

SEmen PARAMETERS AND THEIR SEASONAL VARIATIONS OF LOCAL ARBIA BREED BUCKS IN WESTERN ALGERIA

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Abstract

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Our study aims to investigate semen parameters of Arbia breed goats living in western Algeria. This experiment was conducted at the University experimental farm in Tiaret on one year. Semen of three bucks were collected once a week using an artificial vagina. Semen parameters seem to be influenced by season: ejaculation volume, sperm motility and sperm viability (1.1 ± 0.39 ml; $81.79 \pm 13.42\%$; $73.73 \pm 9.07\%$, respectively) were high in autumn and summer and low during spring (0.11 ± 0.14 ml, $71.07 \pm 11.28\%$, and $52.38 \pm 20.34\%$, respectively). This difference between seasons is mainly significant when comparing autumn and spring ($p < 0.05$). Semen concentration followed an opposite trend to that of other parameters, it is high during winter and spring ($7.87 \pm 2.38 \times 10^9$ spz (spermatozoon) / ml, $7.61 \pm 1.81 \times 10^9$ spz / ml, respectively) and low in autumn ($6.4 \pm 2.62 \times 10^9$ spz / ml), with no significant difference between seasons ($p > 0.05$). The seminal pH fluctuates with high values in winter and summer and low in spring and autumn. In conclusion, Arbia breed bucks at latitude of $35^\circ 15'N$ display seasonal variation in sperm production with a peak in autumn and minimum level in spring.

Key words: bucks; semen characteristics; Arbia breed; season; photoperiod

Abbreviations: ml: milliliter, spz: spermatozoon, h: hour, min: minute, g: gram, LH: Luteinizing Hormone

Introduction

Sexual behavior and semen quality are the main parameters limiting male reproductive efficiency (Karagiannis et al., 2000). In goats, the increase in production is achieved by improving their reproduction (Aboul-Ela and Chemineau, 1988). Some goat breeds exhibit significant seasonal variations in sexual activity with a maximum and minimum sexual activity periods (Baril et al., 1993). In temperate areas, photoperiod is the main environmental cue regulating the reproductive activity of goats (Chemineau et

al., 1999). However, several external factors could modify the andrological characteristics of these seasonal breeders (Zarazaga et al., 2005). At intermediate and higher latitudes, the breeding season begins when the duration of daylight becomes shorter (autumn) and ends in winter, when the photoperiod is increasing. Even in breeds reared in subtropical climates photoperiodic cues can entrain the seasonal rhythms of reproductive parameters (Delgadillo et al., 2004). Previous studies have reported significant seasonal variation in the semen characteristics of small ruminants living at higher latitudes (Ritar, 1993). The studies carried by Hammoudi et

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al. (2010) on two parameters of the sexual activity of Arabia bucks (scrotal circumference and sexual behavior) and those of Ait Amrane et al. (2013) on plasma testosterone levels show that the intensity of sexual activity varies according to the season of the year with a maximal activity during summer and autumn.

The objective of the present work is to complete researches carried on Algerian Arbia male goat. Therefore, it was undertaken to investigate seasonal variation in semen production of these males in relation to photoperiod during the four seasons of the year.

Materials and Methods

Location and experimental period

The study was conducted for a period of 14 months (from January 2015 to February 2016) on the experimental Animal Farm of Ibn-Khaldoun University of Tiaret. The latter is situated in western Algeria at latitude of 35°15' N and longitude of 1°26' E. Climatologically, this region is a semi-arid area characterized by cold and humid winter and hot and dry summer. The daily photoperiod varies between 9 h 37 min during the winter solstice and 14 h 23 min during the summer solstice.

Animals

Three healthy, mature Arbia bucks were used in the study whose animal's age ranges between 03 and 04 years at the beginning of the experiment. The bucks were kept in individual stalls and subject to a natural lighting with no physical contact with female, except the time of semen collection. They receive a daily diet of 500 g barley, and have free access to straw and water. A vitamin and mineral supplement are incorporated in the ration in the form of a salt lick. A general management schedule for deworming, disease prevention, and hoof trimming was followed.

Semen collection

Semen samples were collected from each buck once a week using an artificial vagina ($n = 176$). Immediately after collection, the ejaculates were placed in a water bath (37°C) and taken to the laboratory for the semen characteristics assessment according to methods described by Evans and Maxwell (1987) and Baril et al. (1993).

