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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

# Effects of ambient temperature, season, breed and age on spermiogram of sire in Tunisia

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## Abstract

The objectives were to determine the effects of ambient temperature, season, breed and age on spermogram of 13 AI sires (3 Holstein, 5 Tarentaise and 5 Brune des Alpes) raised in Tunisia whose age varied from 24 to 46 months and the body weight varied from 500 to 900 Kg. Study was undertaken from June 2007 to July 2008 on 654 fresh ejaculates. Results showed that volume, concentration and progressive motility decreased when ambient temperature increased. However, when ambient temperature increased, pH, percentages of live spermatozoa and major abnormalities increased. Besides, there was no correlation between ambient temperature, motility and minor abnormalities. There was a significant difference ($P<0.05$) for volume and percentage of live spermatozoa between the two classes of age. A significant difference existed between seasons for the pH of Holstein bulls and the concentration for Brune des Alpes bulls. Furthermore, pH, concentration, motility and minor anomalies did not change ($P<0.05$) within breeds. However, volume, percentage of live spermatozoa and major and total anomalies of ejaculates varied between the three breeds.

**Keywords:** Bull; semen; quality; season; age; breed

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## Introduction

Artificial insemination (AI) is a powerful biotechnology tool that allows producers to use superior sires, promoting faster genetic improvement and increasing profitability. During the last 10 years, production of frozen bull semen in Tunisia has increased to 137153 straws (2010). Fifty two percent of the national cows are artificially inseminated, demonstrating a great potential for the increased production of frozen bull semen and the use of artificial insemination. Studying factors affecting spermogram would be of considerable benefit to the AI industry.

Extremes climatic factors such as ambient temperature, humidity, radiation and wind influence the mammals environment and deleteriously affect reproduction (Gwazdauskas, 1984). Heat stress, in cows, activates physiological mechanisms that maintain an animal's body temperature at normal level (Marai and Haeeb, 2010). Fertility was lowered during heat stress as a result of dysfunctions in reproductive processes and alterations in energy balance (Rensis and Scaramuzzi, 2003). High environmental temperatures affects semen production and fertility of men (Sinclair, 2000), bulls and goats (Murugaiyah, 1992). Exposure to hyperthermia is harmful for spermatogenesis and testosterone levels (Murray, 1997). The present study was conducted to determine the effects of ambient temperature, season breed and age on spermogram in 13 AI bulls in Tunisia.

## Materials and Methods

### Animals

Thirteen Holstein bulls housed in single pens were used for semen collection. Three Holstein, 5 Tarentaise and 5 Brune des Alpes bulls were evaluated from July 2007 to June 2008. Bulls were 15 to 42 months old and weighing 500 to 900 Kg at the beginning of the study.

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**Semen evaluation**

Semen was collected using an artificial vagina from the bulls and immediately transferred to a 37°C water bath. Semen volume was recorded by reading from the graduated tubes. Sperm concentration was measured using a calibrated spectrophotometer. The pH, was measured via a calibrated pH meter. Percentage of progressively motile spermatozoa was assessed immediately following semen collection. A small drop of semen was placed on a pre-warmed slide, covered with a cover slip, and examined with a bright-field microscope with a heated stage. The percentage of spermatozoa that were progressively motile was estimated. Immediately after semen collection, viability (eosin-nigrosin) test were carried out and an aliquot was fixed for subsequent analysis of sperm anomalies. Under phase-contrast microscopy at least 200 spermatozoa from each ejaculate were examined in random fields (Barth and Oko, 1989). Sperm abnormalities were categorized as either minor or major and their sum as total defects according to the classification of Blom (1973).

**Semen dilution and cryopreservation**

Semen was diluted in a commercial diluent (Bioxcell, IMV, France), pre-warmed to 37°C and packaged into 0.5 ml straws (Minitube, Germany). Temperature of the semen was gradually decreased for 4 hours to 4°C. The straws were organized on racks and placed into the freezing chamber of a computer assisted system that controls the temperature inside the chamber and decreases the temperature of the semen from 4°C to the lowest temperature chosen (-120°C). After the freezing process, straws were covered in a liquid nitrogen tank until subsequent analysis.

