*Short Communication/Kort Mededeling*

# Investigation of luteinizing hormone response to GnRH and testes diameter as possible early indicators of adult sexual activity in Merino sheep

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Testes diameter of 110 Merino ram lambs was measured at 100, 260, and 550 days of age. At 80 days of age the LH response to exogenous GnRH was determined in these rams. During the breeding season at 18 months of age the rams were subjected to libido tests. The repeatability of the libido tests was high (0.74 ± 0.030) and it was evident that rams could be divided into a group displaying a high and a group displaying a low libido. Basal plasma LH concentration as well as plasma LH concentration 30 and 50 min following GnRH administration did not differ between the high and low libido groups. Although the LH response to GnRH was higher ($p < 0.05$) in the high libido rams (8.33 ± 0.48 ng/ml) compared to the low libido rams (6.32 ± 0.76 ng/ml), libido as determined in this study could not be accurately predicted from LH response. Contrary to expectation the testes size at 100 and 550 days of age did not differ between the high and low libido groups.

Die testesdeursneë van 110 ramlammers is op 100, 260, en 550 dae ouderdom bepaal. Op 80 dae ouderdom is die LH-respons op die toediening van eksogene gonadotropien-vrystellingshormoon (GnVH) by hierdie ramme bepaal. Tydens die teëlseisoen op 18 maande ouderdom is die ramme aan libidotoetse onderwerp. Die herhaalbaarheid van die libidotoetse was hoog (0.74 ± 0.030) en dit was duidelik dat die ramme in 'n groep met 'n hoë en 'n groep met 'n lae libido verdeel kon word. Basale plasma LH-konsentrasie sowel as plasma LH-konsentrasie 30 en 50 min na toediening van GnVH het nie tussen die hoë en lae libido groep verskil nie. Ten spyte daarvan dat die LH-respons op die vrystellingshormoon hoër was ($p < 0.05$) in die hoë libido ramme (8.33 ± 0.48 ng/ml) teenoor die lae libido ramme (6.32 ± 0.76 ng/ml) was dit nie moontlik om libido, soos bepaal in hierdie studie, akkuraat vanaf LH-respons te voorspel nie. In teenstelling met wat verwag is, het testesgrootte op 100 en 550 dae ouderdom nie tussen die hoë en lae libido groepe verskil nie.

**Keywords:** Libido, LH, GnRH, testes size, sheep

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It has been reported that between 9 and 33% of all adult Merino rams are sexually inactive or display a very low libido (Van Wyk *et al.*, 1984; Fitzgerald & Perkins, 1991). This finding is of great economic importance as it has been shown that sexual activity is associated with female reproduction rate (Van Wyk *et al.*, 1984; Kilgour *et al.*, 1985; Perkins *et al.*, 1993) and ewe productivity (Fitzgerald & Perkins, 1991). In addition, it has been reported that serving capacity is highly heritable (0.59 ± 0.15) in cattle (Kilgour *et al.*, 1985).

Rams however, are normally selected on production and subjective traits while sexual capacity of selected rams is only judged by semen quality and quantity. During the selection process very little, if any, consideration is given to mating ability. In order to optimize production it is essential to eliminate sires displaying poor sexual activity. This is of particular importance when AI is practised, as natural selection against poor sexual activity is eliminated. Although sexual activity can only be assessed after puberty it is rarely applied in practice in spite of the obvious advantages. If it was possible to identify and cull rams with a poor sexual activity at an early age it would result in a substantial reduction in feeding and handling costs.

The interrelationships between LH secretion, testosterone secretion, testes diameter, libido and female reproduction are well documented (Hafez, 1980; Kilgour *et al.*, 1985). However, the possibility of using LH secretion at an early age as an indirect measure of sexual activity at a later stage, has not been investigated. The objective of the present study was to investigate the possibility of using the LH response to exogenous GnRH and testes size at an early age as a means of predicting adult sexual activity.

The study was conducted on rams maintained on irrigated lucerne and wheat pastures. One hundred and ten Merino ram lambs born between the 1st and 15th of September 1993 were used. Animals were managed as a single flock from birth until completion of the study. The diameter of both testes was measured with a calliper at 100, 260 and 550 days of age. All testis measurements were performed by the same person to minimize experimental error. Body weight was recorded on the same days as testes diameter.