Semen evaluation

One time in the laboratory, semen samples undergo the following examinations:

Volume: the volume of ejaculates was recorded from the graduated collection vials.

pH: a drop of undiluted fresh sperm is placed on a pH-meter strips. The semen pH is assessed by comparing the color change of the pH-meter strips with a color scale provided by the manufacturer.

Individual sperm motility: motility evaluation was performed on a sample of the diluted spermatozoa with normal saline by focusing the binocular microscope at the center of the cover slip at 40 × magnification (B – 350, OPTIKA MISCROSCOPES, ITALY). Slide and cover slip temperature was maintained at 37°C by using a heating stage fitted to the microscope. Motility rate was determined by estimating the proportion of motile and non-motile cells.

Concentration: the concentration of spermatozoa in semen sample was estimated by haemocytometer using improved Malassez chamber following dilution of an aliquot of semen with saline added with formalin at 1%.

Sperm viability: to evaluate the live/dead spermatozoa rate (viability), eosin-nigrosin staining was used (Kit Vita-Eosine, RAL Diagnostics). The smears were prepared routinely by mixing one drop of diluted semen sample with two drops of the stain on a warm slide and immediately spreading the stain with one edge of a second slide. The smears were air-dried and the viability was assessed by counting 200 sperm cells with a bright-field microscopy (40 × magnification) (B – 350, OPTIKA MISCROSCOPES, ITALY). Stained sperm cells were regarded as dead.

Our work plan was read and approved by the Ethics Committee of the Veterinary Science Institute of Tiaret.

Statistical data analysis

An analysis of variation (ANOVA) was applied to study the homogeneity of semen parameters according to months on one hand, and seasons on the other. For comparison of the mean values of each parameter studied, the Tukey (HSD) test was used at the 5% error threshold. Statistical analyzes were performed using the R software version 3.3.0 (2016 – 05 – 03).

Results

Monthly variations in seminal parameters

The monthly average semen volume increase significantly between June and September (0.03 ± 0.07 ml and 1.08 ± 0.22 ml, respectively). Thereafter, its increase becomes less important between October and November until the maximum value in December (1.18 ± 0.4 ml). The monthly average volume of sperm begins to decrease markedly between January and March (0.93 ± 0.45 ml and 0.32 ± 0.17 ml, respectively) until the minimum values in May and June (0.03 ± 0.08 ml and 0.03 ± 0.07 ml, respectively).

Sperm concentration follows a reverse trend compared to

the semen volume, it is high from January to July ($8.1 \pm 5.17 \times 10^9$ spz / ml and $9.77 \pm 4.66 \times 10^9$ spz / ml) and decreased between July and December ($5.32 \pm 2.82 \times 10^9$ spz / ml).

Monthly average sperm motility does not show great variation during the year. But it should be mentioned that the monthly average of sperm motility takes values higher than 80% in August, September and October and minimum values from March to July ($73.36 \pm 3.78\%$ and $74.18 \pm 17.97\%$, respectively).

The study of sperm viability shows that the monthly rate of sperm viability is high from September to December ($75.73 \pm 13.56\%$ and $70.36 \pm 8.34\%$, respectively) with a maximum in September. Instead, the sperm viability rate is low from January to July with a minimum of $63 \pm 11.31\%$ in April.

Semen pH varies during the year, between 6.5 and 7 with a different trend from that of the other parameters. The monthly average of seminal pH is 6.92 ± 0.10 in January, it decreases to a minimum of 6.63 ± 0.15 in April and then again increases and reaches a value of 6.85 ± 0.19 in July. The same evolution occurs the rest of the year: a decrease from July to October and an increase from October to January (Figure 1).

