ÇUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES

MSc THESIS

Dehouegnon Jerry AGOSSOU

EFFECTS OF ARTIFICIAL INSEMINATION ON SOME PERFORMANCE CHARACTERISTICS OF GOATS

DEPARTMENT OF ANIMAL SCIENCE

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We certify that the thesis titled above was reviewed and approved for the award of degree of the Master of Science by the board of jury on 21/02/2018.

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ABSTRACT

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EFFECTS OF ARTIFICIAL INSEMINATION ON SOME PERFORMANCE CHARACTERISTICS OF GOATS

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: Prof. Dr. Mahmut KESKIN

This study aims to determine the effect of artificial insemination on some reproductive performances in Alpine does. This study was carried out from August 2016 to September 2017 in the Çukurova University, Faculty of Agriculture and Dairy Goat Research Farm located in the province of Adana in the East Mediterranean Region of Turkey. Sixty reproductive Alpine females have been involved in this study. Goats were divided into two: 30 does were naturally bred using well performing males. Throughout this trial does were synchronized using vaginal sponge impregnated with 20 mg of progestagen FGA (fluorogestone acetate, Chronogest CR, Intervet, France). Heat animals inseminated by intra-uterin method in trial group while normal methods in controls'. The physiological adaptation parameters were also detected in two groups.

The work showed that the oestrus synchronization followed by natural mating achieved higher fecundity, survival rate and birth live weight (3.83 ± 0.23 vs. 3.15 ± 0.11) than the use of intrauterine AI after oestrus synchronization in goats. However, the AI animal allows an acceptable twining rate (1.82 vs. 2.14). The physiological parameters of both groups are similar but different during the day.

Key Words: Goat, Reproduction, Frozen Semen, Çukurova

ÖZ

YÜKSEK LISANS TEZI

SUNİ DÖLLEME KEÇİLERDE BAZI ÜREME PERFORMANSI ÜZERİNE ETKİLERİ

Dehouegnon Jerry AGOSSOU

ÇUKUROVA ÜNİVERSİTESİ
FEN BİLİMLERİ ENSTİTÜSÜ
ZOOTEKNI ANABİLİM DALI

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: Prof. Dr. Mahmut KESKIN

Bu çalışma Doğu Akdeniz bölgesinde bulunan Çukurova Üniversitesi, Ziraat Fakültesi Döner Sermaye İşletmesi Süt Keçiciliği Araştırma ve Uygulama Çiftliğinde yetiştirilen keçilerde, dondurulmuş teke semeninin suni döllenme yöntemi ile çiftleştirilen keçilerde üreme performansları üzerine etkisini belirlemeyi amaçlamaktadır. Çalışma Ağustos 2016 ve Eylül 2017 arasında, 60 Alpin keçisi üzerinde yürütülmüştür. Keçiler 30'ar baş olmak üzere iki gruba ayrılmıştır. Bir grup normal prosedür uygulanarak, diğer grup ise nar suyu ile zenginleşitirilmiş spermalar ile suni tohumlama yöntemi kullanılarak çiftleştirilmiştir. Bu çalışma boyunca, her iki grup vajinal sünger (20 mg progestagen, flororestone asetat, Chronogest CR, Intervet, Fransa) kullanılarak östrus senkronize edildi. Kızgınlık gösteren hayvanların kontrol grubu normal, deneme grubu ise intra-uterin yöntemi ile suni olarak tohumlandı. Her iki grubun fizyolojik adaptasyon parametreleri de belirlendi.

Çalışma sonunda östrüs senkronizasyonu sonrası normal çiftleştirme prosedürü uygulanan keçilerin suni tohumlananlara göre döl veriminin (%93; % 70), yaşama gücünün ve oğlakların doğum ağırlığının (3.83 ± 0.23; 3.15 ± 0.11) daha yüksek olduğu belirlenmiştir. Ancak suni tohumlama metodu ile çiftleştirilen keçilerin ikizlik oranlarının diğer gruptan daha yüksek olduğu belirlenmiştir (1.82 ; 2.14). Her iki grubun fizyolojik parametreleri benzer ancak gün içinde farklı olarak saptanmıştır.

Anahtar Kelimeler: Keçi, Üreme, Dondurulmuş Sperma**,** Çukurova

EXTENDED SUMMARY

The important industrialization of milk and cheese production has led to an intensification goat farming that involves the use of highly productive breeds subjected to new production strategies such as improved reproductive methods and selection programs. Recently several assisted reproductive technologies have been developed to control and improve reproductive characteristics and productivity in goats. Two of the most commonly methods used in biotechnology of reproduction are semen cryopreservation and AI. All these processes allow an accelerated production of genetically valuable offspring and improvement reproductive performances. Different factors affecting the success semen cryopreservation and AI have been indentified and include: type of extender and cryoprotectant, cooling rate, thawing rate and packaging, as well as the individual animal variation.

This study compare the effect of artificial insemination using the frozen semen buck semen and natural mating on reproductive performances in Alpine does at the Dairy Goat Research Farm located, Çukurova University, Faculty of Agriculture. Sixty reproductive Alpine females have been involved in this study. Goats were divided into two groups: 30 does were naturally bred using well performing males and 30 goats were artificial inseminated using cropreserved semen. Oestrus in all experimental goats has been synchronized using vaginal sponge impregnated with 20 mg of progestagen FGA. The physiological data were recorded during weekly through the trial period. They were collected four times per day: in morning (06:00- 07:00 am), afternoon (12:00-01:00 pm), evening (04:00-05:00 pm) and night (12:00- 01:00 am). The rectal temperature (RT) of goat was taken by introducing the digital thermometer into the rectum for 30 seconds and the displayed value was recorded. It was observed that does natural mated achieved better fecundity (93 vs. 70%), twining rate (1.82 vs. 2.14) and birth live weight (3.83 ± 0.23 vs. 3.15 ± 0.11) than the use of intrauterine AI after oestrus synchronization in goats.

Experimental animals displayed a higher thermal humidity index (THI) in afternoon (89) than morning (78). This exhibits the thermal stress of does during trials. The thermoregulatory response is directly expressed by an increasing RR, RT, PR, respiratory evaporation and sweating rate.

