The Effect of Rocuronium and Sugammadex on Progesterone Levels in Pregnant Rabbits Under General Anesthesia [1][2]

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[2] The preliminary result of the study was presented at 22nd Annual of the European Society for Domestic Animal Reproduction (ESDAR) Conference, 27-29 September 2018, Cordoba/Spain

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Abstract

The purpose of this study was to investigate the effect of rocuronium and sugammadex on progesterone (P4) levels in pregnant rabbits under general anesthesia. Twenty-one pregnant New Zealand rabbits were used in the study. After the rabbits were divided into three groups of three (Control, Group I and Group II), each animal was given 0.5 mg/kg midazolam and 6 mg/kg propofol and then put under general anesthesia with sevoflurane on the 21st day of pregnancy. No procedure was performed on the control group apart from anesthesia. Rocuronium was administered to GI at the onset of anesthesia, and in GII, sugammadex was administered 60 min after general anesthesia + rocuronium. All of the rabbits were monitored during the anesthesia procedure. A sample of venous blood was taken and biochemically analyzed to test P4 levels. The administration of rocuronium was determined to have caused an increase in the serum progesterone level in all recorded min. Sugammadex was found to cause a quantitative decrease in the level of progesterone. In conclusion, it was found out that rocuronium and sugammadex administration did not have a negative effect on progesterone levels in pregnant rabbits receiving general anesthesia.

Keywords: Pregnant, Progesterone level, Rabbit, Sugammadex, Rocuronium

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Genel Anestezi Altındaki Gebe Tavşanlarda Rocuronium ve Sugammadex’in Progesteron Düzeylene Etkisi

Öz

Sunulan çalışmada, genel anestezi uygulanan gebe tavşanlarda, rocuronium ve sugammadex uygulamasının progesteron düzeyine etkisinin saptanması amaçlandı. Çalışmada, 21 adet gebe Yeni Zelanda tavşanı kullanıldı. Her grupta 7 tavşan olan üç gruba ayrıldı (Kontrol, Grup I ve Grup II). Her tonucunun 0.5 mg/kg midazolam ve 6 mg/kg propofol verildi ve sonra genel anesteziye sevofluranla 21. gününe kadar yürüttüldü. Kontrol grubunda hiçbir işlem yapılmadı. Grup I’de, genel anesteziye açılışında rokuronium verildi. Grup II’de ise, genel anesteziye açılışından 60 dk sonra sugammadex verildi. Tüm tavşanların genel anestezi süresince monitöre edildi. Tavşanların venöz kan örnekleri alınarak genel anesteziye açılışından 30 dk süren 4 gruba ayrıldı. Sonuç olarak, genel anesteziye açılışından sonra, rocuronium ve sugammadex uygulamasının progesteron düzeyine olumlu etkisi saptandı.

Anahtar sözcüller: Gebe, Progesteron düzeyi, Tavşan, Sugammadex, Rocuronium
INTRODUCTION

In addition to events that occur in pregnancy like appendicitis, ovarian diseases and trauma [1], surgical operations are performed in intensive care units [2] for various indications. These surgical operations are usually performed under general anesthesia.

Rocuronium is one of the steroid-type non-depolarizing neuromuscular blocker muscle relaxants with a short duration of action [3]. Sugammadex, on the other hand, is a new generation reversal agent used to terminate the effect of neuromuscular blockers (vercuronium and rocuronium). The mechanism of action is that it creates a complex with the circulating muscle relaxant at the nerve juncture to terminate its effect [4]. The sugammadex-rocuronium interaction reduces the amount of free rocuronium in plasma, thus altering rocuronium in plasma by significantly reducing the levels of rocuronium at the neuromuscular juncture. This ensures that muscle activity resumes because it quickly releases the acetylcholine receptors [5]. However, there have not been enough studies conducted on rocuronium and/or sugammadex's effect on pregnancy [6].

Progesterone prevents the endometrium from breaking down in pregnancy and suppresses the stimulating effect of estrogen on uterine contractions to ensure that pregnancy continues [7]. The decline in P4 levels causes the relaxing effect on the uterus to disappear, thus terminating pregnancy [8,9].

