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RESEARCH ARTICLE

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Effect of sugammadex administration on neural tube development in 48-h chick embryos

Elif Büyükerkmen[1] | **Emre Atay**[2] | **Fatma Firat**[3] | **Ahmet Yüksek**[4] | **Abdülkadir Bilir**[2] | **Gülan Albaş Kurt**[2] | **Alperen Saritaş**[2]

[1]Clinic of Anesthesia and Reanimation, Afyonkarahisar State Hospital, Afyonkarahisar, Turkey

[2]Department of Anatomy, Faculty of Medicine, Afyonkarahisar Health Sciences University, Afyonkarahisar, Turkey

[3]Department of Histology and Embryology, Faculty of Medicine, Afyonkarahisar Health Sciences University, Afyonkarahisar, Turkey

[4]Department of Anesthesia and Reanimation, Faculty of Medicine, Yozgat Bozok University, Yozgat, Turkey

Correspondence
Emre Atay, Department of Anatomy, Faculty of Medicine, Afyonkarahisar Health Sciences University, Afyonkarahisar, Turkey.
Email: eemreatay@gmail.com

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Abstract

Sugammadex is a new generation drug that has led to significant changes in the practice of anesthesia. However, its effects on fetal development are not yet fully known. The aim of this study is to investigate the teratogenic effects of sugammadex on neural tube and embryonic development in early chick embryos. In this study, 50 0-day fertile specific non-pathogenic (SPF) eggs were used. Fifty eggs were divided into 5 different groups, each consisting of 10 pieces. While no substance was given to the control group at the 28th hour of the study, 4 different doses of sugammadex were administered to the experimental groups, respectively 2, 4, 8, 16 mg/kg. Cranio-caudal lengths of embryos, somite numbers, average number of argyrophilic nucleolar regulatory regions (AgNOR) per nucleus, total AgNOR area/total nuclear area (TAA/NA) ratios, Caspase-3 *H*-Score results, and presence of neural tube defect were compared among the groups. While the mean cranio-caudal lengths, somite counts, TAA/NA ratios and AgNOR counts of the embryos were found to be statistically significantly lower than the control group, Caspase-3 *H*-Score mean results were found to be significantly higher ($p < .05$). In addition, it was observed that there was an increase in neural tube patency and developmental delay. As a result, sugammadex crossing the placenta was revealed to increase the release of proapopitotic molecules and disrupt the developmental stages of embryos. Thus, it was determined that sugammadex in increased developmental delay and incidence of neural tube defects in early chick embryos with increased dose dependent. Despite these results, the effects of sugammadex on fetal development in in vivo and in vitro environments should be studied with further studies.

Research Highlights

- Sugammadex is a new generation drug that has led to significant changes in the practice of anesthesia. However, its effects on fetal development are not yet fully known. It has been observed that different doses of sugammadex increase the risk of neural tube defect development on chick embryos and slow the embryo development in a dose-dependent manner.

KEYWORDS
argyrophilic nucleolar regulatory region, caspase-3, neural tube, sugammadex

1 | INTRODUCTION

Sugammadex is a modified cyclodextrin that antagonizes aminosteroidal neuromuscular blockers such as rocuronium, vecuronium. It is separated from traditional decurarization methods by its original mechanism of action. The sugammadex forms the sugammadex-noromuscular blocker complex at the neuromuscular junction. It then moves away from the neuromuscular junction with the concentration gradient and quickly returns muscle function. Compared to neostigmine, sugammadex both provides faster antagonism and carries a lower risk of residue block (Carron et al., 2013). Its clinical practice has also changed significantly since it became available throughout Europe and Australia in 2008 and throughout the United States in 2015. It has many advantages such as providing rapid neutralization in difficult intubation situations, reducing the risk of postoperative reintubation, wide therapeutic index and limited drug interactions. However, although it has been well studied in surgical patients, including children, the elderly, those with kidney and liver disorders, those with lung disease, and pregnant women who had a recent cesarean delivery, it has been used in early and late pregnancy, lactating women, women of childbearing potential, and little is known about its use in newborns (Hemmerling et al., 2010; Jahr et al., 2015; Mitchell et al., 2016; Partownavid et al., 2015; Richardson & Raymond, 2020). However, the pregnancy category of sugammadex has been determined as B2 according to the Australian Therapeutic Goods Administration. There are no clinical data on use in pregnancy for drugs in category B2. However, sugammadex may be recommended for use during pregnancy when its benefits outweigh its potential effects on the fetus (Australian Product Information Bridion®, 2021; The Drugs.com editorial team, 2021). According to the report published by the American Society of Obstetric Anesthesia and Perinatology (SOAP) on April 22, 2019, it was stated that the use of sugammadex should be avoided during early pregnancy (Society for Obstetric Anesthesia and Perinatology Statement on Sugammadex, 2019). Given the literature data on sugammadex and the category of pregnancy, it can be predicted that its use, especially in early pregnancy, may have a risk of negatively affecting the development of the nervous system.

