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### Original Article

## Evaluation of Possible Effects of Hyoscine in Xylazine-Induced Fetal Death in Pregnant Rats

Mona Zohani-Ghayeni<sup>1</sup>, Siamak Kazemi-Darabadi<sup>1\*</sup>, Abolfazl Hajibemani<sup>1</sup>, Ghasem Akbari<sup>2</sup>

<sup>1</sup> Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran. <sup>2</sup> Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.

ARTICLE INFO	ABSTRACT
<p><i>Article History:</i></p> <p>Received 3 January 2023            Revised 17 January 2023            Accepted 6 March 2023            Online 6 March 2023</p>	<p>Although xylazine is widely used in domestic animals as a sedative, analgesic, and muscle relaxant, its side effects on the uterus prevent its utilization in pregnant animals or in embryo transfer. Although the effects of xylazine on increasing uterine contractions have been confirmed, no reliable report of fetal death due to xylazine administration has been published. Hyoscine is an anticholinergic medication that has antimuscarinic and antispasmodic effects in the uterine tissue of pregnant cattle during in vitro studies, therefore, we investigated if administration of xylazine in the last third of pregnancy could increase fetal death and if hyoscine could prevent its adverse effects. Twenty adult female rats, after mating with four adult male rats and confirming pregnancy, were randomly divided into two equal control and treatment groups. On the 18<sup>th</sup> day of pregnancy, the number of fetuses per rat was determined using ultrasonography. Rats in the treatment group received hyoscine (1 mg/kg, intraperitoneally) for 3 days. Subsequently, all rats were administered xylazine (10 mg/kg, intraperitoneally) for 3 days. On the 21<sup>st</sup> day of pregnancy, the number of living and dead fetuses was counted after laparotomy. Also, the weight and dimensions of the fetuses were measured. The results showed that although more fetuses lost their lives in the treatment group compared to the control group, the statistical difference in the percentage of fetal mortality in the two groups was not significant (<math>p &gt; 0.05</math>). In addition, the comparison of the mean weight, body length, and body width of living and dead fetuses in both groups showed that there was no statistically significant difference between these groups (<math>p &gt; 0.05</math>). It could be concluded that maternal xylazine intake in rats could cause about 18-25% of fetal mortality. However, the use of hyoscine to prevent fetal death induced by xylazine is not recommended.</p>
<p><i>Keywords:</i></p> <p>Xylazine            Fetal death            Rat            Hyoscine</p>	

### Introduction

Alpha-2 adrenoceptor agonists, including xylazine, are clinically important medications used in animals.

These are the most reliable sedatives licensed for use in livestock. Their other beneficial effects include creating analgesia, muscle relaxation, reducing the required dose of anesthesia induction and maintenance drugs,

\*Correspondence to: Siamak Kazemi-Darabadi, Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran. Email: [s.kazemi@tabrizu.ac.ir](mailto:s.kazemi@tabrizu.ac.ir)

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and weakening the stress response to pain and surgery. These effects are mainly due to interactions with  $\alpha$ -2 adrenoceptors in the central and peripheral nervous system. Despite the widespread use of these drugs, there are many unwanted clinical effects that must be overcome. Bradycardia, ileus and prolonged sedation leading to ataxia are among their most common clinical side effects. Other unwanted effects are vasoconstriction followed by increased blood pressure, decreased bowel movements, hyperglycemia, increased urination, increased uterine tonus, sweating, muscle tremors, increased salivation and tachypnea.<sup>1</sup>

Xylazine is widely used as a sedative, analgesic and muscle relaxant in domestic animals, and its side effects on the uterus prevent its use in pregnant animals or in cases where it is important not to change the uterine tonus, for instance in embryo transfer. The administration of xylazine in mammals increases the number and intensity of uterine contractions. This effect is mediated through selective stimulation of postsynaptic  $\alpha$ -2 adrenergic receptors in the uterus. Smooth muscle contraction induced by activation of these receptors is mediated by inhibition of adenylate cyclase activity. This leads to a decrease in the synthesis of cAMP, which increases the availability of cytoplasmic calcium by opening calcium channels.<sup>2</sup> After examining bovine uterine tissue *in vitro* at different times of pregnancy using a tissue bath, researchers found that xylazine increased the intensity of contractions in almost all stages of pregnancy.<sup>3</sup> A previous study showed that xylazine increased uterine tonus and decreased uterine arterial blood flow in pregnant goats by stimulating postsynaptic  $\alpha$ -2 receptors in the uterus,<sup>4</sup> therefore, despite the fact that the effects of xylazine in increasing uterine contractions have been confirmed, so far no reliable report has been published on the rate of fetal death due to the administration of xylazine to the dam.

