Research Articles

Effects of atropine 0.25 mg/kg on goat sexual desire, semen quality and seminal fluid volume

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Abstract

This experiment compared effect of atropine 0.25 mg/kg between before and after injection in eight male goats. The trial was conducted for 3 experiments in 3 consecutive weeks. At the first week, all male goats were injected intramuscularly with normal saline 1 ml (NSS) as a pre-control group. At the second week, all goats were injected with atropine 0.25 mg/kg as an atropine group. At the third week, all goats were injected with NSS as a post-control group. Pupillary light reflex and heart rate were evaluated 15 minutes before and after injection. Sexual desire was evaluated by libido score. Semen was collected within 40 minutes after injection for assessing semen quality and seminal fluid volume. In atropine group, all goats had mydriasis and significantly higher heart rate after injection. All groups could copulate and ejaculate within 1 minute after contacting the female. Sexual desire, semen parameters and seminal fluid volume were not significantly different among three groups. In summary, injecting atropine 0.25 mg/kg inhibited parasympathetic nervous system and did not change sexual desire, semen quality and seminal fluid volume.

Keywords: atropine, sexual desire, semen quality, seminal fluid, goat

ผลของอะโทรปีน 0.25 มิลลิกรัม ต่อ กิโลกรัม ต่อความต้องการทางเพศ คุณภาพน้ำเชื้อ และปริมาณน้ำเลี้ยงอสุจิในแพะ

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บทคัดย่อ

การทดลองนี้จัดทำขึ้นเพื่อศึกษาผลการฉีดอะโทรปีนขนาด 0.25 มิลลิกรัมต่อกิโลกรัม ต่อความต้องการทางเพศ คุณภาพน้ำเชื้อ และการหลั่งน้ำเลี้ยงอสุจิในแพะ โดยศึกษาในแพะเพศผู้จำนวน 8 ตัว แบ่งออกเป็น 3 การทดลอง ใน 3 สัปดาห์ สัปดาห์แรก แพะเพศผู้ทุกตัวถูกฉีดด้วยน้ำเกลือปริมาณ 1 มิลลิลิตร เข้ากล้ามเนื้อ จัดเป็นกลุ่มควบคุมก่อนทดลอง สัปดาห์ที่สอง แพะทุกตัวถูกฉีดด้วยอะโทรปีน ขนาด 0.25 มิลลิกรัมต่อกิโลกรัม จัดเป็นกลุ่มอะโทรปีน และสัปดาห์ที่สามแพะถูกฉีดด้วยน้ำเกลือ จัดเป็นกลุ่มหลังการทดลอง การตอบสนองของม่านตาและอัตราการเต้นของหัวใจถูกตรวจวัดก่อนและหลังฉีดสาร 15 นาที ความต้องการทางเพศถูกวัดจากคะแนนความต้องการทางเพศ เก็บน้ำเชื้อหลังฉีดสารภายใน 40 นาที เพื่อประเมิณคุณภาพน้ำเชื้อและปริมาณน้ำเลี้ยงอสุจิถูกประเมินทุกครั้งหลังรีดเก็บน้ำเชื้อ ผลการศึกษาพบว่า แพะในกลุ่มอะโทรปีนพบม่านตาขยายและอัตราการเต้นของหัวใจหลังฉีดสูงขึ้นเมื่อเทียบกับก่อนฉีด แพะทุกกลุ่มสามารถขึ้นทับเพศเมียและหลั่งน้ำเชื้อภายใน 1 นาที หลังปล่อยให้เข้าหาเพศเมีย โดยไม่มีความแตกต่างของความต้องการทางเพศ คุณภาพน้ำเชื้อและปริมาณน้ำเลี้ยงอสุจิ ระหว่างกลุ่มอะโทรปีนและกลุ่มควบคุมทั้งก่อนและหลังฉีดอะโทรปีน โดยสรุป การฉีดอะโทรปีนในแพะขนาด 0.25 มิลลิกรัมต่อกิโลกรัม มีผลยับยั้งระบบพาราซิมพาเทติก โดยไม่มีผลต่อความต้องการทางเพศ คุณภาพน้ำเชื้อ และการหลั่งน้ำเลี้ยงอสุจิของแพะ

