Histological Assessment of Placental Development Following Intrauterine Exposure to Carbon Tetrachloride (CCl_4) in Pregnant Wistar Rats

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Abstract
Background/Objectives: In recent years, attention has been drawn to concerns regarding human reproductive disorders, particularly in relation to optimal fetal development during pregnancy. Several studies on carbon tetrachloride (CCL_4) toxicity have focused on maternal health with very little attention on placental and fetal health. Placenta is a vital organ that facilitates the exchange of nutrient between mother and fetus. This study assessed placental development following intrauterine exposure to CCL_4 in pregnant Wistar rats. **Materials and Methods:** Thirty (30) adult Wistar rats weighing between 170 g -180 g were utilized, paired overnight at an estrous cycle with sexually active males at 2:1 ratio. Pregnancy was confirmed by the presence of vaginal plug and/or sperm in the vaginal smear vaginal lavage and the day was recorded as gestational day (GD) 0. The pregnant rats were divided into two (2) groups (A and B) with fifteen (15) rats per group. Group A served as Control and was administered with a single intra-peritoneal injection of 2 ml of olive oil on GD 11, while Group B served as the treated group and was administered with a single intra-peritoneal injection of 2 ml of CCl_4/olive oil (1:1, volume for volume) on GD11. On GD15, GD17 and GD19, five rats were sampled from each group and their placentas were harvested for histological assessment.
Result and conclusion: Histological studies showed normal placental development in the control group and varying alterations in placental histomorphology, ranging from vacuolar degeneration of the glycogen cells in the junctional zone and discoloration indicating necrotic changes as well as dilated sinusoids in the labyrinth zone. In conclusion, histomorphological evidence suggests that CCl_4 administration adversely affects placental development in Wistar rats.
Keywords: *Carbon tetrachloride, Placenta, Wistar rats, intrauterine*

Introduction

Early pregnancy loss, known as spontaneous abortion or miscarriage, is the most common complication of pregnancy, affecting an estimated 15-20 % of recognized pregnancies (1, 2). However, this rate increases to 50-70% when considering early, unrecognized losses (2, 3). Factors contributing to miscarriage are diverse, including chromosomal abnormalities in the offspring (observed in about 50% of early miscarriages) (4, 5), maternal health conditions, and substance use during pregnancy (6). Environmental factors, such as exposure to toxins like DDT and lifestyle choices, can also influence the risk of miscarriage (7).

Estimating the impact of the approximately 80,000 chemicals on the market on miscarriage risk remains challenging due to the complexity of assessing adverse pregnancy outcomes and reproductive toxicity endpoints in both in vitro and in vivo models (3, 8, 9). Despite the insight gained from animal models such as mice and rats, significant anatomical and physiological differences between animal models and humans complicate the translation of findings (10, 11).

Epidemiological studies, like those linking exposure of bromodichloromethane to miscarriage, provide essential insights into

the potential risks of specific chemicals (8, 12). However, accounting for the multifaceted nature of identifying hazards is complex. Limiting studies to live births can skew connections between a toxicant and pregnancy outcome due to miscarriages occurring before pregnancy awareness (13). Abortion is a significant public health issue, affecting a considerable number of pregnancies globally (14). Factors such as environmental contaminants like carbon tetrachloride (CCl_4) have been linked to abortion in pregnant animals (15). Carbon tetrachloride (CCl_4) is a colorless, volatile, non-inflammable liquid that is produced by the mixture of chlorine with chloroform in the presence of light. It is structurally a chlorinated hydrocarbon called tetrachloromethane (16). Although carbon tetrachloride has been employed in the creation of chlorofluorocarbons (CFCs), its main application was as a dry-cleaning agent, fabric-spotting fluid, solvent, and reagent in chemical synthesis. It has also been used as a precursor of refrigerants and propellants, fire extinguisher fluid, and grain fumigant (17). Annual use and production have generally decreased since the mid-1970s. Most of its usage is currently prohibited due to its extreme toxicity and detrimental effects. However, some industries still make use of it (18). However, the mechanism by which CCl_4 causes abortion is not fully understood. Therefore, this study aims to investigate the effects of CCl_4 exposure on the placenta in Pregnant Wistar rats.

