J. Afr. Ass. Physiol. Sci. 3 (2): 110 – 117, December, 2015.

Journal of African Association of Physiological Sciences
Official Publication of the African Association of Physiological Sciences
http://www.jaaps.aapsnet.org

Research Article

Cigarette smoke pollution promotes oxidative stress imbalance and hormonal changes affecting pregnancy outcome in rats

F.O. Awobajo[1], O.A. Oyesola[2], G.O. Amah[1], H.A. Olakitan[2] and T.M. Akinyemi[1],

[1]Endocrinology Laboratory, Departments of Physiology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, and [2]Faculty of Basic Medical Sciences, College of Health Sciences, Olabisi Onabanjo University, Ikene campus, Sagamu, Ogun State, Nigeria

Keywords:
Cigarette smoke, implantation, corpus luteum, pregnancy, female sex hormone, thyroid hormone, oxidative stress

ABSTRACT

Background: The deleterious effect of cigarette smoke on several health parameters due to pollution of air is of international concern. How these affect all living organisms and their wellbeing is a major research area. The development of oxidative stress, alteration in lipid peroxidation, thyroid and pregnancy hormone pattern was examined in pregnant rats exposed to cigarette smoke throughout the gestation period. **Methods:** Cigarette smoke exposed and control pregnant rats were sacrificed at gestational day 6, 12 and 20. Serum was collected for hormonal assay. Some vital organs were also dissected out, homogenized and used for oxidative stress and lipid peroxidation assay. Gestational weights, corporal luteum and implanted embryos were also recorded. **Results:** There was a significant increased loss of corpora lutea, embryonic implants and a disruption of the hormonal pattern of LH, progesterone and estradiol during pregnancy in cigarette smoke exposed rats. A significant increase in serum cortisol and brain tissue level of MDA, SOD and a significant decrease in GSH in cigarette smoke exposed rats was recorded. **Conclusion:** Exposing pregnant rats to cigarette smoke precipitated oxidative stress, early loss of corpora lutea, disruption in hormonal pattern and an increasing loss of embryonic implants.

INTRODUCTION

The use of tobacco continues to be the leading global cause of preventable death (CDCP, 2005, WHO 2011), accounting for the death of nearly six million people and causing hundreds of billions of dollars of economic damage worldwide each year (Peto 2001; Mathers 2006). Most of these deaths were reported to occur in low-and middle-income countries, with propensity towards widening of the disparity over the next several decades (WHO 2011). Among the documented health risks associated with smoking are respiratory impairments, lung cancer, coronary heart disease (Lee et al., 2006). Substantial harmful effects of cigarette smoke on fertility have become apparent, but are not generally appreciated. Other documented effects of active or passive exposure to cigarette smoke on pregnancy include intra-uterine growth retardation (IUGR), sudden infant death syndrome, neuro-developmental and behavioural problems (DiFranza et al. 2004). In Nigeria, tobacco smoking cut across different spheres of society; the rich, the poor, men and women, old and young. Omokhodion and Faseru (2007) reported a smoking prevalence of 3.4 % in a study among cross section of secondary school students in Ibadan, southwest Nigeria, with smoking initiation age range of between 9-21 years. Salawu et al. (2009), reported smoking prevalence of 23 % among female young adults and 40 % among male young adults of a cross section of youth in the northeast Nigeria, with over half of these smokers recording their first experience of smoking between 13-15 years of age. Odey et al. (2012), also reported a prevalence of cigarette smoking of 6.4 % among healthy young adult in south-south Nigeria town of Calabar. They further reported that the prevalence of cigarette smoking is

Address for correspondence:
E-mail: funmi_bajo@yahoo.com

higher among male adult (13.0 %) than females (2.1 %).

There is no doubt about increasing smoking habit among the adolescence and in particular female adolescence in Nigeria population. Cigarette smoking studies by socioeconomic status in developing countries revealed that cigarette use is highest among urban men and women who are less educated and economically disadvantaged (Bobak et al. 2000, Pampel 2005). In a research work on smoking and placental function, Page et al., (2002) reported that there was marked reduction in foetal capillary volume, alteration in the sodium-dependent transportation of alanine across the membrane of the microvillus of the placenta, increase in the thickness of trophoblastic epithelium and hypoxic stress both in the maternal and foetal blood. Ibeh and Ele (2003) put the prevalence of smoking among young Nigerian females at 7.7 %, with age range of smoking initiation at 12.6 ± 3.8 years. Smoking may precipitate epigenetic consequences on the health of the new born latter in adult life.

Therefore, this research work intends to further elucidate possible mechanisms through which cigarette smoke initiates adverse effects on female fertility, and sustenance of pregnancy using experimental rat model.

