

The Influence of Climatic Factors on the Motility, Velocity and Morphology of Merino Rams Spermatozoa

(Pengaruh Faktor Iklim terhadap Karakteristik Motilitas, Kecepatan dan Morfologi Spermatozoa Domba Merino)

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Abstrak

Penelitian ini bertujuan untuk mengetahui pengaruh bulan demikian pula perubahan musim (panjang hari, sinar terang, curah hujan, temperatur, penguapan dan kelembaban) terhadap motilitas, kecepatan dan morfologi spermatozoa domba Merino. Enam domba Merino jantan fertil digunakan dalam penelitian ini. Sperma domba ditampung setiap bulan, mulai Juni 2002 sampai Mei 2003 dengan menggunakan elektroejakulator. Data kecepatan dan morfologi kepala spermatozoa didalam medium TALP dianalisis dengan menggunakan *computer-aided semen analysis* (CASA). Analisis varian, klasifikasi satu arah digunakan untuk mengetahui pengaruh kecepatan dan morfologi kepala spermatozoa. Total rerata VAP, VSL dan VCL selama setahun masing-masing adalah 75,40 $\mu\text{m}/\text{detik}$, 64,00 $\mu\text{m}/\text{detik}$ dan 113,50 $\mu\text{m}/\text{detik}$. Total rerata STR, LIN, dan ELO masing-masing adalah 75,20%, 51,30%, 35,50%. Total rerata ALH, BCF dan AREA masing-masing adalah 5.2 μm , 17.0 Hz, dan 3.0 μm^2 . Sebagai kesimpulan, iklim berpengaruh terhadap kecepatan dan morfologi kepala spermatozoa domba Merino.

Kata Kunci : spermatozoa domba Merino, Kecepatan, Morfologi

Introduction

The photoperiodic mammal undergoes quite remarkable changes in physiology as part of its natural adaptations to seasonal fluctuations in the environment. Seasonal variation in reproduction in mammals is an adaptation to annual changes in the environment and minimizes the energy cost of their reproductive efforts. Seasonality of breeding is a major limiting constraint on efficiency of sheep production, although strict seasonal breeding can be partially overcome by selection of breeding stock (Lewis *et al.*, 1996). Also, increasing or decreasing the photoperiod or providing implants of melatonin (Boland *et al.*, 1985; Fitzgerald and Stellflug, 1991) have been used to alter seasonal reproductive performance in

rams. The pineal hormone melatonin is the common link between photoperiod and reproduction. An increase in the daily diurnal period of melatonin secretion is associated with decrease in GnRH release in long-day breeders, but an increase in GnRH in short-day breeders. Melatonin influences GnRH release within or close to the mediobasal hypothalamus in rams. Opioids are at least, in part responsible for the decrease in testicular function during winter. An opiodergic inhibition of LH release is present during the breeding season in rams, but dopaminergic pathways inhibit LH release during long daylight hours (Gerlach and Aurich, 2000).

In mammals, including rams semen can be collected using artificial vagina or by electroejaculation. Artificial vagina is used routinely to collect semen from farm animals. In

the present study, semen was collected using an electroejaculation. Electroejaculation has been used in deer (Goeritz *et al.*, 2003), cattle (Fitzpatrick *et al.*, 2002).

Conventionally, sperm motility was observed using microscope, in recent studies by using computer-aided semen analysis (CASA) to define velocity characteristics in a number of species including sheep (Bag *et al.*, 2002), boars (Abaigar *et al.*, 1999; Vasquez *et al.*, 2002), mouse (Si and Olds-Clarke, 2000), and camels (Al-Qarawi *et al.*, 2002).

The objective of this study was to investigate the effect of months as well as seasonal changes (day-length, bright sunshine, rainfall, temperature, evaporation and humidity) on the motility and velocity of sperm and morphology of sperm head.

Research Methods

Climatic Conditions

Climatic conditions were obtained from the meteorology station in Townsville (altitude: 9° 14' 52" S; longitude: 146° 46' 01" E; elevation: 7.5 m) the area in which the sheep were located. Parameters relevant for this study were the mean of total bright sunshine, day-length, maximum air temperature, precipitation, evaporation and relative humidity (see Table 1).

