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# The Effects of Sugammadex on Progesterone Levels in Pregnant Rats

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**Background:** Sugammadex has been shown to decrease the efficiency of progesterone-containing oral contraceptive drugs which possess a steroid structure.
**Aims:** The aim of the present study was to evaluate the effects of sugammadex on progesterone levels in pregnant rats as well as on the physiological course of the pregnancy.
**Study Design:** Animal experiment.
**Methods:** This study was approved by the Selçuk University Ethical Committee for Experimental Animal Research. Pregnant Winster Albino rats (n=26) were divided into three groups and administered with various intravenous injections on the 7th day of pregnancy. The control group (Group K, n=6) received 1.5 mL serum physiologic, the sugammadex group (Group S, n=10) received 30 mg/kg sugammadex and the sugammadex + rocuronium group (Group SR, n=10) received 30 mg/kg sugammadex and 3.5 mg/kg rocuronium. Progesterone levels were measured and the offspring were monitored for morphologic status.
**Results:** Mean progesterone levels were 94.16±15.54 ng/mL in Group K, 87.86±12.48 ng/mL in Group S, and 94.53±16.10 ng/mL in Group SR ($p>0.05$). No stillbirth or miscarriage was observed in the rats. The mean number of offspring was 6.8±1.47 in Group K, 6.5±1.35 in Group S, and 6.4±1.17 in Group SR. The offspring appeared macroscopically normal.
**Conclusion:** Sugammadex does not appear to affect the progesterone levels in pregnant rats in the first trimester and the clinical course. Successful completion of pregnancy and the absence of stillbirth or miscarriage will guide future studies about the use of sugammadex, particularly in the first trimester of the pregnancy.
**Keywords:** Pregnant, progesterone level, rat, sugammadex

Sugammadex is a modified gamma-cyclodextrin. The molecule contains eight glucose molecules (1). Human and animals studies have shown the efficiency and safety of sugammadex in when used for reversal of the effects of neuromuscular blockade by the agent rocuronium (2-5). Sugammadex has been designed to encapsulate rocuronium and vecuronium molecules, which are aminosteroid agents that are commonly used in general anesthesia (6). Sugammadex encapsulates and increases the water-solubility of appropriately-sized lipophilic drugs. The side chain of the sugammadexin molecule extends the cavity size to encapsulate rocuronium molecules, and negatively charged carboxyl groups on the end of the chain electrostatically bind to the positively charged nitrogen of the rocuronium molecule (6). Potential interactions of sugammadexin with other drugs include binding affinity to the existing drugs, similarity and *in vitro* applications; however, related data remains limited (7).

Following the use of sugammadex in a woman receiving progesterone-containing oral contraceptives (combined or progesterone-only), the daily dose of contraceptive should be considered to have been missed. The use of 4 mg/kg sugam-

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madex decreases progesterone levels by 34% and causes a reduction in the efficacy of progesterone. In general laboratory tests, sugammadex did not affect serum progesterone analyses; however, the studies did not evaluate the precise clinical relations (7). There are no data on the use of sugammadex in pregnant women.

The regular increase in progesterone levels throughout pregnancy, particularly in the first trimester, is of particular importance for conception and for the maintenance of pregnancy. Progesterone withdrawal results in the termination of pregnancy by preventing relaxation (8,9). Our aim was to determine the effects of the use of Sugammadex on progesterone levels and to explore how this effect could change the course of pregnancy.

## MATERIALS and METHODS

The present study was conducted in the Experimental Animals Research Center of the Selçuk University after obtaining approval from the Experimental Animals Ethics Committee in Selçuk University Faculty of Medicine.

A total of 26 healthy and pregnant Wistar albino rats, weighing 240-400 grams, were included in the present study. Special attention was paid to include healthy rats and not to include rats that had been previously included in another study or exposed to any drugs. The rats were maintained on a 12-hour light and 12-hour dark regimen at a constant temperature of 20-24ºC. The rats were fed *ad libitum* with free access to food and water until 2 hours before the drug administration.