METHODS

Animal grouping treatment and data collection

Thirty-six matured female *Sprague-Dawley* rats weighing between 135-140 g and with regular oestrous cycling were used for this study after acclimatization to the laboratory. Standard rat housing was provided at room temperature and humidity with 12 hours light and 12 hours dark cycle. They were fed with standard rat pellet and clean tap water *ad libitum* during the study period. The study was carried out in pregnant rats divide into control (CON) and cigarette smoke exposed (CSE) groups with each group subdivided into three sub-groups; gestational day (GD) 6, 12, and 21 of pregnancy respectively.

Rat's estrous cycle was studied under the microscope to establish proper cycling according to Marcondes et al. (2002). Pregnancy was achieved by cohabiting cycling female rats with a male of proven fertility (weight matched) overnight, at a ratio of 2 females to a male. Presence of sperm cells in the vaginal smear on the second day of cohabiting confirmed pregnancy and it was recorded as the first day of pregnancy. Pregnant rats were randomly assigned into two groups; CONT and CSE. Each rat in CSE group was exposed to ¼ of a cigarette stick over a period of 20 minute daily. This dose was chosen with reference to doses used in other experiment involving male animals and with consideration for the state of pregnancy (Paiva, 2005). The duration of exposure for the different sub-groups ranges from day one of pregnancy to termination day for the different sub-groups (e.g 6, 12 and 21 days), while the control rats were not exposed and were kept in separate room from the exposed group to prevent passive smoking experience. Exposure was carried out using Galeazzi et al. (1999) modified smoking chamber.

Termination of exposure marked the end of the experiment for each sub-group at which the animals and their corresponding control were sacrificed by cervical dislocation (IACUC 2013). Blood sample were collected through cardiac puncture into a sterile sample bottle, centrifuged and serum withdrawn and stored at - 4^0 C for thyroid hormone, cortisol and pregnancy hormone assay. The ovaries and the uterus were dissected out and the number of corpora lutea (cl) and embryonic implants counted. Resorption sites were also noted. The liver, brain tissue, lung and the heart were also carefully dissected out over ice parks, weighed and homogenized in cold phosphate buffer using glass homogenizer inserted into ice box. The homogenates were cold centrifuged, the supernatant separated and stored frozen at - 40^0 C until used for antioxidant and lipid peroxidation assay.

Hormonal and oxidative stress assay

The following hormonal assays were also carried out using enzyme-linked immunoassay method (ELISA assay kit); Luteinizing hormone (LH), Follicle stimulating hormone (FSH), progesterone, estradiol, thyroid hormones and cortisol according the manufacturer protocols. Oxidative stress assay was carried out as follow; malondialdehyde (MDA) according to Uchiyama and Mihara (1978) method and superoxide dismutase (SOD) according to Sun and Zigman (1978) method. Catalase level in the homogenates was determined by measuring the exponential disappearance of water (H_2O_2) at 240 nm and expressed in units/mg of protein as described by Aebi (1984) while glutathione peroxidase (GSH) level in the homogenates was determined according to Van Doorn et al. (1978). All procedures employed in this study were approved by the Institutional experimentation and ethics Committee (CM/COM/08/VOL.XXIV) of College of Medicine, University of Lagos and are in conformity with International ethical standards on biomedical experimentation and animal care.

Statistical analysis

The results were analysed using Biostat software. All results were presented as Mean ± Standard Error Mean (SEM) and analysed using one-way Analysis of variance (ANOVA). Level of significant was placed at $p < 0.05$.

RESULTS

Table 1: Number of corpora lutea and embryonic implants during different days of pregnancy in cigarette smoke exposed and control rats

Day of pregnancy	Corpora lutea		Number of embryonic implants/ foetus	
	Control	CSE	Control	CSE
6	13.00 ± 1.00	7.00 ± 2.00*	6.50 ± 0.50	0.00 ± 0.00*
12	10.00 ± 1.00	8.50 ± 0.50*	5.50 ± 0.50	1.00 ± 1.00*
21	11.50 ± 1.50	6.50 ± 0.50*	6.00 ± 1.00	1.50 ± 1.50*

† Significantly increased, * significantly decreased

Table 2: Levels of reproductive hormones during different days of pregnancy in cigarette smoke exposed and control rats

