

Submitted: 26/02/2025

Revised: 06/05/2025

Accepted: 11/05/2025

Published: 30/06/2025

Luteal insufficiency in canines: Assessment of progesterone dynamics and efficacy of combined hormonal treatment

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ABSTRACT

Background: Progesterone, primarily produced by the corpus luteum, is essential for maintaining pregnancy in dogs by promoting endometrial receptivity and supporting fetal development. Hypoluteoidism, defined as inadequate progesterone production, significantly increases the risk of pregnancy loss and reproductive failure. This study evaluated the role of progesterone in canine pregnancy, the risks associated with hormonal deficiency, and the effectiveness of therapeutic interventions.

Aim: This study emphasized the role of progesterone supplementation combined with magnesium and tocopherol in supporting pregnancy maintenance and restoring hormonal homeostasis.

Methods: Progesterone levels were monitored across the estrous cycle in French Bulldog bitches ($n = 20$). The control group was divided into two subsets. In the first stage of the study, control group C1 ($n = 10$) consisted of dogs in the anestrus phase. In the second stage, control group C2 ($n = 10$) included dogs on days 18–22 of pregnancy. Animals in both control groups (C1 and C2) did not receive any pharmacological treatment throughout the experiment. The experimental group (dogs with luteal insufficiency, days 18–22 of pregnancy; $n = 10$) exhibited significantly lower serum progesterone levels (9.90 ± 0.19 nmol/l) compared with the control group (55.60 ± 2.23 nmol/l, $p < 0.001$). Serum progesterone levels were measured using the Immulite® system (Siemens Health Care Diagnostics GmbH, Eschborn, Germany). The experimental group received treatment with exogenous progesterone [medroxyprogesterone acetate (MPA), Perlutex® 5 mg, Dechra, UK], administered orally at a dose of 0.1 mg/kg/day. The treatment was combined with tocopherol acetate (2.0 ml, 500 IU) for 21 days and MagiCalm® (1 tablet per 10–25 kg/day for 10–12 days). Blood sampling for hematological and hormonal analysis was conducted as follows: for the control group on gestational days 18–22. In the experimental group (luteal insufficiency), samples were collected on the day treatment was initiated (day 0; gestational days 18–22, prior to treatment initiation), and subsequently on treatment days 5 (23–27 days– 20.03 ± 0.75 nmol/l; $p < 0.001$), 10 (33–37 days– 37.76 ± 0.40 nmol/l; $p < 0.001$), 20 (43–47 days– 43.25 ± 0.57 nmol/l; $p < 0.001$), and 30 (53–57 days– 33.37 ± 0.87 ; $p < 0.001$).

Results: During the anestrus phase (control group C1), the progesterone concentration was minimal (1.53 ± 0.07 nmol/l). Progesterone levels showed significant variation across different phases of the estrous cycle, measuring 4.56 ± 0.20 nmol/l ($p < 0.001$) in proestrus, 19.54 ± 0.17 nmol/l in estrus ($p < 0.001$), and peaking at 37.61 ± 1.96 nmol/l ($p < 0.001$) in metestrus. On gestational days 18–22, healthy bitches in control group C2 exhibited a plasma progesterone concentration of 55.60 ± 2.23 nmol/l, indicating normal luteal function. In contrast, the experimental group (bitches with luteal insufficiency on days 18–22 of gestation) demonstrated a significant decrease in progesterone levels (9.95 ± 0.27 nmol/l; $p < 0.001$), accompanied by clinical signs such as restlessness, dark red vaginal discharge, and increased abdominal muscle tone. Throughout the treatment period, no critical clinical symptoms indicative of pregnancy loss were observed in animals with luteal deficiency. The clinical condition of all patients remained satisfactory. Pregnancies progressed with a dynamic normalization of plasma progesterone levels and culminated in normal, complication-free parturition.

Conclusion: Progesterone therapy, when combined with tocopherol and magnesium, effectively addresses luteal insufficiency in dogs, improving implantation success rates and reducing pregnancy loss. This study highlights the importance of progesterone in canine reproduction and supports its supplementation as a viable treatment for hormonal imbalance.

Keywords: Canine reproduction, Hormonal therapy, Hypoluteoidism, Luteal insufficiency, Progesterone.

