Original Article

Small dosages of alpha1 adrenoceptor antagonist on goat semen quality and seminal fluid volume

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Abstract

The frozen goat semen process must wash seminal fluid from goat semen. A small dosage of alpha1 adrenoceptor antagonist or tamsulosin may reduce seminal fluid in goat. A latin square 4x4 was applied to twelve male goats to receive either normal saline (NSS), tamsulosin 3, 6 and 9 µg/kg intramuscular injection at one-week intervals. Semen collection by artificial vagina and libido scoring was performed within one-hour post injection. Semen quality and seminal fluid were evaluated after semen collection The results showed that none of the libido scores in the groups was different. Anejaculations occurred in 1 (8.3%), 3 (25%) and 4 (33.3%) using tamsulosin 3, 6 and 9 µg/kg, respectively. Only, the volume of seminal fluid in tamsulosin 9 µg/kg has significantly lower than the NSS group. Other semen quality did not show any significant difference. Although, in this experiment, the alpha1 adrenoceptor antagonist or tamsulosin could reduce the amount of seminal fluid in goats anejaculation was promoted in all dosages of tamsulosin one hour after injection. Therefore, this drug may not suitable in reducing seminal fluid when producing goat frozen semen.

Keywords: alpha1 adrenoceptor antagonists, tamsulosin, seminal fluid, semen quality, goat

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Introduction

In many mammal species seminal fluid has an important role for spermatozoa metabolism, function, survival and transportation in the female reproductive tract (Juyena and Stelletta, 2012). Nevertheless, seminal fluid in goats may become toxic and decrease spermatozoa survival in the frozen semen process (Ferreira et al., 2014). The goat seminal fluid washing procedure can increase the spermatozoa survival rate in the frozen semen process (Memon et al., 1985; Kucuk et al., 2014). The seminal fluid washing procedure uses a centrifuge to pack spermatozoa down at the bottom of a centrifuge tube. A packed spermatozoa does not facilitate the evaluation of viable spermatozoa. In addition, the dead spermatozoa are included in the pack of spermatozoa packed pellets. These dead spermatozoa release a reactive oxygen species (ROS) within the packed pellets which causes live spermatozoa membrane damage (Aitken and Clarkson, 1987). Moreover, the washing procedure increases the time of the freezing process and loses spermatozoa during spinning. Interestingly, if we can reduce seminal fluid in ejaculated goat semen, the washing procedure may not be essential in the frozen goat semen process which may lead to an increase in the viability and number of spermatozoa.

Alpha1 adrenoceptor antagonist drugs are used to treat benign prostatic hyperplasia (BPH) in humans (Welliver and Essa, 2016). Tamsulosin is a drug of the alpha1 adrenoceptor antagonist type with a high affinity for alpha1a and 1d adrenoceptors in the human prostate gland. This drug induces smooth muscle relaxation in the human prostate (Kawachi, 1998). In goats, a high dosage of tamsulosin between 60 - 120 μg/kg can suppress ejaculation within 3 hours of injection (Kimsakulvech et al., 2015a,b). Noticeably in the recovery period or 12 hours after tamsulosin 120 μg/kg injection, all goats had normal ejaculation while a higher spermatozoa concentration than in the control group was recorded. It is suggested that the increasing spermatozoa concentration may result from the accessory sex gland smooth muscle remaining relaxed because of the small tamsulosin dosage remaining in the body. Decreasing the seminal fluid in goats by alpha1 adrenoceptor antagonist drug or tamsulosin may be possible with a small dosage. A lower dosage of tamsulosin on semen quality had been shown in humans (Hisasue et al., 2006). Tamsulosin 0.2 and 0.4 mg/ person reduced semen volume and fructose in male volunteers. The reduction of these parameters receiving small dosage tamsuloin may have resulted from alpha1 adrenoceptor antagonist inhibiting accessory gland secretion (Mann, 1974; Marconi et al., 2009). However, the effect of small dosage of tamsulosin on reducing seminal fluid in goats has not been revealed. The present study investigates small dosages of tamsulosin on seminal fluid volume and semen quality in goats.