Hormone	Day of pregnancy	Control	CSE
LH (miu/ml)	6	10.50 ± 1.50	5.10 ± 1.10*
	12	5.30 ± 0.60	4.80 ± 1.20
	21	10.70 ± 2.20	4.50 ± 0.10*
FSH (miu/ml)	6	6.65 ± 0.35	3.85 ± 1.25*
	12	8.65 ± 3.35	7.95 ± 1.75
	21	7.40 ± 2.10	8.20 ± 3 .80
Progesterone (nmol/l)	6	22.55 ± 12.25	96.10 ± 14.40†
	12	12.65 ± 7.25	25.90 ± 3.60†
	21	39.95 ± 4.75	16.25 ± 11.05*
Estrogen (pg/ml)	6	9.65 ± 2.65	7.00 ± 2.10*
	12	14.90 ± 0.08	4.95 ± 0.45*
	21	17.85 ± 0.25	4.40 ± 0.10*
Cortisol (ng/ml)	6	9.55 ± 1.95	17.75 ± 0.15†
	12	7.28 ± 2.43	16.95 ± 0.05†
	21	11.20 ± 3.00	20.15 ± 2.15†

† Significantly increased, * significantly decreased

DISCUSSION

Cigarette smoking has long been attributed to a multitude of poor health outcomes and there are increasing undeniable evidences to show that smoking is detrimental to human health. In women, smoking has been linked to accelerated follicular depletion and occurrence of menopause earlier than in non-smokers (Augood and Duckitt 1998; El-Nemr et al. 1998, Gold et al. 2001), low birth length and weight (Floyd et al, 1993; NCI 1999; Albuquergue et al. 2001) and cognitive impairment, behavioural and psychological defects at adulthood (Orlebeke et al. 1997, Shacka et al. 1997). In this study, we recorded a significant decrease in gestational weight in CSE rats compared with control starting from gestational day (GD) 12 till 21 day of pregnancy (GD 12; control 142.06 ± 1.30, CSE 136.50 ± 3.10, GD 21; control 151.63 ± 1.50, CSE 145.15 ± 3.32 g respectively (Fig 1). Although, feed consumption was not measured in this work and other researchers have reported a decrease in feed consumption and weight gain (Grunberg et al. 1984) which is similar to reduction in weight gain observed in this study. This has been attributed to the effects of the nicotine content of cigarette (Grunberg et al. 1986; Miyata et al. 1999). Nicotine is the addictive component of cigarette. Yoshinaga et al. (1979) reported that sub-cutaneous injection of nicotine significantly reduced progesterone production with resultant delay in implanatation and cleavage of ferlized oocytes at the different stages of development.

Table 3: Levels of thyroid hormones during different days of pregnancy in cigarette smoke exposure and control rats

Hormone	Day of pregnancy	Control	CSE
T3 (nmol/l) ($x10^{-2}$)	6	0.92 ± 0.01	1.39 ± 0.15 †
	12	3.08 ± 1.85	2.08 ± 0.23
	21	2.70 ± 0.23	1.46 ± 0.85
T4 (nmol/l)	6	30.00 ± 6.40	30.80 ±04.70
	12	27.90 ± 0.50	27.85 ± 0.75
	21	43.40 ± 0.80	36.75 ± 5.35*
TSH (miu/ml)	6	0.01 ± 0.00	0.01 ± 0.00
	12	0.00 ± 0.00	0.00 ± 0.00
	21	0.00 ± 0.00	0.00 ± 0.00
T3/T4 ratio ($x10^{-2}$)	6	0.03 ± 0.01	0.06 ± 0.03
	12	0.15 ± 0.12	0.07 ± 0.01
	21	0.06 ± 0.00	0.04 ± 0.02

†significantly increased; *significantly decreased

Table 4: Oxidative stress and lipid peroxidation in selected tissues during pregnancy in cigarette smoke exposed and control rats