The aim of this study was to demonstrate how the use of rocuronium, an effective neuromuscular blocker, and its antagonist sugammadex in pregnant rabbits given general anesthesia affects P4 levels at different times in pregnancy.

MATERIAL and METHODS

Ethics Approval

This study was conducted after obtaining approval from the Kafkas University Local Experimental Animals Ethics Committee (Approval no: KAÜ-HADYEK: 2016-096).

Animals

This study used twenty-one pregnant New Zealand rabbits with an average weight of 2.7-3.3 kg obtained from the Firat University Experimental Research Center. The veterinarian was delivered with a referral report. The rabbits were transported in a single cage and vented for 10 min per hour by transit minibus. Rabbits that were bred on the same day were included in the study, and the day of mating was considered day 0. Rabbits were housed in individual cages where they received 12 h of sunlight and 12 h of darkness. Rabbits were fed ad-libitum with a daily average of 250 g of pellet feed and 100 mL of water.

Ultrasonography Examination

Pregnancy was confirmed with an ultrasound examination immediately before anesthesia was administered 21 days after mating. A maximum of four at least one fetus was detected in the ultrasound examination of rabbits. The rabbits were shaved up to their rib cage and a trans-abdominal examination was performed on the abdominal region of the rabbits as they were held on their back. The ultrasound device used for the ultrasonography procedure was a B Mode real-time device with a 7.5 mHz linear probe (DRAMINSKI iScan, Poland).

Anesthesia Procedure

All of the rabbits were sedated with 0.5 mg/kg midazolam (Zolamid®, 5 mg/5 mL, Defarma Pharmaceutical Industry and Trade Company., Turkey). Propofol Intravenous (IV) (Propofol-Lipuro 10 mg/mL 20 mL, Braun Pharmaceuticals, Germany) was administered slowly at a dose of 6 mg/kg. Anesthesia maintenance was started in the first five min with a mixture of sevoflurane (AbbVie Pharmaceutical Industry and Trade Company, Turkey) 3-4% + oxygen 4 L/min. Because the surgical procedure would not be performed five min later, inhalation anesthesia was reduced and continued with a mixture of sevoflurane 2% + oxygen 4 L/min. All the rabbits were provided with respiration support via ventilation mask. Spontaneous breathing was blocked in the group, which had been given rocuronium, due to the medication. Breathing was administered in head extension via mask ventilation. No respiratory arrest or a complication occurred in any of the groups.

Sevoflurane was cut off sixty min later. All of the rabbits were monitored prior to anesthesia 0 and at 5, 30, 60 and 90 min after the onset of anesthesia to measure physiological parameters. Furthermore, venous blood was collected from the marginal ear vein at each of these time intervals.

Experimental Groups

The anesthesia procedures were performed on pregnant rabbits 21 day after mating.

Control Group: The rabbits in the control (C: n=7) group were only given general anesthesia.

Group I: The rabbits in group 1 (GI: n=7) were given rocuronium (Esmeron®, 50 mg/5 mL, Merck Sharpoo Dohme (MSD) Pharmaceuticals Ltd., Germany) at an intravenous dose of 0.6 mg/kg.

Group II: Unlike the rabbits in Group I, the rabbits in this group were given sugammadex (Bridion® 200 mg/2 mL, Merck Sharpoo Dohme (MSD) Pharmaceuticals Ltd., The Netherlands) at the 60th min mark via IV.

Measurements

Pulse (P), systolic (SAP), diastolic (DAP) and median artery pressure (MAP), oxygen saturation (SPO2), respiration (R)
A P value of <0.05 was considered statistically significant. Friedman’s test. The results were analyzed as mean ± S.D. differences on day 21 of pregnancy were established with examined with the Mann Whitney U test. The internal Shapiro Wilk normality test. Paired comparisons were achieved using fewer less anesthetic. If the neuromuscular junction does not adequately recover after anesthesia, post-operative pulmonary complications may develop, and mortality rates can rise [10]. Therefore, sugammadex, a neuromuscular blocker antagonist has been used in recent years to eliminate residual muscle relaxation [12].

