Effects of sevoflurane on female reproductive functions in Wistar rats

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Abstract

Objective: To determine the effects of sevoflurane by inhalation on female reproductive hormones and ovarian tissues.

Methods: This experimental study was conducted at the Gaziosmanpasa University, Tokat, Turkey, and comprised Wistar-Albino female rats. The rats were divided into six groups; one control and five study groups. The control group (C) received 2 L/min O_2 in 18 min/day for seven days; the first study group (S1) received 1 minimum alveolar concentration sevoflurane + 2 L/min O_2 in 18 min/day for seven days; the second group (S2) received 1 minimum alveolar concentration sevoflurane + 2 L/min O_2 in 18 min/day for seven days and no treatment for the following seven days; the third group (S3) received 1 minimum alveolar concentration sevoflurane + 2 L/min O_2 in 18 min/day for 14 days; the fourth group (S4) received 1 minimum alveolar concentration sevoflurane + 2 L/min O_2 in 18 min/day for 14 days and no treatment for the following seven days; and the fifth group (S5) received 1 minimum alveolar concentration sevoflurane + 2 L/min O_2 in 18 min/day for 14 days and no treatment for the following 14 days. The duration of the study was 28 days in February 2015. Reproductive system hormone levels were analysed and histological assessment of the ovaries was performed. SPSS 20 was used for data analysis.

Results: Of the 30 rats, there were 5(16.7%) in each group. Histological injury scores in S2, S3, S4, and S5 were significantly higher than in C (p=0.016, p=0.008, p=0.016 and p=0.032, respectively). The hormone levels belonging to follicle stimulating hormone, luteinising hormone, estradiol and progesterone revealed significant alterations in all groups (p<0.05).

Conclusion: Chronic exposure to sevoflurane negatively affected the histological structure of the ovary and hormonal regulation.

Keywords: Follicle stimulating hormone, Luteinising hormone, Estradiol, progesterone, Sevoflurane. (JPMA 67: 877; 2017)

Introduction

Along with the technological advancements in industry, chemicals are becoming an indispensable part of our daily lives. In contrast with the advantages that make life easier for human beings, chemical compounds may interfere with the organs or the systems of the body and lead to undesirable effects.

There are several chemicals in the environment that can have adverse effects on the human body. Many of these chemicals are integral factors in the production of substantial substances. Of the 87,000 chemicals registered for commerce in the United States (US), unfortunately only 10% of these compounds have been analysed for potential health effects.[1] Of these, only a portion has been assessed for reproductive health effects.

Inhalation anaesthetics are one of the subgroups of these substances which have been widely used in medical field. Various experimental and clinical studies showed that the routine use of inhalation anaesthetics may have nephrotoxic, hepatotoxic, neurotoxic and genotoxic effects.[2-8] In contrast with the excessive amount of the data on toxicity, limited studies exist on the effects of volatile anaesthetics on the reproductive system.

Sevoflurane, which is a clear, non-flammable liquid at room temperature with no pungent odour and is well-suited for use in ambulatory anaesthesia, is a highly fluorinated substance named fluoromethyl hexafluoroisopropyl ether (C4H3F7O) that was first studied in humans in the US in the late 1970s.[9] It has a low blood-gas partition coefficient of 0.69, providing faster balance on the alveolar concentration with the inspired (delivered) concentration that results in rapid induction of, and recovery from, anaesthesia. Although the main elimination occurs in the lungs, a 5% of administered dose of sevoflurane undergoes metabolism by the liver.[10] Literature review revealed that there has been no data collected on the effects of sevoflurane on the female reproductive system.

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The present study was planned to determine the effects of repeated exposure to sevoflurane on rat ovary tissue and reproductive hormones.

Material and Methods

This experimental study was conducted at the Gaziosmanpasa University, Tokat, Turkey, and comprised rats. Exposure to sevoflurane was performed in an anaesthesia chamber measuring 40 x 50 x 60 cm. Ketamine and xylazine were obtained from Alfasan International B.V. (Woerden, Netherlands). Formaldehyde was purchased from Histomed (Montenegro, BR). Harris Haematoxylin and Eosin (H&E) Y 1% alcoholic were purchased from Atom Scientific Ltd (Manchester, United Kingdom [UK]).

