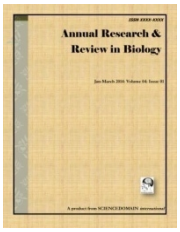

Annual Research & Review in Biology
4(18): 2823-2834, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Defects in Wistar Rat's Embryo and Placenta Development: A [C]^{14}-Morphine Study

Masoomeh Kazemi[1], Elaheh Tekieh[1], Sahar Golabi[2] and Hedayat Sahraei[1*]

[1]Neuroscience Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.
[2]Department of Physiology, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Original Research Article

Received 4th March 2014
Accepted 21st April 2014
Published 7th May 2014

ABSTRACT

Background: In previous studies it has been emphasized that morphine effectiveness site may be either in the embryo or the placenta. In this study, we perused the morphine effect site in embryo with placenta, simultaneously. Animals treated by C^{14}-Morphine.
Material and Methods: Experimental groups received daily doses of 0.05mg/ml C^{14}-morphine in their drinking water. On the 9th and14th embryonic days, the rats were anesthetized, embryos with the placenta removed and were fixed in 10% formalin for two weeks. Then, processed, sectioned in 5 and 25µm thicknesses, and fixed on glass slides for further evaluation. The 25µm sections were delivered to black and white film for three days. Films processed and evaluated with a digital inverse microscope for possible radiological impression. The 5µm sections were processed for H&E staining, and evaluated by light microscope and MOTIC software.
Results: Results showed that total surface of 9th- day placenta and embryo, increases simultaneously in compare with control one. 14th- day placenta, showed a significant decrease. Labeled morphine consumption data, revealed that maximum location of morphine effect aggregation is simultaneously on the placental villi and whole embryo.
Conclusion: Morphine effect to detect embryo's normal growth can be result from its effect on normal placental development and its passage from placental barrier.

**Corresponding author: Email: hsahraei1343@gmail.com;*

Keywords: Placenta development; C14-morphine; rat; embryo development.

1. INTRODUCTION

Opiates production and consumption, is growing on and also the routine stress and problems increase the tendency for opiates consumption. All human beings are in risk for opiates dependency and addiction. Mothers also are in dependency risk to opiates. In pregnant mothers, addiction side effects will involve them and the next generation [1,2,3] .Because placenta is most important part in nutrient exchange between embryo blood and mother, its size is directly relevance to nutrient transmission. This transfer occurs in superficial regions of placenta via simple diffusion and active transport [3,4]. Seepage of blastocyst to uterus is from embryo blast pole and does by trophoblastic cells. Trophoblastic cells compose of two layers: An inner layer, named as cytotrophoblast. These cells are mono nuclear and have cellular cleavage potency. Outer layer, consists of Syncytiotrophoblast cells. They are poly nuclear and have not any distinct membrane bound [4,5]. Syncytiotrophoblast cells force to deeper portions of endometrium and destroy endothelial cover of capillaries. Syncytiotrophoblast cells congestion forms some cavities that will be filling with blood. In this way, blood lacuna forms [1,4,5]. Previous studies indicated that morphine consumption causes a delay in blood lacuna formation. As a result the embryo will be hypoxic. Hypoxia, causes a deficiency in development of embryo [6,7,8]. Morphine illustrates its effect on mu, kappa and delta opioid receptors and activation of these receptors cause a decrease in C-AMP, an increase in K^+ exodus and a reduction in Na^+ entrance [2,9]. Embryo blast cellular bulk also differentiates to two cellular layers. One layer composes of small cubic cells beside blastocyst cavity, called hypoblast layer. Second layer consists of tall cylindrical cells beside amniotic cavity, named epiblast layer [4,7,10]. Previous studies demonstrated that in 9th- day of pregnancy, morphine consumption causes a delay in normal development of embryonic cavities (amniotic and chorionic cavities) of rat embryo [10,11]. Calcium ion has an important role in placental hormones secretion (estrogen and progesterone). These hormones have a significant role in stability and genesis of embryo [1,12]. With advancement of pregnancy, placenta can act as a source for secretion of dominant part of estrogen and progesterone and also other hormones that are necessary for embryonic growth. In this way, placenta can be act as a replacement for ovarian hormones [13,14]. Therefore, morphine can acts as a meddlesome and causes a tribulation in secretory function of placenta .Also, causes a delay in development of embryo [13,14]. Valence of placenta for nutrient transmittance and release, depends on placenta and also profusion, size and shape of transmitter factors. Due to its small molecular size, non-polarity and high solubility in fat, morphine can easily passes through placental barrier and affects embryonic cells [14,15]. Also, morphine disposal causes an increase in corticosterone concentration of mother's plasma [16]. By shortening interphase stage, corticosterone causes cellular abnormal proliferation. This blemish can disrupt normal function of embryonic cells and can be main cause in embryonic normal development [16,17,18]. This is confirmed that opiates can affect placental function via opioid receptors of placental villi. On the other hand, because placenta acts as a conservative barrier, any disruption in placental development causes a deficiency in placental functions such as endocrine, exchanger and conservative functions [2,15,16]. Other studies showed that edible morphine consumption by mother causes a disruption in development of maternal and embryonic portions of placenta .It also affects cytotrophoblast and Syncytiotrophoblast cells development [19,20]. Based on prior studies, edible morphine can passes from placental barrier and has mortal effects on development of embryonic nervous system such as choroid plexus, brain ventricles, ependymal duct, neural tube, spinal cord and visual system [11,21,22,23,24]. Previous studies indicated that morphine causes a delay in placenta and in embryo's nervous system

