



Ovulation and ovulation rate in ewes under grazing conditions: factors affecting the response to short-term supplementation



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ABSTRACT

The relationships between ovulation rate and nutrition remain confused, probably because of uncontrolled variation in experimental conditions. To help resolve the problem, we analyzed data from 20 experiments conducted between 2002 and 2016, in Uruguay with grazing ewes. All experiments were carried out by a single laboratory under comparable conditions of experimental design and measured variables. The studies used a total of 3 720 ewes, of purebred Corriedale, Polwarth, or East Friesian x Polwarth genotypes. In all experiments, a control group grazed native pastures and extra nutrition was provided to the treatment groups using either improved pastures or supplements. Ovulation rate was measured by counting corpora lutea using laparoscopy or rectal ultrasound or by counting foetuses at ultrasound on day 45 of gestation. For statistical analysis, data were grouped according to nutritional treatment (control or supplemented) and, within these groups, type of supplement to provide energy or protein (protected or not from rumen degradation). Across all experiments, 92–99% of the ewes ovulated and the effects of diet, length of supplementation, and initial live weight and genotype are reported. Within diets, ovulation was most affected by overall energy intake during supplementation ($P < 0.01$). Ewes that grazed native pastures supplemented with protein supplements had higher ovulation rates ($P < 0.05$) than control ewes grazing only native pastures. The addition of tannins to the protein supplement, to protect it from degradation in the rumen, did not further increase the ovulation rate. In unsupplemented ewes that had access to legume pastures, ovulation rates did not increase when the legume pasture was rich in tannins although only ewes that grazed tanniniferous legumes had marginally higher ovulation rates than the control ewes ($P < 0.05$). When ewes grazing native pastures were supplemented with energy, their ovulation rate did not increase above those of nonsupplemented ewes. Live weight at the start of supplementation also affected ovulation rate. We conclude that ovulation was most affected by overall energy intake, whereas the factors that affected ovulation rate during short-term nutritional supplementation were intake of protein from highly digested supplements or dietary protein protected from ruminal degradation.

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Implications

Under extensive grazing systems low fertility (ovulation) and low prolificacy (ovulation rate) often limit production efficiency from wool-type ewes. The nutrition of the ewe has a major influence on ovulation because of its accumulative effect on ewe body condition and weight at the time of mating. An increase in ovulation rate is the next step to lift the number of lambs conceived by those ewes that ovulate. This outcome can be achieved either by feeding supplements with

highly digestible crude protein or by grazing improved pastures high in crude protein protected from rumen degradation.

Introduction

Under extensive grazing conditions, wool-type ewes commonly have pregnancies with fewer than 20% of twins, but if they are given enough time after weaning to gain weight before mating, prolificacy can increase (Lindsay et al., 1975; Morley et al., 1978; Ganzabal and Echevarría, 2005). Alternatively, prolificacy can be increased by a short-term nutritional supplement, the ‘flushing’ phenomenon documented by Heape (1899) and subsequently explained by effects on ovulation rate (Clark, 1934).

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Despite this long history of research, the field remains confused, primarily because the increment in ovulation rate above control values is highly variable, with reported differences ranging from -14 to $+40\%$ (Croker et al., 1985; Leury et al., 1990). Explanations for this variability have included the type of supplement fed and the duration of supplementation (early studies reviewed by Smith and Stewart, 1990). For example, in ewes supplemented with high-quality pastures or silage, ovulation rate increased gradually until the third week of treatment (Smith et al., 1983). Ewes therefore needed to be fed a high-energy supplement for at least one oestrous cycle. On the other hand, there are exceptions to this apparent need for a long-term nutritional treatment. Teleni et al. (1989) found that infusion of nutrients was effective after only nine days, and many other laboratories consistently reported that lupin grain or soybean meal can be fed for periods as short as 4–6 days, (Lindsay, 1976; Radford et al., 1980; Fletcher, 1981; Gherardi and Lindsay, 1982; Oldham and Lindsay, 1984; Stewart and Oldham, 1986; Nottle et al., 1990; Molle et al., 1997; Viñoles et al., 2009; Banchemo et al., 2012). Moreover, ovulation rate seems to be further increased when the protein in the supplement was partially protected from rumen degradation (Nottle et al., 1988; Viñoles et al., 2009; Banchemo et al., 2012).

These discrepancies are difficult to explain – indeed, the lack of predictability in the response has made it difficult to recommend the practice to farmers (Croker et al., 1985). It seems likely that much of the confusion arises because the seemingly contradictory experiments were done by many laboratories, and with a variety of techniques and experimental designs, often with insufficient replicates and inappropriate statistical methods, under a variety of experimental conditions. By contrast, between 2002 and 2016, on two experimental stations in Uruguay, 20 experiments were conducted in which the major factors were similar: genotype, dietary treatment, and methodologies. Together, the data from these experiments offered us an opportunity to analyze a large body of information with the aim to better understand the roles of protein and energy in the effects of nutritional treatments on ovulation and ovulation rate.

A critical factor in our analysis was an assessment of the impact of apparently anovulatory ewes in the treatment groups on the overall response in ovulation rate. It is clear that ewes must be ovulating before they can express their ovulation rate so, anovulatory ewes should not be included in the calculation of ovulation rate. It is important to remember that an increase in ovulation rate is not simply a reflection of a stronger physiological drive to ovulate (Martin et al., 1981) – the brain controls the ‘decision’ to ovulate whereas ovulation rate is largely determined at the level of the ovary (Martin et al., 2008; Scaramuzzi et al., 2011). However, the frequency of apparently anovulatory ewes might contribute to our analysis by indicating reproductive capacity in the experimental animals, or perhaps the accuracy of the techniques used to detect corpora lutea in our experiments.

