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Induction of Estrus Using Various Protocols in Female Doges

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Abstract | This study aimed to compare the efficacy of a dopamine agonist (cabergoline) and a GnRH agonist (buserelin) in inducing estrus in anestrous local Iraqi female dogs. A total of 21 bitches were randomly assigned into three groups (n=7 per group): Group A received buserelin acetate at a dose of 1.5 µg/kg subcutaneously for 11 days followed by 0.75 µg/kg for the next 3 days; Group B received cabergoline orally at 5 µg/kg once daily until the onset of clinical proestrus or for a maximum of 30 days; Group C served as the untreated control. Examinations were conducted every 48 hours to assess vaginal cytology and serum progesterone concentrations. Additional parameters including behavioral signs, vulvar swelling, and presence of bloody vaginal discharge were monitored twice daily. The results showed a statistically significant difference ($P \leq 0.01$) in estrus induction rates between the treated groups, with cabergoline showing higher efficacy. However, the onset of proestrus and estrus signs occurred significantly earlier in the buserelin-treated group compared to the cabergoline group.

Keywords | Estrus, Hormone, Cabergoline and buscerelin

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INTRODUCTION

Domestic dogs are non-seasonal monoestrous animals, typically exhibiting two estrous cycles per year due to a prolonged anestrus phase (Concannon *et al.*, 1989; Johnston *et al.*, 2001). This reproductive pattern limits breeding opportunities, especially in cases of conception failure or the need for precise timing in mating or whelping (Kutzler, 2005). Estrus induction is therefore clinically valuable for managing primary or prolonged anestrus, synchronizing ovulation, and facilitating reproductive technologies such as artificial insemination and embryo transfer (Johnston *et al.*, 2001; Jaafar and Al-Mutar, 2024a).

Various hormonal protocols have been developed for estrus induction in bitches, including the use of estrogens, gonadotropins (FSH, LH, eCG, hCG), dopamine agonists (cabergoline, bromocriptine), and GnRH agonists (buserelin, deslorelin) (Nagashima and Songsasen, 2021). Among these, dopamine and GnRH agonists have shown particularly promising outcomes (Verstegen *et al.*, 1999; Beijerink *et al.*, 2003; Lanna *et al.*, 2010). GnRH agonists act by stimulating LH and FSH secretion but may be limited in practice due to high cost and complex administration (Fontaine and Fontbonne, 2010; Bolghanabadi *et al.*, 2023). Dopamine agonists, especially cabergoline, offer a more practical and cost-effective alternative by increasing basal FSH levels and shortening the interestrous interval (Ohtaki *et al.*, 2020; Belala *et al.*, 2025).

Despite their global use, studies on estrus induction in local Iraqi bitches remain limited. This study aims to compare the efficacy of a dopamine agonist (cabergoline) and a GnRH agonist (buserelin) in inducing estrus in anestrous local Iraqi female dogs.

MATERIALS AND METHODS

This study was conducted in Heet City, Anbar Governorate, Iraq, from September 2024 to May 2025. Twenty-one healthy, multiparous local bitches aged 2–4 years and weighing 16–25 kg were selected. All animals were confirmed nonpregnant via ultrasonography and isolated for three months prior to treatment. Bitches were randomly divided into three groups (n=7 per group): Group A received buserelin acetate subcutaneously at 1.5 µg/kg for 11 days, followed by 0.75 µg/kg for the next 3 days (Rota *et al*., 2003). Group B received cabergoline orally at 5 µg/kg once daily until the onset of proestrus or for a maximum of 30 days (Rota *et al*., 2003). Group C served as the untreated control.

Clinical assessments were performed every 48 hours, including vaginal cytology and serum progesterone measurement. Additional daily observations included behavioral signs, vulvar swelling, and presence of bloody vaginal discharge.

Estrous phase progression was determined based on established criteria (Ekambaram *et al*., 2017; Skliarov *et al*., 2022; Kumar *et al*., 2023; Belala *et al*., 2025). The onset of proestrus was defined as the first appearance of sanguineous vaginal discharge. Estrus was marked by female receptivity, while the end of estrus was determined by cessation of male acceptance.