**Statistical analyses**

All statistical analyses were performed using the Statistical Analysis System (SAS 9.0). Initially, Pearson's correlation coefficients were calculated. GLM procedure with Duncan test was used to determine the effect of breed, age, season, ambient temperature on sperm production and semen quality. The model used:

$Yij = \mu + Ti + Eij$

Yij: volume/concentration/pH/Motility/Percentage of live spermatozoa/Major anomalies/Minor anomalies /Total anomalies

μ: mean

Ti: effect of ambient temperature/breed/season/age

Eij: error

## Results

**Relationship between ambient temperature, semen production and semen quality**

Correlation coefficient (Table 1) between ambient temperatures (AT) and volume (Vol), concentration (Conc) and progressive motility indicate that they decrease when ambient temperature increases. However, when ambient temperature increases, pH, percentage of live spermatozoa (% live) and percentage of major abnormality (Major anom) increase also. Besides, there was no correlation between ambient temperatures, motility and minor abnormalities (Minor anom).

**Effect of month on semen traits**

Monthly variation of semen traits (Fig. 1, 2, 3, 4 and 5) shows that there was a fluctuation between months. Indeed, July is marked by the minimum level of pH, the maximum level of major anomalies. August is marked by the minimum level of volume and the minimum level of percentage of minor anomalies. September is marked by the minimum level of major anomalies. October is marked by the maximum value of percentage of live spermatozoa and the maximum value of percentage of minor anomalies. November is marked by the maximum value of concentration. In January, the maximum value of pH was recorded. In March, occurred the maximum value of volume and the minimum value of percentage of live spermatozoa and June, is marked by the minimum value of concentration.

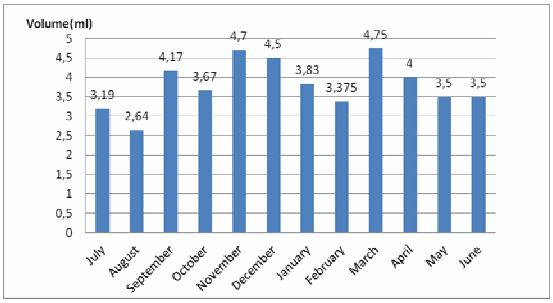


**Fig. 1: Monthly variation of mean volume of ejaculate**

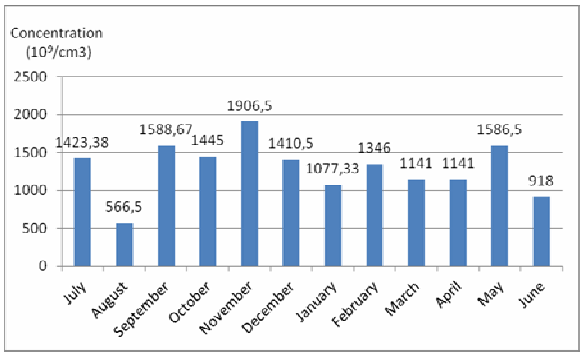


**Fig. 2: Monthly variation of mean concentration of semen**

**Effect of age on semen traits**

The comparison between two classes of age (Table 2), shows that there was a significant difference for volume (P<0.05) and percentage of live spermatozoa. But the others traits didn't differ between the two class of age.