Materials and Methods

The protocol was approved by the Animal Usage and Ethics Committee of the Veterinary Science Faculty, Mahidol University (ID no. MUVS 2016-05-19). Twelve mixed breed male goats aged 1 to 3 years and weighing 30 to 45 kg were bought from the farmer. Male goats were kept in a single pen and separated from female goats at the Veterinary Medical Center for Livestock and Wildlife Animal Hospital, Faculty of Veterinary Science, Mahidol University, Kanchanaburi, Thailand. Before any testing, all goats were evaluated for their health and judged to be brucellosis disease free by the Rose-bengal test. They were confirmed to have a good libido and a normal ejaculating ability using an artificial vagina (AV) producing and normal semen quality.

This experiment was designed as a 4 x 4 Latin square. Each male was administered normal saline (NSS, control), Tamsulosin 3 μg/kg or 6.743 nmol/kg (TAM3), Tamsulosin 6 μg/kg or 13.485 nmol/kg (TAM6) and Tamsulosin 9 μg/kg or 20.229 nmol/kg (TAM9) intramuscularly at one-week intervals.

Solution preparation: Tamsulosin was purchased from Sigma-Aldrich Pte Ltd. TAM (50 mg) was dissolved in 1 ml dimethylsufoxide (DMSO) to generate a concentrated stock solution which was then diluted with additional DMSO (200 μl) prior to administration. Normal saline (200 μl) was administered during the control trial.

Libido scoring and semen collection: Semen was collected using an AV and an estrous female to trigger mating behavior at and after drug administration for 15 - 60 minutes. Libido was scored by a modified version of Frydrychova (Frydrychova et al., 2011), each male had the chance to copulate once or twice. In the first attempt, if no semen was found in the AV collecting tube after thrusting, the male with ejaculatory suppression was allowed to have a second chance of mounting within 10 mins. Subsequently, if the goat did not copulate in 10 mins, the ejaculation of this goat was classified as anejaculation.

Ejaculatory scoring: Ejaculation was classified into two groups: anejaculation and complete ejaculation. Anejaculation was defined as a lack of semen in the AV collecting tube and complete ejaculation as semen volume containing spermatozoa in the collecting tube.

Semen quality assessment: The semen volume, mass spermatozoa movement score, percentages of motile, live spermatozoa, spermatozoa concentration, and total spermatozoa per ejaculate were measured to assess semen quality. Seminal fluid volume was evaluated and calculated according to the percentage of seminal fluid for assessed tamsulosin affecting accessory sex organ function.

Semen volume was measured using a tuberculin syringe. Mass spermatozoa movement was scored from 0 (immotile) to 5 (high), and the percentage of motile spermatozoa was measured using light microscopy. Spermatozoa concentration was estimated by hemocytometer. The percentage of live spermatozoa was based on the hypo-osmotic swelling test (Fonseca et al., 2005). The total number of spermatozoa was calculated from spermatozoa concentration and semen volume.

After semen measurement, the semen of each

goat was centrifuged at 5,000 rpm for 15 minute. Seminal fluid was separated from spermatozoa and was measured by tuberculin syringe. The percentage of seminal fluid was calculated by semen volume.

Statistical analysis: The semen volume, percentages of motile and live spermatozoa, spermatozoa concentration, total spermatozoa, seminal fluid volume and the percentage of seminal fluid were analyzed using analysis of variance for Latin square designs. The libido score and mass spermatozoa movement score were examined by the Chi-square test. Mean values were considered statistically significant with a difference of $p < 0.05$. All values were shown as the mean and standard error of the mean (SEM).

Results

All goats copulated at the first attempt within 2 minutes of face to face contact. The libido score of each group is shown in table 1. Anejaculation is shown in the tamsulosin injection group. Tamsulosin 3, 6 and 9 μg/kg had 1, 3 and 4 anejaculation, respectively. The semen quality of anejaculation goats was not include in the statistical calculation. Only seminal fluid and the percentage of seminal fluid in the tamsulosin 9 μg/kg group was significantly lower than the control groups. Other semen parameters did not show any statistically significant difference as is shown in table 2.