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Introduction

Pregnancy is accompanied by a complex set of physiological, biochemical, and hormonal changes that affect various organs and systems. Hormonal homeostasis ensures fetal development throughout gestation (Kautz *et al.*, 2015; Feng *et al.*, 2017; Graubner *et al.*, 2020; De Geyter *et al.*, 2024). Among the key regulators of pregnancy is the steroid hormone progesterone, which plays a central role in initiating pregnancy, maintaining gestation, and creating optimal conditions for embryonic development (Kautz *et al.*, 2014; Graubner *et al.*, 2017; Kowalewski *et al.*, 2020b). Primarily synthesized by the corpus luteum and to a lesser extent in the uterus, progesterone regulates numerous biological functions. It induces changes in the uterine lining, preparing it for implantation and fetal growth, and stimulates mammary gland development (Ren *et al.*, 2019; Zhelavskiy, 2021).

While its essential role in pregnancy has been well established, current studies continue to investigate optimal concentration thresholds and their impact on outcomes. Understanding these levels is crucial for managing infertility, recurrent pregnancy loss, and other complications linked to hormonal imbalances (Krachudel *et al.*, 2013; Salazar *et al.*, 2016; Graubner *et al.*, 2018; Zhelavskiy *et al.*, 2020; Kowalewski *et al.*, 2021a).

Veterinary research highlights that a serum progesterone level above 15.9 nmol/l after day 30 of gestation is critical for sustaining pregnancy (Hinderer *et al.*, 2021). However, successful pregnancies have been observed below this threshold, suggesting that compensatory mechanisms may support gestation under suboptimal conditions (Nowak *et al.*, 2017; Kowalik *et al.*, 2019; Pecci and Marengo, 2021; Zhelavskiy *et al.*, 2024a).

This variability underscores progesterone's dynamic role in the reproductive system. It is involved in endometrial preparation, gestational support, and pregnancy stability (Friel *et al.*, 2015; Tavares *et al.*, 2022a). Its interaction with other hormonal and cellular factors maintains a delicate balance essential for reproduction. Clarifying these interactions through ongoing research is vital for developing targeted reproductive therapies (Salazar *et al.*, 2016; Graubner *et al.*, 2018).

Additionally, factors such as immune, metabolic, and placental adaptations may contribute to gestational maintenance despite low progesterone levels. This highlights significant gaps in our understanding of its mechanisms and thresholds, stressing the need for further study (Friel *et al.*, 2015; Garrido-Gomez *et al.*, 2020; Diessler *et al.*, 2023).

Greater insight could improve diagnostic and therapeutic approaches, enhance pregnancy outcomes, and address hormonal imbalance-related complications. This study was motivated by ongoing clinical challenges related to luteal insufficiency (hypoluteidism) in canine pregnancy (Granger *et al.*, 2018; Lyzikova *et al.*, 2020;

Kowalewski *et al.*, 2021b; Kazemian *et al.*, 2023). Inadequate progesterone levels in the corpus luteum increase the risk of early embryonic loss, preterm labor, and pregnancy termination.

Overcoming these challenges requires accurate diagnostics and precise therapeutic interventions (McElrath *et al.*, 2020; Zhelavskiy *et al.*, 2023a). Advancing these protocols is essential for reducing progesterone-related risks and improving reproductive management (Kaluka *et al.*, 2015; Guo *et al.*, 2016; Ives *et al.*, 2020; Kazemian *et al.*, 2023).

Tocopherol plays an important role in reproductive health. Hormonal imbalances, exacerbated by free radicals (reactive oxygen species), can damage reproductive tissues, including gametes and the endometrium. Antioxidants like tocopherol neutralize free radicals, maintain cellular balance, and support fertility (Tan *et al.*, 2019; Shastak *et al.*, 2023).

It is hypothesized that monitoring and managing progesterone levels can mitigate the risks associated with luteal insufficiency, thereby ensuring successful pregnancy outcomes. This study emphasizes the role of progesterone supplementation combined with magnesium and tocopherol in supporting pregnancy maintenance and restoring hormonal homeostasis.

By addressing these objectives, this study aimed to enhance the management of pregnancy in veterinary practice and reduce associated risks.