Semen Collection

Six Merino rams (ram 5, 9, 10, 12, 13 and 16) of proven fertility were available for use in this study. Rams semen were collected monthly from June 2002 through May 2003. Semen was collected by electroejaculation (Electrojec, Ratex Instruments, Australia) by using standard procedures (Evans and Maxwell, 1987). The ram was manually restrained on its side within a building out of direct sunlight and the penis extruded. The penis was kept extruded by placing a piece of gauze posterior to the glans penis to hold the extended penis and to direct the glans

into a 15 ml sterile plastic centrifuge tube (Rohre/tube; Sarstedt, Germany). The collection tubes were kept in a polystyrene box at about 39°C. Electroejaculation was achieved by stimulation of the internal male accessory glands and nerves to the penis with a rectal probe connected to a mobile electrical stimulator. The electrical stimuli were given in a three seconds on and three seconds off pattern, with a gradual increase in voltage from zero volts to the optimum desired peak (five volts) then returning to zero volts. An electroejaculation attempt was terminated if semen was not obtained after 16 stimulations. Semen was collected no more than twice from a particular ram within a 7 day period. At the completion of semen collection a small amount of antiseptic cream was applied to the glans penis before allowing the penis to retract into the prepuce. The prepuce and penis was gently massaged for about one minute to reduce any swelling that may have developed and to reduce any discomfort the ram may have experienced.

Media

Tyrodé's albumin lactate pyruvate (TALP) medium was used in this study and the composition of TALP is shown in Table 2.

Semen Analysis

Motility, Velocity and morphology of sperm head in TALP medium were analyzed using computer-aided semen analysis, CASA (Hamilton Thorne Research-IVOS version 10, Beverly MA, USA). Consensus on operating procedures for the use of CASA as well as definition of terminology (see Table 3) have been achieved. Whereas, Elongation is the ratio of the head width to head length, expressed as percentage, for the selected spermatozoa. Head area is the calculated head area of the selected spermatozoa in μm^2 (Yeung *et al.*, 1997).

Table 1. Mean climatic data for seasons during the experiment**

Months	Bright * Sunshine (hours)	Day- length (hours)	Maximum air temperature (°C)	Precipitation (mm)	Evaporation (mm)	Relative humidity (%)
June	8.40	13.10	26.20	0.60	5.30	62.40
July	8.80	13.10	25.60	0.00	4.80	65.40
August	8.60	12.48	26.10	0.30	5.50	70.20
September	10.20	12.15	28.70	0.00	7.00	67.40
October	10.80	11.43	30.20	0.00	8.60	65.30
November	11.70	11.74	30.90	0.00	10.60	62.20
December	10.70	10.74	32.70	0.80	9.80	66.90
January	8.90	11.06	32.60	0.60	9.80	64.40
February	8.10	11.30	32.00	11.30	8.20	76.70
March	8.60	11.85	31.60	2.40	7.50	70.10
April	8.90	12.35	30.50	0.70	6.60	69.70
May	8.10	12.88	28.90	0.20	5.80	63.40
Overall [#]	9.30 ± 1.20	11.96 ± 0.80	29.70 ± 2.50	1.41 ± 3.60	7.46 ± 1.90	67.40 ± 3.80
Range	8.10 - 10.80	10.74 - 13.00	25.60 - 32.60	0.00 - 11.30	4.80 - 10.60	62.20 - 70.20

** Data recorded by the Townsville Meteorology [#] Mean ± SEM

* Bright Sunshine is solar radiation intense enough to cast distinct shadows

Table 2. Composition of modified Tyrode's albumin-lactate-pyruvate medium (TALP)

Constituents	Concentration (mg/ 100 ml)	Supplier
	TALP *	
NaCl	100.00 mM (584.4)	Sigma, USA
KCl	3.10 mM (23.11)	BDH Chemicals, Australia
CaCl ₂	2.00 mM (29.4)	Ajax Chemicals, Australia
MgCl ₂	0.40 mM (3.81)	Sigma, USA
NaHCO ₃	25.00 mM (210.1)	Ajax Chemicals, Australia
NaH ₂ PO ₄ ·2H ₂ O	0.30 mM (4.68)	BDH Chemicals, Australia
L-Lactic acid	21.60 mM (242.14)	Sigma, USA
HEPES	10.00 mM (238.3)	Sigma, USA
Sodium pyruvate	1.00 mM (11)	Sigma, USA
BSA-V	6.00 mg/ml (600)	Sigma, USA
Distilled water	100.00 ml	