Approximately 2–5 mL of blood was collected from the cephalic vein, centrifuged at 3000 rpm for 15 minutes, and serum was stored at 8 °C for hormonal analysis. Progesterone concentrations were measured every 48 hours using a commercial chemiluminescence immunoassay (CLIA) kit (Mindray CLIA Progesterone Reagent Kit), following the manufacturer's instructions, conducted the tests at Al-Majd Central Laboratory, located in Hit, Anbar.

Behavioral evaluation included indicators such as mating posture (standing/flagging), increased urination, restlessness or lethargy, depending on individual temperament and hormonal changes (Costa *et al*., 2023).

Vaginal cytology samples were collected every 48 hours using saline-moistened swabs inserted into the anterior vagina. Slides were prepared, fixed in methanol for 1 minute, stained with Giemsa for 5 minutes, rinsed, and air-dried. Microscopic examination was conducted at 100× and 400× magnification to determine the estrous stage and calculate the cornification index (Labib *et al*., 2018).

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS, version 25, IBM Corp., 2019). The Least Significant Difference (LSD) test was employed to compare group means, while the Chi-square test was used to evaluate differences in estrus response rates among groups. Statistical significance was set at $P \leq 0.01$.

Estrus response (%) was calculated using the formula:

Estrus response (%) = (Number of bitches that exhibited estrus / Total number of treated bitches) × 100

RESULT

Estrous response rate in treated and control groups

The estrus induction rate varied significantly among groups. Group B (cabergoline) showed the highest response rate at 71.4% (5/7), followed by Group A (buserelin) at 28.6% (2/7), while no response was observed in the control group (0/7). The difference between groups was statistically significant ($P \leq 0.01$), as shown in Table 1.

Table 1: Estrous response rates in treated and control groups.

Group	N0	Treated	Induce estrus	Induce estrus rate
A	7	Buserelin	2	28.57 %
B	7	Cabergoline	5	71.42 %
C	7	Control	0	0
χ2 (P-value)	** (P≤0.01)			8.26** (0.016)

Onset and duration of the proestrus and estrus phases based on clinical signs

The clinical observations revealed that the onset of the proestrus phase occurred significantly earlier in Group A (3.75 ± 1.06 days) compared to Group B (16.20 ± 2.84 days), with no signs observed in the control group (Group C). The proestrus phase ended on day 9.25 ± 1.77 in Group A and on day 24.00 ± 1.96 in Group B, resulting in a proestrus duration of 6.00 ± 0.71 and 8.30 ± 2.19 days respectively (P ≤ 0.01) as shown in Table 2.

Similarly, the onset of estrus occurred on day 9.75 ± 1.77 in Group A and 24.50 ± 1.97 in Group B, with durations of 6.25 ± 1.06 and 7.10 ± 1.29 days respectively. Although the onset of estrus differed significantly between the two groups (P ≤ 0.01), the duration did not show a significant difference (P > 0.05) as presented in Table 2.

Onset and duration of proestrus and estrus phases based on progesterone levels

Based on serum progesterone concentrations, the onset

of the proestrus phase was recorded at 3.50 ± 1.41 days in Group A and 16.10 ± 2.61 days in Group B. No hormonal signs of proestrus were detected in the control group (Group C). The end of the proestrus phase occurred at 8.50 ± 1.41 days in Group A and 22.70 ± 2.28 days in Group B, resulting in a proestrus duration of 5.50 ± 0.00 and 7.10 ± 1.67 days, respectively. A statistically significant difference (P ≤ 0.01) was observed between Groups A and B in the onset of both the proestrus and estrus phases. However, no significant difference (P > 0.05) was noted between them regarding the duration of the proestrus phase (Table 3).

The onset of the estrus phase was observed at 9.50 ± 1.41 days in Group A and 23.70 ± 2.28 days in Group B, showing a statistically significant difference (P ≤ 0.01) (Table 3). However, since progesterone levels remained elevated beyond the onset of estrus, it was not possible to determine the end of the estrus phase based on hormonal data alone.

Onset and duration of the proestrus and estrus phases determined by vaginal cytology

Based on vaginal cytology, the onset of the proestrus phase was recorded at 5.50 ± 1.41 days in Group A and 16.90 ± 2.61 days in Group B. No significant changes in the proportions of vaginal epithelial cells were observed in the control group (Group C). The end of the proestrus phase occurred at 10.50 ± 4.24 days in Group A and 22.70 ± 2.28 days in Group B. Consequently, the duration of the proestrus phase was 5.50 ± 2.83 days and 6.30 ± 1.09 days in Groups A and B, respectively. A statistically significant difference (P ≤ 0.01) was observed between Groups A and B in the onset of the proestrus phase; however, no significant difference (P > 0.05) was found in its duration (Table 4).