development [1,21]. But, there are no researches in the base of whether highest density of morphine effect site is simultaneously effective on embryo and placenta? For identification of highest density of morphine effectiveness simultaneously on the embryo and placenta, we used labeled C^{14}-Morphine. By use radio drug (radioactive morphine), we could recognize maximal absorption in embryonic and placental tissues simultaneously.

2. MATERIALS AND METHODS

Female Wistar rats with an average weight of 170 to 200grams were used in this research. The rats were kept in pair cages, under the ambient temperature (24±1°C) and with natural photoperiods (12hours light and 12hours dark). During the experiment, the rats were given adequate food and water. The ethical standards established with laboratory animals were compiled (under the supervision and approval of the ethics committee of Baqiyatallah University of Medical Sciences).

2.1 Drug

In this study, C^{14}-Morphine sulfate provided by the Iran Atomic Agency (Tehran, IRAN) was used orally.

2.2 Animals

We divided 24 rats into three groups (I, II, and III). Group I was the control group (n=12). Group II was the 9th day pregnancy group (n=6), and group III was the 14th day pregnancy group (n=6). A total of 12 female rats in dual groups copulated with adult male rats. After confirmation of pregnancy (observation of a vaginal plug and the existence of sperm in the vagina), the following morning they were separated from male rats and kept in the same dual-groups. Thereafter, the experimental group (first group from day 0 until 9 of pregnancy and second group from day 0 until 14 of pregnancy) received a daily dose of 0.05mg/ml (5mg morphine in 1000ml tap water for six rats). The amount of consumed morphine in 10ml water for every 100g of the rat's weight was computed and attempts were made to give the rats the amount they needed. Control groups were treated with normal tap water. After treatment, all groups were anesthetized by chloroform. Embryos with the placenta were separated from the mother rats and transmitted to a 10% formalin solution for two weeks. Next, Embryos with the placenta were processed, molded, sectioned in 5µm and 25µm thicknesses, and fixed on glass slides for additional evaluation. The prepared sections were stacked on wooden sheets (30cm long and 8cm wide). The slides were covered by photographic strips (black and white photography) and allow remaining in a dark room for three days, after which they were transferred to the photographic archives to prepare the negatives. Films were assayed after appearance. The slides that were prepared with 5µm sections were stained using the hematoxylin and eosin (H&E). Samples were then examined by light microscope and MOTIC software. The MOTIC software was used for tissue measurement, which consists of a microscope which is connected to a computer and a monitor through a software program. This software provides the possibility off scanning the slides and is capable of performing different measurements.

2.3 Statistical Analysis

Data were reported as mean ± SEM. Differences between group means were assessed by a one-way analysis of variance(ANOVA) and unpaired sample *t*-test using the SPSS/PC computer program (version 18.0). (a) P value of <0.01 and (b) value of <0.05 were considered as significant.

3. RESULTS AND DISCUSSION

Our results showed that the effect of edible morphine use by pregnant mother is quite obvious in pregnancy 9th- day. Morphological results of this study on sections with 5µm thickness using H and E staining method revealed that in pregnancy 9th- day, morphine causes an increase in total surface of placenta simultaneously. In14th- day of pregnancy, a decrease in placental total surface was seen (Figs. C1, M1, C2, M2). Assessment of highest location of C^{14} -Morphine effect density on 25µm tissue sections by radioactive morphine showed that the effective situation of morphine is simultaneously on opioid receptors of placental villi, 9th- day embryo surface and 14th- day placenta and embryo (Figs. R1, R2). Morphological data from Morphometric aspect revealed that in morphine group, total surface of embryo and placenta have a significant increase in size compare with 9th- day control group. Again, total surface of 14th- day placenta had a significant decrease in size compare with control group (Fig. 3).