Neural tube defect is one of the congenital anomalies that develop due to irregular neurulation in the early period of central nervous system development. The most common type is spina bifida, which is the most common developmental problem after cardiovascular abnormalities. Its incidence is 6/10,000, and the rate of miscarriage or curettage due to neural tube defect is unknown. Drugs that are sometimes required to be used during pregnancy cause such congenital malformations, but nowadays, the pregnancy category of newly developed drugs and what kind of malformations they may cause are not fully known due to the lack of studies (Robert, 2000; Rowland et al., 2006; Song et al., 2012; Tureci et al., 2011). However, many drugs that are thought to cause congenital malformations have been questioned in terms of neural tube defects by experimental animal model studies (Atay et al., 2020; Ertekin et al., 2019; Ertekin et al., 2021; Rakip et al., 2021).

An average of 5.2 million surgeries is performed annually in Turkey (Bora Başara et al., 2021). From this point of view, anesthetic drugs can be considered one of the most commonly exposed drugs. For this reason, it should be questioned whether there are effects of anesthetic drugs related to neural tube development. Evidence for maternal-fetal placental transfer, which is a prerequisite for causing fetal exposure, is controversial. Sugammadex has a large polar structure with a molecular weight of 2178 g/mol. However, there are publications suggesting that there is a limited passage to the placenta. It is known that some drugs during early pregnancy can stop or delay embryo development, even if they do not cause neural tube defects. Sugammadex has not been adequately investigated in this respect. Another controversial group in the use of sugammadex is nursing mothers and newborns. Due to insufficient evidence, the use of sugammadex in newborns is still not recommended (Bridion® (sugammadex), 2015; Munro et al., 2019; Sarı et al., 2013; Satomoto et al., 2016).

Accordingly, the study was carried out on the chick embryo model, which is a cheap and convenient model in which the effects of teratogenic factors on the neurulation process can be easily examined and drugs can be easily administered. Furthermore, since the neuronal and spinal development of the chick embryo is very similar to the development of the human embryo, we aimed to determine the potential/possible consequences of sugammadex exposure during human pregnancy.

In our literature search, we found no studies investigating the toxic effects of sugammadex on neural tube development and the relationship between AgNOR proteins and the effects of sugammadex exposure in a chick embryo model. This study was designed to fill this gap in the literature and to determine the possible toxic effects of sugammadex using different doses. In this study, we aimed to reveal the effects of sugammadex on neural tube (midline) closure in chick embryos by morphological and histopathological examination.

2 | MATERIALS AND METHODS

2.1 | Study design

Ethics committee approval was obtained for this study from Afyon Kocatepe University Animal Experiments Local Ethics Committee with the decision dated 06/03/2020 and numbered 49533702/270. This study was conducted in the Departments of Anatomy and Histology and Embryology of the Faculty of Medicine of Afyonkarahisar Health Sciences University. Within the scope of the study, 50 White Leghorn, fertile and 0-days specific non-pathogenic (SPF) eggs weighing 60 ± 5 g were used and were obtained from the İzmir Bornova Veterinary Control Institute (İzmir, Turkey). SPF eggs were divided into 5 groups of 10 eggs each: control group (group 1); 2 mg/kg sugammadex (group 2); 4 mg/kg sugammadex (group 3); 8 mg/kg sugammadex (group 4); and 16 mg/kg sugammadex (group 5).

2.2 | Obtaining embryos

Suitable temperature and humidity (37.5 ± 0.5 C temperature and 60 ± 5% humidity) conditions were provided for SPF eggs and incubated for 48 h. Within 28 h of the study, the fertilized eggs were removed from the incubator. Under sterile conditions, with the help of forceps, a window of about 0.5 cm was opened and the embryo was seen. Sugammadex (Bridion 200 mg/2 mL ampoule, Merck Sharp & Dohme Corp., New York, USA) at the doses specified in the working procedure with a Hamilton micro-injector was injected subblastodermically in 30 μL solutions to all experimental groups through the opened shell window. No agents were injected into the control group. After injection, the holes in all eggs were closed with sterile drapes, and the closed eggs were turned 180° and replaced in the incubator. At the end of the 48th hour, the eggs were removed from the incubator. The shells of the eggs were broken and the yolk was placed in a glass container containing sterile saline (0.9% sodium chloride). A watch glass was placed in the container to collect blastoderm. Then, using fine forceps and fine-tipped scissors, the vitelline membrane was cut from the embryonic membrane on the yolk. The vitelline membrane was separated from the egg yolk by holding it carefully at both ends, and the blastoderm adhering to the membrane was advanced in the liquid and placed on the watch glass. The resulting embryos were examined under a light microscope. These embryos were used for morphological and histological evaluation (open or closed neural tube, cranio-caudal length, embryological developmental disorder and somite count).

