THE INFLUENCE OF CLIMATIC FACTORS ON SEMEN PARAMETERS OF MERINO RAMS IN A TROPICAL ENVIRONMENT

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ABSTRACT

The objective of this study was to investigate the effect of rams and seasonal changes on rams semen quantity and quality. Six Merino rams 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. To determine the effect of rams and seasonal changes on the semen characteristics were used analysis of variance univariate. Analysis of variance one way classification was used to determine the effect of season on the sperm motility. Results in this study showed that, the highest in semen volume was a ram 10 and the lowest was a ram 5. Although the best in semen color, sperm motility and sperm concentration were a ram 9. Whereas ram 16 and ram 12 had the lowest in sperm motility and sperm concentration, respectively. The highest in semen color was in July (creamy) and the lowest in semen color was in March (clear). Moreover the lowest in sperm motility and sperm concentration were also in March. Therefore, the best in sperm motility and sperm concentration was in November, 83.3 % and 3773 million/ml, respectively. Maximum air temperature was significantly related with semen color and sperm motility and bright sunshine was significantly related with sperm concentration. In conclusion, semen volume, semen color, sperm motility and sperm concentration of Merino rams varied between ram. More over performance of reproductive of Merino rams was affected by seasons (months).

(Key words: Merino rams, Electroejaculation, Quantity and quality of semen, Seasonal changes).

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PENGARUH FAKTOR-FAKTOR MUSIM TERHADAP PARAMETER SPERMA DOMBA MERINO DI DAERAH TROPIS

INTISARI

Tujuan penelitian ini adalah untuk mengetahui pengaruh pejantan dan perubahan musim pada kuantitas dan kualitas sperma domba Merino. Enam pejantan Merino digunakan dalam penelitian ini. Pejantan ditampung spermanya dengan menggunakan elektroejakulator setiap bulan dari bulan Juni 2002 sampai dengan Mei 2003. Untuk mengetahui pengaruh pejantan dan musim terhadap karakteristik sperma, dianalisis dengan analisis varian univariat. Hasil penelitian menunjukkan bahwa pejantan nomor 10 menghasilkan volume sperma tertinggi, pejantan nomor 5 terendah. Pejantan nomor 9 menghasilkan warna sperma, motilitas dan konsentrasi spermatozoa yang terbaik. Pejantan nomor 16 dan 12 masing-masing mempunyai motilitas dan konsentrasi spermatozoa terendah. Warna sperma terbaik (krem) adalah pada bulan Juli dan warna terjelek (bening) adalah bulan Maret. Walau demikian motilitas dan konsentrasi spermatozoa terbaik adalah pada bulan November, masing-masing adalah 83,3% dan 3773 juta/ml. Temperatur maksimal mempunyai hubungan positif nyata terhadap warna sperma dan motilitas spermatozoa, sedangkan panjang hari mempunyai hubungan yang positif nyata terhadap konsentrasi spermatozoa. Sebagai kesimpulan, isi dan warna sperma, motilitas dan konsentrasi spermatozoa bervariasi diantara pejantan domba Merino. Lebih dari itu, penampilan reproduksi domba Merino dipengaruhi oleh musim (bulan).

(Kata kunci: Pejantan domba Merino, Elektroejakulator, Kuantitas dan kualitas sperma, Perubahan musim).

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).

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-deReviers *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 (82.3%) and lowest in proportion in February (57.8%). Libido and serving capacity scores were highest from September through November and lowest in March (Mickelsen *et al.*, 1982).

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).

The objective of this study was to investigate the effect of rams and months as well as seasonal changes (day-length, bright sunshine, rainfall, temperature, evaporation and humidity) on rams semen quantity and quality and also to determine the most suitable time for ram semen collection, for help to improve the efficiency of artificial insemination organizations in Townsville, Australia.

Material and 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 (Table 1).

Animal

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).

