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Revista Científica, Vol. XIV, Núm. 1, febrero, 2004
Universidad del Zulia
Venezuela

Disponible en: <http://redalyc.uaemex.mx/src/inicio/ArtPdfRed.jsp?iCve=95911219008>



Revista Científica
ISSN (Versión impresa): 0798-2259
revistafcvc@gmail.com
Universidad del Zulia
Venezuela

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Prediction of the day of ovulation in mares through physiological parameters measured during estrous

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RESUMEN

Con el fin de predecir el día de ovulación, fueron estudiados 120 períodos preovulatorios en 80 yeguas. Para tal fin, se analizaron parámetros como el comportamiento sexual, tamaño del folículo, textura del folículo, apariencia ecográfica del folículo, tono uterino y apariencia ecográfica del útero. Entre el cuarto y segundo día previo a la ovulación, el tamaño folicular incrementó, la textura folicular a la palpación presentó un ablandamiento folicular progresivo y la apariencia ecográfica demostró una triangulización del folículo. Los otros parámetros estudiados no presentaron variación estadísticamente significativa, sin embargo, algunos obtuvieron propensión numérica favorable. El procedimiento Stepwise seleccionó la textura folicular a la palpación y el tamaño folicular como las variables que presentan mayor significancia estadística y que predicen la probabilidad de ovulación en los días que la preceden. La función logit con sus 3 interceptos estimados (-4,35; -3,06 y -1,59; 1, 2 y 3 días para la ovulación) y las pendientes, 0,495 para tamaño folicular y 1,05 para la textura del folículo, permite calcular las probabilidades acumuladas de ovulación en los días posteriores. Si encontramos un folículo muy blando y mayor de 45 mm de diámetro se obtiene un 79% de probabilidad de que la yegua ovule en 24 horas. Sin embargo, varias combinaciones de tamaño folicular y textura del folículo ofrecen probabilidades mayores del 80% de que la yegua ovule dentro de las próximas 48 horas.

Palabras clave: Yegua, celo, folículo ovárico, predicción de ovulación, ultrasonido.

Predicción del día de ovulación en yeguas utilizando parámetros fisiológicos medidos durante el estro

ABSTRACT

Behavioural, echographic and exploratory characteristics of the estrous period were studied daily for 120 preovulatory periods in 80 mares. The ovulation across preovulatory days of estrous behaviour, follicular size, follicular texture, echographic follicular appearance, uterine tone and echographic uterine appearance were analyzed. Follicular size increased between fourth and second day before ovulation. Follicular texture showed a progressive softening before ovulation. Echographic follicular appearance evidenced a triangularization of follicle near ovulation. The variability of the rest of studied parameters showed tendencies but did not reach statistical significance. In the other hand, the stepwise procedure selected ovarian texture and follicular size, in this order, as the variables that influence significantly the probability of ovulation in the next days. A logit link function with three intercept estimates, -4.35, -3.06 and -1.59 (1,2 and 3 days to ovulation), and the slopes, 0.495 for follicular size and 1.05 for follicular texture, calculate

the cumulative probabilities of ovulation in the following days. With a combination of a very soft follicle and a follicle larger than 45 mm in diameter we have 79% of probabilities that the mare ovule in 24h. But several combinations of follicular size and follicular texture offer probabilities higher than 80% that mare ovule in 48h.

Key words: Mare, estrous, ovarian follicle, ovulation prediction, ultrasound.

Recibido: 08 / 05 / 03. Aceptado: 22 / 11 / 03.

INTRODUCTION

Estrous period of the mare usually lasts 4-7 days, but is very variable, and usually ends approximately 24 hours (0-48 hours) after ovulation [3]. Ovulation is the culmination of a complex series of events, endocrine, biochemical and cytologic changes, that results in the collapse of the preovulatory follicle and the expulsion of the oocyte from the follicle [13].

Predicting the day of ovulation would have considerable use in co-coordinating the time of breeding, both by natural service or artificial insemination, with the expected time of ovulation [7]. Breeding after ovulation decreases the possibility of a successful pregnancy [9]. Equine practitioners use various symptomatic and exploratory parameters to determine the time of ovulation; nevertheless, the complexity of the ovulation mechanisms and the estrous duration, estrous symptomatology and time of ovulation variability are responsible that none of these parameters brings forward evidence with security that the ovulation is close.

A continuous examination, with 4 or 6 hours of interval, during the estrous period could predict ovulation with relative reliability; however, this continuity normally is not practical and economically feasible. As it was referred previously, estrous symptomatology normally ends 24h after ovulation, although some mares ovule when estrous symptomatology is finished. Preovulatory follicle becomes soft 12 hours before ovulation in 40% of the mares [4, 11], this approach, however, is not reliable to predict ovulation in individual mares. The tone of the uterus by rectal palpation during estrous is fair or poor (not tonic), but there are no specific changes at the time of ovulation [3]. Follicular size increases in diameter linearly over the seven days at an average rate of 2.7 mm per day [4, 5]; although Palmer and Driancourt [10] report that 24-48h before ovulation this increase stops. Preovulatory follicles exhibit a pronounced change in shape from a spherical to a conical or pear-shaped structure in 84% of the preovulatory periods, the remaining follicles retaining a spherical shape [4]. Furthermore, the degree of endometrial edema and the amount of luminal fluid generally decrease prior to ovulation, although this is not always the case [3].