At approximately 80 days of age 5 µg GnRH (Fertagel, Intervet, Boxmeer, Holland) was injected as a bolus into the left jugular vein of each lamb. Blood samples were collected into heparinized vacuum tubes (Radem Laboratory Supplies (Pty) Ltd, Sandton S.A.) from the right jugular vein 30 and 5 min prior to treatment and 30 and 50 min following treatment. Within 10 min after collection, the blood samples were centrifuged at 2000G for 20 min, after which the plasma was aspirated and stored at –20°C for LH assay.

LH in plasma was analysed by radio-immunoassay according to the method of Kritzinger (1982). The primary LH antibody (NIADDK-anti-oLH-1), ovine reference preparation (NIADDK-oLH-1-3) and the ovine LH used for iodination were kindly donated by the National Institute of Diabetes and Digestive and Kidney Diseases, 210 West Fayette Street, Baltimore, Maryland, USA. The LH antibody was used at a final tube dilution of 1 : 625 000. Samples containing less than 0.5 ng/ml LH were considered non-detectable as the sensitivity of the assay, determined according to the technique described by Midgley *et al.* (1969), was 0.49 ng/ml. For statistical calculations the undetectable levels were allotted a concentration of 0.5 ng/ml. The intra- and inter-assay coefficient of variation for the assays were 9.7 and 14.8% respectively. Samples taken at the same time were assayed in one batch to minimize experimental error. The LH response to exogenous GnRH was calculated by subtracting the mean LH concentration prior to treatment from the concentration obtained for samples taken 30 min following treatment.

During the breeding season at 18 months of age, the rams were subjected to libido tests. Each ram was exposed for a 10-min period to 10 ewes in which oestrus was artificially

induced. The time lapse from introduction until the first successful mating as well as the number of unsuccessful mating attempts were recorded. A mating was considered to be successful when a violent pelvic thrust was followed by a sudden backward movement of the head. A mounting without intromission was counted as an unsuccessful mating attempt. Each ram was tested six times over a period of four days.

During the libido test each ram was scored according to the following criteria; a score of zero was allotted if no mating attempts were made; one when between one and five attempts were made; two when 6–10 attempts were made or the ram mated successfully between 6 and 10 min after introduction; three if the ram mated between 3 and 5 min or attempted to mate more than 10 times; four if the ram mated within 2 min following exposure to ewes. For statistical analyses the six scores of each ram were summed to represent the ram's libido score. The GLM procedure of SAS (Littell *et al.*, 1991) was used for statistical analysis. Parameters recorded and the repeatability of libido was estimated by intra-class correlation coefficients.

Although the repeatability of the libido test was high (0.74 ± 0.030) it should be mentioned that all the tests were performed within one week and that the rams had no previous heterosexual experience. It has been reported that serving capacity may be influenced by previous sexual experience, year (Purvis, *et al.*, 1984) and season (Mattner, 1977) and therefore it is possible that the repeatability of libido tests may be lower if tested at different ages and seasons.

The distribution of libido is illustrated in Figure 1. It is evident that rams could be divided into two distinct groups, namely those with a libido score below nine (low libido) and those with a libido score above nine (high libido). Thirty one rams had libido scores of less than nine (7.4 ± 0.5) of which 18 rams exhibited no libido compared to 79 rams with scores above nine (23.5 ñ 0.3). These results are in accordance with the findings of Van Wyk *et al.* (1984) and Fitzgerald & Perkins (1991) who reported that between 9 and 33% of Merino rams are sexually inactive or display very little libido.

The parameters recorded for the high and low libido groups are presented in Table 1. Basal LH plasma concentration as well as plasma LH concentration 30 and 50 min following GnRH administration did not differ ($p > 0.05$) between the high and low libido groups. It is evident that the LH response (difference between basal LH conc. and LH conc. 30 min after exogenous GnRH administration) was higher ($p < 0.05$) in the high libido rams (8.33 ± 0.48 ng/ml) compared to the low libido rams (6.32 ± 0.76 ng/ml). Similarly, it has been reported that rams with a high sexual capability secrete more LH in response to female stimulation than rams with a low sexual capability (Perkins *et al.*, 1992). When considering the relationship between LH response and libido (Figure 2) it is evident that although LH response was lower in the low libido group many rams with high libido also had a below average LH response. Therefore libido, as determined in this study could not be accurately predicted from LH response.