Materials and Method

Protocols for this experiment were in accordance with the guide for the National Research and Ethics Committee, College of Medical Sciences, University Of Benin, Benin City, Edo State Nigeria, With REC approval number CMS/REC/2023/462. Thirty (30) adult Wistar rats weighing between 170g and 180g were used for this experimental study. The animals were kept in polypropylene cages at room temperature. The animals were acclimatized for two weeks before the commencement of the experiment. The animals were fed with Growers Pellets and clean tap water. The animals were weighed daily throughout the duration of this experiment. The animals were paired overnight at the estrous cycle with sexually active males in the ratio of 2:1. Estrous cycle was examined by vaginal smearing. Successful mating was confirmed by the presence of vagina plug and/or sperm in the vagina smear the following morning between 9:00 and 10:00 hours. The day sperm cells were found in the vagina smear was considered Gestational Day (GD) 0. The pregnant rats were divided into two groups (A & B) of 15 rats each. Group A served as the control that was administered with a single intraperitoneal injection of 1 ml of olive oil on GD11, in addition to free access to feed and water. Group B received an intra-peritoneal injection of 2ml/kg CCl_4/olive oil (1:1; volume for volume) on GD11 as previously described by (19). Five animals were sampled from each group and sacrificed on GD15, GD17 and GD19, their placenta(s) and fetuses were harvested, and certain parameters of the placenta and fetus were taken which included the number of fetuses, maternal weight, fetal crown-rump length, placenta weight, placenta diameter major, placenta diameter minor and fetal weight. The harvested placenta and fetus were fixed with 10% neutral buffered formalin before they were taken to the laboratory for histological assessment (20).

Results

Morphometric Findings

Placental weight was significantly decreased ($P<0.05$) on GD17 and significantly increased ($P<0.05$) on GD19 in the CCl_4-treated group compared with the control. Fetal weight was significantly decreased ($P<0.05$) in the CCl_4-treated group compared with the control on GD17 and GD19. Fetal:placental weight ratio was significantly decreased ($P<0.05$) in the CCl_4-treated group compared with the control on GD19. Crown rump length was significantly decreased ($P<0.05$) in the CCl_4-treated group compared with the

control on GD19. Major placental diameter was significantly decreased ($P<0.05$) in the CCl_4-treated group compared with the control on GD17. Minor placental diameter was significantly decreased ($P<0.05$) in the CCl_4-treated group compared with the control on GD17.

Histological Findings

Photomicrographs Junctional zone of the placenta of the control group showed normal development clusters of glycogen cell islands. CCL_4-treated group presented vacuolar degeneration of the glycogen cells (Plate 1). The labyrinth zone of the placenta of the control group showed evidence of normal development with maternal sinusoid filled with blood and normal fetal capillaries. The CCL_4-treated group presented discoloration indicating necrotic changes as well as dilated sinusoids.

Discussion

This study describes the possible effects of carbon tetrachloride on the placental development of adult Wistar rats. The results obtained during this study indicate that there were significant changes in the placenta and fetus due to the administration of CCl_4. These include a notable reduction in placenta weight on GD17 in the treated group compared to the control group. Conversely, the placenta weight of the treated group on GD19 displayed a significant increase compared to the control group. These findings suggest the potential occurrence of placental hypertrophy, likely stemming from the uteroplacental unit's compromised ability to transfer glucose to fetuses due to CCl_4 administration. This agrees with Lewis *et al.* (21) and Furukawa *et al.* (22) who reported that an increase in placenta weight was indicative of certain maternal health conditions or certain infections.

