The effect of circadian rhythm changes on fetal and placental development (experimental study)

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Abstract

OBJECTIVE: To investigate the effect of circadian rhythm changes on fetal-placental development in pregnant rats.
MATERIAL AND METHOD: Pregnant rats were randomly allocated to 4 groups with 10 rats in each group. Group 1: 12 hours light 12 hours dark period (n:10, Control Group); Group 2: 6 hours light 6 hours dark period (n:10); Group 3: constant light group (n:10); Group 4: constant dark group (n:10). Rats were made to deliver by cesarean birth at the end of their pregnancy and their placenta, number and weight of the offspring, and placental histopathological changes were examined.
FINDING: Fetal number in the constant dark group was found significant in comparison to constant light group ($p<0.01$). Fetal weight in all groups with altered circadian rhythm was significantly lower than that in the control group ($p<0.01$). The decrease in fetal weight was more evident in the constant light group than in the constant dark group ($p<0.01$). Comparison of the histopathological findings of the placenta showed that placental edema, fibrin accumulation and leukocyte infiltration were significantly higher in constant light and constant dark groups than in other groups ($p<0.01$).
CONCLUSION: Circadian rhythm change in pregnancy adversely affects the fetal-placental development. More detailed studies are needed to clarify this mechanism.

INTRODUCTION

Due to their rapidly changing roles in the society, women can now work in heavy jobs like men do. A corollary of this life style is that women have to work during their pregnancy. The effects of work stress on pregnancy in women working during pregnancy have been examined in previous studies (Spinillo et al. 1986; Ortayli et al. 1996).

Particularly women who work in shifts are exposed not only to work stress, but also to changes in their circadian rhythms during pregnancy. As it is known, hormones like estriol, progesterone, human chorionic gonadotropin, human placental

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lactogen, growth hormone, cortisol and prolactin, which play a role in fetal growth and development, have a circadian rhythm (Houghton et al. 1982; Liggins, 1994; Christian & Morris, 2002; Bassett et al. 1988; McMillen & Walker,1991). Light and dark periods are particularly important in circadian rhythm changes. Some of the hormones with circadian rhythm are secreted during dark periods, while others are released in light periods.

It was reported in the literature that pregnant women who had shift jobs where the circadian rhythm was altered had more pregnancy complications like small gestational age (SGA), intrauterine growth restriction (IUGR), preterm action and spontaneous abortus than women who work only during the day (Spinillo et al. 1986; Ortayli et al. 1996; Bishnupuri & Haldar,1999). It was claimed in these studies that such conditions as work stress and tiredness led to adverse pregnancy results. However, a shift job may affect pregnancy in a dual way, causing work stress, on one hand and changes in the circadian rhythm, on the other. There is no study in the literature sufficiently elucidating this topic.

In the present study, a pregnant-woman-with-a-shift-job model was formed by creating different light-dark periods in order to investigate the effects of this life style on fetal-placental development.

MATERIAL AND METHOD

This study was conducted on 40 Wistar Albino species adult female rats of 18 weeks after the approval of the local ethics committee of Fırat University Medical School was obtained. "Ethical principles for medical research involving animals" set out in the Helsinki Declaration were observed at all stages of the study.

Care and impregnation of the animals:

All rats were kept in a setting with 23±2 centigrade temperature and 55%±15% humidity in 12 hours light and 12 hours dark periods before the experiment. Their feed and water were given ad libitum. Then rats were placed in cages in 5-rat groups, with one Wistar Albino species male rat for four female rats. After a one-night period, rats that were found to have spermatozoa in the vaginal smear the next morning were accepted to be on the first day of their pregnancy.

Groups of study:

Rats that were accepted as pregnant were randomly allocated to four groups with 10 rats in each group. Group 1: 12 hours light 12 hours dark period (n:10, Control Group); Group 2: 6 hours light 6 hours dark period (n:10); Group 3: constant light group (n:10); Group 4: constant dark group (n:10).