2.3 | Histologic examination

Embryos separated in 10% formol were kept in fixation solution for 48 hours and then washed with distilled water to remove fixatives. After washing, it was passed through graded alcohol series from 50% to 100% for dehydration. Then, it was cleared with xylol and paraffin-block was made. Embryos used in the study were placed on polylysine-coated slides. Standard histological staining method was used for the prepared slides. Tissues dyed with Hematoxylene-Eosin (H&E) were displayed under a light microscope to determine the general histological structure.

2.4 | Indirect immunocytochemical staining

Embryo sections that were sectioned and prepared for immunohistochemical staining were washed 3 × 5 times with phosphate buffer saline (PBS). Endogenous peroxidase activity was inhibited by the application of 3% hydrogen peroxide (H_2O_2). Washed 3 × 5 times with PBS. Antigen retrieval was performed with heat in a microwave oven with 10× Citrate Buffer (pH 6.0) for 20 min and cooled at room temperature for 20 min. After protein block (TP-125-HL, Thermo Fisher Scientific, Waltham, Massachusetts, USA) application for 1 h, Mouse anti-Caspase 3 (NB100-56708, Novus Scientific, Uppsala, Sweden) antibody was diluted 1:200 and incubated at +4°C for 1 night. Washing was done 3 × 5 times with PBS. It was incubated with biotinylated secondary antibody (Thermo, TP-125-HL) for 30 min. After washing with 3 × 5 PBS, HRP-conjugated streptavidin (TP-125-HL, Thermo Fisher Scientific, Waltham, Massachusetts, USA) for 30 min incubation was provided and the visibility of immunoreactivities was provided with DAB (1855920, Thermo Fisher Scientific, Waltham, Massachusetts, USA) chromogen application. Mayers' hematoxylin (TA-125-MH, Thermo Fisher Scientific, Waltham, Massachusetts, USA) was used for counterstaining. After alcohol and xylene, sections were covered with alcohol-based occlusion medium and photographed with a light microscope (Nikon E600W, Image Analysis Program NIS elements, Japan). The immunoreactivity results of the cells in the groups (Figure 1) were evaluated and counted as (+), (++), and (+++) respectively, according to the staining intensity as low, moderate and strong. *H*-score analysis was performed (Numata et al., 2013).

2.5 | Argyrophilic nucleolar regulatory regions (AgNOR) staining procedure

Sections of 5 μm thickness containing the somites and the neural tube were taken. Deparaffinized and rehydrated sections were subjected to AgNOR staining.

Two solutions are required in the preparation of NOR silver staining:

> 1st solution (solution A) is a 2% solution of gelatin dissolved in ultrapure water. Then, by adding formic acid, a 1% gelatin solution is obtained.
>
> 2nd solution (solution B) is a 50% solution of silver nitrate in ultrapure water.

The staining solution is prepared fresh. One volume of solution A is combined with two volumes of solution B, and it is dripped to completely cover the sections at room temperature and stained for 15–30 min away from direct sunlight. After staining, the solution is poured and the sections are washed with distilled water, kept in 5% thiosulfate solution for 10 min and then closed (Lindner, 1993).

Silver-stained samples (Figure 2) were evaluated under a light microscope (Eclipse E-600W, Nikon, Japan) using an image analysis

