Received: 3 April 2019 | Revised: 28 May 2019 | Accepted: 11 June 2019

DOI: 10.1111/and.13458

ORIGINAL ARTICLE

First International Journal of Andrology
aNDROLOGIa
WILEY

Semen collection by trans-rectal digital stimulation and insemination campaign in goat

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Abstract

The overall purpose of this study was to describe a method of semen collection via trans-rectal digital massage (TDM) and to carry out a related fertility trial in Angora goat. Sixteen Angora bucks (ranging 1–4 years) and 28 nulliparous does (1–2 years) were used in this study. Semen samples were collected via trans-rectal massage from 85.71% of the bucks in multiple attempts (18/21). The mean values of volume, pH, mass motility, total motility, concentration, viability, abnormal spermatozoa rate and ejaculation time were 0.64 ± 0.09 ml, 6.3 ± 0.21, 2.7 ± 0.34, 58.18 ± 5.1%, 3.68 ± 0.31 × 10^9/ml, 71.38 ± 7.12%, 18.22 ± 2.48% and 3.4 ± 0.33 min respectively. Oestrus was detected with teaser buck and confirmed by using infrared thermography and ultrasonography (US). The success rate of synchronisation was found as 71.4% (20/28). On Day 21, pregnancy diagnosis was performed trans-rectally with US and the pregnancy rate was determined as 78.57% (11/14). TDM method of semen collection seems to be easily applicable to the buck and it could be a good alternative to collect semen as well as its use in artificial insemination campaign. Thermal monitoring is found to be a valuable tool to monitor the response to hormonal driven ovulatory synchronisation in Angora does during timed artificial insemination.

KEYWORDS

ampulla massage, angora goat, fertility, infrared thermography, semen, trans-rectal massage

1 | INTRODUCTION

The complex process from erection to ejaculation occurs through a cascade of neurologic, vascular and humoral events. However, it can be mimicked through the rectal mucosa and can be initiated with a vigorous back and forth motion over the vesicular glands and ampullae to move the sperm from the ampullae into the pelvic urethra. After manipulation of the accessory glands through trans-rectal digital massage (TDM), parasympathetic output from the S2–S4 spinal segments initiates the reflexogenic erection and the pulsatile expulsion of the semen during ejaculation, as well as the innervation of the pudendal nerve and the dorsal nerve of the penis (Ibrahim, Brackett, & Lynne, 2016).

There are numerous reports that describe the technique for semen collection in bucks with artificial vagina (AV) (Leboeuf, Restall, & Salamon, 2000; Memon, Bretzlaff, & Ott, 1986; Ritar, Mendoza, Salamon, & White, 1992) and electroejaculation (EE) (Santiago-Moreno et al., 2009; Jiménez-Rabadán et al., 2012), even though these two methods of sperm collection have been established and the fertility results were approved, they required preliminary preparation and numerous equipment and generally they are depending on extensive training of the males.

Few studies have been reported about the TDM, particularly in wild animals such as Asian elephants (Schmitt & Hildebrandt, 1998), mouflons (*Ovis orientalis)* and Iberian ibexes *(Capra pyrenaica)* by Ungerfeld et al., (2015) and Sumatran rhinos (Agil, Supriatna,

Purwantara, & Candra, 2008). Recently, this method has been identified in Angora bucks as well (Tekin et al., 2017). The TDM method is preferable in conditions such as low libido, lameness, as well as for bucks, which are refusing the AV and/or are experiencing erectile problems.

Besides the well-known oestrus detection tool, ultrasonography, recently an alternative has been identified. With the use of infrared thermography (IRT) which is a technology and a valuable tool for oestrus detection and determination of the insemination time (Stelletta, Gianesella, Vencato, Fiore, & Morgante, 2012; Stelletta et al., 2017; Stelletta, Vencato, Fiore, & Gianesella, 2013), Angora goat fertility can be improved even out of the breeding season. There are definite differences among regional temperatures and these can be easily measured by IRT. This method can be the solution to the fundamental challenges of Angora goat breeding since it is accurate but not time-consuming for identifying the animals answering well to the synchronisation protocol.