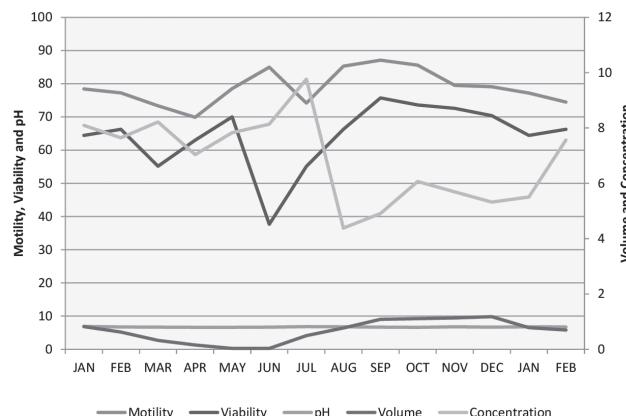


Fig. 1. Monthly variations in semen characteristics of Arbia bucks

Concentration: $\times 10^9$ ml $^{-1}$; Motility and Viability: %; Volume: ml

Table 1
Correlation between monthly means of semen parameters

	Volume	Concentration	Motility	Viability	pH
Volume	1.000000	-0.6389458 p = 0.0139	0.395821 p = 0.1612	0.677198 p = 0.0078	0.2132307 p = 0.4642
Concentration	-0.63894 p = 0.0139	1.0000000	-0.53393 p = 0.0492	-0.59655 p = 0.0243	0.1744583 p = 0.5508
Motility	0.395821 p = 0.1612	-0.5339339 p = 0.04923	1.000000	0.155788 p = 0.5948	-0.063683 p = 0.8288
Viability	0.677198 p = 0.0078	-0.5965500 p = 0.02433	0.155788 p = 0.5948	1.000000 p = 0.6614	-0.128563 p = 0.6614
pH	0.213230 p = 0.4642	0.17445836 p = 0.5508	-0.06368 p = 0.8288	-0.12856 p = 0.6614	1.0000000

Comparison between monthly semen parameters

Statistical comparison between sperm parameters studied shows the existence of a positive correlation between the volume, sperm motility and viability and seminal pH, on the one hand, and between sperm concentration and semen pH, on the other share. This correlation is significant only between sperm volume and rate of live sperm (**p < 0.01). A significant negative correlation was observed between sperm concentration and semen volume and sperm motility and viability (*p < 0.05). Also, seminal pH was negatively correlated with motility and sperm viability, but it is, in any case, significant (p > 0.05) (Table 1).

Seasonal variations in seminal parameters

The seasonal average sperm production is highest in autumn (1.1 ± 0.39 ml) and lowest in spring (0.11 ± 0.14 ml). The seasons of winter and summer are marked by intermediate productions (0.64 ± 0.44 ml and 0.67 ± 0.49 ml, respectively).

Multiple comparison of means using the Tukey (HSD) test shows that the average sperm production recorded in autumn is significantly different from that obtained in summer, winter and spring (**p < 0.001). Also, the difference was very highly significant between the sperm average production of spring and the other seasons (**p < 0.001). However, there is no significant difference between the average seasonal sperm volume of summer and winter (p = 0.9896).

Seasonal values of sperm concentration follow a different distribution of that volume; the highest values are recorded in winter and spring while the minimum values are observed in summer and autumn.

The average values of sperm motility expressed by season show that sperm motility is higher in summer and autumn ($81.79 \pm 13.42\%$ and $79.16 \pm 10.41\%$, respectively) and low in winter and especially in spring ($74.94 \pm 8.43\%$ and $71.07 \pm 11.28\%$, respectively).

The average values of sperm viability, expressed by season show that sperm viability is high in autumn ($73.73 \pm 9.07\%$) and lowest in spring ($52.38 \pm 20.34\%$).

Seasonal averages pH of semen goats shows a seasonal variation characterized by high values in Winter and Summer (6.81 ± 0.20 and 6.81 ± 0.19 , respectively) and low values in the Spring and Autumn (6.65 ± 0.15 and 6.66 ± 0.19 , respectively) (Figure 2).

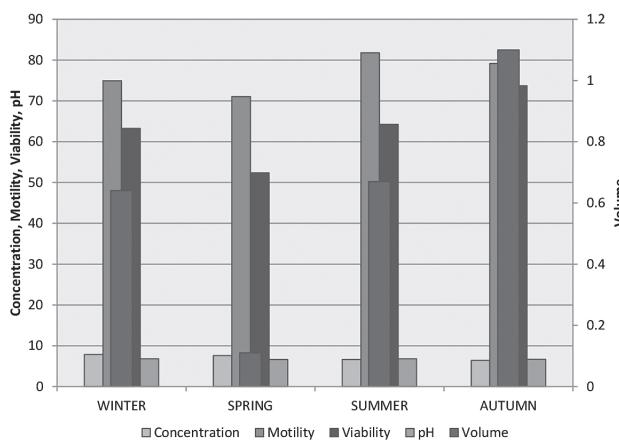


Fig. 2. Seasonal variations in semen characteristics of Arbia buck.