คำสำคัญ : อะโทรปีน ความต้องการทางเพศ คุณภาพน้ำเชื้อ น้ำเลี้ยงอสุจิ แพะ

Introduction

Ejaculation is a process for transporting semen during mating and is controlled via both sympathetic and parasympathetic nervous systems (Coolen et al., 2004; Hsieh et al., 2014). Semen consists of spermatozoa from testis and seminal fluid. Spermatozoa were developed within seminiferous tubules of the testis. Seminal fluid in goats was secreted from accessory sex glands, including seminal vesicle, prostate, and bulbo-urethral glands, which contribute to major volume of the ejaculate (Juyena and Stelletta 2012). Spermatozoa delivery is mainly controlled by sympathetic system (Coolen et al., 2004). Seminal fluid modulates sperm function during spermatozoa movement within reproductive tract such as nutrient supply and spermatozoa movement facilitation (Juyena and Stelletta 2012; Leahy and de Graaf 2012). Parasympathetic system is the main system for controlling seminal fluid secretion and sexual desire process in various male animals (Coolen et al., 2004; Calabró et al., 2019). In boar, an inhibition of the parasympathetic system using atropine could reduce the seminal fluid secretion to semen (Dziuk and Norton 1962; Dziuk and Mann 1963). Moreover, atropine 0.125-0.25 mg/kg decreased seminal fluid volume of the boars. In rats, atropine 100-125 mg/kg disturbs semen transport and reduces pregnancy rate (Ban et al., 2002; Sato et al., 2005). In bull, atropine 200 mg per 1,600-1,900 lb of weight (or about 0.23-0.27 mg/kg) decreases semen volume but increase spermatozoa concentration and mounting time (Baker et al., 1964).

Atropine is a competitive muscarinic cholinergic antagonist drug (Krishnaiyan and Thompson 2013). Generally, atropine has been used in medication such as a pre-anesthetic medication, an antidote of organophosphate and carbamate poisoning in goats (Giadinis et al., 2009; Flecknell et al., 2015; Dalefield 2017). Atropine concentration can be detected in sheep blood at 1 minute after 0.02 mg/kg intramuscular injection and reached peak concentration at about 10 minutes later (Mundie et al., 1988). The dosage of atropine was variable from 0.02-0.4 mg/kg in different therapeutic regimens (Taylor 1991; Flecknell 2009; Flecknell et al., 2015; Dalefield 2017). The recommended dose of atropine for pre-anesthesia in goats was 0.02-0.4 mg/kg that it could help in bradycardia case (Tranquilli et al., 2007; Flecknell et al., 2015;) The initial dose of atropine (0.3 mg/kg) was suggested for carbamate poisoning antidote (Giadinis et al., 2009). These data show that atropine at least 0.02 mg/kg could induce parasympathetic inhibition. To reproductive system, the dosage range of atropine could disturb male reproductive function that had been showed in many previous studies (Dziuk and Mann 1963; Dziuk and Norton 1962; Ban et al., 2002; Sato et al., 2005). In boars, for example, the maximum dose of atropine (0.25 mg/kg) decreases seminal fluid volume (Dziuk and Mann 1963). These data demonstrate that high dose of atropine effects on the male reproductive function. However, high dosage atropine at 0.25 mg/kg has not been studied on sexual desire, semen quality and seminal fluid volume in goats. Therefore, this data aimed to investigate the effect of high dose of atropine (0.25mg/kg) on sexual desire, semen quality and seminal fluid volume in goats.

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). Eight

male mixed breed goats aged 2 to 4 years with 30 to 45 kg body weight were kept separately from the female goats. They had negative brucellosis before entering the housing. Goats were fed with concentrate feed and hay one time per day. The water was freshly replaced in a large basin on a daily basis.

Experimental design

The experiment was designed to investigate the effects of atropine 0.25 mg/kg (1mg /1ml, Union drug laboratories ltd.) before and after intramuscular administration. This experiment was conducted for 3 consecutive weeks. At the first week, all goats were injected with normal saline (NSS) 1 ml at thigh as a pre-control group. At the second week, all goats were injected with atropine 0.25 mg/kg as an atropine high dosage group. At the third week, all goats were injected with 1 ml NSS again as a post-control group. The drug was administered at one-week intervals.

Physiological change assessment

Drug for parasympathetic inhibition such as atropine, could increase heart rate and decrease pupillary light reflex response. For assessing the parasympathetic inhibition, both changes were evaluated 15 minutes before and after drug injection. Pupillary light reflex was considered a response into either normal response with pupil constriction and no parasympathetic response with pupil dilation. Heart rate was counted per minute by a stethoscope.