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SYMBOLS AND ABBREVIATIONS

AI	: Artificial Insemination
CAT	: Catalase
DDGS	: Distiller's dried grains with solubles
DM	: Dry Matter
DNA	: Deoxyribonucleic Acid
GSH	: Glutathione
GSHPx	: Glutathione Peroxidase
HS	: Heat Stress
mg	: Milligram
min	: Minute
ml	: Millilitre
NaB	: Natural Breeding
mm	: Millimetre
° C	: Degree Celsius
Kg	: Kilogramme
%	: Percentage
ROS	: Reactive Oxygen Species
PGF2α	: Prostaglandin F2alpha
PJ	: Pomegranate Juice
PMSG	: Pregnant Mare Serum Gonadotropin
THI	: Thermal Humidity Index

1. INTRODUCTION

High reproductive performances play a fundamental role for the sustainable improvement of animal productivity and are critical factors influencing the profitability of goat keepers. The performances of goat flocks depend directly on the genetic potential, management practices and environmental conditions in which animals are reared. Additionally, the interaction between these factors determines the adaptation and reproduction efficiency of the animals (Aguiar et al., 2013). Although large dependence to these factors, the control of reproduction in goats can be performed using different methods known as assisted reproductive technologies developed in recent years (Abecia et al., 2012; Paramio and Izquierdo, 2014). Semen cryopreservation and artificial insemination (AI) are two of these methods used in biotechnology of reproduction. Semen cryopreservation consists to cool and store male reproductive cells in liquid nitrogen at low temperature of -196°C, where all metabolic processes are arrested (Kundu et al., 2011; Sikarwar et al., 2015). By this technique sperm is indefinitely stored, widely used and can easily be exported facilitating international exchange of genetic materials. It promotes the reproduction of animal in reproductive and non reproductive seasons through the application of AI methods. Ultimately, it extends the effectives reproductive life of a valuable male beyond its own life (Nazar et al., 2008).

The most commonly used extenders for buck semen cryopreservation are TRIS-glucose or skim milk (Dorado et al., 2007). Many factors contribute to a successful semen cryopreservation. They include the interactions between cryoprotectant, type of extender, cooling rate, thawing rate and packaging, as well as the individual animal variation (Lemma, 2011). Studies showed that the oxidation of various unsaturated fatty acids contained in semen results in reactive oxygen species (ROS) during the freeze-thawing process. These oxygen species related to synthesis of hydrogen peroxide are detrimental to post-thawed goat semen motility, DNA fragmentation and acrosome integrity (Baumber et al., 2003;

Peris et al., 2007; Üstüner et al., 2015; Zeitoun and Al-Damegh, 2015; Gürler et al., 2016).

Bansal and Bilaspuri (2011) stated that, spermatic abnormalities such as axonemal phosphorylation failure, lipid peroxidation, and the loss of motility and viability of spermatozoa are due to high concentrations of the ROS. In addition, many evidences demonstrate that sperm physiological processes such as capacitation, acrosome reaction for fertilization can be ensured by low and controlled concentrations of these ROS (Bansal and Bilaspuri 2011). The use of antioxidants has been positively tested on the reduction of oxidation and control of the level of ROS to promote sperm motility and survival of sperm (Gordon, 2004). Categorized as of enzymatic and non-enzymatic molecules, antioxidants dispose, scavenge, and suppress the formation of ROS by maintaining frozen sperm quality (Bansal, 2014). Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx) and reduced glutathione (GSH) constitute the group of enzymatic antioxidants (Kadirve et al., 2014). While non-enzymatic group includes vitamin C, vitamin E, vitamin A, pyruvate, glutathione, taurine and hypotaurine (Agarwal and Allamaneni, 2004). These antioxidants inhibit the oxidative chain reaction-eliminating and reduce the formation of ROS.

Pomegranate juice (PJ) and peel possess a marked antioxidant capacity with a high content in polyphenols, in particular, ellagitannins, condensed tannins and anthocyanins. Some of these antioxidant molecules have been shown to be bioavailable and safe (Al-Olayan et al., 2014). Türk et al. (2008) investigating effects of PJ consumption on sperm quality, spermatogenic cell density, antioxidant activity and testosterone level in male rats found, a significant decrease in malondialdehyde level and marked increases in GSH, GSH-Px, and CAT activities, and vitamin C level were observed in rats treated with different doses of PJ. They concluded that spermatogenic cell density and sperm quality increased because increased antioxidant capacity protected spermatozoa against peroxidative damage in healthy rats. Additionally, studies demonstrated that supplementation of extender with 10-20% PJ significantly increases the post-thaw motility and viability in the chilled extended bull semen. Though, the addition of 40% and 50% PJ failed to preserve motility all over the 10 days (El-Sheshtawy et al., 2016).

On another hand studies demonstrated that heat stress results in decreased milk quantity and quality especially in goat of high genetic value (Al-Dawood, 2017).

This study aims to determine the effect of artificial insemination on some performance characteristics in Alpine does at Çukurova University, Faculty of Agriculture, and Dairy Goat Research Farm. Specifically it has been question to:

- Review the ameliorating effect of the extender enriched with PJ on frozen and post-thawed semen parameters;
- Assess the susceptible environmental factors affecting the reproductive and productive performances of Alpine goats;
- Evaluate the efficiency of AI application using the semen processed spermatozoa in pellets and straws on the reproductive and dairy performances in Alpine goats.

2. LITERATURE OVERVIEW

2.1. Semen cryopreservation in bucks

Cryopreservation is the method that preserves structurally intact living cells and tissues at very low temperatures (Pegg, 2007). It is an important method used in biotechnology of reproduction to preserve male sperm and store it in a bank for future use. It is a process during which all metabolic processes of a cell are arrested due to the cooling and the low temperature of storage (Sikarwar et al., 2015). The advantages of this technique include: the indefinitely and widely used of frozen semen, the easier exportation and facilitation of international exchange of genetic material, reproduction of animals in reproductive as well as non-reproductive seasons (Nazar et al., 2008).

The semen cryopreservation consists of: semen samples collection, the assessment of the collected sample for concentration, motility, and normal morphology, the dilution of semen sample, the diluted semen loading into plastic straws and seal with polyvinyl acetate powder and the straws cooling and freezing into liquid nitrogen.

2.1.1. Semen samples collection

Semen collection can be performed using electrical stimulation or artificial vagina to ensure the ejaculation. Semen collection with the artificial vagina is obviously dependent on the libido of the buck and the skill and experience of the operator. Bucks are usually trained to mount a restrained oestrous teaser doe and to ejaculate into the artificial vagina. Training may take from two days to several weeks, but some bucks may prove impossible to train. Once trained, the buck can be collected from once or twice daily during the breeding season and collections can be taken from some all year round (Gordon, 1997).

As for the semen collection using sine-wave stimulator, it consists to stimulate buck using rectal probes with three longitudinal, surface mounted electrodes. The probe is lubricated, gently inserted into the rectum, and then orientated so that the electrodes are positioned ventrally. The penis is prolapsed beyond the prepuce, and semen can be collected using a sterile plastic container, which is kept warm by covering it with the hand (Garde et al., 2003). Bucks are able to ejaculate after reaching 3-5 volts of electrical stimulation that can be applied for intervals of 3-5 s and alternated with rest periods of similar duration (Ortiz-de-Montellano et al., 2007; Sundararaman et al., 2007).