Concentration: $\times 10^9 \text{ ml}^{-1}$; Motility and Viability: %; Volume: ml

Comparison between seasonal semen parameters

Statistical comparison between sperm parameters shows the existence of a strong positive correlation between the volume, motility and sperm viability and a negative correlation between sperm concentration and other parameters. The seminal pH is positively correlated with all sperm parameters studied (Table 2).

Table 2
Correlation between seasonal means of semen parameters

	Volume	Concentration	Motility	Viability	pH
Volume	1.00000	-0.6565773 p = 0.3434	0.73029 p = 0.2697	0.985204 p = 0.0148	0.1653130 p = 0.8347
Concentration	-0.65657 p = 0.3434	1.0000000	-0.81522 p = 0.1848	-0.77430 p = 0.2257	0.145357 p = 0.8546
Motility	0.730298 p = 0.2697	-0.8152228 p = 0.1848	1.000000	0.809580 p = 0.1904	0.451288 p = 0.5487
Viability	0.985204 p = 0.0148	-0.7743020 p = 0.2257	0.809580 p = 0.1904	1.000000	0.132614 p = 0.8674
pH	0.165313 p = 0.8347	0.1453572 p = 0.8546	0.451288 p = 0.5487	0.132614 p = 0.8674	1.000000

Comparison between sperm parameters evolution and photoperiod

Comparing the studied sperm parameters evolution and the day-length, we see that they move inversely to photoperiod, except for sperm concentration that follows a similar pattern to it. Unlike sperm concentration, ejaculate volume, sperm motility and viability are high values, especially when the photoperiod is less than 12 hours and low values when the photoperiod is greater than 12 hours.

Discussion

The results of our work show that sperm production of Arbia breed goats varies during the year. Sperm characteristics, namely, volume, motility and viability are high in autumn, average in winter and summer and too low in the spring. Instead, the sperm concentration follows an opposite trend to the aforementioned parameters; sperm pH fluctuates between seasons.

Considering that summer and autumn are seasons with decreasing day-length and winter and spring are seasons with increasing day-length, it was agreed that the reproduction of Arbia breed goats is sensitive to photoperiod changes.

In the male, the photoperiod affects, too, endocrine balance characterized by decreases gonadotropins and testosterone secretion which influences testicular development, libido and semen quality (Motlomelo et al., 2002).

Respectively, Hammoudi et al. (2010) and Aït Amrane et al. (2013) reported that in the Arbia male goat the scrotal circumference and sexual behavior on the one hand and testosterone on the other hand are high in autumn and low in spring.

Semen volume

In our study the peak volume spermatic of Arbia breed goats is obtained last with respect to reproductive parameters

of the same breed studied by Hammoudi et al. (2010) and Ait Amrane et al. (2013).

This sequence of physiological processes can be explained by the opinion that suppose a latency which always flows between the testicular initiation process and its full expression at the semen. In addition, the spermatogenic cycle is, in most mammals, about 9 to 12 days, while the total duration of spermatogenesis takes about 40 to 54 days (Yan Cheng., 2008). Chemineau and Delgadillo (1994) reported that increased pulsatile LH activity (amplitude in June-July, frequency in September) results in early testicular growth (July-August) and the testosterone release (September) that stimulates sexual behavior (increased coupling of the sexual behavior test, decreased latency to ejaculation) and semen quality (October).

The seasonal pattern of reproductive activity in the Iranian Markhoz buck is associated with changes in the day length (Farshad et al., 2012). An increase in semen volume, total sperm count per ejaculate was observed during the natural breeding season (summer and autumn) (Talebi et al., 2009).

The average volume values of Baladi and Chami breed goats semen tend to increase significantly from the cold period to the hot period (Abi Saab et al., 2005).

For Spanish Payoya and Verata bucks, there was a clear influence of photoperiod on semen production with an increase in ejaculation volume during the decreasing photoperiod (Perèz, Mateos., 1996; Zarazaga et al., 2009).

Seasonal variations in fresh semen of Serrana breed goat were seen in volume, normal sperm and mid piece abnormalities with better performances in the autumn (Barbas et al., 2006).

