Ano II, Nº 1 (2013)
ISSN: 2182-4975

INVESTIGATING FOLLICLE GROWTH, UTERINE OEDEMA AND OTHER FACTORS AFFECTING REPRODUCTIVE SUCCESS IN THE LUSITANO MARE

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ABSTRACT

A total of n=159 Lusitano mares and n=309 reproductive cycles were analysed to model follicle sizes at the time of ovulation and reproductive success in dependency of several variables. Uterine oedema was found to correlate positively with probability of successful pregnancy ($p<0.05$). Follicle sizes at the time of ovulation are affected by treatment with hCG ($p<0.05$), with lower sizes found for treated mares. Follicle size at the time of ovulation correlates negatively with the mares' age ($p<0.05$) (Pearson's $r = -0.168$). This has implications for the way we monitor follicular growth and intervene in breeding, specific to the Lusitano breed, to accurately time artificial insemination.

Keywords: Lusitano mare, reproductive success, follicle development, uterine oedema

INVESTIGANDO O CRESCIMENTO FOLÍCULAR, O EDEMA UTERINO E OUTROS PARÂMETROS REPRODUTIVOS QUE INFLUENCIAM O SUCESSO REPRODUTIVO EM ÉGUAS DE RAÇA LUSITANA

RESUMO

Um total de n=159 éguas de raça Lusitana e n=309 ciclos reprodutivos foram analisados para modelar o tamanho dos folículos na ovulação e o sucesso reprodutivo em dependência de várias variáveis. O edema uterino tem uma correlação positiva com a probabilidade de sucesso reprodutivo ($p<0.05$). O tamanho dos folículos na ovulação são afetados pelo tratamento com gonadotrofinas coriónicas humanas ($p<0.05$), sendo menores em éguas tratadas. O tamanho dos folículos aquando da ovulação tem uma correlação negativa, decrescendo com a idade das éguas ($p<0.05$) (Correlação de Pearson, $r = -0.168$).

Estes resultados têm impacto sobretudo na monitorização do crescimento folícular especifico da raça Lusitana, tendo em vista uma inseminação artificial sincronizada com a ovulação

Palavras-Chave: Égua Lusitana, sucesso reprodutivo, desenvolvimento folicular, edema uterino

INTRODUCTION

The Lusitano is the most important native equine breed in Portugal, with 2500 registered mares in Portugal alone, and 5000 mares registered worldwide (APCCPSL, 2012). The Lusitano is the product of thousands of years of selection, steeped in the tradition of its historical military links, bullfighting and the classical training methods of the “Haute École” (Gamboa *et al.*, 2008).

Lusitano mares and stallions are selected, not on fertility traits, but almost entirely upon a combination of body conformation and performance, similarly to other breeds (Gamboa *et al.*, 2008). Selective breeding aims to produce a foal with qualities similar to the parents. Therefore it is important to employ efficient breeding practices. The prediction of the time of ovulation to allow for optimum breeding timing is paramount, especially in artificial insemination. The importance of elucidating factors affecting reproductive performance and efficiency is of paramount concern to the equine practitioners and to all equine industry. Furthermore, breeding programmes may benefit from an understanding of the breed specific reproductive physiology of the Lusitano mare.

In modern breeding management, transrectal ultrasonography and rectal palpations are routinely used to determine the stage of the mare’s estrous cycle from the first examination. Through the use of ultrasound, several parameters such as number of developing follicles, presence of fluid in the uterus, presence of a *Corpus Luteum* (CL) and uterine oedema (UO) can be monitored during the entire cycle helping to identify oestrus and predict the time of ovulation (Brinsko *et al.*, 1998). The scoring system for UO developed by Samper (2009) has been a useful tool to determine the appropriate time for determining ovulation in mares (Table I). Follicular development is routinely monitored and changes that include growing, ovulation, regressing follicles and sometimes anovulatory haemorrhagic follicles are recorded; monitoring these changes helps predict ovulation time and can greatly contribute to the success of any reproductive program (Evans, 2003).