* Modified from Parrish *et al.* (1988)

Table 3. Definitions of sperm kinematics measures

Symbol	Name	Definition	Units
VAP	Average path velocity	Time-average velocity of the sperm head along its average trajectory	µm/s
VSL	Straight-line velocity	Time-average velocity of the sperm head along a straight line from its position to its last position	µm/s
VCL	Curvilinear velocity	Time-average velocity of the sperm head along its actual trajectory	µm/s
STR	Straightness	Straightness of the average path (VSL/VAP)	%
ALH	Amplitude of lateral head displacement	Amplitude of variations of the actual sperm head trajectory about its average trajectory (the average trajectory is computed using a rectangular running average)	µm
BCF	Beat cross frequency	Time-average rate at which the actual sperm trajectory crosses the average path trajectory	Hz
LIN	Linearity	Linearity of the curvilinear trajectory (VSL/VCL)	%

Statistical Analysis

All data were analyzed using SPSS (Statistical Product and Service Solution) software program (SPSS 11.0 Brief Guide, New Jersey, USA). Analysis of variance one way classification was used to determine the effect of season on the motility, velocity and morphology of sperm head characteristics whereas the level of significance was considered $P \leq 0.05$.

Results and Discussion

Months were significantly affected the percentage of motile, progressive and rapid of sperm. In January and March the motile, progressive and rapid of sperm were significantly decreased. Percentage of motile, progressive and rapid were significantly increased in June through October (81 - 95%, 38 - 67%, 52 - 80%, respectively).

The total average of average path velocity (VAP), straight-line velocity (VSL) and curvilinear velocity (VCL) of sperm for one year

were 75.40 $\mu\text{m/s}$, 64.00 $\mu\text{m/s}$ and 113.50 $\mu\text{m/s}$, respectively. Although VAP, VSL and VCL were slightly varied between month for one year, in March VAP, VSL and VCL were significantly decreased (Figure 1).

Straightness (STR), linearity (LIN) and elongation (ELO) of sperm were slightly varied between month for one year, except in March STR, LIN and ELO were decreased significantly (Figure 2). The total average of STR, LIN and ELO were 75.20%, 51.30% and 35.50%, respectively.

Amplitude of lateral head displacement (ALH), beat cross frequency (BCF) and head area of sperm were fluctuated except in March ALH, BCF and head area were decreased significantly (Figure 3). ALH and head area were not significant different between month, whereas BCF was significantly different between month. The total average of ALH, BCF and head area of sperm were 5.2 μm , 17.0 Hz and 3.0 μm^2 , respectively.

