mediated uterine artery vasoconstriction[24].Thus it results in reduced uterine artery blood flow and placental hypoxia and the fetus receives fewer nutrients and restricted levels of oxygen which may result in fetal growth delays (lower birth weight)[25] and this could be the reason underlying our observation.

It was observed in our experiment that prenatal stress caused a failure of postnatal catch up growth which was evident by the reduction in weight gain by the stressed rats (both male and female). Retarded postnatal catch up in body weight may be due to the involvement of (corticotrophin – releasing factor) CRF in the hypothalamic region[26].CRF receptors in the hypothalamus mediate acute response to stress that can lead to permanent changes in hormonal or metabolic process that determine the body weight and composition[27].Pathways projecting from limbic areas to hypothalamus could stimulate CRF secretion into pituitary adrenal axis. Increased CRF could mediate this stress- induced suppression of body weight and other growth parameters. It has been reported that growth hormone gene expression in the brain is significantly suppressed by exposure to restraint stress which may also cause growth retardation[28].Circulating growth hormone level has been shown to be decreased in animals exposed to stress during neonatal period[29].Decrease in body weight exhibited in stressed females towards later stages of growth is suggestive of late onset of stress effects in females. This can be further explained as the response of neural substrate for stress might change with the advancing age.

These research findings have important implications for clinical obstetric practice and maternal fetal medicine. Our results reinforce the hypothesis that much psychopathological affection has their origin with early developmental influences. This study reveals the pivotal significance of nine months of pregnancy for the optimal physical growth in the child's life and its consequences on adult health.

References:-

1. Firth EC, Rogers CW, Vickers M, Kenyon PR, Jenkinson CM, Blair HT. The bone-muscle ratio of fetal lambs is affected more by maternal nutrition during pregnancy than by maternal size. Am J Physiol Regul Integr Comp Physiol. 2008; 294(6):1890-94, http://dx.doi.org/10.1152/ajpregu.00805.2007.
2. Gilbert ME, Kelly ME, Samsam TE,Goodman J. H. Chronic developmental lead exposure reduces neurogenesis in adult rat hippocampus but does not impair spatial learning. Toxicol Sci.2005; 86(2):211-13, http://dx.doi.org/doi: 10.1093/toxsci/kfi156.
3. Wormser U, Izrael M, Van der Zee, Brodsky B,Yanai J. A chick model for the mechanism of mustard gas neurobehavioral teratogenecity. Neurotoxicol Teratol 2005; 27(1): 65-71,http://dx.doi.org/ doi:10.1016/j.ntt.2004.09.006
4. Igosheva N, Klimova O, Anischchenko T, Glover V. Prenatal stress alters cardiovascular responses in adult rats. J. Physiol.2004; 557(1):273- 85, http://dx.doi.org/ doi:10.1113/jphysiol.2003.056911.
5. Bowman RE**,** MacLusky NJ, Sarmiento Y, Frankfurt M, Gordon M, Luine VN. Sexually dimorphic effects of prenatal stress on cognition, hormonal responses, and central neurotransmitters. Endocrinology. 2004; 145: 3778–87
6. Roman E, Nylander I. The impact of emotional stress early in life on adult voluntary ethanol intake – results of maternal separation in rats. Stress 2005; 8(3): 157-74.
7. Coe CL, Kramer M, Czeh B, Gould E, Reeves AJ, Kirschbaum C. Prenatal stress diminishes neurogenesis in the dentate gyrus of juvenile rhesus monkeys. Biol Psychiatry.2003*;* 54*:* 1025–*34.*
8. Tegethoff M, Greene N, Olsen J, Meyer AH, Meinlschmidt G. Maternal psychosocial adversity during pregnancy is associated with length of gestation and offspring size at birth: Evidence from a population-based cohort study. Psychosom Med.2010;72(4):419-426. http://dx.doi.org/doi: 10.1097/PSY.0b013e3181d2f0b0.
9. **Vall'ee M, Maccari S,Dellu F, Simon H, Le Moal M ,Mayo W. Long-term effects of prenatal stress and postnatal handling on age-related glucocorticoid secretion and cognitive performance: a longitudinal study in the rat. Eur J of Nerosci 1999; 11:2906-16.** http://dx.doi.org/doi: 10.1046/j.1460-9568.1999.00705.x
10. Hauser J, Feldon J, Pryce CR. Direct and dam-mediated effects of prenatal dexamethasone on emotionality, cognition and HPA axis in adult Wistar rats. Horm Behav.2009;56(4):364-7, http://dx.doi.org/ doi: 10.1016/j.yhbeh.2009.07.003.
11. Kanitz E, Otten W, Tuchscherer M, Manteuffel G. Effects of prenatal stress on corticosteroid receptors and monamine concentrations in limbic regions of suckling piglets (Sus scrofa) at different ages. J Vet Med A Physiol Pathol Clin Med. 2003; 50(3):132-9. http://dx.doi.org/ doi: 10.1046/j.1439-0442.2003.00513.x
12. Sayers SM. Indigenous newborn care. Paediatr Clin North Am.2009; 56(6):1243-61. http://dx.doi.org/ doi: 10.1016/j.pcl.2009.09.009.
13. D'mello AP, Liu Y. Effects of maternal immobilization stress on birth weight and glucose homeostasis in the offspring. PNEC 2006;31:395-406, http://dx.doi.org/ doi:10.14336/AD.2014.05001
14. Lesage J, Del- Favero F, Leonhardt M. Prenatal stress induces intrauterine growth restriction and programmes glucose intolerance and feeding behavior disturbances in the aged rat. J Endocrinol.2004; 181:291-96
15. Igosheva N, Klimova O, Anishchenko T and Glover T. Prenatal stress alters cardiovascular responses in adult rats. J Physiol 2004; 557(1):273-285. , http://dx.doi.org/ doi:10.1113/PHYSIOL.2003.056911.
16. Sunanda, Rao M.S & Raju T.R.Effect of chronic restraint stress on dendritic spines and excrescences of hippocampal CA3 pyramidal neurons- a quantitative study. Brain res.1995*;* 694: 312.
17. Gué M, Bravard A, Meunier J, Veyrier R, Gaillet S, Recasens M . Sex differences in learning deficits induced by prenatal stress in juvenile rats. Behav Brain Res. 2004;150 (1-2):149-57, http://dx.doi.org/doi:10.1016/S0166-4328(03)00250-X
18. Becker J. B., Breedlove S. M., Crews D., McCarthy M. M. *Behavioral endocrinology.* 2nd Edition, MIT Press, Cambridge, MA 2002
19. Mastorakos G., Ilias I. Maternal and fetal hypothalamic-pituitary-adrenal axes during pregnancy and postpartum. *Annals of the New York Academy of Science.2003*; 997: 136–149, 1290.016
20. Jones SA, Brooks AN, Challis JR. Steroids modulate corticotrophin-releasing hormone production in human fetal membranes and placental. *Journal of Clinical Endocrinology & Metabolism. 1989;* 68: 825–830, http://dx.doi.org/doi:10.1210/jcem-68-4-825
21. Glover V., Teixeira J., Gitau R., Fisk N. Mechanisms by which maternal mood in pregnancy may affect the fetus. *Contemporary Review of Obstetrics and Gynecology*. 1999;20: 1–6
22. Kandel ER , Schwartz JH, Jessell TM.Principles of Neuroscience, 4th ed. New York: Mc Graw- Hill;2000