Sexual desire assessment

Goats were brought to meet the estrous female goats to collect semen by artificial vagina. Time to copulation was recorded and assessed for libido score (adapted from Frydrychova) (Frydrychova et al., 2011). Male sexual desire was an attention to female and a need to mate, estimated as libido score. The libido score can be divided into 6 classes, 5 = high sexual desire or copulate female within 1 minute after attaching female and 0 = no sexual desire or not copulate female more than 10 minutes. Normal range of goat was scored at 4 or 5.

Semen quality evaluation

Semen was collected within 40 minutes after drug injection and kept in 15 ml centrifuge tube. Semen quality parameters were composed of semen volume, mass spermatozoa movement score, percentages of motile spermatozoa, spermatozoa membrane integrity, spermatozoa concentration and seminal fluid volume.

Semen volume was estimated by drawn the semen by tuberculin syringe (Nipro Brand, Thailand). Two microliters were drop in glass slide for mass spermatozoa movement and percentage of spermatozoa motility assessment. Mass spermatozoa movement was ranged from 0 (no motile) to 5 (excellent). Percentage of spermatozoa motility was estimated from moving spermatozoa under light microscope at 400x magnification. Semen was diluted in buffer formalin at 1:400 for spermatozoa concentration assessment. Ten microliters of diluted semen were counted number of spermatozoa on hemocytometer (Neubauer counting chamber, produce from Boeco, Germany) and calculated to spermatozoa concentration. Hypo-osmotic swelling test is integrity of spermatozoa plasma membrane test (Fonseca et al., 2005). Bent-tailed goat spermatozoa showed normal integrity of plasma membrane under hypo-osmotic solution at 125 mOsm/l. Semen was diluted in HOS solution at 1:400 and incubated at 37 ^{o}C for 30 minutes. Bent tail spermatozoa were counted from total

two hundred spermatozoa and calculated to percentage of normal integrity of spermatozoa plasma membrane.

To evaluate seminal fluid volume, semen was centrifuged at 5,000 rpm for 15 minutes for isolating seminal fluid. The supernatant was drawn to estimate the volume by tuberculin syringe. Seminal fluid volume was calculated and shown as percentage of seminal fluid volume per semen volume.

Statistical analysis

The number of animals were obtained by calculation in G power program version 3.1 using F tests-ANOVA (Fix effects, special, main effect and interactions). Heart rate was compared between before and after injection with student paired t-test. Semen quality was compared between pair groups of pre-control, post-control and atropine group with student paired t-test except libido score and mass spermatozoa movement score, which were examined by the Chi-square test. Differences of mean values were considered statistically significant at $P < 0.05$. All values were shown as the mean and standard error of the mean (SEM). Data were analyzed using SPSS program version 21.

Results

For all goats in pre- and post-control groups, pupils responded to light and heart rate were not different from each other. For atropine group, pupillary light reflex had normal before injection. After injection, the pupils constricted to light but all goats did not response to light or have mydriasis. The heart rate after atropine injection was significantly higher than that before injection as shown in Table1.

All male goats needed to attach and thrust when met the female. Moreover, all goats from all groups had libido score 5 or all goats could or all goats could copulate in 1 minutes after contact female. Semen quality including semen volume, mass spermatozoa movement score, percentage of spermatozoa motility, percentage of normal spermatozoa membrane integrity, spermatozoa concentration and seminal fluid volume after atropine injection was not difference from both pre- and post-control groups as shown in Table 1. Only the goats in pre-control group had significantly high semen concentration than those in both atropine and post-control groups.

Discussion

In this study, atropine 0.25 mg/kg could interrupt pupillary light reflex and increase the heart rate after 15 minutes of injection in goats. These results indicated parasympathetic inhibition after atropine administration. Semen was collected and assessed within 40 minutes after injection. Agreeable with previous study, atropine 0.2 mg/kg could increase heart rate in goats (Pablo et al., 1995). In small ruminants such as sheep, atropine was measured from blood after administration at least 60 minutes (Mundie et al., 1988). In addition, buffalo calves, atropine 0.04 mg/kg had been reported to increase the heart rate at least 60 minutes after injection (Khan et al., 2007). These studies supported the dosage and duration of atropine to inhibit parasympathetic system by increasing functionality of the sympathetic system in ruminants. These previous studies had supported the duration for sexual desire and semen quality assessment in this study that under the parasympathetic inhibition by atropine.

