

Determination of Semen and Sexual Behavior Parameters of Arabian Stallions to Be Selected for an Artificial Insemination Program under Tunisian Conditions

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Abstract: The present study aimed to evaluate the sexual behavior and semen parameters quality of Tunisian Arabian stallions leading in natural breed and sheltered under Tunisian conditions, to select them for an artificial insemination (AI) program. Semen was collected and analyzed from 9 stallions aged between 7 to 23 years during the breeding season (young stallions: age <15 years, n = 5, number of ejaculates = 25; old stallions: age ≥15 years, n = 4, number of ejaculates = 18). Sexual behavior, seminal qualitative and quantitative parameters were investigated immediately after collection. The percentage of motile sperm was studied in the semen stored at 4°C in the semi skimmed milk extender and antibiotics at 0 hour and 24, 48 and 72 hours after collection. ANOVA was carried to compare variables between the two age classes, using a software RATS (version 6.10). We found that the semen quality of young Arabian stallions was acceptable to be used in an (AI) program with fresh, refrigerated and frozen semen. However, the semen of old stallions had a low quality and consequently they were allowed only to be used in AI program with fresh semen. Our study provided us the knowledge of sexual behavior and seminal parameters of our Arabian stallions and the distinction between young stallions having high quality of semen and old stallions having low quality of semen.

Key words: AI • Sexual behavior • Semen quality • Tunisian Arabian stallions

INTRODUCTION

During the breeding season, stallions in natural service mate a great number of mares. This carried the transmission of sexual diseases and decrease fertility. Therefore, to understand the causes for decreased fertility in stallions, an evaluation of fresh semen quality collected by an artificial vagina can be a mean to reveal these causes and to predict fertility of semen stallion [1, 2]. It could also suspect low fertility and select animals for artificial insemination (AI) program [3]. Besides, semen evaluation could be utilized to determine if the semen of the stallion can tolerate handling procedures such as cooling or freezing [4]. Semen examination is recommended prior to breed mares at the beginning of the breeding season or when we suspect infertility or

subfertility of the stallion. This recommendation is important for a good management of stallions used in AI program [1].

The aims of the present study were to evaluate the sexual behavior parameters during collection and the semen quality collected for the first time from Tunisian Arabian stallions known for their potential performances and practiced exclusively natural service, in order to select them for an AI program.

MATERIALS AND METHODS

General: The study took place in the stud farm of Sidi Thabet, situated in the north of Tunisia at 36° 54' 35. 16'' N, 10° 02' 31. 94'' E. Semen was collected from 9 Arabian stallions (young stallions: age <15 years, n = 5, number of

ejaculates = 25; old stallions: age ≥ 15 years, n = 4, number of ejaculates = 18). The collection undertook during the officially Tunisian breeding season, starting from 15 February to 15 June 2007.

Semen collection: Ejaculates were collected once every day for six consecutive days from each stallion using a Missouri artificial vagina (IMV, France). The artificial vagina was lubricated immediately with a non spermicidal lubricant (NETTEX, U.K) prior to semen collection. An estrus mare was used for semen collection. Sexual behavior such as preparation duration, number of mounting in the artificial vagina and collection duration were measured during each collect as described by Clément *et al.* [5] and Haras Nationaux [6].

Semen evaluation: The collected fresh semen was evaluated for color and volume. When the gel fraction was present, it was removed by a suitable filter as gauze with hydrophilic stitch to estimate the volume of sperm (ml) as described by Haras Nationaux [6]. Sperm concentration (10^6 spermatozoa / ml) was determined by the use of Thoma cell chamber after 1:40 dilution of raw semen using formaldehyde (1.85%) as reported by Haras Nationaux [6]. Moreover, sperm morphology features were studied from unstained sample semen fixed previously by the same rate of dilution formaldehyde solution and observed by a phase-contrast microscope [1].

Total sperm was also determined in the ejaculate (10^9 spermatozoa) by multiplying sperm concentration by semen volume [6]. A subject appraisal of sperm motility was achieved by optic microscopy as the same used by Rousset *et al.* [3] and Haras Nationaux [6] to estimate the percentage of motile spermatozoa in the raw semen samples immediately after filtration. Then, the semen was extended with a semi skimmed milk and antibiotics Gentamicin and Penicillin G. The percentage of dead spermatozoa was estimated by the use of an eosin-nigrosin stain at 6.9 pH and counting a sample of 150 spermatozoa over the slide [3, 6]. The percentage of motile spermatozoa was evaluated again in the semen extended under the optic microscope at 0 hour and after 24, 48 and 72 hours of cool storage (4°C) after semen collection [6].

Statistical analysis: The statistical analysis was developed on a Panel data [7]. The statistical software RATS (version 6.10) was used for descriptive statistics. ANOVA was carried to compare variables between the two age classes. The Fisher's test was used to compare

the individual and temporal parameters of the semen. Student's *t*-test was used to compare the percentage of motile spermatozoa in raw and in diluted semen. The threshold of significance was 5%.