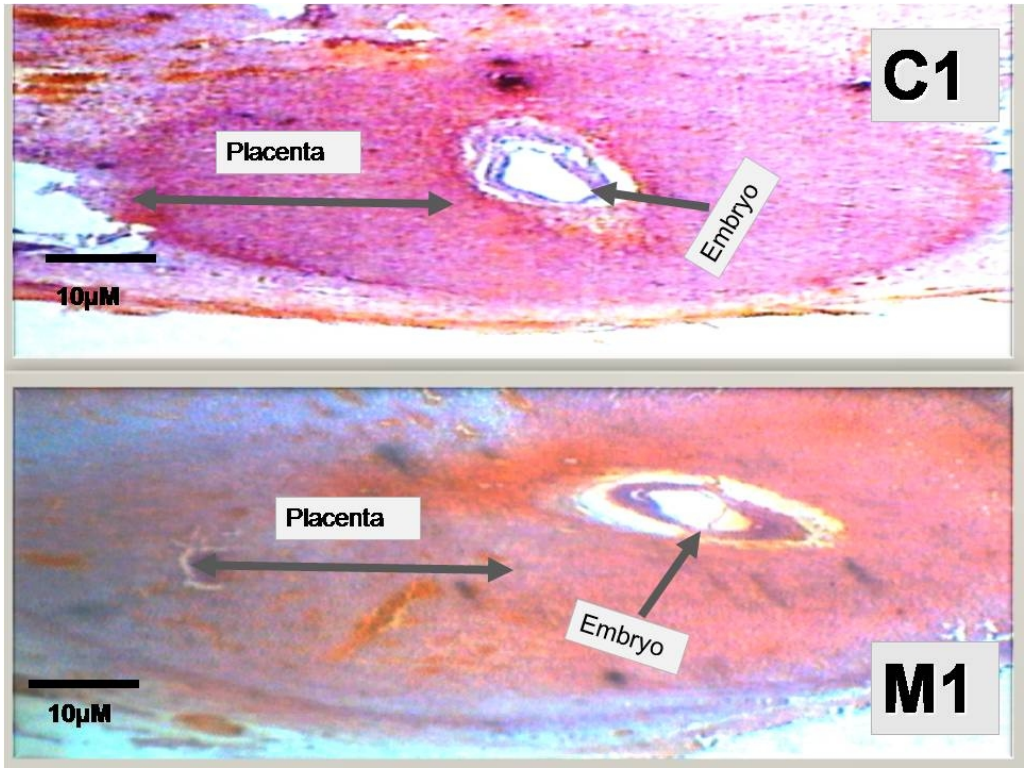

Fig. 1. Microscopic images, placenta and embryo of morphine group (M1) and control group (C1) (X10, longitudinal section) Shows changes of embryonic and placental surface size in 9th day pregnancy