Furthermore, the study's findings indicated a significant reduction in fetal weight, crown rump length, and fetal-placenta weight on GD17 and GD19 in the treated groups compared to the control group. This decline in measurements is anticipated due to the observed enlargement in the placenta, a condition known as placental hypertrophy, which hinders the exchange of nutrients and oxygen between the fetus and the mother. Consequently, this situation contributes to intrauterine growth restriction (IUGR). The observed notable decline in crown-rump length on GD19 in the treated group serves as evidence for the evident occurrence of miscarriage or fetal demise, which can be attributed to intrauterine growth restriction (23). These results further support the studies conducted by Saghir *et al.* (24), Pinson *et al.* (25) and Teng *et al.* (26), all of who also reported related markers of IUGR, specifically decreased fetal weight and morphometric indices of cranial growth.

The study results indicate that on GD15, the junctional zone of the treated group exhibited cystic degeneration of glycogen cell islands and a congested, dilated blood vessel. On GD17 and GD19, cystic degeneration of glycogen cell islands was also observed. This degeneration of glycogen cells significantly affected fetal growth and development, leading to fetal distress and potentially even fetal demise. The underlying cause can be attributed to compromised nutrient and oxygen supply to the developing fetus. Factors such as insufficient blood supply, hormonal imbalances, or genetic abnormalities are responsible for this cystic degeneration. Congestion and dilation of blood vessels in the placenta may suggest a disruption in normal blood circulation, potentially leading to inadequate delivery of oxygen and nutrients to the fetus; this can compromise fetal growth and development and increase the risk of various complications, such as intrauterine growth restriction (IUGR) or fetal hypoxia. This is similar to the study by Padmanabhan *et al.* (27) and Furukawa *et al.* (28).

Evidence of necrotic labyrinth tissues, mildly dilated and congested fetal vessels, and cystically dilated maternal sinusoids were observed in the treated groups on GD15 and GD19, in comparison to the control group. Additionally, on GD17,

necrotic labyrinth tissues and mildly dilated maternal sinusoids were noted. The presence of necrotic labyrinth tissues suggests compromised placental function and impaired exchange of nutrients and oxygen between the mother and fetus. This can lead to inadequate fetal growth, developmental abnormalities, and an increased risk of fetal distress or demise (27, 28, 29). The mild dilation and congestion of fetal vessels have the potential to disrupt normal blood circulation, which in turn can lead to decreased delivery of oxygen and nutrients to the fetus (24). This disruption may impair fetal growth and development and increase the risk of complications, including IUGR or fetal hypoxia (25). The presence of cystically dilated maternal sinusoids suggests a disruption in normal blood flow, compromising the efficient exchange of nutrients and waste products between the mother and the fetus. This disruption can lead to impaired fetal nutrition and oxygenation, which in turn can result in adverse fetal outcomes.

In conclusion, findings from this study provide, histological evidence of placenta toxicity following intrauterine exposure to CCl_4 in Pregnant Wistar rats.