The experiment:

The groups were kept in separate rooms, which had the same physical and comfort conditions, but differed in terms of light periods only. The lighting system used in the light period consisted of 5 light bulbs with 1050 luminance in each room. Measures were taken to block all light in the dark period. Feed and water of the rats were regularly given and light arrangements were checked throughout the study. On the 20th gestational day, caesarean operations were conducted under general anesthesia induced by intra-muscular 60 mg/kg ketamine hydrochloride and 5 mg/kg Xylazine hydrochloride. Fetuses delivered by caesarean and the placentas were individually weighed using fine scales and the weights were registered. Newborns were left with their mothers in the post-operative period. Placenta of each rat was put into 10% formaldehyde in separate boxes and transported for histopathological evaluation to the pathology laboratory where they were buried into paraffin blocks after routine monitoring procedures. Cross-sections of 4-micron thickness were prepared and stained with hematoxylin eosin. In addition, different preparations arranged for a more detailed examination of the vascular structures were stained with Verhoeff elastic stain.

Evaluation of data and Statistics

At the evaluation stage after the completion of all procedures, fetal number, fetal and placental weights were assessed with a nominal scale. Edema, fibrin accumulation, leukocyte infiltration and vascular changes from the histopathological findings of the placenta were used to form the ordinal scale (absent:0; slight:1; normal:2 points). Findings were compared among groups. Kruskall Wallis and Mann Whitney U tests were used in the statistical analyses. Level of significance was set at $p<0.01$.

RESULTS

No death, activity slow-down, preterm birth or abnormal newborns were seen in any of the cases throughout the study. Weight measurements performed before the operation showed that all rats had put on weight (minimum:11, maximum 19 grams) and it was found that all rats were impregnated after mating (pregnancy rate: 100%).

Fetal numbers, fetal weight, placental weight and values related to histopathological features like placental edema, fibrin accumulation and leukocyte infiltration are presented in a table (Table 1).

Double comparisons in terms of fetal number revealed that fetal number was higher in the constant darkness group than all other groups, including the control group, but the difference was significant only relative to the constant light group ($p<0.01$). It was found that fetal weight decreased significantly in constant darkness, constant light and 6 hours light – 6 hours dark groups, where circadian rhythm was altered, when compared to the control group ($p<0.01$). The decrease in fetal weight was more marked in continuous light group, relative to the constant dark group ($p<0.01$). It

Table 1: Fetal numbers, fetal weights, placental weights and values related to histopathological features like placental edema, fibrin accumulation and leukocyte infiltration in all groups.

Groups	n	Fetal numbers	Fetal weight (gram)	Placental Weight (gram)	Placental Odema (Score)	Fibrin Accumulation (score)	Leukocyte Infiltration (score)
6 hours light 6 hours dark	10	5.8±1	3.8±0.2*	1.4±0.1	1.5±0.8*	1.1±0.7*	2.3±0.4*
Constant Light	10	4.8±0.6	3.5±0.2*	1.1± 0.1*	3.1± 1*	2.6± 1.2*	5.5±0.7*
Constant dark	10	6.7±0.8*	4.2±0.3*	1.3 ±0.2*	1.4±0.7*	0.5±1.2	3.3±1.1*
12 hours light 12 hours dark period (Control)	10	5.8±1	4.6±0.2	1.5±0.2	1.1±0.8	0.3±0.5	2.1±0.9

*: <0.01, Significance: Comparisons with the control group

was found that placental weight decreased significantly in constant darkness and constant light groups when compared to the control and 6 hours light – 6 hours dark groups (p<0.01).

Placental edema, fibrin accumulation and leukocyte infiltration among the histopathological findings in the placenta were found to have significantly increased in the constant light group, in comparison to all the other groups (p<0.01). Comparisons with the control group showed that placental pathological changes increased even in the 6 hours light – 6 hours dark group, where the circadian rhythm was impaired only slightly and it was established that the increase was significantly higher in constant light and constant dark groups, where the circadian rhythm was completely altered (p<0.01).

DISCUSSION

The present study examined the effect of changes in light-dark periods during pregnancy on fetal-placental development. It was found that offspring of the rats exposed to constant light had low birth weight and that all rats exposed to constant light or constant dark periods had more marked pathological findings in the placenta.