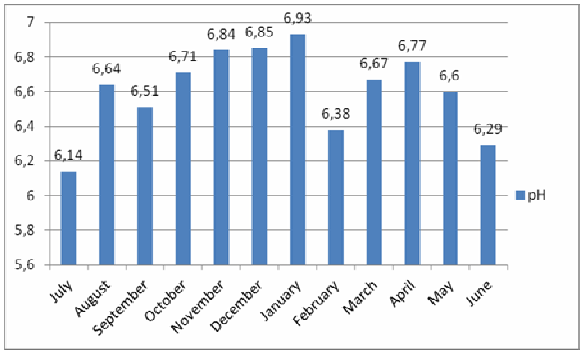


**Fig. 3: Monthly variation of mean pH of ejaculate**

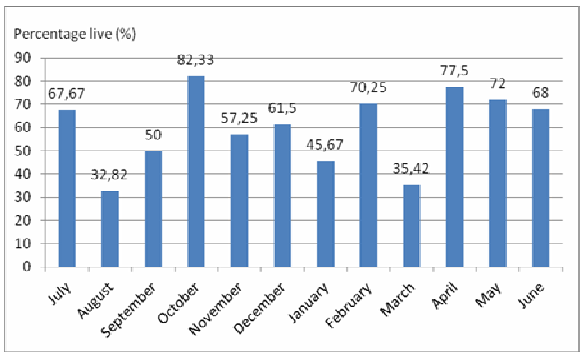


**Fig. 4: Monthly variation of mean percentage of live spermatozoa of ejaculate**

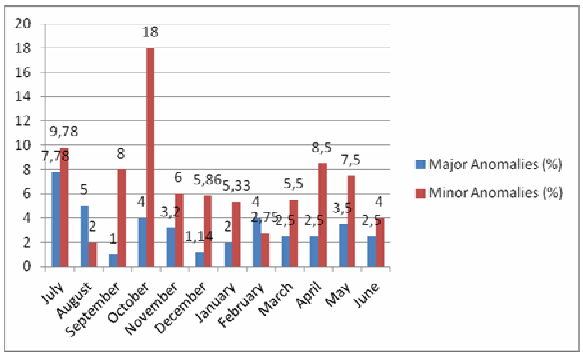


**Fig. 5: Monthly variation of mean percentage of major and minor anomalies of ejaculate**

### Effect of season on semen traits

Comparison of semen collected in summer (June, July and August) and in winter (December, January and February) (Table 3) shows that there was a significant difference for pH for Holstein bulls and concentration for Brune des Alpes bulls. But the others traits did not vary significantly between the two seasons.

### Effect of breed on semen traits

Statistical analysis of semen traits within breeds (Table 4) demonstrated that pH, concentration, motility and minor anomalies did not change (P<0.05) within breeds. However, volume, percentage of live spermatozoa and major and total abnormalities of ejaculates varied between the three breeds. Brune des Alpes bulls have the highest percentage of major and total abnormalities and the least mean of volume. Besides, Holstein bulls have the highest volume and percentage of live spermatozoa.

## Discussion

### Effect of ambient temperature on semen traits

The result of our study confirmed that ambient temperatures affect on all semen traits except motility and minor abnormalities. Different from our finding, studies conducted with Holstein AI bulls in temperate environments show that there were no significant effects of ambient temperature or humidity on sperm production and semen quality (Everett and Bean, 1982). Taylor et al. (1985) demonstrated that extreme temperatures (−24 to −19ºC and 27–32ºC) had only small effects on sperm production, causing a 0.3 ml decrease in semen volume and a small decrease in the total number of spermatozoa ($0.3\times10^9$/ml) compared with optimal temperatures (16 to 21°C). Chacon et al. (1999) reported that ambient temperature and rainfall were positively correlated with the percentage of abnormal sperm tails and proximal cytoplasmic droplets in *B. indicus*, crossbred, and *B. taurus* bulls extensively managed in Costa Rica.

Fuerst-Waltl et al. (2006) analysed semen data from two Austrian AI centres regarding the temperature, either on day of semen collection or during epididymal maturation or spermatogenesis. Temperature had important but inconsistent effects on semen production and sperm quality. Overall, however, an ambient temperature in the range of 5 to 15°C was found to be optimal for semen production.

Chacon et al. (2002) found no relationship between sperm motility and environmental temperature (P>0.05). The frequency of bent tails with cytoplasmic droplet entrapped fluctuated between monthly ejaculates. However, there was no relationship between these fluctuations and environmental temperature (P>0.05). Our study focused on real ambient temperatures measured directly in the sires pens. Others studies artificial or controlled temperatures on semen production and quality such as Vogler et al. (1991) who studied the effect of 48-h scrotal insulation on

