Oral Morphine Consumption Reduces Lens Development in Rat Embryos

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Article info:
Received: 22 January 2012
First Revision: 30 January 2012
Accepted: 10 June 2012

ABSTRACT

Introduction: Consumption of morphine, during pregnancy, in addition to inducing defects in the mother's nervous system function, caused defects or delays in the formation and evolution of embryonic visual system. In the present study, changes in lens development were assessed in embryos exposed to morphine in utero.

Methods: Female Wistar rats (250-300 g) were mated with male rats and pregnancy was determined by sperm observation in vaginal smear. This day was considered as embryonic day zero (E0). The females were then divided randomly into the experimental and the control groups. The control group received tap water and the experimental group received morphine (0.05 mg/ml) in their water. On embryonic day 13 (E13), blood samples were collected from the retro-orbital sinus of all animals for plasma corticosterone detection. On embryonic day 17(E17), the animals were killed by an overdose of chloroform and the embryos were taken out surgically. The embryos were fixed in 10% formalin for 30 days. At this time, the head of the embryos were removed for tissue processing and Hematoxylin- Eosin (H&E) staining. The samples were evaluated using light microscope and MOTIC software.

Results: Our data indicated that plasma corticosterone level was dramatically increased and the lens was thinner in the experimental group. (Although the proliferation of lens cells increased in the experiment group but that lens had delay in removing the proliferated and elongation cells with abnormal density in the lateral part of the lens in comparison with the control group). Moreover, the opening of the eyelids was delayed in the off springs of the mothers who received morphine.

Discussion: This study showed that morphine consumption during pregnancy leads to defects in fetal visual system development, particularly in the lens, and eyelids.

Key Words:
Morphine,
Visual System,
Lens,
Eyelids,
Rat.

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1. Introduction

Visual system is considered one of the important sensory organs, on which life depends(Mithchel et al., 2007). Visual system has a complex structure composed of numerous cell types, that have to generate correctly in interacting with different tissues. (Fishbach et al., 2008). Any defect in each step of visual system development may result in abnormal organism function (Rossant et al., 2002).

According to the fetal programming hypothesis, the environment of mothers' body and uterus can alter the development of the fetus during particular sensitive periods, with a permanent effect on the set point of physiologic systems and the phenotype in later years (Gutteling et al., 2007). One of the most common drugs used in the medicine is morphine as an analgesic (Harrison et al., 1993). Previous studies have shown that morphine consumption can lead to delay in neural tube (Nasiraei-Maghadam et al., 2005), neural plate (Nasiraei Moghadam et al., 2009), basal ganglia (Soleimani M et al., 2006), olfactory bulb (Saeedabadi S et al.,2008), olfactory cortex Fahanik-babaei J et al., 2010), hippocampus (Ramazani M et al., 2010), amygdaloid complex Ramazani M et al., 2010) and cerebellum (Sadraie SD et al.,2008) in rat. However, the effect of morphine on lens development has not been studied earlier.

The vertebrate ocular lens is composed of two cell types, epithelial and fiber cells, encased in a collagenous basement membrane (the capsule) (Kuszak et al., 2004). The anterior of the lens has a single layer of simple epithelial cells located between the anterior lens capsule and the outermost layer of lens fiber cells (Perng et al., 2007). One function of these epithelial cells is to serve as the reservoir of cells from which the lens grows during development and throughout life. The bulk of the lens is composed of lens fiber cells. These cells are formed from the epithelial cells at the lens equator by differentiation, a process that involves the degradation of all membrane-bound intracellular organelles and the expression of lens fiber cell–specific proteins, including the β- and γ-crystallins (16); Since the lens is a closed system and newly differentiating fiber cells form on top of older layers, the oldest lens cells are located at the very center of the lens, termed the lens nucleus. These cells were formed around 2–3 months after conception. Factors in shaping the visual system are very effective; most notably include the correct expression of genes induced and establishing effective communication among the various different parts. Teratogenic foreign substance during pregnancy causes impaired embryonic development of various systems including the visual system.