RESULTS AND DISCUSSION

The overall means for sexual behavior are presented in Table 1. The number of mounting in the artificial vagina was higher in the young stallions than in the old ones ($p < 0.05$). We noted that times of collection stallions have been less than 200 seconds in the two age classes of stallions which was in agreement with Noue *et al.* [9]. But this parameter was higher in the young stallions than the old ones ($p < 0.05$).

A significant individual effect found for these sexual behavior parameters ($p < 0.01$). We also found that the number of mounts and the collection time affected the fertility of fresh semen such as the number of dead spermatozoa and the percentage of motile spermatozoa in the raw semen ($p < 0.05$). This result was not in agreement with those reported by Clément *et al.* [5] who showed that there wasn't any determined relation between fertility and sexual behavior. However, Sieme *et al.* [8] found that semen volume, sperm concentration and longevity of spermatozoa were influenced by the number of mounts.

Our results showed an individual effect on seminal parameters such as percentage of motile spermatozoa in raw semen, abnormal sperm, total sperm, volume of gel fraction and semen volume ($p < 0.01$). The individual variability between stallions for seminal parameters found is in agreement with the previous reports [10, 11]. This large difference in semen parameters among individuals showed that random fluctuations over time are involved [12].

We also noted that 16% of total collected ejaculate represented a volume of gel fraction which varied from 0.4 ± 0.2 ml for young stallions to 0.7 ± 0.6 ml for old stallions ($p < 0.01$) (Table 2).

Table 1: Sexual behavior of Tunisian Arabian stallions (means \pm sem)

Stallions	Number of mounts	Preparation time	Collection time
		(seconds)	(second)
Age < 15 years (n = 5)	1.6 \pm 0.1 ^a	107 \pm 16 ^a	136 \pm 16 ^a
Age ≥ 15 years (n = 4)	1.2 \pm 0.1 ^b	89 \pm 14 ^b	117 \pm 14 ^b

a, b: $p < 0.05$

Table 2: Semen characteristics of Tunisian Arabian stallions (means±sem)

Stallions	Filtered volume (ml)	Volume of gel fraction (ml)	Sperm concentration (millions spermatozoa / ml)	Total sperm (billions spermatozoa)	Percentage of dead spermatozoa (%)	Percentage of motile spermatozoa in raw semen
Age < 15 years (n = 5)	38±3 ^a	0.4±0.2 ^a	259±26 ^c	9±0.8 ^e	29±2 ^e	69±3 ^a
Age = ≥15 years (n = 4)	28±11 ^b	0.7±0.6 ^b	247±41 ^d	7±1.1 ^f	41±4 ^f	53±5 ^b

a, b: $p < 0.01$; c, d: $p < 0.1$; e, f: $p < 0.05$

Table 3: Abnormal sperm of Tunisian Arabian stallion (means±sem)

Stallions	Abnormal head (%)	Abnormal flagella (%)	Abnormal mid-piece (%)	Abnormal principal piece (%)	Cytoplasm droplets (%)	Abnormal sperm (%)
Age < 15 years (n=5)	3.4±0.5	2.2±0.3	15.8±1.8	2±0.4	2.7±0.5	25.7±2.2 ^a
Age = ≥15 years (n=4)	7.7±1.1	2.1±0.3	17.7±1.5	1±0.3	3.5±0.5	31.4±2.4 ^b

a, b: $p < 0.05$

The sperm volume filtered (Table 2) was higher in young stallions than in the old stallions (38±3 ml vs 28±11 ml, $p < 0.01$). The volume of old stallions has been inferior to the minimum value indicated by Tibary *et al.* [13] which was 30±7.1 ml for Arabian stallions. Nevertheless young stallions passed beyond this value. In his several studies on seminal characteristics of stallions, Jasko [1] found that semen volume varied from 45±30 ml to 58±24 ml. In this case, the semen volumes that our Arabian stallions got were inferior to those reported by Jasko [1]. This indicated a variability of animals sheltered under different climatic conditions.

The sperm concentration was inclined to go up in the young stallions compared to the old stallions (259±29 vs 247±41 millions of spermatozoa / ml, $p < 0.1$). We found also that the total sperm was higher in young stallions than the old stallions (9±0.8 vs 7±1.1 billions spermatozoa; $p < 0.05$) (Table 2). However, the percentage of dead spermatozoa and the percentage of motile spermatozoa in raw semen were higher in the old stallions than the young ones (41±4 vs 29±2%, $p < 0.05$; 69±3 vs 53±5%, $p < 0.01$) (Table 2). We also observed that there was a light increase in motility when the semen was extended in the two age classes, but our statistical analysis showed no difference between motility in raw and in extended semen.

Tibary *et al.* [13] found that the Arabian stallions can have in their semen a concentration from 149.9±100 to 286.8±86 10⁶ spermatozoa / ml and Jasko [1] found that it was varied from 173±118 to 335±232 10⁶ spermatozoa / ml. However, Chevalier-Clément *et al.* [14] in her study on infertile stallions defined stallion having normal fertility with a mean sperm concentration of 194±106 10⁶

spermatozoa / ml and reported that infertile and abnormal males had low concentrations (93±130 10⁶ spermatozoa) and low percentage of motile spermatozoa in diluted semen.