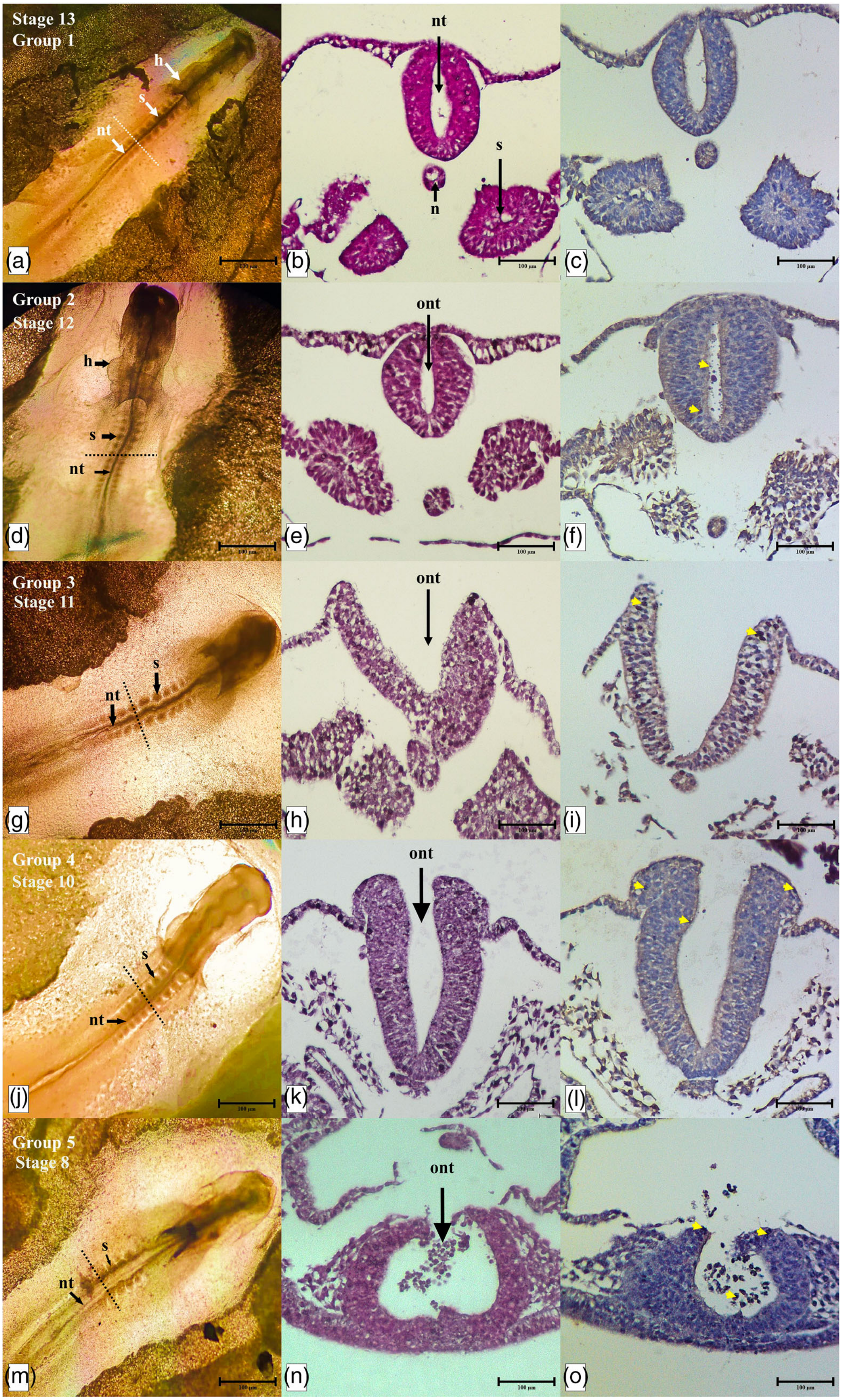

FIGURE 1 The appearance of embryos under the light microscope (a, group 1; d, group 2; g, group 3; j group 4; m, group 5), tissue samples stained with H&E (b, group 1; e, group 2; h, group 3; k group 4; n, group 5), tissue samples with indirect immunocytochemical staining (c, group 1; f, group 2; i, group 3; l, group 4; o, group 5), cells with indirect immunocytochemical staining are indicated by the yellow arrow, the dashed line indicates the level at which the preparation was sectioned. H&E, Hematoxylin–Eosin staining; h, heart; n, notochord; nt, neural tube; ont, open neural tube; s, somite.

system (NIS Elements Nikon, Japan). Fifty nuclear AgNOR protein images per sample were evaluated with the Image-J program. The average AgNOR number and total AgNOR area/total nuclear area (TAA/NA) ratio were calculated for each nucleus.