In this research, the effects of maternal oral morphine consumption on lens development were investigated in the Wistar rat embryos.

2. Methods

Twelve female Wistar rats (250-300 g, pasture institute, Tehran, Iran) divided into experiment and control groups. Rats were housed 2/cage with 12/12 light cycle, constant room temperature (22±2◦C), and ad-lib food and water available.

2.1. Procedure

Female rats were housed with males (2/1) overnight for mating. On the next morning, the male rats were separated from females and embryonic day 0 was considered if sperms were observed in vaginal smear. Control group received tap water and experiment group received morphine solution (0/05 mg/ml). Blood samples were taken from rats' retro-orbital sinus on the 13th embryonic day (E13) for blood corticosterone concentration measurement. On the 17th embryonic day (E17), the pregnant rats died of chloroform then the embryos were taken out surgically, cleaned and immersed in formalin 10%

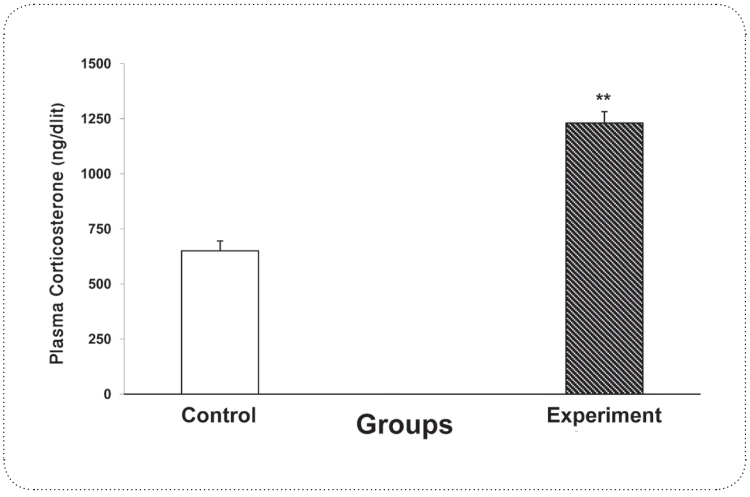

Figure 1. Effects of morphine consumption on plasma corticosterone level in pregnant rats. Data is the mean±SEM for 6 animals, **p < 0.01