Tocolytic drugs prevent myometrial smooth muscle contractions. These medications include  $\beta$ -adrenergic agonists, calcium channel antagonists, oxytocin antagonists, non-steroidal anti-inflammatory drugs (NSAIDs) and magnesium sulfate. However, some of these medications are not paid much attention due to severe side effects (e.g., NSAIDs) or lack of clear positive effects (e.g., magnesium sulfate). Beta-agonists are also responsible for frequent adverse effects in the mother such as tachycardia, dyspnea, and anxiety and even rare fatal complications such as pulmonary edema, and today their use is more limited.<sup>5</sup> Among  $\beta$ -2

adrenoceptors, terbutaline, ritodrine, salbutamol, clenbuterol, and fenoterol have been used as potential inhibitors of uterine contractions that prevent both prostaglandin- and oxytocin-induced contractions in mid-pregnancy or during labor. The administration of clenbuterol and nifedipine (a calcium channel antagonist) 5 minutes before the administration of xylazine in pregnant goats had uterine relaxing effects and prevented the increase of uterine contractility due to xylazine.<sup>2</sup> In another study, the addition of lidocaine to xylazine caused the intensity of contractions of bovine uterine tissue in laboratory conditions to be lower than when xylazine was used alone.<sup>3</sup> Jang *et al.* concluded that *Clostridium botulinum* toxin A reduced the duration and intensity of uterine contractions caused by mifepristone.<sup>6</sup>

Hyoscine (also known as scopolamine) is an anticholinergic drug that is well absorbed after oral or intravenous administration. Studies have shown that this medication is not teratogenic. The duration of its effect is about 40 minutes after intramuscular injection, however, its metabolites remain in the body for several hours. In a report, the administration of hyoscine to three women in whom attempts at *in vitro* fertilization (IVF) were unsuccessful due to uterine motility led to their conception.<sup>7</sup> This antimuscarinic and spasmolytic substance has reduced the tonic effect of xylazine in the uterine tissue of pregnant cows in an *in vitro* study,<sup>8</sup> therefore, in the current study, the possible effects of hyoscine in reducing fetal mortality caused by myometrial contraction due to xylazine were investigated. Our hypothesis was that the administration of xylazine in the last third of pregnancy could cause fetal death by increasing uterine contractions, and hyoscine might prevent the adverse effect of xylazine by reducing uterine contractions.

## Materials and Methods

### Animals

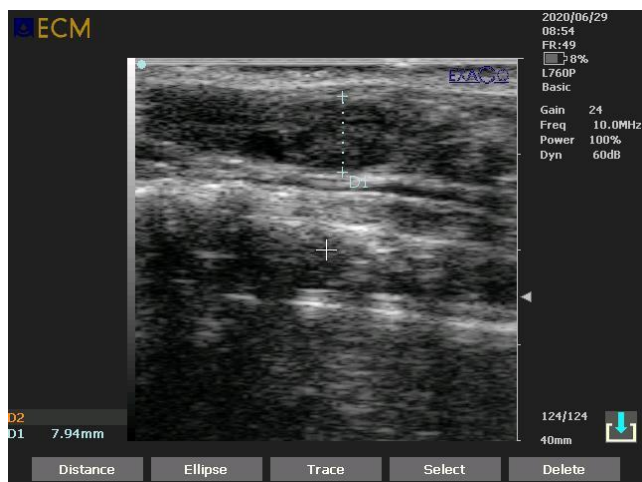
In this research, 20 adults female Wistar rats of 2 to 3 months old with an average weight of  $210 \pm 28$  g and 4 adult male rats were used. All procedures were in accordance with the WMA Statement on Animal Use in Biomedical Research (reaffirmed by the 203<sup>rd</sup> WMA Council Session, Buenos Aires, Argentina, April 2016). Also, the research was approved by the Academic/Regional Committee of Ethics in Biomedical Research of University of Tabriz (IR.TABRIZU.REC.1399.047). Every five female rats

were kept in one cage. Before the beginning of the research, rats were given one week to get acclimatized to their environment. The light/dark cycle had 12-hour intervals, and the rats had free access to commercial pellets, corn, and tap water.