The aims of the present study were to validate different parameters used to determine ovulation, evaluate the relationship between these parameters, and to obtain a possible equation for predicting ovulation with relative accuracy after a daily exploration.

MATERIAL AND METHODS

A number of 80 healthy sports-bred mares (*Selle Française*, Anglo-arabians, Andalusian and Thoroughbred mares), aged 3-13 years old, were used, during the breeding season, from February to July 2000 and 2001, in the northeastern region of Spain. One hundred and twenty estrous periods were evaluated, including postpartum estrous, maiden estrous and estrous of non-gravid adults mares in the last year.

Maiden and non gravide mares were kept in an individual box with artificial lighting

(200w, from 17:00h to 23:00h) since november 15th to induce ovarian activity. Mares were teased, palpated transrectally and examined by ultrasound with 5 MHz linear-array transducer (Aloka 210) morning (10:00h to 12:00h) daily during estrous by two experienced practitioners. Six different parameters were evaluated and categorized as is seen below.

1. Estrous behaviour

0 points - no estrous, resistance

1 point - indifference

2 points - interested, elevated tail and winks vulva

3 points - profuse urination and vulvar activity with immobilization response

2. Follicular size

0 points - <35mm

1 point - 35-39mm

2 points - 40 – 44mm

3 points - >45mm

3. Follicular texture (by transrectal palpation)

0 points - fibrous ovary

1 point - turgid

2 points - elastic

3 points - soft

4 points - edematous and dolorous

4. Echographic follicular appearance

0 points - round

1 point - oval

2 points - triangular with ovulation conus

3 points - irregular

5. Uterine tone (by transrectal palpation)

0 points - poor

1 point - fair

2 points - good

6. Echographic uterine appearance

0 points - grey and diffuse

1 point - irregular black spaces

2 points - regular black spaces

The evolution along preovulatory days of estrous behaviour, follicular texture, follicular size, echographic follicular appearance, uterine tone and echographic uterine appearance were analyzed through a model containing the preovulatory day and the mare. As most of the mares were measured two or more times, data were analyzed through a mixed model including the effect of the mare as a random factor. The relationship between estrous parameters and time to ovulation in days was studied by assuming a logistic regression model for ordinal responses (1, 2, 3 and 4 days to ovulation), with the logit function used as the link function. The independent variables that influence ovulation were selected by a stepwise procedure. The logit function is defined as

$$P(x_i) = \frac{1}{1 + e^{-x_i' \beta}}$$

Where, $P(x_i)$ is the probability of occurrence (ovulation or no ovulation in a given term) against the regressor variables, and $x_i' \beta$ is the product of the vectors of observed variables (x_i') in each individual and the partial regression coefficients (β). All computations were performed by means of the SAS statistical package [14].

RESULTS

Evolution of different parameters among days before ovulation

The evolution of the parameters analyzed is presented in TABLE I. Estrous behaviour scores increased along estrous, since the mares tended to show more interest and receptivity to the stallion, although the differences did not reach statistical significance.

TABLE I
VARIATION OF PARAMETERS BETWEEN DAYS
BEFORE OVULATION

Parameters	Day of Oestrus			
	4	3	2	1
Estrous behaviour	1.91 ^a	1.93 ^a	2.13 ^a	2.15 ^a
Follicular Size	1.64 ^b	2.24 ^a	2.56 ^a	2.56 ^a
Follicular texture	1.50 ^c	1.97 ^b	2.25 ^{a,b}	2.49 ^a
Echographic follicular appearance	1.37 ^c	1.38 ^{b,c}	1.56 ^{a,b}	1.79 ^a
Uterine tone	1.36 ^a	1.17 ^a	1.16 ^a	1.30 ^a
Echographic uterine appearance	1.18 ^a	1.17 ^a	1.28 ^a	1.33 ^a

(a,b,c): Different letters within the same row imply statistically significant differences (P<0.05).

Follicular size tended to increase during the second day prior ovulation, without differences between the third, the second and first days before ovulation. Follicle diameter scores were between 35-39 mm in the fourth day before ovulation and over 42 mm two days before ovulation, then growth stopped.

The consistency of the preovulatory follicle, measured by rectal palpation, decreased from the fourth to the first day prior to ovulation. Follicle experienced a softening before ovulation.

Echographic follicular appearance score tended to increase with estrous. Preovulatory follicles tended to elongation and triangularization along the estrous.

Echographic uterine appearance and uterine tone by transrectal palpation did not evidence significant variations between the last four days of estrous. Echographic uterine appearance showed black spaces during the last four days of estrous with a tendency of a regular radial disposition of these spaces. Uterine tone showed a tendency to decrease the third and the second day prior ovulation and to increase the last day.