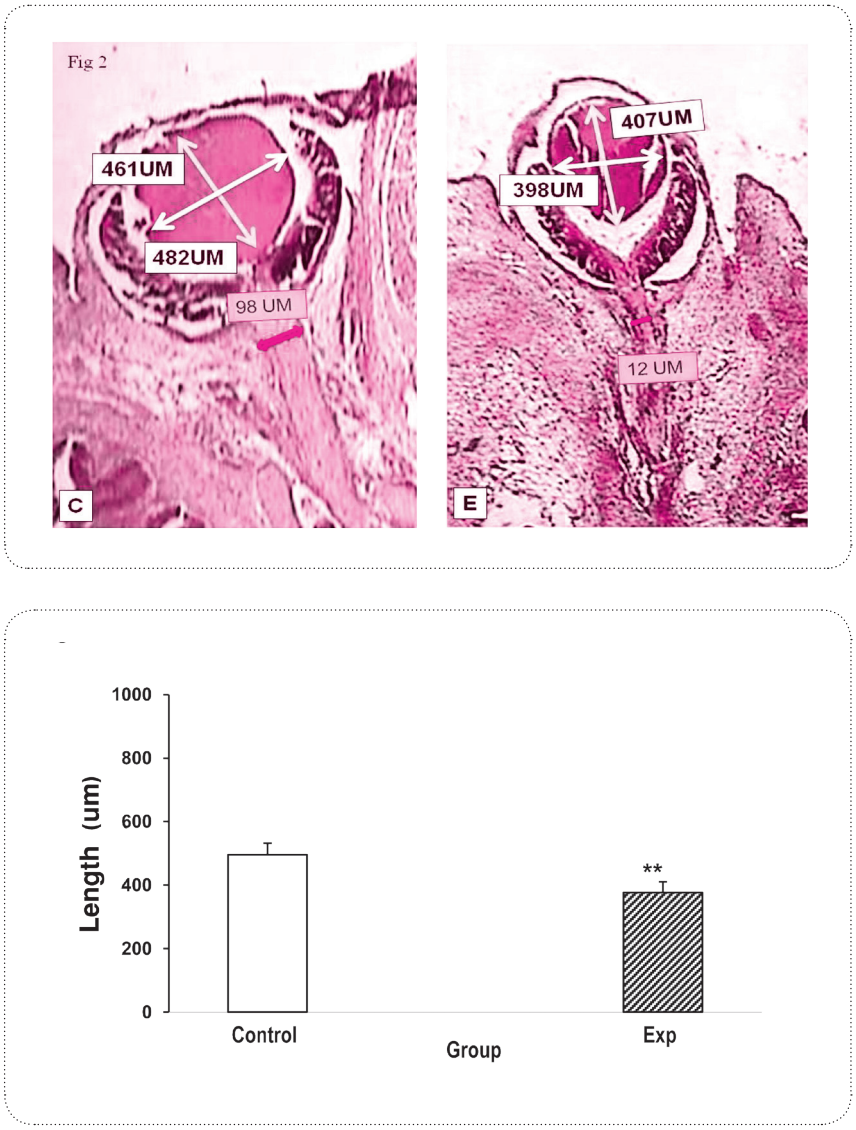

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Figure 2. Transverse section of eye in 17-day-old Wistar rat embryos. C: Control group E: Experimental group. The size of eye in the experimental group significantly decreased as compared to control group. (100X) (a). Decrease of lens size in embryos of experimental group in compared with control group. Data are shown as mean±SEM of 18-22 slide/group, **p < 0.01 (b).

for 30 days for fixation. The weight and length of the embryos were measured by a digital balance (0/0001 g) and a caliper (0/05 mm). The fixed embryos were sectioned using the paraffin embedded method which formed serial sections (5 µm). The sections were chosen as 1 of 5 sections for staining. Hematoxylin and eosin (H&E) staining processing was according to (Bancroft JDet al., 2002). The sections then were studied by MOTIC software under light microscopy.

2.2. Drugs

Morphine sulfate (TEMAD-Iran) was used in this research. The drug (0.01 mg/ml) was dissolved in tap water in a volume of 100 ml/rat/cage for the animal to drink.

2.3. Blood Sampling and Corticosterone Assessment

One ml blood was collected in an Ependorf tube containing 5 µl heparin (5000 IU/ml) and centrifuged at 3000×g for 5 min. Plasma was removed and kept at -74 °C for measuring the corticosterone. Plasma corticoste-

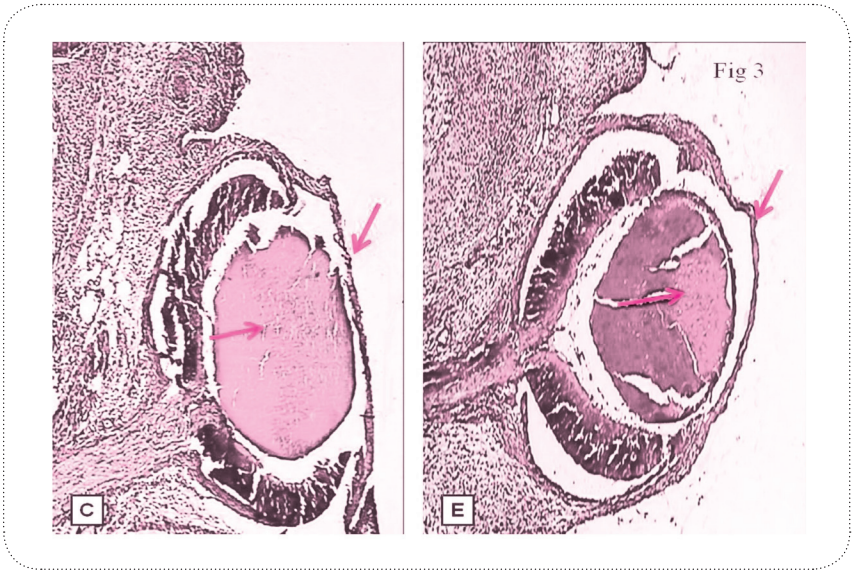

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Figure 3. Comparison of eyelid diameter and lens cells immigration in control and experimental groups of 17-day-old Wistar rat embryos. Eyelid diameter in experimental group is markedly more than the control group. (100X). C: control group, E: experimental group.

