

Clinical Determination of Bromocriptine 2.5 mg (Parlodel®) Induced Ovulation in Atlas Shepherd Bitch

Slimani Khaled Mabrouk^{1*} and Niar Abdellatif²

¹Canine Clinical Pathology, Institute of Veterinary Sciences, University Ibn Khaldoun, Tiaret 14000, Algeria

²Laboratory of Animal Reproduction, Institute of Veterinary Sciences, Ibn Khaldoun University, Tiaret 14000, Algeria

Corresponding author: Slimani Khaled Mabrouk, Canine Clinical Pathology, Institute of Veterinary Sciences, University Ibn Khaldoun, Tiaret 14000, Algeria, Tel: +213 (0)551527755; E-mail: mabroukslimani15@gmail.com

Rec date: February 06, 2018; **Acc date:** February 26, 2018; **Pub date:** February 28, 2018

Copyright: © 2018 Mabrouk SK, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

Eight atlas shepherd bitch tow years old and weighing between 14 and 16 kg have received a heat induction treatment with bromocriptine (Parlodel® 2.5 mg Breakable tablet Box of 30 tablets). The dosage adapted for the dog was 1.25 mg/15 kg body weight per day for a heat induction. The heats were followed from the 5th day of proestrus and during estrus to the canine clinical pathology of veterinary institute Ibn Khaldoun University of Tiaret between January 2014 and January 2015. For the diagnosis of ovulation the vaginal smears and ultrasound of the ovaries were combined with the proportioning of progesterone. No structural anomaly of ovaries and no genital infection were observed on clinical examination. The aim of this study is: To answer the question: Can Bromocriptine treatment induce ovulatory heat? All bitches (8/8) showed vulvar loss between day 21 and day 40 of Bromocriptine treatment. 89% (7/8) bitches exhibited a significant positive chronological evolution between the progesterone blood level and eosinophilic index and diameter of ovaries in. The ovarian diameter in 100% (8/8) bitches was 19 +/- 2.07 mm at the moment of ovulation.

Keywords: Heats; Ovulation; Atlas shepherd dog; Ovary; Ultrasonography; Cytology

Materials and Methods

Ethical approval process

This article represents part of a doctoral study under the theme: Use of ultrasound and vaginal cytology in the diagnosis of ovulation and pregnancy in the bitch.

The experiment carried out at the Institute of Veterinary Sciences of Tiaret. The Algeria, This research work, approved and validated by the Scientific Committee of the Faculty of Sciences of Nature and Life. University Mustapha Stambouli of Mascara, Algeria. And recorded under the number: 12 / FSNV / 2014.

Animals: 8 apparently healthy shepherds of the atlas bitches of about two year old and weighing between 14 and 16 kg. The animals were dewormed, vaccinated, and maintained in an iso managemental condition. All the bitches were nulliparous and had the first pubertal heat since 8 months on overage and consulted at canine clinical pathology of Veterinary Institute, Ibn Khaldoun University of Tiaret between January 2014 and January 2015.

Heats induction: The molecule chosen for induction of heat was Bromocriptine available in the human pharmacy known as Parlodel 2.5 mg, which was the only antiprogestin molecule to be commercialized in Algeria in box 30 tablet form. All the dogs selected for this study had a confirmed anoestrus by vaginal cytology examination which showed a clean smear; poor in cells with a few clusters of parabasal cells on smear colored then with the trichrome of Harris-Shorr (Kits Diagnosestrus-RAL A & B). The hormonal analyzes showed a very low blood level (less than 2 nmol/L) of the progesterone in the serum analyzed using the Elecsys1010 Roche® analyzer.

Each bitch received the prescribed oral dose of 1.25 mg/15 kg body weight per day until the appearance of vulvar losses; In order to

prevent the vomiting side effects often observed during the first week of the beginning of the treatment the molecule was administered 6 hours before the distribution of the food.

Follow-up of heats: Once the bitch shows vulvar losses, which indicates the onset of proestrus, a cytological follow-up was started every 1 to 2 days from the 5th day of proestrus (the day of the onset of heat was determined by the onset of vulvar loss) and during estrus. Included ultrasound of the ovaries and proportioning of progesterone. The results were recorded in paper folder for each bitch.

Vaginal cytology: A vaginal smear was prepared on blades (P-O blades) pack of 50 slides (Reproduction Canine.eu, EARL le paragon du King, Bouhey-France). Fixed with the cyto-fixer (cyto-RAL) aerosol of 75 ml (Reproduction canine.eu, EARL le paragon du Roy, Bouhey-France). Colored then with the trichrome of Harris-Shorr (Kits Diagnoestrus-RAL R & B, Reproduction canine, EARL le paragon du Roy, Bouhey-France).