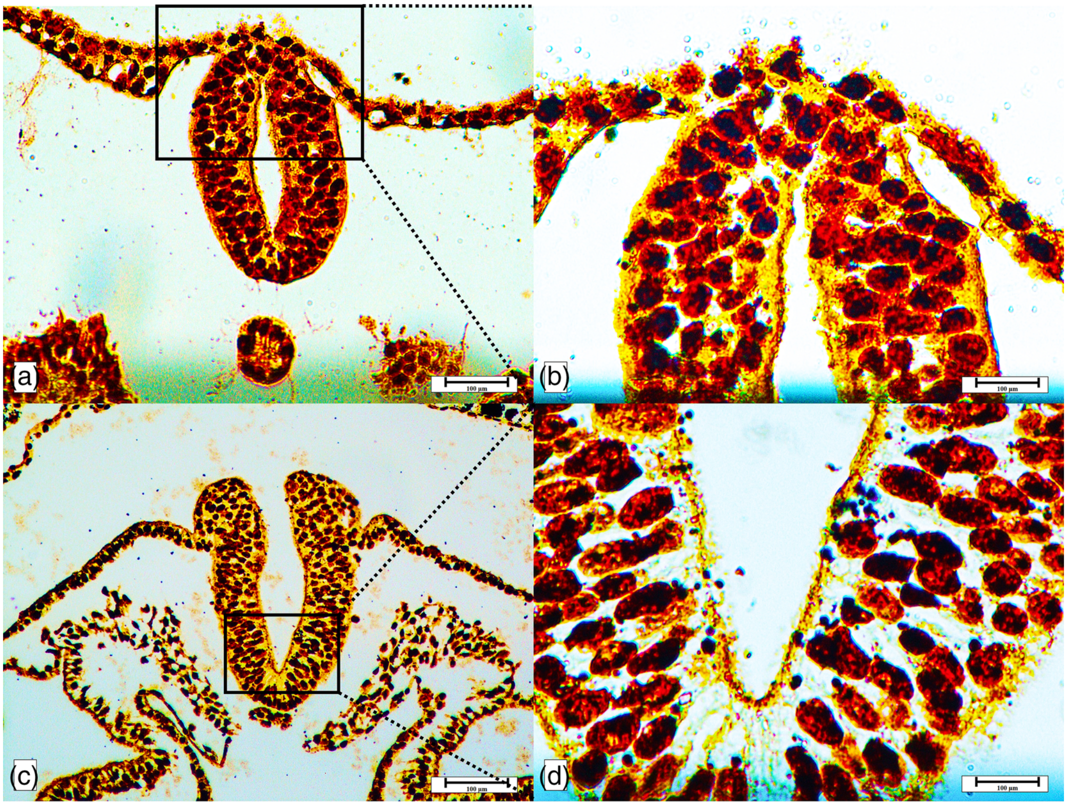

FIGURE 2 Tissue samples with AgNOR staining, (a) embryo sample with closed neural tube, (b) calculation of AgNOR area/total nuclear area (TAA/NA) ratio in a randomly selected area (10× magnification), (c) embryo sample with open neural tube, (d) calculation of the AgNOR area/total nuclear area (TAA/NA) ratio in a randomly selected area (10× magnification). AgNOR, argyrophilic nucleolar regulatory regions; NA, total nuclear area; TAA, total argyrophilic nucleolar regulatory regions area.

TABLE 1 Experimental groups statistical analysis results.

	Group 1	Group 2	Group 3	Group 4	Group 5
NT position (on/off)	0/10	3/7[a]	10/0[a]	10/0[a]	10/0[a]
Developmental retardation (no/yes)	10/0	10/0	10/0	5/5[a,b,c]	4/6[a,b,c]
Length	766.91 ± 48.51	710.59 ± 84.1	684.64 ± 58.72	613.83 ± 56.07[a]	580.38 ± 79.59[a,b]
Somite	16.1 ± 0.88	13.6 ± 1.43	12.1 ± 1.45	5.6 ± 2.80[a,b]	5.1 ± 4.12[a,b]
TAA/NA	0.33 ± 0.04	0.29 ± 0.01	0.25 ± 0.02	0.21 ± 0.07[a,b]	0.18 ± 0.02[a,b]
AgNOR Number	4.33 ± 0.54	3.11 ± 0.39	2.96 ± 0.17[a]	2.9 ± 0.51[a]	2.8 ± 0.24[a]
Caspase-3	583 ± 3	661 ± 11	1782 ± 4	2322 ± 10[a]	2474 ± 5[a,b]

Abbreviations: AgNOR, argyrophilic nucleolar regulatory regions; NT, neural tube; TAA/NA, total AgNOR area/total nuclear area.
[a]Statistically significant when compared with Group 1.
[b]Statistically significant when compared with Group 2.
[c]Statistically significant when compared with Group 3.

2.6 | Statistical analysis

Statistical analysis of the data was performed using the SPSS (Statistical Package for the Social Sciences) program. The open/closed state of the neural tube was determined using the corresponding statistical chi-square test. Means of cranio-caudal length, somite numbers, AgNOR numbers, TAA/NA ratios and *H*-score analysis results of the embryos were analyzed using the nonparametric Kruskal–Wallis test. Dunn's test was used as a post-hoc test and $p < .05$ values were considered significant. Data were expressed as mean ± standard deviation (SD).

3 | RESULTS

In our study, the effect of sugammadex in different doses on neural tube development was examined. The findings of the control group along with the groups given sugammadex were presented under separate headings. Mean ± SD values and statistical analysis results are shown in Table 1.

Group 1: No agent was injected into the control group. The neural tube was observed to be closed in all of the embryos and no developmental delay was observed. Under a light microscope, the macroscopically and morphologically assessed the control group embryos. The embryos' heads had started to tilt slightly to the left. Because the cervical bending created a wide angle, the head of some embryos seemed fully turned to the left. The cranial and cervical areas developed twisted, broad curves. The telencephalon was noticeably enlarged, and the head fold covered the anterior, middle, and posterior brain. Some of the embryos had the identifiable Rathke's sac. The Hamburger-Hamilton (H&H) classification of these observations placed them in Stages 13 (Hamburger & Hamilton, 1992). When the embryos are examined macroscopically; it was observed that the primary optic vesicles and optic sac were well defined, the anterior neuropores were closed, and the heart took a slight S shape (Figure 1). When the embryos are examined morphologically; It was determined that the mean cranio-caudal length was 766.91 ± 48.51 μm, the mean somite number was 16.1 ± 0.88, TAA/NA 0.33 ± 0.04, the mean AgNOR numbers 4.33 ± 0.54, mean Caspase 3583 ± 3 (Table 1).