Materials and Methods

The control group was divided into two subsets. In the first stage of the study, control group C1 ($n = 10$) consisted of dogs in the anestrus phase. In the second stage, control group C2 ($n = 10$) included dogs on days 18–22 of pregnancy. Animals in both control groups (C1 and C2) did not receive any pharmacological treatment throughout the experiment. The subjects were French Bulldog bitches aged 3–5 years, weighing 9.5–11.2 kg. The research was conducted at a veterinary clinic in Kamianets-Podilskyi, Ukraine, between 2021 and 2025. The animals were subjected to regular deworming (Drontal Plus, Bayer HealthCare LLC, Germany) and vaccination with combination vaccines (Nobivac® DHPP, Merck & Co., Inc., Rahway, NJ). Before breeding, the bitches underwent a breeding evaluation, including an examination of external genitalia, vaginoscopy, vaginal cytology, bacterial culture, ultrasound of the uterus and ovaries, and serological testing [detection of Canine Herpesvirus (CHV, Qiagen, Germany) and Mycoplasma (IDEXX Mycoplasma PCR, USA)].

Clinical and laboratory examinations

Clinical and laboratory examinations were conducted during anestrus, proestrus, estrus, and metestrus. Treatment was started on the day of pregnancy on the 18th–21st day of pregnancy (day 0 of treatment). The criteria for diagnosis were the detection of vaginal discharge, increased abdominal tone, and determination

of progesterone ($P4\ 9.95 \pm 0.27\ \text{nmol/l}$). Blood samples were collected during the first phase of the study at various stages of the reproductive cycle: anestrus (day 3), proestrus (day 2), estrus (day 3), and metestrus (day 3). In the second phase, samples were collected on the day of treatment initiation (day 0, corresponding to days 18–22 of gestation) and subsequently on days 5 (gestation days 22–27), 10 (33–37), 20 (43–47), and 30 (53–57) of treatment. Progesterone concentrations were measured using Immulite® (Siemens Health Care Diagnostics GmbH, Eschborn, Germany). Ovulation was based on serum progesterone levels of $15.9\text{--}25.44\ \text{nmol/l}$ ($5\text{--}8\ \text{ng/ml}$). Bitches were mated once or twice within 1–3 days postovulation with a fertile male of the same breed. The first ultrasound for pregnancy diagnosis was conducted 22 days postovulation (Concannon, 2011).

Laboratory research methods

At the initiation of the study, a blood sample volume of 50 ml was collected from each subject via the cephalic vein (v. *cephalica*), following strict aseptic techniques to prevent contamination and ensure sample integrity. The blood samples were subjected to detailed biochemical profiling to evaluate a comprehensive range of physiological parameters. Hematological analysis included erythrocyte count, leukocyte count, leucogram values, hemoglobin concentration, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and thrombocyte count using the Abaxis Vetscan HM5 Hematology Analyzer (USA).

Furthermore, the concentration of progesterone ($P4$, nmol/l) was meticulously evaluated to monitor the reproductive status and hormonal dynamics (Conley et al., 2023). This was achieved using the Catalyst Dx Chemistry Analyzer (IDEXX Laboratories, Inc., USA), a reliable platform widely recognized for its precision in hormone assays (Constantin and Posastiuc, 2024). The microbiological assessment included the culture of meat peptone agar and meat peptone broth (Merck KGaA, Germany) to identify bacterial spectra.

Visual diagnostics

Ultrasound diagnostics were conducted using the Sonosite SonoSite 180 Plus (FUJIFILM Sonosite, USA) with a C60/5-2 transducer and Parker Laboratories Ultrasound Gels (USA). Radiography was performed using a Vet Ray Digital DR (Vet Ray Technology, by IDEXX, USA).

Differential diagnoses included abortion, vaginitis, pyometra, endometrial hyperplasia, endometritis, ovarian cysts, canine herpesvirus infection, mycoplasmosis, brucellosis, and vaginal/vulvar tumors, such as leiomyomas, fibromas, lipomas, and canine transmissible venereal tumors.