### *Mating and Pregnancy Detection*

For mating, every five female rats were housed overnight with one male rat in the same cage. The observation of mucous plaque in the vulva in the early morning after mating was considered a confirmation of successful mating. Where the mating was successful, the female rat was transferred to a new cage and the other rats were allowed to stay with the male for another night. This work continued until the mating of all female rats was confirmed. The gestational age of rats was considered from the confirmed day of mating. It should be mentioned that pregnancy lasts 22 days in rats.<sup>9</sup>

It has been reported that the correct diagnosis of pregnancy in rats by ultrasonography is 100% from the 10th day of pregnancy onwards,<sup>10</sup> therefore, in the present study, in the middle of the last third of pregnancy, i.e., on the 18th day after mating, pregnancy was diagnosed using an ultrasound device and the number of fetuses of each rat was counted. For this purpose, restraining was done by hand and the hair of the abdominal area was clipped, then ultrasonic gel (Polygel ultrasonic gel, Palizteb Co., Iran) was used and abdominal ultrasound was performed with a suitable transducer (Figure 1).



**Figure 1.** Examining the pregnancy status and counting the number of fetuses in female rats on the 18th day after mating using an ultrasound machine. The dashed line shows the dorsal-ventral distance of the fetus. There are three fetuses in the picture.

### *Grouping and Examining the Effects of Drugs*

After the diagnosis of pregnancy, the female animals were randomly divided into two equal groups including the control and the treatment groups. Rats in the treatment group received 0.2% hyoscine (Hyoscine Chimidarou, 20 mg/ml Amp, Iran), at a dose of 1 mg/kg,<sup>11</sup> and rats in the control group were given the same volume of normal saline intraperitoneally. In a study, intraperitoneal administration of 3 mg/kg scopolamine to Wistar and Sprague-Dawley rats caused convulsive movements,<sup>12</sup> therefore, a higher dose of scopolamine was not used in this study. After that, all rats in both groups were administered xylazine hydrochloride (2%, Alfasan, Woerden, Holland), at a dose of 10 mg/kg intraperitoneally. Administration of hyoscine and xylazine continued for three days, i.e., days 18, 19, and 20 of pregnancy. On the 21st day of pregnancy, laparotomy was performed under general anesthesia with an intraperitoneal injection of ketamine hydrochloride (10%, Alfasan, Woerden, Holland), and xylazine (100 mg/kg and 20 mg/kg, respectively), and after performing a hysterectomy in both uterine horns, the number of live and dead fetuses were counted. Also, after removing the fetuses and separating the placenta, the weight and dimensions of the fetuses were measured using a digital scale (EK6100i precision digital scale, A&D, Tokyo, Japan), and digital vernier caliper (Guanglu Digital Caliper, China). After delivery, the rats were euthanized under general anesthesia by cervical vertebrae dislocation according to AVMA Guidelines for the Euthanasia of Animals.

### *Statistical Analysis*

Statistical analysis was done using Minitab software (Minitab 16.2.0 Statistical Software, Minitab Inc., State College, PA, USA). The parameters of weight, body length and width of fetuses and their mortality rate were analyzed by student's t-test statistical method. The results of weight and body dimensions were presented as mean  $\pm$  standard error of the mean (SEM) and the results of fetal mortality were presented as percentages.  $p < 0.05$  was considered a statistically significant difference.

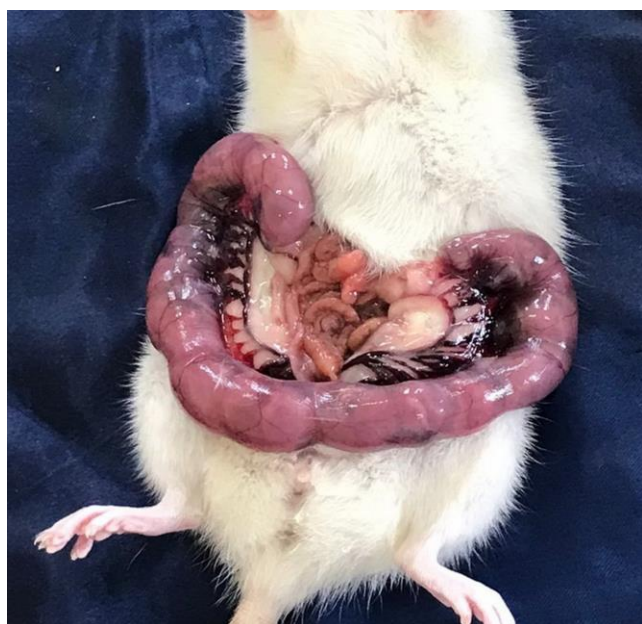
### *Results*

There was no difference between the total number of fetuses counted using ultrasound on the 18th day and the number of fetuses observed during