Therefore, this study was conducted with the objectives of (a) establishing a novel collection method in Angora bucks, (b) assessing the quality parameters and fertility results of the trans-rectally collected ejaculates, (c) integrating TDM and IRT-timed artificial insemination (TAI) detection to obtain a more successful artificial insemination campaign.

2 | MATERIAL AND METHODS

2.1 | Animals and facilities

This study was carried out with 16 clinically healthy Angora bucks (1–4 years of age) and twenty-eight nulliparous Angora does, 1–2 years of age in the nonbreeding season (July, 2016). All animals received the general clinical examination and routine sanitary measures against local contagious diseases before the experimental design. Animals were kept under uniform conditions with ad libitum feeding materials and water. Experiments were conducted according to ethical principles, and this study was approved by the animal ethics committee in Ankara University (2015-21-230).

2.2 | Semen collection, evaluation and buck selection

Before the collection, preputial area was trimmed and disinfected using a chemical agent. Preputium was gently cleansed by washing with a syringe containing 10,000 IU gentamicin diluted in 0.9% NaCl solution. Preliminarily, the faeces were manually evacuated. Extension of the penis was accomplished by rectal massage. After extension, the penis was cleaned and dried to reduce environmental contamination of the semen sample. A vigorous back and forth motion over the vesicular glands and ampullas was applied through the rectal mucosa. After detection of the ejaculatory response, massage was continued in an attempt to move the sperm from the ampullae into the pelvic urethra. Massage was limited to a maximum time of 5 min in order to avoid traumatisation of the rectal mucosa.

Semen was collected into a graduated tube, placed in a warm water bath (33°C) and evaluated immediately for volume, concentration, mass motility and percentage of total motile sperm as previously reported (Memon, Mickelsen, & Goyal, 2007). Spermatozoa concentration was measured according to the hemacytometric method. Sperm cell morphology was assessed by the examination of eosin–nigrosin stain-fixed samples (Memon et al., 2007).

Among eleven out of fourteen bucks answering well to the manipulation, seven, which had the highest semen quality, were selected for the insemination campaign. A tris-based extender (30.7 g of Tris, 16.4 g of citric acid, 12.6 g of fructose and 1,000 ml of bidistilled water at a pH of 6.8 without cryoprotectant) was used for the dilution of pooled semen to 500 × 10^6 motile spermatozoa/ml as insemination dose.