2.1.2. Assessment of semen samples

The assessment of semen characteristics consists to evaluate the seminal attributes of semen sample in order to determine the fertilizing potentialities of the spermatozoa. Generally, the major parameters include in semen characteristics assessment in term of quality are spermatozoa morphology, sperm concentration and spermatozoa motility (Tardif et al., 1999; Sutkeviciene et al., 2009). This microscopic examination of sperm morphology, concentration and motility is subjective as the total integrity of spermatozoa is not analyzed (Söderquist et al., 1991; Sutkeviciene et al., 2009). Studies demonstrated that semen quality and characteristics are affected by many factors such as body weight, body condition score, age, scrotal circumference measurements and testicular parameters, the breed, management, climatic and seasonal conditions, nutrition, the method of semen collection, and degree of sexual stimulation (Zamiri and Heidari, 2006; Rajuana et al., 2008; Zarazaga et al., 2009; Hammoudi et al., 2010; Akpa et al., 2013; Elhammali et al., 2013; Perumal et al., 2015; Raji and Ajala, 2015).

2.1.3. Semen dilution and packaging into plastic straws

Collected semen samples after evaluation are diluted using diluents containing a complex extender (egg-yolk, milk or milk-whey), cryprotectant (e.g., glycerol) (Kundu et al., 2011). TRIS-glucose or skim milk extenders are most commonly used for cryopreserving goat sperm (Dorado et al., 2007). Semen is diluted to a final concentration of approximately 250-500 x 106/ml; if this involves a dilution of less than 5:1, semen can be washed once before resuspending to the final concentration in the extender (Curry, 2007). After dilution, the semen is load into plastic straws and seal with polyvinyl acetate powder.

2.1.4. Cooling and freezing of semen

The straws containing the diluted semen are kept in a 500 ml beaker of water at ambient temperature and placed in a refrigerator for 1-2 h to allow slow cooling to 5°C. The straws are introduced into the tank of liquid nitrogen where they are horizontally suspended 5 cm above the surface of the liquid nitrogen for 7 min and then plunge rapidly into the liquid nitrogen. In the case of long-term storage, the straws are kept submerged in liquid nitrogen. Sperm samples may be stored indefinitely without any further loss of viability at -196°C. However, degradation of the sample can occur as a result of a partial thawing and refreezing (Curry, 2007).

The nature of the extender, cooling and thawing rate, packaging, interactions between cryoprotectant, as well as the individual animal variation have been identified as potential factors influencing the success of sperm cryopreservation (Lemma, 2011).

2.2. Factors affecting semen cryopreservation

2.2.1. Effect of medium used for semen dilution

The success of semen cryopreservation largely depends upon the type, composition as well as concentration of semen extender used during the process. In general, the medium used for semen dilution is constituted by a non-penetrating and a penetrating cryoprotectant, a buffer, and one or more sugars. Non-penetrating cryoprotectants are milk or egg yolk while penetrating cryoprotectants comprise glycerol, ethylene glycol, or dimethyl sulfoxide. As for buffer, it is Tris or Test and sugars are glucose, lactose, raffinose, saccharose, or trehalose (Büyükleblebici et al., 2014). Literature revealed that the commonly used extenders for cryopreserving goat sperm is made off Tris-glucose, egg yolk, skim milk, and glycerol. This has pH values ranging from 6.75 to 7, as mammalian semen has a pH ranging from 7.2 to 7.8 (Dorado et al., 2007; Barbas and Mascarenhas, 2009; Lemma, 2011). Skim milk based extender can maintain higher sperm quality as compare to semen stored in egg yolk based extender during liquid storage at 4ºC for 72 hours for fruitful AI and maximum for 96 hours (Mushtaq et al., 2015).

On the other hand, studies showed that the levels of non-penetrating and penetrating cryoprotectants in extender have a critical role in freeze-thawed goat semen. For instance, 10% egg yolk has exerted better functional membrane integrity, acrosome intactness than the presence of 20% egg yolk after freezing and thawing of Jakhrana goat semen (Priyadharsini et al., 2011). Moreover, glycerol concentration is an important factor affecting freezability of goat semen from different breeds (Kulaksiz et al., 2013). Thus, the optimal values of glycerol concentration providing success of semen cryopreservation in Markhoz, Saanen, Angora and Kilis is 7%, 7%, 5 % and 5-9% respectively (Farshad et al., 2009; Kulaksiz et al., 2013; Büyükleblebici et al., 2014). Additionally, at 37°C the motility, progressive motility and viability of spermatozoa increase with an increasing of glycerol concentrations while the rate of morphological normal acrosome decreases significantly (Farshad et al., 2009).

We can conclude that diluents used for freezing semen and fresh semen must have similar biochemical composition and must contain cryoprotective agents in order to reduce intra and extra-cellular, biochemical and physiological changes and protect spermatozoa against cold shock leading to membrane damage during freezing.

2.2.2. Effect of temperature and freezing rate

During cryopreservation sperm plasma membrane suffers a series of changes in fluidity due to changes in temperature. Evidences demonstrated the detrimental effects of cryopreservation on sperm motility, integrity of membrane, DNA function, and mitochondrial function (Üstüner et al., 2015; Garcia-Olivares et al., 2016). Buck sperms do not seem to be well-adapted to long-term cooling at low temperatures, A reduction of their post thaw viability and consequently low fertility rate is observed. According to results obtained by Ahmad et al. (2014) in Beetal buck, the cooling (from 37°C to 4°C in 90 min) of diluted sperm (37°C) followed by a freezing and thawing causes considerable damage after freezing and thawing to motility (89.8±1.26% vs 42.3±7.5%), plasma membrane integrity (85.3±0.92% vs 50.1±8.7), acrosomal cap (87.7±1.3% vs 45.2±8.4%), live-dead ratio (92.6±0.68% vs 56.0±10.5) and morphology (96.8±0.36% vs 81.0±5.7) of the sperm. Additionally, Üstüner et al. (2015) reported that in Saanen goats, the increase of freezing rate from 10°C/min to 24°C/min between +5°C and -150°C progressively reduced sperm motility and acrosome integrity. In terms of DNA fragmentation, they have found a non-significant difference between the freezing stages, except for freezing rate at 24°C/min and 15°C/min, where DNA fragmentation was 32.5% 19.1% respectively. They also reported that DNA damage observed for the lowest freezing rates (10°C/min) and highest (24°C/min) was higher than the other freezing rate groups (12°C/min and 15°C/min). They concluded that post-thawed DNA damage increases as the freezing rate from 10°C/min to 24°C/ min between +5°C and -150°C.

In conclusion, the survival of spermatozoa can be significantly influenced by cooling rate from temperature just above 0°C after freezing and thawing (Sarma et al., 2015). For this, some have proposed to cool down the sperm beyond the traditional cooling temperature (4-5°C) to allow sperm plasma membrane to accommodate those changes in fluidity without losing selective permeability (Garcia-Olivares et al., 2016).