Parameter	Day of pregnancy	Liver		Brain		Lung		Heart	
		Control	CSE	Control	CSE	Control	CSE	Control	CSE
MDA ($x10^{-6}$ unit µmol/ml)	6	1.28 ± 0.05	0.71 ±0.00*	5.26 ± 0.84	1.03 ± 0.01*	1.19 ± 0.01	2.95 ± 0.39†	2.45 ± 0.10	1.57 ± 0.65*
	12	1.36 ± 0.31	1.73 ± 0.79†	2.19 ± 0.58β	2.62 ± 0.93α	1.26 ± 0.05	1.41 ± 0.24	1.73 ± 0.41	1.12 ± 0.00*
	21	NA	1.24 ± 0.47	NA	1.34 ± 0.29	NA	1.00 ± 0.06 β	NA	0.85 ± 0.11β
SOD ($x10^{-3}$ unit µmol/ml)	6	12.31 ± 0.04	4.73 ± 0.21*	7.41 ± 1.33	5.97 ± 0.27	3.79 ± 0.05	8.74 ± 2.71†	8.77 ± 0.05	3.48 ± 0.17*
	12	4.77 ± 0.25β	5.28 ± 0.95	5.91 ± 0.74	13.21 ± 0.55†α	5.56 ± 0.10α	13.46 ± 0.26†α	5.36 ± 0.99	13.21 ± 0.01†α
	21	NA	12.24 ± 0.15α	NA	13.09 ± 1.08	NA	12.81 ± 0.91	NA	13.21 ± 1.01
CAT (unit µmol/ml)	6	0.45 ± 0.02	1.00 ± 0.20†	0.69 ± 0.22	0.64 ± 0.07	0.41 ± 0.00	0.86 ± 0.23†	1.31 ± 0.01	0.66 ± 0.01*
	12	0.52 ± 0.14	1.22 ± 0.06†	0.49 ± 0.01β	0.66 ± 0.10†	0.53 ± 0.01α	0.36 ± 0.01*β	0.73 ± 0.33β	0.36 ± 0.01*β
	21	NA	1.15 ± 0.61	NA	0.65 ± 0.18	NA	0.96 ± 0.10α	NA	0.58 ± 0.00α
GSH (unit µmol/ml)	6	0.28 ± 0.00	0.10 ± 0.00*	0.41 ± 0.12	0.21 ± 0.01*	0.23 ± 0.00	0.07 ± 0.06*	0.71 ± 0.00	0.08 ± 0.01*
	12	0.29 ± 0.01	0.27 ± 0.21	0.34 ± 0.01	0.29 ± 0.01*α	0.33 ± 0.01	0.00 ± 0.00*β	0.29 ± 0.26β	0.01 ± 0.00*β
	21	NA	0.14 ± 0.01	NA	0.25 ± 0.13	NA	0.19 ± 0.08α	NA	0.10 ± 0.04

† significantly increased compared to corresponding control value *significantly decreased compared to corresponding control value β Significantly reduced compared with earlier days of pregnancy. α Significantly increased compared with earlier days of pregnancy. NA: Not available

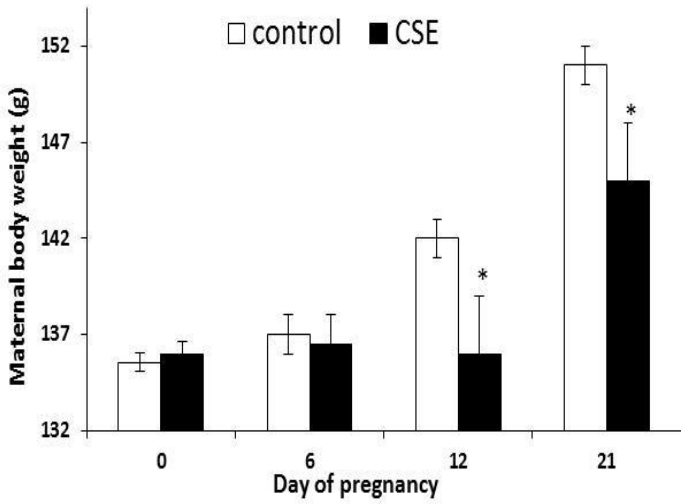

Fig. 1. Body weight changes during different days of pregnancy in cigarette smoke exposed and control rats * indicates significant level of decrease