**Table1: Correlations of Pearson between ambient temperature and semen traits**

| AT (°C) | Vol | pH | Conc | Gross mot | Ind mot | % live | Major anom | Minor anom | Tot anom |
|---|---|---|---|---|---|---|---|---|---|
| Cor pear | -0.36 | 0.35 | -0.17 | 0.07 | -0.13 | 0.31 | 0 .19 | 0.04 | 0.02 |
| Probability | 0.0010 | 0.0024 | 0.1074 | 0.6443 | 0.3438 | 0.0085 | 0.0941 | 0.4837 | 0.9725 |

**Table 2: Effects of age on quantitative traits of semen**

| Group of age | 15 < age < 20 months | Age ≥ 21 months |
|---|---|---|
| Volume (ml) | 3.43 ± 1.72[b] | 6.42 ± 1.57[a] |
| Concentration ($10^3$ spermatozoids / $cm^3$) | 1561.34 ± 527.28[a] | 1619.71 ± 446.55[a] |
| pH | 6.50 ± 0.32[a] | 6.49 ± 0.31[a] |
| Gross motility | 3.32 ± 0.96[a] | 3.51 ± 0.73[a] |
| Progressive motility | 61.17 ± 11.79[a] | 62.50 ± 12.16[a] |
| Percentage live (%) | 65.64 ± 22.44[b] | 74.91 ± 11.39[a] |
| Major Anomalies (%) | 4.53 ± 5.69[a] | 4.02 ± 3.24[a] |
| Minor Anomalies (%) | 7.58 ± 4.63[a] | 6.52 ± 4.32[a] |
| Total Anomalies (%) | 11.52 ± 8.34[a] | 10.58 ± 6.49[a] |

The numbers of the same line that don't have the same letters are significantly different (P < 0.05)

spermatozoal viability (motility and acrosomal integrity), before and after semen cryopreservation, in six young Holstein bulls. This scrotal insulation caused elevated testicular temperatures. Semen viability before and after freezing was lower during scrotal insulation than in control period (P<0.05). These differences coincided with the appearance of abnormal sperm morphology and depressed semen motility.

Meyerhoeffer et al. (1985) showed the effects of elevated ambient temperature on semen characteristics of sixteen yearlings. Their results showed that percentage of motile sperm decreased (P<0.01). Heat stress also resulted in more abnormal cells. These data indicate that exposure of bulls to elevated ambient temperatures results in decreased semen quality as evidenced by a reduced percentage of motile sperm, reduced sperm output and an increased percentage of abnormal and aged sperm.

**Effect of season on semen traits**

Our results indicated that season affected pH and concentration of semen. Several authors reported changes in ejaculate quality associated with changes in the season (Igboeli and Rakha, 1971; Everett et al., 1978; Fields et al., 1979; Everett, 1982; Everett and Bean, 1982; Kim et al., 1983; Orji et al., 1983). Muturu ( *Bos brachyceros)* bull semen collected during the dry season ( December- March) showed rather low motility, poor sperm concentration and high percentages of morphologically abnormal spermatozoa and was devoid of fructose (Orji et al., 1984). In the tropics, sperm production and semen quality decreased during summer (Fields et al., 1979; Rekwot et al., 1987). Taylor et al. (1985) reported that sperm production in Holstein bulls (ejaculate, volume, sperm concentration and total sperm number) was greatest during the summer in temperate environments. Higher semen quality in dairy *B. taurus* bulls during summer was also reported (Chandler et al., 1985; Soderquist et al., 1996). However, Mathevon et al. (1998) reported that Holstein bulls produced more sperm (higher sperm concentration and total sperm number) with greater motility during the winter and the spring. Diarra et al. (1997) observed an inverse seasonal variation in ejaculate volume and sperm concentration that resulted in non-significant variation in sperm production during the year. Godfrey et al. (1990) reported that in a temperate climate, *B. indicus* (but not *B. taurus*) bulls suffered from cold stress that was reflected in decreased sperm production and semen quality during the winter. In the tropics and semi-tropics, sperm production and semen quality decreased during the hot season only in *B. taurus* and crossbred bulls, but *B. indicus* bulls were not affected (Fields et al., 1979; Kumi-Diaka et al., 1981). However, some authors showed that in Africa, sperm production (ejaculate volume, sperm concentration and total sperm number) and percentage of normal sperm cells decreased during the hot season in *B. indicus* bulls (Igboeli and Rakha, 1971; Rekwot et al., 1987). Wildeus and Hammond (1993) did not observe any seasonal effect on sperm production and semen quality in Senepol and Holstein (*B. taurus*) bulls in St. Croix, US Virgin Islands. In the present study, environmental conditions did not vary sufficiently over the year to affect sperm production and semen quality.