The findings we obtained can be interpreted in at least two ways on the basis of the previously reported literature data. The first interpretation is that when the circadian rhythms of the hormones playing a role in fetal development change, fetal-placental development is adversely affected due to the changed hormonal status (Bassett et al. 1988; Bishnupuri & Haldar, 1999). Although no hormonal parameter was examined in this study, it is known that such hormones as estriol, progesterone, human chorionic gonadotropin, human placental lactogen, growth hormone, cortisol and prolactin, which play a role in fetal growth and development, have a circadian rhythm (Houghton et al. 1982; Liggins, 1994; Christian & Morris, 2002; Bassett et al. 1988; McMillen & Walker,1991).

Similarly, one hormone that is mostly affected by light and dark orders and that is released according to the circadian rhythm is melatonin. It was demonstrated in the literature that pregnant sheep exposed to light had a decrease in melatonin and a parallel decrease in fetal respiratory movements (McMillen et al. 1990). Besides, melatonin supports fertilization and embryo development, besides influencing the circadian rhythm of adrenocortical hormones (Ishizuka et al, 2000). The fact that the fetus number in the constant light group was found lower relative to the constant dark group can be possibly explained by early embryo resorption due to decreased melatonin in constant light. Given that the pineal gland can send photoperiodical data to the endocrine system, this can be attributed to changes in both melatonin levels and melatonin sensitivity of the edge organs. Likewise, melatonin has a direct effect on the circadian rhythm of the hormones that have a part in fetal growth and weight increase (Sirotkin,1994).

It was found in another study carried out on monkeys that placental blood flow decreased in the light period and increased in the dark period (Harbert et al. 1979). The fact that the weights of the offspring of rats exposed to constant darkness were higher relative to the constant light group can be explained by ultra-placental blood flow that increased due to hormonal influences caused by the biorhythm change.

Another condition that is as important as the fetal and placental weight changes in our study is that such placental histopathological findings as edema, leukocyte infiltration, fibrin accumulation in the inter villous distance were greater in rat groups with altered light orders. As it is known, these histopathological findings are commonly found in the placentas of pregnant women with IUGR, SGA, preeclampsia and preterm activity (Salafia et al.1992). It is known that free radical activity is particularly high in IUGR (Okatani et al. 2001). Lymphocyte infiltration's being more marked as a placental pathological finding in rats exposed to constant light than in those exposed to constant darkness can be attributed to the fact that the activity of melatonin, which has an important place in the antioxidant defence, is inhibited in constant light and its diurnal secretion is impaired in constant

darkness. Similarly, leukocyte infiltration lessens due to the inhibiting effect of progesterone, another hormone that is significant in terms of fetal development in pregnancy, on the maternal immune system (Junkermann et al. 1982). The finding in our study that leukocyte infiltration, a placental histopathological finding, was greater in the group with altered light order suggests that progesterone secretion may have been affected, as well.

Another interpretation of our findings is that the change in light-dark periods, which have an important place in the lives of animate beings, causes stress in them. It is well known that maternal stress is one of the major environmental factors in fetal development. Animal studies have shown that prenatal stress caused low birth weight offspring (Paarlberg et al. 1999). Maternal anxiety and stress lead to spontaneous abortus, preterm birth, malformation and developmental retardation in the fetus (Fenster et al.1995). In our study, rats exposed to altered light periods can be considered, in one sense, as being subjected to stress. Likewise, studies carried out on sheep exposed to chronic stress showed that their hPL and progesterone levels decreased and their fetuses had IUGR (Regnault et al. 1999).

It was reported that more incidences of low birth weight newborns were seen in case of exposure to stress in the first trimester of pregnancy (Piko, 1999). Low fetal placental weight found in the offspring of rats exposed to altered light periods can be evaluated as a consequence of stress and stress-related metabolic-hormonal effects.

The rat model we developed in our study, though a preliminary one, largely reflects the situation of pregnant women working in night shifts. A clinical interpretation of our results on the basis of this information demonstrates that findings of the studies including women working in night shifts to the effect that fetal development is affected unfavorably are consistent with the findings of our study.

In conclusion, it was shown in this study that there was an absolute connection between light,dark, stress and maternal circadian rhythm change, and fetal-placental development. However, more detailed studies are needed to clarify the causes of this connection. The most important point we would like to draw attention to, in terms of the planning of the study and the results we obtained, is that in cases of pregnant women who work in shift jobs without regular light-dark periods both stress and irregular light-dark periods adversely influence fetal-placental development, and thus, working conditions of pregnant women with shift jobs may be rearranged.

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