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Recovery of ovulation rate in ewes following their removal from an oestrogenic lucerne forage

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Analysis of the corpora lutea count data

The data consisted of the live weight at mating, change in live weight during the experiment, grazing treatment and the subsequent number of corpora lutea identified by laparoscopy of 59 ewes.

Initially the data were graphed to gain a general, if heuristic, impression of the data. A naïve approach was to calculate a simple exponential regression of number of corpora lutea (CL) against grazing days on grass prior to ovulation. This gave a highly significant result (P = 0.017; R^2adj. = 0.104; Fig. S1) but it also violates a number of the criteria for a valid regression analysis rendering the quantification of the response suspect.

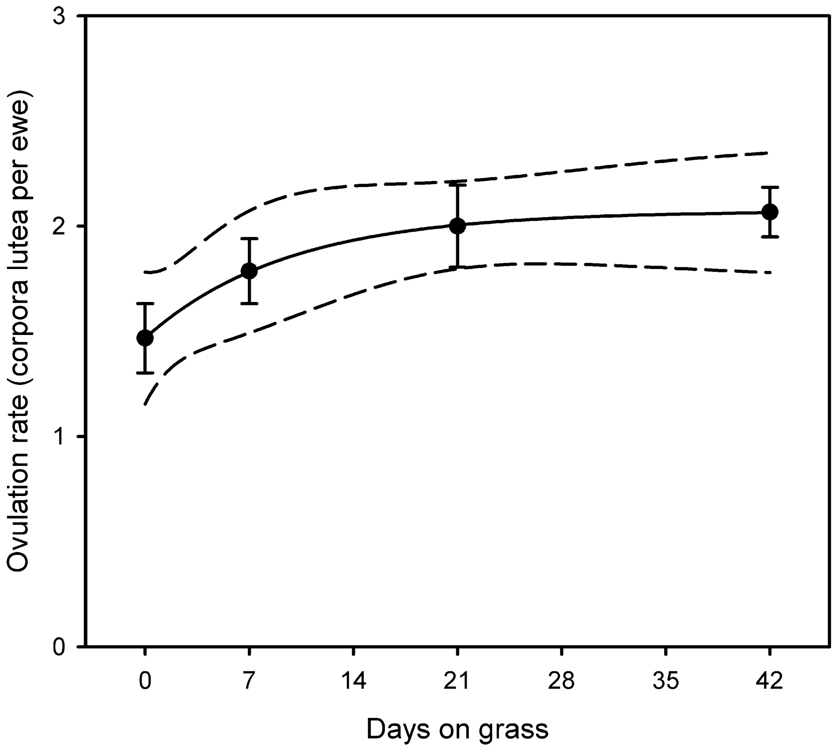

Fig. S1. Exponential regression relationship (P = 0.017; $R^2_{adj.}$ = 0.104) of average ovulation rate per ewe against the number of days on grass prior to ovulation. Error bars are standard error of the mean ovulation rate for each group of ewes (n = 14/15). The relationship is described by the equation: $y = 1.47 + 0.603(1 - 0.899^x)$. Dashed lines are 95% confidence intervals for the mean predicted values calculated from this equation.

The appropriate method is ordinal regression (McCullagh and Nelder 1989) and the implementation in Genstat was used here. In place of 'grass days' the transformed variate, 'exp(-0.11*Grass days)', was used; the coefficient chosen to minimise the residual deviance. Graphs were drawn with the independent (X) variable back transformed to days. Live weight and change in live weight were found to be very non-significant so the analysis was continued to study the effect of diet alone.

The residual mean deviance was high (1.89), indicating over-dispersion, but this may be the norm (McCullagh and Nelder (1989), p.124, p.193), so no adjustment has been made to the default dispersion (1.0) but the reader should note that the variances may be biased downwards by an unknown degree. The estimated parameters and their respective standard errors are listed in Table S1.

Table S1. Table of coefficients for the logistic response of ovulation rate to the main effect of days on grass (using the transformed variate). The residual mean deviance was greater than 1 (1.89) and therefore variances and P values are approximate.

	Value	Standard Error (SE)	t-value	P value
Coefficients:				
Days on grass	-2.296	0.743	-3.09	<0.002
Intercepts:				
0|1	-5.40	1.14	-4.72	<0.001
1|2	-2.058	0.500	-4.12	<0.001
2|3	1.392	0.446	3.12	< 0.002

Genstat allows the extraction and storage of the coefficients (B) and the associated variance-covariance matrix (Σ_1). Logits corresponding to ewes of various days on grass prior to ovulation ranging from 0 to 42, and probabilities of 0 CL, 'at most 1' CL and 'at most 2' CL were calculated from B by linear combinations. These are conveniently held in a matrix (L_1) so that the logits are obtained by the matrix product, L_1B and the corresponding variance matrix (Σ_2) by $L_1 \Sigma_1 L_1^T$. (The superscript indicates the transpose of the matrix.)

The logits are then transformed into cumulative probabilities (Cp) by $Cp_i = (1+ \exp(-logit_i))^{-1}$. The corresponding variance matrix is obtained by statistical differentials (see, for example, Kempthorne and Folks (1971)). These terms simplify to $Cp_i(1 – Cp_i)$ and may be placed in a diagonal matrix, D_1. The variance matrix (Σ_3) for the cumulative probabilities is calculated as $D_1 \Sigma_2 D_1$.(D_1 is its own transpose.)

The probabilities of a ewe having 0, 1, 2 or 3 CL are obtained from the cumulative probabilities by appropriate subtraction. I.e., the probability of having none P(0) is the same as the cumulative probability, Cp(0); that of having 1, P(1) = Cp(1) – Cp(0); P(2) = Cp(2) – Cp(1); and P(3) = 1 – Cp(2). Again, these linear combinations may be placed in a matrix (L_2) and the vector of cumulative probabilities augmented by '1' so that the P(.) values are calculated by L_2Cp_A, where Cp_A is the augmented vector. The variance matrix of the probabilities is then calculated by $L_2\Sigma_{3A}L_2^T$, where Σ_{3A} indicates the matrix Σ_3 augmented by a row and a column of zeros, corresponding to the variance of the unit probability of 'at most 3'.

While the SEs for the probabilities P(.) can be taken by extracting the diagonal and taking its square root, this statistic is inappropriate for calculating confidence intervals for the probabilities as they are asymmetrically distributed. The probabilities P(.) were therefore transformed to logits, and again the method of statistical differentials used to calculate the appropriate variance matrix. The diagonal of this matrix was extracted and its square root taken to give the SEs of the logits. Hence, the 95% confidence intervals were calculated for P(0), P(1), P(2) and P(3). The expression, $(1 + \exp(-logit))^{-1}$, was then used with these values and the result plotted on the graphs.

Using the probabilities of each corpora luteal value the expected number of corpora lutea were then calculated by $\Sigma(P(x).x)$ for various days on grass prior to ovulation. Expected variance (Var) was calculated from the variance covariance matrix (Σ_2) for the predicted cumulative probabilities of 0, 1, 2 and 3 corpora lutea for each grazing date (t). The lower and upper confidence levels were calculated for each grazing date by extracting the diagonal and taking its square root from the expected corpora lutea[t] ± 2.004*sqrt(Var[t]).

References

Kempthorne, O, Folks, L (1971) 'Probability, Statistics, and Data Analysis.' (Iowa State University Press: Ames, Iowa)

McCullagh, P, Nelder, JA (1989) 'Generalized linear models.' (Chapman and Hall: London; New York)