Matos & Thomas (1992) reported that testicular size was correlated with plasma LH, FSH and testosterone concentration. It has also been reported that plasma testosterone con-

**Table 1** Measurements on rams with low and high libido

| | Libido < 9 | Libido > 9 | *p* |
|---|---|---|---|
| Number of rams | 31 | 79 | |
| Libido score at 550 days | 1.4 ± 0.50 | 17.2 ± 0.31 | |
| Body weight at 100 days (kg) | 24.6 ± 0.69 | 25.5 ± 0.43 | 0.2635 |
| Body weight at 260 days (kg) | 46.4 ± 0.87 | 45.2 ± 0.55 | 0.2589 |
| Body weight at 550 days (kg) | 73.2 ± 1.25 | 74.8 ± 0.79 | 0.2602 |
| Testes diam. at 100 days (cm) | 2.43 ± 0.09 | 2.47 ± 0.06 | 0.6856 |
| Testes diam. at 260 days (cm) | 5.39 ± 0.08 | 5.21 ± 0.05 | 0.0494 |
| Testes diam. at 550 days (cm) | 6.42 ± 0.09 | 6.41 ± 0.06 | 0.9484 |
| Change in testes diam. between 100 and 260 days (cm) | 39.98 ± 0.08 | 3.80 ± 0.05 | 0.0699 |
| **GnRH stimulation test at 80 days of age** | | | |
| Basal LH conc. (ng/ml) | 3.34 ± 0.38 | 2.71 ± 0.24 | 0.1591 |
| LH conc. 30 min after GnRH stimulation (ng/ml) | 9.66 ± 0.79 | 11.04 ± 0.50 | 0.1407 |
| LH conc. 30 min after GnRH stimulation (ng/ml) | 3.41 ± 0.25 | 3.61 ± 0.25 | 0.6775 |
| Mean LH conc. after GnRH stimulation (ng/ml) | 6.53 ± 0.52 | 7.33 ± 0.33 | 0.2011 |
| Response in LH conc. after GnRH stimulation (ng/ml) | 6.32 ± 0.76 | 8.33 ± 0.48 | 0.0274 |

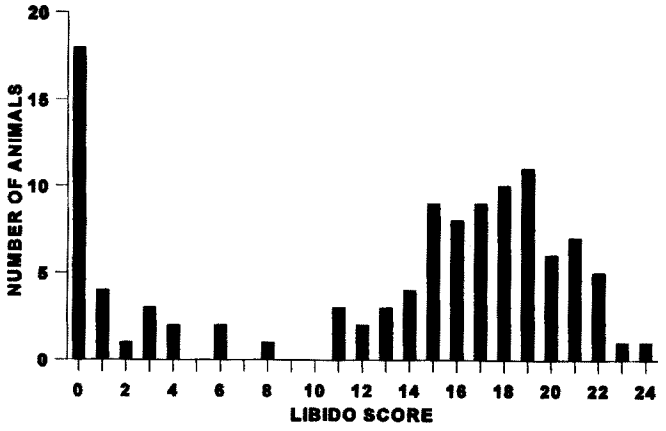


**Figure 1** The distribution of libido in Merino rams at 18 months of age.

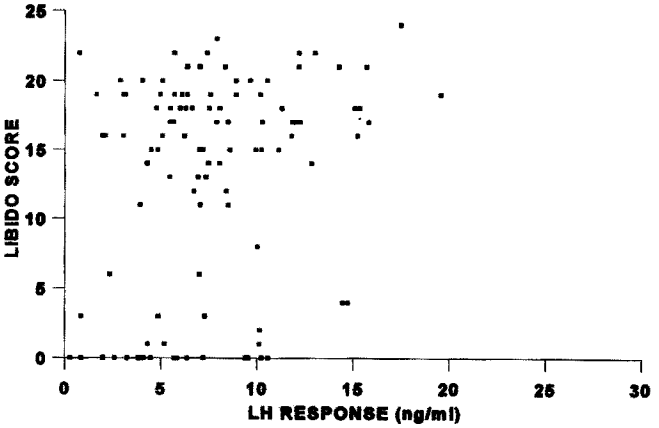


**Figure 2** The relationship between LH response following GnRH stimulation and libido.

centration is correlated with libido within breed (Dufour *et al.*, 1984). As testosterone secretion, which is also responsible for testicular growth (Hafez, 1980), is controlled by LH it was expected that the testes of the high libido group would be larger than those of the low libido group. From Table 1 it is evident that contrary to expectation, the testes size at 100 and 550 days of age did not differ between the high and low libido groups while at 260 days of age the low libido group had larger testes ($p < 0.05$). Although not significant ($p = 0.07$), testes growth from 100 to 260 days of age was also higher in the low libido group. From the present results it is concluded that testes size and growth is not a reliable parameter for libido.

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