Table 1. Mean climatic data for seasons during the experiment[1]

Months	Bright[3] Sunshine (hours)	Day-length (hours)	Maximum air temperature (°C)	Precipitation (mm)	Evaporation (mm)	Relative humidity (%)
June	8.40	13.10	26.2	0.6	5.3	62.4
July	8.80	13.10	25.6	0.0	4.8	65.4
August	8.60	12.48	26.1	0.3	5.5	70.2
September	10.20	12.15	28.7	0.0	7.0	67.4
October	10.80	11.43	30.2	0.0	8.6	65.3
November	11.70	11.74	30.9	0.0	10.6	62.2
December	10.70	10.74	32.7	0.8	9.8	66.9
January	8.90	11.06	32.6	0.6	9.8	64.4
February	8.10	11.30	32.0	11.3	8.2	76.7
March	8.60	11.85	31.6	2.4	7.5	70.1
April	8.90	12.35	30.5	0.7	6.6	69.7
May	8.10	12.88	28.9	0.2	5.8	68.4
Overall[2]	9.3 ± 1.2	11.96 ± 0.8	29.7 ± 2.5	1.41 ± 3.0	7.46 ± 1.9	67.4 ± 3.8
Range	8.1 - 10.8	10.74 - 13	25.6 - 32.6	0.0 - 11.3	4.8 - 10.6	62.2 - 70.2

[1]: Data recorded by the Townsville Meteorology; 2: Mean SEM; 3: Bright Sunshine is solar radiation intense enough to cast distinct shadows.

The collection tubes were kept in a polystyrene box at about 39°C. Electro-ejaculation was achieved by stimulation of the internal male accessory glands and nerves to the penis will 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

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

Semen analysis

Semen volume and semen color observed by macroscopic, sperm motility and sperm concentration observed by microscopic. Whereas, sperm velocity observed by computer-aided semen analysis (CASA).

Statistical analysis

All data were analyzed using SPSS software program (SPSS 11.0 Brief Guide, New Jersey, USA). To determine the effect of rams and months on the semen characteristics were used analysis of variance univariate. Analysis of variance one way classification was used to determine the effect of season (bright sunshine, day-length temperature, precipitation, evaporation, and humidity) on the motility, whereas the level of significance was considered $P \leq 0.05$. To determine the correlation between season (bright sunshine, day-length temperature, precipitation, evaporation, and humidity) and semen quantity and quality was used correlate bivariate.

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

Constituents	Concentration (mg/ 100 ml) TALP[1]	Supplier
NaCl	100 mM (584.4)	Sigma, USA
KCl	3.1 mM (23.11)	BDH Chemicals, Australia
$CaCl_2$	2 mM (29.4)	Ajax Chemicals, Australia
$MgCl_2$	0.4 mM (3.81)	Sigma,USA
$NaHCO_3$	25 mM (210.1)	Ajax Chemicals, Australia
$NaH_2PO_4.2H_2O$	0.3 mM (4.68)	BDH Chemicals, Australia
L-Lactic acid	21.6 mM (242.14)	Sigma, USA
HEPES	10 mM (238.3)	Sigma, USA
Sodium pyruvate	1 mM (11)	Sigma, USA
BSA-V	6 mg/ml (600)	Sigma, USA
Distilled water	100 ml	

[1]: Modified from Parrish *et al.*, 1988.

Results and Discussion

Effects of rams and months on the semen characteristics for one year

Results in this study showed that semen volume, semen color, sperm motility and sperm concentration of Merino rams varied between ram (Table 3). Average semen volume and semen color were 0.77 ± 0.03 ml with range 0.2 ml to 1.60 ml and 2.6 ± 0.16 with range 0.0 to 5.0, respectively. Average sperm motility and sperm concentration were 66.3 ± 2.6% with range 0.0% to 90% and 2044 ± 164 with million/ml with range 10 million/ml to 6210 million/ml, respectively. The highest in semen volume was a ram 10 and the lowest was a ram 5. Although the best in semen color, sperm motility and sperm concentration were a ram 9. Whereas ram 16 and ram 12 had the lowest in sperm motility and sperm concentration, respectively.