One day before the administration, female rats (n=60), which showed two normal consecutive reproduction cycles, were placed in cages as quadruplets to conceive. One male rat (n=15) was placed in each cage for mating with the four female rats. One day later, male rats were removed from the cages. After inserting a pipette into the vagina of the rats, 0.5 mL physiological saline solution (SF) was administered and vaginal secretions of the rats were collected. The samples were examined by light microscopy at 10x and 40x magnification, and a rat was considered to be pregnant and on Day 0 of pregnancy if the microscopic examination showed sperm. Rats were considered pregnant without the need for microscopic examination of the vaginal secretion if the vaginal membrane was observed on vaginal inspection (n=26). Rats which were considered to be pregnant underwent abdominal examination on the 7th day of gestation.

Pregnant rats were randomly divided into three groups: control (Group K, n=6), Sugammadex (Group S, n=10), and Sugammadex+Rocuronium (Group SR, n=10). The drugs were administered in a double-blind fashion. The weight of the rats was measured using a precision balance. Ketamine (Ketalar, Parke Davis Eczacıbaşı, İstanbul, Turkey) (50 mg/kg, intraperitoneal) and xylazine (Rompun, Bayer, Toronto, Canada) (10 mg/kg) were administered to provide sedation and analgesia.

The rats in Group K were administered with 1.5 mL %0.9 NACI.

The rats in Group S were administered with 30 mg/kg sugammadex (bridion, Merck Sharp & Dohme, İstanbul, Turkey) at a volume of 1.5 mL.

The rats in Group SR were administered with 3.5 mg/kg rocuronium (Esmeron, Schering-Plough, İstanbul, Turkey) followed by 30 mg/kg sugammadex at a volume of 1.5 mL.

A 1.5 mL blood sample was obtained 35 minutes after the injections.

All samples were centrifuged at 3000 rpm for 10 minutes. Serum samples were separated and stored at 2-8$^{o}$C. Progesterone levels were measured in the serum samples using Unicel DXI 800 Access Immunoassay System after 1/10 dilution with Beckman Coulter solution.

All rats underwent daily abdominal examination during their pregnancy. On the 7$^{th}$ day of pregnancy, the rats were monitored for 2 hours after the administration of sedatives and analgesic drugs, and blood samples were collected. All rats were assigned a unique number, and each pregnant rat was placed in a separate cage. On the 8th day of pregnancy, all rats underwent abdominal and vaginal examination. During vaginal examination, the rats were examined for the presence of vaginal discharge or bleeding. Time of delivery and the number of offspring delivered were recorded. The offspring were evaluated for the presence of macroscopic anomalies. After delivery, mother rats and the offspring were sacrificed by cervical dislocation under anesthesia.

SPSS 16 for Windows (SPSS Inc., Chicago, IL, USA) was used to analyse all data obtained in the study. The difference between the calculated parameters of experimental groups was compared using one-way analysis of variance (ANOVA). Tukey's multiple comparison test was performed in the presence of significant differences between the groups ($p<0.05$). All experimental parameters are expressed as number, day, and mean ± standard deviation.

## RESULTS

There was no significant difference between the groups in terms of mean weight measured on the 7$^{th}$ day of pregnancy, progesterone levels, duration of pregnancy, and the number of offspring ($p>0.05$) (Table 1). In all of the study groups, no stillbirth or miscarriage was observed in the rats. All

**TABLE 1.** Mean weights of the rats and mean progesterone levels on the 7th day of pregnancy, mean duration of pregnancy and mean number of offspring

| | Group K (n=6) | Group S (n=10) | Group SR (n=10) | p |
|---|---|---|---|---|
| Weight (gr). (mean±sd) | 280.66±26.68 | 322.30±37.75 | 320.70±48.77 | 0.118 |
| Progesterone level (ng/mL). (mean±sd) | 94.16±15.54 | 87.86±12.48 | 94.53±16.10 | 0.552 |
| Mean duration of pregnancy (day). (mean±sd) | 21.33±0.51 | 22.10±0.87 | 22.10±0.73 | 0.115 |
| Mean number of offspring. (mean±sd) | 6.83±1.47 | 6.83±1.47 | 6.40±1.17 | 0.812 |

Group K: control group; Group S: sugammadex group; Group SR: sugammadex+ rocuronium group

of the offspring in the three groups appeared to be macroscopically normal without any deformity or malformation.