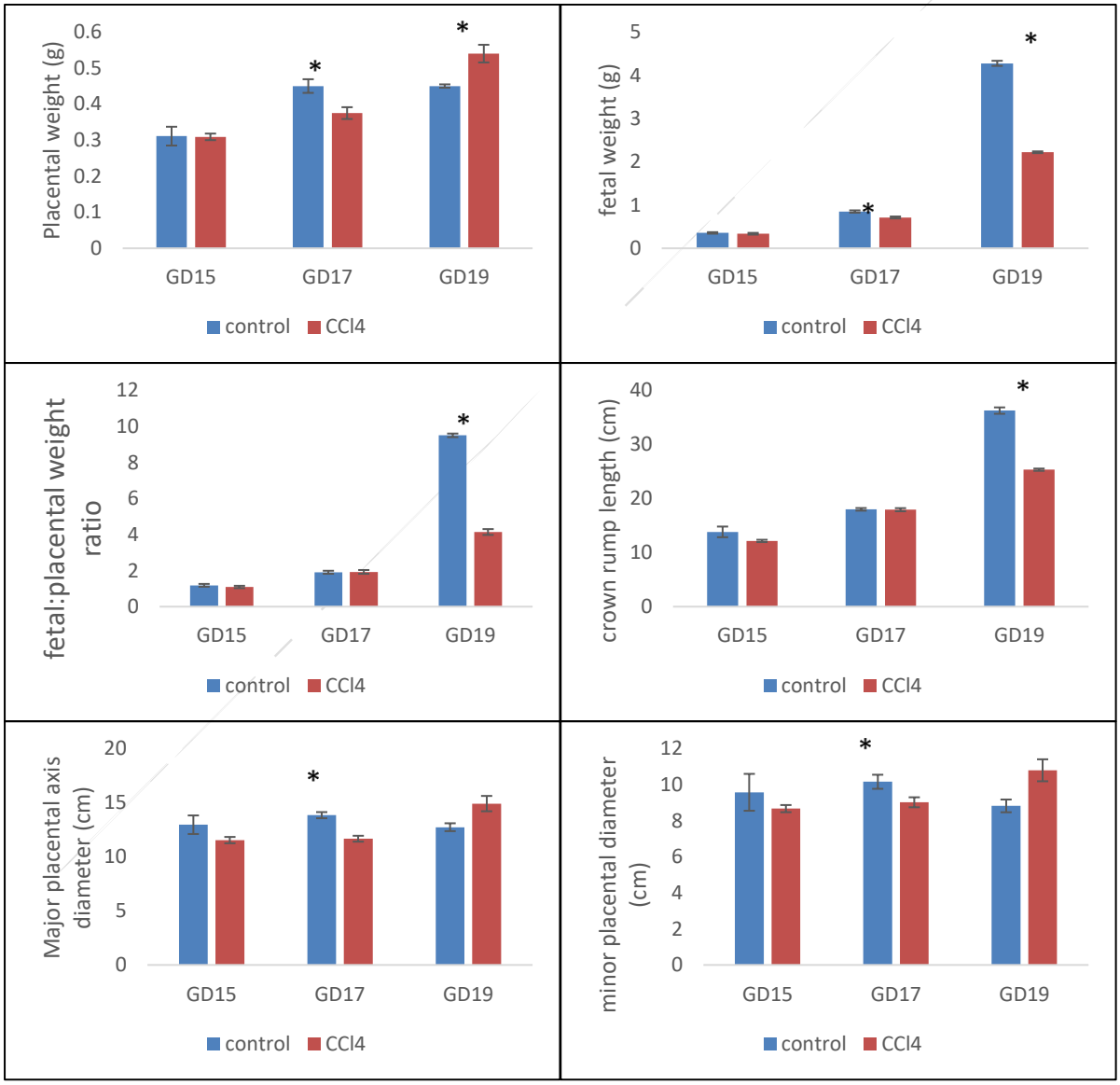

Figure 1: chart showing Placental weight (upper left), fetal weight (upper right) fetal:placental weight ratio (middle left), Crown rump length (middle right), Major placental diameter (lower left) and Minor placental diameter (lower right) on GD 15, GD 17 and GD 19. (* means statistically significant, P<0.05)