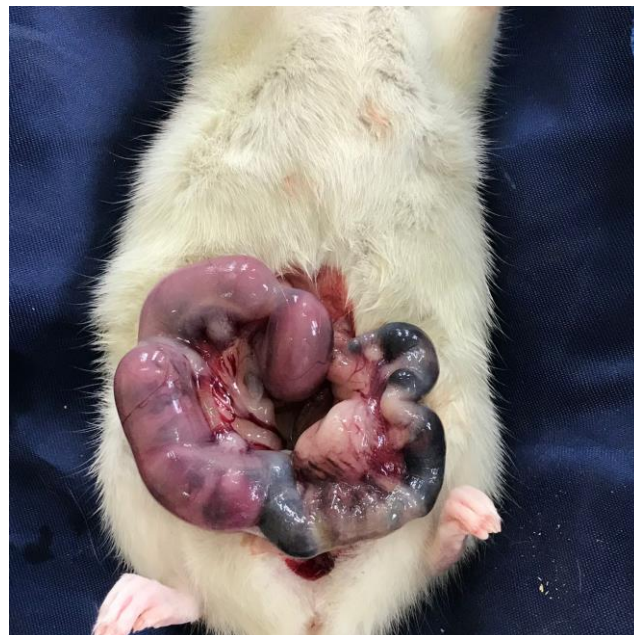
laparohysterectomy on the 21st day, therefore, the diagnosis of pregnancy in rats with the ultrasound device was successful. Although more fetuses died in the treatment group (16 fetuses out of 58 fetuses counted) compared to the control group (9 fetuses out of 62 fetuses counted), the statistical difference in the percentage of fetuses lost in the two groups was not significant ( $p > 0.05$ ) (Figures 2 and 3). The total number of live fetuses was 42 out of 58 fetuses in the treatment group and 53 out of 62 fetuses in the control group, and the percentage of live fetuses in the two groups was not statistically significant ( $p > 0.05$ ). Table 1 shows the percentage of live and dead fetuses in each group.

In addition, the comparison of the average weight, body length, and body width of live and dead fetuses that had preserved their anatomical structure in both groups also showed that there was no statistically significant difference between these groups ( $p > 0.05$ ). The average body weight  $\pm$  SEM of the fetuses was  $1.86 \pm 0.09$  g in the control group and  $1.88 \pm 0.14$  g in the treatment group. The average body length  $\pm$  SEM of fetuses was  $26.14 \pm 0.44$  mm in the control group and  $25.74 \pm 0.77$  mm in the treatment group. Finally, the average body width  $\pm$  SEM of the fetuses was  $10.76 \pm 0.16$  mm in the control group and  $10.80 \pm 0.27$  mm in the treatment group, as shown in Table 2.

It should be noted that among the live fetuses in the treatment group, 5 fetuses were abnormal in appearance and hyperemic. In addition, among the



**Figure 2.** The condition of the rat fetuses of the control group on the 21st day of pregnancy.



**Figure 3.** The condition of the rat fetuses in the treatment group on the 21st day of pregnancy.

**Table 1.** The mean of live and dead fetuses' percentage in rats of control (receiver of xylazine) and treatment (receiver of hyoscine and xylazine) groups.

	Control	Treatment	<i>p</i> -value
<b>Live fetuses (%)</b>	81.5	75.1	0.659
<b>Dead Fetuses (%)</b>	18.5	24.9	0.658

**Table 2.** Comparison of weight, body length, and body width of live and dead fetuses that had preserved their anatomical structure (mean  $\pm$  SEM) in rats of control (receiver of xylazine) and treatment (receiver of hyoscine and xylazine) groups.

	Control	Treatment	<i>p</i> -value
<b>Weight (g)</b>	$1.86 \pm 0.09$	$1.88 \pm 0.14$	0.886
<b>Length (mm)</b>	$26.14 \pm 0.44$	$25.74 \pm 0.77$	0.655
<b>Width (mm)</b>	$10.76 \pm 0.16$	$10.80 \pm 0.27$	0.885

dead fetuses in the treatment group, 13 fetuses were absorbed and deformed, and this number was 1 in the control group. Due to the fact that the body anatomy was not detectable in the absorbed fetuses and compared to the living or dead fetuses that had preserved their anatomical structure, they had a very small volume. The measurements of their body dimensions and weight were not included in the statistical analysis. Therefore, it could have been said that if these absorbed fetuses had been considered in the final results, the average weight and dimensions of the fetuses in the treatment group would have been much lower than the control group (Figures 4 and 5).



**Figure 4.** Rat fetuses of the control group after Cesarean section on the 21st day of pregnancy. Most fetuses are alive.



**Figure 5.** Rat fetuses of the treatment group after Cesarean section on the 21st day of pregnancy. Some fetuses are absorbed.

