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Estrus Induction in Belgian Malinois Dogs Using Pregnant Mare Serum Gonadotropin-Human Chorionic Gonadotropin Protocol

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Abstract

THE induction of estrus in females, particularly in valuable breeds such as the Belgian Malinois dog, is a practice increasingly adopted in veterinary medicine. The aim of this study was to reduce the duration of the anestrus phase using a protocol based on the administration of pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG). A total of one hundred and five multiparous bitches, with an average age of 3.41 ± 0.96 years and an average weight of (24.25 ± 4.39) kg Prior to induction, a gynecological and cytological examination was performed to confirm that the bitches were in anestrus state. Each bitch received a daily intramuscular injection of PMSG (Folligon®) at 500 IU for five days, followed on the fifth day by an injection of hCG (Endo 5000®) at 500 IU. Induced heat was observed by monitoring blood flow and vaginal smears. Ultrasound examination confirmed pregnancy at 4 weeks. The results showed that 81.3% of the females responded favorably to the induction treatment, with over 72.0% of them carrying a pregnancy to term. In addition, statistical analysis showed a significant correlation between the onset of heat after induction and the number of puppies per litter. However, the weight parameters and body condition score of the females showed no impact on the onset of heat. This study concludes that heat induction is an effective tool for optimizing reproduction in specialized Belgian Malinois Shepherd dog farms in Algeria.

Keywords: Dog, Belgian Malinois, heat induction, PMSG-hCG protocol, Algeria.

Introduction

The estrous cycle of the bitch is very different from that of other domestic species, in particular because of the spontaneous ovulation process and the variable duration of anestrus [1]. The bitch estrous cycle comprises the phases of proestrus, estrus, diestrus and anestrus, with irregular intervals that can complicate reproductive management, particularly in the context of breeding or disciplines requiring precise synchronization of heats. Anestrus, the phase during which the bitch shows no signs of heat, can last for several months and make it difficult to plan matings and breeding programs [2].

In order to overcome these obstacles, various hormonal protocols have been put in place to induce and synchronize estrus in the bitch. Among these, the most recent, such as the administration of cabergoline (CAB), a dopamine agonist, is a treatment commonly used to inhibit prolactin, a hormone involved in the maintenance of the lactation phase and ovulation, thus favoring the resumption of the estrous cycle [3]. The combination of CABand pregnant mare serum gonadotropin (PMSG) is particularly useful for inducing more rapid oestrus in anestrus bitches, thereby reducing the duration of induction [4]. The use of prostaglandin F2α (PGF2α)

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represents one of several pharmacological strategies that have been evaluated for inducing estrus in the bitch [5]. The combination of gonadotropin realizing hormone, PMSG and human chorionic gonadotropin (hCG) has been another widely studied treatment to induce oestrus and stimulate follicular growth. These gonadotropins, which mimic the action of luteinizing hormone (LH) and follicle stimulating hormone (FSH) [6].

Based on the collected data, the aim of this study is to evaluate the efficacy of a protocol combining PMSG and hCG to induce estrus in Belgian Shepherd Malinois bitches. The results of the present study, conducted on a larger sample, confirm the effectiveness of the treatment and support previous findings obtained from a much smaller sample. A study conducted in Algeria by Djoudi et al. (2023)[7], involving a small sample of eight bitches of different breeds and using the same protocol (PMSG followed by hCG), showed that 60% of the bitches exhibited signs of estrus 12 days after the start of treatment, compared to 20% on days 13 and 14. While these findings support the efficacy of the hormonal protocol, the limited sample size highlights the need for further investigation. In this context, the current study offers added value by expanding the sample size, refining estrus detection criteria, and potentially improving the timing of hormonal administration. It may therefore be considered as a preliminary or pilot step towards optimizing estrus induction protocols in bitches under field conditions. This study involved a population of ten bitches of various breeds—including German Shepherd, Rottweiler, and Pitbull—aged between 2.5 and 4.5 years, allowing for more robust observations and greater breed diversity compared to previous studies

The Belgian Malinois, due to its specific physiological characteristics and its frequent use in contexts requiring precise reproductive management (e.g., working dogs, competition), represents an excellent model for assessing the performance of hormonal treatments.