Day-length, precipitation, evaporation and relative humidity were not correlated with semen volume, semen color, sperm motility and sperm concentration. Whereas, maximum air temperature was significantly related with semen color and sperm motility and bright sunshine was significantly related with sperm concentration (Table 4).

Table 3. Effect of rams on the semen characteristics for one year

Rams No.	Semen volume (ml)	Semen color[1]	Sperm motility (%)	Sperm concentration (x 10^6 / ml)
5	0.61 ± 0.08^a	3.0 ± 0.43^a	70.4 ± 6.8^a	2551 ± 551^a
9	0.85 ± 0.04^b	3.7 ± 0.24^b	81.5 ± 2.0^b	3089 ± 324^a
10	0.99 ± 0.08^c	2.8 ± 0.37^a	65.4 ± 6.9^a	2045 ± 435^b
12	0.80 ± 0.07^b	2.1 ± 0.37^c	63.5 ± 5.7^c	1521 ± 280^c
13	0.65 ± 0.06^a	2.1 ± 0.33^c	60.6 ± 6.9^c	1537 ± 306^c
16	0.70 ± 0.05^a	2.1 ± 0.40^c	56.9 ± 6.8^c	1564 ± 336^c
Overall	0.77 ± 0.03	2.6 ± 0.16	66.3 ± 2.6	2044 ± 164
Range	0.2 - 1.60	0.0 - 5.0	0.0 - 90	10 - 6210

[1]0 = clear, 1 = cloudy, 2 = milky, 3 = thick milky, 4 = creamy, 5 = thick creamy
Data value represents the mean (± SEM) of 12 replicates (every month for one year)
Data with different ^{abc} superscripts within column were significantly different.

Table 4. Correlation (r) between seasons parameters and rams semen characteristics

Parameters	Semen volume	Semen color	Sperm motility	Sperm concentration
Day-length	- 0.253	0.034	0.243	0.007
Maximum air temperature	0.310	-0.621*	-0.541*	-0.222
Bright sunshine	0.134	0.298	0.383	0.585*
Precipitation	0.403	-0.277	-0.327	-0.259
Evaporation	0.202	-0.283	-0.235	0.100
Humidity	0.311	-0.335	-0.281	-0.325

* Significant ($P \leq 0.05$).

Performance of reproductive of rams was affected by seasons (months). Although there was no effect of months on the semen volume, however semen color, sperm motility and sperm concentration were varied between months (Table 5). The highest in semen color was in July (creamy) and the lowest in semen color was in March (clear). Moreover the lowest in sperm motility and sperm concentration were also in March. Therefore, the best in sperm motility and sperm concentration was in November, 83.3 % and 3773 million/ml, respectively.

Effect of months or season on the semen characteristics

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 (Figure 1). Percentage of motile, progressive and rapid were significantly increased in June through October (81% - 95%, 38% - 67%, 52% - 80%, respectively). Whereas, percentage of medium and slow of sperm relatively low no more than 20% for one year.

Seminal output and quality (motility and percentage of live spermatozoa) 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).