Cigarette smoke and changes in reproductive and thyroid hormones

The hormonal profile of pregnant rats exposed to cigarette smoke showed a significant reduction in the LH (GD 6; control 10.50 ± 1.50, CSE 5.10 ± 1.10, GD 21; control 4.50 ± 0.10, CSE 10.70 ± 2.20 miu/ml), progesterone (GD 6 control 22.55 ± 12.25, CSE 96.10 ± 14.40, GD 12 control 12.65 ± 7.25, CSE 25.90 ±3.60, GD 21 control 39.95 ± 4.75, CSE 16.25 ±11.05 nmol/l) and estradiol (GD 6; control 9.65 ± 2.65, CSE 7.00 ± 2.10, GD 12; control 14.90 ± 0.08, CSE 4.95 ± 0.45, GD 21; control 17.85 ± 0.25, CSE 4.40 ± 0.10 pg/ml) (with progression of pregnancy (Table 2). The reported pattern for estrogen during normal pregnancy was a steady increase with progression of pregnancy with a peak toward late gestation, and a final fall three to two days to paturition (Khanum et al. 2008). Other authors have reported the inhibitory effects of cigarette smoke on follicular growth and estradiol production (Neal et al. 2007). Progesterone level is known to increase with progression of pregnancy (Luisi et al. 2000; Khanum et al. 2008). Reduction in progesterone level during pregnancy except towards parturition is a recognized diagnostic sign of preterm birth and thus administration of progesterone is used as prevention in women at risk (DaFonseca et al. 2003; Meis et al. 2003). In addition, the maternal thyroid hormone assay results at different stages of pregnancy revealed a significant increase in maternal serum level of T3 on GD6 (GD 6; control 0.92 ± 0.0, CSE 1.39 ± 0.15 $x10^{-2}$ nmol/l), and significant decrease in T4on GD21 (GD 21; control 43.40 ± 0.80, CSE 36.75 ± 5.35 nmol/l) (Table 3). However, TSH which is an indicator of hypothalamic positive feedback towards increasing thyroid hormone production was not significantly affected. Cigarette smoke is known to suppress hypothalamic activities through the activities of the addictive components. Further work will however be required in elucidating the mechanism via which exposure to cigarette smoke initiated suppression of maternal thyroid hormones synthesis. Thyroid hormones have been established to be an important contributor to implantation processes, sustenance of pregnancy, proper growth and development of the foetus (Henry 1996). Increased maternal thyroxine level has also been shown to stimulate placenta growth thus promoting proper foeto-placetal communication and enhanced foetal development (Spencer and Robinson 1993). Exposure to endocrine disruptor chemicals such as Nitrofen, an herbicide has been reported to distrupt thyroid hormone synthesis during pregnancy (Manson et al. 1984). Nicotine, one of the major constituent of cigarette has long been shown to interact in vivo with primate brain aromatase in regions involved in mood, aggression, and sexual behavior (Weathersbee 1980; Biegon et al. 2010).

Nicotine is known to affect the hypothalamo-pituitary-ovarian axis activity responsible for ovulation and early tropic support for pregnancy till placenta funtions are properly developed (Weathersbee 1980). A significant decrease in the weights of embryonic implats in CSE rats was recorded in this study. Other authors have reported the visceral organ weight reduction effect of nicotine in female rats (Iranloye and Bolarinwa 2009). Apart from the significant decrease in weights of embryonic implant, we also recorded a significant progressive reduction in number of corpora lutea (CSE; 7.0 ± 2.00, 8.5 ± 0.50, and 6.5 ± 0.50, control; 13.0 ± 1.00, 10.0 ± 1.00, 11.5 ± 1.50 on pregnancy day 6, 12 and 21 respectively) and embryonic implants in CSE rats (CSE; 0.0 ± 0.00, 1.00 ± 1.00 and 1.50 ± 1.50, control; 6.50 ± 0.50, 5.50 ± 0.50 and 6.00 ± 1.00 on day pregnancy day 6, 12 and 21 respectively) (Table 1). The loss of the embryonic implants can be partly atributed to the significant reduction in progesterone level (Table 2) as a result of significant reduction in the number of surviving corpora lutea. Corpus luteum is responsible for early production of progesterone before the eventual take over of this role by the placenta. Previous report by Nepomnaschy et al. (2004) have established a negative association between cortisol and progesterone level around implantation. The reduction in progesterone level during pregnancy is one of the factors promoting parturition and when it occurs before term, preterm birth is the result. This may have partly contributed to the recorded significant loss of embryonic implant in the CSE group compare with the control.

Cigarette smoke stress and cellular oxidative stress

In this study, rats that were exposed to cigarette smoke during pregnancy also recorded increased level of cortisol with progression of pregnancy (GD 6, 12 and 21 respectively; control; 9.55 ± 1.95, 7.275 ± 2.425, 11.20 ± 3.00, CSE; 17.75 ± 0.15, 16.95 ± 0.05, 20.15 ± 2.15 (ng/ml) (Table 2). Cortisol is a stress maker produced from the adrenal cortex in response to stress and it modifies the preparedness of the body to accommodate new challenges. Its effect transverse the entire systems modifying hormonal and metabolic activities going on in the various cell of the body. Maternal stress is commonly cited as one of the potential cause for at least part of pregnancy losses that remain unexplained in human (Ezechi et al. 2003, Nepomnaschy et al. 2006). Increased experience of stress during pregnancy has been associated with increased maternal cortisol level (Canals et al. 1997, Nepomnaschy et al. 2006,) very early pregnant loss or preterm birth and reduction of birth weight (Valladares et al. 2009). The recorded increase in cortisol level in rats exposed to cigarette smoke during pregnancy therefore, indicated an increased stress which may partly explain the corresponding reduction in number of embryonic implants and in some other cases complete loss of embryonic implants depending on the duration of exposure. In addition, the reported discovery of corticotrophin-releasing factor receptors on the ovary (Tilbrook et al. 2002) also buttressed the possibilities of the existence of a down-regulatory effect of stress on steroidogenesis and production of sex hormones.