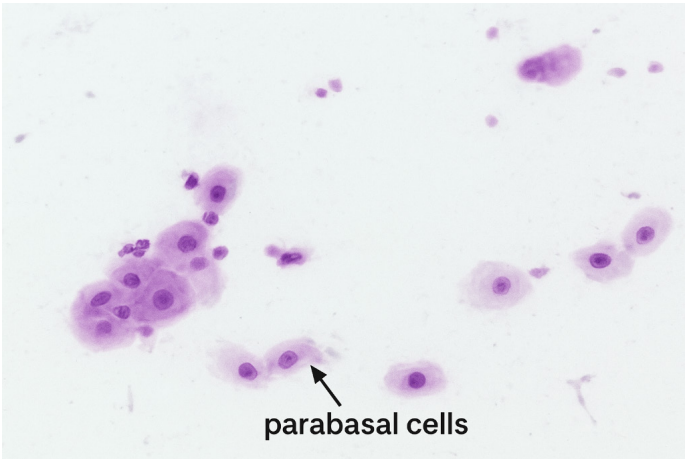

Figure 1: A vaginal smear from a female dog shows a cytological profile characteristic of the anestrus stage.

Similarly, the estrus phase began at 11.50 ± 4.24 days in Group A and 23.70 ± 2.28 days in Group B, with no detectable cytological changes in Group C. The estrus phase ended at 18.00 ± 3.53 days and 29.50 ± 0.01 days in Groups A and B, respectively. The duration of the estrus phase was 7.00 ± 0.71 days in Group A and 6.30 ± 2.28 days in Group B. A statistically significant difference (P ≤ 0.01) was observed between Groups A and B in the onset of the estrus phase, whereas no significant difference (P > 0.05) was found in its duration (Table 4).

Table 2: Comparison of different treatment groups based on clinical signs of onset and duration of proestrus and estrus phases (in days).

Group	Number of animals	The onset of proestrus phase (day)	The end of proestrus phase (day)	Duration of the proestrus phase (day)	The onset of estrus phase (day)	The end of estrus phase (day)	Duration of the estrus phase (day)
A	7	3.75 ±1.06 b	9.25 ±1.77 b	6.00 ±0.71 a	9.75 ±1.77 b	15.50 ±0.71 b	6.25 ±1.06 a
B	7	16.20 ±2.84 a	24.00 ±1.96 a	8.30 ±2.19 a	24.50 ±1.97 a	31.10 ±0.89 a	7.10 ±1.29 a
C	7	0 ±0 b	0 ±0 c	0 ±0 b	0 ±0 c	0 ±0 c	0 ±0 b
L.S.D. (P-value)		4.288 ** (0.0001)	3.201 ** (0.0001)	3.301 **(0.0008)	3.202** (0.0001)	1.426** (0.0001)	2.075** (0.0001)

Means having with the different letters in same column differed significantly. ** (P≤0.01).

Table 3: Comparison of treatment groups based on progesterone levels at the onset and duration of proestrus and estrus phases (Days).

Group	Number of animals	The onset of proestrus phase (day)	The end of proestrus phase (day)	Duration of the proestrus phase (day)	The onset of estrus phase (day)	The end of estrus phase (day)	Duration of the estrus phase (day)
A	7	3.50 ±1.41 b	8.50 ±1.41 b	5.50 ±0.00 a	9.50 ±1.41 b	-	-
B	7	16.10 ±2.61 a	22.70 ±2.28 a	7.10 ±1.67 a	23.70 ±2.28 a	-	-
C	7	0 ±0 b	0 ±0 c	0 ±0 b	0 ±0 c	-	-
L.S.D. (P-value)		4.009 ** (0.0001)	3.542 ** (0.0001)	2.483 ** (0.0004)	3.542 ** (0.0001)	-	-

Means having with the different letters in same column differed significantly. ** (P≤0.01).