Table 5. Effect of months on the semen characteristics for one year

Months	Semen volume (ml)	Semen color[1]	Sperm motility (%)	Sperm concentration (x 10^6 / ml)
June	0.73 ± 0.06	3.1 ± 0.26^{b}	73.57 ± 2.3^{b}	1640 ± 180^{ab}
July	0.78 ± 0.06	3.7 ± 0.21^{b}	80.00 ± 0.0^{c}	2950 ± 262^{bc}
August	0.73 ± 0.09	3.2 ± 0.48^{b}	75.83 ± 5.2^{b}	1988 ± 359^{ab}
September	0.73 ± 0.09	2.5 ± 0.50^{ab}	76.67 ± 4.0^{b}	2017 ± 416^{ab}
October	0.69 ± 0.10	3.0 ± 0.51^{b}	67.92 ± 7.8^{b}	2261 ± 430^{ac}
November	0.82 ± 0.09	3.3 ± 0.61^{b}	83.33 ± 4.2^{c}	3773 ± 702^{bc}
December	0.84 ± 0.08	2.7 ± 0.42^{ab}	80.83 ± 3.2^{c}	2377 ± 277^{ac}
January	0.69 ± 0.09	1.5 ± 0.56^{a}	42.50 ± 11.2^{a}	903 ± 413^{a}
February	0.85 ± 0.13	2.3 ± 0.42^{ab}	56.67 ± 7.1^{b}	1694 ± 551^{ab}
March	0.76 ± 0.19	0.8 ± 0.31^{a}	31.67 ± 15.1^{a}	315 ± 193^{a}
April	0.89 ± 0.10	2.7 ± 0.62^{ab}	66.22 ± 6.3^{b}	2627 ± 723^{bc}
May	0.68 ± 0.12	2.4 ± 0.61^{ab}	60.71 ± 7.1^{b}	1810 ± 592^{ab}
Overall	0.77 ± 0.03	2.6 ± 0.16	66.34 ± 2.5	2045 ± 164
Range	0.20 - 1.60	0.0 - 5.0	0.0 - 90.0	10 - 6120

[1]0 = clear, 1 = cloudy, 2 = milky, 3 = thick milky, 4 = creamy, 5 = thick creamy

Data value represents the mean (± SEM) of 6 replicates

Data with different ^{abc} superscripts within column were significantly different.

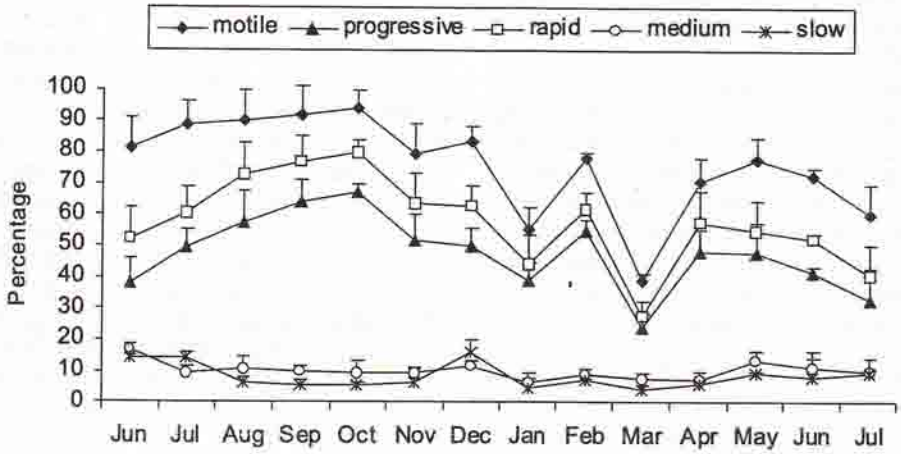

Figure 1. Effect of months on the percentage of motile, progressive, rapid, medium and slow of ram spermatozoa in TALP medium and analyzed using computer aided semen analysis (CASA). The results are the mean (± SEM) of the data from six rams.

The decreases in natural light during summers is 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 circanual cycle which drives an endogenous rhythm somewhat independent of photoperiod (Boland *et al.*, 1985; Jackson and Jansen, 1991).

Conclusion

Semen volume, semen color, sperm motility and sperm concentration of Merino rams varied between ram and performance of reproductive of Merino rams was affected by seasons (months). The best semen collection of Merino rams was in November.

References

Abaigar, T., W.V. Holt, R. A. P. Harrison and del G. Barrio. 1999. Sperm subpopulations in boar (Sus scrofa) and Gazelle (Gazella damamhorr) semen as revealed by pattern analysis of computer-assisted motility assess-ments. Biol. Reprod. 60 : 32-41.