After obtaining approval from the institutional ethics committee, adult female Wistar-Albino rats were obtained from the experimental medicine unit of the university. All animals were 90 days old, weighed 250-300 grams, and were selected in the same period of oestrus cycle as assessed through vaginal smears. The animals were kept in a room maintained at 20-24°C with a 12-hour light-dark cycle (lights on from 0600 to 1800 hours) and a constant humidity of 40-50%. All rats were housed in polycarbonate cages with tap water ad libitum.

For sevoflurane administration, rats were placed into a glass anaesthesia chamber measuring 40 x 50 x 60 cm, which was connected to an anaesthesia system (Prima SP Alpa, Penlon Limited, Oxon, UK). As previously described by Ceyhan et al., two holes, one at the top left side of the chamber and the other at the upper right side of the chamber, were opened for anaesthetic gas inlet and outlet.[11] Rats were randomly divided into six groups, including one control and five study groups.

The control group (C) received 2 L/min O_2 in 18 min/day for seven days; the first group (S1) received 1 minimum alveolar concentration (MAC) sevoflurane + 2 L/min O_2 in 18 min/day for seven days; the second group (S2) received 1 MAC sevoflurane + 2 L/min O_2 in 18 min/day for seven days and no treatment for the following seven days; the third group (S3) received 1 MAC sevoflurane + 2 L/min O_2 in 18 min/day for 14 days; the fourth group (S4) received 1 MAC sevoflurane + 2 L/min O_2 in 18 min/day for 14 days and no treatment for the following seven days; the fifth group (S5) received 1 MAC sevoflurane + 2 L/min O_2 in 18 min/day for 14 days and no treatment for the following 14 days. The duration of the study was 28 days in February 2015.

All rats were anaesthetised by intra-peritoneal injection of ketamine 90 mg/kg and xylazine 10 mg/kg (Alfasan

International B.V.), and were killed performing cervical dislocation at the end of the 7th day in C and S1 groups, the 14th day in S2 and S3 groups, the 21st day in S4 group, and the 28th day in S5 group. Intra-cardiac blood samples (5cc) were collected for hormonal biochemical analysis. Bilateral ovaries were subsequently removed, and the right ovary of all animals was fixed quickly upon collection in a 10% neutral buffered formalin solution. Tissues were dehydrated and embedded in paraffin for histopathological evaluation. The left ovary of each animal was placed on ice and then transferred to a -70°C freezer where they remained frozen until biochemical analysis.

Five μm thick sections were prepared from paraffin-embedded ovary tissue and mounted on glass slides. For histological assessment by light microscope (Nikon Eclipse E600W, Japan), the mounted sections were stained with H&E. Ovary sections from each of the five animals per group were randomly numbered. Thereafter, all slides were coded to perform a blind semi-quantitative analysis on the ovary sections. Histopathological injury was evaluated by an investigator who was initially blinded to the experiment. At least five microscopic fields of each ovary in each group were randomly selected for the evaluation. A modified five-level grading scale based on oedema, follicular cell degradation, vascular congestion, haemorrhage and infiltration by inflammatory cells was used to determine the histopathological injury score of the ovary. Normal ovarian architecture was defined as grade 0; mild oedema, mild follicular cell degradation, mild vascular congestion, no haemorrhage and no leukocyte infiltration was grade 1; moderate oedema, moderate follicular cell degradation, moderate vascular congestion, no haemorrhage and no leukocyte infiltration was grade 2; severe oedema, severe follicular cell degradation, severe vascular congestion, minimal haemorrhage and minimal leukocyte infiltration as grade 3, and severe oedema, severe follicular cell degradation, severe vascular congestion, haemorrhage and leukocyte infiltration as grade 4.[12,13]

The blood samples were given 20 minutes to form clotting, and the serum was separated by centrifugation at 1,500g (4°C) for 15 minutes. Then, the serum was transferred into separate eppendorf tubes and stored at -20°C until analysis. Serum luteinising hormone (LH), follicle stimulating hormone (FSH), progesterone (PG) and oestrogen (ES), and ovarian tissue LH, PG and ES levels in all groups were obtained by using LH (YH Biosearch, Shanghai, China), FSH (YH Biosearch, Shanghai, China), ES (Cayman Chemical Company, Michigan, US), and PG (Cayman Chemical Company, Michigan, US) rat enzyme-

linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. The outcomes of follicle stimulating hormone and luteinising hormone were calculated as mIU/mL, and estradiol and progesterone as pg/mL.