The eosinophilic index (EI), the presence or absence of erythrocytes and leukocytes, the percentages of parabasal, small intermediate, large intermediate and superficial cells were determined. The smear was then numbered and preserved.

The blades were read using a binocular microscope (S-350 OPTIKA) with magnification x40, x100.

This gave us the following information:

- The majority coloring of the smear (acidophilic or basophilic cells);
- Cellular density;
- The presence of erythrocytes and polymorphonuclear neutrophils (PNN);
- The aspect of the bottom of the smear.

For cellular "typing" on a minimum of 10 to 20 cells; the slides were then observed at a higher magnification x100, in the areas of the smear where the cells formed less clusters. When reading vaginal smears a particular interest was reserved for the determination of the eosinophilic index (EI). It is the percentage of the really keratinized cells compared to those which could be but are not it (large intermediate polychromatophiles and basophilic cells). The small intermediate cells and parabasales cells are not taken into account [6-8]. EI=number of acidophilic cells × 100/number of basophilic cells.

Echographic examination: After a broad shearing of the abdomen the bitch was placed in *dorsal decubitus*. The appearance and the measurement of the diameter of the ovaries were made at the base of the echographic images obtained by longitudinal and transverse sections by using of an electronic sectoral probe with a frequency of 6 MHz and an IMAGO.S transportable echograph. The echography of the ovaries began from the 5th day of the proestrus (the day of the beginning of heat was determined by the beginning of the vulvar loss) and during the estrus. On average the examinations were practiced during 9 days. During the echographic examinations, the following steps were respected:

- Topographic identification of the two ovaries.
- Evaluation of the size of each ovary on longitudinal sections.
- Determination of the ultrasound aspect of the ovaries.

Progesterone assay: The determination of the progesterone blood level was achieved with a frequency of 4 to 5 blood sample per bitch every 24 to 48 hours from the 5th day of the proestrus until the day of the Ovulatory peak of the progesterone. The blood was collected from

the radial vein, once collected in a tube containing heparin; the blood sample was dated and identified by assigning a corresponding number to the bitch file. The serum was analyzed using the Elecsys1010 Roche® analyzer from a private laboratory for quantitative determination.

Results

Before the start of treatment all our bitches had a confirmed physiological anoestrus by vaginal cytology and plasma progesterone assay. Cytology revealed small clusters of parabasal cells with absences of red blood cells and leukocyte cells.



Figure 1: Vaginal swab for the realization of a cytological smear.

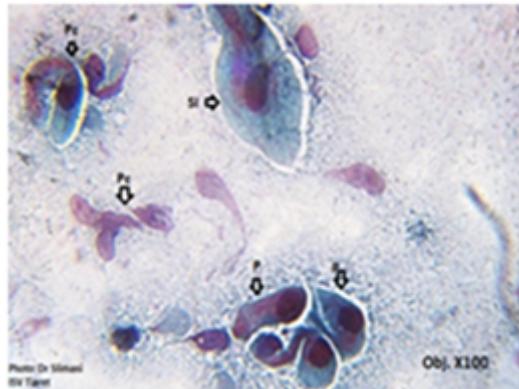


Figure 2: A view of a vaginal smear of anoestrus at magnification x100 which shows a cluster composed of parabasal cells and parabasal cells "in column form" (deep layer of the vaginal mucosa) in a smear stained with Harris Shorr and a few small intermediate cells with a slightly dirty background. Leukocytes are absent. Eosinophilic index is less than 10%. P: parabasal cells; SI: small intermediate cells.

The rate of progesterone was on average less than 2 nmol/L. Abdominal ultrasound of the genital system revealed no particular abnormality was detected on the ovaries and no anomalies in the uterus (Figures 1 and 2).

The proestrus was systematically induced in 100% (8/8) bitches. The time of onset of vulvar losses was variable from one bitch to another,

so 25% (2/8) bitches showed heat after 21 days of treatment. 37.5% (3/8) showed heat after 35 days. 37.5% (3/8) showed heat after 40 days of treatment, with an average of 32 ± 10 days (Figure 3).



Figure 3: Vulvar loss observed in a bitch after 35 days of treatment with Bromocriptine (Parlodel 2.5 mg).