Treatment

MPA (Perlutex® 5 mg, Dechra, UK) was administered to stabilize pregnancy. We used blood progesterone

levels of $9.90 \pm 0.19\ \text{nmol/l}$ (day 0). The medication was given orally at a dose of $0.1\ \text{mg/kg}$ body weight once daily (Romagnoli et al., 2024). Treatment was initiated on the day of luteal insufficiency detection. Treatment initiation and dose adjustment were based on laboratory analysis of blood progesterone levels (experimental group). Subsequently, from day 48 of pregnancy, the dose was gradually reduced ($0.05\text{--}0.025\ \text{mg/kg}$) over the final week of treatment, concluding by day 52 of pregnancy. Dogs in the experimental group were orally administered tocopherol acetate (vitamin E for dogs, Ivitamins, USA) at a dose of 2.0 ml (500 IU) for 21 days. The complex supplement MagiCalm® (Healthspan, Holistic Pet Organics, USA) was used to reduce uterine and abdominal muscle tone. The formulation contained magnesium (magnesium citrate) 200 mg, taurine 50 mg, valerian extract 30 mg, and vitamin B₆ (pyridoxine) 5 mg. The dosage was one tablet per 10–25 kg body weight, administered orally once daily for 10–12 days until muscle tone normalized (Humphrey et al., 2015).

Statistical analysis

Results were expressed as mean $\pm$ SD to summarize variability within each dataset (MANOVA). A p -value of less than 0.05 was used as the criterion for statistical significance, ensuring a reliable interpretation of the data. All computations and analyses were conducted using Statistica® 12.6 software (StatSoft, USA), a comprehensive tool for statistical and data visualization tasks.

Ethical approval

The clinical investigations were carried out in compliance with the Law of Ukraine «On Protection of Animals from Cruel Treatment» (21/02/2006, 3447-IV) and followed the European Commission's guidelines on the treatment of vertebrates, ensuring protection from thirst, hunger, malnutrition, discomfort, fear, pain, and suffering. All studies adhered to bioethics standards, with written owner consent, local animal protection guidelines, and national legislation.

Results

Progesterone concentrations varied significantly depending on the stage of the estrous cycle (Table 1). During anestrus, baseline progesterone levels were measured at $1.53 \pm 0.07\ \text{nmol/l}$. In the proestrus phase, progesterone concentrations gradually increased, reaching approximately $4.56 \pm 0.20\ \text{nmol/l}$ in peripheral blood ($p < 0.01$).

During estrus (3-day stage), a significant rise in progesterone levels was observed ($19.54 \pm 0.17\ \text{nmol/l}$; $p < 0.01$), indicating the onset of luteal activity after ovulation. At this point, the corpus luteum begins to actively synthesize progesterone to support the early phases of a potential pregnancy. The highest progesterone concentrations were recorded during the metestrus phase (3-day stage), reaching $37.61 \pm 1.96\ \text{nmol/l}$.

Hematological analysis (Table 2) revealed significant alterations in blood parameters in dogs with luteal insufficiency. In the experimental group, a decline in progesterone levels was associated with a moderate but statistically significant increase in leukocyte count ($12.50 \pm 0.30 \times 10^9/l$; $p < 0.05$). There was also a moderate increase in neutrophils ($8.59 \pm 0.08 \times 10^9/l$; $p < 0.05$) and a marked elevation in both the absolute count ($3.40 \pm 0.18 \times 10^9/l$; $p < 0.01$) and percentage ($27.43 \pm 0.18\%$; $p < 0.01$) of lymphocytes. Additionally, significant increases in hemoglobin (183.2 ± 2.09 g/l; $p < 0.01$) and hematocrit values (0.44 ± 0.02 l/l; $p < 0.01$) were observed, reflecting altered oxygen transport capacity. Other hematological parameters such as MCV, MCH, MCHC, and platelet

counts also changed notably, with a significant increase in platelet levels in peripheral blood ($466.4 \pm 3.56 \times 10^9/l$; $p < 0.001$).

In pregnant dogs in the experimental group (18–22 days of gestation), clinical signs indicative of luteal insufficiency included restlessness, dark-red vaginal discharge, and increased abdominal tone. Hormonal analysis showed (Table 3) significantly lower progesterone levels in this group (9.90 ± 0.19 nmol/l) compared with controls (55.60 ± 2.23 nmol/l; $p < 0.001$), which was considered a risk factor for pregnancy loss (day 0 of treatment). These animals received a treatment regimen including MPA and tocopherol.