2.2.3. Effects of length of semen storage

Another factor that might alter post-thawed sperm characteristics are the duration of storage and concentration of sperm in a sample. In effect, it was well documented that sperm morphology, motility, viability and low fertility of frozen semen in buck are decreased progressively with increasing the period of storage. In a study on West African Dwarf buck raised in Sudano-Guinean area of Cameroon, Ngoula et al. (2012) have found that during storage at 4°C, the sperm mobility declined with storage time that was 105 hours and 65 hours after semen preservation in Tris-based extender (from score 4 to score 0) and skimmed milk-based extender (from 3.57 at the time of dilution to a score 0) respectively. Similar results have been found by Wahjuningsih et al. (2012) reporting a decreasing of sperm motility with increasing length of storage in 5°C. Also, the same authors have stated that concentration of spermatozoa affect the motility of individual and they pinpointed the concentration of sperm 40 x 10^6/ ml and length of storage 0 h in 5°C showed the highest motility of spermatozoa.

2.3. Pomegranate juice: Protective effects of reproductive cells against damage during cryopreservation

Commonly known as pomegranate, the *Punica granatum Linn* (Punicaceae) is a shrub or a small tree, native to the Mediterranean region (Celik et al., 2009). The *Punica granatum Linn* produce fruits extensively used as based dietary supplements, cosmetic and pharmaceutical products (Al-Olayan et al., 2014; Kahramanoğlu and Usanmaz, 2016). It has immense content of essential nutrients and medico-chemicals proprieties including antioxidant, anti-inflammatory with beneficial or therapeutic effects on human health (Rahimi et al., 2012; Kahramanoğlu and Usanmaz, 2016).

Several studies indicate the protective role of the use pomegranate peel, juice, and seed as antioxidants source against oxidative stress and preventing DNA damage (Guo et al., 2007; Al-Olayan et al., 2014). Recent studies shown that the addition of pomegranate extracts including peel, juice, or seed improved the output quality of sperm in male rats, rabbit bucks, roosters, cattle (Türk et al., 2008; Zeweil et al., 2013; Al-Daraji, 2015; Nikseresht et al., 2015; El-Sheshtawy et al., 2016; Türk et al., 2016). The study results of Türk et al. (2008) demonstrated a positive effect of the supplementation of concentrated PJ on rats' semen quality by increasing spermatogenic cell density, epididymal sperm concentration, sperm motility and decreasing spermatic abnormalities rate and preventing DNA damage (Nikseresht et al., 2015). In roosters, the addition of 6 and 8 ml PJ per 100 ml of the semen diluents significantly improved the seminal quality of roosters. This positive effect of PJ is directly exhibited at semen collection or during the *in vitro* storage of the semen for up to 72 h. In heat stressed rabbit bucks, Zeweil et al. (2013) found that the supplementation with 1.5, 3.0, or 4.5% pomegranate peels in the diet increased ejaculate volume by 19, 18 and 12%, and seminal plasma fructose by 7, 18, and 24%. In addition the results demonstrated an improvement of sperm motility by 28, 34 and 49%, and an increase of spermatic total output by 37, 69 and 102%, while the dead rate of spermatozoa was 24, 32 and 64% respectively (Zeweil et al., 2013).

On another hands, the supplementation of bull semen extender with 10% and 20% of PJ have been demonstrated to have a significant increasing effect on the post-thawed sperm motility and viability (El-Sheshtawy et al., 2016). This good effect of PJ is due to the powerful and very potent antioxidants activity, which depends on vitamin C content of juice, rich tannins and polyphenolic flavonoids compounds (Al-Daraji, 2015). The consumption of PJ enhances sperm resistance to lipid peroxidation by reducing the CCl4- induced damages in male reproductive organs and cells. It also increases the testosterone concentration in conjunction with protein oxidation (Türk et al., 2016). PJ contains a large amount of vitamins notably A, C and E, all of which have boosting effect on sexual libido in men and women (Al-Daraji, 2015). The same author pinpointed that the high amount of Vitamin A results in increase of blood concentration of testosterone and estrogen, while a deficiency of vitamin E in the diet could lead to a lower sex drive and reduced fertility. As for vitamin C, it increases men's semen volume with the expression of high sexual appetite (Al-Daraji, 2015).

2.4. Reproductive and genetic improving effect of Artificial insemination in goats

Artificial insemination consists to transfer of the collected semen (fresh or frozen) from buck to the reproductive tract of the doe. Important technology for improving animal production and preservation of indigenous breeds in many parts of the globe, the consistent use of AI allows better control of reproduction and sexually transmitted diseases, the advance of herd genetics through the rapid dissemination of high valuable genetics (Lehloenya et al., 2005; Cseh et al., 2012). For instance in dairy cow the genetic selection that is the result of transferring superior genes of progeny tested sires through AI has successfully improved by increasing the milk components and milk yield with 113-136 kg more per cow (Overton and Sischo, 2005; Stevenson, 2014). In literatures, many factors including nutrition, breeding season, climatic conditions, parity, breed, farm, depth of semen deposition, extenders composition or hormonal treatments have been indentified to affect AI success (Baril et al., 1996; Menchaca and Rubianes, 2007; Arrebola et al., 2013). The most important factor determining the success of the AI is the timing (Baril et al., 1996). Generally, in dairy goats the heat lasts 12 to 36 hours. Six hours after the oestrus onset the ovum are released. Therefore, a successful AI must be carried 12 to 18 hours after the goat comes into standing heat. Arrebola et al. (2013), assessing the factor affecting fertility in Payoya goats artificially inseminated at farm level, reported six driven factors including farm, age, group size, trans-cervical AI, number of kidding and milk production significantly influencing the fertility rate which was averagely 59%. In a comparative study to examine the effect of the used of two short-term synchronization protocol at different moments of timed artificial insemination (TAI) on pregnancy rate, Menchaca and Rubianes (2007) set two trials detailed as follow:

- Trial 1: 250 IU of equine chorionic gonadotrophin (eCG) has been administered to goat following of progestogen exposure. The cervical TAI was performed using fresh semen at 48 h or 54 h later.
- Trial 2: goat received 250 IU of eCG at sponge withdrawal or 200 lg of oestradiol benzoate (ODB) 24 h later. 54 h after the end of progestogen treatment timed artificial insemination was performed.

The results demonstrated that in trial 1, the pregnancy rate for goats artificial inseminated at 54 h (63.7%) was significantly higher than for those inseminated at 48 h (49.4%) after sponge withdrawal. In trial 2, the goat treated with ODB achieved a low pregnancy rate (40.3%) compared to those who received eCG hormonal treatment (61.0%). Authors concluded that achievement the highest pregnancy rate can be possible using short-term protocol using eCG followed by the insemination 54 h later.

