บทความวิจัย

สัตวแพทย์มหานครสาร

JOURNAL OF MAHANAKORN VETERINARY MEDICINE

Available online: www.tci-thaijo.org/index.php/jmvm/

ผลของยากลุ่มปิดกั้นการจับตัวรับชนิดมัสคารินิก โคลิเนอจิก แบบไม่เลือก (อะโทรปีน) ต่อคุณภาพน้ำเชื้อและปริมาณน้ำเลี้ยงอสุจิแพะ

Sakdichod Kimsakulvech[1,#], Tuempong Wongtawan[2], Sookruetai Boonmasawai[3], Arpron Leesombun[3], Chowalit Nakthong[4], Yupaporn Lanamtiang[5], and Waraluk Oupala[5]

[1]Department of Preclinic and Applied Animal Sciences, Faculty of Veterinary Science, Mahidol University, Salaya Campus, 999 Phuttamonthon 4 Road, Salaya, Nakhon Pathom, Thailand. 73170.; [2]Akkhararatchakumari Veterinary College, Walailak University, Thai Buri, Tha Sala, Nakhon Si Thammarat, Thailand 80160; [3]Department of Preclinic and Applied Animal Sciences, Faculty of Veterinary Science, Mahidol University, Salaya Campus, Nakhon Pathom, Thailand. 73170; [4]Department of Clinical science and Public health, Faculty of Veterinary Science, Mahidol University, Salaya Campus, Nakhon Pathom, Thailand. 73170; [5]Veterinary Medical Center for Livestock and Wildlife Animal Hospital, Faculty of Veterinary Science, Mahidol University, Kanchanaburi Campus, Sai-yok, Kanchanaburi, Thailand. 71150

บทคัดย่อ: ระบบประสาทพาราซิมพาเทติกมีบทบาทสำคัญในการควบคุมคุณภาพน้ำเชื้อผ่านการจัดการต่อมสร้างน้ำเลี้ยงอสุจิในสัตว์หลายชนิด น้ำเลี้ยงอสุจิมีผลเสียต่อการเก็บรักษาน้ำเชื้อแพะแช่แข็ง เราตั้งสมมติฐานว่าการลดการหลั่งของน้ำเลี้ยงอสุจิระหว่างการเก็บน้ำเชื้ออาจช่วยปรับปรุงคุณภาพของการแช่แข็งของน้ำเชื้อแพะได้ ในการศึกษานี้ใช้อะโทรปีนเพื่อยับยั้งระบบพาราซิมพาเทติก ด้วยการทดสอบที่ความเข้มข้นแตกต่างกัน 6 ระดับ (0.02, 0.04, 0.08, 0.1, 0.15 และ 0.2 มก./กก.) ในแพะเพศผู้ 12 ตัว ความเข้มข้นสุดท้าย (0.2 มก./กก.) ถูกทดสอบในการรีดเก็บน้ำเชื้อติดต่อกันสองครั้ง โดยบันทึกการเปลี่ยนแปลงทางสรีรวิทยาทั้งอัตราการเต้นของหัวใจและการตอบสนองของรูม่านตาทั้งก่อนได้รับและหลังการฉีดอะโทรปีน ผลการศึกษาพบว่าอะโทรปีนไม่ส่งผลกระทบต่อระบบสืบพันธุ์ส่วนใหญ่ รวมถึงการมีเพศสัมพันธ์ การหลั่งน้ำเชื้อ คุณภาพน้ำเชื้อ และปริมาณของน้ำเลี้ยงอสุจิ รวมถึงความเข้มข้นและความแตกต่างของโปรตีนในน้ำเลี้ยงอสุจิ อย่างไรก็ตามการให้ยาอะโทรปีนบางขนาดส่งผลต่อระบบอื่นๆ ของร่างกาย เช่น เพิ่มอัตราการเต้นของหัวใจเมื่อขนาดยามากกว่า 0.1 มก./กก. และการขยายรูม่านตาเมื่อขนาดยามากกว่า 0.04 มก./กก. โดยสรุปการยับยั้งพาราซิมพาเทติกโดยอะโทรปีนไม่ส่งผลกระทบต่อระบบสืบพันธุ์เพศผู้แพะ หรือระบบพาราซิมพาเทติกอาจไม่มีความสำคัญในการควบคุมระบบสืบพันธุ์เพศผู้แพะ

คำสำคัญ: อะโทรปีน แพะ การยับยั้งระบบพาราซิมพาเทติก คุณภาพน้ำเชื้อ น้ำเลี้ยงอสุจิ

[#,*]ผู้รับผิดชอบบทความ

สัตวแพทย์มหานครสาร. 2565. 17(1): 49-59.