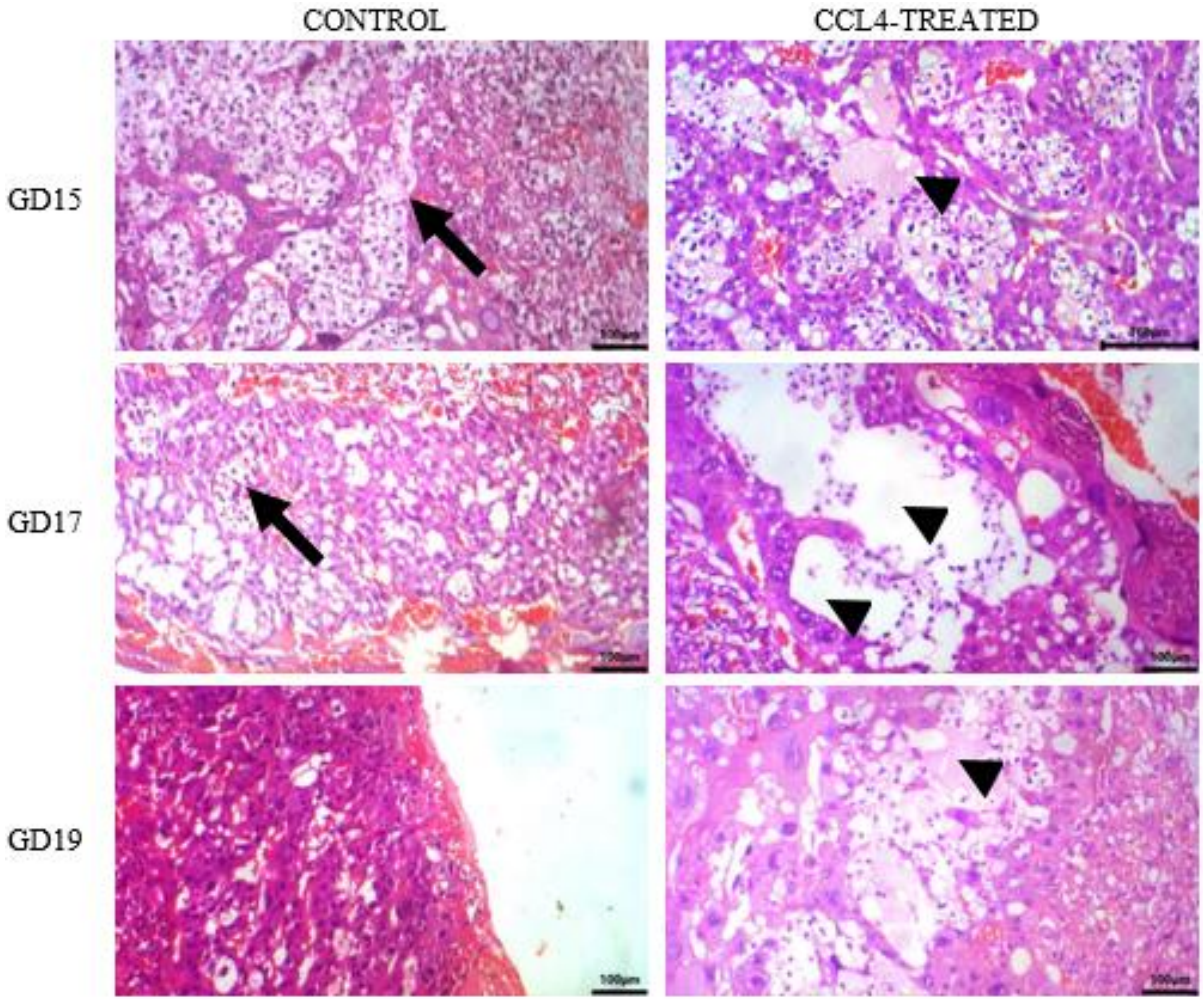

PLATE 1: Photomicrograph of a section of the Junctional zone of the placenta of the control group (on the left) and CCl_4-treated group (on the right) on GD15 (first row), 17 (second row) and 19 (third row). The control groups shows developing junctional zone at GD15 and 17 with clusters of glycogen cell islands (arrow) and well-developed junctional zone on GD 19. CCL_4-treated group presents vacuolar degeneration of the glycogen cells (arrow head) (H&E; 100×).

