Available online at www.sciencedirect.com
ScienceDirect
Theriogenology 68 (2007) 56–66
Theriogenology
www.theriojournal.com

The introduction of rams induces an increase in pulsatile LH secretion in cyclic ewes during the breeding season

P.A.R. Hawken [a,b,*], A.P. Beard [b], T. Esmaili [a], H. Kadokawa [c], A.C.O. Evans [d], D. Blache [a], G.B. Martin [a]

[a] School of Animal Biology, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia
[b] School of Agriculture, Food and Rural Development, University of Newcastle, Newcastle upon Tyne NE1 7RU, United Kingdom
[c] Department of Veterinary Medicine, Faculty of Agricultural Science, The University of Yamaguchi, Yoshida 1677-1, Yamaguchi 753-8515, Japan
[d] School of Agriculture, Food Science and Veterinary Medicine, Conway Institute for Biomolecular and Biomedical Research, College of Life Science, University College Dublin, Belfield, Dublin 4, Ireland

Received 5 February 2007; accepted 17 March 2007

Abstract

Application of the ram effect during the breeding season has been previously disregarded because the ewe reproductive axis is powerfully inhibited by luteal phase progesterone concentrations. However, anovulatory ewes treated with exogenous progestagens respond to ram introduction with an increase in LH concentrations. We therefore tested whether cyclic ewes would respond to ram introduction with an increase in pulsatile LH secretion at all stages of the estrous cycle. We did two experiments using genotypes native to temperate or Mediterranean regions. In Experiment 1 (UK), 12 randomly cycling, North of England Mule ewes were introduced to rams midway through a frequent blood-sampling regime. Ewes in the early (EL; $n = 6$) and late luteal (LL; $n = 6$) phase responded to ram introduction with an increase in LH pulse frequency and mean and basal concentrations of LH (at least $P < 0.05$). In Experiment 2 (Australia), the cycles of 32 Merino ewes were synchronised using intravaginal progestagen pessaries. Pessary insertion was staggered to produce eight ewes at each stage of the estrous cycle: follicular (F), early luteal (EL), mid-luteal (ML) and late luteal (LL). In all stages of the cycle, ewes responded to ram introduction with an increase in LH pulse frequency ($P < 0.01$); EL, ML and LL ewes also had an increase in mean LH concentration ($P < 0.05$). In conclusion, ram introduction to cyclic ewes stimulated an increase in pulsatile LH secretion, independent of ewe genotype or stage of the estrous cycle.

Keywords: Ram effect; Breeding season; Estrous cycle; Ewes; Luteinising hormone

1. Introduction

The ram effect is a well-established phenomenon that induces ovulation in anovulatory ewes. In brief, the introduction of rams stimulates an increase in pulsatile secretion of LH within minutes and, in some breeds, this is sufficient to induce a pre-ovulatory LH surge, ovulation and, subsequently, estrus and conception [1–3]. One of the subsidiary benefits is that the response to the ram effect is synchronous among the flock so it can be used as a non-pharmacological alternative to conventional methods of estrus synchronisation, an important issue in today's consumer-driven climate [4].

However, the ram effect cannot induce ovulation in cyclic ewes, so is considered ineffective for estrus synchronisation during the breeding season. Conversely,

* Corresponding author at: School of Animal Biology M085, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia. Tel.: +61 8 6488 3588; fax: +61 8 6488 1040.
E-mail address: phawken@animals.uwa.edu.au (P.A.R. Hawken).

0093-691X/$ – see front matter.
doi:10.1016/j.theriogenology.2007.03.023

there is some evidence that cyclic ewes can respond to stimuli from the ram during the breeding season. For example, during the follicular phase, the continuous presence of rams advances the LH surge [5], accelerates the onset of estrus, and reduces the duration of sexual receptivity [6–11]. During the luteal phase, progesterone produced by the CL strongly inhibits pulsatile LH secretion [12], but ram introduction elicits an increase in pulsatile LH secretion in ovariectomised ewes treated with progesterone during the breeding season [13]. The same applies to seasonally anovulatory ewes undergoing progesterone treatment [14]. More recently, Evans et al. [15] found that ram introduction towards the end of a progestagen synchronisation protocol advanced the LH surge and the onset of estrus after withdrawal of the progestagen. Furthermore, as the rams were removed at progestagen withdrawal, this observation infers a residual effect of rams on the timing of subsequent endocrine events.