100% (8/8) of our bitches presented a positive chronological correlation between the eosinophilic index and the increase in the diameter of the ovaries during the period located between the 5th day of the proestrus and the middle of the estrus which coincides with the period of ovulation. The diameter of the ovaries was equal to 19 ± 2.07 mm and corresponded to an eosinophilic index equal to or higher than 80% in 100% (8/8) of the bitches at the time of ovulation (Figure 4).

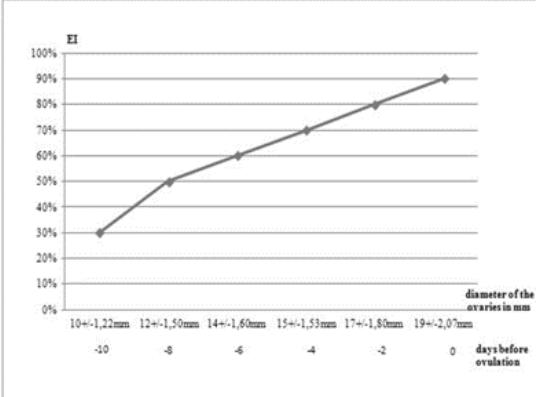


Figure 4: The chronological evolution of the eosinophilic index (EI) and the diameter of the ovaries from the 5th day of proestrus to the day of ovulation.

87.5% (7/8) bitches presented a positively correlation between the progression of the progesterone level and the eosinophilic index (EI) during the period located between the 5th day of the proestrus and the middle of the estrus which coincides with the period of ovulation (Figure 5).

The progesterone level at the time of ovulation was located between 21.04 ± 14.53 nmol/L and 33.15 ± 15.16 nmol/L in 7 bitches (Figure 5). Except for one bitch which showed a stable progesterone level of 9

nmol/L during all the period of heats independently of the increase of the éosinophilique index.

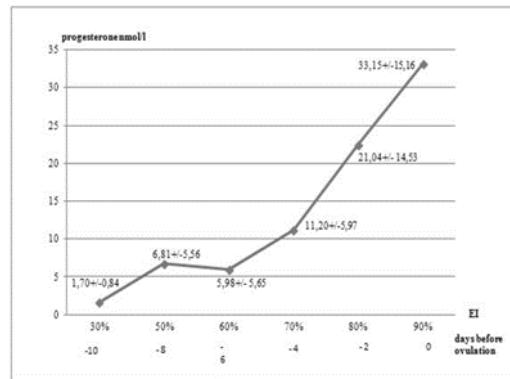


Figure 5: Chronological evolution of progesterone and eosinophilic index (EI) from the 5th day of proestrus to the day of ovulation.

It should be noted that keratinized acidophilic cells that reflect the eosinophilic index (EI) were already present when progesterone was at basal values in all bitches. When the progesterone level was at 1.70 ± 0.84 nmol/L the éosinophilique index presented an approximate percentage of 30% (Figure 5).

87.5% (7/8) bitches presented a positive chronological evolution between progesterone level and diameter of the ovaries from the 5th day of proestrus to the middle of the estrus (coinciding with the day of ovulation) (Figure 6). Except for a bitch which had a stable progesterone level of 9 nmol/L independently of the increase of the ovary diameter and the éosinophilique index.

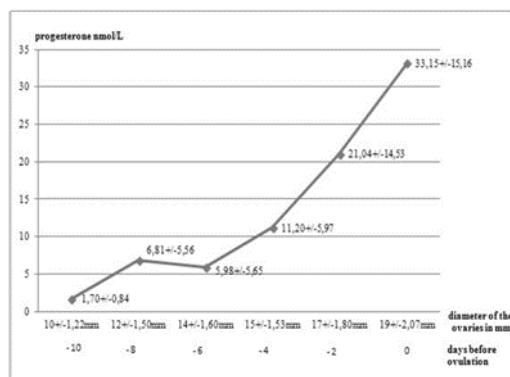


Figure 6: Chronological evolution of progesterone and ovary diameter from the 5th day of proestrus to the day of ovulation.

Variability in the moment of expression of the peak of the eosinophilic index was observed. 75% (6/8) of the bitches expressed this peak between the 12th and the 15th day of the heats, 12.5% (1/8) of the bitches at the 10th day of the heats and 12.5% (1/8) of the bitches at the 20th day of the heats. 75% (6/8) of the bitches ovulated between 12th and the 15th day of the heats (Figures 7 and 8).