Although, there were fluctuations in the level of the antioxidants GSH, SOD and CAT measured during the duration of pregnancy in this study (table4), the overall effect was a significant increase in the level of these antioxidants with continuous exposure to cigarette smoke and with progression of pregnancy (Liver; MDA 12^{th} week control 1.36 ± 0.31, CSE 1.73 ± 0.79 ($x10^{-6}$ unit µmol/ml), CAT 6^{th} week; control 0.45 ± 0.02, CSE 1.00 ± 0.02, 12^{th} week control 0.52 ± 0.41, CSE 1.22 ± 0.06 (unit µmol/ml), Brain SOD 12^{th} week control 5.91 ± 0.74, CSE 13.21 ± 0.55 ($x10^{-3}$ unit µmol/ml), Lungs MDA; control 1.19 ± 0.01, CSE 2.95 ± 0.39 ($x10^{-6}$ unit µmol/ml), SOD control 3.79 ± 0.05, CSE 8.74 ± 2.71, 12^{th} week control 5.56 ± 0.10, CSE 13.46 ± 0.26 ($x10^{-3}$ unit µmol/ml), CAT 6^{th} week; control 0.41 ± 0.00, CSE 0.86 ± 0.26 (unit µmol/ml). This gave an indication of increased generation of reactive oxygen species and increased oxidative stress in the CSE rats. Nampothiri et al. (2007) have reported that intra peritonea injection of cadmium one of the metabolites of cigarette smoke inhibited SOD activities while increasing catalase activities in rat ovarian granulosa cells.

Exposure of pregnant laboratory rats to cigarette smoke adversely affected sterodogenesis, T3 and T4 synthesis, potentiated stress and oxidative stress with early loss of cl and embryonic implants.

ACKNOWLEDGEMENT

We acknowledge the assistance of Mr. Dada kayode and Mr Adekunle Daniel both of the department of Physiology, University of Lagos for their assistance at various stages of the bench work. We also acknowledge the assistances of Dr. Oyelowo in proof reading and suggestion of possible title.

REFERENCES

Aebi H. (1984). Catalase *in vitro*. *Methods in Enzymology*. 105:121-126.

Albuquerque C., Doyle W., Hales K., Harding R. (2001). Influence of cigarette smoking during pregnancy on maternal body mass index and foetal growth. *Obst. Gyne*. 97 (4): S70-S71.

Augood C., Duckitt K., Templeton A. A. (1998). Smoking and female infertility a systematic review and meta-analysis. *Hum. Reprod*. 13:1532-1539.

Biegon A., Kim S. W., Logan J., Hooker J. M., Muench L., Joanna S. (2010). Nicotine Blocks Brain Estrogen Synthase (Aromatase): In Vivo Positron Emission Tomography Studies in Female Baboons Fowler. *Biol. Psychiatry*. 67(8):774-777.

Bobak M., Prahab J., Nguyen S., Jarvis M. (2000). Poverty and smoking. In Prahab J. and Chaloupka, F., edit. Tobacco control in developing countries. Oxford University Press.

Canals J., Colomina M. T., Domingo J. L., Doménech E. (1997). Influence of smoking and drinking habits on salivary cortisol levels. *Person Individ Diff*. 23:593-599.

Centre for Disease Control and Prevention "CDCP" (2009). Tobacco Use: Targeting the nation's leading killer at a glance. National Center for Chronic Disease Prevention and Health Promotion 4770 Buford Highway NE, Mail Stop K–50, Atlanta, GA 30341-3717.

DaFonseca E. B., Bittar R. E., Carvalho M. H .B., Zugaib M. (2003). Prophylactic administration of progesterone by vaginal suppository to reduce the incidence of spontaneous preterm birth in women at increased risk: a randomized placebo-controlled double-blind study. *Am J Obstet Gynecol* 188:419-24.

DiFranza J. R., Aligne C. A., Weitzman M. (2004). Prenatal and postnatal environmental tobacco smoke exposure and children's health. *Pediatrics*. 113 (3):1007-1015.

ElNemr A., ALShawaf T., Sabitini L., Wilson C., Lower A. M. (1998). Effect of smoking on ovarian reserve and ovarian stimulation in in vitro

fertilization and embryo transfer. *Hum. Reprod*. 13:2192–2198.

Ezechi O. C., Makinde O. N., Kalu B. E. K., Nnatu S. N.N. (2003). Risk factors for preterm delivery in South Western Nigeria. *J Obstet Gynaecol* 23(4):387–391.