Table 1 Libido score (mean ± SEM) between control (NSS), TAM3, TAM6 and TAM9

	n	NSS	TAM3	TAM6	TAM9
Libido score	12	5.0	4.92±0.08	5.0	4.92±0.08

Table 2 Comparison of goat semen quality and seminal fluid volume (mean ± SEM) between NSS, TAM3, TAM6 and TAM9

Parameters	n	NSS	n	TAM3	n	TAM6	n	TAM9
Semen volume (ml)	12	0.65±0.07	11	0.552±0.08	9	0.439±0.08	8	0.419±0.09
Seminal fluid volume (ml)	12	0.48±0.05[a]	11	0.36±0.05	9	0.291±0.05	8	0.261±0.06[b]
Sediment semen (ml)	12	0.17±0.03	11	0.19±0.03	9	0.15±0.04	8	0.16±0.04
Percentage of seminal plasma per semen volume	12	75.18±3.5[a]	11	67.88±3.6	9	64.52±4.1	8	59.51±4.3[b]
Mass spermatozoa movement score	12	4.75±0.17	11	4.73±0.18	9	4.56±0.20	8	4.50±0.21
Percentage of spermatozoa motility	12	91.7±4.3	11	80.91±4.5	9	85.56±5.0	8	81.25±5.3
Spermatozoa concentration ($x10^9$ cells)	12	4.31±0.5	11	3.97±0.6	9	3.65±0.6	8	3.50±0.7
Percentage of live spermatozoa	12	49.21±4.4	11	40.23±4.6	9	40.50±5.1	8	35.5±5.4
Number of spermatozoa per ejaculate ($x10^9$ cells)	12	2.94±0.5	11	2.60±0.6	9	1.84±0.6	8	1.47±0.7

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

Discussion

Tamsulosin, alpha1 adrenoceptor antagonist, 3 - 9 μg/kg, IM caused anejaculation in goats within 60 minutes of injection. Tamsulosin 9 μg/kg promoted a higher anejaculation than other groups. The number of anejaculation in goats increased with an increase of the tamsulosin dosage. In previous reports, tamsulosin 0.2 mg/person (about 3 μg/kg) in humans caused ejaculatory dysfunction. The tamsulosin decreased the capacity of seminal vesicle contraction leading to a reduction in semen volume (Hisasue et al., 2006). The higher dosage of tamsulosin 0.8 mg/person (about 11.42 μg/kg) promoted 35 percent anejaculation (Hellstrom and Sikka, 2006b). In goats, tamsulosin 9 μg/kg caused 66.6 percent of anejaculation. These results suggest that the suppressed ejaculation with tamsulosin seemed to effect goats more than humans. However, the effect on anejaculation with tamsulosin dosage in this study was dose dependent, consistent with a previous study (Kimsakulvech et al., 2015a).

In addition to seminal fluid volume, semen quality parameters did not show a significant difference between groups. Concentration of the tamsulosin 6 - 9 μg/kg tended to reduce the mean semen volume, spermatozoa concentration and number of spermatozoa per ejaculate more than the tamsulosin 3 μg/kg. Tamsulosin 9 μg/kg tended to effect semen quality similar to volunteers receiving tamsulosin 0.8 mg/person (Hellstrom and Sikka, 2006a; Hellstrom and Sikka, 2006b). In a previous study, in the recovery period 12 hours after tamsulosin 120 μg/kg injection, all goats had normal ejaculation with a higher spermatozoa concentration than control group (Kimsakulvech et al., 2015a). In the recovery period, the remaining tamsulosin in the body that was received at a high dosage of tamsulosin could result in only the inhibition of the accessory sex glands function. However, the small dosage of the tamsulosin in this experiment did not demonstrate any spermatozoa concentration difference. The rate of drug delivery

dependent on blood flow in each organ may have been the cause of this difference (Kok-Yong and Lawrence, 2015). The possibility is that accessory sex glands may have a lower blood flow than the epididymis or vas deferens. Also, in the recovery period of tamsulosin 120 μg/kg, the tamsulosin might also affect only accessory sex glands. Seminal fluid decreased from accessory sex glands dysfunction. In this experiment, one hour after injection, the duration period might not have been enough to diffuse tamsulosin from the vas deferens and epididymis, also it might have inhibited the contraction of these organs.