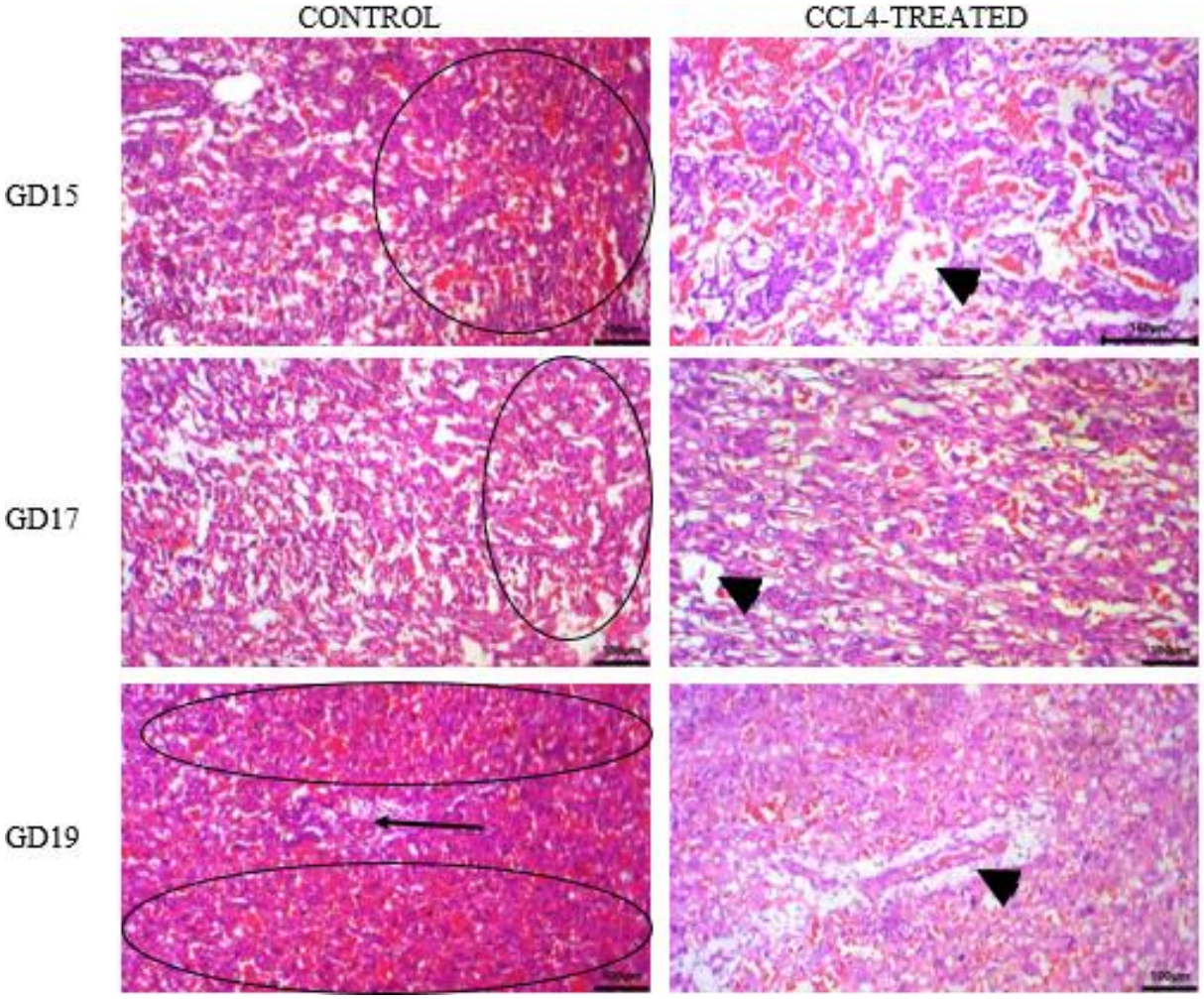

PLATE 2: Photomicrograph of a section of the labyrinth zone of the placenta of the control group (on the left) and CCl_4-treated group (on the right) on GD15 (first row), 17 (second row) and 19 (third row). The control groups shows well-developed labyrinth zone at GD15, 17 and 19 with maternal sinusoid filled with blood (encircled) and normal fetal capillaries (arrow). CCL_4-treated group presents discoloration indicating necrotic changes as well as dilated sinusoids (arrow head) (H&E; 100×).