The apparent ability of ewes to respond to the ram, despite the presence of progesterone, suggests that it may be possible to use the ram to control estrous cycles during the breeding season. We therefore tested whether ram introduction could increase LH pulse frequency in ewes at all stages of the estrous cycle. The responsiveness of ewes to the conventional ram effect is highly dependent on genotype and region of origin [16], so we have done two experiments, one using cyclic Merino ewes, native to Mediterranean regions, and a second using cyclic North of England mule ewes, native to temperate regions.

2. Materials and methods

2.1. Experiment 1

2.1.1. Animals and experimental procedures

The experiment was carried out in accordance with the Animals (Scientific Procedures) Act 1986 and was approved by the University of Newcastle Animal Ethics Committee and the UK Home Office.

During October (mid-breeding season; Northern Hemisphere), primiparous North of England mule ewes (2 y; Scottish Blackface × Bluefaced Leicester; $n = 12$) that had been previously isolated from ram contact (i.e. more than 500 m away from rams for a minimum of 2 mo) were selected and housed in groups of three in 2 m × 1.8 m pens under a photoperiod equivalent to the natural day length (8 h) in an animal house at Cockle Park Research Farm, Newcastle upon Tyne (55°13′N). Each pen of ewes was given 2.5 kg hay daily. The rams ($n = 1$ per three ewes) were adult, sexually experienced, vasectomised, crossbreds (6–7 y; Suffolk-Scottish Blackface × Bluefaced Leicester).

2.1.2. Blood sampling

Blood was sampled via jugular cannula every 12 min for 6 h before and 6 h after ram introduction (Day 0) and every 2 h from 19 to 43 h after ram introduction (Day 1). This second period was considered best for detecting an LH surge based on observations that, in anovulatory ewes, the ram-induced LH surge occurs 20–40 h after ram introduction [1]. Blood collected for LH was left to clot for a minimum of 16 h at room temperature. Samples were then centrifuged at 2000 g for 15 min and serum was decanted into plastic tubes and stored at −20 °C until analysis.

Blood was sampled twice weekly for progesterone for 2 weeks before ram introduction to determine retrospectively the stage of cycle on the day of ram introduction (Day 0). Blood samples were taken daily on Days 3 to 6 after ram introduction, and twice weekly for 3 weeks after ram introduction, to profile estrous cycle dynamics. Blood collected for progesterone was centrifuged immediately at 2000 g for 15 min. Plasma was then decanted into duplicate plastic tubes that were capped, immediately frozen and stored at −20 °C until analysis.

2.1.3. Immunoassay

Progesterone was assayed in duplicate samples of plasma using a commercial enzyme linked immunoassay (ELISA) kit (Ridgeway Science Ltd., Gloucester, UK) in accordance with the manufacturer's instructions, as detailed by Madgwick et al. [17]. The sensitivity of the assay was 0.23 ng/mL. For low (1.85 ng/mL), medium (3.02 ng/mL) and high (7.43 ng/mL) concentration samples, mean intra-assay coefficients of variation were 7.6, 11.4, and 2.7%, and inter-assay coefficients of variation were 10.5, 7.4, and 8.0%.

Serum LH concentrations were determined using a previously validated double-antibody radioimmunoassay [18]. The sensitivity of the assay was 0.1 ng/mL. Low (0.28 ng/mL), medium (1.53 ng/mL) and high (3.47 ng/mL) concentration samples were used to estimate mean intra-assay coefficients of variation (17.1, 16.2, and 7.8%) and mean inter-assay coefficients of variation (8.2, 8.4, and 18.6%).

2.1.4. Data analysis

The progesterone profile for each ewe was used to determine the stage of the estrous cycle at the time the