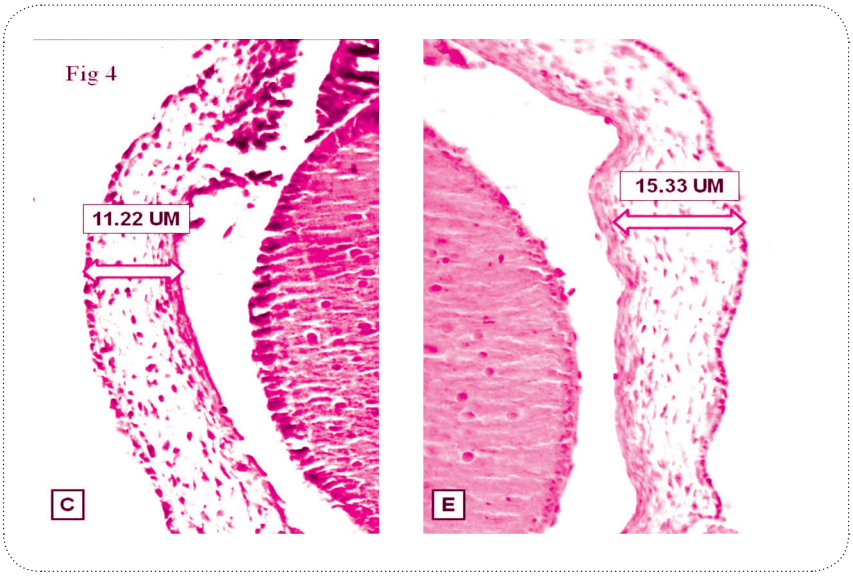

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Figure 4. Comparison of eyelid diameter in control (C), and experimental (E) groups of 17-day-old Wistar rat embryos (400X).

rone was analyzed by the corticosterone Eliza kit (Bio Activa, Germany).

2.4. Statistical Analysis

The Data were expressed as mean ±SEM. Un-paired student T-test was used in which $P<0.05$ was considered a significant level.

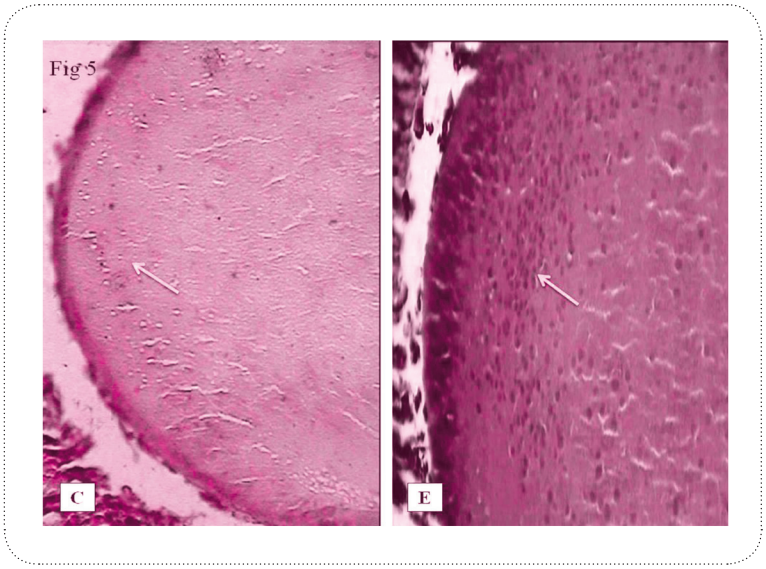

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Figure 5. Comparison of lens cells immigration in 17-day-old Wistar rat embryos in control (C) and experimental (E) groups. Immigration of lens cells from peripheral to center area in experimental group significantly delayed as compared with control group. Cell density in lens peripheral area in experimental group is obvious (400X).