We have therefore used the INIA data to re-test the hypothesis that ovulation rate can be increased by a short period of supplementation with either a protein-rich supplement in ewes grazing native pastures or by allowing ewes a short period of access to legume-rich pastures. We additionally expected that, if the protein in the supplement was protected from rumen degradation by tannins either by adding exogenous tannins or by allowing the ewes to graze tanniniferous legumes, ovulation rate would be increased compared to the same diets lacking such protection. Finally, we assessed the potential role of the percentage of anovulatory ewes as a determinant of the experimental outcome.

Material and methods

Experimental settings

Twenty experiments were conducted over 14 years between 2002 and 2016 at INIA La Estanzuela and INIA Treinta y Tres, experimental

stations of the National Institute of Agricultural Research of Uruguay ($30\text{--}35^\circ$ South; $53\text{--}58^\circ$ West) involving 3 720 maiden and adult ewes of three breeds: Corriedale, Pure Polwarth, and East Friesian by Polwarth (‘Polwarth cross’) in the peak breeding season; March to May. Adult ewes had been weaned since December and both adult and maiden ewes were kept apart from males since the previous year service. Oestrus was synchronized in most of the ewes (Table 1) with either one or two doses of 160 mg d-Cloprostenol (Veteglan Laboratorio Calier, Barcelona, Spain), and the treatments were applied during the subsequent oestrous cycle (Fig. 1). For those ewes that were not synchronized, 6% of fertile rams with harnesses were introduced into each group of ewes eight days before the end of treatments (Table 1; experiments 7 to 10) and remained with the ewes for 16 days.

Ovulation and ovulation rate were measured one week after the oestrus following treatment by counting corpora lutea either by laparoscopy or rectal ultrasound or later by pregnancy ultrasound. Ovulation was recorded as the percentage of the total animals in each treatment with corpora lutea, and ovulation rate was assessed as the number of corpora lutea as a proportion of the ewes with corpora lutea. Within the ewes that were not synchronized, success of pregnancy and litter size were used as indirect measures of ovulation and ovulation rate. Every experiment had a control group of ewes grazing native pasture (Table 1).

Nutritional treatments

The treatments were either improved pastures or supplements of either grain or concentrate. For statistical analysis, the data were divided into two groups. The first, designated ‘dietary treatment’ were used to evaluate the effect on ovulation and ovulation rate of energy or type of protein (protected or not from rumen degradation), from either pasture alone or pasture plus the supplement. The second, designated ‘length of supplementation’ was used to evaluate the duration of supplementation on ovulation and ovulation rate. The groups described below were analyzed separately to avoid confounding the effects of supplementation, but the same model was used for all analyses. In summary, we had seven dietary treatments that are shown in Table 2:

- 1) *Control* ($n = 1\ 087$) – access to only native pasture (forage allowance of 12 kg of dry matter (DM)/100 kg liveweight (LW); 7.6 MJ of metabolizable energy (ME)/kg DM and 86 g of crude protein (CP)/kg DM);
- 2) *Legumes without tannins* ($n = 354$) – access to legumes with little or no tannins but with a high-protein content (forage allowance of 12 kg DM/100 kg LW; 8.3 MJ ME/kg DM and 172 g CP/kg DM);
- 3) *Tanniniferous legumes* ($n = 581$) – access to legumes high in protein and with moderate levels of endogenous tannins (forage allowance of 12 kg DM/100 kg LW; 8.4 MJ ME/kg DM and 177 g CP/kg DM);
- 4) *Protein supplementation of native pasture* ($n = 1\ 073$) – access to native pasture (forage allowance of 12 kg DM/100 kg LW; 7.6 MJ ME/kg DM and 86 g CP/kg DM) plus a high-protein supplement (11.6 MJ ME/kg DM and 346 g CP/kg DM) at 1 kg/100 kg LW;
- 5) *Energy supplementation of native pasture* ($n = 114$) – access to native pasture (forage allowance of 12 kg DM/100 kg; 7.5 MJ ME/kg DM and 69 g CP/kg DM) plus a high-energy supplement (13.7 MJ ME/kg DM and 90 g CP/kg DM) at 1 kg/100 kg LW);
- 6) *Energy supplementation of tanniniferous legumes* ($n = 119$) – access to legumes rich in protein and with a moderate tannin content (forage allowance of 12 kg DM/100 kg LW; 6.3 MJ ME/kg DM and 185 g CP/kg DM) plus a high-energy supplement (14.2 MJ ME/kg DM and 96 g CP/kg DM) at 1 kg/100 kg LW);
- 7) *Native pasture supplemented with protein protected by exogenous tannins* ($n = 392$) – access to native pasture (forage allowance of 12 kg DM/100 kg LW; 7.8 MJ ME/kg DM and 79.6 g CP/kg DM) plus a high-protein supplement protected by exogenous tannins (10.9 MJ ME/kg DM; 353 g CP/kg DM) at 1 kg/100 kg LW.

Table 1
General description of the experiments on ovulation and ovulation rate of the ewes included in the study.

