COMPARISON OF DIFFERENT METHODS USED FOR OESTRUS EXAMINATION IN THE BITCH

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The aim of this study was to determine the reliability of oestrus symptoms of the bitch (vulvar swelling, vaginal fluid, attraction of the male, teasing) and that of the most frequently used examination methods (vaginal smear, vaginoscopy, serum progesterone determination) which indicate the stage of the cycle and allow to predict the optimal day of mating (ODM). The determination of the cycle stage was based upon the number of days left from the day of examination to the day of parturition (DTP). Our results indicate that none of the above-mentioned symptoms and examination methods is reliable alone for the determination of DTP, and even the complex picture obtained from the integrated results of different methods will not enable an exact estimation. ODM can be predicted with a higher reliability, and 1–2 days before it, it can be detected very safely. Since most bitches are presented for timing to practitioners, a scheme was created based upon a scoring system, which gives a point value to each individual symptom and examination result, and judges the bitch according to the total amount of points.

Key words: Ovulation detection, cycle staging, bitch, progesterone

The prediction of the optimal day for mating (ODM) has belonged to the routine veterinary work for a long time. To assess the actual heat cycle stage may be important, too. Exclusively from the clinical symptoms that can be observed by the breeder it is difficult to determine the 2- to 3-day period of the 3- to 4-week-long heat when the bitch should be bred. The sexual hormone profiles during heat have almost completely been clarified (Zöldág, 1993), along with their detection methods (Haaften et al., 1989, Johnston et al., 1994, Zöldág et al., 1994). Vaginoscopy and vaginal smear have been applied for a long time, too (Phemister et al., 1973; Lindsay, 1983; Olson et al., 1984*a, b*). To monitor the plasma progesterone (P4) levels quantitative ELISA tests have become available only in recent years, but today they are the easiest and least expensive methods of sufficient efficacy (Nagy et al., 1998).

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Nevertheless, it is very important to analyse statistically the methods used for timing of bitches, and to examine their efficacy in the determination of the heat stadium, and their accuracy in predicting ODM, since in most publications only one method was examined separately, predominantly on experimental dogs, at a well-equipped university clinic. These valuable results are not always useful for the clinicians, as they are working with heterogeneous dog populations and do not have access to expensive equipment. However, the majority of cycling bitches are presented to practitioners, so it can be important to assess the efficacy of their examination methods.

The aim of this study was to detect how safely the visible symptoms and the examination methods available at most clinics can estimate the number of days to parturition (DTP), the actual stage of the heat, and the number of days left until the ODM.

To determine the cycle stage the number of days left from the day of examination until the day of parturition was observed. Authors in different publications frequently relate their results to the day of the LH surge, which precedes parturition by 64–66 days (Yeager and Concannon, 1990; Wallace et al., 1992; Nishiyama et al., 1999), or to the day of the dioestral shift in the vaginal smear, when the cornification index decreases at least 20% in one day (this precedes parturition with 57–58 days; Phemister et al., 1973). These methods are excellent for comparison, because the interval from their detection until parturition is more or less constant, but we could not use them for several reasons: (*i*) both require a daily examination, which is impractical in the daily practice; (*ii*) they do not show the result of the breeding, so there is no follow up about the effectiveness of timing; (*iii*) LH tests are quite expensive and thus cannot be used in daily practice; (*iv*) the dioestral smear gives a retrospective result only, therefore it cannot be used for timing.

It has to be remarked that the day of parturition may fluctuate by 1–2 days, when the number of puppies is very high or in case of elective caesarean section. In the recent study only those bitches are involved which got pregnant and whelped; all others were excluded. Thus the evaluation of the chance for pregnancy with the examined methods (although it is possible) will not be examined now.

Materials and methods

During a two-year period 207 bitches were examined. The examined population consisted of bitches from different breeds and of different age. The fertilisation occurred usually through natural breeding, 1–4 times per bitch. Out of these dogs 133 whelped (64.3%) and 74 did not (35.7%). Since the examined population contained many infertile, 'problem' bitches, determination of the ODM was not always sufficient to obtain pregnancy. The effect of several factors

influencing the pregnancy rate should be considered: (*i*) very little information was available about the sire dogs used for breeding; (*ii*) the owners exceptionally presented their bitch for pregnancy check 21–28 days after breeding, so it was not possible to detect the rate of fetal resorption or abortion; (*iii*) some other factors were found to decrease the pregnancy rate, e.g. two of the examined bitches died during the 2-month period after breeding and one disappeared.