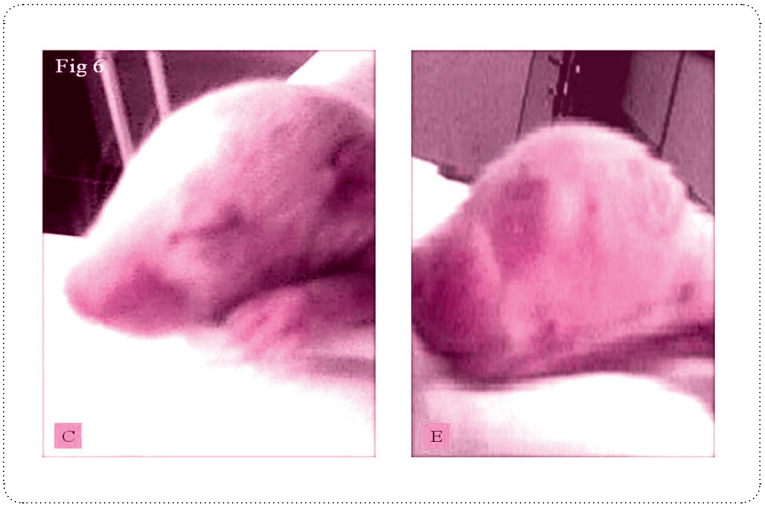

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Figure 6. Comparison of eyelid opening time in neonatal rats in control (C) and experimental (E) groups.

3. Results

3.1. Effects of Oral Morphine Consumption on Plasma Corticosterone

In the first part of our study, the plasma corticosterone level in the experimental and control groups were evaluated. Our data indicated that plasma corticosterone level increased significantly in the experimental group (2121 ± 212 ng/l) as compared with the control group (843.6 ± 85.3 ng/l). Analysis revealed that the plasma corticosterone level increment is statistically significant [Unpaired T-test; $t11=6.34$, $P<0.001$, (Fig.1)].

3.2. Effects of Oral Morphine Consumption on Lens Thickness in Rat Embryos

As shown in figure 2a, 2b, 3 and 4, when the diameter of lens and optic nerve were determined, it became clear that lens diameter, and optic nerve thicknesses also were reduced in the experimental group (the embryos of morphine consumed mothers) as compared with the mothers used tab water. (control group). Statistical analysis also showed that the differences are significant [Un-paired T-test; $t39=4.23$, $P < 0.01$], (Fig 2a, b). However, eyelids thickness was decreased in the experimental group in comparison with control group (Fig 3, 4).

3.3. Evaluation of Morphine Effects on the Lens Cells Immigration in Rat Embryos

The cell number and density was the indicator chosen for determining lens abnormal proliferation. Our observations indicated that although the proliferation of lens cells increased in the experiment group as showed by the increment in the number of the cells in the lens, but, a delay in removing the proliferated and elongation cells with abnormal density in the lateral part of the lens was observed in the experimental group as compared with the control group. However, even thought the number of cell bodies in the lens of the control group was rear (Fig 3,5).

3.4. Effects of Oral Morphine Consumption on Eye Lids Opening in the Off Springs

The results obtained in our experiments indicated that the offspring rats whose mothers used tab water, opened their eyes 10 or 12 days after birth, but on the other hand the morphine receiver rats couldn't open their eyes not less than 14 or 12 days after birth. .. This difference was statistically significant as Un-paired T-test revealed [$t7=7$, $P<0.01$] (Fig 6).