E-mail address: sakdichod.kim@mahidol.edu

Impact of Nonselective Muscarinic Cholinergic Antagonist (Atropine) on Goat Semen Quality and Seminal Fluid Volume

Sakdichod Kimsakulvech[1,#], Tuempong Wongtawan[2], Sookruetai Boonmasawai[3], Arpron Leesombun[3], Chowalit Nakthong[4], Yupaporn Lanamtiang[5], and Waraluk Oupala[5]

[1]Department of Preclinic and Applied Animal Sciences, Faculty of Veterinary Science, Mahidol University, Salaya Campus, 999 Phuttamonthon 4 Road, Salaya, Nakhon Pathom, Thailand. 73170.; [2]Akkhararatchakumari Veterinary College, Walailak University, Thai Buri, Tha Sala, Nakhon Si Thammarat, Thailand 80160; [3]Department of Preclinic and Applied Animal Sciences, Faculty of Veterinary Science, Mahidol University, Salaya Campus, Nakhon Pathom, Thailand. 73170; [4]Department of Clinical science and Public health, Faculty of Veterinary Science, Mahidol University, Salaya Campus, Nakhon Pathom, Thailand. 73170; [5]Veterinary Medical Center for Livestock and Wildlife Animal Hospital, Faculty of Veterinary Science, Mahidol University, Kanchanaburi Campus, Sai-yok, Kanchanaburi, Thailand. 71150

Abstract: The parasympathetic nervous system plays an important role in controlling the semen quality via manipulation of accessory glands in many species. Seminal fluid has a negative impact on semen cryopreservation in goats. We hypothesized that reducing the secretion of seminal fluid during semen collection may help to improve the quality of semen cryopreservation. In this study, we used atropine to inhibit the parasympathetic pathway. Six different concentrations (0.02, 0.04, 0.08, 0.1, 0.15 and 0.2 mg/kg) of atropine were tested in twelve male goats. The final concentration (0.2 mg/kg) was tested in two consecutive semen collections. Physiological changes, including heart rate and pupil response, were evaluated before and after the atropine injection. The results revealed that atropine did not affect most of the reproductive system, including copulation, ejaculation, semen quality and seminal fluid volume, protein concentration and protein profile. However, some dosages of atropine affected other body systems, such as increased heart rate at a dose of more than 0.1 mg/kg and pupil dilation at a dose greater than 0.04 mg/kg. In conclusion, we proposed that parasympathetic inhibition by atropine did not impact the goat's male reproductive system and suggested that the parasympathetic system may not be important for regulating the male reproductive system in goats.

Keywords: Atropine, Goat, Parasympathetic inhibition, Semen quality, Seminal fluid

[#,*]Corresponding author

E-mail address: sakdichod.kim@mahidol.edu

J. Mahanakorn Vet. Med. 2022 17(1): 49-59.

Introduction

The seminal fluid has an important role in the supply of nutrients for spermatozoa through the female reproductive tract (Evans and Ganjam, 2011; Leite-Browning, 2009). However, seminal fluid is a major obstacle to cryopreservation of goat semen because it can cause poor quality of frozen-thawed spermatozoa (Ferreira et al., 2014). One of the factors is a lipase in seminal fluid that can affect the acrosome integrity and motility of caprine spermatozoa during the semen freezing process (Sias et al., 2005). The process of washing goat semen could increase the improved spermatozoa survival rate in the frozen semen process (Kucuk et al., 2014; Memon et al., 1985). However, this process increases the freezing time and loss of spermatozoa. Therefore, the decrease of seminal fluid in ejaculated goat semen during semen collection may improve frozen-thawed semen quality.