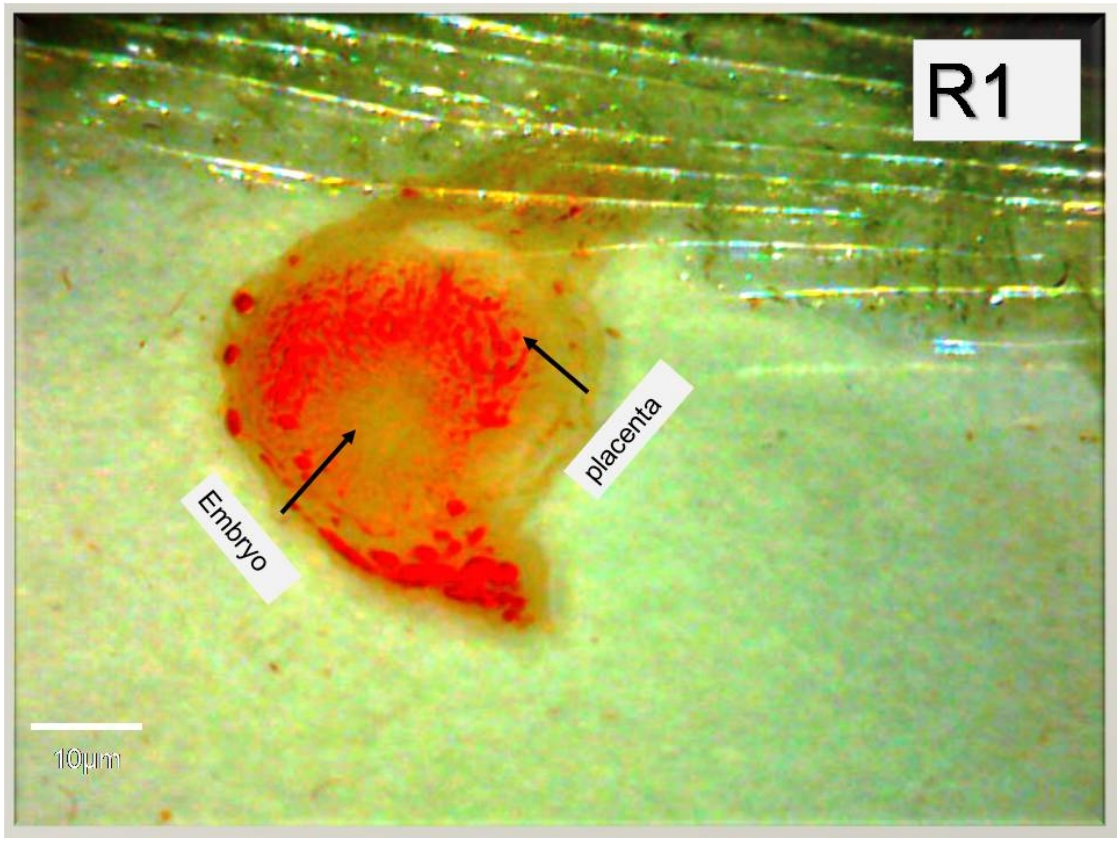

Fig. R1. The picture shows appointment of highest density of morphine effect simultaneously on the under development embryo and placenta 9th- day. Note location of C^{14} -Morphine effect on blood vessels of under development placenta that was shown by arrows (imaged by Dino capture x50)

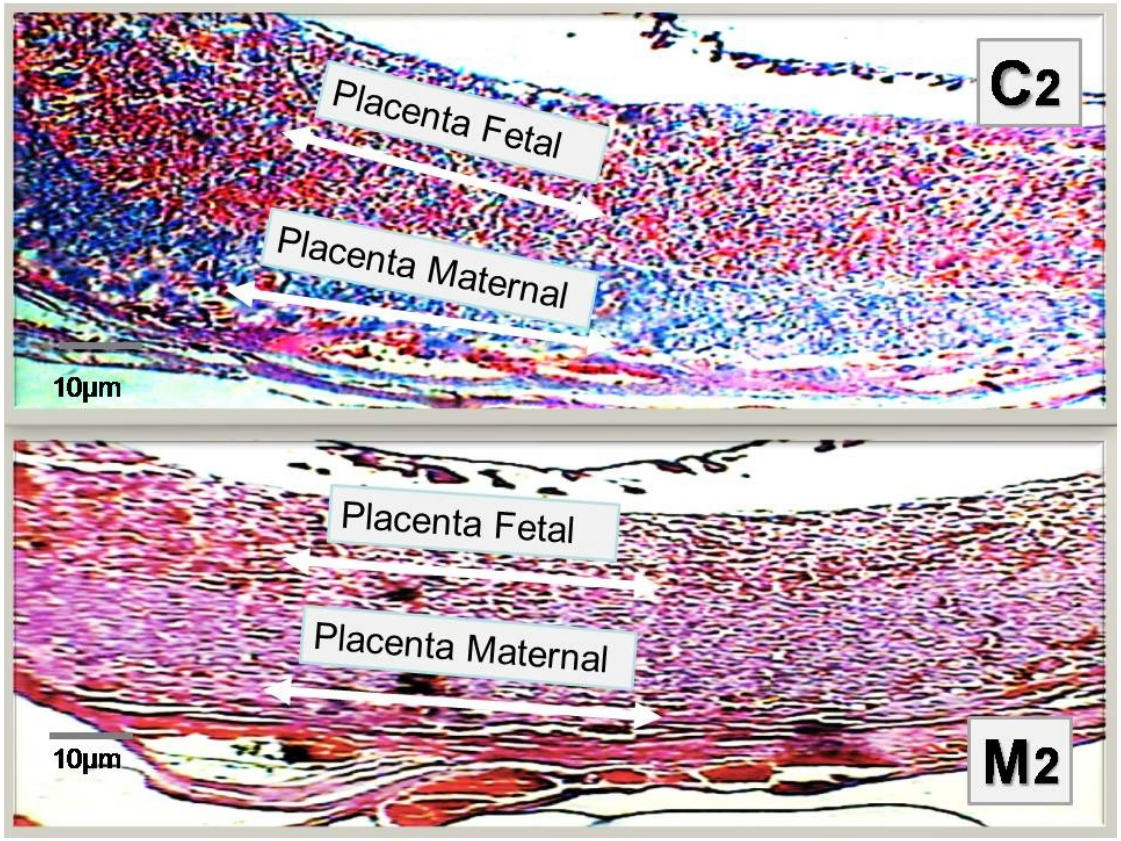

Fig. 2. The effect of edible morphine on development of 14th- day placenta. A comparison between control group (C) and morphine group (M), for total size of placenta and embryo surface in pregnant rat