Clinical improvement was observed during therapy: vulvar discharge subsided, and abdominal tone

Table 1. Plasma concentrations of progesterone (P4, nmol/l) during the estrous cycle of the canines [mean ($\pm$ SE)].

Stages	Concentrations P4
Anestrus ($n = 10$, 3 day), control group C1	1.53 ± 0.07
Proestrus ($n = 10$, 2 day)	$4.56 \pm 0.20^{***}$
Estrus ($n = 10$, 3 day)	$19.54 \pm 0.17^{***}$
Metestrus ($n = 10$, 3 day)	$37.61 \pm 1.96^{***}$

Significance $^{***}p < 0.001$ relative to the anestrus stage (compared to the control group C2).

Table 2. Hematological parameters of luteal insufficiency canines [mean ($\pm$ SE)].

Parameter	Units	18–22 th day of pregnancy (control C2, $n = 10$)	Luteal insufficiency Canine ($n = 10$)
Erythrocytes (RBS)	$\times 10^{12}/l$	7.16 ± 0.04	7.11 ± 0.04
Leukocytes (WBS)	$\times 10^9/l$	11.50 ± 0.24	$12.50 \pm 0.30^*$
Neutrophil (NEU)	%	68.80 ± 1.47	$65.70 \pm 1.56^*$
	$\times 10^9/l$	7.71 ± 0.15	$8.59 \pm 0.08^*$
Lymphocyte (LYM)	%	23.33 ± 0.86	27.43 ± 0.18
	$\times 10^9/l$	2.78 ± 0.10	$3.40 \pm 0.18^{**}$
Eosinophils (EOS)	%	2.19 ± 0.16	2.19 ± 0.16
	$\times 10^9/l$	0.46 ± 0.02	0.26 ± 0.03
Monocytes (MON)	%	2.81 ± 0.12	3.12 ± 0.17
	$\times 10^9/l$	0.48 ± 0.04	$0.54 \pm 0.08^*$
Basophils (BAS)	%	0.10 ± 0.01	0.10 ± 0.01
	$\times 10^9/l$	0.01 ± 0.001	0.01 ± 0.001
Hemoglobin (HGB)	g/l	154.90 ± 3.14	$183.2 \pm 2.09^{**}$
Hematocrit (HCT)	l/l	0.37 ± 0.02	$0.44 \pm 0.02^{**}$
MCV	fl	68.11 ± 0.73	$71.04 \pm 0.97^*$
MCH	fmol	1.27 ± 0.02	$2.57 \pm 0.10^{**}$
MCHC	mmol/l	19.81 ± 0.51	$37.17 \pm 0.29^{**}$
Thrombocyte (PLT)	$\times 10^9/l$	367.60 ± 12.96	$466.4 \pm 3.56^{**}$

MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; Significance $^*p < 0.05$; $^{**}p < 0.01$ (compared to the control group C2).

normalized. Progressive increases in progesterone concentrations following MPA with tocopherol and magnesium supplementation were documented. No signs of uterine hypertonicity or vaginal discharge were recorded in treated pregnant dogs. In all cases, the pregnancy progressed without complications. However, two dogs developed mammary gland hyperplasia (Fig. 1), requiring dose adjustments of MPA with tocopherol and magnesium supplementation (Table 4). The mean serum progesterone (P4) concentrations in canines during treatment for luteal insufficiency were evaluated. In the control group (C2), the progesterone concentration at 18–22 days of gestation was 55.60 ± 2.23 nmol/l. In the group with luteal insufficiency ($n = 10$), the concentration was significantly lower at 9.90 ± 0.19 nmol/l ($p < 0.001$ compared to control). Following treatment, progesterone levels increase progressively. On treatment day 5 (gestational days 23–27), the concentration rose to 20.03 ± 0.75 nmol/l ($p < 0.001$ compared to untreated luteal insufficiency group). On day 10 (33–37 days of gestation), progesterone levels reached 37.76 ± 0.40 nmol/l ($p < 0.001$). By day 20

(43–47 gestational days), the concentration further increased to 43.25 ± 0.57 nmol/l ($p < 0.001$). On day 30 (53–57 gestational days), a decrease in progesterone concentration to 33.37 ± 0.87 nmol/l was observed ($p < 0.001$). Deliveries occurred on days 62–63 of gestation, and all litters consisted of anatomically and physiologically normal puppies (Fig. 2).