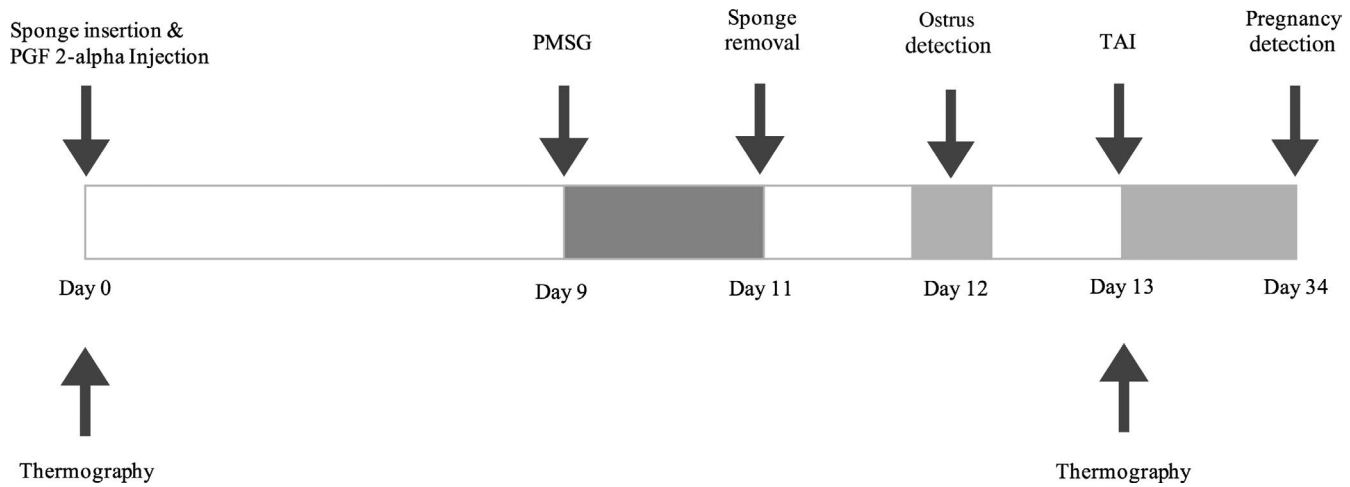

FIGURE 1 Synchronisation and infrared thermography at timed artificial insemination detection protocol. PGF_2, prostaglandin $F_2\alpha$; PMSG, pregnant mare serum gonadotropin; TAI, timed artificial insemination

TABLE 1 Spermatological parameters of semen collected through trans-rectal digital stimulation for the selection and insemination

Parameter	Total number of collection	Mean ± *SEM*	Number of ejaculates used for insemination	Mean ± *SEM*
Volume (ml)	18	0.64 ± 0.09	7	0.49 ± 0.15
pH	18	6.3 ± 0.21	7	6.88 ± 0.25
Mass motility	18	2.7 ± 0.34	7	2.42 ± 0.78
Total motility (%)	18	58.18 ± 5.1	7	60 ± 7.3
Concentration (sperm x 10^9/ml)	18	3.68 ± 0.31	7	3.87 ± 0.26
Viability (%)	11	71.38 ± 7.12	–	–
Abnormal spermatozoa (%)	11	18.22 ± 2.5	–	–
Ejaculation time (min)	21	3.4 ± 0.33	7	4.04 ± 0.41

2.3 | Synchronisation

Oestrus synchronisation protocol for does in the nonbreeding season (from January to August) was selected considering the success in our previous study and carried out administering 125 μg $PGF_2\alpha$ (d-cloprostenol, Gestavet, Hipra-Turkey) intramuscularly and introducing an intravaginal sponge (20 mg fluorogestone acetate, Chronogest®, Hipra-Turkey) for 11 days (Stelletta et al., 2017). Does were treated with intramuscular PMSG (Freeze-dried equine chorionic gonadotropin, Oviser, Hipra-Turkey) with a dose of 300 IU, 48 hr before sponge removal (Figure 1).

2.4 | Oestrus detection

Oestrus was detected with a teaser buck (Tirpan et al., 2019), verified both by using IRT and by ultrasonography to identify the presence of a peri-ovulatory follicle (MyLab-VetOne, Linear ESAOTE). Variations of surface temperature between the vulvar and perivulvar area during the synchronisation protocol were performed by collecting a range of infrared rays from labial and perivulvar (perilabial, perianal, glabrous area of tail base) surfaces at the time of sponge insertion (Day 0) and just before the TAI at Day 13, using a thermal camera (Flir Systems TM; Flir Systems, E60) positioned at a distance of 100 cm.