With a standard deviation difference of 1.06 in FSH levels with a two-sided type I error of 0.05, and a power of 0.80, five rats in each group were required to find a significant difference.

SPSS 20 was used for data analysis. Normality and variance were evaluated using the one sample Shapiro-Wilk test for each variable. Quantitative data was presented as means and standard deviation and

qualitative data as frequency and percentage. The hormonal levels and histopathological injury scores of all groups were analysed using the Kruskal-Wallis analysis of variance (ANOVA) and post-hoc comparisons were conducted by Tukey's honest significant difference (HSD) test with Bonferroni correction. P<0.05 was considered significant.

Results

Of the 30 rats, there were 5(16.7%) in each group. All rats were anaesthetised after the 10th minute of exposure. No death or any complications occurred during the experimental exposure period.

After H&E staining, all ovarian tissues were analysed for

Table-1: Serum hormone levels.

	Control	S1	S2	S3	S4	S5	p
FSH	8,69±1,60[a]	9,24±0,93[b]	9,22±0,78[c]	8,30±1,25[d]	8,88±0,50[e]	10,52±0,19[a,b,c,d,e]	0.016*
LH	5,32±1,00[f]	5,45±0,68[g]	5,60±0,38[h]	5,08±0,86[i]	5,36±0,47[k]	6,41±0,12[f,g,h,i,k]	0.025*
ES	6,11±1,58[m,n,p]	15,00±18,41	18,25±12,43[m]	15,07±8,06[n]	16,48±13,10	20,85±18,89[p]	0.164
PG	15096,00±8698,71	8074,00±3190,51[q]	18466,00±20929,15	17080,00±12041,69	19626,00±6420,56[q]	14612,00±7589,52	0.274

FSH: Follicle stimulating hormone
LH: Luteinising hormone
ES: Estradiol
PG: Progesterone
HSD: Honest significant difference

*Kruskal-Wallis test (p<0.05), intergroup comparisons were performed by Tukey's HSD test.
Significant changes for FSH:
aC-S5: p = 0.009; bS1-S5: p = 0.009; cS2-S5: p = 0.009; dS3-S5: p = 0.009; eS4-S5: p = 0.009
Significant changes for LH:
fC-S5: p = 0.012; gS1-S5: p = 0.009; hS2-S5: p = 0.009; iS3-S5: p = 0.009; kS4-S5: p = 0.009
Significant changes for estradiol:
mC-S2: p = 0.026; nC-S3: p = 0.015; pC-S5: p = 0.026
Significant changes for progesterone:
qS1-S4: p = 0.009.

Table-2: Ovarian tissue hormone levels.

	Control	S1	S2	S3	S4	S5	p
LH	2,24±0,59[a]	2,18±0,89	1,49±0,26[a,b]	2,88±1,07[b,c]	2,12±1,24	0,98±0,76[c]	0.077
ES	8,94±1,57[d,e]	16,90±24,90	21,75±11,50	20,72±8,41[d]	30,57±30,56[e]	22,71±8,62	0.175
PG	1611,80±881,18[f]	1517,46±468,25[g,h,k]	2434,82±345,71[g]	2780,36±690,89[f,h,k]	2295,30±632,36	1837,27±1154,01	0.071

LH: Luteinising hormone
ES: Estradiol
PG: Progesterone
HSD: Honest significant difference