Discussion

Hypoluteoidism is a reproductive dysfunction characterized by inadequate production or premature decline of progesterone, a hormone critical for maintaining early pregnancy. This endocrinological deficiency typically results from suboptimal activity of the corpus luteum following ovulation, impairing its ability to sustain the hormonal environment necessary for embryo implantation and gestational progression (Kowalik *et al.*, 2018; Kowalewski *et al.*, 2020c; Gualdoni *et al.*, 2022; Zhelavskiy *et al.*, 2024b). In canines, the corpus luteum is the primary source of progesterone during the luteal phase of the estrous cycle (Peluso, 2022). A failure to achieve or sustain

Table 3. Plasma concentrations of progesterone (P4) in luteal insufficiency canines [mean ($\pm$ SE)].

Parameter	Units	18–22 th day of pregnancy (control C2, n = 10)	Luteal insufficiency Canine (n = 10)
Concentrations progesterone (P4)	nmol/l	55.60 ± 2.23	$9.90 \pm 0.19^{***}$

Significance $^{***}p < 0.001$ (compared to the control group C2).

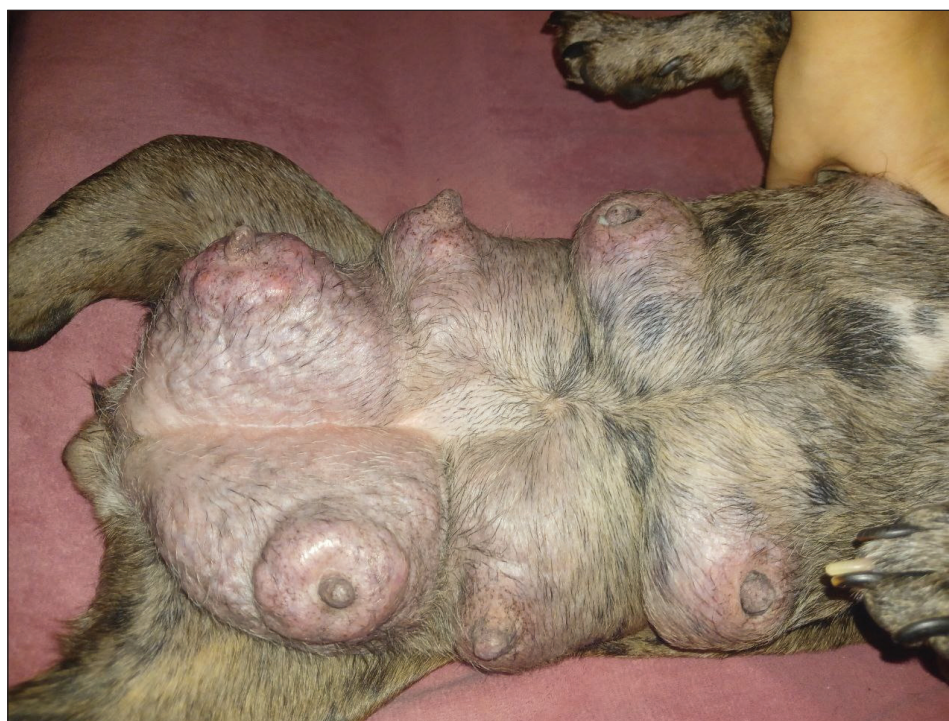


Fig. 1. Signs of mammary gland hyperplasia in a 2-year-old French bulldog.

Table 4. Mean ($\pm$ SE) serum progesterone (P4) concentration (nmol/l) during dynamic treatment for luteal insufficiency of canines.

Parameter	Days pregnant	Concentrations progesterone (P4)
Control (C2, $n = 10$)	18–22	55.60 ± 2.23
Luteal insufficiency Canine ($n = 10$)	18–22	$9.90 \pm 0.19^{\diamond***}$
Treatment: days 5 ($n = 10$)	23–27	$20.03 \pm 0.75^{\Delta***}$
Days 10 ($n = 10$)	33–37	$37.76 \pm 0.40^{\Delta***}$
Days 20 ($n = 10$)	43–47	$43.25 \pm 0.57^{\Delta***}$
Days 30 ($n = 10$)	53–57	$33.37 \pm 0.87^{\Delta***}$

Significance $^{\diamond***}p < 0.001$ (compared to the control group C); $^{\Delta***}p < 0.001$ (compared to the Luteal insufficiency canine).