Thus the 64.3% pregnancy rate does not reflect the possible effectiveness of the timing, but, since all bitches were clinical patients and not experimental dogs, there was no chance for more detailed examinations.

Under our conditions several factors limited the number of examinations performed on one bitch: (*i*) financial restrictions; (*ii*) the owner lived far from the clinic, so the time requirement of transportation further limited the number of examinations; (*iii*) the owner had no time to present the bitch at the required appointment; (*iv*) in different cases the owners required the timing with a different accuracy.

Examination methods

Vulvar swelling was observed visually while the amount of vaginal fluid by vaginoscopy. Information about attraction of the male and the teasing reflex were obtained from the owner.

Vaginal smear collection. Sampling was performed from the craniodorsal part of the vaginal lumen. Staining was performed with J 322 Dip Quick Stain set (Jorgensen Laboratories Inc., Colorado, USA).

Vaginoscopy. The cranial vagina was visualised by introducing a metal tube of the appropriate size. The light source was a fibreoptic instrument.

Blood sampling, progesterone (P4) testing. P4 was assayed by a quantitative ELISA test (Quanticheck Progesterone, Szent István University, Faculty of Veterinary Science, Budapest, Hungary). Six standards were used, with a P4 content of 0, 1, 2, 4, 7, and 10 ng/ml, respectively. Since there was no ELISA-reader available, the P4 results were judged visually. When the colour of the sample fell between two standards, it was possible to judge with a good accuracy to which it will be closer, since human eyes are relatively sensitive to blue colour intensity. So the values were evaluated with an accuracy of 1.0 ng/ml. The accuracy of the assay is about 0.5 ng/ml with P4 values lower than 4 ng/ml, and 1 ng/ml at values higher than that. When the P4 content was expected to be above 10 ng/ml, the serum sample was diluted with PBS in a ratio of 1:1 or 1:3. Each judgement was made by two different persons, difference between the results of judgement was found in about 10% of the cases, in a value of max. 1 ng/ml, mostly at the values above 4 ng/ml. The accuracy of the examination was further increased by the fact that each sample was examined in duplicates, so the subjective failures were easily noticeable. There are other methods for P4

detection which are much more accurate than this ELISA test, but for our purposes this accuracy was satisfactory.

To decrease the costs of examining one bitch, when it seemed obvious from the vaginoscopy and smear that the bitch was in an early cycle stage, no blood was collected for P4 assay. When the stage was not so obviously early by vaginoscopy and smear, blood for progesterone assay was collected and the assay was completed as well. When the hormone level was 4 ng/ml and it was in accordance with the results of other examinations, the first breeding was recommended two days later and further matings on every alternate day as many times as possible. If the P4 value was below 4 ng/ml, another examination was recommended two days later. When the P4 level was above 4 ng/ml, supported by the other results, an immediate mating was recommended, and then mating was repeated at two-day intervals as many times as possible.

Nomenclature used

To present the results a new nomenclature was established, which was based partly on the literature (Lindsay, 1983; Olson et al., 1984*a*; Verstegen, 1999*a, b*), partly on our own clinical experience. It is simple, easy to use and sufficiently accurate for the routine practice. The meaning of the nominations and signs is presented below.

Attraction of the male: signed with +, +/− and −. With '+' the male would mount, with +/− he only smells the bitch, plays with her, and with '−' he does not show any interest towards the bitch.

Teasing reflex: signed with + and −. '+' means that the bitch stands for the male, with '−' she refuses standing.

Vulvar swelling: scored from 0 to 3. A score of 0 means that the size of the vulva is like that of the non-cycling bitch, while at a score of 3 it is maximally swollen, oedematous.

Amount of vaginal fluid: scored from 0 to 3. A score of 0 means that there is no fluid in the vaginal vestibule and the vaginal wall is dry, while at a score of 3 a large amount of reddish fluid can be detected, the bitch leaves drops on the floor, by introduction of the vaginoscope at least 1–2 ml fluid can be obtained.

Cycle stage according to vaginal smear: P = prooestrus, O = oestrus, M = metoestrus, the numbers beside the letters sign the early (1) and late stage (2). Determination of the cycle stage from smear was based on Olson et al. (1984*a*).