Experiment	Year	Site	Breed	Number		OS	Treatments	LPL	SL	OR
				A	M					
1	2002	TT	C	111	37	1Pg	NP; NP + corn; LU	16	16	LP
2	2003	TT	C	208		1Pg	NP; NP + corn; LU; LU + corn	14	10	LP
3	2004	TT	C	406		2Pg	NP; NP + SFM; NP + SFM + corn; NP + lick; LU; Lu + corn	21	9	LP
4	2005	LE	P	178		1Pg	NP; LU; LC	26		PR
5	2005	LE	PX	127		1Pg	NP; LU; LC	26		PR
6	2005	TT	C	182	28	1Pg	NP; NP + lick; SB crop	15	15	PR
7	2006	LE	PX	140		NS	NP; NP + SB grain; LU	26	21	PR
8	2006	LE	P	176		NS	NP; NP + SB grain; LU	26	21	PR
9	2007	LE	PX	109		NS	NP; AA	12		PR
10	2007	LE	P	400		NS	NP; AA; RC; LC	12		PR
11	2008	LE	PX	71		2Pg	NP; NP + SBM; NP + SBM + 1.5% Tn; NP + SBM + 2.5% Tn		9	OUS
12	2008	LE	P	275		2Pg	NP; NP + SBM; NP + SBM + 1.5% Tn; NP + SBM + 2.5% Tn		9	OUS
13	2009	LE	P	158		2Pg	NP + SBM; NP + SBM + 0.9% Tn; NP + SBM + 1.5% Tn		9	OUS
14	2010	LE	P	160		2Pg	NP; NP + SBM		9	OUS
15	2010	TT	C	203		2Pg	NP; NP + SBM + RB; NP + SBM + RB + 1.0% Tn		9	OUS
16	2011	LE	P	49	42	2Pg	NP; NP + SBM + RB		9	OUS
17	2013	LE	P	150		2Pg	NP; NP + corn; NP + SBM; NP + lick; NP + lick + 1.2% Tn		8	OUS
18	2014	LE	P	152		2Pg	NP; NP + SBM; NP + SBM + DEMP®		9	OUS
19	2015	LE	P	148		2Pg	NP; NP + SBM		9	OUS
20	2016	LE	P	126	84	2Pg	NP; NP + SBM; NP + lupins		9	OUS

Sites: TT = Treinta y Tres; LE = La Estanzuela. Breeds: C = Corriedale; P = Polwarth; PX = Polwarth cross. Number: A = Adult; M = Maiden. OS: method used for synchronization of oestrus: 1Pg = single injection of prostaglandin F2 α ; 2Pg = 2 injections of prostaglandin F2 α ; NS = not synchronized. Treatments: NP = native pasture; LU = *Lotus uliginosus* cv. Maku; SFM = sunflower meal; LC = *Lotus corniculatus* cv. Draco; SB = soybean; SBM = soybean meal; AA = Alfalfa (lucerne); RC = red clover; RB = rice bran; DEMP® = microbial protein; Tn = tannins. LPL = pasture legume length (days). SL = supplementation length (days). Method to measure OR (ovulation rate): LP = laparoscopy, PR = pregnancy rate by ultrasound; OUS = ovarian ultrasound.

For the duration of supplementation, the groups were: i) unsupplemented; ii) supplemented for 8–10 days, or iii) supplemented for 15–21 days. Groups ii and iii differed in type of synchronization: Group ii was synchronized with two injections of synthetic prostaglandin, whereas Group iii was either not synchronized or synchronized with a single injection. The three breeds were represented in all treatments except for Treatment 5 (only Corriedale and Polwarth) and Treatment 6 (Corriedale only). Ewes were weighed and condition scored (1 to 5; Russel et al., 1969) one month before and immediately before and after the treatments were applied. All ewes grazed native pasture *ad libitum* until allocated to each treatment on Day 1 when they were offered pasture daily at 12 kg DM/100 kg of LW. The ewes in each experiment grazed the same paddock with the same pasture allowance per ewe but were separated into treatment groups using electric fences. The supplement was gradually introduced over 4 days to avoid acidosis (0.1, 0.2, 0.3, 0.4 kg/ewe daily) after which 1 kg/100 kg LW was provided each day for the designated days of supplementation treatment (Fig. 1). The tannins added to the protein supplement in Treatment 7 were condensed extracts from the Quebracho tree (*Schinopsis balansae*), with the amount provided calculated as described by Banchemo et al. (2012). The forage in the experimental paddocks was sampled as described by Haydock and Shaw (1975) one week before

the treatments were applied. Pastures and supplements were analyzed for CP at the Nutrition Laboratory of INIA La Estanzuela and the ME content was calculated in MCal according to (Association of Official Analytical Chemists (AOAC), 1984; Table 2) and converted to MJ. The intakes of forage and supplement were estimated and ME and CP intake were calculated as described by Banchemo et al. (2012) with descriptive results presented as the mean and SD (Table 2). The levels of tannin in the pastures were not measured due to the absence of an appropriate technique at the time of the experiments. We discussed the results assuming an average concentration of 15–25 g condensed tannins/kg legume DM, (A. Mederos, unpublished) by using the butanol/HCl technique (adapted from Porter et al., 1986).

Statistical analyses

Generalized linear mixed models (GLMM) were used for the analysis of the two response variables of interest, ovulation, and ovulation rate. For ovulation, the error distribution was assumed binomial and the logit link function was used. For ovulation rate, Poisson distributed errors were considered, and the logarithmic (log) link function was used. An Akaike Information Criterion (AIC/AIC_c or QAIC/QAIC_c in cases of over-dispersion) was used in the process of the model selection,