4. Discussion

The present study demonstrates that oral morphine administration during pregnancy, could lead to delay in visual system development. The delay was observed in all compartments and could be attributed to more complex response to morphine in embryos. Since the eye is considered as a complex organ composing of different parts with different embryonic initials (Wolf Louise V et al., 2009),(Gilbert Scott S, 2000). This study shows that morphine consumption can increase plasma corticosterone level in pregnant rats. The increment was statistically significant for the defects were clearly observed in the embryos visual system.

In agreement with previous studies which focuses on the effects of oral morphine consumption by the pregnant rats on embryonic neural tube (Nasiraei-Moghadam S et al., 2005), neural plate (Nasiraei-Moghadam S et al., 2009), cerebellum (Sadraie SD et al., 2008), basal ganglia (Soleimani M et al., 2006), olfactory bulb (Saeedabadi S et al., 2008), olfactory cortex (Fahanik-babaei J et al., 2010), amygdaloid complex (Ramazani M et al., 2010) and hippocampus (Ramazani M et al., 2010) development, present data also shows that rat's embryo eye development could be affected by morphine. Our data showed that the experimental group has thickened eyelids than controls which emphasis their eye opening delay(fig 4,6). Since for fully eye development, eyelid must be opened, the lower thickness of eyelid is a good advantage and may help the visual system to be operational in an appropriate time. The eyelid thickened may delay the eye opening for more additional time, which in fact can reduce the animal's chance for survival Harrison LM et al.,1993),(Dorfman AL et al., 2009). According to our data one can conclude that morphine consumption during pregnancy reduces the animals' ability to adapt with their environment because of the delay in eye opening due to increasing the eyelid thickness. Moreover, decreased cell size also may be contributed in defect eye development. At least, morphine consumption reduces lens diameter in experimental group. Lens is the most important part of the eye and delay in its development may result in complete eye dysfunction (Rossant G et al., 2003; Gilbert Scott S, 2000).

The real reason for destroying effects of morphine is not completely understood. According to previous studies, morphine can readily crosses the placenta and reach the embryos (Kopcky EA et al., 1999). In addition, opioid receptors are recognized on placenta villi and their activation resulted in placenta vessel contraction which in turn reduces embryos blood supply (Ahmed MS et al., 1989). These studies all show that morphine itself may increase the delay observed in previous and present study. However, these findings did not discuss the increase in cell number and decrease in cell size which we observed in our study. Our results on plasma corticosterone level of pregnant rats might lighting this corner. We found out that oral morphine consumption by pregnant rats significantly increases their plasma corticosterone level. Corticosterone is an important glucocorticoid in rodents which supports the animals against stressful life events (Derijk Roel H and Ron de Kloet E, 2008),(Timpl P et al., 1998),(Mastorci F et al., 2009). Its plasma level increases when an animal perceives stressful occasions (Nikisch G,2009). However, sev-

eral studies have revealed that stress during pregnancy, could lead to an abnormal embryonic central nervous system and endocrine system development both in human and animals (Mulder EJH et al., 2009; Weinstock M, 2008). In rats, however, stress can increase plasma corticosterone level and could lead to abnormal cell growth and proliferation (Xio L and Chen Y,2008; Jenkins S,2007). It is interesting that morphine can induce corticosterone release in pregnant rats. The main site for morphine action in this regard is adrenal cortex and several studies indicated that morphine can interact with its mu-opioid receptors located on the adrenal cortex cell surface and induces corticosterone release (Nikisch G, 2009; Pascoe John E et al., 2008). However, Pirnik and co workers have shown that morphine may interact with glutamatergic system for induction of corticosterone release (Pirnik Z et al., 2001). Moreover, the corticosterone, affects gene expression in target cells and abnormal increment in corticosterone plasma level could lead to change in expression of genes that play an important role in cell development and proliferation Weinstock M,2008). One important gene, the PAX6, expressed in surface ectoderm cells which is important in making ectoderm competent for respond to the inductive signal from the optic vesicle for the forming lens (Wolf Louise V et al., 2009; Kozmik Z,2008).

Whether corticosterone interacts with this gene and affects its expression or not, is a question which must be investigated in future studies, but eyelid thickness which observed in experimental group can be concluded in this line.