Cycle stage according to vaginoscopy: it was based on the description of Lindsay (1983).

Serum P4 level: was expressed in ng/ml.

Results

I. Estimation of the number of days till parturition (DTP) from our results

1. Examination of results on different days before parturition. Table 1 demonstrates how many dogs produced the examined result on a particular day between day 57 – day 70 before parturition. The numbers in the columns represent the number of dogs producing the given result on the examined day. Since it was not always possible to perform every examination on each dog, the lines are completed with the actual sample size (n).

Table 1

Number of dogs producing the examined result on a particular day between day 57 and day 70 before parturition

DTP	Male "+"	Male "-"	n	Teasing "+"	Teasing "-"	n	Smear p1	p2	p2-o1	o1	o2	n	Vag. p1	p2	p2-o1	o1	o2	n	P4 0	1	2	3	4	5	6	7	8	9	10	n	Swell 0	1	2	3	n	Fluid 0	1	2	3	n
57	1	-	1	1	-	1	-	-	-	1	-	1	-	-	1	1	-	2	-	-	-	-	-	1	-	1	-	-	-	2	2	-	-	-	2	2	-	-	-	2
58	2	-	2	2	-	2	-	-	-	1	-	1	-	-	-	1	-	1	-	-	-	-	2	1	-	-	-	-	1	4	3	-	1	-	4	3	-	1	-	4
59	5	1	6	4	1	5	-	-	-	3	4	7	-	-	-	3	4	7	1	-	1	-	3	-	1	-	1	1	-	8	1	1	4	2	8	2	1	4	1	8
60	5	-	5	5	1	6	-	-	1	5	2	8	-	-	1	3	3	7	1	-	1	-	2	2	1	2	1	-	1	11	2	3	4	2	11	3	1	4	3	11
61	10	1	11	10	2	12	-	1	1	9	3	14	-	2	4	6	3	15	-	2	2	1	8	2	3	2	-	-	1	21	7	3	9	2	21	7	5	6	3	21
62	14	3	17	12	6	18	-	3	7	11	1	22	-	2	8	8	1	19	1	2	6	5	13	-	1	2	2	-	1	33	9	4	16	5	34	10	8	9	7	34
63	15	4	19	14	7	21	-	3	1	18	1	23	-	2	5	15	2	24	-	-	9	4	10	1	1	5	-	-	-	30	5	9	13	5	32	4	13	12	3	32
64	6	-	6	3	4	7	-	2	5	4	1	12	-	3	1	8	1	13	2	-	7	-	4	-	1	4	-	-	-	18	6	4	7	3	20	6	4	5	5	20
65	11	5	16	9	8	17	-	4	3	15	3	25	-	3	7	16	2	28	1	6	15	3	5	-	-	-	-	-	1	31	5	8	10	8	31	8	9	6	8	31
66	13	5	18	10	5	15	1	2	2	14	2	21	1	2	5	14	2	24	3	1	7	5	5	-	2	-	1	-	-	24	3	5	10	7	25	6	7	7	5	25
67	9	3	12	7	4	11	-	6	4	9	-	19	-	6	6	8	-	20	3	2	8	3	3	1	2	-	-	-	-	22	7	6	8	7	28	9	5	7	7	28
68	7	3	10	7	5	12	-	7	4	2	-	13	-	3	4	1	1	9	4	3	6	-	2	2	-	-	-	-	-	17	3	4	6	5	18	3	6	4	5	18
69	7	4	11	6	5	11	1	7	2	6	-	16	-	6	3	6	-	15	4	3	4	1	2	-	-	-	-	-	-	14	1	2	8	7	18	3	2	2	11	18
70	1	1	2	-	2	2	1	1	1	-	-	3	-	-	1	-	-	1	-	-	2	-	-	-	-	-	-	-	-	2	1	-	2	-	3	-	2	-	1	3

The reasons why not all data could be recorded at each examination were:

– the owner could not always tell us data about the attraction of the male or the teasing reflex of the bitch;

– vaginoscopy and smear collection would have caused too much stress for the bitch (mostly virgin bitches and bitches in a P1 or P2 stage);

– to decrease the expenses, blood for P4 determination was collected only when the smear and vaginoscopy results indicated that the bitch is over the P1-P2 phase.

– the bitches having P4 above 4 ng/ml were sent to breed, and afterwards they rarely returned for further examination.