Nichi et al. (2006) reported significantly higher percentages of major sperm defects during the summer than the winter for Simmental bulls. There was an interaction of breed and season for minor sperm defects (P = 0.037; highest in Nelore bulls in the summer) and an effect of season on total defects (P = 0.066; higher in summer). The greater increase in major defects in the summer was consistent with previous reports. Kumi-Diaka et al. (1981), conducted on bulls raised in the north of Nigeria, and Chacon et al. (1999), studied bulls from Costa Rica. Both reported higher percentages of sperm head and intermediary piece defects during the summer in *B. taurus* bulls compared to *B. indicus* bulls. No seasonal differences were found for sperm concentration/ml (overall mean 1.92+0.16×10 s) and for sperm output/ejaculate (overall mean 4.14±0.39) <108) (Igboeli and Rakha, 1971).

The influence of season on the ejaculate characteristics of Zebu, Friesian and their crossbred bulls in an A.I. programme in Nigeria was investigated over a 2-year period. Ejaculate volume, sperm concentration, percent morphologically normal

**Table 3: Effect of season on quantitative traits of semen**

| | HL | | SL | | TL | |
|---|---|---|---|---|---|---|
| Variables | Summer | Winter | Summer | Winter | Summer | Winter |
| Volume | 6.08 ± 1.83[a] | 5.58 ± 1.28[a] | 2.80 ± 0.83[a] | 3.24 ± 0.90[a] | 3.81 ±2.42[a] | 4.67±1.80[a] |
| pH | 6.31 ± 0.26[b] | 6.61 ± 0.20[a] | 6.24 ± 0.28[a] | 6.65 ± 0.32[a] | 6.46± 0.22[a] | 6.65±0.26[a] |
| Concentration | 1706.09±451[a] | 1277.67±343.70[a] | 1644.89±508.54[a] | 1376.77±295.87[b] | 1590.46±638.19[a] | 1476.24±475.68[a] |
| Gross motility | 2.88 ± 0.99[a] | 3.75 ± 0.35[a] | 3.29 ± 1.12[a] | 3.13 ± 0.94[a] | 3.70 ± 0.98[a] | 3.19±0.56[a] |
| Progressive motility | 56.25± 17.06[a] | 65.50 ± 5.5[a] | 60.16 ± 14.34[a] | 62.50 ± 7.75[a] | 65.28± 11.18[a] | 57.89±9.76[a] |
| Percentage live (%) | 78.33 ± 4.93[a] | 77.54 ± 8.55[a] | 63.10 ± 19.82[a] | 50.59 ± 29.18[a] | 64.60± 18.18[a] | 68.05±14.77[a] |
| Major Anomalies (%) | 2.42 ± 1.93[a] | 2.12 ± 1.41[a] | 11.45 ± 10.60[a] | 3.37 ± 1.89[a] | 1.82 ± 1.54[a] | 2±1.31[a] |
| Minor Anomalies (%) | 4.07 ± 5.42[a] | 4.25 ± 1.74[a] | 11.09 ± 6.24[a] | 6.84± 4.75[a] | 7.36 ± 3.29[a] | 6.23±3.37[a] |
| Total Anomalies (%) | 6.43 ± 7.04[a] | 5.90 ± 2.77[a] | 22.55 ± 13.03[a] | 10.26 ± 5.53[a] | 9.18 ± 3.54[a] | 8.60±3.33[a] |

The numbers of the same line that don't have the same letters are significantly different (P<0.05)