In conclusion, although the exact mechanism by which morphine induces its effects on embryos visual system are not well understood, our results indicated that at least one mediator of morphine effect may be the hormone corticosterone. Our data showed that experimental group shows lens growth, lens differentiation, and more cell numbers in their visual system which also were observed previously in studies regarding stress and corticosterone effects on embryos development.

5. Acknowledgement

This work was supported by Neuroscience Research Center, Baqyiatallah University of Medical Sciences.

References

Administration of morphine delay neural plate development of rat embryos. Physiology and Pharmacology .12 : 314-319.

Ahmed MS, Schoof T, Zhou DH, Quarles C. (1989). Kappa opioid receptors of human placental villi modulate acetylcholine release. Life Sci.45: 2383-93.

Bancroft JD, Gamble M. (2002).Theory and practice ofhistological techniques. 5th ed. London: Churchill Livingston.

Bloemendal H., et al. Ageing and vision: structure, stability and function of lens crystallins. Prog. Biophys. Mol. Biol. 2004;86:407–485.

Derijk Roel H, Ron de Kloet E. (2008). Corticosteroid receptor polymorphisms: determinants of vulnerability and resilience. J PHARMACOL SCI.583:303-311.

Dorfman AL, July S, Haedy P, Chemtolo S, Lachapelle P. (2009). The effects of oxygen and light on the structure and function of the neonatal rat retina. Doc Ophthalmol.118(1):37-54.

Fahanik-babaei J, sadooghi M, zardooz H, sahraei H. (2010). maternal oral morphine consumption delays olfactory cortex development in wistar rats during embryonic period. Journal of rafsanjan university of medical sciences.9(1): 3-14.

Fishbach KF., Hiesinger, PR. Optic lobe development. Adv Exp Biol. 2008; 628, 115-36

Gilbert S. (2000). The central nervous system and cell-cell communication in development.in: Gilbert SS, Editor. Developmental biology. Massachusetts: Sinauer Associates Inc .143-148.

Gutteling Barbara M., De Weerth C, Buttelaar J K. (2007). Prenatal Stress and Mixed-Handedness. PEDIATR RES.62(5): 586-590.

Harrison LM, Zadina JE, Banks WA, Ksstin AJ. (1993). Effects of neonatal treatment with Try-MIf-1, morphiceptin, and morphine on development, tail flick,and blood-brain barrier transport. Brain Res.75(2): 207-12.

Jenkins SA, Muchow M, Richards MP, McMurtry JP, Porter TE. (2007). Administration of adrenocorticotropic hormone during chicken embryonic development prematurely induces pituitary growth hormone cells.148(8):3914-21.

Kopcky EA, Simone C, Knie B, Koren G. (1999).Transfer of morphine across the human placenta and its interaction with naloxone. Life Science.65: 2359-71.

Kozmik Z. (2008). The role of pax6 genes in eye evolution. Brain Res Bull.75: 335-9.

Kuszak JR, Zoltoski RK, and Tiedemann CE.(2004). Development of lens sutures. Int. J. Dev. Biol.48: 889-902.

Mastorci F, Vicentini M, Virat O, Mangi M, Graiani G, Quaini F, Meerlo P, Nalivaco E, Maccarti S, Sgoifo A. (2009). Long-term effects of prenatal stress: Changes in adult cardiovascu-

lar regulation and sensity to stress. NEUROSCI BIOBEHAV R. 33:191-203

Mithchel DC, Bryan BA, Liu JP, Liu WB, Zhang L, Li Dawid W. (2007). Developmental expression of three small GTPases in the mouse eye. Mol Vis.13: 1144-1153.

Mulder EJH, Robles de Medina PG, Huizink AC, Van den Bergh BRH., Buitelaar JK, Visser GHA. (2002). Prenatal maternal stress: effects on pregnancy and the(unborn)child. Early Human Development.70:3-14.

Nasiraei-Maghadam S ,Shiva SH, Bahadoran H, Sadoogi M. (2005). Effects of maternal oral morphine consumption on neural tube development in wistar rats. Brain Res.159:12-17.