2. Correlation between the complex picture and DTP. The correlation between the complex clinical picture obtained from the examination results and DTP was tested by linear regression (the countings listed here do not follow from Table 1, but the source of both is the same database). Since the P4 level increases exponentially over the days, the logarithm of P4 level was involved in the regression, too.

The different symptoms and examination results explain the change in DTP with the determination coefficients demonstrated in Table 2 (higher values mean a stronger correlation). It seems that the complex clinical picture explains the change in DTP by far the best, but the determination coefficient (47%) is still low to make a correct estimation, so it can be concluded that the DTP and so the expected day of parturition cannot be determined from the results of our examinations, it can only be estimated.

Table 2

Correlation between DTP and different symptoms and methods

Examined relation	Correlation
DTP – swelling	0.006
DTP – fluid	0.003
DTP – smear	0.25
DTP – vaginoscopy	0.17
DTP – log P4	0.29
DTP – complex clinical picture	0.47

II. The change of symptoms and examination results other than P4 in relation to the P4 level

Since the P4 level and the other results show a relatively large deviation related to the DTP, next it was examined how the symptoms and results other than P4 would change in relation to the P4 level. Table 3 shows the distribution of the results at P4 levels of 2, 4 and 7 ng/ml. In this case the numbers mean how high percentage of the dogs produced the examined symptoms on the same day, when the P4 level of the same dog was 2, 4 or 7 ng/ml, respectively. It can be seen that the data follow the expected tendencies much more closely than in the case of DTP.

Table 3

Correlation between P4 level and other parameters

P4 level	Male		Teasing		Smear					Vaginoscopy					Swelling				Fluid			
	+	–	+	–	p1	P2	p2-o1	o1	o2	p1	P2	p2-o1	o1	o2	0	1	2	3	0	1	2	3
P=2	71	29	62	38	2	25	29	40	4	0	19	27	52	2	18	19	34	29	16	26	36	22
P=4	90	10	74	26	0	8	15	64	13	0	7	17	62	14	16	29	43	12	22	35	31	12
P=7	100	0	88	12	0	0	20	70	10	0	0	12	63	25	38	37	25	0	44	37	7	12

III. Prediction of the optimal day for mating (ODM)

The optimal day for mating (ODM) was determined only for those bitches which became pregnant and were bred only once (ODM = the day of breeding), or twice with a maximal difference of two days between the two matings, when

the day between the two mating days was regarded as the ODM. ODMs were calculated from our examinations as well (CALC), and were compared to the clinically obtained ones (OBT).

1. Determination of ODM from our data. The connection between the examination results and ODM was reviewed with linear regression. When the P4 and the smear were considered, the data explained the change in ODM in 57%, which is a much better correlation than that between our data and the DTP. However, when examining the accuracy of ODM estimation, Fig. 1 shows that the closer the ODM was to the day of estimation, the more accurate estimation could be obtained.
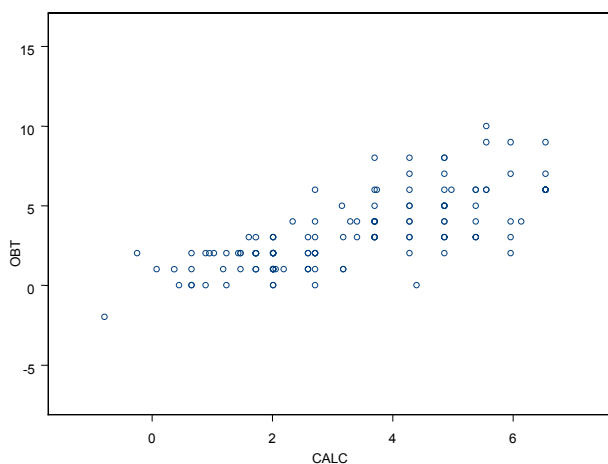

Fig. 1. Correlation between calculated (CALC) and clinically obtained (OBT) ODMs

Figure 1 shows the correlation between the calculated (CALC) and clinically obtained (OBT) ODMs. It is obvious that with low values of CALC (< 1.5) OBT changes in a narrow interval, which indicates that they show a strong correlation in this case.

