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PROGESTERONE IS AN INDIRECT INDICATOR OF REPRODUCTIVE OUTCOMES FOR YEARLING EWES

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SUMMARY

Ewes joined to first lamb as yearlings in industry ram breeding flocks were sampled for progesterone concentration (PROG) exactly 14 days after first exposure to males. The heritability of PROG was 0.22±0.06. PROG had positive genetic ($P>0.05$) and phenotypic ($P<0.05$) correlations with yearling, but not two-year old (2yo) ewe, reproductive traits affected by fertility. Pubertal status assigned using PROG was a significant ($P<0.0001$) factor for yearling fertility and related traits. Systematic effects, such as birth-rear type and dam age groups influenced yearling outcomes but were generally not significant for reproductive performance of 2yo ewes.

INTRODUCTION

The reproductive performance of ewes joined to lamb as yearlings is highly variable across flocks and years (Fogarty *et al.* 2007), even when weight and condition at joining are sufficient. A similar situation exists with beef heifers first joined to calve as two-year olds in Northern herds, where failure to attain puberty during the joining period has been identified as a contributing factor (Johnston *et al.* 2009). In that study, serial ovarian scanning was used to identify attainment of puberty based on the age when the first *corpus-luteum* (CL) was observed. However, this strategy is costly and time consuming and an alternative could be to evaluate physiological status based on reproductive hormone levels, such as progesterone. Circulating progesterone is potentially suitable as a marker for puberty, because it is produced post-puberty by the CL and is maintained at relatively high levels throughout most of the reproductive cycle (Foster and Jackson 2006).

In this study we investigate the use of progesterone sampling in the field during the first joining event for ewe lambs (<1 year old), under a controlled protocol of ram exposure and timing of sampling. The implications of systematic effects for progesterone and the subsequent reproductive performance of yearling and 2yo ewes are evaluated, along with the association between sire breeding values for yearling reproductive performance traits and progesterone level.

MATERIALS AND METHODS

Ewes used in this study were sourced from industry ram-breeding flocks representing a range of production environments and breeds recorded across nine sites in Southern Australia. Pedigree and birth details were available, along with accompanying growth and reproductive data. Ewes represented Merino (MER), maternal (MAT) and terminal (TERM) breed types. Flocks commenced joining predominantly in February and March when ewes averaged 7.5 months of age, but ewes ranged from 152 to 321 days of age at the commencement of joining. Blood samples from all sites were collected exactly 14 days after the introduction of ewes to males (teasers or rams). Plasma from these samples was assayed for progesterone concentration using a commercial ELISA for human samples, following the manufacturer instructions (Demeditec 2009). Ewes were classified as not pubertal (<0.95 ng/ml), of uncertain pubertal status (0.95-1.05 ng/ml) or pubertal (>1.05 ng/ml) at D14 of joining based on previous studies defining the threshold at which puberty is indicated

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(Sangha *et al.* 2002), while allowing for variation due to ELISA procedures. Ewes without progesterone recorded were classified as unknown status. Accompanying reproductive data from these flocks were extracted from the Sheep Genetics database for four years (2013-2016). Reproductive traits included fertility (FERT), number of lambs born (NLB) and weaned (NLW) for ewes joined, litter size at lambing (LSIZE) and weaning (LWEAN) for ewes which lambed. Fertility and litter size traits were inferred from lambing data, or scanning data otherwise.

Reproductive traits were analysed fitting contemporary group (CG: 44 levels), which was a combination of site, year of joining and joining sub-group, and flock-dam breed group as the base model (M0). The M0 contemporary group for progesterone reflected site, date of bleeding and assay plate (PCG: 39 levels). Additional systematic effects were then investigated through a series of analyses. Dam age group (AGD: 4 levels; 1, 2, 3-5, 6+ years), month of birth (MON: 9 levels; March - November) and birth-rear type (BRT: 7 levels; 11, 2-, 21, 22, 31, 32, 33) were added simultaneously to M0 (M1). Pubertal status (PUB: 4 levels) was added to M1 for yearling traits, or after accounting for whether the ewe was previously joined as a yearling (YJOIN) for 2yo ewes traits (M2). Heritability estimates and genetic correlations between progesterone values and reproductive traits were estimated from a series of bivariate analyses under M1 fitting an animal model, using ASREML (Gilmour *et al.* 2009). Pearson correlations between sire breeding values for yearling reproductive traits (obtained from Sheep Genetics) and progesterone concentration (for sires with daughters recorded for progesterone) were calculated.