Seminal fluid is produced from both the epididymis and the accessory sex glands (Rodriguez-Martinez et al., 2011). It contains various substances such as fructose, calcium, phosphorus and proteins (Aguiar et al., 2013). Protein in the seminal fluid plays an important role in sperm motility, acrosome reaction, protection, capacitation and fertilization (Moura et al., 2018). However, relevant knowledge of seminal fluid secretion in goats is also limited. In current data, the parasympathetic system is believed to stimulate seminal fluid secretion (Coolen et al., 2004). Inhibition of the parasympathetic system has been reported to reduce seminal fluid secretion in some animals (Dziuk and Norton, 1962). Atropine, a nonselective muscarinic cholinergic antagonist, is a commonly used drug to inhibit the parasympathetic nervous system (Flecknell, 2009). It can decrease the volume of seminal fluid in boars and bulls (Baker et al., 1964; Dziuk and Norton, 1962). Therefore, we hypothesized that inhibiting the parasympathetic nervous system by atropine before semen collection may help improvement the cryopreservation of goat semen.

Materials and Methods

Animals

The protocol was approved by the Animal Usage and Ethics Committee of Veterinary Science Faculty, Mahidol University (ID no. MUVS 2017-09-24). Twelve mixed-breed male goats weighing 30 to 45 kg and aged 2 to 4 years were kept apart from the female goats.

Atropine dosage and experimental design

Atropine (product by Union drug laboratories Ltd, Thailand) was divided into two experiments based on the dose of atropine injection, the low dosage of 0.02-0.08 mg/kg and the high dose (0.1-0.2 mg/kg). Previously, these atropine doses have been shown to cause physiological changes in goats (Kumar, 1977; Tranquilli et al., 2007).

Two experiments were performed on different days and the 4×4 Latin square experimental design was used as examined in

Table 1. In the low dosage experiment, 4 treatments were used, including 3 different doses of atropine injection (0.02, 0.04, 0.08 mg/kg) and 1 ml of normal saline (NSS) was injected as a control. The high dose experiment was composed of 0.1, 0.15 and 0.2 mg/kg of atropine and 1 ml of NSS as well as the control group.

At the present time, the process of seminal fluid production in goats is unclear. Seminal fluid may be produced before the atropine injection. Also, seminal fluid volume and protein profile after the first time of semen collection may not represent the effects of atropine injection. The effects of atropine on protein secretion in goat seminal fluid were confirmed by twice semen collection in a short period of time. After finishing the high dose experiment, a cross over design with double semen collection was tested. Goat semen was collected twice after an atropine (0.2 mg/kg) injection. The first semen was collected approximately 60 minutes and later 120 minutes after injection. Semen was divided into 4 groups as follow: first semen collection in the NSS group (NSS1), the second semen collection in the NSS group (NSS2), the first semen collection in the atropine group (A1) and second semen collection in the atropine group (A2).

Libido scoring and semen collection

Semen was collected using a goat's artificial vagina and an estrous female to trigger mating behavior after drug administration. Libido was scored as described by Frydrychova (Frydrychova et al., 2011) with modification.

Table 1 Experiment design

Drug / Week	NSS	0.02 mg/kg	0.04 mg/kg	0.08 mg/kg
Week 1	Group 1	Group 2	Group 3	Group 4
Week 2	Group 2	Group 3	Group 4	Group 1
Week 3	Group 3	Group 4	Group 1	Group 2
Week 4	Group 4	Group 1	Group 2	Group 3

Semen quality assessment

Semen volume, mass spermatozoa movement score, percentage of spermatozoa motility, sperm viability and spermatozoa concentration were measured. Semen volume was measured using a tuberculin syringe. Mass spermatozoa movement was evaluated by sperm waves with whirlpools, which were scored from 0 (immotile) to 5 (high). The percentage of spermatozoa moving forward was evaluated for spermatozoa motility. Spermatozoa concentration was estimated by using a hemocytometer. The hypo-osmotic swelling test was used to determine the integrity of the spermatozoa plasma membrane, which was presented as a percentage of sperm viability (Fonseca et al., 2005).