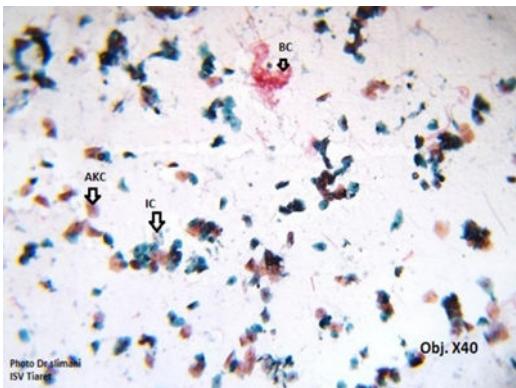


Figure 7: A view of a vaginal smear of proestrus (beginning) at magnification x40 which shows majority intermediate cells that become progressively acidophilic (cells undergoing keratinization), dirty bottom and presence of red blood cells in a smear stained with Harris Shorr. Eosinophilic index is greater than 40%. AKc: Acidophilic keratinized cells; Ic: large intermediate cells; Bc: blood cells.

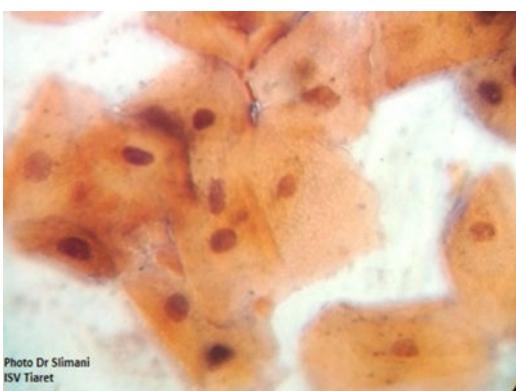


Figure 8: A view of a vaginal smear of estrus at magnification x100 which shows clusters of Large, very angular, acidophilic cells (keratinized cells) with small nucleus, ± pycnotic. Eosinophilic index is great than 60%.

Between the 5th day of proestrus and the middle of the estrus 87.5% (7/8) of the bitches showed an increasingly anechoic appearance of their ovaries with an irregular surface accompanied by an increase in their volume. Except for a bitch in this group who presented an anovulatory cycle (Figure 9).



Figure 9: Longitudinal echographic view of the ovary in pre-ovulatory period showing parenchyma of homogeneous texture dominated by a hypoechoegenous ovarian stroma and presence of a small hypoechoic cavity (pre-ovulatory follicles). This echographic aspect indicates the presence of a follicular activity related to an estrus. OV: ovary; F: follicle.

Discussion

The protocol of the study

First, the bitches included in the study were all breeds of atlas shepherds dog. This choice is based on the fact that there is little or no study concerning the reproduction in this race of dog in Algeria.

However, our sample seems restricted (8 bitches) and is certainly not representative of the canine population. During this study 80 blades were collected, adjustments had to be made. Smears were eliminated due to coloring problem and some due to defective swab. Some bitches have been agitated even aggressive which makes handling difficult. These two facts led us to eliminate 19 blades. The unavailability of necessary equipment for the proportioning of progesterone in our institute we have to realize this proportioning in a private laboratory.

The resolution of the ultrasound screen used for visualization of the ovaries did not allow to properly observing the ovarian follicle, so we referred to the determination of the diameter of the ovary in its entirety rather than to determine that of the ovulatory follicle.

Duration of heat and time of ovulation

Vaginal cytology (Figure 1) revealed small clusters of parabasal cells with absences of red blood cells and leukocyte cells which indicated an anoestrus for all the bitches on smear read using a magnification of x100 (Figure 2). However the clinical examination showed that the bitches were in good health. The smear of an anoestrus is poor in cells [9]. Parabasal cells and small intermediate cells are predominant [2,10].

Ultrasound examination revealed no particular abnormality was detected on the ovaries. No anomalies in the uterus were detected before the start of treatment. During the anoestrus the ovaries have an echogenicity similar to that of the neighboring structures and the follicular structures are small, often less than 1 mm [11]. The ovaries are small and not very visible. They have a homogeneous echogenicity

similar to that of the renal cortex. However, at the end of the anoestrus, at 30 days or more from the beginning of the pro-estrus, the hypoechoic ovaries tend to increase and small follicular cavities of one to two millimeters in diameter can be detected [12].

Blood level of the progesterone in the serum was less than on average 2 nmol/l which confirmed the presence of an anoestrus for all bitches.

The induction of heat in a bitch is realized in the case of impubérisme for 24 months or in the case of absence of heat for more than 10 months. The use of dopaminergic substances is recommended as a first-line treatment for pathological anoestrus (>7 months interoestrus) [1].