**RESULTS**

Signs of sedation were observed in all of the individuals given midazolam, and the animals were then given propofol for induction, followed by problem-free general anesthesia for 60 min with sevoflurane.

On day 21 after mating, there was no statistically significant difference among the groups in terms of (P/min, SAP/mmHg, DAP/mmHg, MAP/mmHg) and (sPO2/%) values (P>0.05).

On day 21 of pregnancy, respiratory values of control group at the 5th, 30th and 60th min were found out to be significantly lower than those of Group II (P<0.05). Respiratory values of both the Control Group and Group II were also significantly lower at the 90th min than they were at the 5th, 30th and 60th min (P<0.05) (Table 1).

There was a statistically significantly difference in body temperature between the control group and Group I on day 21 of pregnancy (P<0.05). There was a statistically significant difference within the control group between min 5 and 60 (P<0.05). There was a statistically significant difference within Group I between baseline and 60 min and 0 and 5 min (P<0.05) (Table 1).

In Group I, it was observed that TOF went down to zero approximately 45.4 sec after administering rocuronium. In Group II, TOF went down to zero 43.2 sec after the same process and it took TOF an average of 73.6 sec following sugammadex administration to reach 100%.

P4 levels (ng/mL) at 60 and 90 min in Group I were significantly higher than in Group II (P<0.05). Although there was a statistically significant difference in Group I between 0 and 5 min with 30 and 60 min (P<0.05), in Group II, P4 levels at 30 min were significantly higher than at 60 and 90 min (P<0.05) (Table 2). None of the groups in the study experienced abortus, early birth or still birth. The births took place when due. Each rabbit delivered 4 to 6 babies.

**DISCUSSION**

It is a known fact that the rate of non-obstetrical operations in pregnancy is quite high [10]. Anaesthesia means, loss of sensation in the entire body or any part of the body [11]. Modern anesthesia methods are used in many of the stated operations. One of the most important aspects of modern anesthesia methods is the use of neuromuscular blocker agents. These agents create better intubation conditions by preventing voluntary and/or reflexive muscle movements. As a result, muscle relaxation sufficient for the operation is achieved using fewer less anesthetic. If the neuromuscular junction does not adequately recover after anesthesia, post-operative pulmonary complications may develop, and mortality rates can rise [10]. Therefore, sugammadex, a neuromuscular blocker antagonist has been used in recent years to eliminate residual muscle relaxation [12].

It has been established that the TOF ratio, which is a neuromuscular recovery parameter in the adductor pollicis muscle, must be higher than 0.90 to lower the risk of aspiration after intubation and to avoid postoperative atelectasis and pneumonia [13,14]. However, Eriksson et al. [14] demonstrated that upper esophageal sphincter tonus decreased noticeably in 14 conscious patients when the TOF ratio was less than 0.90, and that muscle coordination also decreased when the TOF ratio was less than 0.60. Studies with humans found that it took 70 sec for TOF to reach 80% after administering sugammadex 2 mg/kg to reverse neuromuscular block induced with rocuronium (0.5 mg/kg) [15]. A study conducted on rabbits found that it took an average of 123 sec for TOF to reach 90% after administration of sugammadex 2 mg/kg to reverse neuromuscular block induced with rocuronium (0.6 mg/kg) [16]. It has been determined that TOF was 100% after approximately 73.6 sec following the administration of...
sugammadex (2 mg/kg), which was used in the process of reversing the neuromuscular block induced with rocuronium in Group II. It is considered that the fact that it takes less time for TOF to get to 100% might correlate with the dose of rocuronium.

All animal studies have shown that sugammadex effectively antagonizes the neuromuscular blockage caused by rocuronium without having any significant effect on arterial blood pressure or heart rate [5,17-19]. One study found that systolic, diastolic and mean arterial pressures and heart