Following semen measurements, the semen of each goat was centrifuged at 5,000 rpm for 15 min. Seminal fluid was separated out from spermatozoa and was measured with a tuberculin syringe. The percentage of seminal fluid was calculated by the proportion of seminal fluid volume to the semen volume.

Seminal fluid protein analysis

The protein concentration was quantified using the Bradford protein assay (nach Bradford).

Electrophoresis was done following a previous report (Poltep et al., 2018). Briefly, seminal fluid was mixed with the loading buffer containing 0.2 M Tris (Bio Basic, Markham, ON, Canada; pH 6.8), 20% glycerol (Bio Basic), 10% SDS (Bio-Rad, Hercules, CA, U.S.A.), and 5% β-mercaptoethanol (EuroClone, Pero, MI, Italy) to yield 0.2 µg/µl and then boiled at 95°C for 10 min. Ten microliter of mixtures were loaded into 8% SDS gels in a Mini-PROTEAN system (Bio-Rad) at 90 V for 90 min. The gels were washed with distilled water and stained with 1% Coomassie brilliant blue R250 (Thermo Fisher Scientific, MA USA) for 2 hours. Excess dye was removed with a destaining solution (10% methanol and 7% glacial acetic acid) for 2 hours. The protein bands were imaged using a scanner (Fuji Xerox, Bangkok, Thailand).

Physiological evaluation for parasympathetic pathway

Pupillary light reflex and heart rate were evaluated before (15 minutes) drug injection and before each semen collection (15 minutes). The pupillary light reflex was classified into two characters: normal constriction response and dilation. The heart rate was monitored using a stethoscope and presented at a beat per minute.

Statistical analysis

Semen volume, percentages of motile and sperm viability, spermatozoa concentration, seminal fluid volume and percentage of seminal fluid were represented as mean and standard error of the mean (SEM). The statistical difference was analyzed using analysis of variance (ANOVA) and a post hoc test with the Tukey test. Libido score and mass spermatozoa movement score were examined by the Chi-square test. Differences in mean values were considered to be statistically significant at $p<0.05$. Data was analyzed using SPSS program version 23.

Results

Goat behavior

Goats displayed typical male goat sexual behavior for all control groups and treatment groups. Libido scores were not different among the groups (Table 2, Table 3 and Table 5).

Physiological changes after atropine injection

Before the atropine injection and a control group (NSS injection), the pupils displayed normal constriction after performing the pupil light reflex test. The number of goats that exhibited abnormal pupil light reflex (pupil dilation) depended on the dose: 33% (n=4), 58% (n=7), 66% (n=8), 75% (n=9), 91% (n=11), and 100% (n=12) of goats displayed dilated pupils after injection with 0.02, 0.04, 0.08, 0.1, 0.15, 0.2 mg/kg, respectively.

The heart rate in the low doses of atropine injections was not significantly different between the treatments for both pre and post injection (Table 2). Although atropine injection at doses of 0.04 and 0.08 mg/kg tended to increase the heart rate after injection, there was no statistical difference. For the high dose group, the heart rate was significantly increased ($p<0.05$) after

injection at all doses (Table 3). The heart rate after injection with 0.2 mg/kg atropine was the highest and was significantly different ($p<0.05$) compared to other treatments. Semen quality, seminal fluid volume and percentage of seminal fluid volume were not significantly different among the treatments (Table 3).

Two consecutive semen collections

For this experiment, an atropine dose of 0.2 mg/kg was chosen because it demonstrated the most potent effect of atropine on inhibiting the parasympathetic nervous system (which resulted in the highest heart rate and the greatest number of goats exhibiting pupil dilation). Therefore, we hypothesize that this dose may clearly display the high impact of seminal fluid secretion.

During the first and second semen collections, all goats injected with atropine exhibited dilated pupils after testing the light reflex. The heart rate at first semen collection was significantly increased ($p<0.05$) from the pre-injection (Table 4). For the control group, the pupil response was normal, and the heart rate was not different between pre and both post injections.