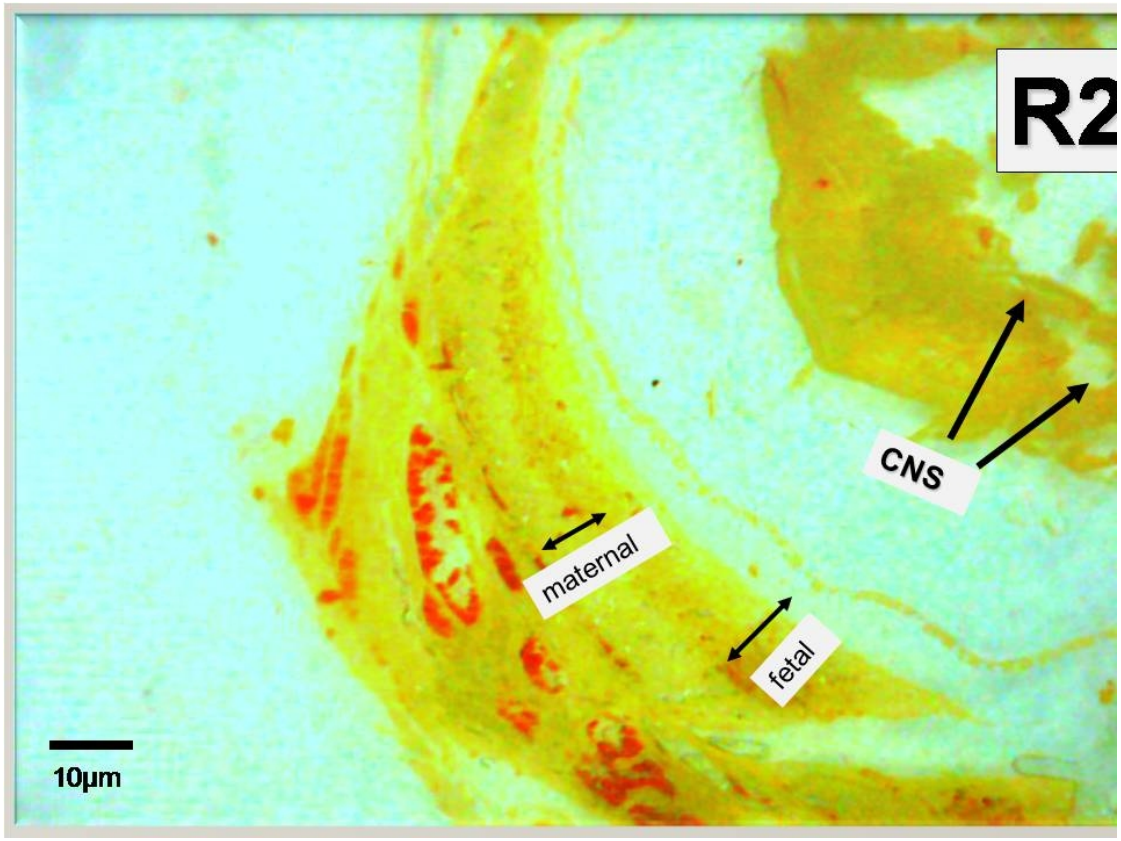

Fig. R2. Expression of maximum density of morphine simultaneously on the placenta and embryo in consumer mothers by radioactive C^{14} -Morphine in 14^{th}- day of pregnancy. Note morphine effect site on the placental blood vessels, especially in the embryonic as well as on nervous system that was shown by arrows (imaged by Dino capture x50)