### Table 1. Values for parameters measured on day 21 after mating

<table>
<thead>
<tr>
<th>Groups</th>
<th>Values Measured</th>
<th>0th min. Mean±S.D.</th>
<th>5th min. Mean±S.D.</th>
<th>30th min. Mean±S.D.</th>
<th>60th min. Mean±S.D.</th>
<th>90th min. Mean±S.D.</th>
<th>Statistical Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>P (min)</td>
<td>242.57±39.93</td>
<td>228±38.39</td>
<td>254±23.58</td>
<td>232.86±26.67</td>
<td>256.29±16.72</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>SAP (mmHg)</td>
<td>157.57±45.85</td>
<td>145.86±36.07</td>
<td>179.57±46.72</td>
<td>129.7±8.5</td>
<td>152.57±30.66</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>DAP (mmHg)</td>
<td>130.86±48.21</td>
<td>143.71±51.57</td>
<td>107.86±41.25</td>
<td>84.57±12.58</td>
<td>124±36.31</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>MAP (mmHg)</td>
<td>137.57±45.37</td>
<td>154.57±47.73</td>
<td>126.86±39.77</td>
<td>99.71±12.49</td>
<td>132.71±30.96</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>sPO2 (%)</td>
<td>93.43±2.99</td>
<td>94.14±3.8</td>
<td>95.71±3.25</td>
<td>96.14±2.48</td>
<td>94.86±3.13</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>T (°C)</td>
<td>38.13±0.48</td>
<td>38.36±0.39</td>
<td>37.83±0.56</td>
<td>37.66±0.39</td>
<td>37.81±0.38</td>
<td>a:b:0.000</td>
</tr>
<tr>
<td>Group I</td>
<td>P (min)</td>
<td>258.29±31.9</td>
<td>248±25.92</td>
<td>248.57±20.15</td>
<td>241.86±8.51</td>
<td>237.57±26.47</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>SAP (mmHg)</td>
<td>144.86±23.86</td>
<td>154±36.1</td>
<td>152.14±25.63</td>
<td>88.71±34.09</td>
<td>124.43±29.63</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>DAP (mmHg)</td>
<td>120.29±29.49</td>
<td>112.86±37.48</td>
<td>115.43±48.17</td>
<td>88.71±34.09</td>
<td>124.43±29.63</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>MAP (mmHg)</td>
<td>128.57±22.11</td>
<td>125.7±30.22</td>
<td>130.29±46.95</td>
<td>103.14±32.06</td>
<td>133±26.2</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>sPO2 (%)</td>
<td>95.71±2.29</td>
<td>96±2.94</td>
<td>95.71±2.69</td>
<td>95.86±2.61</td>
<td>94.43±2.76</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>T (°C)</td>
<td>38.87±0.39</td>
<td>38.66±0.99</td>
<td>38.36±0.57</td>
<td>37.94±0.53</td>
<td>38.01±0.6</td>
<td>a:b:0.002</td>
</tr>
<tr>
<td>Group II</td>
<td>P (min)</td>
<td>250.86±16.07</td>
<td>264±31.3</td>
<td>239.71±17.17</td>
<td>230.71±11.57</td>
<td>261.86±36.29</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>SAP (mmHg)</td>
<td>123.43±19.05</td>
<td>147.43±22.31</td>
<td>155.71±31.53</td>
<td>131.43±22.91</td>
<td>161±35.57</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>DAP (mmHg)</td>
<td>100.86±16.47</td>
<td>110.71±43.43</td>
<td>105.86±25.96</td>
<td>94.86±31</td>
<td>134.14±36.24</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>MAP (mmHg)</td>
<td>112.57±18.95</td>
<td>129±34.18</td>
<td>105.29±47.11</td>
<td>105.29±28.34</td>
<td>138.86±35.8</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>sPO2 (%)</td>
<td>95.71±1.63</td>
<td>94.71±1.5</td>
<td>94.14±2.73</td>
<td>95.86±2.67</td>
<td>93.14±2.48</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>T (°C)</td>
<td>38.56±0.49</td>
<td>38.63±0.83</td>
<td>38.5±0.76</td>
<td>38.3±0.43</td>
<td>38±0.43</td>
<td>ns</td>
</tr>
</tbody>
</table>

