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Short Communication

LH response of seasonally anovular Corriedale ewes acutely exposed to rams and estrous ewes

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Abstract

Serial blood sampling before and after exposing four anovular Corriedale ewes to a group of rams and estrous ewes during the non-breeding season revealed a pattern of LH secretion similar to that previously observed in Merinos. Mean LH values doubled ($P < 0.001$) from 0.24 ± 0.06 μg L^{-1} (mean ± s.e.m.) before to 0.55 ± 0.05 μg L^{-1} after 2 h of visual, auditory, and odor exposure to rams and estrous ewes in an indoor facility. A non-significant ($P < 0.17$) increase of LH pulses per hour was also observed (0.7 ± 0.3 pulses per hour before compared with 1.3 ± 0.3 during stimulation). All four ewes had recently formed corpora lutea by five days after stimulation. Results are consistent with the pattern of sudden increase and sustained release of LH observed in other sheep breeds, particularly the Merino.

Keywords: Sheep – ram effect; Ovulation; Anestrus; Seasonal breeding; LH

1. Introduction

The reproductive stimulation triggered in anestrous ewes by a sudden exposure to rams after a period of isolation has been recognized at least since early in the nineteenth century (Girard, 1813). Similar phenomena have been reported for a number of ungulates (goat: Amoah and Bryant, 1984; cattle: Alberio et al., 1987; red deer: Moore and Cowie, 1986; moose: Whittle et al., 2000;

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springbok: Skinner et al., 2002). Most research on this phenomenon, termed the 'male effect', has been done in Merino sheep, a breed that is not highly seasonal in breeding pattern. A rapid increase of LH secretion is the first endocrine response detectable in ewes acutely exposed to rams (Martin et al., 1980; Poindron et al., 1980). An attenuation of the negative feedback of estradiol-17β on the hypothalamus and the pituitary may contribute to the sustained LH response (Martin et al., 1986). The effectiveness of the 'male effect' varies depending on a number of factors. Although establishing valid comparisons is difficult, response to the male depends on extent of breed seasonality (Knight, 1983; Martin et al., 1986; Ungerfeld, 2006). While Merino ewes readily respond to a sudden introduction of rams during most of the anestrous period, breeds with more distinct seasonal breeding patterns usually do not, except shortly prior to the start of the breeding season. Merino-derived moderately seasonal breeds such as the Corriedale have an intermediate response to introduction of rams. In this latter breed, rams alone do not evoke a full response (Rodríguez Iglesias et al., 1992), but the presence of estrous ewes at ram introduction seems to provide the additional variety and intensity of stimulus required for a reliable response throughout the anestrous period (Rodríguez Iglesias et al., 1991). The immediate ovarian response observed in Corriedales (ovulations without expression of behavioral estrus within 48 to 60 h of exposure to rams and estrous ewes) is consistent with what has been observed in ram-exposed Merinos (Martin et al., 1986). The timing of the ovarian response suggests that the pattern of increased LH release after ram-stimulation is similar to that observed in Merinos, and most likely underlies the ovarian response. The limited evidence available on the timing of LH surges after ram exposure in Corriedale ewes (Ungerfeld et al., 2002; samples collected every 4 h for a period of 60 h) also indicates that a similar LH pattern is likely to exist with this breed. Such a pattern, however, remains hypothetical. To the best of our knowledge, a single LH profile from samples collected at 15 min intervals after ram exposure (Ungerfeld, 2003) is the only available information on the immediate LH response of Corriedale ewes. This single profile, however, does not fit well with the assumption that there is a sudden increase in the frequency of LH pulses; it rather suggests a progressive and sustained increase over a period of several hours. Establishing the nature of the immediate LH response of Corriedale ewes would contribute to the characterization of between-breed variation for a trait of considerable importance for non-pharmacological out-of-season breeding in sheep. The pattern of LH release in response to a ram-induced stimulus, however, is also a needed benchmark for evaluating immediate hormonal responses to other possible surrogate triggers such as sound recordings, images, or videos. This type of research, already underway (Hawken et al., 2006; Ferrería et al., unpublished results), is required to elucidate the relative importance of and possible interactions between different sensory inputs in triggering hormonal and ovarian responses in anovular ewes.

We report results from an observational study in which mature anovular Corriedale ewes were longitudinally sampled for LH before and after being exposed to the presence of rams and estrous ewes during anestrus.