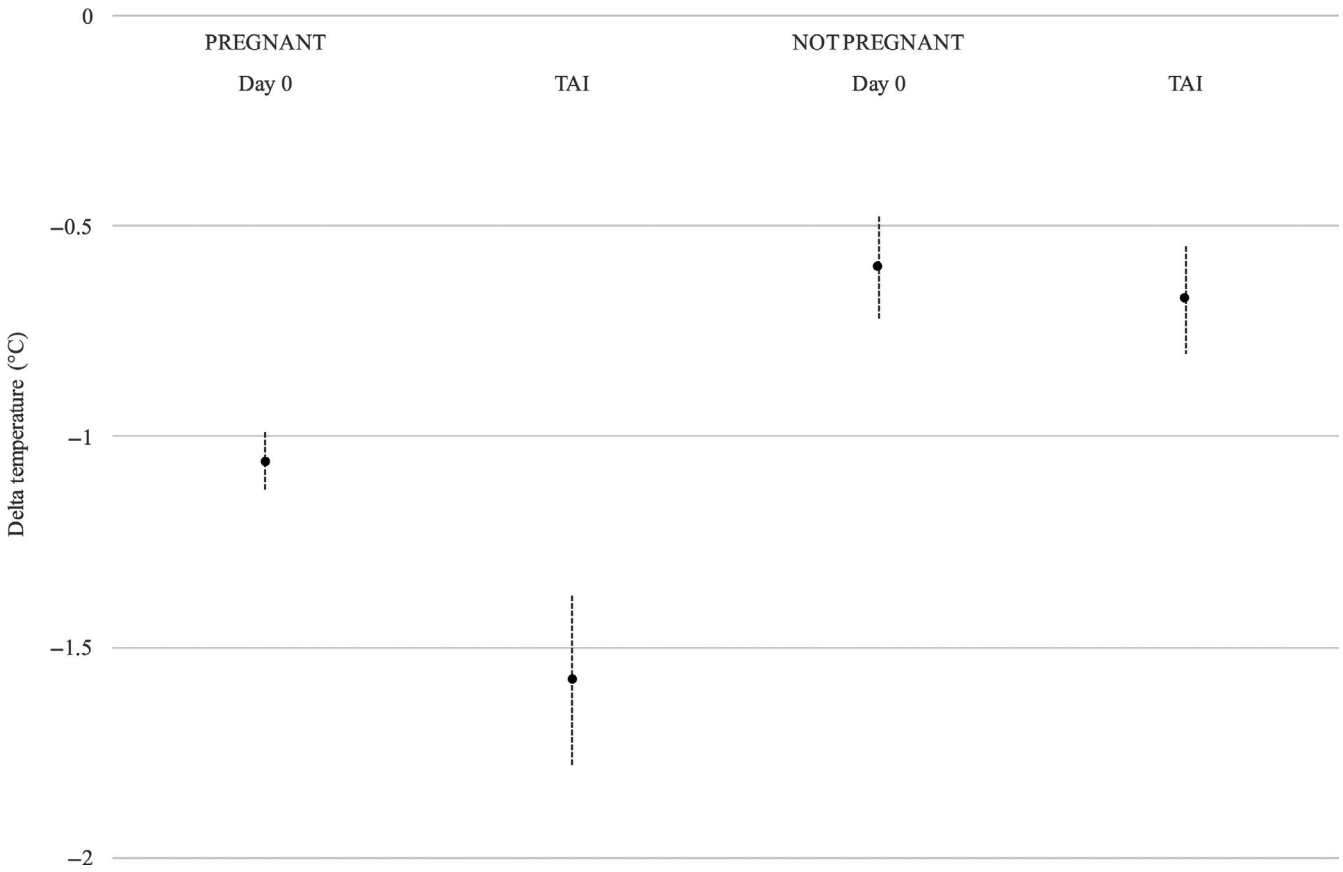

FIGURE 2 Difference between vulvar and perivulvar temperature (Delta T) at Day 0 and at timed artificial insemination in pregnant and not pregnant selected does. TAI, timed artificial insemination

Environmental parameters were set in the basic software set-up to avoid creating an influence on the acquisition of infrared rays. The vulvar (VST) and perivulvar (PST) surface temperatures were measured and their differences (ΔT) were calculated (Stelletta et al., 2017). Only the females showing an evident ΔT (around −0.5°C) with a colder vulvar surface received the insemination service.

2.5 | Insemination and pregnancy detection

Timed artificial inseminations were carried out at 43–45 hr after sponge removal in does with detected oestrus. Females were restrained gently by two technicians, and perianal disinfection was done before the vaginal insemination. The hindquarters of does were raised over a rail, and semen was deposited as deeply as possible into vaginal canal, or close to first cervical ring. Twenty-one days after the insemination, does were positioned in dorsal recumbences and monitored trans-rectally by US using a 10 MHz linear probe.

2.6 | Statistical analysis

Collected data were analysed using the GLM procedure of the statistical software Sigmastat 2.03. Descriptive statistics were performed identifying average values and standard errors. Dependent variables as vulvar and perivulvar temperatures, Δ-average, Δ-minimal and Δ-maximal temperatures were compared for the independent variable pregnancy diagnosis's group (pregnant and not pregnant) considering $p < .05$ as level of significance.