Table I - THE UTERINE OEDEMA (UO) SCORING SYSTEM DEVELOPED BY SAMPER (2009)

Score	Characteristics
UO1	Moderately soft uterus. Presence of 25-35 mm follicle. Shown in mares in transitional season.
UO2	First real sign of oedema, mare is coming into estrus. Endometrial folds enlarge, easy identifiable. The uterus softens and has a fishbone appearance. Follicles >35 mm are detected
UO3	Endometrial folds are easily observed. Follicles >38 mm. Appearance of cart wheel pattern at ultrasound. Indicates a close ovulation.
UO4	Endometrial folds increase. Cartwheel pattern of the uterus is still maintained. Follicles >40 mm. At this point a normal mare starts decreasing oedema as it approaches to ovulation.
UO 5	Considered abnormal. Endometrial folds abnormally thick. Cartwheel pattern is lost. Oedema persists even after ovulation. A mare with this score may accumulate uterine fluid.

Predicting the day of ovulation would have considerable use for coordinating the time of breeding and pregnancy success (Roig *et al.*, 2004). Time of ovulation may be influenced by factors such as breed, age, size of pre-ovulatory follicles and score of the UO (see Table I), which might affect subsequently the pregnancy success (Ginther, 1982). It has been suggested that external factors such as month, side of ovulation and number of follicles ovulated might also affect pregnancy rates (Morris and Allen, 2002).

This study will focus on the identification of factors capable of affecting the follicle size and the subsequent success of pregnancy focussing on the Lusitano breed. Contributing factors that will be analysed include the size of the follicle in the different growing stages and at ovulation, hormonal treatment, UO, side of ovulation, season and age of the mare.

MATERIAL AND METHODS

Data was collected retrospectively by kind permission from Uson Olaso, Lda, a veterinary clinic established in Portugal in the region of Alentejo.

Records were complete from the initial scan during the routine breeding soundness examinations through to confirmation of pregnancy and successful foaling the following year. These details are recorded for each mare in a season book. A total of n=190 mares and n=309 reproductive cycles were included in the sample used, covering the 2006-2011 breeding seasons.

Data available from the sample included: *age of the mare* (in years), *follicle size* (measurements at ovulation and during the previous week), *ovary side of ovulation* (left or right) and, *degree of UO* (Samper scale, with the following observations 1, 1+, 2-, 2, 2+, 3- and 3 transformed into a continuous variable as 1, 1.3, 1.7, 2, 2.3, 2.7 and 3 respectively). Pregnancy management data available included: *reproductive technique applied* (natural mounting, artificial insemination with chilled semen, artificial insemination with frozen semen), *hCG treatment* (yes or no), *$PGF_{2\alpha}$ treatment* (yes or no), *oxytocin treatment* (yes or no), *antibiotic treatment* (yes or no), *season* (month of insemination) and *pregnancy outcome* (pregnant or not). The dates of the measurements of follicle size were also available.

From the n=309 reproductive cycles sampled, n=243 cycles were analysed after exclusion of incomplete data or confounding factors (multiple ovulations, and mares treated for embryo transfer), which resulted in the use of data from n=159 mares.

To model the probability of successful pregnancy, as dependent variable, a logistic regression was fitted using a backwards stepwise approach to select the significant variables from: *follicle size at ovulation, UO, age of the mare* as covariates *and follicle side, season, hormonal treatment ($PGF_{2\alpha}$, hCG, Oxytocin)*, *antibiotic treatment* and *reproductive technique*, as factors. Independent samples t-tests were used to analyse the mare's follicle size at ovulation in dependency of *hormonal treatment ($PGF_{2\alpha}$, hCG, Oxytocin)*, *antibiotic treatment* and *side of ovulation*. A simple linear regression was used to relate *age* and *follicle size at ovulation*. The logistic regression was found to be the most parcimonious model to model follicular growth (Mata, 2012) and was therfore used to model folicular growth with and without use of hCG. Data was analysed setting the significance level in 95% and using the statistical package IBM SPSS® Statistics 19.

RESULTS

The logistic regression was found not to fit most of the variables (p>0.05) but the covariate *UO* (p<0.05). An intercept was also found to be significant (p<0.05) and the full description of the parameters is presented in Table II, where the odd ratios and respective 95% confidence intervals are also stated.