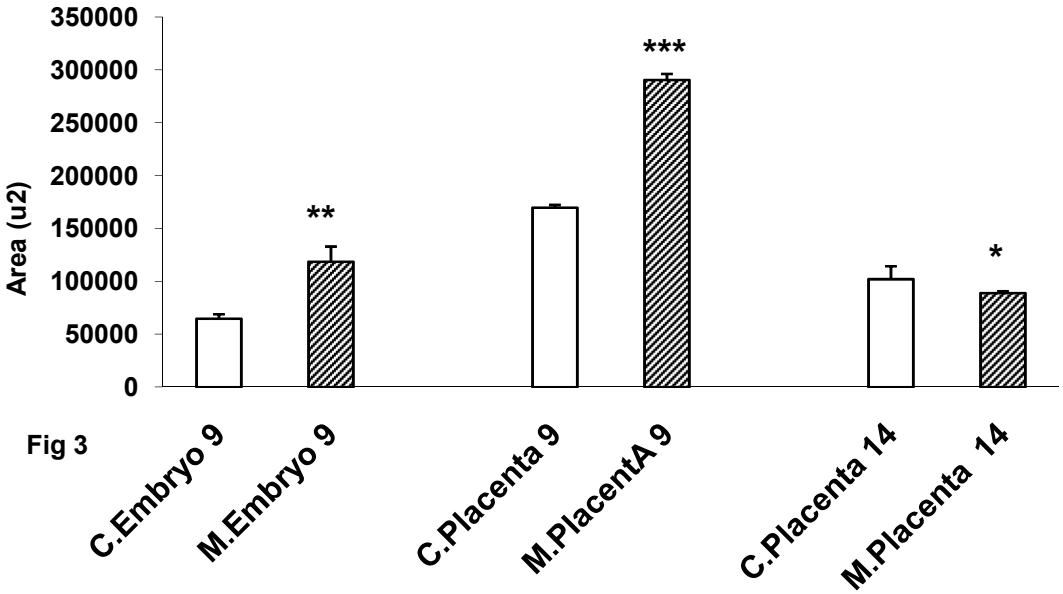

Fig. 3. The effect of edible morphine on development of 9th and 14th- day placenta. A comparison between control group (C) and morphine group (M), for the total size of placenta and embryo surface in pregnant rat (significant *p<0.05, mean ± SEM). There are 6 rats in each group

***p< 0.01 shows a significant increase in total surface of 9th- day embryo in morphine group. ***p<0.001 shows a significant increase in total surface of 9th- day placenta in morphine group and *p< 0.05 demonstrates a significant decrease of 14th- day placenta in morphine group in compare with control one*

Various studies were done about morphine effect on embryo or placenta and results from researches showed that morphine causes a delay in normal development of embryo or placenta [1,21]. Based on previous studies, opiates such as morphine cause a decrease in number and surface of placental blood lacuna [11,25]. Previous researches confirmed that mu, kappa and delta opioid receptors are present on blood vessels of placental villi. Morphine interact with membranous opioid receptors to, shows its effect. It is revealed that maximum effect site of morphine in consumer mothers placenta is on the receptors of blood vessels endothelium of placenta's embryonic part [2,26]. In previous studies for localization of morphine effect in embryonic nervous system, radio drug was used. Results showed that highest density of morphine effect is on the opioid receptors of blood vessels endothelium of embryonic choroid plexus [22]. In present study, we studied consumer mother's embryo and placenta simultaneously. Our results were in line with previous findings. our data by using radioactive morphine demonstrated that maximum density of morphine effects on consumer mother's embryo and placenta, is observable simultaneously on placental and embryonic tissues [R1, R2],this finding was according to data from previous studies. Indeed, anywhere that there are more blood vessels, we have more opioid receptors and in these regions the effect of morphine is more. Therefore, resultant malformations are more in these zones [1,22,27]. According to our results, especially in 14th- day of pregnancy that placenta and embryo is more developed, location of morphine effect is on the both parts (placenta and embryo). Previous findings indicated that highest density of morphine simultaneously

relevant to embryonic part of placenta and embryonic nervous system [R2]. Prior studies revealed that malformative factors such as drugs are more effective in first half of pregnancy. Because in this period, different systems of embryo are in development process, they are more sensitive to malformative factors. On the other hand, in the start of embryonic period, developmental malformations are more [28,29]. Based on studies, morphine motives cellular division especially in low differentiates cells. Increment in total surface of placenta and embryo in 9^{th}- day of pregnancy is a result from morphine effect. With its effect on placenta and passage from placental barrier, morphine causes abnormal embryonic development in first half of pregnancy [Fig. 3].Prior studies confirmed that morphine administration causes release of stress hormones such as corticosterone. Corticosterone activity in this situation causes an elevation in blood pressure and fill the CP with blood in rat [30,31].This is also demonstrated that with progression in pregnancy, corticosterone concentration increases in pregnant mother's plasma. Again, morphine administration causes an elevation in corticosterone concentration of pregnant mother's blood [16,32]. Scientists acclaimed that glucocorticoids increment causes weakness of embryo and placenta that directly occurs with change in cellular cycle from proliferation phase to differentiate phase [32,33]. The results from this study revealed that with placental development, there is a decrease in morphine effects on placental surface size in 14^{th}- day of pregnancy. These findings are in line with data from previous researches that confirms that with pregnancy progression, there is an elevation in corticosterone concentration. Time length of morphine administration can increase this concentration. As a result, abnormal increase of corticosterone causes abnormal increment of cellular division by shortening the interphase stage. This incompetence in cellular development causes repeatedly division of cell without its normal growth [33]. Therefore, regard to surface size of placenta, total surface of placenta inverses with placental development [Fig. 3]. Based on previous studies, corticosterone can induces proliferation of cytotrophoblast cells in the rat embryos. These cells are low differentiating cells and have high potency for division. Regard to this point that morphine administration causes an elevation in corticosterone concentration, we can say that morphine and corticosterone potentiate each other function [16]. Blood flow is a principal factor for placental function and embryonic growth. Morphological studies demonstrated that changes that occur in physiological conversions of spiral vessels during pregnancy can cause an increase in invasion of trophoblast cells and resultant elevation in high speed of blood flow causes destruction of placental villi and other malformations [31,32]. Spiral vessels are supplier of placenta. Regard to this point that more blood vessels are in embryonic portion of placenta, therefore, maximum opioid receptors and highest destructive effects of opioids are in this portion. On the other hand, most important portion of nutrient exchange between mother and embryo is embryonic part of placenta. Any disruption in normal growth and development of this part causes irreparable malformations in embryo. Studies confirmed that morphine by virtue of its action on placental villi opioid receptors can causes vessel constriction and decreases blood flow from placenta to embryo (hypoxia), [16,19]. Embryonic part of placenta chiefly composed of cytotrophoblast and Syncytiotrophoblast cells. These cells have a significant role in secretory function of placenta such as estrogen and progesterone secretion that are pivotal in embryonic growth and development. Disruption of secretory function of these cells, can delay placental genesis and as a result, incompetence in embryo genesis [14,33]. In a general, our results by using labeled morphine, demonstrated that highest density of morphine effect site on embryo and placenta simultaneously is on the blood vessels of placental villi and in 9^{th}- day and 14^{th} -day embryos.