3 | RESULTS

Trans-rectal semen collection was applied to fourteen bucks with multiple attempts, and collection success rate was 85.71% (18/21). Two males were excluded before the collection attempts because of their young age (1-year-old) and low reproductive physical scores. Mean values (means ± *SEM*) of volume, pH, mass motility, total motility, concentration, viability and abnormal spermatozoa rate were in the acceptable range (Table 1). However, it was noted that semen volume was less than in other collection methods. The time required for TDM procedure took an average of 3.4 ± 0.33 min for the total of 21 attempts. However, three males have shown lower response to the digital manipulation (no penile protrusion) than others, and solely seminal plasma was present in the collection tubes. Average values of pooled ejaculate motility were acceptable enough to perform a vaginal AI campaign (Table 1).

Regarding the female selection process, the delta temperature differences of pregnant and nonpregnant does were −1.58 ± 0.2°C ΔT and −0.67°C ± 0.07 ΔT respectively. All pregnant does clearly showed the thermal differences with a colder vulvar surface than not pregnant animals at the time of insemination (Figure 2, Figure S1) ($p < .05$). Does were selected to TAI application considering their thermal relationship of vulvar and perivulvar surfaces. Twenty does (71.4%) answered to the synchronisation protocol and showed an evident oestrus behaviour pattern. Only sixteen does had a colder vulvar surface (−0.5°C) and these received the insemination service. Eleven out of fourteen does (78.57%) were detected as pregnancy positive at Day 21 with US monitoring.

4 | DISCUSSION

The present study demonstrated that AI campaign with TDM and IRT monitoring resulted with an acceptable pregnancy rate in Angora goat breeding programme at the field level. Therefore, the IRT technology reveals a bright future for an optimised AI time detection method which is less time-consuming and easily applicable at the field level. The TDM sperm collection method may present an alternative approach to AV and EE methods, not only for AI, but also for the andrological examination systems and for semen cryopreservation.

Our self-imposed limitation to achieve a successful collection within five minutes of stimulation could affect the mean of total duration. A higher threshold in terms of stimulation time could give more successful collection rate. Moreover, the success of TDM sperm collection and the required time were not affected by the performing physician.

To our knowledge, this is the first study reporting on the establishment of pregnancy with TDM-collected semen in Angora goat.

In the present study, the innovative integration of thermal monitoring for TAI protocol in Angora goat represents an applicable and noninvasive tool to optimise the artificial insemination timing. Eighty per cent (16/20) of the does showed not only the oestrus behaviour but also an evident thermal difference between vulvar and perivulvar surfaces. The −1.58 Δ°C temperature of vulvar area lower than perivulvar area at the time of AI has displayed a similar pattern with our previous work (Stelletta et al., 2017). Lower level of Δ°C could be attributable to a strict selection method of inseminated does (Δ°C: −0.5°C) out of breeding season and the young age of the group. A selection method of the does involved in a synchronisation protocol depending on the response can improve the pregnancy rate. In the present study, a relatively high (78.57%) early pregnancy rate was obtained, considering that a vaginal approach was used to perform the insemination. The accuracy of IRT method was verified by cross-checking with US of ovaries for existing preovulatory follicles. The use of IRT in the field is more feasible when compared to US because IRT provides a faster and easier animal's response detection without hard restrain and related stress. The IRT method captures physiological changes in the body without being invasive like US or any other hormone detection methods and does not have the error margin of behavioural change observation when there is silent oestrus.

The commonly used semen collection protocols are based on natural copulation or erection mimicking with artificial vagina or electrical stimulation of pudendal nerves in male ruminants. The approach used in this study considers the initiation of reflexogenic

erections through TDM, and by this way, the pudendal nerve, and dorsal nerve of the penis, which controls the extension of the penis are innerved, thereby leading to pulsatile release of the semen.