References

1. Gupta, S., Agarwal, A., Banerjee, J., and Alvarez, J.G. The role of oxidative stress in spontaneous abortion and recurrent pregnancy loss: a systematic review. *Obstet Gynecol Survey*. 2007; 62(5): 335-347.
2. Agenor, A. and Bhattacharya, S. Infertility and miscarriage: common pathways in manifestation and management. *Women's Health (Lond)*. 2015; 11(4): 527-541.
3. Griebel, C.P., Halvorsen, J., Golemon, T.B. and Day, A.A. (2005). Management of spontaneous abortion. *Am Fam Physician*. 2005; 72(7): 1243-1250.
4. Ramien, C., Taenzer, A., Lupu, A., Heckmann, N., Engler, J.B., Patas, K. and Gold, S.M. Sex effects on inflammatory and neurodegenerative processes in multiple sclerosis. *Neurosci Biobehav Rev*. 2016; 67: 137-146.
5. Okada, H., Tsuzuki, T. and Murata, H. Decidualization of the human endometrium. *Reprod Med Biol*; 2018; 17(3): 220-227.
6. Verbrugghe, E., Boyen, F., Gaastra, W., Bekhuis, L., Leyman, B., Van Parys, A. and Pasmans, F. (2012). The complex interplay between stress and bacterial infections in animals. *Vet Microbiol*. 2012; 155(2-4): 115-127.