Group 2: Embryos in this group were injected with 2 mg/kg sugammadex. Three out of 10 embryos had neural tube open. The neural tubes of the remaining 7 embryos were closed and there was no developmental delay. Examination under light microscopy showed that the embryos were compatible with Stage 11–12 according to H&H classification. In stage 11 embryos, the heart was bent to the right. In stage 12 embryos, the heart had a slightly S-shaped appearance. In general, mild cranial curvature was detected in the embryos. The anterior neuropore of all embryos was closed and the telencephalon was well prominent (Hamburger & Hamilton, 1992). When the embryos are examined macroscopically; It was observed that there was a slight cranial fold, the anterior neuropore began to close, the optic vesicles became prominent and the heart was located to the right (Figure 1). The mean cranio-caudal length was 710.59 ± 84.1 μm, the mean somite number was 13.6 ± 1.43, TAA/NA 0.29 ± 0.01 and the mean AgNOR numbers were 3.11 ± 0.39, and the mean Caspase 3 was 661 ± 11 (Table 1).

Group 3: Embryos in this group were injected with 4 mg/kg sugammadex. The neural tubes of all embryos were open, but developmental delay was not detected in any of them. Compatible with Stage 11 according to the H&H classification. In general, the somite number of embryos was in accordance with the H&H classification. Brain vesicles (three basic brain vesicles) clearly visible. Optic vesicle visible, but no constriction at the base of the vesicle. The heart was inclined to the right. In addition, the macroscopic appearance of some of the embryos was similar to that of Group 2 embryos (Figure 1) (Hamburger & Hamilton, 1992). It was determined that the mean cranio-caudal length was 684.64 ± 58.72 μm, the mean somite number was 12.1 ± 1.45, TAA/NA 0.25 ± 0.02, the mean AgNOR numbers were 2.96 ± 0.17, the mean Caspase 3 was 1782 ± 4 (Table 1).

Group 4: In this group, 8 mg/kg sugammadex was injected. The neural tube of all embryos was open and developmental delay was detected in 5 embryos (Figure 3). The macroscopic appearance of the embryos varied between Stage 8 and Stage 10 according to the H&H classification (Hamburger & Hamilton, 1992). Embryos with developmental delay were generally compatible with Stage 8. The neural folds of these embryos were fused at the midbrain level. In other embryos (Stage 10), the primordium of the heart had begun to fuse and the heart of some embryos was slightly bent to the right. Primary optic sacs and heart were visible in some embryos. It was determined that the mean cranio-caudal length was 613.83 ± 56.07 μm, the mean somite number was 5.6 ± 2.80, the TAA/NA 0.21 ± 0.07, and the mean AgNOR numbers were 2.9 ± 0.51, the mean of Caspase 3 was 2322 ± 10 (Table 1).

Group 5: Embryos in this group were injected with the highest dose (16 mg/kg) of sugammadex. The neural tubes of all embryos were open and developmental delay was detected in 6 embryos (Figure 3). The macroscopic appearance of the embryos varied between Stage 6 and Stage 10 according to the H&H classification (Hamburger & Hamilton, 1992). While the first somite initiation could be seen in some embryos, the formation of primary optic sacs and rightward placement of the heart had begun in others. It was

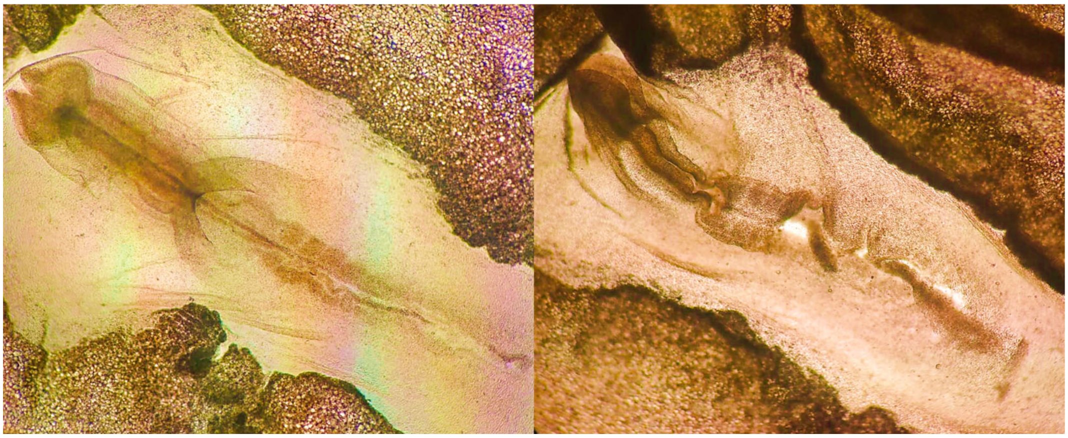

FIGURE 3 Examples of embryos showing developmental delay.

observed that neural folds began to merge at the midbrain level and islands of blood appeared. It was determined that the mean cranio-caudal length was 580.38 ± 79.59 μm, the mean somite number was 5.1 ± 4.12, TAA/NA 0.18 ± 0.02, the mean AgNOR numbers were 2.8 ± 0.24, the mean Caspase 3 was 2474 ± 5 (Table 1).