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3. MATERIALS AND METHODS

3.1. Study area

This study was carried out from August 2016 to October 2017 in the Dairy Goat Research Farm Unit of Çukurova University located in the province of Adana, in the East Mediterranean region of Turkey (40 m in altitude; 36 59' North, 35 18'East). It is characterized by a typical sub-tropical climate conditions with mild and wet winters and hot and dry summers. The annual precipitation of is 450 mm (Koluman and Göncü, 2017). The extreme temperatures can reach -8.1°C (in January) and 45.6°C (in August) with an average of 28.4°C and 9.4°C in winter (December-March) and summer (May-August) respectively. The average relative humidity is 66%. The dominant winds move from north to south. However, due to change in atmospheric pressure during seasons, the winds can blow in both directions (Kaliber, 2012).

Figure 3.1. Dairy Goat Research Farm of Çukurova University

3.2. Selection and management of experimental animals

Sixty reproductive Alpine females being 2 to 4 years old have been involved in this study. Goats were divided into two: 30 does were naturally bred (NaB) using well performing males. The other 30 goats were artificially inseminated (AI) using frozen semen. Animals with a body condition score (BSC) of 3-3.5 on a scale of 1 to 5 (1 being very thin and 5 being overweight) have been selected. The determination of the BSC has been performed by three different technicians. The animals are identified by ear tags with given numbers. Animals were healthy without any diseases include abscesses, pink eye, pneumonia, and internal or external parasites and being able to move pain-free without limping. Throughout this trial the animals were collectively kept in pens and fed based on different rations formulated according their physiological stage and based on daily requirements of nutrients (notably protein and energy) recommended by National Research Council (2007) containing forage (hay and alfalfa) and concentrate feed. The ration contained 40% of concentrate fed and 60% of forage. The composition of the feed is given in Table 3.1.

Table 3.1. The composition (DM %) total mixed ration used in the study

Feed Ingredients	%	Chemical compositions	%
Barley	5.2	Dry Matter	89.9
Corn	14.2	Ash	6.8
Wheat bran	15.0	Ether extract (EE)	2.4
DDGS	9.1	Crude Protein	14.8
Corn bran	6.0	Acid Detergent Fiber	23.0
Cotton Seed Meal	2.8	Neutral Detergent Fiber	38.0
Sunflower Meal	6.0	Crude fiber	16.2
Lime	1.2		
Salt	0.5		
Vitamin-Mineral Premix*	0.1		
Alfalfa hay	40.0		

*Vitamin Mineral premix contains 15.000000 IU vitamin A, 3000000 IU Vitamin D, 60000 mg vitamin E, 50000 mg Fe, 10000 mg Cu, 50000 mg Mn, 150 mg Co,150000 mg Zn, 800 I, 150 mg Se, 50000 mg Mg per kg.

Clean water has been provided ad libitum for them. Also, the routine management practices including preventive health measures have been observed to maintain the animals active and healthy.

Figure 3.2. Experimental animal materials (Alpine goat and buck)

3.3. Environmental data collection

With dry bulb thermometer the climatic data including minimum and maximum values of ambient temperature (ºC), and relative humidity were recorded. These data helped to calculate the Thermal Humidity Index (THI) according to following formula:

$$THI = db - (0.55 - 0.55\ RH)\ (db - 58).$$

Where db is the dry bulb temperature in and RH is the relative humidity (RH %)/100. THI values of less than 72 are probably not stressful, 72 to 78 are stressful, and over 78 extreme distresses occurred and animals were unable to maintain thermoregulatory mechanisms or normal body temperature (Abdel-Samee, 1996).

3.4. Processing of cryopreserved semen

French straws (0.25 ml) were stored in liquid nitrogen (-196° C) until use. The thawing process consists to place individually straws into a warm water bath at 37°C for 30 seconds. The post thawed semen quality was microscopically assessed by the experts. Semen with good attributes (high score of mobile spermatozoa: over 55%, sperm motility equals or above to 3.0 with a concentration of 55 x 10^6 spermatozoa/ml has been used.

3.6. Oestrus synchronization and Artificial Insemination of does

The oestrous synchronization in does have been performed based on the timed artificial insemination protocol carried out with 11 days and using vaginal sponge impregnated with 20 mg of progestagen FGA (fluorogestone acetate, Chronogest CR, Intervet, France). The sponges have been inserted in vagina and maintained for 11 days, 48 hours before removal of sponges an intramuscular injection, separately, of 2.5 mg of PGF2α and 300 I.U. of PMSG have been applied on animals (Figure 3.3.). 24 hours after removal of sponges, the goats have been observed for the standing heat detection by using bucks equipped with an apron to avoid penetration. Detection of heat onset has been performed in morning at 08:00-09:00 am and evening at 05:00-06:00 pm. 48 to 52 hours after the end of sponge removal; the intrauterine AI method has been performed in does using the prior thawed semen and as described by Tsuma et al. (2015). Only semen with high score of mobile spermatozoa (over 55% of mobile spermatozoa), sperm motility (3.0 or above) and concentration of 55 x 10^6 spermatozoa/ml has been used. The oestrus synchronization and AI have been performed by the end of August and beginning September 2017 respectively.

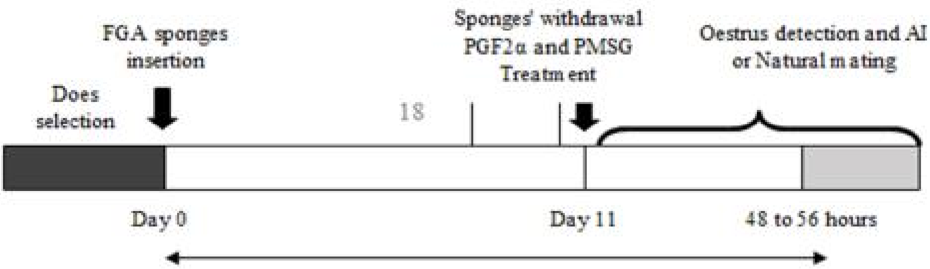

Figure 3.3. Oestrous synchronization protocol: Adapted from Yotov et al. (2016)

For naturally mated goats a ratio of 1 male for 5 females was applied in addition. All females were mated by intrauterine methods within 48 hours following oestrus detection. Eight hours after the first mating, they were mated for the second time with the same buck.

Pregnancy has been confirmed by trans-abdominal ultrasound (5.0 MHz.) 35-40 days after the insemination. Reproductive parameters including pregnancy rate, fecundity rate, litter size, weight of kid have been determined. Kidding has been recorded from February to March.

Figure 3.4. Preparing the speculum and inserting the sponge into the cervix

Figure 3.5. Performing the intrauterine AI in experimental animals

Figure 3.6. Performing the pregnancy detection in experimental animals using by trans-abdominal ultrasound scanner

Figure 3.7. Experimental Female giving birth

3.7. Physiological data collection

The rectal temperature (RT) of goat was taken by introducing the digital thermometer into the rectum for 30 seconds and the displayed value was recorded. After recording of data, the thermometer was disinfected before the next one. Pulse rate (PR) and respiration rate (RR) were recorded by using stethoscope and counting the movements of flank. Skin temperatures of hindquarter (STH) and skin temperatures udder (STU) were recorded by using a non-contact thermometer. The thermometer was kept 10 cm away from skin. These data were recorded during weekly through the trial period. They were collected four times per day: in morning (06:00-07:00 am), afternoon (12:00-01:00 pm), evening (04:00-05:00 pm) and night (12:00-01:00 am).