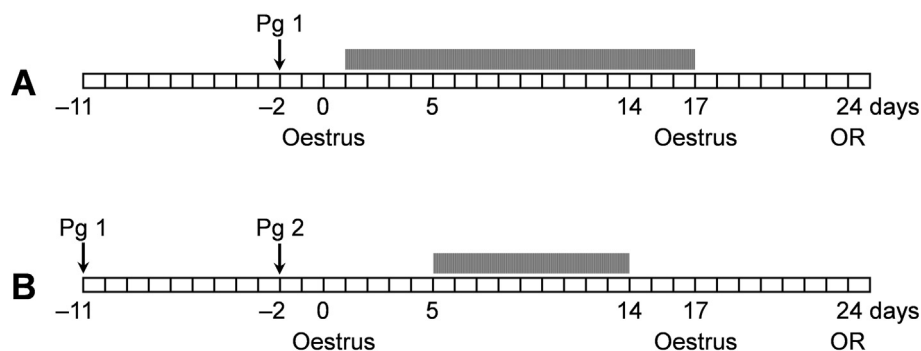


Fig. 1. Timing of experimental protocols that used oestrus synchronization with prostaglandin F2 α (Pg), applied to the ewes as either as A) a single injection or B) as two injections 9 days apart. Shaded area: nutritional treatments. OR: measurement of ovulation rate.

Table 2

General description of the ewes, pasture, and supplement quality for the grouping of nutritional treatments for the analysis.

Treatment	Ewes				Pasture quality		Supplement quality		Ewe's estimated intake	
	n	Breed	Initial BW kg ± SD	Initial BCS (1–5) ± SD	Crude protein g/kg DM	Energy MJ/kg DM	Crude protein g/kg DM	Energy MJ/kg DM	Crude protein g/d	Energy MJ/d
1	1 087	C,P;PX	48.4 ± 6.8	2.1 ± 0.42	86 ± 25	7.6 ± 1.10			130 ± 38.6	11.6 ± 1.73
2	354	C,P;PX	48.7 ± 7.3	2.2 ± 0.35	172 ± 17	8.3 ± 1.10			267 ± 20.7	11.9 ± 2.39
3	581	C,P;PX	48.3 ± 6.9	2.2 ± 0.46	177 ± 33	8.4 ± 1.30			263 ± 67.1	12.8 ± 2.71
4	1073	C,P;PX	47.8 ± 6.2	2.1 ± 0.37	89 ± 27	7.6 ± 1.10	346 ± 88	11.6 ± 1.00	253 ± 56.4	13.3 ± 1.83
5	114	C,P	44.4 ± 3.9	2.4 ± 0.30	74 ± 3.0	7.5 ± 1.29	90 ± 4.40	13.7 ± 0.08	109 ± 12.7	13.4 ± 1.01
6	119	C	47.6 ± 5.8	2.3 ± 0.30	185 ± 13	6.3 ± 0.56	96 ± 4.00	14.2 ± 0.52	237 ± 1.30	13.6 ± 0.60
7	392	C,P;PX	49.9 ± 7.3	1.9 ± 0.34	80 ± 13	7.8 ± 0.62	353 ± 44	10.9 ± 0.57	252 ± 32.7	13.6 ± 1.24

Dietary treatments: 1) control (NP = native pastures); 2) legumes without tannins; 3) tanniniferous legumes; 4) protein supplementation of native pastures; 5) energy supplementation of native pastures; 6) energy supplementation of tanniniferous legumes; 7) native pasture supplemented with protein protected by exogenous tannins. BW: body weight. BCS: body condition score. Breeds: C = Corriedale; P = Polwarth; PX = Polwarth cross. Values are Mean ± SD.

aiming at getting an adequate fit without over-fitting. The QAIC/QAIC_c was obtained by using two different methods for estimating the GLMM parameters – Schall's (1991) approach for ovulation and Breslow and Clayton's (1993) approach for ovulation rate.

The aim of the analyses was to assess the effects of two factors, dietary treatment (seven types) and length of supplementation (three types), on ovulation and ovulation rate. Due to confounding between the two factors, two separate analyses were performed for each of the response variables. The model for ovulation also included parity, year, and site as fixed effects with two covariates, initial live weight and initial body condition score. The effect of genotype was included in the model as a random factor. The model for ovulation rate was the same except that genotype was fitted as a fixed effect and site was a random factor. Pairwise comparisons of means were made on the transformed means, assuming a significance level of 0.05.

The above-described analyses were conducted using the PROC GLIMMIX procedure of SAS, version 9.3 (SAS Institute, Cary, NC, USA) and GenStat, version 20 (VSN International, Hemel Hempstead, UK).

The analyses of ovulation and ovulation rate included assessing the correlation between the accumulated rainfall from 1 December to 15 April (www.inia.uy/gras), coinciding with the period from weaning to flushing (re-breeding), with both variables, the ovulation rate of the control ewes and the dietary treatment response, for each year.

To evaluate the association between pre-mating traits and those taken during mating for ovulation and ovulation rate, we conducted discriminant multivariate analyses using Statistica software (Statsoft Inc., 7.0, CA, USA). In this analysis, traits before treatment and during treatment were considered in order to identify those that performed with a clear discrimination or separation for ewes that ovulated or not and for ewes that had single or multiple ovulation.

The pretreatment traits included body condition score and live weight one month before and at the onset of treatment. The traits during treatment included: body condition and live weight at the end of treatment, daily energy and protein intake of the ewes during treatment, duration of supplementation, and the length of time spent grazing the pasture treatments. The analysis allowed to determine which of the variables are more representative in the classification of ovulation and ovulation rate. Basically, the linear combinations of the independent variables, as described above, were identified in order to discriminate between the categories of the response (dependent) variables, i.e. to determine which of the independent variables had more power to discriminate for success of ovulation and ovulation rate.