Material and Methods

Animals

One hundred and five (105) Belgian Berger Malinois bitches aged between 2 and 6 years with regular estrous cycles and an average body weight (24.25 ± 4.39 kg) were selected for this experiment. The bitches were observed regularly for 20 days. The study was conducted in the wilaya of Blida, Algeria.

Methods

At each visit, detailed information was collected using data sheets including the history, physiological stage of the females and number of parameters (age, weight, identification number, body condition score of the animal).

Weight and body condition score (BCS)

The weighing technique was based on a BLET-type balance. All animals were dewormed and vaccinated. The body condition score (BCS)was estimated by observing the morphology of each bitch prior to treatment using the 1 to 9 scoring system described by [8].

Vaginal smears

A total number of 315 vaginal smears were taken, with each bitch being examined every five days. The protocol included one smear to confirm the anestrus phase, followed by two further smears post-induction. The technique consisted of lifting the bitch's tail, then taking samples from the vaginal walls using a swab soaked in physiological water, while taking care to avoid any contamination. Sampling is carried out using a gentle, rotating rubbing motion, ensuring adequate harvesting of epithelial cells. The sample taken is immediately transferred to a glass slide in preparation for the smear. After freeze-drying, the slides are ready for cytological analysis using the May-Grunwald Giemsa staining technique. This method, which uses acidic (eosin) and basic (methylene blue) stains, allows precise observation of the cells under the microscope at 400 x magnification, providing information on the different phases of the estrous cycle [9].

*Hormonal Protocol for Estrus and Ovulation Induction in Bitches (Fig. 1)***:**

This protocol is designed to induce estrus in anestrous or acyclic bitches by stimulating follicular development followed by ovulation, with the ultimate goal of achieving pregnancy.

From Day 1 to Day 4, a daily injection of PMSG (Pregnant Mare Serum Gonadotropin), is administered. This hormone has FSH-like activity, promoting the recruitment and maturation of ovarian follicles.

On Day 5**,** a combined injection of PMSG (to maintain follicular stimulation) and h**CG** (human Chorionic Gonadotropin) is given. The hCG**,** with its LH-like activity, triggers ovulation of the previously developed follicles.

This protocol mimics the physiological role of endogenous gonadotropins, by this means synchronizing the reproductive cycle and facilitating subsequent fertilization and pregnancy [9]

Signs of estrus were observed based on bleeding, vulval swelling and male attraction. The bitches were then presented to adult males for natural mating. The number of puppies was counted for each female after whelping.

Diagnosis of pregnancy

After the females had been inseminated by 21 days, pregnancy was diagnosed by transabdominal ultrasound using a Mindray dp 10 convex probe 35C50EB 7MHz ultrasound system.

Statistical analysis

The data collected were analyzed using XLSTAT software (vers. 2016.02.28451). Quantitative variables, means, standard deviations and extreme values were calculated for the 105 bitches, according to age and weight. A correlation analysis was performed between age, weight, body score condition and onset of heat. The error level used was 5%. Data presented as (Mean ± SD)

Results

Vaginal smears

Vaginal smears taken during the experiment using MGG monochrome staining showed a dirty smear with a low cell count (parabasal cells with large nuclei) during the anestrus phase. The rate of keratinization is estimated at 10%. The estrus smear is clean, with superficial anucleated cells grouped in clusters. The nucleus condenses in the center of the cell. Keratinization of superficial cells, estimated at 80% (Fig. 2).

Sixty-two out of 85 females showed heat after induction treatment were diagnosed as pregnant *Descriptive analysis and gynaecological exam*

The gynaecological examination, which is fundamental for assessing the physiological state of bitches during the reproductive period, was based mainly on two signs: swelling of the vulva and the presence of vulval blood secretions. These signs are essential in determining the onset of estrus, the first day of bleeding being defined as the first day of estrus. This indicates not only responsiveness to treatment, but also optimal reproductive management for older bitches.

Descriptive statistics for the BSC revealed a mean score of 5.3±1.

The study revealed significant variation in heat matching after induction treatment, with an average of 19.7 days. Descriptive analysis of the number of puppies per litter (Table 1) showed that females had an average of 5 puppies per litter.

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The present results showed that the 3–4-years-old age group is more receptive to induction treatment.

It is remarkable to note that in the population studied, more than 80% (Table 2) of the females were observed in heat after induction.