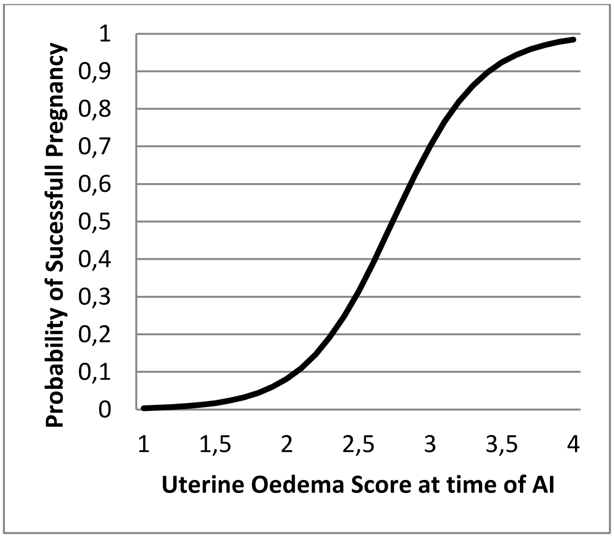
For *UO* there is an increase of 26.417 in the odds ratio of pregnancy success per UO unit increase, therefore a positive relation between pregnancy success and UO is shown. The curve fitted to the model is represented in Fig. 1.

TABLE II – LOGISTIC REGRESSION PARAMETERS TO MODEL PROBABILITY OF SUCCESSFUL PREGNANCY IN DEPENDENCY OF UTERINE OEDEMA AT THE TIME OF INSEMINATION.

Variables in the equation	β	SE (β)	P-value	95% CI (β)		OR (e^{β})	95% CI OR (e^{β})	
Intercept	-8.967	3.932	0.023	-16.663	-1.261	$1.3x10^{-4}$	$5.8x10^{-8}$	0.283
Uterine oedema	3.274	1.483	0.027	0.366	6.181	26.417	1.442	483.475

Note: SE standard error, CI confidence interval, OR odds ratio. The adjusted final model has Deviance 12.8 and AIC 17.6. The model is graphed in Fig. 1, using the equation: *P*(successful pregnancy) = $(\exp(\beta_0 + \beta_1 X_1)) / (1 - \exp(\beta_0 + \beta_1 X_1))$, where: *P*(successful pregnancy) is the probability of successful pregnancy for a given mare; β_0 is the intercept; β_1 is the regression coefficients calculated for uterine oedema; and X_1 is the uterine oedema of the given mare.

Figure 1 - Probability of successful pregnancy given by the fitted model. This model is valid for uterine oedema scores between 1 and 3, respectively the minimum and maximum values observed in the data collected and used to fit the model.



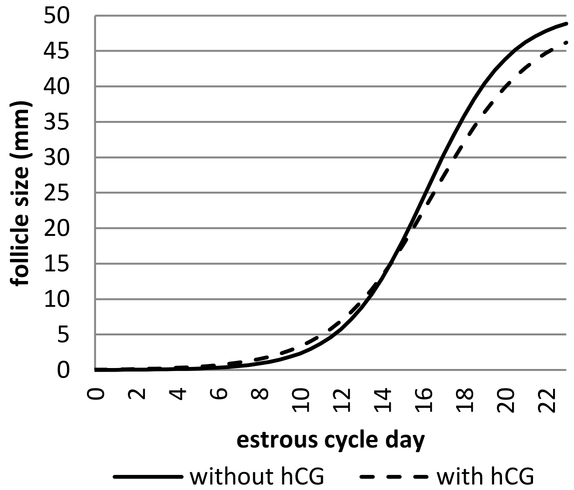
Follicle sizes at the time of ovulation where found to be significantly different when hCG was used ($p<0.05$). No significant differences were found for $PGF_{2\alpha}$ ($p=0.051$), oxytocin ($p>0.05$) and antibiotics ($p>0.05$). Respective mean and 95% confidence interval were found as follows: for follicle size with hCG treatment 47.89 mm (45.89; 49.91) and without hCG treatment 45.16 mm (44.31; 46.01). This difference is evident in the logistic growing curves with parameters in Table III and represented in Fig. 2.

TABLE III – LOGISTIC REGRESSION PARAMETERS FOR THE FITTED MODELS OF FOLLICLE GROWTH WITH AND WITHOUT HCG.

Parameters in the equation	a	b	c
With hCG	49.476	798.578	0.405
Without hCG	50.603	2772.528	0.490

Equation: *S(t) = a / {1 + b exp[-c(t)]}*, where: *a*, *b* and *c* are the parameters and *S(t)* stands for size at time *t*

Figure 2 – Growing curve of follicles in mares with and without hCG treatment.



No significant difference (p>0.05) was found between left and right ovaries with regards to *follicle sizes at the time of ovulation*.