Table 4

Point values given for different symptoms and examination results

Smear	Point	Vagino-scopy	Point	Male	Point	Teasing	Point	Swel-ling	Point	Fluid	Point
P1, P2	–20	P1, P2	0	+	10	+	25	0–1	5	0–1	–5
P2–O1	10	P2–O1	5	+/–	0	–	0	2–3	0	2–3	0
O1, O2	20	O1, O2	15	–	–10						
M	–20	M	0								

P4 (ng/ml)	0	1	2	3	4	5	6	7	8	9	10
Point	50	50	60	100	120	130	140	150	160	170	190

2. Complex evaluation by scoring system. Since the most frequent problem is the prediction of ODM, an easy-to-use scheme without difficult mathematical calculations was created. Each symptom was paired with a point value, and the bitch was judged according to the total amount of points obtained (Table 4).

3. Correlation between points and ODM. The correlation between the total points obtained and the days to ODM was checked by linear regression (Fig. 2).

Figure 2 shows the correlation between the points obtained on the day of examination (POINT) and the deviation of the same day from the clinically proven ODM. Linear regression shows an even stronger correlation (63.85%) between the two parameters than between the calculated and clinically proven ODMs (56.84%). It is remarkable in Fig. 2 that the values above 190 points change only in a 2-day interval (2 and 0), so the same result is obtained as in Chapter III/2, but the estimation is more exact here. Since the spermatozoa maintain their fertility in the female genital tract for 3–7 days, it is obvious that if the points of a bitch exceed 190, she should be mated. Under 190 points the interval to ODM is more variable, therefore it is better to re-examine the bitch 2 days later again, until her points reach 190, than to give estimations with a low reliability.
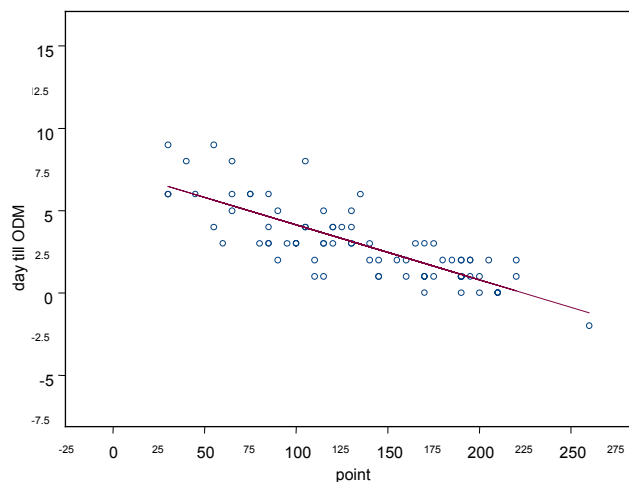

Fig. 2. Correlation between the total points obtained and the days to ODM

4. Examination of days left from the beginning of the heat until parturition and ODM. The bitches exhibited the first signs of oestrus on average 74 days before parturition, 50% of them between days 76 and 72. The 95% confidence interval is 65.75 to 82.97. If the first signs of oestrus are compared to the ODM, the average is 13 day, the 95% confidence interval is 6.26 to 20.39. Since the deviation is great in both cases, it is apparent that neither the DTP nor the ODM can be predicted from the 1st day of oestrus.

Discussion

With the examined methods it was possible to estimate the optimal day for breeding (ODM) with a good accuracy, but not the number of days till parturition (DTP). The reason is not the low reliability of these methods, rather the fact that it is hard to find an event throughout the cycle and pregnancy, to which the time of other events can be related. The time of particular events is calculated in the literature only in periods, and it is related to the time of other events (e.g. LH surge, dioestral smear, implantation of the embryos, first heartbeat, etc.). Usually no absolute day or cycle stage is determined. The same deviation exists at the determination of the heat cycle periods and the events limiting them. Now it has been proven that the parturition date itself is also not an absolute basis for comparison, as it may change in an unpredictable way. This is probably one reason why the correlation between the examination results and DTP is relatively weak.

To predict the ODM one single symptom or examination result was not sufficient; the diagnosis should be based on a complex picture rather than on one examination. Different examinations may allow to identify different causes of possible infertility. In case of serial examinations the change among results obtained on different days will further improve the chance to determine the ODM.

The detection of LH from serum has been described to improve further the accuracy of timing (Concannon et al., 1989; Zöldág et al., 1994; Nishiyama et al., 1999). It improves the chance to find the ODM, and mostly enables a better estimation of the DTP, but it is still not widely used in the general clinical practice, so it was not included in this study. According to our experience ODM can be determined with a satisfactory accuracy using only our methods as well.

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