In Sudan, the mean semen volume of mature crossbred bucks (Nubian × Saanen) collected during autumn was significantly high compared to those collected during winter and summer (Elsheikh, Elhammali., 2015). Results concerning monthly changes in ejaculate volume of indigenous bucks (Baghdad – Iraq) showed a significant effect of months, which is highest in October being significantly higher than in other months (Hussain et al., 2012).

Instead of our results, the best semen volume and individual motility of local Pakistani dairy goats were recorded during the spring and the highest concentrations were recorded during the winter (Qureshi et al., 2013). In equatorial area, semen volume of non-defined breed goats in Brazil did not differ between the periods of the year (Aguiar et al., 2013).

Semen concentration

In our study, we found that sperm concentration was negatively correlated strongly and significantly with ejaculate

volume. It is high at a time when semen volume is low. This result can be explained by the following points:

The accessory glands are involved in the production of 3 / 4 of ejaculate volume, the remaining 25% being produced by the epididymis. These glands are more active when the testosterone concentration is high during the breeding season and less active when this one is low during the non-breeding season.

Talebi et al. (2009), report in their study that sperm concentration follows an opposite trend to ejaculate volume reflecting seasonal variations in the secretion and release of seminal plasma by the accessory glands.

Sperm concentration was lower during the breeding than the non-breeding season in Alpine, Saanen and Damascus bucks, Payoya, Verata and Malagueña Spanish male goats (Ritar., 1993 ; Perèz, Mateos., 1996 ; Karagiannidis et al., 2000).

During autumn, the sperm concentration of semen Markhoz Iranian bucks remained low and higher concentrations were recorded in spring, winter and summer (Talebi et al., 2009).

In contrast to our study, Abi Saab et al. (2005) and Elsheikh and Elhammali (2015) report that semen concentration of Chami and Baladi breed goats, Nubian and Saanen crossbred, respectively, was higher in autumn than in spring. Similar results were reported by Ahmed et al. (1997) and Hussain et al. (2012).

As well as ejaculate volume, semen concentration in equatorial area (Brazil) did not show seasonal variations during the year (Aguiar et al., 2013).

Sperm motility and viability

Individual motility and spermatozoa viability of Arbia breeds goats are positively correlated with the ejaculate volume. This correlation is very significant between the ejaculate volume and sperm viability. Individual sperm motility and spermatozoa viability appear to be influenced by season, their rate is high in summer and autumn and low in spring. Both parameters change in a similar way during the year, but with low values of sperm viability in relation to their motility. This finding may be explained by a slightly toxic dye owns action and or the time to smear preparation (Hanzen., 2009).

Recent studies have shown that in tropical climates, the season of the year affects the biochemical composition of goat's seminal plasma (Catunda et al., 2009).

Akpa et al. (2013) report that a good concentration of sodium, potassium and calcium in the semen improves sperm concentration and motility of the Nigerian Red Sokoto goat breed.

A similar trend of these semen parameters was found by Karagiannidis et al. (2000), Abi Saab et al. (2005), Talebi et al. (2009) and Elsheikh and Elhammali (2015) who reported that progressive sperm motility and sperm viability rate are high in summer and autumn and low in winter and spring.

A clear influence of photoperiod on the percentage of motile was observed only in Verata bucks that showed an improvement during the decreasing photoperiod (Perèz, Mateos., 1996).

The percentage of motile spermatozoa in semen of non-defined breed goats did not differ between the dry and the rainy period of the year (Aguiar et al., 2013).

Seminal pH

The seminal pH of Arbia breed goats evolves differently from the other parameters studied with a high seasonal average in winter and summer alternating with weak seasonal average in spring and autumn, during the same year. The highest seasonal averages coincide with an increase in sperm concentration and an increase in sperm motility in spring and autumn, respectively. This decrease in pH during these seasons can be explained by its relationship with concentration and sperm vitality. Battaglini (1992) reported the existence of a negative correlation ($r = -0.47$) between sperm concentration and seminal pH.

The pH of pure semen follows a reverse development of that which was observed for the ejaculate concentration (Barrell, Lapwood., 1979). This reflects, probably, changes in lactic acid production, itself a function of the number of sperm per unit volume and also those of the cellular glycolytic activity (Mann., 1964).

In conclusion, Arbia local breed bucks, at latitude of $35^{\circ}15'N$, display a clear seasonal variation in sperm production. Superior semen quality and quantity were observed in late summer and throughout autumn, while inferior semen characteristics were observed in spring.

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