## DISCUSSION

Progesterone levels are of particular importance in the first trimester of pregnancy, and reduced or the lack of a gradual increase in progesterone levels of rats is associated with miscarriages and still births. The present study aimed to demonstrate the effects of sugammadex, which decreases the efficiency of oral contraceptives, on endogenous progesterone levels, as reported in the literature. We investigated the effects of the changes in progesterone levels on the physiological process of pregnancy in rats.

Mirakhur et al. (10) highlighted the presence of two theoretical drug interactions: substitution and encapsulation. Such interaction mechanisms reduce the plasma levels of the drug. Drugs such as toremifene, flucloxacillin, and fucidic acid exhibit substitution-type interactions. Encapsulation-type interactions are potentially exhibited by hormonal contraceptive drugs. Pharmacological simulations estimate that the interaction reduces unbound progesterone levels by 34%. Following the use of sugammadex in a woman receiving progesterone-containing oral contraceptives, the daily dose of contraceptive should be deemed to have been missed. This interaction has been documented by pharmacological simulation and microcalorimetric methods (10,11). Rezonja et al. (12) evaluated the effects of the interaction between corticosteroid and sugammadex on human myocytes and reported a decreased efficacy for sugammadex with the administration of high doses of corticosteroids.

Eikermann et al. (13) employed 3.5 mg/kg rocuronium in their study which examined the impaired activity of upper airway and breathing by sugammadex and neostigmin; in the same study, they used incremental doses of sugammadex (5, 10 and 15 mg/kg). Kalkan et al. (14) employed 16 mg/kg and 96 mg/kg sugammadex in their study investigating the effects of sugammadex on the immunoreactive efficiency of calcineurin in rat testes after neuromuscular blockade.

In the present study, we employed 30 mg/kg sugammadex as indicated by the European Medicines Agency and the European Public Assessment Report for Bridion (7), and 3.5 mg/kg rocuronium in parallel with the literature (14).

Williamson et al. (15) conducted a study in pregnant women using sugammadex; however, this study included pregnant women in the third trimester and women who planned to undergo cesarean section. There is no study in the literature regarding the use of sugammadex in the first trimester of pregnancy.

In the present study, we used pregnant rats during the first trimester, which is of particular importance for the continuation of pregnancy, and is characterized by a gradual increase in progesterone levels.

Dao et al. (16) emphasized the importance and necessity of progesterone in pregnant rats, and administered pregnant rats with three different anti-progesterone drugs, either alone or in combination with anordiol, at Days 7, 8, 9 and 16 of the pregnancy. The rate of pregnancy termination was 0% with 1 and 2 mg/kg mifepristone but the rates were 40% and 100% with the doses of 2.5 mg/kg and 4 mg/kg, respectively. The rate of pregnancy termination was 83% with 4 mg/kg onapristone and 100% with 8 mg/kg onapristone. Progesterone levels are important for conception and the continuation of pregnancy, particularly in the pre-implantation and peri-implantation periods. The rate of pregnancy termination reached 100% with increasing doses of anti-progesterone drugs.

Chwalisz et al. (17) highlighted the importance of sufficient interactions between trophoblastic residual connections and sufficient blood flow across these interactions to maintain pregnancy and showed the importance of nitric oxide (NO) synthesis in endometrial functions during pre-implantation (1-4 days) and peri-implantation (4-7 days) stages of the pregnancy in rats. NO synthesis is either directly or indirectly controlled by progesterone. The administration of pregnant rats with anti-progesterone and amino-guanidine drug combinations between Days 1-4 and Days 4-7 prevented pregnancy in all (100%) rats.