2. Materials and methods

2.1. Location and animals

The present study was conducted at the Argerich Experiment Station (latitude: 38° 44′S) during late December and early January 2006. Four 4.5 to 6.5-year-old multiparous anovular Corriedale ewes with body weights (66 ± 4 kg; mean ± s.d.) and body condition scores (2.8 ± 0.5; mean ± s.d.; scale 1 [emaciated] to 5 [obese]; Jefferies, 1961) representative of an experimental

Corriedale flock ($n = 150$) maintained at the station, were randomly chosen for the study. Mid-ventral laparoscopy, performed under local anaesthesia, was used to determine both anovular status before (absence of corpora haemorrhagica, corpora lutea or corpora albicantia) and occurrence and timing of ovulation (on the basis of color; Oldham and Lindsay, 1980) after the stimulation period.

Another group of ten mature ewes and five vasectomized sexually experienced Corriedale rams was available to act as the stimulus for the experimental animals. Ewes in this latter group were brought into standing estrus by intramuscular injections of 500 μg of oestradiol benzoate after a 5-day progestogen (MAP; Gador Argentina) priming period. The five vasectomized rams have been successfully used before for the induction of ovulation in seasonally anovular ewes.

2.2. Experimental procedure

The present study was conducted indoors in a well-ventilated facility, and under prevailing photoperiod. To limit unnecessary handling of the animals and facilitate blood sampling, experimental ewes were individually restrained in elevated (1 m over floor level) wooden crates with a meshed opening allowing full vision of a 5 × 7 m fenced (1 m tall) arena. Crates were arranged in a single row against the longer side of the arena thus allowing the exposure of experimental ewes to the sight, sound, and smell (but not touch) of the stimulus group. Ewes were allowed to acclimate to such an environment for one week and then fitted with indwelling jugular vein cannula on the day before the start of the sampling period. Water and alfalfa hay were available to the animals *ad libitum* during both acclimation and sampling.

A before–after sampling protocol was adopted to take advantage of the within-animal variance, thus limiting the number of animals required to detect the expected effect. Given the nature of the live stimulus required (sight, sound, and smell from a group of conspecifics), replicated facilities and stimulation groups would have been required to remove possible time and location effects should a treatment–control approach had been adopted.

The stimulus group of ten ewes and five rams was housed in a different facility (250 m away downwind) until required; the occurrence of sexual behavior in this group was visually verified shortly after estrogen treatment and again immediately before they were moved into the experimental arena to initiate the stimulation period.

Duplicate blood samples (5 ml) were collected at 10 min intervals during a prestimulation period of 1 h, discontinued during a 3.5-h resting period, and then collected again during a stimulation period of 2 h. Imposing a resting period in between collection periods was an attempt to minimize potential carry-over effects of the prestimulation bleeding on LH secretion during the stimulation phase (Adams et al., 1993). Blood samples were collected into heparinized tubes, immediately centrifuged, and plasma stored at –18 °C until assay. Plasma LH concentration was assessed in duplicate blood samples using a commercial immunoenzymatic kit (LH Detect®; Pelletier et al., 1982). The minimal detectable concentration of the assay was 0.04 $\mu g\,L^{-1}$ and the within-assay coefficient of variation was 13%.

After blood sampling, the experimental ewes remained in full contact with the stimulus group for the next four days and were then subjected to laparoscopy to assess the occurrence of ovulation. Sampling protocol and animal care were consistent with standards set in the Consortium guide (Consortium for Developing a Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching, 1999). In particular, laparoscopies were performed by a very experienced individual and demanded less than 1 min on average; a very thin cannula (1 mm external diam-

eter; Tyco Electronics Australia) was used for repeated blood sampling in order to minimize discomfort.

2.3. Statistical analyses

The LH concentration data were log-transformed to improve variance homogeneity ($P < 0.25$ for lack of homogeneity after transformation) and then subjected to repeated measures analysis. A linear mixed-effects model (Pinheiro and Bates, 1996, 2000) was fitted to the longitudinal data with a before–after factor adjusted as the only fixed effect. Several covariance matrices were tested for modeling within-subject variation but they only produced marginal differences in the fitting. The analysis reported is based upon an unstructured covariance matrix. A non-parametric test (Wilcoxon, 1945) was used to evaluate before versus after mean LH concentrations in individual ewes. The four longitudinal LH datasets were submitted to a computerized peak detection algorithm (Veldhuis and Johnson, 1986) to assess the number of LH peaks occurring per hour; before and after frequencies were then compared using Wilcoxon's test on paired data. S-Plus (MathSoft Inc., 2000) was used for data management and statistical calculations.

3. Results

Mean LH concentration values of experimental ewes doubled ($P < 0.001$) from $0.24 \pm 0.06\ \mu g\ L^{-1}$ (mean ± s.e.m.) before to $0.55 \pm 0.05\ \mu g\ L^{-1}$ after being acutely exposed to rams and estrous ewes for a 2-h period. Responses of individual ewes were fairly homogeneous with mean LH concentrations increasing in all four sheep ($P < 0.04$ to $P < 0.001$).