RESULTS AND DISCUSSION

Reproductive data were dominated by MAT breed types (~85% of all data) contributing to the relatively high mean litter size (Table 1). Fertility, NLB and NLW were substantially higher for ewes bred to lamb as 2yo ewes compared to yearling ewes, as expected. The heritability for progesterone concentration was moderate (0.22±0.06, Table 1). Heritability estimates for FERT, NLB and NLW were higher for yearling than 2yo ewes. Negligible heritability for yearling LWEAN (Table 1) indicates that culling on yearling LWEAN will be ineffective.

Table 1. Raw data characteristics along with heritability estimates (h^2) and the phenotypic variance (σ^2_p) for progesterone concentration and the reproductive traits (model M1)

	Yearling ewes				2yo ewes			
	N	**Mean (SD)**	**h^2**	**σ^2_p**	**N**	**Mean (SD)**	**h^2**	**σ^2_p**
FERT	10998	0.59 (0.49)	0.18±0.02	0.21	6494	0.92 (0.27)	0.03±0.02	0.02
NLB	10998	0.89 (0.86)	0.13±0.02	0.63	6494	1.46 (0.76)	0.08±0.02	0.54
NLW	9422	0.64 (0.78)	0.13±0.02	0.52	5913	1.07 (0.86)	0.07±0.02	0.69
LSIZE	6201	1.49 (0.54)	0.09±0.02	0.26	5972	1.58 (0.65)	0.08±0.02	0.39
LWEAN	4850	1.13 (0.67)	0.03±0.02	0.42	5421	1.17 (0.83)	0.07±0.02	0.63
PROG	1894	0.98 (0.13)	0.22±0.06	0.009	na	na	na	na

Contemporary group was very highly significant (P<0.0001) but explained less than 10% of variation for all reproductive traits (R^2(M0), Table 2). The addition of MON, AGD and BRT in combination increased model R^2 by up to 70% for YFERT, YNLB and YNLW (M1, Table 2), although overall R^2 remained relatively low, as expected for reproductive traits. Month of birth was the most significant factor (P<0.0001) affecting YFERT and therefore YNLB and YNLW, and remained significant for reproductive traits of 2yo ewes. Month of birth was more significant than month of joining when fitted concurrently (not presented). Birth-rear type was significant (P<0.05) for reproductive outcomes of yearling but not 2yo ewes, while dam age group was only significant for fertility (not litter size or lamb survival) outcomes and progesterone levels (M1, Table 2). Month of birth remained significant for yearling (but not 2yo ewes) reproductive traits even when age at the commencement of joining was fitted as a linear covariate (not presented), demonstrating that the effect of MON for yearling outcomes was not solely due to variation in age at joining. Pubertal status assigned using progesterone results was significantly associated with YFERT, YNLB and YNLW, but did not greatly increase model R^2 values due to both limited data for PROG and because systematic effects were common to both reproductive traits and PROG (Table 2). PUB was also significantly associated with litter size traits (but not fertility) of ewes lambing as two-year olds. This suggests that females which attain puberty early may also have higher litter size when more mature, supporting results observed by Edwards *et al.* (2015).