Floyd R., Rimer B., Giovino G., Mullen P., Sullivan S. (1993). A review of smoking in pregnancy: Effects on pregnancy outcomes and cessation efforts. *Annual Review of Public Health*. 14:379-411.

Galeazzi F., Blennerhassett P. A., Qiu B., OByrne P. M., Collins S. M. (1999). Cigarette smoke aggravates experimental colitis in rats. *Gastroenterology* 117(4):877-883.

Gold E. B., Bromberger J., Crawford S., Samuels S., Greendale G. A., Harlow S. D., Skurnick J. (2001). Factors Associated with Age at Natural Menopause in a Multiethnic Sample of Midlife Women. *Am J Epidemiol*. 153:865-874.

Grunberg N. E., Bowen D. J., Winders S. E. (1986). Effects of nicotine on body weight and food consumption in female rats. *Psychopharmacology*. 90:101–105.

Henry J. B. (1996). Clinical diagnosis and management by laboratory methods 19 edt. pub. W.B. Saunders, Philadelphia. 333 -342.

IACUC (2013). The University of Texas at Austin Guidelines for the use of cervical dislocation for Rodent.pg 1-2. Euthanasiahttp://www.utexas.edu/research/rsc/iacuc/forms/guideline04.pdf.

Ibeh C. C., Ele P. U. (2003). Prevalence of cigarette smoking in young Nigerian females. African Journal of Medicine and Medical Sciences. 32(4), 335-338.

Iranloye B. O, Bolarinwa A. F. (2009). Effect of nicotine administration on weight and histology of some vital visceral organs in female albino rats. *Niger J Physiol Sci*. 24(1) 7-12.

Khanum S. A., Hussain M., Kausar R. (2008). Progesterone and estradiol profiles during estrous cycle and gestation in Dwarf goats (Capra Hircus). *Pakistan Vet. J*. 28(1):1-4.

Lee C. R., North K. E., Bray M. S., Avery C. L., Mosher M. J., Couper D. J., Coresh J., Folsom A. R., Boerwinkle E., Heiss G., Zeldin D. C. (2006). NOS3 polymorphisms, cigarette smoking, and cardiovascular disease risk: the Atherosclerosis Risk in Communities study. *Pharmacogenet Genomics* 16(12):891-9.

Luisi S., Petraglia F., Benedetto C., Nappi R. E., Bernardi F., Fadalti M., Reis F. M., Luisi M., Genazzani A. R. (2000). Serum pregnanolone levels in pregnant women: changes during pregnancy, at delivery, and in hypertensive patients. *The Journal of Clinical Endocrinology & Metabolism* 85(7):2429-2433.

Manson J. M., Brown T., Baldwin D. M. (1984). Teratogenicity of nitrofen (2,4-dichloro-4'-nitrodiphenyl ether) and its effects on thyroid function in the rat. *Toxicol Appl Pharmacol* 73:323-355.

Marcondes F. K., Bianchi F. J., Tanno A. P. (2002). Determination of the estrous cycle phases of rats: some helpful considerations. *Brazillian Journal of Biology* 62(4A):609-614.

Mathers C. D., Loncar D. (2006). Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med. 3:e442. doi:10.1371/journal.pmed.0030442.

Meis P. J., Klebanoff M., Thom E., Dombrowski M. P., Sibai B., Moawad A. H., Spong C. Y., Hauth J. C., Miodovnik M., Varner M. W., Leveno K. J., Caritis S. N., Lams J. D., Wapner R. J., Conway D., Osullivan M. J., Carpenter M., Mercer B., Ramin S. M., Thorp J. M., Peaceman A. M., Gabbe S. (2003) Prevention of recurrent preterm delivery by 17 alpha-hydroxyprogesterone caproate. *New Engl J Med* 348:2379-2385.

Miyata G., Meguid M. M., Fetissov S. O., Torelli G. F., Kim H. J. (1999) Nicotine's effect on hypothalamic neurotransmitters and appetite regulation. *Surgery* 126:255-263.

Nampoothiri L. P., Agarwal A., Gupta S. (2007). Effect of co-exposure to lead and cadmium on antioxidant status in rat ovarian granulose cells. *Arch Toxicol*, 81:145-150.

National Cancer Institute "NCI" (1999). Health Effects of Exposure to Environmental Tobacco Smoke: The Report of the California Environmental Protection Agency. Smoking and Tobacco Control Monograph No. 10. NIH Pub. No. 9904645. Bethesda, MD: U.S. Department of Health and Human Services, National Institutes of Health, National Cancer Institute.