## Discussion

Although Stickrod in 1979, during a study on rats, stated that xylazine at a dose of 10 mg/kg in combination with ketamine provided satisfactory anesthesia without adverse effects on pregnancy or fetuses,<sup>13</sup> the possibility of the role of xylazine in abortion, including in cows has been reported for more than forty years,<sup>14</sup> and since then, it has always been confirmed that the intrauterine pressure and uterine tonus are increased due to the administration of this drug in late-pregnant cows.<sup>15</sup> Uterine contraction mediates by several neurotransmitters including catecholamines and acetylcholine. These neurotransmitters enhance uterine contraction when they bind to alpha-adrenergic and muscarinic

receptors.<sup>16,17,3</sup> The expression of these binding sites can be influenced by hormonal status, therefore, it changes during all stages of reproduction including pregnancy.<sup>2,18</sup> Xylazine has systemic and local negative effects on the uterus and its use during labor or Cesarean section can cause critical fetal hypoxia.<sup>19</sup> From this point of view, the use of xylazine in pregnant cow surgery is dangerous, because there is a risk of reabsorption of the fetus, abortion or premature delivery.<sup>3</sup> Eesa stated in 2007 that xylazine did not affect buffaloes in the last stages of pregnancy, however, caused abortion in pregnant cows by 3.65%.<sup>20</sup> In a study conducted by Blanchard *et al.* in 2010, injection of a combination of acepromazine, xylazine, and butorphanol immediately before mating in Thoroughbred mares did not have significant negative effects.<sup>21</sup>

Central  $\alpha$ -2 adrenergic receptors are involved in many functions including pain,<sup>22</sup> locomotion,<sup>23</sup> cardiovascular function,<sup>24</sup> and stress response.<sup>25,26</sup> Xylazine, which is an  $\alpha$ -2 adrenergic receptor agonist, has an oxytocin-like effect in the uterus of ruminants.<sup>27</sup> Its administration in non-pregnant cows in the ovulation stage has the same effect on increasing intrauterine pressure as oxytocin and the greatest increase in intrauterine pressure occurs in proestrus.<sup>28</sup> Data from Pirnik *et al.*'s study in 2005 showed that peripheral administration of xylazine was a strong stimulant for oxytocin.<sup>29</sup> Xylazine-induced uterine contraction in cows is directly mediated by  $\alpha$ -2 adrenoreceptors in the myometrium.<sup>30</sup> In mares, xylazine increases uterine myoelectric activity,<sup>31</sup> and, where administered during estrus, causes a tetanic increase in intrauterine pressure.<sup>32</sup> It has been suggested that the inotropic effect of xylazine is entirely dependent on extracellular calcium ions and that xylazine induces its uterine effects by opening calcium channels.<sup>33</sup>

In mice, the use of the combination of ketamine and xylazine on days 0, 4, 12, and 15 of pregnancy caused a significant decrease in fetal growth. Of course, the same researchers attributed this effect to xylazine and stated that ketamine had no effect on the growth of embryos.<sup>34</sup> The results of a study conducted by Hodgson *et al.* in 2002 showed that the administration of xylazine significantly reduced arterial blood flow and oxygen pressure in the uterus of cows that were in the last stages of pregnancy, and as a result, oxygen intake by the fetus was lowered by 59%. A decrease in oxygen pressure can also be due to a decrease in hematocrit.

Also, the vascular resistance of the uterine artery increases by 156%, without increasing blood pressure, which leads to a decrease in blood pumping.<sup>19</sup> In another study, the number of maternal pulses in the umbilical arteries was decreased by 17% and the volume of blood flow in the umbilical arteries was decreased after administration of xylazine in the tail vein. Also, the number of fetal pulses was decreased by 6%. These researchers concluded that xylazine should be given to cows in the final stages of pregnancy or parturition only after extreme caution due to the risk of transient fetal hypoxia.<sup>35</sup> No group was considered in the present study to evaluate the characteristics of fetuses in natural conditions and to compare it with groups receiving drugs, however, the loss of 18.5% of fetuses in rats receiving xylazine showed that this sedative drug was not very safe for use in pregnant rats.