3.8. Weaning process and Milk collection and analysis

During the first week, kids freely suckled and from the second week to four week (one month) alfalfa hay is supplemented to them in addition to the milk. From six weeks to concentrate feed has been served; restricted suckling were applied to kids and of milking was carried out once a daily. From sixty days, kids were progressively weaned until seventy five days when they were completely weaned. During this period goats were milked twice daily. Milk samples were individually collected monthly (from March to September 2017) at second milking throughout the automatic milking system. From sixty days, kids were partially weaned and goats were milked twice daily. Milk samples were individually collected monthly at second milking throughout the automatic milking system. Milk samples collected into 50 mL plastic tubes were carried to the laboratory for analysis of milk dry matter, crude protein, crude fat and pH using Milkoscan FT-120 (FT-120; Foss, Hillerød, Denmark). Milk yield of animals were recorded were weekly recorded.

3.9. Statistical analysis

The study was conducted according to the experimental set as randomized blocks design. Statistical analyzes of the data were performed using the SPSS 20 package program. Difference between physiological parameters' means was analyzed by Duncan's multiple range tests and the mathematical model of the experiment for is as follows:

$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}$

Where:

Y_{ijk} = observation k in group i and time j.

μ = the overall mean

α_i = the effect of group i

β_j = the effect of time j

$\alpha\beta_{ij}$ = the interaction effect of group i and time j.

εijk = random error;

i = the number of groups; j = the number times; k = the number of observations in each group for each time.

The statistical analysis of data related to reproductive parameters, milk production and composition was performed using the procedure of the generalized linear model using of SPSS 20. The difference between reproductive groups was assessed using t-student procedure.

4. RESULTS AND DISCUSSION

4.1. Climatic measurement during experimental

The recorded values for climatic parameters including ambient temperature (Ta), Relative Humidity (RH) and Temperature Humidity Index (THI) in the morning (06:00-12:00 am) and evening (03:00-07:00 pm) are shown in Table 4.1. The results demonstrated that Ta (33.23 ± 0.12 vs. 37.15 ± 0.25), RH (62.81 ± 0.56 vs. 69.53 ± 0.98) and THI (78 vs. 89) were significantly ($P< 0.001$) higher from afternoon (03:00 pm) to evening (07:00 pm) than morning (06:00 am) to noon (12:00 am). THI is the most common stress index used for thermal stress assessment in animal (Spiers et al., 2004). Silanikove (2000) suggested that THI values of 70 or less are considered comfortable, 75-78 stressful, and values higher than 78 are distressful. In the present study, the obtained value of THI in morning (78) showed that goats are under stressful conditions in morning through the trial period. This is exacerbated in the afternoon and evening when the THI reached 89. These findings are consistent with those of Koluman et al. (2009), who recorded higher values for Ta (32.25±0.16 vs. 35.23±0.12), RH (62.88±0.56 vs. 64.89±0.56) and THI (84 vs. 89) in evening (06:00-07:00 pm) compared to morning (07:00-08:00 am). Silanikove and Koluman (2015) argued that dairy goats are more adapted than dairy cows as the THI for expression of an extreme heat stress are respectively superior to 90 and 84.

Table 4.1. Climatic data and Thermal Heat Index values

Traits	Hours	Average Values
Ambient Temperature (°C)	06:00-12.00 am	33.23 ± 0.12
	03:00-07.00 pm	37.15 ± 0.25
Relative Humidity (%)	06:00-12.00 am	62.81 ± 0.56
	03:00-07.00 pm	69.53 ± 0.98
THI	06:00-12.00 am	78
	03:00-07.00 pm	89

The study of all the environmental indices such temperature, air velocity, relative humidity, solar radiation and the level of rainfall is fundamental to understand the potential future impacts of climate change on agriculture especially livestock production and human welfare.

Geographically, Turkey is located in a transitory zone between arid and temperate climates, characterized by Mediterranean and continental climate types. Adana is located in the Eastern Mediterranean region of Turkey. This region is highly vulnerable to climate change impacts, where, during the day maximum temperatures appear to increase most rapidly (Lelieveld et al., 2012; IPCC, 2013; Turunçoğlu et al., 2018). The assessment of climate evolution and projection in Turkey indicates that, the average annual temperatures are expected to increase between 1°C-2°C, 1.5°C-4°C, 1.5°C-5°C, in the period 2016-2040, 2041-2070, 2071-2099 respectively. For the last 30 years of the 21st century (2071-2099), the thermal projection in winter would be higher for the eastern and inner parts of Turkey, where increasing temperature would be reached by 3°C (Demircan et al., 2017). Over the same period of time, the precipitation amount will decrease especially in winter following an irregular regime (Demircan et al., 2017). All organisms can be exposed to heat stress especially in the Mediterranean regions where the humidity and average temperatures are very high (Kibar et al., 2017). Heat stress affects reproductive and productive performances in both male and female animals for all species. Heat stress impairs reproductive organs functioning, affects lactation process in dairy animals, decreases growth and makes them vulnerable to diseases.

4.2. The physiological responses of experimental goat to environmental features

The daily recorded data on the physiological responses: Rectal Temperature (RT), Respiration Rate (RR) and Pulse Rate (PR), Skin Temperatures of Hindquarter (STH) and Skin Temperatures Udder (STU) are presented in the

Tables 2. The result showed that, during the day (from 06:00 am to 05:00 pm), the RR (breaths/min), PR (beats/min), RT (°C), STH (°C) and STU (°C) significantly (P< 0.05) increased in both groups: goats naturally bred (NaB) and goats inseminated (AI) as the ambient temperature augmented. Statistically, there were not significant differences between the experimental groups (goats naturally bred and goats inseminated) for RT, PR, RR, STH, and STU.