Each bitches received the prescribed oral dose of 1.25 mg/15 kg body weight per day until the appearance of vulvar losses; Antiprogestins are dopaminergic agonists that inhibit the secretion of prolactin via dopamine secretion or suppression of serotonin secretion. The use of certain antiprogestins during pseudo-lactation decreases the inter-estral interval of the treated bitches [1,13,14]. The use of dopaminergic agonists in anticipation of estrus (less than 7 months after the last heat) is very disappointing [15].

During the treatment period the bitch were consulted every 15 days; this control included the realization of vaginal smears in order to detect any change in the cellular structure of the vaginal mucosa.

In the last 15 days before the onset of the heats of cell clusters of small intermediate and large intermediate cells appeared gradually on the smear, indicating clear changes in the vaginal mucosa that are evidence of the existence of ovarian rearrangement induced by the administered molecule (Figure 7).

Guerin et al. [16] report that vaginal smears are good indicators in the monitoring of the heats but are insufficient for the determination of the ovulatory period. Similarly Van Haaften et al. [17] points out that the eosinophilic index allows estrus to be well detected but not for ovulation.

In contrast to bromocriptine (Parlodel®, dopaminergic molecule) or cabergoline (Galastop®, an anti-serotonergic and dopaminergic molecule), metterolol (Contralac®, an anti-serotonergic molecule) does not induce estrus. The central dopaminergic effect of antiprogestins that seems to act favorably during this heat-inducing effect. Their efficacy in the onset of estrus depends on dose, duration of treatment [1,13,14].

We have seen the appearance of vulvar loss before the bitch is in heat at different times of treatment as well 25% (2/8) bitches showed heat after 21 days of treatment. 37.5% (3/8) showed heat after 35 days. 37.5% (3/8) showed heat after 40 days of treatment, with an average of 32 +/- 10 days (Figure 3). Proestrus and estrus were confirmed by vaginal cytology and the increase in the eosinophilic index measured during heat monitoring was consistent with the manifestation of the heat (Figure 8).

Dumon [18] reports that heat is obtained after 30 to 40 days of treatment with bromocriptine (Parlodel®) 20 µg/kg twice day. Their effectiveness in the onset of estrus depends on the dose, the duration of the treatment and the anoestrus stage.

The two most widely used anti-progestin are cabergoline at the dose of 5 µg/kg/day until proestrus is obtained (usually for 3 weeks) and bromocriptine 20-50 µg/kg twice daily until postestrus was obtained (usually for 4 weeks) [1,13,18].

A positive chronological correlation was observed between the eosinophilic index and the increase in the diameter of the ovaries during the period located between the 5th day of the proestrus and the middle of the estrus which coincides with the period of ovulation (Figure 4).

The diameter of the ovaries was equal to 19 ± 2.07 mm and corresponded to an eosinophilic index equal to or higher than 80% in 100% (8/8) of the bitches at the time of ovulation (Figure 9).

A positively correlation was observed between the progression of the progesterone level and the eosinophilic index (EI) during the period located between the 5th day of the proestrus and the middle of the estrus which coincides with the period of ovulation (Figure 5).

The progesterone level at the time of ovulation was located between 21.04 ± 14.53 nmol/L and 33.15 ± 15.16 nmol/L in 7 bitches (Figure 5). Except for one bitch which showed a stable progesterone level of 9 nmol/L during all the period of heats independently of the increase of the eosinophilic index.

England and Concannon [19] report that ovulation can occur between the 5th and 30th day after the beginning of the heat. The use of the vaginal smears must be supplemented by the determination of the level of progesterone [2,8].

Fontbonne and Malandin [20] show that the echographic examination on the ovaries increases only 10% the precision of detection of ovulation compared to the proportioning of progesterone. They also estimate that the detection of ovulation can be realized with an echograph of standard quality by practicing daily examination for the bitches of weight lower than 25 kg. On the other hand for the large or the obese bitches, it seems necessary to use an echograph of higher quality.

Marseloo et al. [21] reports that there is a good correlation between the values of progesterone and the echographic aspect of the ovaries during the follow-up of heats.

Vomiting was the only treatment-related side effect observed in five bitches, this symptom disappears after 10 days of treatment. Pierson and Samuel [1] report that the most observed side effects are vomiting occur in 3 to 25% following treatment with anti-progestins.

On the other hand, we did not observe cases of hair discoloration related to the use of bromocriptine, in contrast to cabergoline, which induces transient skin discoloration in 25% of bitches that receive this molecule for 14 to 45 days [1,14].

Ovulations occurred between the 12th and the 15th day of heats. 75% (6/8) of the bitches ovulated between 12th and the 15th day of the heats on average on the 13, 5th day of the heats. The bitches ovulate on average on the 12th day of the heats [17].