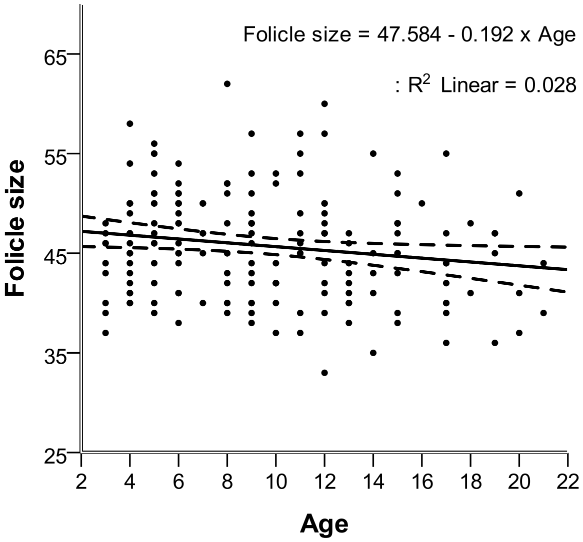
The regression between *age of the mare* and *follicle size at the time of ovulation* was found to be significant (p<0.05). The full description of the parameters is presented in Table IV, where the 95% confidence intervals are also stated. *Age of the mare* shows, therefore, to have a negative correlation with *follicle size at ovulation* (Pearson's r = -0.168) (Fig. 3).

TABLE IV – REGRESSION PARAMETERS TO MODEL PRE-OVULATORY FOLLICLE SIZE FUNCTION OF AGE.

Variables in the equation	***β***	**SE (*β*)**	**P-value**	**95% CI (*β*)**	
Intercept	47.584	0.927	<0.001	45.753	49.414
Age	-0.192	0.086	<0.05	-0.363	-0.022

Note: SE standard error, CI confidence interval. The adjusted final model has $R^2 = 0.028$. The model is graphed in Figure 3, using the equation: $Y = \beta_0 - \beta_1 X$, where: Y is the follicle size for a given mare; β_0 is the intercept (47.584); β_1 is the regression coefficient for age (-0.192); and X is the age of the mare.

Figure 2 – Regression line (with 95% confidence envelope) between *age of the mare* and *follicle size at ovulation*.



DISCUSSION

Uterine oedema

Previous studies have investigated the use of UO score to predict the time of ovulation (Samper, 1997; Pelehach *et al.*, 2002; Samper, 2009). Samper (1997) reports that detection of the pattern of UO with ultrasonography, combined with rectal palpation of the follicles, helps technicians to determine the optimal time for breeding of a normal mare. Low UO (1–2) and the presence of a large follicle (often >40 mm) is a good indication of imminent ovulation

(Samper, 1997). The appearance and disappearance of UO is related to the onset of oestrus in mares (Samper, 1997). UO is a powerful tool when controlling the mare to improve time of ovulation and detect a possible uterine infection (Samper, 1997; 2009). However, this tool has to be combined with rectal palpation of follicles and is not a good indicator when used alone. The presence of UO is therefore a mechanism under the influence of other events; it is a management tool for monitoring progress towards ovulation and does not have any effect on how follicle development performs. Results from the present study show that UO can also be a reliable parameter to predict pregnancy success in Lusitano mares. When modelling pregnancy success, *UO* was found to be significant ($p<0.05$); mares with a UO score of 2 prior to ovulation, have a probability of getting pregnant ~0.1, while mares with a UO score of 3 increase this probability to ~0.75.

Pregnancy success

Follicle size has been reported to be a good parameter to determine the timing of breeding in horses (Roig *et al.*, 2004; Samper, 2009), which obviously relates to the maturity of the follicle (with tendency to be bigger approaching ovulation). The size of the mature follicle (at ovulation) shows some variability, but findings from the current study suggest that the size of the follicle does not affect the pregnancy rate as long as it is a mature follicle.

Age was not found to have a significant effect on pregnancy success in this study ($p>0.05$), although the effect of increasing age on oocyte and subsequent embryo quality has been reported. Ginther (1993) reported that pregnancy rates were significantly reduced in aged Standard bred mares (30% vs. 57%). In an early field survey it was indicated a decline in fertility with increasing in age. Although lower fertility was suggested in older mares, gross inspection of ovaries and reproductive tracts showed many old mares to be reproductively active (Wesson and Ginther, 1981).