Table 4.2. Mean values ± SE of physiological parameters for experimental units

Traits	**Hours**	**Natural Breeding**	**AI Goat**	**Sig.**
RT (°C)	06:00-07:00 am	38.12 ± 0.03[a]	38.81 ± 0.03[a]	NS
	12:00-01:00 pm	38.74 ± 0.03[ab]	39.01 ± 0.03[ab]	NS
	04:00-05:00 pm	39.16 ± 0.03[b]	39.88 ± 0.04[b]	NS
	12.00-01.00 am	39.15 ± 0.04[b]	39.98 ± 0.05[b]	NS
	Sig.	0.05	0.05	
RR (breath/min.)	06:00-07:00 am	46.33 ± 1.28[a]	48.24 ± 1.36[a]	NS
	12:00-01:00 pm	73.32 ± 1.48[b]	75.56 ± 1.83[b]	NS
	04:00-05:00 pm	72.04 ± 2.03[b]	73.81 ± 1.94[b]	NS
	12.00-01.00 am	65.34 ± 1.72[b]	67.20 ± 1.77[b]	NS
	Sig.	0.006	0.005	
PR (beat/min.)	06:00-07:00 am	81.24 ± 1.15[a]	80.90 ± 0.99[a]	NS
	12:00-01:00 pm	92.61 ± 1.17[b]	94.85 ± 1.20[b]	NS
	04:00-05:00 pm	89.98 ± 1.39[b]	91.64 ± 1.34[b]	NS
	12.00-01.00 am	82.43 ± 1.25[a]	84.36 ± 1.04[a]	NS
	Sig.	0.004	0.004	
STH (°C)	06:00-07:00 am	27.99 ± 0.18[a]	28.75 ± 0.19[a]	NS
	12:00-01:00 pm	33.37 ± 0.21[b]	32.46 ± 0.18[b]	NS
	04:00-05:00 pm	34.46 ± 0.17[b]	34.76 ± 0.16[b]	NS
	12.00-01.00 am	29.10 ± 0.17[ab]	30.20 ± 0.21[a]	NS
	Sig.	0.034	0.032	
STU (°C)	06:00-07:00 am	29.65 ± 0.19[a]	29.74 ± 0.27[a]	NS
	12:00-01:00 pm	34.85 ± 0.19[b]	34.45 ± 0.19[b]	NS
	04:00-05:00 pm	35.68 ± 0.13[b]	35.73 ± 0.15[b]	NS
	12.00-01.00 am	30.79 ± 0.23[a]	30.93 ± 0.27[a]	NS
	Sig.	0.012	0.023	

Differences were illustrated on the hours for RT, RR, PR and ST as a,b,ab

These results are similar to those obtained by Hooda and Upadhyay (2014) who found in crossbred (Alpine x Beetle) goat exposed to high temperature (40° C to 44° C) an increasing trend of the RR (from 19.50 ± 0.23 to 20.44 ± 0.28 breaths/min), PR (from 57.39 ± 0.26 to 60.05 ± 0.26 /min) and RT (101.40 ± 0.18° F to 101.40 ± 0.20° F). Additionally, the present results were in agreement with those of Koluman et al. (2009), who demonstrated that heat stress significantly increased the RT, RR and RR in Saanen goats. Animals kept under elevated ambient temperature and high THI are unable to maintain thermoregulatory mechanisms or normal body temperature (Silanikove 2000). The physiological responses of animals under uncomfortable thermal conditions are expressed by an increasing RR, RT, PR, respiratory evaporation and sweating rate. This thermoregulatory response directly affects water consumption and feed intake thereby, reduces reproductive and productive performances (growth rate, milk yield), and even death in extreme cases. Dairy breeds are typically more sensitive to HS than meat breeds, and higher producing animals are, furthermore, susceptible since they generate more metabolic heat (Brasil et al., 2000; Das et al., 2016).

4.3. Reproduction and dairy performances of experimental goat

The data on reproductive traits of Alpine goats after using natural mating and artificial insemination by frozen semen and following the oestrus synchronization are given in the Table 4.3. The obtained fecundity rates for natural mated and artificial inseminated goat were 93 and 70% respectively. The fecundity rate in NaB does is higher than that recorded in AI does. The pregnancy rate, fecundity rate and number of newly born kids are the most important indicators to evaluate the success of reproductive performance in animal agriculture (Yotov et al., 2016). The fecundity rate calculated in NaB does groups in this research is higher to 85.7% recorded by Fonseca et al., (2005) in Alpine goats treated with intravaginal sponges (MAP, 60 mg) for 9 days plus 200 IU eCG and 22.5 μg d-cloprostenol 24 h before sponge removal and similar to 93.44% reported by

Duričić et al. (2012) in Boer goats reared in semi-intensive environment in Croatia. If the results showed that mortality rate (4 vs. 2%) and kidding interval (463.965 ± 142.4 vs. 453.85±154.56 days) were significantly higher in AI goat than NaB females, this is not the case for the twining rate (2.14 vs. 1.82 %) for which, the obtained value was not significant different.

Table 4.3. Reproduction Traits of natural bred and AI inseminated Alpine goats

Traits	Natural Breeding	AI	Sig.
BCS	3.25	3.01	NS
Live weights at mating(kg)	53.10	52.80	NS
Twining rate	1.82	2.14	NS
Litter weight at birth (kg)	3.83 ± 0.23	3.15 ± 0.11	NS
Litter weight at weaning (75 days) (kg)	12.44 ± 1.25	11.16±1.39	0.05
Mortality rate of Kids (birth/weaning. %)	2	4	NS
Kidding interval (days)	453.85±154.56	463.965±142.4	0.05
Litter size (no. of kids)	51	45	
Fecundity rate (%)	93	70	

The goats artificially inseminated have longer kidding interval days compared to natural bred goats. Chawla and Bhatnagarv (1984) reported an overall average twining rate of 39.4%. The research of Duričić et al. (2012) stipulated that litter size in adult Boer does was 1.38 kids (Duričić et al., 2012). With the objective to assess the reproductive performance of Turkish Saanen goats subjected to oestrus synchronization (20 mg fluorogestone acetate sponges + IM administration of 125 mg of cloprostenol) and after intrauterine laparoscopic AI, Kulaksiz and Daşkin (2010), found that pregnancy and kidding rate of 66 and 53%, 100 and 100 % in primiparous and multiparous respectively.

Several reasons may justify this low pregnancy, fecundity and kidding rate obtained in AI group when compared to NaB. Firstly, goats must be inseminated

12 h after the onset of oestrus and 5-10 h before the expected ovulation (Lehloenya et al., 2005). In this trial AI was performed 48-52 h after the withdrawal of vaginal sponge, i.e. 24-28 h after onset of oestrus was detected. Secondly, the detrimental effect of the freeze-thawing process on frozen semen characteristics due to the over-formation of ROS can also influence the success of the AI in does. The ROS resulting from the oxidation of various unsaturated fatty acids contained in semen reduces sperm motility, mitochondrial activity, and generates sperm membrane and acrosomal damage (Mazzilli et al., 1995; Lucio et al., 2016; Zhu et al., 2017). HS is another factor important susceptible cause of poor development and the low survival rate, quantity and quality of goat embryos exposed to extreme hot weather within the first month after breeding (Rekik et al., 2012). Indeed, oocytes are damaged by HS during follicular growth and oocyte maturation. Following fertilization, the embryo itself is susceptible to maternal hyperthermia. This way HS further enhances preimplantation embryonic mortality (Chandra et al., 2015). Effects of HS on fertility involve actions during cleavage-stage embryos (Hansen, 2013) where heat shock reduce developmental potential of embryos by reducing competence to complete cleavage divisions after first cleavage (Sakatani et al., 2012) causing cellular damage that triggers the cascade of apoptosis (Santos Junior et al., 2013).