Table 4: Comparison of treatment groups based on vaginal cytology for the onset and duration of proestrus and estrus phases (Days).

Group	Number of animals	The onset of proestrus phase (day)	The end of proestrus phase (day)	Duration of the proestrus phase (day)	The onset of estrus phase (day)	The end of estrus phase (day)	Duration of the estrus phase (day)
A	7	5.50 ±1.41 b	10.50 ±4.24 b	5.50 ±2.83 a	11.50 ±4.24 b	18.00 ±3.53 b	7.00 ±0.71 a
B	7	16.90 ±2.61 a	22.70 ±2.28 a	6.30 ±1.09 a	23.70 ±2.28 a	29.50 ±0.01 a	6.30 ±2.28 a
C	7	0 ±0 c	0 ±0 c	0 ±0 b	0 ±0 c	0 ±0 c	0 ±0 b
LSD (P-value)		4.009 ** (0.0001)	4.621** (0.0001)	2.653 ** (0.0012)	4.621 ** (0.0001)	2.622 ** (0.0001)	3.423 ** (0.0031)

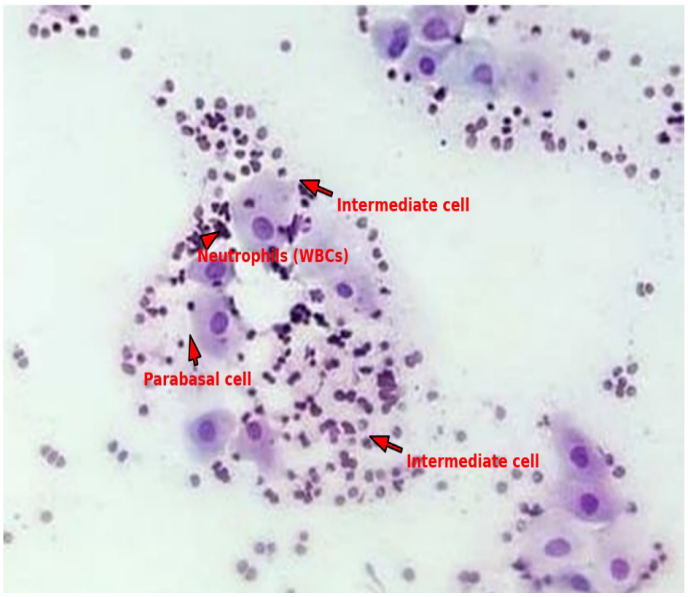

Figure 2: A vaginal smear from a female dog shows a cytological profile characteristic of the prostrus stage.

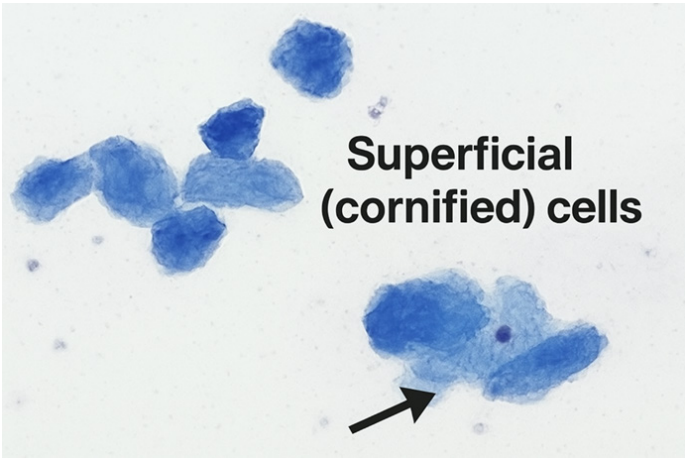

Figure 3: A vaginal smear from a female dog shows a cytological profile characteristic of the estrus stage.

This Table 5 shows the serum progesterone levels (in ng/mL) in several animals (A1, A2, A3, A4, A5, A6, A7) measured on different days (1, 3, 5, 7, 9, 11, 13, 15) after treatment with Buserelin.

It also categorizes the reproductive phase of each bitch (A = Anestrus, P = Proestrus, E = Estrus) according to the progesterone concentration measured on each day.