23. Yousif MH, Chandrasekhar B, Kadavil EA, Oriowo MA.Nor-adrenaline-induced vasoconstriction in the uterine vascular bed of pregnant rats chronically treated with L-NAME:Role of prostanoids. Journal of Cardiovascular Pharmacology.2000; 42:428-435
24. Xiao DL, Huang XH, Pearce WJ, Longo LD, Zhang L. Effect of cortisol on norepinephrine-mediated contractions in ovine uterine arteries. American Journal of Physiology:Heart and Circulatory Physiology.2003; 284:1142-1151
25. Dugoff L, Lynch AM, Cioffi-Ragan D, Hobbins JC, Schultz LK, Malone FD, D'Alton ME. 2005. FASTER Trial Research Consortium. First trimester uterine artery Doppler abnormalities predict subsequent intrauterine growth restriction. American Journal of Obstetrics and Gynecology, 2005;193:1208-1212, , http://dx.doi.org/10.1016/j.ajog.2005.06.054
26. Delbende C,Bunel TD,Tarozzo G,Grino M, Oliver C,Mocaer E et al. Effect of chronic treatment with the antidepressant tianeptine on the hypothalamo-pituitary-adrenal axis. Eur J of Pharmacol 1994;251(2-3):241-51
27. Smagin GN, Howell LA, Stephen RJ, Ryan DH, Harris RBS. Prevention of stress- induced weight loss by third ventricle CRF receptor antagonist. Am J Physiol. 1999;276(5):1461-68
28. Zhang YS, Du JZ. The response of growth hormone and prolactin of rats to hypoxia. Neurotransmitters .2000; 279(3):137-40
29. Khun CM, Pauk J, Shanberg SM. Endocrine responses to mother-infant separation in developing rats. Dev Psychobiol. 1990;23:395-410