All parameters of semen quality were not statistically different ($p>0.05$) between the control group and the atropine injection group. The first and second semen collections were not significantly different (Table 5). Semen volume and seminal fluid tended to be reduced after atropine injection in the first and second semen collection, but there was no significant difference ($p>0.05$). The protein concentration tended to decrease in the second semen collection, but there was no significant difference ($p>0.05$) between collection times and groups (Table 6). The protein bands after electrophoresis showed similar bands of proteins among treatments (Figure 1).

Discussion

The present study demonstrated that atropine injection did not affect male libido and behavior, semen quality and seminal fluid volume and protein in goats. This is in contrast to other species, such as bulls which atropine has been shown to reduce the reaction time to mount (Baker et al., 1964).

In some goats, the lowest dose that caused physiological changes was 0.04 mg/kg. At the maximal dose in this study (0.2 mg/kg), all goats displayed clear pupil dilatation and increased heart rate, this suggest that atropine may already affect the whole body system, but not the reproductive system. Similar to our study, reports also showed that 0.2 mg/kg of atropine can increase the heart rate of mature goats (Pablo et al., 1995). However, in other ruminants, such as buffalo calves, it requires a lower dose of 0.04 mg/kg of atropine to increase the heart rate (Khan et al., 2007).

In the present study, atropine injection did not affect the semen quality and seminal fluid volume in the goats. Differences from the findings in other studies revealed that semen and seminal fluid were significantly decreased

Table 2 Comparison of libido score, heart rate, semen qualities and seminal fluid volume in low atropine dosage

Parameters	N	NSS	A0.02	A0.04	A0.08
Pre heart rate (bpm)	12	78.33±5.67	71.00±4.00	67.67±2.12	72.67±3.68
Post heart rate (bpm)	12	70.00±5.31	69.33±4.07	75.67±4.63	77.33±4.36
Libido score	12	5.00	5.00	4.75±0.18	5.00
Semen volume (ml)	12	0.68±0.06	0.71±0.05	0.74±0.08	0.68±0.09
Seminal fluid volume (ml)	12	0.45±0.06	0.45±0.05	0.49±0.06	0.44±0.06
Sediment semen (ml)	12	0.24±0.02	0.25±0.02	0.25±0.02	0.24±0.03
Percentage of seminal fluid volume	12	61.68±4.76	63.63±3.57	65.92±1.47	63.62±3.22
Mass spermatozoa movement score	12	4.50±0.26	4.17±0.37	4.33±0.33	4.58±0.19
Percentage of spermatozoa motility	12	85.00±3.59	74.17±8.92	80.83±6.68	65.83±4.52
Spermatozoa concentration ($x10^9$ cells)	12	5.71±1.0	4.91±0.7	7.1±1.2	4.4±1.3
Percentage of sperm viability	12	37.08±4.04	30.58±2.80	34.92±3.80	33.08±4.14

Table 3 Comparison of libido score, heart rate, semen qualities and seminal fluid volume in high atropine dosage

Parameters	N	NSS	A0.1	A0.15	A0.2
Pre heart rate	12	78.33±4.16	$70.00±3.39^{2}$	$71.00±2.88^{2}$	$74.00±6.87^{2}$
Post heart rate	12	$73.67±3.39^{a}$	$84.33±4.04^{1ab}$	$97.33±6.83^{1bc}$	$105.67±6.20^{1c}$
Libido score	12	4.92±0.08	4.83±0.11	5.00±0.00	5.00±0.00
Semen volume (ml)	12	0.65±0.06	0.49±0.06	0.49±0.05	0.59±0.08
Seminal fluid volume (ml)	12	0.42±0.02	0.31±0.03	0.29±0.02	0.38±0.03
Sediment semen (ml)	12	0.24±0.02	0.18±0.03	0.19±0.02	0.21±0.03
Percentage of seminal fluid volume	12	63.75±1.87	62.73±4.34	58.86±2.52	64.80±2.35
Mass spermatozoa movement score	12	4.42±0.19	3.83±0.37	4.58±0.19	4.42±0.34
Percentage of spermatozoa motility	12	73.33±4.14	73.33±7.91	73.33±7.11	68.25±9.08
Spermatozoa concentration ($x10^9$ cells)	12	3.53±0.42	4.54±0.58	4.78±0.74	5.38±0.54
Percentage of sperm viability	12	27.83±3.53	33.21±4.87	30.33±6.21	31.42±4.37