*Kruskal-Wallis test (p < 0.05), intergroup comparisons were performed by Tukey's HSD test.
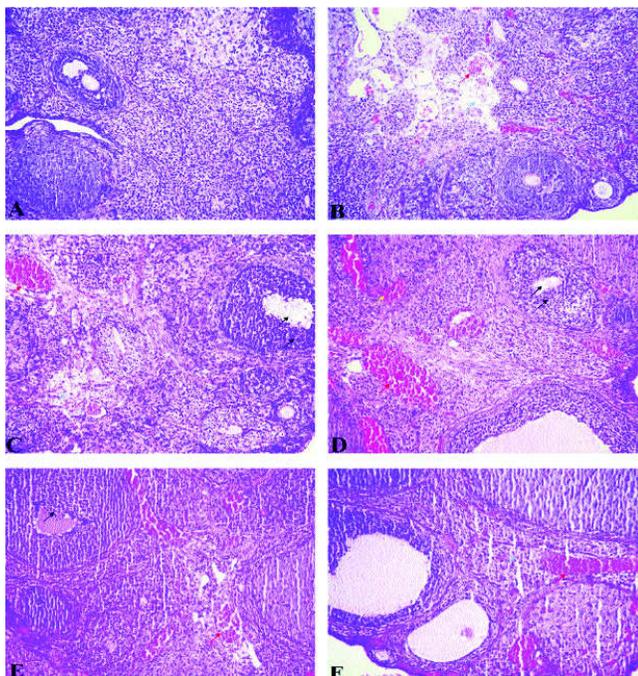Significant changes for LH:
aC-S2: p = 0.021; bS2-S3: p = 0.02; cS3-S5: p = 0.049
Significant changes for estradiol:
dC-S3: p = 0.009; eC-S4: p = 0.028
Significant changes for progesterone:
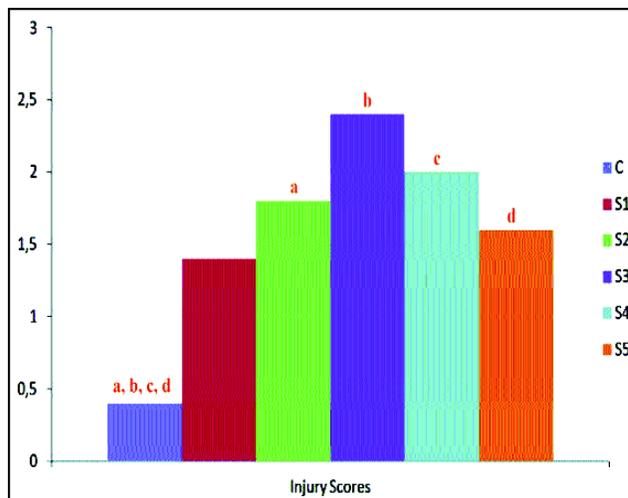fC-S3: p = 0.036; gS1-S2: p = 0.021; hS1-S3: p = 0.021; kS1-S3: p = 0.047.

H&E: Haematoxylin and eosin.

Figure-1: Histological sections.
Histological sections of rat ovary tissues staining by H&E. (A) Control; (B) S1; (C) S2; (D) S3; (E) S4; (F) S5. Red arrows displayed congestion areas, blue arrows showed oedema, the yellow arrow indicated bleeding sites, and follicular cell degeneration was presented with black arrows. Five animals were included in each group, and six ovary sections from each of the five animals per group were assessed (n = 30). Magnification: 100×.

HSD: Honest significant difference.

Figure-2: Histopathological injury scores.
The scoring system reported by Kurt et al. (2011) and Guven et al. (2010), which ranged from 0 to 4 due to the injury severity, was used. The intergroup comparisons were completed by the Tukey's HSD test.
Significant changes between groups:
aC-S2: p = 0.016; bC-S3: p = 0.008; cC-S4: p = 0.016; dC-S5: p = 0.032.