It was concluded in the study of Toma *et al.* in 2013 that hyoscine did not have the ability to bind to the nicotinic receptors of the motor end plate and did not affect the physiological parameters of skeletal muscles.<sup>36</sup> Other pharmacological studies have shown that this substance has a high dependence on muscarinic receptors in smooth muscle cells including in the digestive system and its anticholinergic activity has a muscle relaxant and spasmolytic effect.<sup>37</sup> The mechanism of action of hyoscine is competitive antagonism with acetylcholine in muscarinic receptors.<sup>38</sup> The parasympathetic branch in the uterus regulates contractile activity, increases angiogenesis and stimulates secretions from cervical glands,<sup>39</sup> therefore, hyoscine can reduce these activities. In a study, intravenous administration of hyoscine immediately induced reticular atony in cattle that lasted only three to nine minutes.<sup>40</sup> Sciorsci *et al.* in 2018, after adding carbachol (an agonist of muscarinic receptors) and xylazine to the tissue strips prepared from the uterus of pregnant cows in different stages of pregnancy in the tissue bath, used hyoscine and concluded that this drug reduced the tonic effects of xylazine in the bovine uterus *in vitro* and therefore possibly reduced the risk of fetal resorption or premature delivery due to xylazine *in vivo*. They attributed the reduction of the tonic effect observed in xylazine-treated strips after the addition of hyoscine to the blockade of the parasympathetic system. Based on these findings, these researchers recommended the use of hyoscine in cases where xylazine is needed to administer pregnant cows.<sup>8</sup> Of course, the results of our study did not show a statistically significant difference

in the rate of fetal death due to xylazine with and without hyoscine administration. Even the number of absorbed and deformed embryos was higher in the group receiving hyoscine in our study, which shows that hyoscine not only does not prevent fetal death due to xylazine but can even have more negative effects on the fetus. Therefore, our results were not consistent with the suggestion of Sciorsci *et al.* To explain this difference, it should be said that although xylazine increases myometrial contractions and hyoscine also has spasmolytic effects in the uterus, probably the cause of the death of fetuses due to the administration of xylazine does not depend only on uterine contractions so that hyoscine can prevent it. Because, as mentioned above, xylazine has other effects, such as reducing blood flow in the uterus and causing hypoxia in fetuses.<sup>19,35</sup> Therefore, it cannot be expected that this factor will prevent fetal death in *in vivo* conditions simply because of the reduction of uterine contractions caused by hyoscine *in vitro*. In addition, the studies of Rizzo *et al.* in 2018 showed that the administration of hyoscine immediately after parturition in cows regulates the contractile activity of the uterus with a rebound effect. This means that hyoscine temporarily blocks organ contractions after parturition for its half-life (2 to 3 hours), however, after the pharmacological effect of hyoscine subsides after two to three hours, the uterus can start contracting more effectively.<sup>41</sup> Before that, Romański showed in 2010 that anticholinergic drugs, which are usually used as spasmolytic drugs, can produce a paradoxical stimulatory effect in the digestive system.<sup>42</sup> Therefore, the results of the present study can also be justified on the basis that the administration of hyoscine may have caused synergism of these contractions in the later stages, despite counteracting the contraction effects of xylazine in the uterus in the first few hours.

Additionally, hyoscine has some side effects that may play a role in the results obtained in this study. After the administration of doses higher than 8.4 µg/kg of hyoscine, transient tachycardia is observed and within 30 minutes it turns into long-term bradycardia. Also, hyoscine easily passes through the placenta, therefore, it is stated that its administration in pregnant women should be done only under close clinical supervision. Symptoms of poisoning with it in infants include tachycardia, fever and lethargy.<sup>38</sup>

According to the results of the present study, it could be stated that receiving xylazine by the dam in rats during heavy pregnancy could cause about 18-25%

of fetal losses, which might be due to fetus hypoxia in addition to increasing uterine contractions. However, although hyoscine has spasmolytic effects, it is not able to prevent the death of fetuses due to xylazine and even endangers the health of fetuses; Therefore, the use of this anticholinergic drug for the prevention of fetal death induced by xylazine is not recommended.

### Conflict of Interest

There is no conflict of interest to declare.