Neal M. S., Zhu J, Holloway A. C., Foster W. G. (2007). Follicle growth is inhibited by benzo-[a]-pyrene, at concentrations representative of human exposure, in an isolated rat follicle culture assay. *Hum Reprod* 22:961-967.

Nepomnaschy P. A., Welch K., McConnell D., Strassmann B. I., England B. G. (2004). Stress and female reproductive function: A study of daily variations in cortisol, gonadotrophins, and gonadal steroids in a rural Mayan population *Am J Hum Biol* 16:523-532.

Nepomnaschy P. A.,Welch K. B., McConnell D. S., Low B. S., Strassmann B. I., England B. G. (2006). Cortisol levels and very early pregnancy loss in humans *PNAS* 103 (10): 3938–3942.

Ockene I. S, Miller N. H. (1997). Cigarette Smoking, Cardiovascular Disease, and Stroke A Statement for Healthcare Professionals from the American Heart Association. *Circulation*. 96:3243-3247.

Odey F. A., Okokon I. B., Ogbeche J. O., Jombo G. T., Ekanem E. E. (2012). Prevalence of cigarette smoking among adolescents in Calabar city, south-eastern Nigeria. *Journal of Medicine and Medical Sciences*. 3(4):237-242.

Omokhodion F. O., Faseru B. O. (2007). Perception of cigarette smoking and advertisement among senior secondary school students in Ibadan, southwest Nigeria. *West African Journal of Medicine*, 26(3): 206-209.

Orlebeke J., Knol D., Verhulst F. (1997). Increase in child behaviour problems resulting from maternal smoking during pregnancy. *Archives of environmental health*, 52:317-321.

Page K. R., Bush P., Abramovich D. R., Aggett M., Burke M. D., Mayhem T. M. (2002). The effects of smoking on placenta membranes. *Journal of memebrane science*. 243-252.

Paiva S. A. R., Novo R., Matsubara B. B., Matsubara L. S., Azevedo P. S., Minicucci M. F., Campana A. I. O., Zornoff L. A. M. (2005). β-Carotene Attenuates the Paradoxical Effect of Tobacco Smoke on the mortality of rats after experimental myocardial infarction1, 2. *Biochemical and Molecular Actions of Nutrients*. 135 (9): 2109-2113.

Pampel F. C. (2005). Diffusion, Cohort Change, and Social Patterns of Smoking. *Social Science Research*. 34:117-39.

Peto R., Lopez, A. D. (2001) Future worldwide health effects of current smoking patterns. In: Koop CE, Pearson C. E., Schwarz M. R., eds. Critical issues in global health. San Francisco, Wiley (Jossey-Bass):154–161.

Salawu F. K., Danburam A., Desalu O. O., Olokoba A. B., Agbo J., Midala . K. (2009). Cigarette smoking habits among adolescents in northeast Nigeria. *African Journal of Respiratory Medicine*, 8-11.

Shacka J., Fennell O., Robinson S. (1997). Prenatal nicotine sex-dependently alters agonist induced locomotion and stereotype. *Neurotoxicology and teratology*, 19:467-476.

Soares, S. (2009). Cigarette Smoking and Fertility. *Reproductive Biology Insights*, 2:39–46.

Spencer G. S., Robinson G. M. (1993). Stimulation of placental, foetal and neonatal growth by thyroxine administration to pregnant rats. *J Endocrinol*. 139(2):275-279.

Sun M., Zigman S. (1978). An improved spectrophotometric assay for superoxide dismutase based on epinephrine autoxidation. *Analytical Biochemistry* 90:81-89.

Tilbrook A. J., Turner A. I., Clarke I. J. (2002). Stress and reproduction: central mechanisms and sex differences in non-rodent species. *Stress*. 5:83-100.

Uchiyama M., Mihara M. (1978). Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Analytical Biochemistry*, 86:271-278.

Valladares E., Pena R., Ellsberg M., Persson L. A., Hogberg U. (2009). Neuroendocrine response to violence during pregnancy–impact on duration of pregnancy and foetal growth. *Acta Obstet Gynecol Scand* 88:818-823.

VanDoorn R., Leijdekkers C. M., Handerson P. T. (1978). Synergistic effects of phorone on the hepatotoxicity of bromobenzene and paracetamol in mice. *Toxicology*, 11(3): 225-233.

Weathersbee P. S. (1980). Nicotine and its influence on the female reproductive system *J. Reprod Med*., 25(5):243-50.

WHO (2011). WHO Report on the on the global tobacco epidemic, 2011, Warning about the dangers of tobacco.

Yoshinaga K., Rice C., Krenn J., Pilot R. L. (1979). Effects of Nicotine on Early Pregnancy in the Rat. *Biology of Reproduction*, 20:294-303.