the effects of sevoflurane on rat ovarian tissue pathology (Figure-1). Rats in C preserved the normal structure of the ovary without showing any sign of oedema or follicular cell degeneration (Figure 1A). The ovarian tissues from S1 displayed mild oedema and mild vascular congestion; however, no significant differences existed between C and S1 (Figure-1B). S2 showed moderate oedema, moderate follicular cell degradation, and moderate vascular congestion (Figure-1C). In S3, the ovarian structure revealed severe oedema, severe follicular cell degradation, severe vascular congestion, and minimal haemorrhage (Figure-1D). The histological grade belonging to S3 was significantly higher than C (2.4±0.54, p=0.008); however, no significant difference was found between S3 and other groups (p>0.05). There were mild follicular cell degradation and mild vascular congestion in S4 (Figure 1E). In addition, mild oedema and mild vascular congestion was found in S5 (Figure 1F). The evaluation under light microscopy revealed that chronic sevoflurane exposure led to follicular cell oedema, degeneration, congestion and bleeding in the ovaries of rats (Figure-1B-1F).

The mean histopathological injury scores were significantly higher in all groups, except S1, compared to C (Figure-2).

There were significant differences found among all groups for serum FSH, LH, estradiol ES and PG (p<0.05). Serum FSH levels were significantly higher in S5 compared to C, S1, S2, S3, and S4 (p=0.009, p=0.009, p=0.009, p=0.009, p=0.009, respectively). In addition, serum LH levels in S5 were significantly higher than in C, S1, S2, S3, and S4 (p=0.012, p=0.009, p=0.009, p=0.009, p=0.009, respectively). The ES levels in S2, S3, and S5 were significantly higher than C (p=0.026, p=0.015, p=0.026, respectively). In S4, the PG levels were detected as significantly higher than in S1 (p = 0.009) (Table-1).

Additionally, there were significant alterations in all groups of ovarian tissue LH, ES and PG levels (p<0.05). The LH levels in S2 were significantly lower compared to C and S3 (p=0.021, p=0.020, respectively). In S3, the LH levels were significantly higher than S5 (p=0.049). S3 and S4 had significantly higher ES values compared to C (p=0.009, p=0.028, respectively). In S3, the mean PG level was significantly higher than C (p=0.036). In addition, the PG levels in S2, S3 and S4 were significantly higher compared to S1 (p=0.021, p=0.021, p=0.047, respectively) (Table-2).

Discussion

The present study revealed that acute and sub-chronic exposure to sevoflurane may lead to several significant hormonal changes in the reproductive system and may lead to ovarian tissue damage, as supported by the histological findings. It is well described that the oestrous cycle of rats lasts four or five days and consists of proestrus, oestrus, metoestrus, and dioestrus, and includes various alterations in the concentrations of gonadal steroids and gonadotropins.[14-17] Briefly, in the proestrus phase, the oestradiol level rises and ovarian follicles grow fast. This stage takes approximately 12 hours and is similar to the follicular phase of humans. After LH surge, ovulation occurs in the night of oestrus. Associated with the absence of a fertilisation during the time of ovulation, the corpora lutea is temporarily functional and excretes a low quantity of progesterone.[18] As mentioned above, the regulation of reproductive functions is controlled by a finely tuned balance between androgens and sex steroids, which can be affected by numerous bio-chemical agents. Various studies have demonstrated the impacts of these agents on the hormone levels of the hypothalamo-hypophyseal axis.[19-23] Similar findings were revealed in the present study. For instance, serum FSH and LH values were significantly increased in S1, S2, S4 and S5. The FSH and LH rise in S1 and S2 suggested that seven days (acute) of exposure may lead to ovarian tissue damage, causing responsiveness to FSH and LH. In addition, the increase of FSH and LH in S4 and S5 with the decrease in S3 suggested that 14 days of exposure (sub-chronic) could damage the hypotalamic-hypophyseal axis and be followed by a progressive rise of hormone levels during the recovery period. The ES showed an increase in S1, S2, S4 and S5, and a decrease in S3. Elevated levels of ES in S1 and S2 suggested that acute exposure may disturb the integrity of follicles containing ES, leading to a hormonal release. In relation, the ES decline in S3 suggested that sub-chronic exposure could disrupt the ES synthesis of theca cells in the ovarian follicles, and the following increase in S4 and S5 confirmed that ES production improved during the recovery period. The ovary tissue levels of ES in S3, followed by an increase in S4 and S5, confirmed this phenomenon. The PG levels showed an inconsistent route compared to the degree of exposure. However, PG has complex production and secretion mechanisms, which can be influenced by many factors.