4. CONCLUSION

Our findings were in compatibility with previous studies. This means that morphine can simultaneously causes a delay in embryo genesis and also can causes different malformations in embryo by its passage from placental barrier. Significant and principal finding of this research demonstrated that maximum density and effect of radio drug simultaneously is on the opioid receptors of placental villi, especially embryonic part and also embryo itself. So, opiate agents simultaneously cause a delay in placental and embryonic development.

ACKNOWLEDGEMENTS

This study has been financially sponsored by Neuroscience Research Center, Baqiyatallah University of Medical Sciences.

COMPETING INTERESTS

The authors have no potential conflict of interests pertaining to this journal submission.

REFERENCES

1. Kazemi M, Sahraei H, Dehghani L. Identification of site of action of morphine in the pregnant Wistar rat's placenta: A [C]^{14}-morphine study Cell Journal. 2012;14:122-129.
2. Dietis N, Rowbotham DJ, Lambert DG. Opioid receptors subtypes: facts or artifacts? Br J Anaesth. 2011;107:8-18.
3. Marion L, Sanford S, Hans H, Vikram P, Laxmaiah M. A comprehensive review of opioid-induced hyperalgesia. Pain Physician. 2011;14:145-161.
4. Dehghani L, Sahraei H, Meamar R, Kazemi M. Time-dependent effect of oral morphine consumption on the development of cytotrophoblast and -syncytotrophoblast cells of the placental layers during the three different periods of pregnancy in Wistar rats. Clinical and Developmental Immunology. 2013;2013:974205.
5. Fowden AL, Forhead AJ, Coan PM, Burton GJ. The placenta and intrauterine programming .J Neuroendocrinol. 2008;20:439-450.
6. Ornoy A, Michailevskaya V, Lukashov I, Bar-Hamburger R, Harel S. The developmental outcome of children born to heroin-dependent mothers.raised at home or adopted. Child Abuse Negl. 1996;20:385-396.
7. Sadler TW. Langman's Medical Embryology. 9th ed. Piladelphia: Lippincott William & Wilkins. 2006;121-154.
8. Nettleton RT, Wallisch M, Olsen GD. Respiratory effects of chronic in utero methadone or morphine exposure in the neonatal guinea Pig. Neurotoxicol Teratol. 2008;30:448–454.
9. Sara E, Peng H, Jashvant D. Drug interactions at the blood-brain barrier: fact or fantasy? Pharmacol Ther. 2009;123:80–104.
10. Kazemi M, Sahraei H, Azarnia M, Bahadoran H. Effect oral morphine consumption on amniotic and chorionic cavities development in the embryo Wistar rat. Journal of Shaheed Sadoughi University of Medical Sciences and Health. 2011;18:444-450.
11. Nasiraei-Moghadam S, Sahraei H, Bahadoran H, Sadooghi M, Salimi SH, Kaka GR, Imani H, Mahdavi-Nasab H, Dashtnavard H. Effects of maternal oral morphine consumption on neural tube development in Wistar rats. Brain Res. 2005;159:12-17.