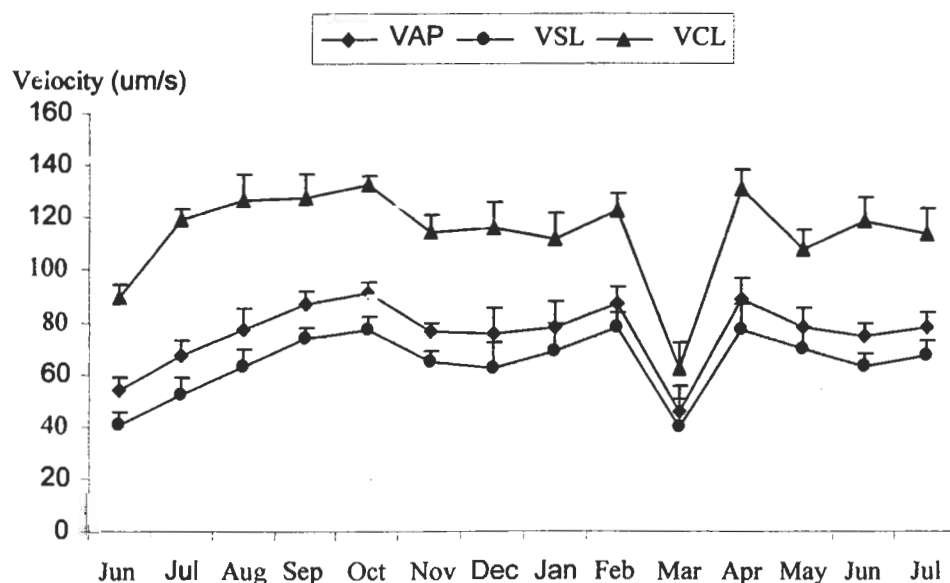


Figure 1. Effect of months on the average path velocity (VAP), straight-line velocity (VCL) and curvilinear velocity (VCL) of ram spermatozoa in TALP medium and analyzed using computer aided semen analyzed (CASA). The results are the mean ($\pm$ SEM) of the data from six rams.

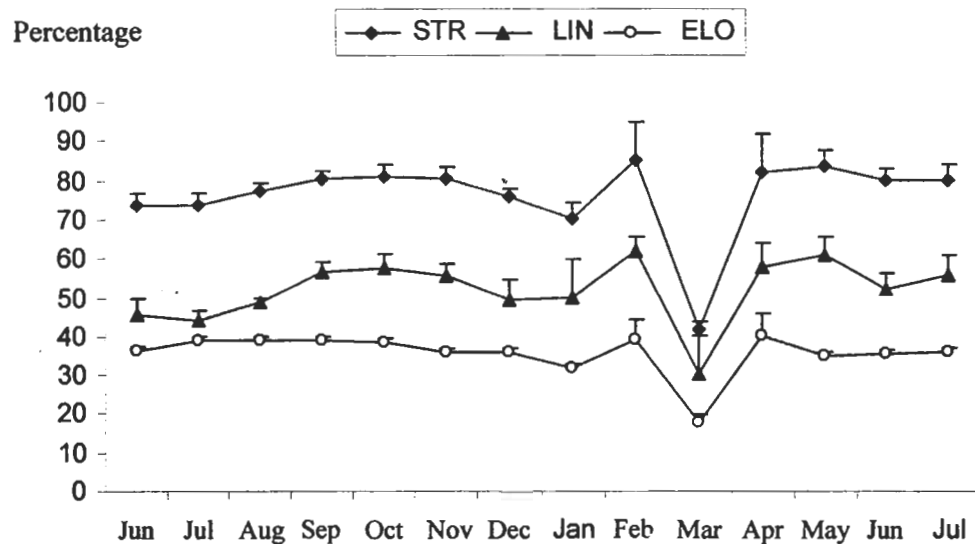


Figure 2. Effect of months on the straightness (STR), linearity (LIN) and elongation (ELO) of ram spermatozoa and analyzed using CASA. The results are the mean ($\pm$ SEM) of data from six rams.