[ab]Values with different superscripts in the same row are significantly different at $p<0.05$

[12]Values with different superscripts between pre and post heart rate in same column are significantly different at $p<0.05$

Table 4 Pre and post heart rate in two consecutive semen collection

Parameters	N	NSS	Atropine
Pre heart rate	12	78.00±4.94	77.33±3.73[b]
Post heart rate before first collection	12	83.67±7.02	102.33±4.58[a]
Post heart rate before second collection	12	72.67±4.59	84.00±4.29[ab]

[ab] Values with different superscripts in the same column are significantly different at $p < 0.05$

Table 5 Comparison of libido score, heart rate, semen qualities and seminal fluid volume in two consecutive semen collection (NSS1 = first collection in NSS group, NSS2 = second collection in NSS group, A1 = first collection in atropine group, A2 = second collection in atropine group)

Parameters	N	NSS1	A1	NSS2	A2
Libido score	12	4.92±0.08	4.92±0.08	4.92±0.08	5.00
Semen volume (ml)	12	0.92±0.11	0.65±0.06	0.78±0.08	0.66±0.07
Seminal fluid volume (ml)	12	0.62±0.07	0.41±0.04	0.56±0.07	0.43±0.05
Sediment semen (ml)	12	0.30±0.05	0.24±0.02	0.22±0.03	0.23±0.03
Percentage of seminal fluid volume	12	68.1±2.7	62.98±1.87	69.70±3.30	64.79±2.22
Mass spermatozoa movement score	12	4.92±0.08	4.33±0.19	4.67±0.19	4.58±0.29
Percentage of spermatozoa motility	12	82.50±4.7	73.75±5.54	81.25±2.83	79.17±6.3
Spermatozoa concentration ($x10^9$ cells)	12	4.11±0.8	3.28±0.61	3.18±0.41	0.31±0.42
Percentage of sperm viability	12	25.0±3.9	27.25±4.5	27.58±5.70	24.92±3.72

Table 6 Protein concentration in twice semen collection

Group	N	NSS1	A1	NSS2	A2
Protein concentration (mg/ml)	12	45.79±5.78	51.96±3.86	55.4±5.41	49.31±3.5

after atropine injection (Dziuk and Mann, 1963). Several previous studies have suggested the possibility of parasympathetic on control of seminal fluid production in animals (Coolen et al., 2004; Hsieh et al., 2014; O'Shaughnessy, 2015). In pigs, only one semen collection after atropine injection was enough to reduce the seminal fluid volume (Dziuk and Mann, 1963). In bull, atropine had affected on increase semen concentration (Evans and Ganjam, 2011).

Currently, the studies of nervous system to control seminal fluid and protein secretion are not distinct data. Secretion of seminal fluid protein in this study could be compared with the study of protein secretion in saliva. Our result was consistent with the study of protein secretion from salivary glands in lamps. Parasympathetic inhibition by atropine did not affect the change in the quantity and protein in saliva (Edwards and Titchen, 2002). For goat