Results

Ewes ovulating

The percentage of ewes ovulating was high, ranging from 92 to 99%, but we still detected the effects of both dietary treatment and duration

of treatment on ovulation (Table 3; $P < 0.05$). Ewes that had access to tannin-rich legume pastures or native pastures and fed an energy supplement were most likely to be ovulating: 98% in Treatment 5 and 99% in Treatment 6 ($P < 0.05$; Table 4). There were no differences among the remaining treatments ($P > 0.05$). The statistical analysis revealed that for ovulation the estimated energy intake was highly significant ($P = 0.0018$), while the estimated crude protein intake was not significant ($P = 0.08$). Each additional 1.0 MJ of estimated ME intake was associated with 2.32% more ewes ovulating ($P < 0.01$). The proportion of ewes ovulating (97%) was greater when the duration of supplementation was equal to or longer than one oestrus cycle (Table 4; $P < 0.001$), compared to shorter durations of supplementation (94%) or no supplementation (93%); the two latter groups did not differ ($P > 0.05$). The live weight of the ewes at the start of treatment, but not body condition score, was positively associated with the percentage of ewes ovulating ($P < 0.001$; Table 3). Genotype also had a significant effect ($P < 0.001$), with less Polwarth ewes ovulating than Corriedale or Polwarth x East Frisian ewes, which were not different ($P > 0.05$). The year of the experiment also affected the percentage of ewes ovulating ($P < 0.001$), but there was no effect of ewe parity (maiden or adult) or experimental site (La Estala Estanzuela or Treinta y Tres) (Table 3).

The discriminant analysis with no adjustments (Table 5) indicates that the variables that discriminated best for success of ovulation were the estimated ME intake during treatment and initial body condition score ($P = 0.0001$).

Table 3Significance (P -values) of the fixed effect for dietary treatment and length of supplementation on ovulation and ovulation rate of experimental ewes.

Responsible variable	Term	P -value	Term	P -value
Ovulation	Dietary treatments	<0.001	Length of supplementation	<0.001
	Year	<0.001	Year	<0.001
	Initial BLW	<0.001	Initial BLW	<0.001
	Initial BCS	0.25	Initial BCS	0.19
	Parity	0.40	Parity	0.65
	Site	0.55	Site	0.33
	Genotype	<0.001	Genotype	<0.001
	Ovulation rate	Dietary treatments	<0.001	Length of supplementation
Year		<0.001	Year	0.008
Initial BLW		<0.001	Initial BLW	<0.001
Initial BCS		0.09	Initial BCS	0.55
Parity		0.30	Parity	0.29
Genotype		0.34	Genotype	0.38

BLW = body live weight; BCS = body condition score.

Table 4Transformed and back transformed means for ewe's ovulation¹ and ovulation rate² with their SE (SEM) and SED (SED between the means).

Term	#	Transformed means	SE	SED	Back transformed means	Term	Transformed means	SE	SED	Back transformed	
Ovulation¹											
Dietary treatments	1	2.47	0.42	0.61	92	Length of supplementation	8–10d	2.79	0.28	0.34	94
	2	2.84			94		15–21 d	3.48			97
	3	2.38			92		Unsupplemented	2.57			93
	4	2.72			94						
	5	2.84			98						
	6	4.21			99						
	7	2.65			93						
Year	2002	5.24	0.44	0.54	99	2002	5.08	0.41	0.53	99	
	2003	3.34			97	2003	3.57			97	
	2004	2.43			92	2004	2.44			92	
	2005	2.13			89	2005	2.00			88	
	2006	2.87			95	2006	2.60			93	
	2007	0.93			72	2007	1.00			73	
	2008	3.71			98	2008	3.62			97	
	2009	3.43			97	2009	3.34			97	
	2010	1.70			85	2010	1.60			83	
	2011	2.81			94	2011	2.68			94	
	2013	3.69			98	2013	3.67			98	
	2014	4.03			98	2014	3.96			98	
	2015	2.91			95	2015	2.84			95	
	2016	3.01				2016	2.89			95	
Parity	Maiden	2.87	0.28	0.33	95	Parity	Maiden	2.86	0.26	0.32	95
	Adult	3.17			96		Adult	3.04			95
Site	La Estanzuela	3.40	0.28	0.28	97	Site	La Estanzuela	3.32	0.26	0.28	97
	Treinta y Tres	2.36			93		Treinta y Tres	2.58			93
Genotype	Corriedale	3.34	0.26	0.22	97	Genotype	Corriedale	3.28	0.24	0.22	96
	Polwarth	2.36			91		Polwarth	2.29			91
	Polwarth X	3.34			97		Polwarth X	3.28			96
Ovulation rate²											
Dietary treatments	1	0.29	0.11	0.04	1.33	Length of supplementation	8–10d	0.40	0.26	0.07	1.49
	2	0.37			1.44		15–21 d	0.25			1.29
	3	0.44			1.55		Unsupplemented	0.32			1.38
	4	0.37			1.45						
	5	0.26			1.30						
	6	0.37			1.45						
	7	0.42			1.52						
Year	2002	0.46	0.11	0.05	1.59	2002	0.47	0.26	0.10	1.59	
	2003	0.33			1.40	2003	0.29			1.33	
	2004	0.29			1.33	2004	0.25			1.29	
	2005	0.35			1.42	2005	0.38			1.46	
	2006	0.25			1.29	2006	0.29			1.33	
	2007	0.19			1.21	2007	0.18			1.20	
	2008	0.48			1.55	2008	0.39			1.48	
	2009	0.42			1.52	2009	0.38			1.46	
	2010	0.23			1.26	2010	0.17			1.19	
	2011	0.45			1.58	2011	0.38			1.47	
	2013	0.46			1.58	2013	0.39			1.48	
	2014	0.36			1.44	2014	0.29			1.34	
	2015	0.45			2.58	2015	0.38			1.47	
	2016	0.35			1.42	2016	0.28			1.32	
Parity	Maiden	0.32	0.11	0.08	1.38	Parity	Maiden	0.28	0.26	0.08	1.32
	Adult	0.40			1.50		Adult	0.37			1.44
Genotype	Corriedale	0.29	0.14	0.15	1.32	Genotype	Corriedale	0.28	0.34	0.34	1.27
	Polwarth	0.44			1.44		Polwarth	0.33			1.39
	Polwarth X	0.36			1.55		Polwarth X	0.40			1.50

Dietary treatments: 1) control (NP = native pastures); 2) legumes without tannins; 3) tanniferous legumes; 4) protein supplementation of native pastures; 5) energy supplementation of native pastures; 6) energy supplementation of tanniferous legumes; 7) native pasture supplemented with protein protected by exogenous tannins.