As a result of the statistical evaluation of neural tube position (open/closed) and developmental status (present, absent) between the experimental groups, a statistically significant difference was determined between all sugammadex dose groups and the control group ($p < .001$). While the mean cranio-caudal lengths, somite numbers, TAA/NA ratios and AgNOR numbers of embryos decreased proportionally from Group 1 to Group 5, Caspase-3 *H*-Score results increased linearly. When the mean cranio-caudal length of the embryos was evaluated statistically, it was found that between Group 1 (Control group) and Group 4 ($p \leq .001$) and Group 5 ($p = .001$), Group 2 and Group 5 ($p = .024$); when the mean somite numbers were evaluated, it was found that between Group 1 and Group 4 ($p \leq .001$) and Group 5 ($p \leq .001$), Group 2 and Group 4 ($p = .004$) and Group 5 ($p = .004$); when the mean TAA/NA ratios were evaluated, it was found that between Group 1 and Group 4 ($p = .001$) and Group 5 ($p \leq .001$), Group 2 and Group 4 ($p = .045$) and Group 5 ($p = .001$); when the averages of AgNOR numbers were evaluated, it was found that between Group 1 and Group 3 ($p = .047$), Group 4 ($p = .002$) and Group 5 ($p = .004$); when Caspase 3 averages were evaluated, significant differences were found between Group 1 and Group 4 ($p = .004$) and Group 5 ($p \leq .001$), Group 2 and Group 5 ($p = .004$) (Table 1).

4 | DISCUSSION

Obstetric and non obstetric surgeries are frequently observed in pregnant women. However, the incidence of difficult intubation, which is an indication for sugammadex, is also high in pregnant women. The frequency of surgeries not directly related to pregnancy in pregnant women is 0.3%–2.2% in the United States (USA). It is seen that 42% of these are done in the first 3 months of pregnancy (1^{st} trimester), 35% are in the second trimester (2^{nd} trimester) and 23% are done in the last 3 months of pregnancy (3^{rd} trimester). The most common surgeries are laparoscopic appendectomy and cholecystectomy, and less frequently brain and heart surgeries (Gunaydin, 2012; Ní Mhuireachtaigh & O'Gorman, 2006; Tolcher et al., 2018). As a result of these ongoing surgeries, sugammadex has become an indispensable part of clinical practice following its clinical use approval in Europe in 2008 and in the USA in 2015 (Carron et al., 2013; Jahr et al., 2015). In addition, the successful use of sugammadex in pediatric cases and the termination of general anesthesia after cesarean delivery, and the successful use of sugammadex in pregnant women without recurarization are positive markers that increase the frequency of sugammadex use. However, studies and evidence on its effects on the fetus during early pregnancy are limited (Pühringer et al., 2010).

There can be many complications in a long process such as pregnancy. Sugammadex cannot be said to be safe without adequate investigation of its possible contribution to these pregnancy complications. It is known that sugammadex interacts with oral contraceptive drugs and affects progesterone levels. This interaction should be kept in mind in women of gestational age (Williams & Bryant, 2018). In different publications, it has been shown that sugammadex does not reduce progesterone levels enough to cause abortion in early pregnancy (Et et al., 2015; Gunduz Gul et al., 2016). However, its effect on early fetal development in known or unknown pregnancy cases is unknown. In our study, in which the teratogenic effects of sugammadex were evaluated, both developmental delay and neural tube defects were observed in embryos treated with different doses of sugammadex. However, while neural tube defects were observed in 30% of the embryos in the low-dose sugammadex group and in all of the other groups; growth retardation is more common in high dose groups (Table 1). These findings show us that sugammadex-related neural tube defect and growth retardation increase in a dose-dependent manner.

Although not supported by sufficient clinical data, it is thought that sugammex may have minimal placental transmission. In a rare and interesting case in the literature, rocuronium was administered to the fetus during intrauterine blood transfusion. Meanwhile, rocuronium was administered to the pregnant woman by accident, and after the dyspnea of the pregnant woman was reversed with sugammadex, additional neuromuscular blocker was required in the fetus to continue the procedure. This case is one of the rare studies suggesting that sugammadex may have limited placental transmission (Munro et al., 2019). The results obtained in our study support this literature information. Because in our study, it was determined that there was a significant developmental delay and a decrease in cranio-caudal length in chick embryos in groups treated with sugammadex. However, it was observed that there was a dose-dependent developmental delay in neural tube development. All these results support that sugammadex exposure during pregnancy may also affect the fetus. Whether the placental passage changes under different conditions can be examined as the subject of another study.

In a vitro study by Palanca et al., it was concluded that sugammadex increased apoptosis and necrosis in cells, possibly by reducing neuronal cholesterol levels. It is known that the increase in cholesterol in cells prevents the release of proapoptitic molecules such as Caspase-3 (Palanca et al., 2013). Aldasoro et al., in their study, which obtained similar results, associated neuronal damage and cell death due to increased Caspase-3 activity with sugammadex administration. They showed that they prevented toxic neuronal damage caused by sugammadex with the addition of Rocuronium and Vecuronium (Aldasoro et al., 2017). In our study, it was determined that Caspase-3 levels increased significantly depending on the dose of sugammadex, consistent with these literature findings. When Caspase 3 averages were evaluated with the *H*-Score method, Caspase-3 levels increased significantly from the control group to the group that received the highest dose of sugammadex. This significant increase in Caspase-3 levels may be due to the change in neuronal cholesterol levels, and the mechanism of this pathology is not yet fully known. However, we think that the neural tube defects may be caused by Caspase-3-induced neuronal damage.