**Table 2. Distribution of progesterone levels on day 21 after mating**

<table>
<thead>
<tr>
<th>Groups</th>
<th>0th min. Mean±S.D. (ng/mL)</th>
<th>5th min. Mean±S.D. (ng/mL)</th>
<th>30th min. Mean±S.D. (ng/mL)</th>
<th>60th min. Mean±S.D. (ng/mL)</th>
<th>90th min. Mean±S.D. (ng/mL)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.77±0.17</td>
<td>5.72±0.36</td>
<td>5.76±0.38</td>
<td>5.74±0.33</td>
<td>5.79±0.23</td>
<td>ns</td>
</tr>
<tr>
<td>Group I</td>
<td>5.51±0.14</td>
<td>5.67±0.2</td>
<td>6.71±0.32</td>
<td>6.67±0.33</td>
<td>6.64±0.22</td>
<td>x:y:0.001</td>
</tr>
<tr>
<td>Group II</td>
<td>5.66±0.37</td>
<td>5.61±0.19</td>
<td>6.59±0.29</td>
<td>4.62±0.23</td>
<td>4.59±0.2</td>
<td>x:y:0.001</td>
</tr>
</tbody>
</table>

**P:** Refers to comparisons between groups; **Pb:** Refers to comparisons within groups. Pulse (P), Systolic Arterial Pressure (SAP), Diastolic Arterial Pressure (DAP), Median Arterial Pressure (MAP), Saturation (SPO2), Respiratory Rate (R), Body Temperature/Centigrade degree (T/°C); **a,b,c** Refers to the statistical difference on each row; **ns:** non significant.

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<table>
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<td>5.76±0.38</td>
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<td>5.79±0.23</td>
<td>ns</td>
</tr>
<tr>
<td>Group I</td>
<td>5.51±0.14</td>
<td>5.67±0.2</td>
<td>6.71±0.32</td>
<td>6.67±0.33</td>
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<td>x:y:0.001</td>
</tr>
<tr>
<td>Group II</td>
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<td>5.61±0.19</td>
<td>6.59±0.29</td>
<td>4.62±0.23</td>
<td>4.59±0.2</td>
<td>x:y:0.001</td>
</tr>
</tbody>
</table>

**P**a,b,c Refers to the statistical difference in each column (P=0.001); **x,y** Refers to the statistical difference on each row (P=0.001); **ns:** non significant.
rate were lower in the sugammadex group than in the neostigmine group, and the difference was statistically significant [20]. Our study did not find significant differences within groups with regard to pulse and systolic or diastolic arterial pressures on day 21 after mating. This suggests that rocuronium and sugammadex do not affect these parameters. The differences observed in parameters such as median arterial pressure, sPO₂, respiration values and body temperature are thought to be associated with changes expected in anesthesia.

It has been shown that the use of sugammadex (4 mg/kg) for contraceptive purposes in women reduces the amount of P₄ by 34% and reduces its efficacy [21]. Progesterone is very important to pregnancy and continuation of pregnancy [8,9]. It ensures that desidual tissues develop, and it facilitates implantation following fertilization. Furthermore, it stimulates uterine growth and prevents the decline in P₄ levels after using sugammadex, but that administration of sugammadex only lowered serum P₄ levels in rats numerically. However, this decline was not statistically significant. The fact that pregnancy continued to develop normally without abortus or stillbirth suggests that sugammadex can be used safely on day 12 of pregnancy. A different study, however, reported that administration of sugammadex had no effect on serum P₄ levels [12]. Because endogenous steroids similar to P₄, like rocuronium, do not contain the ammonium compounds found in steroidal neuromuscular blockers, sugammadex reportedly demonstrates low affinity for these steroids [30,31]. Furthermore, steroid hormones contribute to this low affinity because they bind strongly to special transporter proteins [12,31]. In our study, on day 21 of pregnancy, there was a significant decline in serum P₄ levels at 60 and 90 min in Group II compared to levels prior to sugammadex administration. Gunduz Gul et al. [12] found that there was a temporary decline in P₄ levels after using sugammadex, but that levels rose again when measured 4 h later. None of the rabbits in our study experienced abortus or stillbirth during pregnancy in spite of the decline in P₄ levels. This suggests that the P₄ levels rose again some h later.

In conclusion, our study did not find that the use of the neuromuscular blocker rocuronium and its antagonist sugammadex in rabbits on day 21 of pregnancy had a negative effect on serum P₄ levels during pregnancy.

REFERENCES