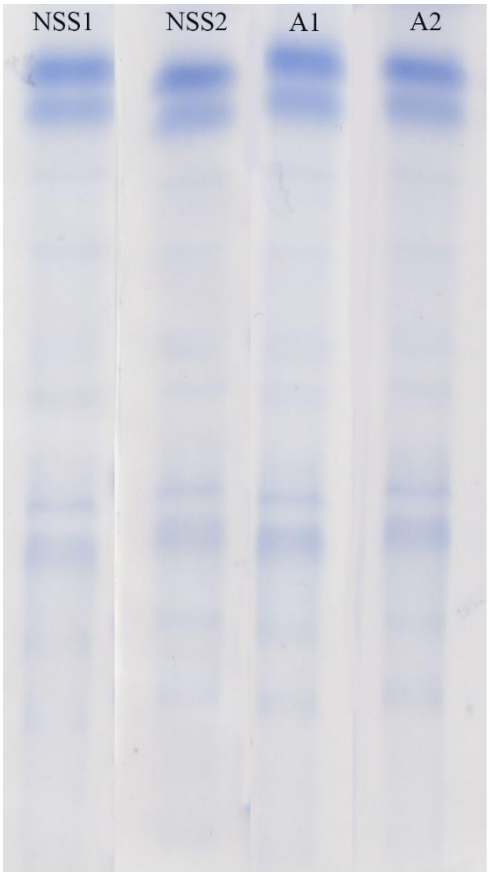

Figure 1 Comparison of protein range in seminal fluid in two consecutive collection experiment (NSS1 = first collection in NSS group, NSS2 = second collection in NSS group, A1 = first collection in atropine group, A2 = second collection in atropine group)

seminal fluid secretion, parasympathetic inhibition did not change the protein concentration and type of protein. Atropine could not change any seminal fluid volume and protein in the goats.

It could be argued that the seminal fluid may have already been reserved for the accessory sex glands before the atropine injection. However, in the consecutive semen collection, the seminal fluid from the second semen collection should be decrease if, the atropine can inhibit seminal fluid secretion. This result suggested that parasympathetic pathway may not essential to control the reproductive system in male goat particularly at accessory organs. Conversely, sympathetic inhibition by tamsulosin could decrease seminal fluid in goats (Kimsakulvech et al., 2018), suggesting the important role of the sympathetic pathway in manipulating the seminal fluid secretion in goats.

Conclusions

This experiment suggested that atropine does not affect the goat's reproductive system, particularly libido, ejaculation, semen quality and seminal fluid secretion. This could imply that the parasympathetic nervous system does not play a significant role in the control of the male reproductive system in goats.

Acknowledgements

The grant was supported by the Mahidol University. The authors declare no other conflicts of interest in this study.

References

Aguiar, G.V., van Tilburg, M.F., Catunda, A.G.V., Celes, C.K.S., Lima, I.C.S., Campos, A.C.N., Moura, A.A.A., and Araújo, A.A. 2013. Sperm parameters and biochemical components of goat seminal plasma in the rainy and dry seasons in the Brazilian Northeast: the season's influence on the cooling of semen. Arq. Bras. Med. Vet. Zoo. 65: 6-12.

Baker, R.D., Vandemark, N.L., Graves, C.N., and Norton, H.W. 1964. Effects of pilocarpine on copulatory behaviour, ejaculation and semen. J. Reprod. Fertil. 8: 297-303.

Coolen, L.M., Allard, J., Truitt, W.A., and McKenna, K.E. 2004. Central regulation of ejaculation. Physiol. Behav. 83: 203-215.

Dziuk, P.J. and Mann, T. 1963. Effect of atropine on the composition of semen and secretory function of male accessory organs in the boar. J. Reprod. Fertil. 5: 101-108.

Dziuk, P.J. and Norton, H.W. 1962. Influence of drugs affecting the autonomic system on seminal ejaculation. J. Reprod. Fertil. 4: 47-50.

Edwards, A,V, and Titchen, D.A. 2002. The effect of parasympathetic postganglionic denervation on parotid salivary protein secretion in anaesthetized sheep. Auton. Neurosci. 100: 50-57.

Evans, T.J. and Ganjam, V.K. 2011. Reproductive anatomy and physiology, In: Gupta RC (Ed.) Reproductive and Developmental Toxicology. Academic Press, San Diego. 7-32.

Ferreira. Vd.S., Mello, M.R.Bd., Fonseca, C.E.Md., Dias, Á.C.F., Cardoso, J.M., Silva, R.B., and Martins Jr. W.P. 2014. Effect of seminal plasma and egg yolk concentration on freezability of goat semen. Rev. Bras. Zootec. 43: 513-518.