Treatments

In a recent study of Gastal *et al.* (2006), mares with and without hCG treatment have been compared; an increase in granulosa thickness and echogenecity and a decrease in circulating estradiol during the 36 hours post treatment were greater in the hCG group than in controls. Furthermore, diameter of the pre-ovulatory follicle was shorter from the hCG group. In the current study, results obtained were similar to Gastal *et al.* (2006). In Lusitano mares the administration hCG causes a reduction in size on the pre-ovulatory follicle. Results also indicate that follicle development when using hCG is slower which causes a reduction in follicle size from day -7 to ovulation (Ovulation = day 0). This reduction in size does not appear to significantly impact the pregnancy rate (p>0.05), and therefore despite its lower size, maturity of the follicle is not affected.

In this study the effect of $PGF_{2\alpha}$ administration in mares may also cause a reduction on the size of pre-ovulatory follicles (p=0.051) close to significance and therefore deserving further attention. Previous studies (Mata, 2012) also indicated that pre-ovulatory follicle slows its growth from day -8 to day -1 (Ovulation = day 0) and it seems that closest to ovulation grows rapidly.

Side of ovulation

The side of ovulation has no influence on pregnancy rates in Lusitano mares as no statistically significant difference was found between ovulations from the left and right ovary in relation to pregnancy success (p>0.05). Ginther *et al.* (2004a; 2004b) also reported no differences in the frequency of ovulation from either ovary in pony mares, which was consistent with previous studies (Ginther *et al.*, 1982).

Season

The spring transition has been studied extensively because of its practical importance and due to comparisons between the events preceding ovulation (Mckinnon *et al.*, 2011). The diameter of the largest follicle gradually increases as day length increases (Ginther, 1979; Carnevale *et al.*, 1997). Furthermore, Driancourt *et al.* (1982) indicated greater follicular activity during the first half of the ovulatory season (May to July) compared to the second half (August to October) in pony mares. The present study did not found significant differences both in follicle size and rate of pregnancy in dependency of season (p>0.05).

Age

If a relationship between follicle size and accentuated poorer reproductive performance does exist it might be expected that pre-ovulatory follicle size decreases with age. The present study supports this theory as in Lusitano mares *age of the mare* correlates negatively with *follicle size at ovulation* (r=-0.168, p<0.05). This finding is in agreement with that of Morel *et al.* (2005); Ginther *et al.* (2008) and Morel *et al.* (2010). Controversially, Ginther (2009) reported that in mixed breeds of large ponies and pony-horse crosses there were no differences among age groups in the day of maximum diameter of the pre-ovulatory follicle. In an earlier study it was reported that age effects involved a reduced number of follicles available and not diameter of the largest follicle (Ginther, 2004b).

Follicle size

Differences in pre-ovulatory follicle size among breeds and mare status have been reported (Gastal, 2009). The pre-ovulatory follicle is larger in Quarter horses than in Arabians but similar with ponies. Limited data suggest that pre-ovulatory follicles are about 5mm smaller in Miniature mares and 10 mm larger in Clydesdales than in Quarter horses and large ponies (Ginther, 1995).

McKinnon and Voss (1993) indicate that in mares with single ovulations pre-ovulatory diameter ranged from means of 48 mm in May and approximately 40 mm in July. Wesson and Ginther (1981) also indicate significant effects of month on paired ovarian weight, diameter of largest follicle and number of follicles. Furthermore, several studies state that the diameter of the pre-ovulatory follicle is greater for the first ovulation of the year than for the second and for later ovulations. The diameter of the pre-ovulatory follicle is about 5mm greater before the first ovulation (Driancourt *et al.*, 1982; Pierson and Ginther, 1987; Ginther, 1990).

CONCLUSION

Understanding the physiology associated to the reproduction of the mare is essential to optimize breeding management practice. While a lot of information is available in other species rather than horses, gaps in knowledge of the mare's physiology persist. Pregnancy rates might be affected by internal and external factors that can affect the physiology of the mare. The current study demonstrated that UO is not only a powerful predictor to time of breeding but also an indicator of pregnancy success; drugs that are used to minimise the number of mattings have an effect on follicle size and, finally; size of pre-ovulatory follicles and also follicle development vary inversely to mares' age in Lusitano mares. Despite the fact that differences in reproductive parameters between breeds have been reported, there is a lack of studies in particular and important breeds. Lusitano mares comprise an important part of the equine industry in Portugal; however studies in this particular breed are few. The data collected in the present study might be useful to compare reproductive parameters between horse breeds, gain a better understanding on the mare's physiology and increase pregnancy rates in reproductive programs.

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