12.5% (1/8) of the bitches ovulated at the 20th day of the heats. In a study on bitches of all races (number not determined) only 13.3% of bitches ovulate between the 16th and the 25th of heats [22]. There is variability in the timing of ovulation from one bitch to another and from one cycle to another for the same bitch [23].

Van Haaften et al. [17] report an interval of 6 to 21 days of the time of ovulation. England and Concannon [19] report that ovulation can occur between the 5th and 30th day after the beginning of the heat. This variability was observed in our bitches with an interval of ovulation of 12th to 20th day of heats.

We did not observe a significant difference between the evolution of the eosinophilic index and that of the progesterone level during the heat, except for a bitch who presented a stable progesterone level between 8.78 nmol/L and 9 nmol/L for all the period of heats despite significant evolution of its eosinophilic index and the diameter of its ovaries which indicated the approach of ovulation.

This leads us to conclude that the eosinophilic index as well as the diameter of the ovaries are a good witnesses for the determination of the phases of heat but not of the ovulation.

Several authors confirm this observation, England and Concannon [19] report that one can have an eosinophilic index near to 100% and are between 9 and 2 days before the ovulation. In some breeds, the smears obtained are never characteristic of an estrus. In other races smears characteristic of the estrus are often obtained very early during heats where as the bitch is far from being ready to ovulate [24].

Guerin et al. [16] report that vaginal smears are good indicators in the monitoring of the heats but are insufficient for the determination of the ovulatory period. Similarly Van Haaften et al. [17] points out that the eosinophilic index allows estrus to be well detected but not for ovulation.

It was easy to follow accurately the evolution of the diameter of the ovaries during proestrus and oestrus, however, the echographic distinction of pre-ovulatory follicles was difficult. The same goes for the differentiation between the preovulatory and post-ovulatory phase. It can sometimes be difficult to distinguish the pre-ovulatory follicles and the *corpus luteum* [12,25]. Of this fact it is necessary to combine these two techniques with the proportioning of progesterone. The use of the vaginal smears must be supplemented by the determination of the level of progesterone [8].

It is important to note that the size and the weight of some bitches have made the localization of the ovaries difficult during the echographic examinations adding to that the quality of the echographic image which did not make it possible to visualize the follicles clearly. This fact we were obliged this to concentrate on the determination of the size and the echographic aspect of the ovaries. During this study we determined the moment of ovulation when the great anechoic spherical forms disappeared and are replaced by small structures which return the ovary less hypoechoic than during the pre-ovulatory period (Figure 9). Concannon et al. [26] recommends to use linear probe or sectoral with a high frequency (8 to 10 MHz) to visualize the change of the ovary during ovulation.

Fontbonne and Malandin [20] note that in some cases the disappearance of the follicular cavities does not observed. They show that the echographic examination on the ovaries increases only 10% the precision of detection of ovulation compared to the proportioning of progesterone. They also estimate that the detection of ovulation can be realized with an echograph of standard quality by practicing daily examination for the bitches of weight lower than 25 kg. On the other hand for the large or the obese bitches, it seems necessary to use an echograph of higher quality. Hayer et al. [25] note that echography is useful only to follow the development of the follicles and the corpus luteum but at the time of ovulation there are very rarely clear and significant modifications of the ultrasound images.

Cell populations observed on vaginal smears perfectly matched the heat phase in all our bitches. All our bitches had appositive chronological evolution of the eosinophilic index and diameter of the ovaries. Guerin [27] indicates the existence of differences between the

cervical smears and the real stage of the cycle of the bitch. In another study Guerin [27] reports that the cervical smears are good indicators of the heat but insufficient for the determination of the ovulatory period. Van Haaften et al. [17] specify that the eosinophilic index makes it possible estrus to be well detected but not ovulation.

According to Hewitt and England [28] there are usually two peaks of keratinization during heats. This has not been observed in our bitches. All our bitches presented during the estrus an evolution of the eosinophilic index similar to the observations of Guerin [27] who reports that the index is from 20 to 50% the first day of the estrus and it becomes higher than 50% during the second day.

The moment of expression of the peak of the eosinophilic index was variable from one bitch to another. 75% (6/8) expressed this peak between the 12th and 15th day of the heats and 12.5% (1/8) of the bitches at the 10th day of the heats and 12.5% (1/8) of the bitches at the 20th day of the heats. In a study, Schutte [6] reports that 89% of bitches expressed this peak at the 14ème day of cycle. 43% presented this peak at the 10th day of the cycle. This peak of the eosinophilic index appears between 8th and the 12th day of the cycle [7].