Pregnancy diagnosis

The results of the pregnancy diagnosis showed that 72.9% were pregnant.

Analysis of correlations between the beginning of hormonal treatment and age, weight, body condition score of the animal)

The correlation analysis was used to identify the relationships between several parameters—namely (table 3), the number of days after treatment, body condition score, weight, and age—and the onset of heat in 105 bitches. The results revealed a highly significant correlation between the number of days after the start of treatment and the onset of heat ($r = 0.899$, $p < 0.001$), indicating that this parameter is strongly associated with the hormonal treatment response. A significant but weaker correlation was observed between body condition score and the onset of heat ($r = 0.203$, $p < 0.05$), suggesting that nutritional status may also influence the hormonal response. In contrast, body weight showed no significant correlation with the onset of heat ($r = 0.068$, $p > 0.05$), indicating that it does not have a notable impact in this context. Finally, age showed a slight negative correlation with the onset of heat ($r = -0.181$), close to the threshold of significance ($p < 0.1$), which may suggest a tendency for a weaker response in older bitches

Discussion

Reproductive management in dogs is often challenging due to irregular estrous cycles and there is a need to synchronize mating to optimize genetic potential [10]. Estrus induction can be achieved through various hormonal approaches, including the use of equine chorionic gonadotropin (eCG, also known as PMSG) and hCG [11].

PMSG/eCG possesses dual activity similar to both FSH and LH. When administered to bitches in the anestrus phase, it promotes follicular development and the onset of estrus [11]. In contrast, hCG primarily exhibits LH-like activity, triggering ovulation of the mature follicles induced by prior PMSG stimulation. For this reason, hCG is frequently used as a complement to PMSG in order to ensure ovulation and improve fertility outcomes [12].

The response to the hormonal treatment was related to the average body weight and age of the used bitches as previously reported [7]. Several studies on estrus induction in bitches have highlighted significant variability in age and weight, two key factors that can influence hormonal response and protocol effectiveness. For instance, Tani et al. (2016) [13] included relatively young bitches (aged

1.5 to 3 years) of two breeds (French Bulldog and English Bulldog), all in good health-conditions that may positively affect the hormonal response. Conversely, the use of CAB in older bitches (aged 4 to 11 years) may be associated with prolonged anestrus, potentially compromising treatment efficacy [5]. Body weight also plays an important role in hormonal treatment response. The study by Tani et al. (2016) [13]is notable for the homogeneity in body weight (between 9.6 and 20 kg depending on breed), which allowed for standardized dosing and minimized the risk of under- or overdosing. Similarly, Kutzer (2020) [14] used a GnRH agonist (buserelin) with a dosage adjusted to body weight, taking into an account individual metabolic variability that may influence drug action.

Body condition score is a key metric in evaluating reproductive potential. A previous study [14] had shown a direct correlation between BCS and reproductive capacity. Poor BCS often leads to decreased ovulation rates, irregular cycles, or even complete reproductive failure.

In our study, the average BCS was $5.3 \pm 1.$-, indicating moderate variability among individuals. A significant correlation ($P < 0.05$) was found between BCS and the onset of estrus, suggesting that BCS may have a slight but noteworthy influence on receptivity to hormonal treatment. Though not a dominant factor, BCS merits consideration in reproductive planning.

Vaginal smears taken during anestrus and after induction showed cytological changes characteristic of the onset of estrus. These findings are consistent with a previous study [15]. Cytology revealed a progressive increase in superficial cells, indicating the transition from anestrus to estrus. After 14 days, 90% of cells were superficial [13]. another related study observed a similar progression from parabasal to cornified cells, with ≥80% indicating estrus [4].

Observable signs of estrus included vulvar swelling, vaginal bleeding, and male attraction. The average time from hormone administration to estrus onset was 19.7 ± 11.2 days. This interval varied across studies depending on the hormones and combinations used. For example, one study reported 27.7 ± 6.5 days for CAB, prolactin inhibitor, alone [3], while another combining PMSG and CAB found intervals of 30.0 ± 3.05 days, 7.67 ± 1.20 days, and 13.0 ± 1.20 days for CAB, PMSG, and CAB + PMSG combination, respectively [3]. In our study, treatment with eCG followed by hCG induced proestrus signs within 5–6 days in 7 out of 10 bitches, as previously reported [15]. Similarly, another study using deslorelin reported estrus onset in all treated bitches within six days [16].