### References

1. Zeiler GE. A review of clinical approaches to antagonism of alpha2-adrenoreceptor agonists in the horse. *Equine Veterinary Education* 2015; 27(1): 48-54.
2. Perez R, Garcia M, Arias P, Gallardo M, Valenzuela S, Rudolph MI. Inhibition of xylazine induced uterine contractility by clenbuterol and nifedipine. *Research in Veterinary Science*. 1997; 63(1):73-76.
3. Piccinno M, Rizzo A, Mutinati M, D'Onghia G, Sciorsci RL. Lidocaine decreases the xylazine-evoked contractility in pregnant cows. *Research in Veterinary Science*. 2016; 107: 267-272.
4. Sakamoto H, Misumi K, Nakama M, AOKI Y. The effects of xylazine on intrauterine pressure, uterine blood flow, maternal and fetal cardiovascular and pulmonary function in pregnant goats. *Journal of Veterinary Medical Science*. 1996; 58(3): 211-217.
5. Tsatsaris V, Cabrol D, Carbonne B. Pharmacokinetics of tocolytic agents. *Clinical Pharmacokinetics*. 2004; 43(13): 833-844.
6. Jang HY, Lee KM, Park YS, Moon MJ, Hahn WB, Ahn EH. Clostridium botulinum toxin A significantly inhibits uterine contractions in rats undergoing mifepristone-induced preterm labor. *Korean Journal of Obstetrics and Gynecology*. 2012; 55(9): 629-635.
7. Kido A, Togashi K, Hatayama H, Nakayama T, Yamamoto A, Kataoka M, Tulandi T. Uterine peristalsis in women with repeated IVF failures: possible therapeutic effect of hyoscine bromide. *Journal of Obstetrics and Gynaecology Canada*. 2009; 31(8): 732-735.
8. Sciorsci RL, Piccinno M, Rizzo A. Scopolamine butylbromide decreases the xylazine-mediated contractility in bovine pregnant uteri. *Theriogenology*. 2018; 108: 348-353.
9. Kirberger RM, Bester EG, Schulman ML, van Rensburg IJ, Hartman MJ. Ultrasonographic evaluation of fetal development in the rat. *Theriogenology*. 2019; 125: 24-29.
10. Inaba T, Inoue A. Use of echography in rats for pregnancy diagnosis. *Japanese Journal of Veterinary Science*. 1985; 47(3): 523-525.
11. Gomar A, Hosseini A, Mirazi N, Gomar M. Effect of Alcoholic Salvia officinalis Extract on Analgesia Induced by Hyoscine in Adult Male Wistar Rats. *Journal of Ardabil University of Medical Sciences*. 15(3): 237-245.
12. Enginar N, Nurten A, Türkmen AZ, Çağla B. Scopolamine-induced convulsions in fasted animals after food intake: Sensitivity of C57BL/6J mice and Sprague-Dawley rats. *Epilepsy Research*. 2015; 112: 150-153.
13. Stickrod G. Ketamine/xylazine anesthesia in the pregnant rat. *Journal of the American Veterinary Medical Association*. 1979; 175(9): 952-953.
14. Knight AP. Xylazine. *Journal of American Veterinary Medical Association*. 1980; 176: 454-455
15. LeBlanc MM, Hubbell JA, Smith HC. The effects of xylazine hydrochloride on intrauterine pressure in the cow. *Theriogenology*. 1984; 21(5): 681-690.
16. Yasuda K, Sumi G, Kanamori C, Nakajima T, Tsuzuki T, Cho H, Nishigaki A, Okada H, Kanzaki H. Effects of ovarian hormone treatment on the gene expression of muscarinic acetylcholine receptors in the ovariectomized rat myometrium. *The Journal of Steroid Biochemistry and Molecular Biology*. 2014; 143: 81-89.
17. Taneike T, Kitazawa T, Funakura H, Asanuma K, Tsuji S, Yamada M, Teraoka H, Ohga A. Smooth muscle layer-specific variations in the autonomic innervation of bovine myometrium. *General Pharmacology: The Vascular System*. 1999; 32(1): 91-100.
18. Kitazawa T, Hirama R, Masunaga K, Nakamura T, Asakawa K, Cao J, Teraoka H, Unno T, Komori SI, Yamada M, Wess J. Muscarinic receptor subtypes involved in carbachol-induced contraction of mouse uterine smooth muscle. *Naunyn-Schmiedeberg's Archives of Pharmacology*. 2008; 377(4): 503-513.
19. Hodgson DS, Dunlop CI, Chapman PL, Smith JA. Cardiopulmonary effects of xylazine and acepromazine in pregnant cows in late gestation. *American Journal of Veterinary Research*. 2002; 63(12): 1695-1699.
20. J Eesa M. The clinical evaluation of the effect of xylazine in pregnant buffaloes and cattle. *Basrah Journal of Veterinary Research*. 2007; 6(2): 37-41.
21. Blanchard TL, Thompson JA, Brinsko SP, Varner DD, Love CC, Ramsey J, O'Meara A. Pregnancy rates in tranquilized maiden Thoroughbred mares. *Journal of Equine Veterinary Science*. 2010; 30(4): 187-190.
22. Holden JE, Schwartz EJ, Proudfit HK. Microinjection of morphine in the A7 catecholamine cell group produces opposing effects on nociception that are mediated by  $\alpha$ 1- and  $\alpha$ 2-adrenoceptors. *Neuroscience*. 1999; 91(3): 979-990.
23. Peričić D, Švob D, Jazvinščak Jembrek M, Mirković Kos K. The involvement of  $\alpha$ 2-adrenoceptors in the anticonvulsive effect of swim stress in mice. *Psychopharmacology*. 2001; 158(1): 87-93.
24. Paris A, Philipp M, Tonner PH, Steinfath M, Lohse M, Scholz J, Hein L. Activation of  $\alpha$ 2B-adrenoceptors mediates the cardiovascular effects of etomidate. *The Journal of the American Society of Anesthesiologists*. 2003; 99(4): 889-895.
25. Prieto M, Gómez FM, Teresa Giralt M. Effects of acute, repeated and chronic variable stress on in vivo tyrosine hydroxylase activity and on  $\alpha$ 2-adrenoceptor sensitivity in the rat brain. *Stress*. 2003; 6(4): 281-287.
26. Finn DP, Hudson AL, Kinoshita H, Coventry TL, Jessop DS, Nutt DJ, Harbuz MS. Imidazoline2 (I2) receptor- and  $\alpha$ 2-adrenoceptor-mediated modulation of hypothalamic-pituitary-adrenal axis activity in control and acute restraint stressed rats. *Journal of Psychopharmacology*. 2004; 18(1): 47-53.