The average birth weight and live at the weaning were 3.83 ± 0.23 and 3.15 ± 0.11, 12.44 ± 1.25 and 11.16 ± 1.39 for NaB and AI groups. Kids born from NaB were heavier than those born by AI. Many studies demonstrate a negative correlation between birth weight and litter size: birth weight decreases with an increase in litter size (Freetly and Leymaster, 2004; Mellado et al., 2011). Therefore, the high observed value of litter size in this study explains the low birth weight recorded in AI group. In addition, Lehloenya et al. (2005) argued that the kids from multiple births tended to be weaker at birth than single kids. This weakness usually leads to a lower survival rate and high mortality rate of multiple kids as a result of physiological starvation in the uterus and are born with lower energy reserves. This support the higher mortality rate observed in AI in the present study.

4.4. Milk yield and composition of experimental goat

Although it is known that it will not be affected by the insemination method, some lactation characteristics are added to the thesis as complementary information. The quantity and composition of milk are given in the Table 4.4. The milk yield and composition did not significantly differ between NaB and AI groups. However the milk yield, crude protein and pH were slightly higher in AI than that collected from NaB group. As for the dry matter and fat contents of the milk, they were lower in AI compared to NaB.

Table 4.4. Average milk yield and milk composition of goats

Traits	Natural Breeding	AI	Sig.
Lactation yield (kg)	342.8 ± 51.9	345.6 ± 48.6	NS
Lactation period (months)	7	7	-
Crude fat (%)	3.66 ± 0.05	3.60 ± 0.09	NS
Crude protein (%)	3.83 ± 0.03	3.84 ± 0.06	NS
Dry matter (%)	12.61 ± 0.09	12.39 ± 0.15	NS
pH	6.17 ± 0.03	6.39 ± 0.02	NS

The lactation milk yields obtained from both NaB and AI groups are very lower compared to 577.20 ± 3.71 Kg over a lactation period of 264.51 ± 0.57 days reported in Alpine goat in Croatia (Mioč et al., 2008). However the crude fat (3.47 ± 0.01) and crude protein (3.08 ± 0.004) contents in the Croatian alpine goat were lower than those recorded in this study. Similar composition for fat (3.44), protein (3.35) and pH (6.57) has been reported by Voutsinas et al. (1990) in herd of lactating Alpine goats imported into Greece. All milk constituents evaluated as well as pH and density significantly depended on the stage of lactation (Voutsinas et al., 1990). In this study, the potential factor affecting the milk yield and composition may be the HS as animal displayed high THI (78-89). High temperatures by reducing feed intake namely dry matter, fibre and net energy intake markedly decrease milk production simultaneously as heat load increased (Peana et al., 2007; Pragna et al., 2017). Given that in heat stressed dairy animal the energetic requirements for maintenance increase by 30%, energy intake would not be enough to cover the daily requirements for milk production (Das et al., 2016). Investigating the effect of heat stress on milk production and composition in Sarda ewes Peana et al. (2007), found that the milk yield reduced by 20% (0.39 kg/d per head) as minimum temperatures changed from 9-12 °C to 18-21°C and THI augmented from 60-65 to 72-75. Similar studies showed that in Murciano-Granadina dairy goats the decline of feed intake by 20 to 30% decreased milk yield by 3 to 10% (Salama et al., 2014). According to Koluman et al. (2016), in Saanen and German improved fawn goats under mild heat stress (THI = 74.88 ± 0.17) and good nutritional conditions any difference and effect was not observed in feed intake, milk yield, milk production efficiency, and weight changes. However, protein and casein contents in milk were lower in Saanen goat than in the German improved fawn goat.

On another hands HS affects the various components of milk such as fat, protein and dry matter content which continuously decrease with increasing THI. Menéndez-Buxadera et al. (2012) reported yearly losses of 1.9 and 3.1% in yield of fat and protein for heat stressed Murciano-Granadina and Payoya goat, respectively. In addition to this, it has been demonstrated that saturated fatty acids from C6 to C12 d monounsaturated significantly decrease, whereas the long-chain fatty acids, Conjugated Linoleic Acid c9,t11 and its precursor increased significantly in a hot summer. Also short-chain fatty acids (C6:0, C8:0, C10:0), which are responsible for the development of unpleasant aromas, decreased (Salari et al., 2016). Moreover, the concentration of calcium (Ca), phosphorus (P), and magnesium (Mg) and high chloride in milk have been found to be lower for heat stressed animals (Bernabucci et al. 2013). In cow (Pragna et al., 2017), ewes (Peana et al., 2007), goat (Salama, 2014) and studies demonstrated that milk somatic cell count, increased by high temperatures. Additionally, heat stress increased the somatic cell count from 236,000 to 375,000 cells/ml as temperature increased from 21-24 °C to 33-36 °C reducing the quality of milk produced in ewe (Peana et al., 2007). Milk from goats exposed to elevated temperature had unexpected behaviour during the curd firming stage of coagulation, which would have a negative impact on cheese making process control operations.

5. CONCLUSION

In this study, the ameliorating effects of pomegranate juice on frozen semen characteristics have been highlighted. The use of pomegranate peel, juice, and seed as antioxidants source play a protective role against oxidative stress and prevent DNA damage. Literature stipulated that there is a good effect of PJ is due to the powerful and very potent antioxidants activity, which depends on vitamin C content of juice, rich tannins and polyphenolic flavonoids compounds. On another hand, this study compared the use of two reproductive methods (natural breeding and artificial insemination using frozen semen) and examined their effect on the efficiency of the reproductive and dairy performance milk-type goats under Mediterranean climatic conditions. Although, statistical significant difference were not recorded, the work showed that the oestrus synchronization followed by natural mating achieved better reproductive performances (high fecundity and survival rate and birth live weight) than the use of AI after oestrus synchronization in goats. However, the AI animal allows an acceptable twining rate. Factors potentially affecting the reproductive are the timing of AI and the exposure to uncomfortable thermal conditions. This later is expressed by a high THI, which is a good indicator to evaluate the HS in animal. HS negatively affects goat embryo development and quality during period prior to conception and the early stages of pregnancy and reduces reproductive outcomes of goat. To express all their milking potentialities, dairy animals should be kept in favourable conditions ideal environmental temperature. In this study goat were under HS, as their displayed THI in morning and evening were 78 and 89 respectively. The decline of dry matter intake is directly associated with negative energy balance, which largely responsible for the reduction in milk synthesis and milk composition. Respiration rate, pulsation rate and rectal temperature are the parameters which illustrate the mechanism of physiological adaptation. Animals increased their respiration rate in response to the increased rate of respiratory evaporation required for thermal balance. PR and RR per minute were found to be increased by the effect of environmental temperature.

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