The Table 6 demonstrates the variability in response of the treated animals to Buserelin administration. Two animals (A2 and A5) showed a successful induction of a cycle, evidenced by the high rise in progesterone (indicating the presence of a corpus luteum) and the progression to the Proestrus and Estrus stages. The remaining five animals (A1, A3, A4, A6, A7) remained in Anestrus with low progesterone, indicating a lack of response to the treatment.

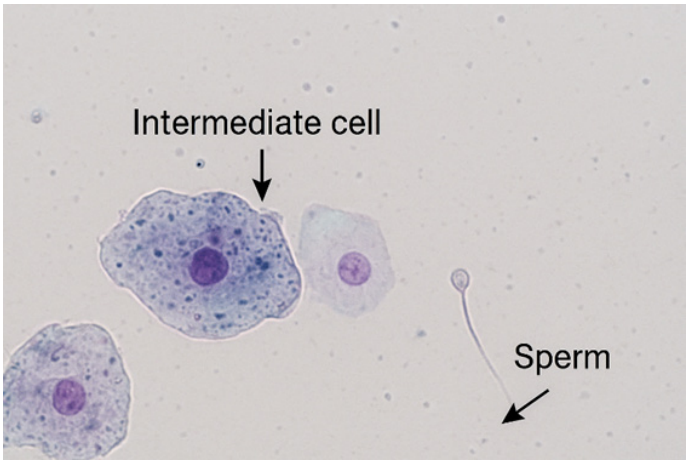

Figure 4: A vaginal smear obtained during the estrus stage from a female dog shows the presence of spermatozoa, indicating that mating has occurred.

Table 5: Serum progesterone concentrations (ng/mL) in the group treated with Buserelin.

No/day	1	3	5	7	9	11	13	15
A1	0.60 A	0.67 A	0.69 A	0.83 A	0.78 A	0.80 A	1.12 A	0.98 A
A2	0.59 A	0.71 A	1.10 P	1.35 P	1.93 P	2.71 E	11.4 E	13.2 E
A3	0.55 A	0.61 A	0.60 A	0.73 A	0.90 A	0.95 A	0.94 A	1.13 A
A4	0.52 A	0.54 A	0.70 A	0.70 A	0.69 A	0.65 A	0.78 A	0.86 A
A5	0.45 A	0.98 P	1.3 P	1.9 P	3.1 E	5 E	7.2 E	14.3 E
A6	0.59 A	0.61 A	0.63 A	0.82 A	0.80 A	0.83 A	0.91 A	0.95 A
A7	0.42 A	0.43 A	0.47 A	0.58 A	0.77 A	0.73 A	0.69 A	0.71 A

Note: A = Anestrus; P = Proestrus; E = Estrus.

Table 6: Serum progesterone concentrations (ng/mL) in the group treated with Cabergoline.

No/day	1	3	5	7	9	11	13	15	17	19	21	23	25	27	29
B1	0.51 A	0.54 A	0.59 A	0.57 A	0.65 A	0.71 A	0.77 A	0.82 A	0.90 P	0.82 P	0.71 P	1 P	1.3 P	3.78 E	8.2 E
B2	0.33 A	0.40 A	0.47 A	0.46 A	0.51 A	0.57 A	0.61 A	0.60 A	0.63 A	0.71 A	0.68 A	0.64 A	0.65 A	0.65 A	0.67 A
B3	0.69 A	0.73 A	0.79 A	0.77 A	0.89 A	0.75 A	0.81 A	0.90 P	0.91 P	1.2 P	1.7 P	2.4 E	7.1 E	12.2 E	16.8 E
B4	0.86 A	0.79 A	0.63 A	0.67 A	0.76 A	0.74 A	0.78 A	0.75 A	0.68 A	0.66 A	0.67 A	0.72 A	0.76 A	0.84 A	0.81 A
B5	0.34 A	0.53 A	0.61 A	0.77 A	0.79 A	0.79 A	0.83 A	0.81 A	0.90 A	1.21 P	1.43 P	1.75 P	3.1 E	4.2 E	11.8 E
B6	0.40 A	0.49 A	0.53 A	0.53 A	0.61 A	0.66 A	0.63 A	0.69 A	0.78 A	0.89 P	1.12 P	1.71 P	2.9 E	3.8 E	10.1 E
B7	0.36 A	0.51 A	0.67 A	0.73 A	0.82 A	0.73 A	0.96 P	0.92 P	1.1 P	1.3 P	2.87 E	3.98 E	5.7 E	8.2 E	13.3 E

Note: A = Anestrus; P = Proestrus; E = Estrus.