Seeing the lowest values of sperm concentration, total sperm and percentage of motile spermatozoa in raw and the highest values of the percentage of dead spermatozoa obtained in old stallions, we considered that these stallions had low fertility in their fresh semen compared to the young stallions.

In the two age classes of stallions, we found that the number of dead spermatozoa was increased by the number of mounting, the presence of gel fraction and the time of collection ($p < 0.01$). The number of mounting and the presence of gel fraction affected the percentage of motile spermatozoa in the raw semen ($p < 0.01$).

The percentage of abnormal sperm was less in the young stallions than in the old stallions (25.7±2.2% vs 31.4±2.4%, $p < 0.05$) (Table 3). The percentage of abnormal head, mid-piece and cytoplasm droplets were higher in the old stallions than in the young stallions (7.7±1.1, 17.7±1.5 and 3.5±0.5% vs 3.4±0.5, 15.8±1.8 and 2.7±0.5%). However, percentage of abnormal principal piece was higher in the young stallion than the old ones (2±0.4% vs 1±0.3). Clément *et al.* [15] and Brito [16] showed when there was a high percentage of abnormal sperm, this led to suspect the case of subfertility. Posor [17] reported also that some stallions having a high percentage of abnormal sperm could present abnormalities in the reproductive tract specially varicocele. Moreover, Jasko *et al.* [18] reported that there was a negative correlation between sperm head defect and the per cycle pregnancy rates. Love *et al* [19] found that a 1% in the percentage of head defect led a reduction

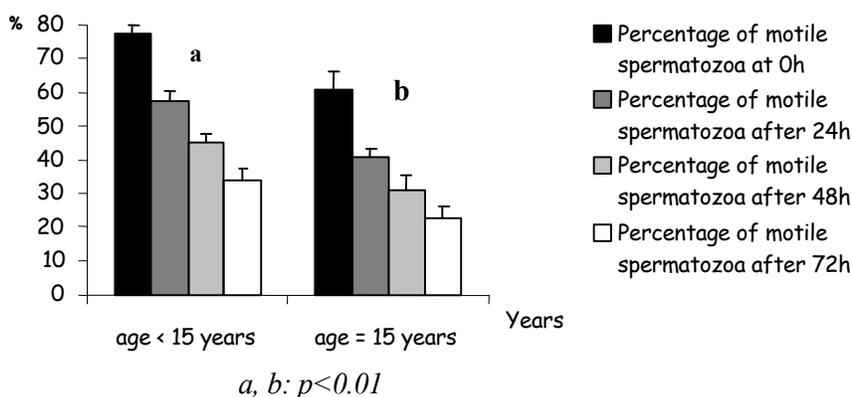


Fig. 1: Percentage of motile spermatozoa during storage for 72 hours after collection at 4°C (means±sem)

of 0.67% in the per cycle pregnancy rates. Other authors [20, 21] reported that the percentage of detached head in a normal ejaculate should be low than 5%. Card [22] affirmed that if there was a high percentage of detached sperm head, it indicated abnormal spermatogenesis or senescence in the tract. For the percentage of cytoplasm droplets, it's also correlated negatively with the per cycle pregnancy rates [23]. Jasko *et al.* [18] showed that there was a negative correlation between proximal cytoplasm droplets and per cycle pregnancy rates. However, Love *et al.* [19] showed that it wasn't a correlation between proximal cytoplasm droplets and fertility. We can add that Colenbrander *et al.* [2] showed when the percentage of normal and motile sperm was below 40% the fertility was compromised.

So, for the old stallions of our study, it was also necessary to examine the organs of their reproductive tract in order to better understand the origin of the lowest fertility in their fresh semen.

The percentage of motile spermatozoa extended in semi-skimmed milk with antibiotics stored at 4°C was decreased during its conservation for 72 hours after collection in the two age classes of stallions (Figure 1). The decrease in the percentage of motile spermatozoa was higher in the old stallions compared to the young ones ($p < 0.01$). Consequently, the semen of old stallions were allowed only to be used for an AI program with fresh semen according to the work of Haras Nationaux [6], Clément *et al.* [15] and Clément *et al.* [5]. For the young stallions under study, the motility of their spermatozoa during 24, 48 and 72 hours allowed them to be exploited for an AI program with fresh, refrigerated or frozen semen and this according to Magistrini [24], who reported that motility of extended semen after 24 and 48 hours is an important parameter in order to select stallions for AI.

CONCLUSION

Our study was taken to prove that semen evaluation is important to identify the fertile Tunisian Arabian stallions and to select them for an AI program. It provided us the knowledge of sexual behavior and seminal parameters of our Arabian stallions sheltered under Tunisian conditions and the distinction between young stallions whose semen can be used in AI program with fresh, refrigerated or frozen semen and old stallions whose semen can be used only in AI program with fresh semen.

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