Nasiraei-Moghadam S, Bahadoran H, Saeedabady S, Shams J, Sahraei H. (2009).Oral administration of morphine delay neural plate development of rat embryos. Physiology and Pharmacology .12: 314-319.

Nikisch G.(2009). Involvement and role of antidepressant druge of the hypothalamic-pituitary-adrenal axix and glucocorticoid receptor function.Neuroendocrinol.30(1): 6-11.

Pascoe JE, Wiliams KL, Mukhopadhyay P, Rice K, Woods JH, Ko Mei-Chuan. (2008). Effects of mu,kappa, and delta opioid receptor agonist on the function of hypothalamic-pituitary-adrenal axis in monkeys.Psychoneuroendocrinoology.33(4): 478-486.

Perng M.D, Zhang Q, Quinlan RA. (2007). Insights into the beaded filament of the eye lens. Exp. Cell Res. 313:2180–2188.

Pirnik Z, Schwendt M, Jezova D. (2001). Single dose of morphine influences plasma corticosterone and gene expression of main NMDA receptor subunit in the adrenal gland but not in the hippocampus. Endocrine Regulations.35: 187-193.

Ramazani M, Ameli H, Hakimi Gilani V, Bahadoran H, Sahraei H . (2010) Reduction of cell size in amygdaloid complex of the Wistar rat embryos after oral morphine consumption. Physiology and Pharmacology.14 (2): 181 -190

Ramazani M, Hamidi E, Haji Moghadam Zadeh A, Bahadoran H, Sahraei H. (2010). the effect of oralmorphine consumption on hippocampus development in Wistar rats embryo. Kowsar Medical Journal . 1(1): 11-15.

Rossant G, Patrick PLT. (2002). Mous development, patterning, morphogenesis, and organogenesis.520-538

Sadraie SH, Kaka GR, Sahraei Hadayat, Dashtnavard H, Bahadoran H, Mofid M, Mahdavi nasab H, Jafari F. (2008). Effects of maternal oral administration of morphine sulfate on developing rat fetal cerebrum: A morphometrical evaluation. Brain Res. 12(45): 36-40.

Saeedabadi S, Sadooghi M, Sahraei H, Bahadoran H, Fahanik Babaei J, Jalili C. (2008) Effects of oral morphine on the development of olfactory bulb in rat embryo. The Scientific Journal of Arak Medical University.11: 1-8.

Soleimani M, Sahraei H, Sadooghi M, Maleki P. (2006). Effectsof prenatal morphine exposure on the basal ganglia development in rat embryo. The Scientific Journal of Arak Medical University.9 ; 53-61.

Taylor, C.C. Soong YI., Wu. D. Yee JS. Szeto, HH. (1997). Morphine stimulates adrenocorticotropin and cortisol release in the late-term ovine fetus. Pediatr Res.41(3): 411-415.

Timpl P, Spangel R, Sillaber I, Kresse A, Reul J, Stalla G, Veronique B, Steckler T, Holsboor F, Wurst W. (1998). Impaired stress response and reduced anxiety in mice lacking a functional corticotrophin-releasing hormone receptor 1. NAT GENET.19:162-166.

Van den Bergh Bea RH, Mulder Eduard JH, Mennes M, Glover V. (2005). Antenatal maternal anxiety and stress and the neurobehavioural development of the fetus and child: links and possible mechanisms. A review. NEUROSCI BIOBEHAV R.29:237-258.

Weinstock M. (2008). The long term behavioural consequences of prenatal stress. NEUROSCI BIOBEHAV R .32:1073-1086.

Wolf Louise V, Yang Y, Wang J, Xie Q, Brounger B, Tam ER, Zavadil J.(2009). Identification of pax6-dependent Gene regulatory networks in the mouse lens. PLOS One.4(1): e4159.

Xio L, Chen Y. (2008). Culture condition and embryonic stage dependent silence of glucocorticoid receptor expression in hippocampal neurons. Steroid Biochem Mol Biol. 111(1-2):147-55.