Natural mating was performed using proven males, and pregnancy was confirmed in 62% of cases. According to Jaafar et al. (2018) [6], pregnancy rates were 80% in the GnRH-treated group and 75% in the PMSG/hCG group [17]. The average litter size observed was 6.1 ± 3 pups per bitch. Litter size was influenced by ovulation rate, fertility, and embryonic loss [18].

Correlation analyses revealed a strong negative relationship between the number of days after treatment and estrus onset ($r = -0.899$, $P < 0.001$), indicating a rapid response to PMSG and hCG. This confirms the efficacy of the protocol in synchronizing estrus. In contrast, age and weight showed weak and non-significant correlations with treatment response, suggesting these factors were not decisive in this cohort.

These findings emphasize the importance of considering BCS and hormonal protocol when managing reproduction in bitches, regardless of age or weight.

Conclusion

This study highlights the efficacy of a protocol combining PMSG and hCG to induce and synchronize heat in Belgian Herd Malinois bitches. The results show that over 80% of bitches respond positively to the treatment, with more than70% of pregnancies confirmed after mating. The hormonal approach therefore proves to be a reliable and effective method for optimizing reproductive management, facilitating mating planning and improving reproductive performance. These results underline the importance of paying particular attention to hormonal protocols, while taking into an account the BCS of the bitches to maximize the chances of reproductive success.

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No financial support was received for the conduct of this research.

Declaration of Conflict of Interest

The authors declare that they have no conflict of interest related to this publication.

TABLE 1. Descriptive statistics on age, weight, body score condition and number of offspring per litter.

Parameters studied	N	Min	Max	Mean	SD
Age (years)	105	2	6	3,41	0,961
Weight (Kg)		10	35	24,25	4,39
Body score (BSC)		1	9	5,3	1
Number of puppies/litters		0	10	6,1	3
Number of days on heat after the beginning of treatment (days)		12	60	19,7	11,2

Avg: mean; SD: standard deviation. Min: minimum; Max: maximum

TABLE 2. Relationship between age and onset of heat

Age (years)	N	Onset of heat		
		Negative	Positive	%p
2	17	7	10	9,5
3	46	6	40	38,1
4	29	6	23	23,9
5	13	1	12	11,4
Total	105	20	85	80,9

TABLE 3. Correlation analysis between the different parameters (weight, age, body score condition, number of days after treatment) and the onset of heat.

Parameteres (N = 105)	Heat		*P*
	N Heat	P Heat	
Number of days on heat after the beginning of treatment (days)	0,899	-0,899	*<0.001*
Body score	0,203	-0,203	*<0.05*
Weight (Kg)	0,068	-0,068	*> 0.05*
Age (years)	-0,181	0,181	*< 0.1*