Flecknell, P.A. 2009. Chapter 2 - Anaesthesia, In: Flecknell PA (Ed.) Laboratory Animal Anaesthesia (Third Edition). Academic Press, San Diego, 19-78.

Fonseca, J.F., Torres, C.A.A., Maffili, V.V., Borges, A.M., Santos, A.D.F., Rodrigues, M.T., and Oliveira, R.F.M. 2005. The hypoosmotic swelling test in fresh goat spermatozoa. Anim. Reprod. 2: 139-144.

Frydrychova S., Opletal, L., Macakova, K., Lustykova, A., Rozkot, M., and Lipensky, J. 2011. Effects of herbal preparation on libido and semen quality in boars. Reprod. Domest. Anim. 46: 573-578.

Hsieh, J.T., Kuo, Y.C., Chang, H.C., Liu, S.P., Chen, J.H., and Tsai, V.F. 2014. The role of sympathetic and parasympathetic nerve systems on the smooth muscle of rat seminal vesicles - experimental results and speculation for physiological implication on ejaculation. Andrology. 2: 59-64.

Khan, I., Kumar, A., Singh, J., Peshin, P., and Singh, S. 2007. Evaluation of Atropine as an Anticholinergic in Buffalo Calves (*Bubalus bubalis*). Ital. J. Anim. Sci. 6: 999-1002.

Kimsakulvech, S., Boonmasawai, S., Gronsang, D., Nakthong, C., and Lanamtiang, Y. 2018. Small dosages of alpha1 adrenoceptor antagonist on goat semen quality and seminal fluid volume. Thai J. Vet. Med. 48: 567-572.

Kucuk, N., Aksoy, M., Ucan, U., Ahmad, E., Naseer, Z., Ceylan, A., and Serin, I. 2014. Comparison of two different cryopreservation protocols for freezing goat semen. Cryobiology. 68: 327-331.

Kumar, A.. 1977. Cardiovascular, respiratory and biochemical effects of atropine sulphate in goats. Indian Vet. J.

Leite-Browning, M.L. 2009. Biology of Reproductive of Goats, Alabama A&M and Auburn Universities. 1-7.

Memon, M.A., Bretzlaff, K.N., and Ott, R.S. 1985. Effect of washing on motility and acrosome morphology of frozen-thawed goat spermatozoa. Am. J. Vet. Res. 46: 473-475.

Moura, A.A., Memili, E., Portela, A.M.R., Viana, A.G., Velho, A.L.C., Bezerra, M.J.B, and Vasconselos, F.R. 2018. Seminal plasma proteins and metabolites: effects on sperm function and potential as fertility markers. Anim. Reprod. 15: 691-702.

O'Shaughnessy, P., 2015. Knobil and Neill's Physiology of reproduction, Vol 1, 4 Edition. Elsevier Inc, Waltham, MA ,United States of Ameirca.

Pablo, L.S., Webb, A.I., and McNicholas, W.T. Jr. 1995. The effects of atropine and glycopyrrolate on heart rates in conscious mature goats. Vet. Surg. 24: 531-534.

Poltep, K., Tesena, P., Yingchutrakul, Y., Taylor, J., and Wongtawan, T. 2018. Optimisation of a serum albumin removal protocol for use in a proteomic study to identify the protein biomarkers for silent gastric ulceration in horses. J. Equine. Sci. 29: 53-60.

Rodriguez-Martinez, H., Kvist, U., Ernerudh, J., Sanz, L., and Calvete, J.J. 2011. Seminal plasma proteins: what role do they play?. Am. J. Reprod. Immunol. 66(Suppl 1): 11-22.

Sias, B., Ferrato, F., Pellicer-Rubio, M.T., Forgerit, Y., Guillouet, P., Leboeuf, B., and Carrière, F. 2005. Cloning and seasonal secretion of the pancreatic lipase-related protein 2 present in goat seminal plasma. Biochim. Biophys. Acta. Mol. Cell Biol. Lipids. 1686: 169-180.

Tranquilli, W.J., Thurman, J.C., and Grimm, K.A., 2007. Lumb and Jones' veterinary anesthesia and analgesia. Blackwell Publishing.