A systematic review of the literature revealed that no study existed on the effects of sevoflurane on the female reproductive system. In contrast, several studies have been conducted on the effects of sevoflurane on males.[11,24-27] In accordance with the study conducted by Ceyhan et al., chronic sevoflurane exposure in male rabbits may lead to testicular dysfunction and directly affects the sperm morphology, resulting in abnormalities in the sperm shapes.[11] In addition, Kaymak et al. used semen samples of 28 volunteers and exposed the samples to various concentrations of halothane, isoflurane, sevoflurane and desflurane, searching for deoxyribonucleic acid (DNA) damage in sperm cells. The investigators reported that the inhalation anaesthetics, with the exception of desflurane, have the potential to cause genotoxicity.[25] Similarly, Wang et al. demonstrated that sevoflurane with a dose from 1.4% to 5.6% shows minimal effect on motility and vitality of human sperm, while expecting impairment in the motor function of human sperm.[26] A recent study conducted by Kaya et al., investigating seven days and 14 days of sevoflurane exposure on the male rat reproductive system, revealed that chronic exposure to sevoflurane can result in testicular tissue damage, decreased sperm count and motility, occurrence of abnormal sperm forms, and disruption in reproductive hormones having a direct role in fertility such as FSH, LH, ES and PG.[24]

Moreover, the mean histological injury scores showed significantly higher values in all study groups, except S1, compared to C. The S3 revealed severe deterioration including severe oedema, severe follicular cell degradation, severe vascular congestion, and bleeding sites. Lower histological injury scores in S4 and S5 suggested that there can be an improvement in ovarian tissue associated with the recovery period (seven days and 14 days). The diminished serum hormone levels in S3 after an increase in S4 and S5 provides a symmetric pattern with the histological scores. In terms of the duration of sevoflurane inhalation, most damage appeared with longer exposure. The existence of a recovery period in S2 caused a decrease in histologic injury score after S1. There can be two speculative explanations for this phenomenon; first, perhaps seven days of exposure (acute) was not sufficient to trigger the repair systems of the tissues or simply mild damage was not enough to initiate a response or recovery in ovary tissues. Second, perhaps seven days of exposure led to higher levels of injury; however, it could not be detected in S1 associated with the euthanasia of rats at the end of seven days in S1, thus no increased level of injury scores was detected. In S2, the injury level could be raised to a higher level and then decreased in seven days of recovery to the present value.

Currently, several investigators are focused on the single issue that anaesthetic agents may cause infertility among

health workers.[28-31] In this context, Guirguis et al. emphasised the association between exposure to anaesthetic gases and abnormal pregnancy outcomes.[28] In addition, Gauger et al. demonstrated that there is a higher incidence of spontaneous abortion in paediatric anaesthesiologists compared to non-paediatric, which might be associated with the occupational exposure to inhalation anaesthetics during induction and the use of un-cuffed endotracheal tubes in paediatric anaesthesia.[29] Various studies attested to the findings of previous authors, reporting that occupational exposure to unscavenged waste and anaesthetic gases may lead to adverse outcomes, or that inhalation exposure can lead to first-born female offspring.[30,31]

This study had several limitations as well. First, the effect of sevoflurane on ovarian cell DNA could not be included in the study design, which might provide more detailed information about cell damage. Second, a new hormone named anti-müllerian hormone level could not be investigated, which shows the functional status of the ovaries and might support the findings of the present study.

To our knowledge, this study was the first experimental investigation on the effects of sevoflurane on the female rat reproductive system.

Conclusion

Acute and sub-chronic exposure to sevoflurane may negatively alter the hormonal regulation and histological structure of the female reproductive system. Through the current study, our understanding of inhalation anaesthetics has developed considerably; however, there is scarcity of useful clinical outcome data to guide this issue. Further studies are required to elucidate the exact mechanisms of the inhalation anaesthetics.

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