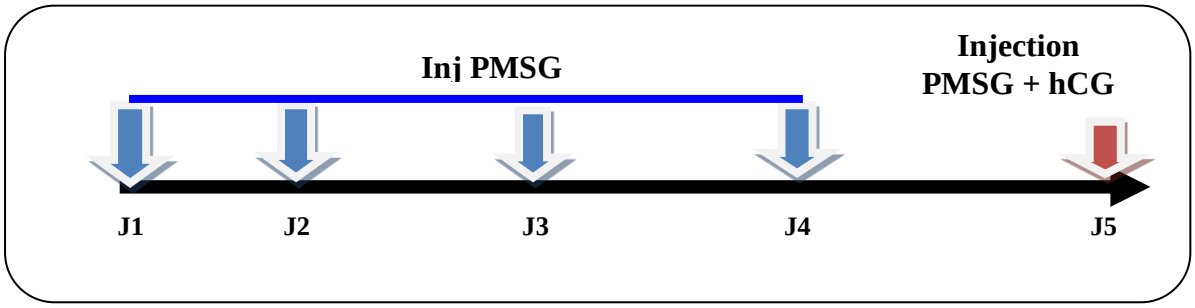
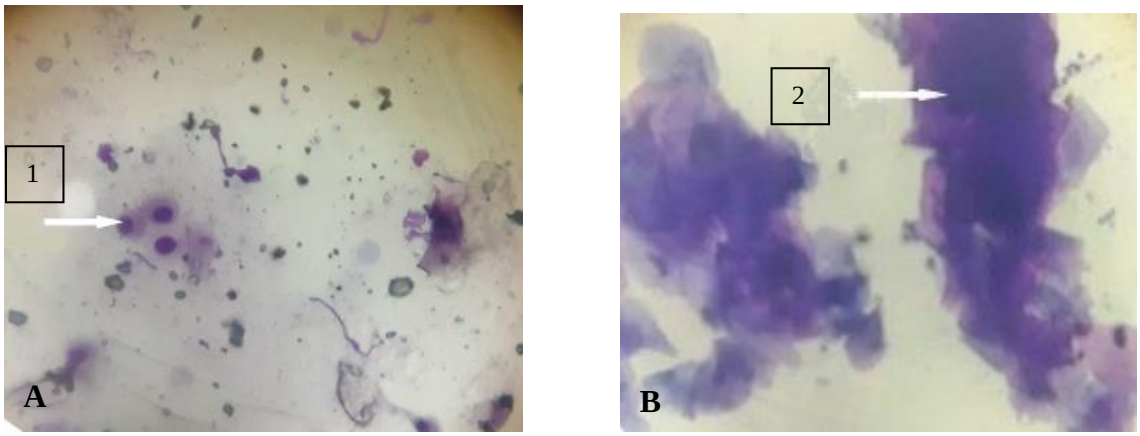


Fig. 1. Estrus Induction Protocol Based on PMSG and hCG Injections



A: Anestrus phase **B: estrus phase**

Fig.2. Morphological Aspects of Vaginal Smears After May-Grünwald Giemsa Staining (400×)

Showing: A-: parabasal cells (no keratinisation) B: Picknose nucleus (keratinisation phase)

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التحفيز الاصطناعي للشبق في كلاب المالينوا البلجيكية باستخدام بروتوكول مصل الفرس الحامل (PMSG) وهرمون موجهة الغدد التناسلية المشيمية البشرية (hCG)

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الملخص

يُعتبر تحفيز الشبق لدى كلاب الإناث، لا سيما في السلالات ذات القيمة العالية مثل كلاب المالينوا البلجيكية، ممارسة متزايدة الاستخدام في الطب البيطري. تهدف هذه الدراسة إلى تقصير مدة مرحلة السكون الجنسي (الأنستروس) باستخدام بروتوكول يعتمد على إعطاء مصل الفرس الحامل (PMSG) وهرمون موجهة الغدد التناسلية المشيمية البشرية .(hCG) شملت الدراسة 105 إناث متعددة الولادات، بمتوسط عمر قدره 3.41 ± 0.96 سنة ووزن متوسط بلغ (24.25 ± 4.39) كغ. قبل بدء التحفيز، تم إجراء فحص نسائي وسيتولوجي للتأكد من أن الإناث في حالة أنستروس. تم حقن كل أنثى يوميًا بحقنة عضلية من (®Folligon) PMSG بجرعة 500 وحدة دولية لمدة خمسة أيام، تلاها في اليوم الخامس حقنة واحدة من (®Endo 5000) hCG بجرعة 500 وحدة دولية. تم رصد ظهور الشبق من خلال مراقبة الإفرازات الدموية والمسحات المهبلية، كما تم تأكيد الحمل باستخدام الفحص بالأمواج فوق الصوتية بعد 4 أسابيع. أظهرت النتائج أن 81.3٪ من الإناث استجبن بشكل إيجابي للعلاج، حيث حملت أكثر من 72.0٪ منهن حتى الولادة. كما بين التحليل الإحصائي وجود علاقة ذات دلالة إحصائية بين بداية الشبق بعد التحفيز وعدد الجراء في كل ولادة. في المقابل، لم تُظهر معايير الوزن وتقييم الحالة البدنية كلاب للإناث أي تأثير على بداية الشبق. وتخلص الدراسة إلى أن تحفيز الشبق يعد أداة فعالة لتحسين التكاثر في مزارع تربية كلاب الراعي المالينوا البلجيكي المتخصصة في الجزائر.

الكلمات المفتاحية :الكلب، المالينوا البلجيكي، تحفيز الشبق، بروتوكول PMSG-hCG الجزائر.