Fig. 2. Newborn (2 days old) French bulldog.

adequate progesterone concentrations during this phase can compromise endometrial receptivity, ultimately leading to implantation failure and early embryonic loss (Wetendorf and Demayo, 2014; Gram *et al.*, 2015; Castelnovo *et al.*, 2019; Conley *et al.*, 2023). Progesterone is indispensable in modulating the uterine environment, notably by transforming the endometrial lining to a state conducive to embryo attachment and implantation (Gram *et al.*, 2014; Kowalewski *et al.*, 2020a; Zhelavskiy *et al.*, 2020; Garrido-Gomez *et al.*, 2022). Its role in the reproductive cycle of dogs cannot be overstated because it ensures favorable conditions for embryo development. In cases in which progesterone

levels are inadequate, the endometrium may not reach the necessary receptivity, potentially resulting in failed implantation (Monsivais *et al.*, 2017; Nowak *et al.*, 2019). This hormonal insufficiency can also contribute to early pregnancy losses or miscarriages because the uterine environment becomes unsupportive of the developing embryo (Kowalik *et al.*, 2014; Ponikwicka-Tyszko *et al.*, 2019; Mistry *et al.*, 2020; Nöthling *et al.*, 2022; Zhelavskiy *et al.*, 2023b).

Hypoluteoidism, characterized by insufficient plasma progesterone levels, is a poorly documented cause of embryo or fetal loss in dogs. Experimental studies suggest that a plasma progesterone concentration of at

least 6–9 nmol/l (2–3 ng/ml) is required to maintain pregnancy, and levels below this threshold for more than 24–48 hours result in pregnancy loss (Zedda *et al.*, 2017 ; Kreis *et al.*, 2019).

In experimental settings, dogs have been observed to maintain pregnancy with plasma progesterone concentrations of 2–5 ng/ml. However, in clinical practice, it is often safer to anticipate potential pregnancy loss by initiating progesterone supplementation when levels decline (Conley *et al.*, 2023). This approach is typically adopted without concrete evidence that the pregnancy is terminated without supplementation. Clinicians and breeders prefer to avoid waiting until progesterone levels drop to 2–3 ng/ml, as preserving pregnancy at this stage may no longer be feasible (Thomas *et al.*, 2014; Sarkar and Mondal, 2020; Hinderer *et al.*, 2021).

Hypoluteoidism is challenging to document clinically, but it is considered a potential cause of pregnancy loss in dogs. Cases in which pregnancy loss is directly linked to low plasma progesterone levels raise questions about whether the failure is solely due to low progesterone levels or other underlying causes affecting the corpus luteum. Hypoluteoidism has been observed around 25–35 days of pregnancy, a period normally marked by increased progesterone secretion in pregnant animals through mechanisms associated with placentation (Kazemian *et al.*, 2022; Kowalewski, 2023).

In cases with low progesterone levels and imminent pregnancy loss risk, progestin supplementation may be recommended, albeit with empirical dosing (Thomas *et al.*, 2014; Sarkar and Mondal, 2020). Progesterone use should be restricted to diagnosed or suspected cases of luteal insufficiency because improper use may lead to adverse effects, including mammary fibroadenoma development (Zhelavskiy, 2024; Zedda *et al.*, 2017) and masculinization of female pups.

Studies have demonstrated that tocopherol can improve oocyte quality and reduce the risk of miscarriage in dogs with luteal insufficiency (Peluso *et al.*, 2014; Gram *et al.*, 2016; Cottrell *et al.*, 2017; Beal *et al.*, 2021). It also mitigates the oxidative stress associated with hormone therapy, thereby enhancing reproductive outcomes. Tocopherol is a natural antioxidant that improves the fertility and functioning of the gonads in both males and females (Krachudel *et al.*, 2013).