¹ Ovulation analyzed using a generalized linear mixed model assuming binomial distribution with logit link.

² Ovulation rate analyzed using a generalized linear mixed model assuming Poisson distribution with log link.

Ovulation rate

Ovulation rate varied from 1.30 to 1.55 across all treatments and was affected by diet ($P < 0.001$; Tables 3 and 4). Among the pasture treatments, ovulation rate was higher for ewes grazing native pastures supplemented with protein (Treatments 4 and 7; $P < 0.001$; Table 4) than for control ewes grazing unsupplemented native pastures (Treatment 1). The addition of tannins to the protein supplement (Treatment

7) did not increase ovulation rate ($P > 0.05$; Treatment 4). However, the tannin-rich legume pasture in Treatment 3 was the only pasture treatment to produce a higher ovulation rate than the control ewes ($P < 0.001$; Table 4) and led to an ovulation rate similar to that observed with low-tannin legume pastures (Treatment 2). Supplementing ewes grazing tannin-rich legumes with energy (Treatment 6) did not increase ovulation rate ($P > 0.05$; Table 4) above that of un-supplemented ewes, but they were still above the values observed for control ewes grazing

Table 5
Discriminant analysis for ovulation and ovulation rate in the ewes.

	Variable	P-value
Ovulation	Estimated energy intake during treatment (MJ)	<0.0001
	Initial BCS	<0.0001
	Ewe LW at the end of treatment (Kg)	0.025
Ovulation rate	Estimated crude protein intake during treatment (g)	<0.0001
	Ewe BLW one month before treatment (Kg)	<0.0001
	Ewe BCS one month before treatment	<0.0001
	Estimated energy intake during treatment (MJ)	0.0195
	Length of the pasture treatment (d)	0.0297

BLW = body live weight; BCS = body condition score.

native pastures ($P < 0.001$; Table 4). When ewes grazing native pastures were supplemented with energy (Treatment 5), their ovulation rate did not increase ($P > 0.05$) compared to the unsupplemented ewes.

Ovulation rate was positively associated with estimated intake of crude protein ($P = 0.0006$), where each additional gram of crude protein consumed increased the ovulation rate by 0.103% ($P < 0.01$), but was not affected by the estimated ME intake ($P = 0.08$). There was no interaction between dietary treatment or length of supplementation and genotype, and neither length of supplementation nor ewe genotype affected ovulation rate (Table 3; $P > 0.05$).

The live weight of ewes at the start of the treatment period positively influenced ovulation rate ($P < 0.001$; Table 3). The year the experiment was conducted also affected ($P < 0.05$) ovulation rate. On the other hand, parity, body condition score at the commencement of the experiment and site had no effect on ovulation rate ($P > 0.05$; Table 3). Across all experiments, there was a negative correlation between ovulation rate and accumulated rainfall ($r = -0.76$; $P < 0.01$). When the ewes were split into control and treatment groups, the correlation was still significant for the control ewes ($r = -0.77$; $P < 0.01$) but not for the treated ewes ($r = -0.41$; $P > 0.05$).

The discriminant analysis with no adjustments (Table 5) indicates that the variables that discriminated best for single or multiple ovulation rate were the estimated crude protein intake during treatment, initial body weight, and condition score ($P < 0.0001$).

Discussion

Our general hypothesis that the ovulation rate of ewes grazing native pastures would be increased by short-term supplementation with protein, not energy, was supported, with each additional gram of crude protein provided by the diet being associated with a 0.103% increase in ovulation rate. Moreover, grazing legume pastures increased ovulation rate more than grazing native pastures, but only when the legumes were rich in endogenous tannins. By contrast, our second hypothesis that ovulation rate would be increased if the protein in the supplement was protected from rumen degradation by tannins, was not supported. Protein supplementation of native pasture or access to tanniferous legumes provided the ewes with twice the concentration of crude protein and slightly more energy than native pastures alone. Ewes that had access to low-tannin legumes also had twice the intake of protein of control ewes, but their ovulation rate did not differ from control ewes fed only native pastures. These observations are consistent with the conclusions drawn by Fletcher (1981) and Davis et al. (1981) that the response in ovulation rate to an acute change in feeding is related to the quality of protein consumed. From the present study, it seems that the threshold amount of protein needed to increase in ovulation rate can be attained with either highly digestible protein, in the case of supplementation of native pasture, or by feeding legumes with less degradable protein that is naturally protected from rumen degradation. This suggests that we should focus on protein rather than energy as a practical means of increasing ovulation rate in wool-type sheep.