27. Marnet PG, Garcia-Villar R, Laurentie MP, Toutain PL. In vivo pharmacological characterization of alpha adrenergic receptors in sheep myometrium and their physiological meaning. *Biology of Reproduction*. 1987; 37(1): 241-248.
28. Rodriguez-Martinez H, McKenna D, Weston PG, Gustafsson BK, Whitmore HL. Uterine motility in the cow during the estrous cycle. III. Effects of oxytocin, xylazine, and adrenoceptor blockers. *Theriogenology*. 1987; 27(2): 359-368.
29. Pirnik Z, Jezova D, Mikkelsen JD, Kiss A. Xylazine activates oxytocinergic but not vasopressinergic hypothalamic neurons under normal and hyperosmotic conditions in rats. *Neurochemistry International*. 2005; 47(7): 458-465.
30. Ko JC, Smith BE, Hsu WH. Xylazine enhances porcine myometrial contractility in vitro: possible involvement of  $\alpha 2$ -adrenoceptors and  $Ca^{2+}$  channels. *Biology of Reproduction*. 1990; 43(4): 614-618.
31. Gibbs III HM, Troedsson MH. Effect of acepromazine, detomidine, and xylazine on myometrial activity in the mare. *Biology of Reproduction*. 1995; 52: 489-493.
32. De Lille AJ, Silvers ML, Cadario ME, Tran TQ, Cage CL, LeBlanc MM. Interactions of xylazine and acepromazine with oxytocin and the effects of these interactions on intrauterine pressure in normal mares and mares with delayed uterine clearance. *Journal of Reproduction and fertility. Supplement*. 2000; (56): 373-379.
33. Ko JC, Hsu WH, Evans LE. The effects of xylazine and alpha-adrenoreceptor antagonists on bovine uterine contractility in vitro. *Theriogenology*. 1990; 33(3): 601-611.
34. Thaete LG, Levin SI, Dudley AT. Impact of anaesthetics and analgesics on fetal growth in the mouse. *Laboratory Animals*. 2013; 47(3): 175-183.
35. Waldvogel D, Bleul U. Effect of xylazine, isoxsuprine, and lidocaine on Doppler sonographic uterine and umbilical blood flow measurements in cows during the last month of pregnancy. *Theriogenology*. 2014; 81(7): 993-1003.
36. Toma VA, Filip A, Farcas A, Mirescu C, Rosioru C. Effect of hyoscine N-butylbromide on the skeletal muscle contraction in Wistar rat. *Annals of the Romanian Society for Cell Biology*. 2013; 18(2): 123.
37. Tytgat GN. Hyoscine butylbromide. *Drugs*. 2007; 67(9): 1343-1357.
38. Renner UD, Oertel R, Kirch W. Pharmacokinetics and pharmacodynamics in clinical use of scopolamine. *Therapeutic Drug Monitoring*. 2005; 27(5): 655-665.
39. Sato Y, Hotta H, Nakayama H, Suzuki H. Sympathetic and parasympathetic regulation of the uterine blood flow and contraction in the rat. *Journal of the Autonomic Nervous System*. 1996; 59(3): 151-158.
40. Braun U, Gansohr B, Hässig M. Ultrasonographic evaluation of reticular motility in cows after administration of atropine, scopolamine and xylazine. *Journal of Veterinary Medicine Series A*. 2002; 49(6): 299-302.
41. Rizzo A, Gazza C, Silvestre A, Maresca L, Sciorsci RL. Scopolamine for uterine involution of dairy cows. *Theriogenology*. 2018; 122: 35-40.
42. Romański KW. Characteristics of phase 3-like activity and rebound excitation triggered by hexamethonium and atropine administration in the ovine small bowel. *Indian Journal of Experimental Biology*. 2010; 48(2): 124-132.