Autonomic nervous system control of seminal fluid secretion in goats is not well understood. Previous reports have explained that the autonomic nervous system is the main control of seminal fluid secretion in rats and boars (Dziuk and Norton, 1962; Dziuk and Mann, 1963; Kepper and Keast, 1997; Coolen et al., 2004). The importance of a sympathetic nervous system on seminal fluid secretion has been shown in rats. Alpha adrenoceptor antagonists such as prazosin reduced the seminal vesicle pressure in rats (Hsieh et al., 1998). This study reveals the inhibition of a sympathetic nervous system by alpha1 adrenoceptor antagonist or tamsulosin 9 μg/kg can significantly reduce seminal fluid in the control group. A sympathetic nervous system could be one part of the seminal fluid secretion control in goat. The sympathetic control ejaculation in goats had an effect on spermatozoa ejection (Kimsakulvech et al., 2015b). Interestingly, the small tamsulosin dosage in this study still had an effect on spermatozoa ejection. One hour after tamsulosin injection at this dosage may not be enough to reduce the effect on vas deferens or epididymis on spermatozoa ejection.

The lowest dosage of alpha1 adrenoceptor antagonist in this study, tamsulosin 3 μg/kg could reduce seminal fluid to 75 percent of control group with other semen quality and was not distinctly different from the control group. However, only one anejaculate occurred at this dosage and this cannot exclude the possibility of using tamsulosin for reducing seminal fluid prior to production in the frozen goat semen process.

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บทคัดย่อ

ผลของ อัลฟ่าวัน อะดรีโนเซ็บเตอร์ แอนตาโกนิส ปริมาณน้อยต่อคุณภาพน้ำเชื้อ และปริมาณน้ำเลี้ยงอสุจิในแพะ

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การผลิตน้ำเชื้อแช่แข็งในแพะจำเป็นต้องมีขบวนการล้างน้ำเลี้ยงอสุจิ (seminal fluid) การลดขั้นตอนนี้โดยใช้สารกลุ่มอัลฟ่าวัน อะดรีโนเซ็บเตอร์ แอนตาโกนิส (alpha1 adrenoceptor antagonist) หรือ แทมซูโลซิน (tamsulosin) อาจช่วยลดปริมาณน้ำเลี้ยงอสุจิได้ โดยไม่ต้องมีขั้นตอนการล้างน้ำเลี้ยงอสุจิ การทดลองนี้ใช้รูปแบบ 4x4 ลาตินสแคว์ โดยใช้แพะเพศผู้ 12 ตัว ฉีด น้ำกลั่น หรือ สารละลาย tamsulosin ขนาด 3 6 และ 9 ไมโครกรัม ต่อกิโลกรัม เข้ากล้ามเนื้อ สลับกัน ฉีดห่างกัน 1 สัปดาห์ จนแพะทุกตัวได้รับสารละลายครบทุกชนิด เก็บน้ำเชื้อด้วยช่องคลอดเทียม และประเมินความต้องการทางเพศหลังฉีดภายใน 1 ชั่วโมง ประเมินคุณภาพน้ำเชื้อและปริมาณน้ำเลี้ยงอสุจิหลังได้น้ำเชื้อ ความต้องการทางเพศไม่มีความแตกต่างระหว่าง การฉีด tamsulosin ขนาด 3 6 และ 9 ไมโครกรัม ต่อกิโลกรัม ทำให้ไม่เกิดการหลั่งน้ำเชื้อ จำนวน 1 (8.3 เปอร์เซ็นต์), 3 (25 เปอร์เซ็นต์) และ 4 (33.3 เปอร์เซ็นต์) ครั้งตามลำดับ ปริมาณน้ำเลี้ยงอสุจิในกลุ่มฉีด 9 ไมโครกรัม ต่อกิโลกรัม น้อยกว่ากลุ่มฉีดน้ำกลั่นอย่างมีนัยสำคัญเท่านั้น ส่วนคุณภาพน้ำเชื้ออื่นไม่มีความแตกต่างกันอย่างมีนัยสำคัญ การทดลองนี้สารกลุ่ม alpha1 adrenoceptor antagonist หรือ tamsulosin มีผลลดปริมาณน้ำเลี้ยงอสุจิในแพะแต่มีผลให้ไม่มีการหลั่งน้ำเชื้อภายหลังฉีด 1 ชั่วโมง จึงอาจยังไม่เหมาะสมสำหรับการนำไปใช้ลดปริมาณน้ำเลี้ยงอสุจิสำหรับผลิตน้ำเชื้อแช่แข็งแพะ

คำสำคัญ: แอลฟ่าวัน-อะดรีโนเซ็บเตอร์-แอนตาโกนิส แทมซูโลซิน น้ำเลี้ยงอสุจิ คุณภาพน้ำเชื้อ แพะ

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