7. Krieg, S.A., Shahine, L.K. and Lathi, R.B. Environmental exposure to endocrine-disrupting chemicals and miscarriage. *Fertil Steril*. 2016; 106(4): 941-947.
8. Hogge, W.A., Byrnes, A.L., Lanasa, M.C. and Surti, U. The clinical use of karyotyping spontaneous abortions. *Am J Obstet Gynecol*. 2003; 189(2): 397-400.
9. Xia, L., Zhao, S., Xu, H., Wu, X., Zhang, A. and Niu, Z. Miscarriage rate is high with frozen-thawed blastocysts arising from poor-quality cleavage stage embryos. *Front Endocrinol.* 2018; 11: 561085.
10. Faas, M.M. and De Vos, P. Maternal monocytes in pregnancy and preeclampsia in humans and in rats. *J Reprod Immunol*. 2017; 119: 91-97.
11. Clark, G.F. Functional glycosylation in the human and mammalian uterus. *Fertil Res Pract*. 2014; 1(1): 1-12.
12. Parker, V.J. and Douglas, A.J. Stress in early pregnancy: maternal neuro-endocrine-immune responses and effects. *J Reprod Immunol.* 2010; 85(1): 86-92.
13. Liew, Z., Ritz, B., von Ehrenstein, O.S., Bech, B.H., Nohr, E.A., Fei, C. and Olsen, J. Attention deficit/hyperactivity disorder and childhood autism in association with prenatal exposure to perfluoroalkyl substances: a nested case–control study in the Danish National Birth Cohort. *Environ Health Perspect*. 2015; 123(4): 367-373.
14. Ganatra, B., Gerdts, C., Rossier, C., Johnson, B.R., Tunçalp, Ö., Assifi, A. and Alkema, L. Global, regional, and subregional classification of abortions by safety, 2010–14: estimates from a Bayesian hierarchical model. *Lancet*. 2017; 390(10110): 2372-2381.
15. Itoh, A., Isoda, K., Kondoh, M., Kawase, M., Watari, A., Kobayashi, M. and Yagi, K. Hepatoprotective effect of syringic acid and vanillic acid on CCl_4-induced liver injury. *Biol Pharm Bull*. 2010; 33(6): 983-987.
16. Smith, A.M., Herbst, A.S. and Olshan, A.F. Carbon tetrachloride exposure and adverse pregnancy outcomes: A retrospective study of women in the National Birth Defects Prevention Study. *Environmental Health.* 2021; 20(1): 1-10.
17. Unsal, V., Cicek, M. and Sabancilar, İ. Toxicity of carbon tetrachloride, free radicals and role of antioxidants. *Rev Environ Health*. 2021; 36(2): 279-295.
18. Rossi, F., Gambelli, A.M., Sharma, D.K., Castellani, B., Nicolini, A. and Castaldi, M.J. Experiments on methane hydrates formation in seabed deposits and gas recovery adopting carbon dioxide replacement strategies. *Appl Therm Eng*. 2019; 148: 371-381.
19. Sert, N.N. and Altinsaat, Ç. Effects of Carbontetrachloride (CCl_4) on some Blood Values in Pregnant and Non-pregnant Rats. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*. 2008; 14(2): 237-242.
20. Drury, R.A.B. and Wallington, E.A. Carleton's histological technique 5th edition. New York: 1980; Churchill Livingstone: 195.
21. Lewis, R.M., Batchelor, D. C., Bassett, N.S., Johnston, B.M., Napier, J. and Skinner, S.J. Perinatal growth disturbance in the spontaneously hypertensive rat. *Pediatr Res*. 1997; 42(6): 758-764.
22. Furukawa, S., Hayashi, S., Usuda, K., Abe, M. and Ogawa, I. Histopathological effect of ketoconazole on rat placenta. *J Vet Med Sci*. 2008; 70(11): 1179-1184.
23. Vorhees, C.V., Acuff-Smith, K.D., Schilling, M.A., Fisher, J.E., Moran, M.S. and Buelke-Sam, J. A developmental neurotoxicity evaluation of the effects of prenatal exposure to fluoxetine in rats. *Fundam Appl Toxicol.* 1994; 23(2): 194-205.
24. Saghir, S.A., Khan, S.A. and McCoy, A.T. Ontogeny of mammalian metabolizing enzymes in humans and animals used in toxicological studies. *Crit Rev Toxicol*. 2012; 42(5): 323-357
25. Pinson, M.R., Tseng, A.M., Adams, A., Lehman, T.E., Chung, K., Gutierrez, J., Larin, K.V., Chambers, C., Miranda, R.C. Collaborative Initiative on Fetal Alcohol Spectrum Disorders. Prenatal alcohol exposure contributes to negative pregnancy outcomes by

altering fetal vascular dynamics and the placental transcriptome. *Alcohol Clin Exp Res*. 2022; 46(6): 1036-1049.
26. Teng, H., Wang, Y., Han, B., Liu, J., Cao, Y., Wang, J. and Yin, J. Gestational systolic blood pressure trajectories and risk of adverse maternal and perinatal outcomes in Chinese women. *BMC Pregnancy and Childbirth*. 2021; 21(1): 155.
27. Padmanabhan, R., Abdulrazzaq, Y.M., Bastaki, S.M. and Shafiullah, M.M. (2008). Intrauterine growth restriction and skeletal variations in mouse fetuses induced by vigabatrin administered at preimplantation stages of development. *Congenital Anomalies*. 2008: 48(1): 29-39.
28. Furukawa, S., Hayashi, S., Usuda, K., Abe, M. and Ogawa, I. The relationship between fetal growth restriction and small placenta in 6-mercaptopurine exposed rat. *Exp Toxicol Pathol*. 2011; 63(1-2): 89-95.
29. Ali, H., Ghareeb, A. E., Taha, E.F., Hamdi, H. Placental toxicity in rats after administration of broncholytic drug (Mucophylline) A morphological, histological, histochemical and immunohistochemical studies. *J Clin Exp Tox*. 2018; 2(1): 1-9.

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