Determination of the progesterone level

We took as a threshold a progesterone level of 23 nmol/L which indicate an ovulation. The progesterone level at the time of ovulation in 7 bitches was between 21.04 +/- 14.53 nmol/L and 33.15 +/- 15.16 nmol/L (Figures 5 and 6). At the time of ovulation, progesterone levels significantly increased from 4 to 10 ng/ml (12.72 nmol/L to 31.8 nmol/L) [24]. Progesterone level at the time of ovulation increased from 5 to 10 ng/ml (15.9 nmol/L to 31.8 nmol/L) [28].

In a study on 35 bitches of various races Marseloo et al. [28] reports that the progesterone at the day of ovulation was at constant levels 6.25 +/- 1.55 ng/mL. This progesterone level is of 7.52 ± 0.33 (or 23.91 nmol/L +/- 1.04 nmol/L) [29]. It is 6 to 8 ng/ml (or 19.08 nmol/L to 25.44 nmol/L) [30]. This rate is 7 to 9 ng/ml (or 22.26 nmol/L to 28.62 nmol/L) [31]. We note that all these values are therefore concordant so ovulation may be considered to have occurred when the maximum threshold value has been exceeded. We also noted that bitches had a clear individual variation of the progesterone level during the proestrus until ovulation. Buff and Salesse [32] report that there exist very great individual variations of the progesterone level in the bitches.

87.5% (7/8) of the bitches of this Group presented a positive chronological evolution of the level of progesterone with the increase of the eosinophilic index and de increase of the Ovary diameter during the heat until the day of ovulation (Figure 5). Marseloo et al. [21] reports that there is a good correlation between the values of progesterone and the echographic aspect of the ovaries during the follow-up of heats.

87.5% (7/8) of the bitches showed a progesterone curve that corresponds to the standard curves of the canine species of all breeds [17,23].

One bitch in this group had unchanged levels of progesterone throughout the duration of the heats (8.44 nmol/L and 9 nmol/L), which corresponds to an anovulatory cycle. Guerin et al. [33] who mentions in some races that vaginal smears are systematically discordant with the progesterone dosage [34].

Conclusion

We were interested in our study to heat induction by the use of bromocriptine and the detection of ovulation using vaginal cytology, ovarian ultrasound and progesterone assay. So the results show that the use of bromocriptine to induce heat in the dog is possible and allows to obtain ovulatory after 3 weeks of treatment on average. The use of vaginal cytology and ultrasound of the ovaries remain techniques inseparable from the use of the progesterone assay when precisely locating the time of ovulation. We did not find any particularities regarding the manifestation of heat and ovulation that can divide this breed from other dog breeds.

Acknowledgments

My thanks goes to the assistants of Canine clinical Pathology, Institute of Veterinary Sciences, University Ibn Khaldoun of the carnivores for their contribution to realize this work.

Conflict of Interest

The author(s) declare(s) that there is no conflict of interest.