Relationship among variables (Logistic Regression)

The stepwise procedure selected follicular texture and follicular size, in this order, as the only variables that influence significantly the probability of ovulation in the next days. No additional variables met the 0.05 significance criterion to enter into the model. The estimates of the intercept and partial regression coefficients were as follows:

a. There were three intercept estimates corresponding each to the cumulative distribution probabilities of the n-1 response categories (1, 2 and 3 days to ovulation). The intercepts were -4.35, -3.06 and -1.59, respectively.

b. The slopes for follicle size and follicular palpation were 0.495 and 1.05, respectively.

From these values, by substituting in the logit link function it is possible to calculate the cumulated probabilities of ovulation of a mare in the following day, in the two next days and in the three next days. It was computed these probabilities for all possible combinations of values for follicular size and follicular texture, and the results are presented in TABLE II.

TABLA III
MEDIAS POR CUADRADOS MÍNIMOS PARA LAS
VARIABLES PSICRÓTROFOS Y TERMODÚRICOS

Marca	Psicrótrofos (ufc/mL)	Termodúricos (ufc/mL)
	Media	Media
General	$8,7 \times 10^2$	$5,9 \times 10^3$
A	$1,3 \times 10^{2b}$	$2,1 \times 10^{3b}$
B	$1,3 \times 10^{3a}$	$5,4 \times 10^{3ab}$
C	$4,3 \times 10^{3a}$	$1,9 \times 10^{4a}$
D	$8,3 \times 10^{2a}$	$5,5 \times 10^{3ab}$
E	$8,1 \times 10^{2ab}$	$6,8 \times 10^{3ab}$

Los valores de una misma columna con diferente superíndice difieren significativamente ($P < 0,005$).

Follicular size score of 2 or 3 and follicular texture score of 4 showed a cumulative probability of ovulation within 24h of over 70%, and several combinations of follicular size and follicular texture showed a cumulative probability higher than 80% that the mare ovule in 48h.

DISCUSSION

Previous reports showed that behavioural signs occurring most frequently during estrous, in decreasing order, are tail raising, remaining calm, winking, posturing and urinating [2]. In this study, those signs increased with estrous in agreement with several previous reports [1, 2, 3, 15], although the differences did not reach statistical significance, possibly due to an individual variations.

The growth rate of follicle during estrous and mean diameter of the preovulatory follicle observed on day-1 was consistent with reported dates [6, 7, 10, 12]. Follicles usually reach 4 cm in diameter before ovulating [3]. No preovulatory follicles are smaller than 35 mm or larger than 58 mm on day-1 [7]. The large preovulatory size variation between mares and estrous cycle would mean that this is a bad predictor of ovulation. However, the combination of a follicle that reach 4 cm in diameter with a softening of this follicle seems to be the best predictor of ovulation.

As it was observed, several authors have described the softening of the preovulatory

follicle in the mare, 90% of preovulatory follicles shows a change in consistency from turgid to soft during estrous [4, 11].

Few time before ovulation the follicle became more soft in 40% of mares [3, 4], and this finding is a good predictor of ovulation. The stepwise selection procedure demonstrates that ovarian texture is the most important parameter to determine ovulation.

Pierson and Ginther [12] classified 85% of the follicles as non-spherical by day-1 to ovulation suggesting a relation between this observation and the softening of the preovulatory follicle. It is possible that the softening of follicles is related with the elongation of the follicle to the ovulation cavity. The variation between days before ovulation observed in our study is in agreement with that report. However, the stepwise procedure did not include this parameter as a predictor of ovulation.

During estrous, endometrial folds enlarge and becomes edematous, in consequence uterine tone becomes not tonic [3]. There was no significant change among days. Perhaps a little increase, not significant, was detected for day-1, possibly related with hormonal changes.

Ultrasonic evaluation of the uterus during estrous shows hyper and hypoechogenic areas. Hypoechogenic regions are the result of submucosal fluid (edema) [7]. The degree of uterine edema increases with follicle growth. Edema generally reduces or stops, commencing one day prior to ovulation, but it is not always the case [3].

The combination of follicular texture and follicular size appeared to be valuable for predicting ovulation in a daily exploration. With a follicle larger than 45 mm and very soft we have 79% probabilities that the mare ovule in 24h. But several combinations of follicular size and follicular texture offer probabilities higher than 80% that the mare ovule in 48h.

After natural breeding or artificial insemination with fresh or refrigerated semen, the survival time of the spermatozoa in the female reproductive tract varies between 72-120h [8]. Perhaps some echographic images could inform that ovulation is imminent, as the follicular wall increases in thickness, follicular fluid increases in echogenicity, follicular irregularity or, in the ovarian palpation, detection of a very soft follicle. Nevertheless, when in a daily exploration there are not these casual findings, the application of the work formula could be a useful tool. However, an experiment must be carried out to test if the proportion of the mares that become pregnant after predicting the time remaining to ovulation is within an acceptable range.

CONCLUSION

The statistic logistic regression is a good tool to select the best parameters that predict the day of ovulation in mares. Several combinations of follicular size and follicular texture offer probabilities higher than 70% that the mare ovule in 24 hours and 80% that ovule in 48 hours.

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