The frequency of LH pulses was numerically increased during the stimulation period (0.7 ± 0.3 pulses per hour before versus 1.3 ± 0.3 during stimulation), but the before versus after comparison did not reach statistical significance ($P < 0.17$). This result was mainly associated with the response of one ewe that produced no pulses before and only one large pulse during the 2-h stimulation period.

All four sheep exhibited one or more recently formed (shiny, deep red) corpora lutea when laparoscopy was conducted on day-5.

4. Discussion

Monitoring ovarian responses to the 'male effect' in real time (i.e., *via* ultrasonography or laparoscopy) is relatively inexpensive, but requires exposing experimental subjects to the stimulus for a period of days. Short term (hours) exposures have failed at evoking consistent ovulatory responses (Signoret et al., 1982/83). Experimental protocols involving surrogate sensory stimuli (e.g. sound recordings, pictures, or video images), however, become impractical when groups of animals have to be maintained indoors for a number of days in order to expose them continuously to a certain amount of stimulation. A full ovarian response to the 'male effect' should presumably be preceded by a rapid and sustained neurohormonal response (Martin et al., 1986). Thus, assessing immediate LH secretion patterns, instead of waiting for ovulation to occur, would be a more feasible experimental paradigm to evaluate response in controlled indoor environments. The quantitative characteristics of the LH response, as opposed to the all-or-none nature of ovulation, would offer additional opportunities to identify possibly significant diversity of LH response patterns. Any protocol successful

on an LH basis, however, should ultimately be assessed as to whether it effectively induces ovulation.

The present study aimed at characterizing the immediate LH response of anovular Corriedale ewes exposed to a stimulus known to consistently induce ovulation (as verified in the current study). Results from the present study revealed LH secretion characteristics (increased mean concentrations, numerically, though not statistically increased release of pulses) similar to those previously reported for Merinos ewes (Martin et al., 1986). Prestimulation mean LH values were typically less and pulses infrequent as expected for anestrual ewes (Martin et al., 1986). However, three out of four ewes initiated pulsatile LH releases within the first 10 min of ram exposure; the remaining ewe did so after a 50 min delay and only released a single LH pulse in 2 h.

Given the observational nature of the present study, the LH patterns observed may have been induced by causes other than the exposure to the stimulus group. Although such a possibility cannot be discounted, the precaution of inserting a resting period in between collection periods, the minimal distress produced by bleeding cannulated animals acclimated to their surroundings, and the rapidity of the LH response observed (typical of the male effect) suggest that the immediate endocrine response of anovular Corriedale ewes to the sudden presence of a group of rams and estrous ewes was effectively characterized.

For three of four ewes sampled, results do not appear to match those of the single LH profile available for Corriedale sheep (Ungerfeld, 2003). However, the reduced number of animals involved and the quasi-fractal nature of LH secretion episodes (pulses within pulses, as time resolution is increased by means of more frequent sampling; see, for example, Martin et al., 1986) makes comparisons across studies difficult; the particular secretion patterns compared are partially dependent upon the time of the sampling protocol. What appears to be a plateau at a particular time scale, may be revealed as a series of pulses if sampling frequency was increased. The particular nature of the male effect LH secretion profile may thus change as technical capabilities (e.g. minimal detectable concentrations, automatic sampling devices) improve. In the mean time, results from the present study indicate that frequent sampling for a period of 2 to 3 h would be required to characterize individual LH responses in Corriedale ewes. Even though an increased frequency of LH pulses seems to be the physiologically significant event associated to the sudden exposure to rams (Martin et al., 1986), on an individual basis, significant changes in mean LH concentration would be feasible surrogates to identify individuals responding to the male effect. Given the discrete nature of the variable, detecting increases in the mean number of pulses per hour would require wider temporal sampling windows both before and during stimulation.

5. Conclusions

Anovular Corriedale ewes exposed to rams and estrous ewes during anestrus exhibited a pattern of LH secretion similar to that previously reported for Merinos. The effect was more noticeable for mean LH concentration than for the number of LH pulses per hour.

On an individual basis, serial blood collection for a period of 2 to 3 h would suffice for the purpose of detecting positive mean LH concentration responses. Detecting increased LH pulse frequencies would probably require longer sampling periods.

Ovulation and LH concentration results suggest that the LH pattern observed is probably characteristic of the response of anovular Corriedale ewes to a sudden exposure to rams and estrous ewes.

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