12. Wallace JM, Aitken RP, Milne JS, Hay WW Jr. Nutritionally mediated placental growth restriction in the growing adolescent: consequences for the fetus. Biol Reprod. 2004;71:1055-1062.
13. Fowden AL, Ward JW, Wooding FP, Forhead AJ, Constancia M. Programming placental nutrient transport capacity. J Physiol. 2006;572:5-15.
14. Behravan J, Piguette-Miller M. Drug transport across the placenta, role of the ABC drug efflux transporters. Expert Opin Drug Metab Toxicol. 2007;3:819-830.
15. Sargeant TJ, Day DJ, Miller JH, Steel RW. Acute in utero morphine exposure slows G2/M phase transition in radial glial and basal progenitor cells in the dorsal telencephalon of the E15.5 embryonic mouse. Eur J Neurosci. 2008;28:1060-1067.
16. Kazemi M, Sahraei H, Azarnia M, Dehghani L, Bahadoran H, Tekieh E. The effect of morphine consumption on plasma corticosterone concentration and placenta development in pregnant rats. Iranian Journal of Reproductive Medicine. 2011;9:71-7.
17. Khalili M, Semnanian S, Fatholahi Y. Caffeine increases paragigantocellularis neuronal firing rate and induces withdrawal signs in morphine-dependent rats. Eur J pharmacol. 2001;412:239-245.
18. Fowden AL, Forhead AJ. Endocrine mechanisms of intrauterine programming. Reproduction. 2004;127:515-526.
19. Kazemi M, Sahraei H, Azarnia M, Bahadoran H. Effect of oral morphine consumption on placenta fetal and maternal portions cells development in the Wistar rat. Ardebil University of Medical Sciences Research and Scientific Journal. 2010;10:145-154.
20. Roloff DW, Howatt WF, Kanto WP, Borker RC. Morphine administration to pregnant rabbits: effect on fetal growth and lung development. Addict Dis. 1975;2:369-379.
21. Kazemi M, Sahraei H, Azarnia M, Dehghani L, Bahadoran H. Effect of oral morphine consumption in female rats on development of brain cavities, central canal and choroid plexus of their embryos. Cell J. 2011;12:489-494.
22. Kazemi M, Sahraei H. The Effect of oral morphine consumption on ependymal duct and Spinal cord development in Wistar rats embryos. Iranin Suoth Madical Journal. 2011;14:16-19.
23. Sahraei H, Rostamkhani F, Tekieh E, Dehghani L, Meamar R, Kazemi M. Identification of morphine accumulation in the rat embryo central nervous system: A C14-morphine administration study. International Journal of Preventive Medicine. 2013;95:203-209.
24. Niknam N, Azarnia M, Bahadoran H, Kazemi M, Tekieh E, Ranjbaran M, Sahraei H. Evaluating the effects of oral morphine on embryonic development of spinal cord in Wistar rats. Basic and Clinical Neuroscience. 2013;4:24-29.
25. Kazemi M, Sahraei H, Azarnia M, Bahadoran H, Salehy M. Effect oral morphine consumption on lacunas development in ten day placenta pregnant Wistar rats. Journal of Zanjan University of Medical Sciences and Health Services. 2010;18;29-36.
26. Redmer DA, Wallace JM, Reynolds LP. Effect of nutrient intake during pregnancy on fetal and placental growth and vascular development. Domestic Anim Endocrinol. 2004;27:199-217.
27. Reynolds LP, Borowicz PP, Vonnahme KA, Johnson ML, Grazul-Bilska AT, Wallace JM, Caton JS, Redmer DA. Animal models of placental angiogenesis. Placenta. 2005;26:689-708.
28. Williams JT, Christie MJ, Manzoni O. Cellular and synaptic adaptations mediating opioid dependence. Physiol Rev. 2001;81:299-343.
29. Fabian G, Bozo B, Szikszay M, Horvath G, Coscia CJ, Szucs M. Chronic morphine-induced changes in mu-opioid receptors and G proteins of different subcellular loci in rat Brain. J Pharmacol Exp Ther. 2002;302:774–780.

30. Wu LY, Chen JF, Tao PL, Huang EY. Attenuation by dextromethorphan on the higher liability to morphine-induced reward, caused by prenatal exposure of morphine in rat offspring. J Biomed Sci. 2009;16:106-115.
31. Ward JW, Wooding FB, Fowden AL. The effects of cortisol on the binucleate cell population in the ovine placenta during late gestation. Placenta. 2002;23:451-458.
32. Collins LR, Hall RW, Dajani NK, Wendel PJ, Lowery CL, Kay HH. Prolonged morphine exposure in utero causes fetal and placental vasoconstriction: a case report. J Matern Fetal Neonatal Med. 2005;17:417-421.
33. Glasel JA. The effects of morphine on cell proliferation. Prog Drug Res. 2000;55:33-80.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=520&id=32&aid=4515