Magnesium is critical for numerous biochemical processes, including hormone synthesis and regulation. Magnesium is a vital cation that plays numerous essential roles in the body, particularly in the nervous and muscular systems. Additionally, magnesium is crucial for muscle function because it is involved in muscle contraction and relaxation processes by interacting with calcium and potassium. Beyond these functions, magnesium also contributes to energy production, as it is a cofactor for many enzymes involved in ATP synthesis and supports bone health by promoting calcium absorption. A magnesium deficiency

can lead to a range of issues, including muscle cramps, fatigue, and neurological disturbances, highlighting its importance for maintaining overall health (Kolanu *et al.*, 2020).

Magnesium deficiency can impair ovarian function, negatively affecting reproductive capabilities. Antistress properties of magnesium are particularly valuable for dogs experiencing stress or anxiety during pregnancy or estrus. Stress can impair reproductive function, underscoring the importance of providing comfortable living conditions and supporting the emotional well-being of animals (Jaripur *et al.*, 2022).

These findings highlight the intricate regulation of progesterone secretion throughout the estrous cycle and reinforce its pivotal role in orchestrating reproductive events (Kautz *et al.*, 2014; Kowalewski *et al.*, 2021a). Hormonal fluctuations in progesterone are tightly linked to sequential physiological transformations that facilitate successful reproduction (Guo *et al.*, 2016; Graubner *et al.*, 2020). Notably, therapeutic strategies involving progesterone supplementation, particularly in conjunction with antioxidant and mineral cofactors such as tocopherol (vitamin E) and magnesium, have shown promise in producing synergistic effects (Tan *et al.*, 2019; Shastak *et al.*, 2023). These combined treatments are associated with improved implantation outcomes and decreased incidence of spontaneous pregnancy loss. Emerging evidence underscores the clinical importance of restoring adequate progesterone levels in individuals diagnosed with luteal phase deficiency, as a prerequisite for achieving a favorable intrauterine environment conducive to embryo implantation and early gestational development (Mistry *et al.*, 2020; Nöthling *et al.*, 2022).

The necessity of rigorous hormonal monitoring and prompt therapeutic intervention upon detection of progesterone insufficiency is further emphasized in the literature. Experimental studies have validated the efficacy of progesterone-based regimens in mitigating endocrine-related causes of recurrent miscarriage. In the broader context of fertility, luteal insufficiency, which is characterized by deficient progesterone output, remains a critical barrier to conception (Thomas *et al.*, 2014; Hinderer *et al.*, 2021). A suboptimal hormonal milieu disrupts the morphofunctional preparation of the endometrial lining, undermining its receptivity to embryonic implantation and thereby elevating the risk of infertility and early pregnancy termination (Zedda *et al.*, 2017; Tavares *et al.*, 2022b). Clinical investigations support the application of targeted hormonal therapies to correct such deficiencies, demonstrating significant improvements in pregnancy retention rates and highlighting the essential role of early diagnosis and timely hormonal correction in reproductive management (Carr *et al.*, 2020; Khalphallah *et al.*, 2022; Kapper *et al.*, 2024).

Conclusion

Progesterone therapy when combined with supplementary agents, such as tocopherol and magnesium, shows considerable promise in addressing luteal insufficiency in dogs. The synergistic effects of these treatments improve implantation success rates and reduce the likelihood of pregnancy loss, providing an effective approach to correcting hormonal imbalances. Future research should aim to refine progesterone-based therapies and investigate innovative supplement combinations to enhance outcomes in animals with reproductive disorders. Such advancements will deepen our understanding of hormonal regulation and improve clinical approaches to managing infertility and pregnancy-related complications in canines.

Acknowledgments

The authors would like to express their sincere gratitude to the faculty and staff of the Vinnytsia National Agrarian University, Vinnytsia, Ukraine, and the National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine, for their invaluable support and assistance throughout this research. Their contributions were essential to the successful completion of this study.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Funding

This research was not funded by any funding organization.

Authors' contributions

MZ: responsible for conceptualizing the study, data acquisition, analyzing the results, and initial manuscript drafting. MM: supervised the overall research process, conducted the analysis of laboratory data, and actively participated in the preparation and revision of the manuscript. DM and VK: conducted the analysis and interpretation of clinical data, and assisted in drafting and refining the manuscript. NB: the author guided the research process and played a key role in manuscript development and revision. TP: statistical analysis was performed and supported the writing and editing of the manuscript. All authors critically reviewed, revised, and approved the final manuscript for submission.

Data availability

All data were provided in the manuscript.

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