Nonetheless, we cannot rule out energy as an important factor in lifting ovulation rate. When low-protein native pastures were supplemented with energy in the form of starch from corn grain, ovulation rate did not increase above control values even when the total energy in the diet was about 20% higher than that of the control diet. By contrast, Teleni et al. (1989) demonstrated a linear relationship between glucose entry rate and ovulation rate in Merino sheep. It seems that the role of protein is to allow this to happen. Teleni et al. (1989) found that ovulation rate and glucose entry rate did not differ between ewes being infused with glucose and ewes fed lupins (a grain rich in energy and protein, with part of the protein escaping from rumen degradation (Hume, 1974)). However, supplementation with starch from cereal grains, that could provide as much glucose as when glucose was infused, did not increase ovulation rate to the same extent as when ewes consumed lupins (Nottle et al., 1988). Moreover, starch infusion into the abomasum failed to increase the blood concentration of glucose (Huber et al., 1961). The reason seems to be that cereal grains, unlike infused glucose, must be eaten and digested and, since they are generally low in protein, they are unable to provide the ewe with enough glucose quickly enough to trigger the physiological mechanisms that increase ovulation rate (Scaramuzzi et al., 2011). Until now, many interpretations have been proposed about the differences in glucose entry rate in ewes supplemented with cereals or protein-rich supplements, but the results presented here suggest another interpretation. Taniguchi et al. (1993 and 1995) showed that the digestibility of starch in the small intestine of sheep improves and the net concentration of glucose in the portal blood of steers increased in direct response to an increased supply of protein reaching the small intestine. With an increased supply of protein available for digestion in the small intestine, there is a greater secretion of all digestive enzymes from the pancreas, including the amylase that is responsible for the initiation of starch digestion in the small intestine (Huntington, 1997). The extra protein reaching the small intestine could be supplied either as microbial protein synthesized in the rumen or as dietary protein protected from degradation in the rumen by tannins. From this we would expect that cereal grain, or supplements rich in energy, especially starch from cereal grains, will not be digested to supply glucose without adequate protein in the diet.

An increased supply of amino acids (protein) alone seems to be able to promptly increase the amount of glucose available to the ovary (Downing et al., 1995). Normally, mature ewes that undergo flushing do not need to consume a diet high in protein to meet their protein requirements for maintenance since they are not growing, in late pregnancy or lactating where the demand for protein is much higher than that for maintenance (National Research Council (NRC), 2007). However, irrespective of the origin of the additional protein reaching the small intestine, once the protein is digested the absorbed amino acids are either used for synthetic processes in the body or are catabolized. The catabolism of a number of the absorbed amino acids in excess of the ewe's requirements for synthetic processes could lead to the production of intermediates from the tricarboxylic acid cycle, or the production of pyruvate which can ultimately be converted to glucose, as described by Lindsay (1980) and cited by Teleni et al. (1989) to help explain how the protein in lupins increases ovulation rate of ewes. For this reason, dietary protein that is undegraded in the rumen and subsequently digested to amino acids for absorption from the small intestine, would explain the increases in ovulation rate we observed when the protein from protein-rich supplements was partially protected from rumen degradation by exogenous tannins or the protein in legumes by their own endogenous tannins (Banchemo and Quintans, 2007; Viñoles et al., 2009; Banchemo et al., 2012). Therefore, when implementing focus feeding to increase ovulation rate, the quantity and quality of the protein fed should be given due consideration despite the predominant role of energy since protein is likely to yield glucose faster and probably more safely than carbohydrate supplements. This proposition is reinforced by our results from the discriminant analysis

where there were no adjustments and the estimated intake of protein and energy were the dominant traits that predicted ovulation rate.

The results of the studies considered here, and those of Banchemo and Quintans (2007), Viñoles et al. (2009), King et al. (2010) and Robertson et al. (2015), confirm the conclusion that ovulation rate can be increased by feeding legumes for 12 or fewer days. In contrast, Smith et al. (1983) reported that a response in ovulation rate required 3 weeks of grazing or supplementation with silage made from good quality pastures. However, not all legumes produced similar responses in our studies. Ewes fed legumes rich in tannins had a higher ovulation rate than control ewes, but this was not the case for legumes with little or no endogenous tannins (Treatment 2) despite the fact that these legumes provided the ewes with the same amount of protein and energy as those rich in tannins.

The lack of difference in ovulation rate between ewes consuming unprotected or protected supplementary protein (produced by treatment with Quebracho tannins) was probably associated with the concentration of tannins used in the protein supplement. Tannins are not very palatable, so ewes fed supplements with an excess of tannins would probably consume less of the supplement (Min et al., 2003; Banchemo et al., 2012). If so, the intake of potential metabolizable protein, as well as energy, would be smaller thus negating any potential increase in ovulatory response (Banchemo et al., 2012). Tannins, either endogenous to the forage or provided in a supplement, can increase ovulation rate to levels above that for the same feedstuffs without tannins, but to do so they must be consumed at appropriate levels.

Tannin-free legumes did not increase ovulation rate over that of control diets even when the legumes had a seemingly adequate protein content. It is possible that the protein in these legumes could not provide enough metabolizable protein to the duodenum to increase glucose entry rate enough to bring about an ovulatory response, as suggested by Poppi and McLennan (1995). Our results differ from those of King et al. (2010) who reported an increase in ovulation rate in Merino ewes that grazed lucerne, a non-tannin legume, for 12 days. The response in the study of King et al. (2010) may be attributed to the lucerne providing a much greater differential in nutrient intake, especially protein, relative to the intake of the control ewes, compared to our study. It is also possible that the apparent difference in ovulation rate in the study of King et al. (2010) was not due to lucerne increasing ovulation rate but rather due to a depression of ovulation rate with the poor-quality control diet. In our experiments, the ovulation rates with non-tannin legumes were not different to the tanniniferous legumes. Since tannin-containing legumes can supply more metabolizable protein post-ruminally, future experiments should include an evaluation of ovulation rate responses with legumes of low tannin content supplemented with feedstuffs that may change the dietary protein:energy ratio, as suggested by Poppi and McLennan (1995) with respect to increasing the growth rate of cattle.