References

1. Pierson A, Buff S (2009) Induction of estrus in dogs and pussies: which molecule and which molecule and which protocol to use?. Colloquium AERA- House-Alfort.
2. Concannon PW (2011) Breeding cycles of the domestic bitch. Anim Repro Sci 124: 200-210.
3. Concannon PW (2000) Canine pregnancy: predicting parturition and timing events of gestation. In: Recent advances in Small Animal Reproduction. International Veterinary Information Service, Ithaca, NY.
4. England G, Yeager AE (1993) Ultrasonographic appearance of the ovary and uterus of the bitch during oestrus, ovulation and early pregnancy. J Repro Fert 47: 107-117.
5. Silva LDM, Onclin K, Verstegen JP (1996) Assessment of ovarian changes around ovulation in bitches by ultrasonography, laparoscopy and hormonal assays. Vet Radio Ultras 37: 313-320.
6. Schutte AP (1967) Canine vaginal cytology: I Technique and cytological morphology. J Small Anim Pract 8: 301-306.
7. Taradach C (1980) Vaginal smear in beagle dog. Determination of the ovulation period using the eosinophilic index. Med Vet Rev 131: 775-782.
8. Wright PJ (1990) Application of vaginal cytology and plasma progesterone determinations to the management of reproduction in the bitch. J Small Anim Pract 31: 335-340.
9. Neveux M (1999) The vaginal smears in the bitch. Item Vet 30: 557-564.
10. Concannon PW, Digregorio GB (1987) Canine vaginal cytology. In: Burke (ed). Small animal reproduction and infertility. Philadelphia, pp: 96-111.
11. Concannon PW, England G, Verstegen J, Forsberg CL (2002) Recent advances in small animal reproduction. International Veterinary Information Service, Ithaca, New York.
12. England G, Yeager A, Concannon PW (2003) Ultrasound imaging of the reproductive tract of the bitch. In: Recent Advances in Small Animal Reproduction. International Veterinary Information Service, Ithaca, NY.
13. Beijerink NJ, Dieleman SJ, Kooistra HS, Okkens AC (2003) Low doses of bromocriptine shorten the interestrous interval in the bitch without lowering plasma prolactin concentration. Theriogenology 60: 1379-1386.
14. Gier JD, Beijerink NJ, Kooistra HS, Okkens AC (2008) Physiology of the canine anoestrus and methods for manipulation of this length. Reprod Domest Anim 43: 157-164.
15. Fontbonne A, Levy X, Fontaine E, Gilson C (2008) Guide practice of canine and feline clinical reproduction. pp: 41-43.
16. Guerin C, Petit C, Badinand F (1996) Fecundity in dog after protrusion or artificial insemination: study on 202 bitches. Point Vet 28: 51-56.
17. Haafken BV, Dieleman SJ, Okkens AC (1989) Timing the mating of dogs on the basis of blood progesterone concentration. Vet Rec 125: 524-526.
18. Dumon C (1992) Vaginal smears in the bitch. In: Indispensable Reproduction, 7508 Paris, pp: 47-52.
19. England G, Concannon PW (2002) Determination of the optimal breeding time in the bitch: basic considerations. In: Recent Advances in Small Animal Reproduction. International Veterinary Information Service, Ithaca, New York, USA.
20. Fontbonne A, Malandain E (2006) Ovarian ultrasonography and follow-up of estrus in the bitch and queen. Waltham Focus 16: 22-29.
21. Marseloo N, Fontbonne A, Bassu G (2004) Comparison of ovarian ultrasonography with hormonal parameters for the determination of the time of ovulation in bitches. In: Proceedings of the 5th International Symposium on Canine and Feline Reproduction pp: 75-77.
22. Doucet F, Vannimenus C (2001) Contribution to the study of the reproduction in the bitch: analysis of the files of the bitches followed at the study center in canine breeding assisted by the National Veterinary School of Alfort 1994 to 1998. Méd Vét Alfort Thesis: n°38.
23. Badinand F, Fontbonne A, Maurel MC (1993) Fertilization time in the bitch in relation to plasma concentration of oestradiol, progesterone and LH and vaginal smears. J Reprod Fertil Suppl 47: 63-67.
24. Fontbonne A (1993) Monitoring of heat and gestation: diagnostic approach in cases of reproductive and therapeutic disorders. In: Proceedings of the CNVSPA Congress, pp: 564-567.
25. Hayer P, Gunzel Appel AR, Luerssen D (1993) Ultrasonography monitoring of follicular development ovulation and the early luteal phase in the bitch. J Reprod Fertil Suppl 47: 93-100.
26. Concannon PW, Tsutsui T, Schille V (2001) Embryo development, hormonal requirements and maternal responses during canine pregnancy. J Reprod Fertil Suppl 57: 169-179.
27. Guerin C (1997) Artificial insemination in the canine species. Point Vét 28: 33-42.
28. Hewitt D, England G (2000) Assessment of optimal mating time in the bitch. In Practice 22: 24-33.
29. Wildt DE, Chakraborty PK, Panko WB (1979) Relationship of serum estrone, estradiol-17 β and progesterone to LH, sexual behavior and time of ovulation in the bitch. Biol Reprod 20: 648-658.
30. Jeffcoate IA, Lindsay FEF (1989) Ovulation detection and timing of insemination based on hormone concentrations, vaginal cytology and the endoscopic appearance of the vagina in domestic bitches. J Reprod Fertil Suppl 39: 277-287.
31. Coster RD, Beckers JF, Ballman PW (1979) Variations of LH, FSH, 17 β estradiol and progesterone during the oestrous cycle of the bitch. Ann Méd Vét 123: 177-184.
32. Buff S, Salesse H (2000) Heat tracking in a bitch. Point Vet 31: 341-344.
33. Fontbonne A (1996) The traps of vaginal smears in the bitch. Point Vét 28: 19-25.
34. Concannon PW, McCann JP, Temple M (1989) Biology and endocrinology of ovulation, pregnancy and parturition in the dog. J Reprod Fertil Suppl 39: 3-25.