**Table 4: Effect of breed on quantitative traits of semen**

| Variables | SL | HL | TL |
|---|---|---|---|
| pH | 6.44 ± 0.36[a] | 6.49 ± 2.13[a] | 6.56 ± 2.26[a] |
| Volume (ml) | 2.91 ± 0.87[c] | 6.42 ± 0.31[a] | 3.97 ± 0.26[b] |
| Concentration ($10^3$ spermatozoids/ $cm^3$) | 1600.85 ± 474.59[a] | 1619.71 ± 468.63[a] | 1713.57 ± 582.82[a] |
| Gross motility | 3.24 ± 1.06[a] | 3.53 ± 0.73[a] | 3.43 ± 0.82[a] |
| Progressive motility | 60.93 ± 12.49[a] | 62.50 ± 12.16[a] | 61.48 ± 10.98[a] |
| Percentage live (%) | 64.23 ± 16.20[b] | 74.91 ± 11.39[a] | 66.96 ± 15.70[b] |
| Major Anomalies (%) | 6.55 ± 7.53[a] | 4.02 ± 3.24[b] | 1.93 ± 1.37[b] |
| Minor Anomalies (%) | 8.4 ± 5.64[a] | 6.55 ± 4.32[a] | 6.60 ± 3.33[a] |
| Total Anomalies (%) | 14.76 ± 10.66[a] | 10.58 ± 6.49[b] | 8.80 ± 3.36[b] |

The numbers of the same line that don't have the same letters are significantly different (P<0.05)

over a 2-year period. Ejaculate volume, sperm concentration, percent morphologically normal spermatozoa and percent live spermatozoa were significantly higher in the rainy season than in the dry season (P<0.05) (Rekwot et al., 1987).

Sixteen Simmental bulls and 11 Nelore bulls were managed extensively in a tropical environment. Semen was collected twice annually (summer and winter) for 2 consecutive years. Simmental bulls had significantly higher percentages of major sperm defects during the summer than the winter. There was an interaction of breed and season for minor sperm defects and an effect of season on total defects (Nichi et al., 2006).

### Effect of age on semen traits

Our study indicated that there was a significant difference (P<0.05) between class of age only for volume and percentage of live spermatozoa. But the others traits did not differ significantly. Similar studies are in agreement with the present findings. Some authors found that sperm motility does not seem to change with age (Everett and Bean, 1982; Diarra et al., 1997; Mathevon et al., 1998) and sperm defects are either unaffected (Chandler et al., 1985) or decreased significantly with age (Soderquist et al., 1996). In agreement with our study, Gamer et al. (1996) found that age was significantly correlated with ejaculate volume but not with the total number of spermatozoa per ejaculate.

Different from other studies, Hoflack et al. (2006), found that volume, concentration, and total sperm output depended largely on age.

Furthermore, Fuerst-Waltl et al. (2006) studied semen data from two Austrian AI centres regarding to ejaculate volume, sperm concentration, percentage of viable spermatozoa in the ejaculate, total spermatozoa per ejaculate and motility. They found that age of bull significantly affected all traits (P<0.01 to P<0.001). Ejaculate volume and total number of spermatozoa increased with age of bull while sperm concentration was lower in higher age classes.

Our results indicated that the incidence of sperm abnormalities (abnormal heads, proximal droplets, total abnormalities) was not affected significantly by age. In agreement with the present findings, Soderquist et al. (1996) studied the effects of age among others factors on the incidence of sperm morphological abnormalities in fresh semen in fifty-two dairy A.I. bulls aged from 14 months to 6 and a l/2 years old. Their results indicate that age influence the sperm characteristics.

### Effect of breed on semen traits

Our comparison of semen traits within breeds demonstrated that pH, concentration, motility and minor anomalies did not change within breeds (P<0.05). However, volume, percentage of live spermatozoa, major and total anomalies of ejaculates varies between the three breeds. In agreement with our finding, Hoflack et al. (2006) found that concentration did not differ between breeds, but primary sperm abnormalities occurred far more in the BB breed than Holstein bulls. Gross, total, and progressive motility, percentage live and percentage normal spermatozoa and volume increased with age in both breeds.

## Conclusions

In conclusion, ambient temperature, season, breed and age affect the majority of the semen traits for the bulls studied. These semen traits are so important to a successful insemination and to a greater fertility.

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