Trans-rectal massage (TM) is generally applies for andrological evaluation in bulls, as an alternative technique to AV and EE methods, in the cases which semen cannot be collected due to impotence coeundi or unwillingness to artificial vagina. However, in small ruminants, traditionally, AV and EE methods have been described as the most common semen collection techniques. There are some advantages and disadvantages of mentioned methods. Sperm collection with EE has become a matter of debate due to animal welfare concern (Palmer et al., 2005). Semen collection by AV is a preferable method when compared to EE due to these concerns. Besides, there are many reports revealing that, with AV method, better spermatological parameters can be obtained compared with EE (Jiménez-Rabadán et al., 2012, 2016; Leboeuf et al., 2000). However, it needs a certain training of the males (Wulster-Radcliffe, Wiliams, Stellflug, & Lewis, 2001), a teaser female and various equipment. Thus, using AV as an instant application in the andrological examination process is limited. On the other hand, the use of EE causes an increase in serum cortisol concentrations, rectal temperature, respiration and heart rate due to the created pain and the stress (Abril-Sanchez et al., 2017; Boussena et al., 2013; Stafford et al., 1996) and may need the sedation or anaesthesia to decrease this side effects (Abril-Sánchez et al., 2018). Furthermore, the semen quality obtained with EE differs in terms of its lower spermatological quality, (Santiago-Moreno et al., 2011), higher pH (Giriboni, Lacuesta, & Ungerfeld, 2017; Ungerfeld et al., 2016) and seminal plasma characteristics (Marco-Jimenez, Vicente, & Viudes-de-Castro, 2008). In addition, after the freezing–thawing processes, sperm quality has been found lower than other collection methods (Freitas-De-Melo, Ungerfeld, Hötzel, Orihuela, & Perez-Clariget, 2017; Ungerfeld et al., 2016). Another critical issue is the presence of contaminants and urine in semen during the application of EE (Palmer et al., 2005).

In the present study, the semen collection with TDM was successful as 85.71% (18/21) of the collection attempts. The general spermatological parameters were between the acceptable ranges compared with other collection techniques (Abril-Sanchez et al., 2017; Jiménez-Rabadán et al., 2012; Marco-Jimenez, Puchades, Gadea, Vicente, & Viudes-De-Castro, 2005). A similar method, trans-rectal ultrasound-guided massage of the accessory sex glands (TUMASG) was reported in bucks by Abril-Sanchez et al. (2017) with the method of massaging the ampullae using a US probe and it was reported that the EE causes a greater increase in cortisol levels although no difference in sperm characteristics than TUMASG. Trans-rectal digital stimulation was applied in other animal species such as bulls and elephants (Palmer et al., 2005; Schmitt et al., 1998; Sylla, Palombi, Stradaioli, Vagniluca, & Monaci, 2015) as well as in humans by Ibrahim et al., 2016.

In terms of fertility results, the pregnancy rate obtained in this study with the use of TDM is found admissible when compared with EE and AV methods (Karatzas, Karagiannidis, Varsakeli, & Brikas, 1997; Leboeuf et al., 1998; Ritar et al., 1992; Ritar & Salamon, 1983). The results of this study also reveal the success and combined applicability of techniques such as out of breeding season synchronisation protocol, infrared thermography for oestrus detection, semen collection via trans-rectal massage and vaginal insemination in goats.

In conclusion, the TDM method is found to be highly applicable in Angora goats. In this study, semen collected with TDM had good quality, without contamination by urine and had high concentration. This procedure might be preferred in cases of low libido, foot diseases and other conditions without erectile dysfunction. Besides, it can be applied to animals with impotentia or unable to mount and copulate, as well as wild animal species. This technique has the most advantage when animal welfare is taken into account, and especially in conditions which semen have to be collected frequently and instantly for breeding soundness examination or to be implemented to AI campaigns.

On the other hand, it was concluded that, thermal monitoring is a valuable, noninvasive tool for monitoring the Angora does in order to identify the appropriate animals and the precise timing at TAI protocol. However, infrared thermography and US methods can be combined to increase timing detection efficiency and fertility outcome.

ACKNOWLEDGEMENTS

The Authors thank to the Angora goat breeder Mesut Eleroğlu for providing the animals and give sincerest appreciations to the Fatih Köksal for his great contributions during the experiment.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Tekin K, Cil B, Alemdar H, et al. Semen collection by trans-rectal digital stimulation and insemination campaign in goat. *Andrologia*. 2019;00:e13458. https://doi.org/10.1111/and.13458