The studies performed to date have employed repeated doses and the combined administration of anti-progesterone drugs. Such regimens increase anti-progesterone drug concentration and prolong the action time. In the present study, we employed a single and high dose of sugammadex. We preferred administering sugammadex specifically on Day 7 as many factors in rats result in the termination of pregnancy within the first 4 days, and progesterone levels increase significantly on Day 7.

Shorter action time for sugammadex compared to other anti-progesterone drugs and single dose administration prevented a significant decrease from occurring. Therefore, we did not observe any miscarriages.

Elgera et al. (18) administered non-pregnant rats with progesterone antagonists and progesterone receptor antagonists, and reported delayed ovulation period in rats. Delayed ovulation becomes prominent following the administration of combinations of progesterone antagonists. The administration of pregnant rats with the anti-progesterone drugs RU 486 and onapristone in the first trimester resulted in a decrease in the hormone levels, and the pregnancy ended with miscarriages.

Vodstrcil al. (19) reported progesterone withdrawal due to stable levels of relaxin hormone in the circulation and decreased levels of relaxin receptors in myometrium, and such effects caused delays in the pregnancy of rats and miscarriages in pregnant animals. Progesterone and 17-beta-estradiol levels of pregnant women who were administered the progesterone antagonist (RU 486) at Days 7, 16 and 19 were found to be lower compared to those of the control group. The administration of RU-486 was followed by progesterone withdrawal, and the rats were unable to maintain pregnancy due to relaxation of the myometrial muscles.

Yang et al. (20) administered rats, mice and hamsters with progesterone antagonists DL111-1T and RU-486 for the early termination of the pregnancy. The rate of pregnancy termination was 40% and 100% after the administration of 9 mg/kg and 100 mg/kg DL111-1T, respectively. Oral administration of 0.3 mg/kg RU-486 did not induce miscarriage in any rats; however, the rate of termination was 100% after the administration of 3 mg/kg RU-486.

In this study, considering its anti-progesterone features, sugammadex was administered in a single dose of 30 mg $kg^{-1}$ at Day 7 involving pre-implantation and peri-implantation periods (7,17). Blood progesterone levels were measured at 30 minutes, which is the closest time point to the slow distribution phase corresponding to the 27 minute distribution half-life (t1/2) (7).

Our aim was to determine the effects of this regimen on progesterone levels and to evaluate changes in the physiologic course of the pregnancy in rats and to make predictions for clinical application based on the current data. Low progesterone levels in Group S were of no statistical significance and did not pose any risks to the pregnancy of rats; all rats completed their pregnancies.

In their study, Chwalisz et al. (17) employed a specific radioimmunoassay for the measurement of progesterone levels. Dao et al. (16) preferred radioimmunoassay for the measurement of progesterone and 17-beta-estradiol. Radioimmunoassay is a widely used method in hormone analysis.

This method has been replaced by the chemiluminescence assay (CLIA) in recent studies. Hui et al. (21) evaluated estradiol and progesterone levels in desert rats using CLIA. Mete et al. (22) studied endocrine functions and effects on the behavior of pre-pubertal rats, and employed CLIA in the analysis of steroid hormones. In the present study, we employed CLIA in the analysis of progesterone levels in pregnant rats.

Sugammadex is often administered to pregnant women during caesarean section, in other words in the third trimester of pregnancy. The application of this agent is generally limited to cases and case reports, and there has been no prospective or retrospective study conducted in larger groups (23-26). There is also no case report or study regarding the use of Sugammadex in the first trimester.

In conclusion, low progesterone levels in Group S were of no statistical significance and did not affect the clinical course. Successful completion of pregnancy and absence of stillbirth or miscarriage will guide future studies about the use of sugammadex, particularly in the first trimester of pregnancy.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the ethics committee of the Experimental Animals Ethics Committee in Selçuk University Faculty of Medicine (Date: 30.03.2011/protocol no: 2011-23).

**Informed Consent:** N/A.

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