In the newborn mouse study of Satamoto et al., they showed that co-administration of sugammadex and sevoflurane caused a 150% increase in neuroapoptosis in the brain compared to 2% sevoflurane exposure alone (Satomoto et al., 2016). These studies reinforce the suspicion that sugammadex may have a negative effect on neuronal development. According to our results, the risk of neural tube defects, embryo length, somite count and Caspase-3 level increase in a dose-dependent manner. Although it has limited placental passage and has been used with some steroidal muscle relaxants, increased sugammadex doses seem to be risky in terms of both neural tube defect and growth retardation. The manufacturer reported no evidence of teratogenicity after intravenous administration in pregnant rats and rabbits at levels equal to and 6 to 8 times greater than the maximum recommended human dose (16 mg/kg). However, fetal growth retardation has been reported in rabbits at 8 times the maximum recommended human dose (Bridion® (sugammadex), 2015). This information supports our results.

Nuclear organizer regions (NORs) are metaphase regions where ribosomal genes form their bonds and many regulatory proteins bind. Staining these regions with silver nitrate enables the metabolic activity in cells to be visualized with a light microscope. AgNOR numbers have also been used to show cancer cells with increased metabolic activity. Conversely, decreased AgNOR numbers may be associated with cell death in the case of decreased activity (Ertekin et al., 2016; Lindner, 1993; Ploton et al., 1983).

In the literature, in neural tube development studies based on the chick embryo model, TAA/NA ratio and AgNOR numbers were used as a measure of the proliferation activity of cells and were measured to be low in cells with growth retardation (Ertekin et al., 2021; Rakip et al., 2021). Our results are also similar to the literature, and the TAA/NA ratio and AgNOR numbers were found to be statistically significantly lower depending on the dose. Again, in the embryos looked at on the same days, it was determined that the control group embryos were compatible with Stage 13 according to the H&H classification, while the group 5 embryos varied between Stage 6 and 10. With these results, it was concluded that embryo development decreased inversely with increasing sugammadex dose.

5 | CONCLUSION

Although long-term sugammadex exposure in chick embryos in our study has been proven to have a negative effect on neural tube development and embryo development, its acceptance in humans may be limited. With the limited placental passage of sugammadexin, its high selectivity to rocuronium and vecuronium, its short half-life for fetal development, its effect of possible exposure may be limited.

In addition, Ertekin et al.'s studies using diclofenac sodium and Rakip et al.'s studies using a similar material method using pethidine showed neural tube defect formation (Ertekin et al., 2019; Rakip et al., 2021). Also, the effects of different drugs such as caffeine, methotrexate, quetiapine on neural tube development were investigated with the chick embryo model (Ma et al., 2012; Ovalioglu et al., 2021; Vatansever et al., 2003). This shows the possibility of similar results with different chemicals depending on the chemical and physical effects of sugammadex as well as its pharmadynamic effect. However, we believe that our findings will contribute to explain the effect of sugammadex on embryo development, since it is a study that cannot be done on human embryos.

Although fetal exposure seems to be limited after sugammadex administered during pregnancy, it has been observed that different doses of sugammadex increase the risk of neural tube defect development on chick embryos and slow the embryo development in a dose-dependent manner. It is difficult to test this condition clinically. However, it is clear that more research with in vitro studies is needed.

AUTHOR CONTRIBUTIONS

Elif Büyükerkmen: Conceptualization; investigation; funding acquisition; writing – original draft; writing – review and editing; project administration. **Emre Atay:** Conceptualization; investigation; writing – original draft; methodology; writing – review and editing; visualization. **Fatma Firat:** Methodology; formal analysis; writing – review and editing. **Ahmet Yüksek:** Writing – review and editing; writing – original draft. **Abdülkadir Bilir:** Writing – original draft; investigation; methodology; writing – review and editing; formal analysis; data curation. **Gülan Albaş Kurt:** Writing – review and editing; methodology; data curation. **Alperen Saritaş:** Data curation; writing – review and editing; methodology.

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CONFLICT OF INTEREST STATEMENT

The authors of this manuscript declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID

Elif Büyükerkmen https://orcid.org/0000-0001-9644-2185
Emre Atay https://orcid.org/0000-0002-2378-1183
Fatma Firat https://orcid.org/0000-0003-0027-5138

Ahmet Yüksek https://orcid.org/0000-0002-7529-2971
Abdülkadir Bilir https://orcid.org/0000-0003-0633-9542
Gülan Albaş Kurt https://orcid.org/0000-0002-4007-9062
Alperen Saritaş https://orcid.org/0000-0003-3523-1936

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