Al-Qarawi, A. A., H. A. Abdel-Rahman, S. A. El-Mougy, M. S. El-Belely. 2002. Use of new computerized system for evaluation of spermatozoal motility and velocity characteristics in relation to fertility levels in dromedary bulls. Anim. Reprod. Sci.74 : 1-9.

Bag, S., A. Joshi, S. M. K. Naqvi, P. S. Rawat, J. P. Mittal. 2002. Effect of freezing temperature, at which straws were plunged into liquid nitrogen, on the post-thaw motility and acrosomal status of ram spermatozoa. Anim. Reprod. Sci. 72: 175-183.

Boland, M. P., A. A. Al-Kamali, T. F. Crosby, N. B. Haynes, C. M. Howles, D. L. Kelleher, I. Gordon. 1985. The influence of breed, season and photoperiod on semen characteristics, testicular size, libido and plasma hormone concentrations in rams. Anim. Reprod. Sci. 9: 241-252.

Dufour, J. J., M. H. Fahmym, F. Minvielle. 1984. Seasonal changes in breeding activity, testicular size, testosterone concentration and seminal characteristics in rams with

long or short breeding season. J. Anim Sci. 58: 416-422.

El-Alamy, M. A., R. H. Foote, E. Hare. 2001. Sperm output and hormone concentration in Finn and Dorset rams exposed to long- and short-day lighting. Ther. 56, 839-854.

Evans, G., W. M. C. Maxwell. 1987. *Salamon's Artificial Insemination of Sheep and Goats*. Butterworths Pty. Limited, Sydney.

Fitzpatrick, L. A., G. Fordice, M. R. McGowan, J. D. Bertram, V. J. Doogan, J. DeFaveri, R.G. Miller, R. G. Holroyd. 2002. Bull selection and use in northern Australia part2. Semen traits. Anim. Reprod. Sci. 71: 39-49.

Fitzgerald, J. A., J. N. Stellflug. 1991. Effect of melatonin on seasonal changes in reproduction of rams. J. Anim Sci. 69: 264-275.

Gerlach, T., J. E. Aurich. 2000. Regulation of seasonal reproductive activity in the stallion, ram and hamster. Anim. Reprod. Sci. 58: 197-213.

Gerlach, T., J. E. Aurich. 2000. Regulation of seasonal reproductive activity in the stallion, ram and hamster. Anim. Reprod. Sci. 58: 197-213.

Goeritz, F., M. Quest, A. Wagener, M. Fassbender, A. Broich, T. B. Hildebrandt, R. R. Hofmann, S. Blooner. 2003. Seasonal timing of sperm production in roe deer: interrelationship among changes in ejaculate parameters, morphology and function of testis and accessory glands. Ther. 59: 1487-1502. 1376-380.

Parrish, J. J., J. L. Susko-Parrish, Winer, M.A., N. L. First. 1988. Capacitation of bovine sperm by heparin. Biol. Reprod. 38: 1171-1180.

Si, Y., P.Olds-Clarke. 2000. Evidence for the involvement of calmodulin in mouse sperm capacitation. Biol. Reprod. 62: 1231-1239.

Schanbacher, B. D., D. D. Lunstra. 1976. Seasonal changes in sexual activity and serum levels of LH and testosterone in Finnish Landrace and Suffolk rams. J. Anim Sci. 43: 644-650.

Tulley, D., P. J. Burfening. 1983. Libido and scrotal circumference of rams as affected by season on the year and altered photoperiod. Ther. 20: 435-448.

Vazquez, J. M., E. A. Martinez, I. Parrilla, M. A. Gil, X. Lucas, J. Roca. 2002. Motility characteristics and fertilizing capacity of boar spermatozoa stained with Hoechst 33342. Reprod. Dom Anim. 37: 369-374.