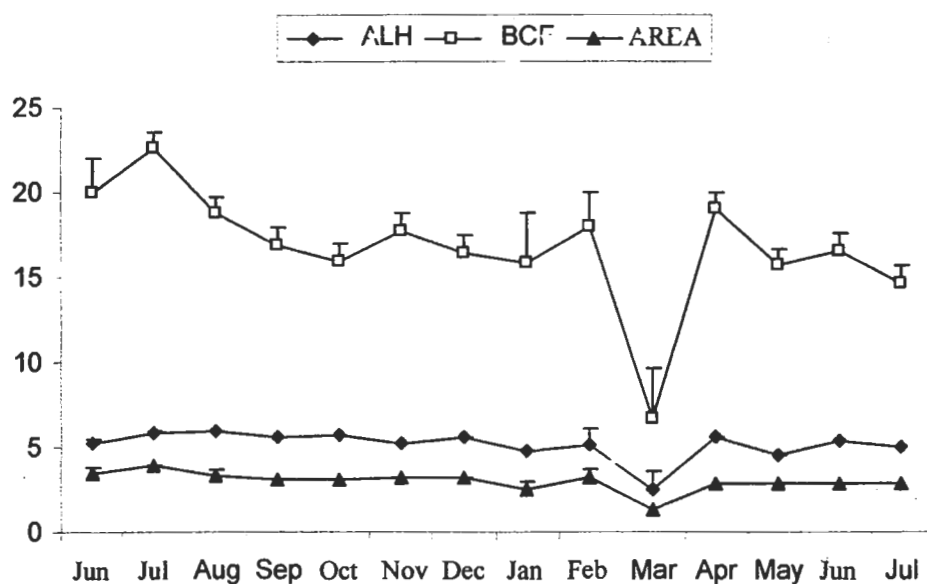


Figure 3. Effect of months on the amplitude of lateral head displacement (ALH, μ m), beat cross frequency (BCF, Hz) and head area (AREA, μ m²) of ram spermatozoa and analyzed using CASA. The results are the mean ($\pm$ SEM) of data from six rams.

Table 3. Correlation (r) between seasons, motility and velocity characteristics of ram sperm

Parameters	Mot	Prog	Rap	VAP	VSL	VCL	ALH	BCF	STR	LIN
Day-length	0.21	-0.11	-0.03	-0.31	-0.37	-0.13	0.08	0.35	0.02	-0.15
Maximum Temperature	0.52*	-0.17	-0.29	-0.22	0.35	-0.08	0.39	0.55*	-0.14	0.13
Bright sunshine	0.33	0.44*	0.47*	0.29	0.24	0.31	0.30	0.01	0.19	0.18
Precipitation	-0.16	-0.12	0.12	0.21	-0.03	-0.18	-0.08	0.07	0.20	0.21
Evaporation	-0.23	0.05	-0.01	0.26	0.33	0.07	-0.11	-0.32	0.01	0.17
Humidity	-0.13	0.08	-0.03	0.24	0.32	0.09	-0.17	-0.20	0.04	0.17

Mot = motile, Prog = progressive, Rap = rapid, VAP = average path velocity, VSL = straight-line velocity, VCL = curvilinear velocity, ALH = amplitude of lateral head displacement, BCF = beat cross frequency, STR = straightness, LIN = linearity

* Significant ($P \leq 0.05$)

In this study showed that maximum air temperature was significantly affected motile of sperm and beat cross frequency. Whereas bright sunshine was significantly affected progressively motile and rapidly motile of sperm (Table 3).

Seasonal changes reported for rams in the fall include increases in testis size (Dufour *et al.*, 1984; Boland *et al.*, 1985; Gerlach and Aurich, 2000) and in spermatogenesis and semen quality (Mickelsen *et al.*, 1981; Boland *et al.*, 1985; Hochereau-de Reviers *et al.*, 1985; Gerlach and Aurich, 2000). Mean scrotal circumference was highest from August through October and lowest in February. Spermatozoa that were morphologically normal were highest in proportion in September (82.30%) and lowest in proportion in February (57.80%). Libido and serving capacity scores were highest from September through November and lowest in March (Mickelsen *et al.*, 1982).

The decreases in natural light during summers are accompanied by an increase in gonadotropic hormones and testosterone, and a decrease in prolactin (Schanbacher and Lunstra, 1976; Dufour *et al.*, 1984; Gerlach and Aurich, 2000). Coincident with the hormonal changes, libido also is affected (Tulley and Burfening, 1983). Breeds of sheep differ considerably in the magnitude of seasonal and experimental photoperiod responses.

Part of the responses may be due to a direct effect of increasing or decreasing day-length or sunshine and part to a circannual cycle which drives an endogenous rhythm somewhat independent of photoperiod (Boland *et al.*, 1985; Jackson and Jansen, 1991).

Seminal output and quality (motility, velocity and viability) were highest in October and November and lowest in April and May. Rams within breeds differed significantly in the seminal characteristics studied (Mickelsen *et al.*, 1982). However, changes in photoperiod did not affect sperm output in Finn rams, but may have affected Dorset rams (El-Alamy *et al.*, 2001).

Conclusion

Climatic factors were slightly affected the motility and velocity characteristics of Merino ram spermatozoa. Maximum air temperature was affected motile of sperm and beat cross frequency. Bright sunshine was affected progressively motile and rapidly motile of sperm.

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