All goats in this study were able to copulate the female and ejaculate every time of semen collection. The present study demonstrated that libido score did not

Table 1. Comparison of heart rate, semen quality and seminal fluid volume between pre-control, atropine and post-control groups

Parameters	Pre-control	Atropine	Post-control
Pre heart rate (bpm)	63.25±2.23	67±4.88	79.0±4.21
Post heart rate (bpm)	66.50±3.74	89.5±2.61	82.75±6.70
Semen volume (ml)	0.39±0.08	0.29±0.05	0.39±0.07
Seminal fluid volume (ml)	0.19±0.04	0.13±0.02	0.19±0.04
Sediment semen volume (ml)	0.20±0.04	0.17±0.03	0.21±0.04
Percentage of seminal fluid volume	48.51±4.88	43.80±3.79	46.3±1.45
Mass spermatozoa movement score	4.88±0.13	4.88±0.13	4.75±0.16
Percentage of spermatozoa motility	86.88±2.66	92.50±2.31	86.25±3.24
Spermatozoa concentration (x10^9 cells)	8.26±0.55^a	5.4±0.53^b	5.44±0.7^b
Percentage of live spermatozoa	62.81±5.45	68.38±4.36	67.50±6.47

^{a,b}Values with different superscripts in the same row are significantly different at $P < 0.05$

^{1,2}Values with different superscripts between pre and post heart rate in same column are significantly different at $P < 0.05$

differ all three groups. Atropine dosage in this study did not decrease copulation time and disturb sexual desire in all goats. In a previous study, atropine could decrease the reaction time to mount in bulls (Baker et al., 1964). The parasympathetic system had been reported as a part of sexual desire process (Calabró et al., 2019). However, our result demonstrated that, inhibition of the parasympathetic system with atropine did not influence on sexual desire in goats.

Spermatozoa concentration in pre-control had significantly higher than that of both atropine and post-control groups. Before starting this experiment, goats had skipped the semen collection for 2 weeks before pre-control test. This may cause the higher spermatozoa concentration for the pre-control group. According to a previous study, in sheep, semen concentration could be decreased according to the amount of ejaculation (Jha et al., 2018). Moreover, abstinence period on human semen more than 5 day could increase the semen concentration while decreasing spermatozoa vitality (Comar et al., 2017).

This study showed that spermatozoa concentration in atropine group was not different from post-control group. Spermatozoa concentration represents the ability to deliver sperm that was regulated by the sympathetic system. (Coolen et al., 2004). Parasympathetic inhibition by atropine in this study, did not affect the sympathetic control and spermatozoa deliver in goats. This was different from atropine effect in boar, which atropine increases spermatozoa concentration (Dziuk and Mann 1963). According to previous study in boars, atropine administration in goats did not change percentage of spermatozoa motility and integrity of plasma membrane (Strzezek et al., 1998). Spermatozoa was developed and mature to obtain full function in epididymis. If normal epididymal function occur, normal semen quality will be found (Joseph et al., 2009). The atropine did not disrupt epididymal functions in goats in this study similar to the effects of atropine in boars (Dziuk and Mann 1963).

Based on a previous study, the maximum dose of atropine 0.25 mg/kg significantly decreased boar semen and seminal fluid volume (Dziuk and Mann 1963).

However, this dosage has never been tested in goat. The similar dosage of atropine in boar did not change goat semen quality and seminal fluid volume. This study revealed the difference of parasympathetic inhibition on seminal fluid secretion between goats and boars. In goats, the inhibition of parasympathetic system with atropine did not interrupt seminal fluid secretion. Atropine was not obviously affected accessory sex glands functions in goats.

Previously, parasympathetic inhibition by atropine was reported to decrease male sex function in rats (Ban et al., 2002; Sato et al., 2005). Atropine 100 mg/kg was administered orally for 4 weeks in the male rats, this could reduce pregnancy rate in the female (Ban et al., 2002). In addition, atropine 125 mg/kg was treated in rats for continuous 10-15 days; it was found that seminal vesicles and seminal plug significantly reduced in atropine group (Sato et al., 2005). The prolongation of parasympathetic inhibition induced the impairment of fertility in male rats by inhibiting sperm delivery and seminal fluid secretion. Therefore, a long-term atropine administration may inhibit those functions in goats as same as in the rat experiment. However, these results in this study showed that a single high dose of atropine 0.25 mg/kg had no effects, in term of sexual desire, semen quality and seminal fluid volume goats.

Conclusions

These results showed that single high dose of atropine (0.25 mg/kg) could induce parasympathetic inhibition but did not disturb goat male sexual desire, spermatozoa delivery, epididymal function and seminal fluid secretion. This suggested that the parasympathetic control may did not influence on goat sexual desire, semen quality and seminal fluid secretion.

Acknowledgements

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

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