Table 2. The significance of systematic effects for yearling (Y) and 2yo ewes (H) reproductive traits and progesterone (PROG) under various models

Model 1 (M1)	Trait	R^2(M0)	R^2(M1)	P(AGD)	P(MON)	P(BRT)	P(PUB)
CG + AGD + MON + BRT	YFERT	8.8	14.3	0.04	<0.0001	0.006	-
CG + AGD + MON + BRT	YNLB	9.0	15.3	0.28	<0.0001	0.0002	-
CG + AGD + MON + BRT	YNLW	9.6	15.0	0.24	<0.0001	0.31	-
CG + AGD + MON + BRT	YLSIZE	7.6	9.3	0.26	<0.0001	0.007	-
CG + AGD + MON + BRT	YLWEAN	5.8	6.7	0.32	0.003	0.75	-
HCG + AGD + MON + BRT	HFERT	7.7	8.4	0.82	0.003	0.02	-
HCG + AGD + MON + BRT	HNLB	5.5	6.4	0.87	<0.0001	0.25	-
HCG + AGD + MON + BRT	HNLW	5.8	6.5	0.58	<0.0001	0.81	-
HCG + AGD + MON + BRT	HLSIZE	7.3	7.9	0.74	0.001	0.43	-
HCG + AGD + MON + BRT	HLWEAN	9.1	9.5	0.63	0.003	0.99	-
PCG + AGD + MON + BRT	PROG	46.4	51.3	0.04	<0.0001	0.07	na
Model 2 (M2)		R^2(M1)	R^2(M2)				
M1 + PUB	YFERT	14.3	14.7	0.05	<0.0001	0.01	<0.0001
M1 + PUB	YNLB	15.3	15.5	0.34	<0.0001	0.0003	<0.0001
M1 + PUB	YNLW	15.0	15.1	0.32	<0.0001	0.31	0.001
M1 + PUB	YLSIZE	9.3	9.3	0.28	<0.0001	0.007	0.70
M1 + PUB	YLWEAN	6.7	6.8	0.33	0.004	0.75	0.51
M1 + YJOIN + PUB(YJOIN)	HFERT	8.4	8.4	0.80	0.01	0.02	0.81
M1 + YJOIN + PUB(YJOIN)	HNLB	6.4	6.9	0.68	0.005	0.37	0.03
M1 + YJOIN + PUB(YJOIN)	HNLW	6.5	6.7	0.39	0.001	0.79	0.07
M1 + YJOIN + PUB(YJOIN)	HLSIZE	7.9	8.5	0.61	0.02	0.67	0.0006
M1 + YJOIN + PUB(YJOIN)	HLWEAN	9.5	9.8	0.41	0.01	0.99	0.01

Least square means show declining fertility outcomes with increasing MON, of large magnitude for yearling ewes (Y) and lesser magnitude for 2yo ewes (H). Relative to lambs reared as singles, lambs reared as multiples had reduced YFERT, but not reduced HFERT. YFERT was lower when progesterone sampling indicated that the ewe was not showing signs of puberty 14 days into the joining period (Table 3). In addition, ewe lambs born to yearling dams had both lower progesterone (0.92 vs 0.95, P=0.02) and poorer fertility outcomes (0.54 vs 0.59, P=0.01) than ewe lambs born to older dams.

Genetic and phenotypic correlations between PROG with YFERT, YNLB or YNLW suggest a positive genetic association between progesterone levels and yearling reproductive traits influenced by fertility (Table 4). Correlations between sire breeding values for PROG with ASBVs for YNLB or YNLW, derived using more extensive data, were positive in two of the three breed groups. For sires with N>10 daughters sampled for progesterone and with an accuracy >30% for the ASBV for YNLB, Pearson correlation coefficients were 0.35 and 0.51 (P=0.02) in Merino's (20 sires), 0.21 and 0.10 in MAT breeds (48 sires) and -0.16 and -0.33 in TERM breeds (12 sires) for YNLB and YNLW. However, yearling reproductive data for TERM breed ewes were affected by a delay in joining following pharmaceutical intervention, whereas MER and MAT ewes were naturally joined. The timing of sampling for progesterone was chosen to minimise false negatives (ie ewes which tested negative because of the phase of their cycle). However, it was also possible for ewes to attain puberty within the joining interval after progesterone sampling and therefore a single sample of progesterone is not a perfect predictor for the early attainment of puberty.