Our observation that ewes that were heavier at the onset of flushing had a higher ovulation rate than ewes of lower live weight is consistent with the data provided in the review by Kenyon et al. (2014). By contrast, we found no effect of ewe body condition score at the onset of flushing on ovulation rate, although the range of body condition scores in all of our experiments was quite narrow. From a practical perspective, this demonstrates that, when studying factors that influence body weight, the diet of the ewes should also be evaluated rather than just relying on the relationship between ewe body condition score and liveweight.

Ovulation rate varied significantly from year to year during our studies and was negatively correlated with accumulated summer rainfall. This result is consistent with the view of farmers in Uruguay that wet summers have a negative impact on the health of their sheep. If this is so, ewes would be expected to be lighter and of lower condition score, so the base ovulation rate should be lower than in drier years. It has been suggested that heavy rainfall events just before ovulation affects

ovulation rate (Doney et al., 1973), but there is currently no scientific evidence to suggest that the total rainfall between weaning and first post-weaning ovulation can affect the ovulation rate at that time. The ewes in our studies had the highest ovulation rate when the accumulated rainfall was below the historical average ('good years') and the lowest ovulation rate when the rainfall exceeded the historical average ('bad years'). Consequently, it is possible that in 'good years' the difference in ovulation rate between treated and control ewes was small because the ovulation rate of the control ewes was already high, whereas in 'bad years' the dietary treatments had a more marked effect on ovulation rate. Live weight and body condition score one month before the start of flushing discriminated strongly for ovulation rate, supporting the view of Uruguayan farmers that high rainfall years adversely affect the condition of their ewes. This suggests that liveweight and body condition score one month before flushing along with the summer rain records, at least in Uruguay, may be useful for alerting farmers that there might be additional value in short-term flushing prior to joining in the humid, summer months. It may be less of a problem where wool-type sheep are typically raised under much drier conditions. These observations may also help explain why the research to date has not provided a better understanding of the underlying mechanisms that are the direct drivers of ovulation rate.

The average percentage of ewes ovulating in our experiments was high, varying from 92 to 99% across the range of dietary treatments. In those experiments where the percentage of ewes ovulating was highest, the dietary treatments that had the most impact on ovulation were those where a high-energy supplement of corn grain was fed regardless of the pasture available. Indeed, the estimated ME intake during flushing had a more marked effect on the percentage of ewes ovulating than their intake of protein. The duration of supplementation also had an important influence on the percentage of ewes ovulating. In fact, ewes supplemented for longer than one oestrus cycle had 2 or 4% more ovulations than ewes that were not supplemented or were supplemented only for a short time. These effects of energy and duration of supplementation on ovulation were not expected. Indeed, we designed the experiments assuming that all ewes would be in positive energy balance and ovulating at the time treatments were applied. Follicular development and ovulation are not themselves energetically expensive, but it is generally accepted that female mammals appear to avoid ovulation, and therefore subsequent pregnancy and lactation, unless they have adequate metabolic reserves for pregnancy and lactation. That said, for sheep, data supporting this conclusion are rare. Early studies originated well before the advent of laparoscopy allowed field studies with large sample sizes and repeated observations (Lindsay et al., 1975) and had relied on expression of oestrus as an indicator of the occurrence of ovulation. Such studies concluded that, in adult ewes, irregular oestrous cycles or acyclicity were only observed with severe undernutrition and thus extremely poor body condition (Allen and Lamming, 1961; Smith, 1965; Hunter and Van Aarde, 1973). Cahill et al., 1984). The consensus seems to be that, during the normal breeding season, anovulation is only observed when the metabolic situation is extremely unfavourable, perhaps to the point of emaciation (Martin et al., 2008). In other words, variations in body condition commensurate with the studies analyzed in the present paper would not affect the occurrence of ovulation.

However, the present analysis brings the power of 3720 observations with ewes of similar genotypes across multiple sites with modern techniques. The outcome is a significant relationship between live weight (and body condition score) at the onset of treatment and the percentage of ewes ovulating. Moreover, in the discriminant analysis with no adjustments, body condition score was one of the better predictors of ovulation, as were estimated energy intake and ewe weight at the end of treatment. These observations challenge our initial assumption that all ewes would be ovulating because they are in positive energy balance.

In conclusion, both ovulation and ovulation rate in ewes are enhanced by an increase in energy intake. This ovulatory response is augmented by an increase in glucose entry rate facilitated by a supply of additional metabolizable protein, either from microbial protein produced from rumen-degradable dietary protein or from dietary protein protected from degradation in the rumen during short-term supplementation.

Ethics approval

All the studies presented in this paper were conducted in accordance with the current directives of the National Institute of Agricultural Research, INIA, Uruguay, regarding the use of animals for experimentation. The institutes policy complies with the law of Uruguay for the care and management of experimental animals (cnea.gub.uy; law 18611).

Data and model availability statement

The model was not deposited in an official repository. Access right to data, software, or model is confidential.

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Georget Banchemo: Conceptualization, data curation, funding acquisition, investigation, methodology, resources, visualization, writing – original draft, writing – review & editing. Graciela Quintans: Funding acquisition, investigation, methodology, resources, project administration. Katia Stefanova and Fernando Baldi: Formal analysis. David Lindsay and John Milton: Visualization, writing – review & editing. Graeme Martin: Conceptualization, supervision, visualization, writing – review & editing.

Declaration of interest

None.

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