Table 3. Least square means for systematic factors affecting progesterone concentrations or yearling (Y) and 2yo ewes (H) reproductive traits

Factor		Month of birth					Birth-rearing group			Pubertal status			
Trait		6	7	8	9	10	SS	MS	MM	0	1	2	U
PROG	Y	0.98^{a}	0.96^{a}	0.96^{a}	0.85^{b}	0.83^{b}	ns	ns	ns	na	na	na	na
FERT	Y	72^{a}	70^{a}	55^{b}	33^{c}	37^{c}	59^{a}	59^{a}	56^{b}	50^{a}	57^{a}	67^{b}	58^{ac}
	H	96^{a}	91^{ab}	90^{ab}	87^{b}	84^{bc}	ns	ns	ns	ns	ns	ns	ns
NLB	Y	1.12^{a}	1.06^{a}	0.74^{b}	0.38^{c}	0.45^{c}	0.85^{a}	0.89^{a}	0.79^{b}	0.75^{a}	0.80^{a}	1.0^{b}	0.83^{ac}
	H	1.56^{abc}	1.48^{ac}	1.47^{ac}	1.38^{b}	1.50^{c}	ns	ns	ns	1.46^{a}	1.52^{ab}	1.68^{b}	1.61^{bc}
NLW	Y	0.84^{a}	0.76^{a}	0.49^{b}	0.21^{c}	0.11^{c}	ns	ns	ns	0.52^{a}	0.57^{ab}	0.68^{b}	0.53^{ac}
	H	1.43^{a}	1.08^{b}	1.11^{b}	0.88^{c}	1.08^{ab}	ns	ns	ns	ns	ns	ns	ns
LSIZE	Y	1.52^{a}	1.44^{a}	1.30^{bc}	1.26^{c}	1.25^{c}	1.35^{ab}	1.39^{a}	1.31^{b}	ns	ns	ns	ns
	H	1.71^{ac}	1.58^{a}	1.63^{a}	1.54^{b}	1.70^{ac}	ns	ns	ns	1.64^{a}	1.69^{a}	1.79^{b}	1.68^{a}
LWEAN	Y	1.15^{a}	1.02^{a}	0.92^{bc}	0.86^{c}	0.75^{abc}	ns	ns	ns	ns	ns	ns	ns
	H	1.60^{ac}	1.15^{a}	1.28^{a}	1.00^{b}	1.20^{ac}	ns	ns	ns	1.25^{a}	1.32^{a}	1.22^{ab}	1.12^{b}

SS: born-reared single; MS: multiple-reared single; MM: multiple-reared multiple; 0: not pubertal; 2: pubertal; 1: intermediate; U: untested; ns: P>0.05; na: not applicable; common superscripts within factor indicate P>0.05 (Month of birth and Birth-rearing type levels simplified for presentation)

Table 4. Genetic (r_g) and phenotypic (r_p) correlations between progesterone concentration and reproductive traits for ewes joined to lamb as yearlings or 2yo ewes

Trait		FERT	NLB	NLW	LSIZE	LWEAN
Yearling	r_g	0.21±0.18	0.25±0.19	0.09±0.19	0.39±0.28	0.05±0.39
	r_p	0.16±0.02	0.12±0.02	0.05±0.02	-0.02±0.04	-0.11±0.04
2yo ewes	r_g	na	0.08±0.21	0.19±0.22	-0.05±0.22	0.11±0.23
	r_p	na	0.05±0.03	0.06±0.03	0.06±0.03	0.08±0.03

CONCLUSIONS

Results from this study suggest that failure to attain puberty is a likely contributor to failed reproductive performance ewes joined to lamb as yearlings. Progesterone measured at D14 after the commencement of joining was a heritable indicator of puberty and fertility. Several systematic effects which contribute to yearling reproductive performance were not significant for outcomes of 2yo ewes, and therefore models used for the genetic evaluation of yearling reproductive outcomes requires refinement for more accurate genetic evaluation of performance in this age class.

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