Table 6 show the serum progesterone levels (in ng/mL) in several animals (A1, A2, A3, A4, A5, A6, A7) measured on different days (1, 3, 5, 7, 9, 11, 13, 15) after treatment with Cabergoline.

It also categorizes the reproductive phase of each bitch (A= Anestrus, P= Proestrus, E= Estrus) according to the progesterone concentration measured on each day.

Table 6 demonstrates the variability in response of the treated animals to Buserelin administration. Five animals (B1, B3.B5, B6 and B7) showed a successful induction of a cycle, evidenced by the high rise in progesterone (indicating the presence of a corpus luteum) and the progression to the Proestrus and Estrus stages. The remaining two animals (B2 and B4) remained in Anestrus with low progesterone, indicating a lack of response to the treatment.

Table 7: Serum progesterone concentrations (ng/mL) in the control group.

No/day	1	3	5	7	9	11	13	15
C1	0.82 A	0.80 A	0.74 A	0.85 A	1.2 A	0.97 A	0.93 A	0.96 A
C2	0.34 A	0.41 A	0.39 A	0.52 A	0.60 A	0.57 A	0.64 A	0.61 A
C3	0.44 A	0.47 A	0.51 A	0.53 A	0.50 A	0.58 A	0.57 A	0.64 A
C4	0.52 A	0.55 A	0.61 A	0.56 A	0.41 A	0.48 A	0.48 A	0.51 A
C5	0.65 A	0.63 A	0.71 A	0.65 A	0.73 A	0.79 A	0.82 A	0.77 A
C6	0.70 A	0.73 A	0.78 A	0.81 A	0.73 A	0.80 A	0.77 A	0.67 A
C7	0.36 A	0.45 A	0.41 A	0.54 A	0.61 A	0.63 A	0.57 A	0.53 A

Note: A = Anestrus; P = Proestrus; E = Estrus.

Table 7 shows serum progesterone concentrations (ng/mL) in untreated (control) animals measured every two days (Day 1, 3, 5, 7, 9, 11, 13, 15). Animals in this group received no hormonal treatment, so their reproductive cycle should remain natural. All animals in this table are labeled A, meaning Anestrus a period of ovarian inactivity with low progesterone levels.

DISCUSSION

The high estrous response rate observed with cabergoline in this study is consistent with the findings of Rota *et al.* (2003) and Rezende *et al.* (2018), who also reported elevated induction rates following cabergoline treatment. In contrast, the moderate response associated with buserelin, approximately 30%, aligns with its previously documented limited efficacy. These outcomes reinforce the potential of cabergoline as a more effective therapeutic option for inducing estrus in anestrous bitches.

Nevertheless, the variability in response to cabergoline has been noted in earlier literature. Concannon (1993) reported inconsistent results, particularly in bitches that had not yet entered their natural estrous phase. Additionally, Feldman *et al.* (2014) highlighted that the success of pharmacological estrus induction can be unpredictable, with several influencing factors such as the animal's hormonal profile, reproductive background, and external environmental conditions.

The observed pattern of delayed onset and prolonged duration of estrus following GnRH agonist administration is consistent with previous findings by Verstegen *et al.* (2001a), Junaidi *et al.* (2009), and Jaafar and Al-Mutar (2024b), who reported similar responses in bitches undergoing hormonal induction. Furthermore, the lack of

observable proestrus and estrus signs in the control group corroborates earlier reports describing the absence of spontaneous cyclicity in untreated anestrous animals.

On the other hand, the present findings differ from those of Johnston *et al.* (2001) and Root Kustritz (2005), who documented comparatively longer durations of the estrous phases. Such discrepancies may be attributed to factors including breed-specific reproductive characteristics, variations in the hormonal induction protocols applied, or differing environmental and management conditions.

The findings of the present study are in agreement with those reported by Kutzler (2007), Noakes *et al.* (2009), and Concannon (2011), who demonstrated that hormonal treatments can significantly affect both serum progesterone concentrations and the timing of estrus onset in bitches. However, a considerable degree of individual variability continues to pose a challenge in clinical and research settings.

Supporting this, Verstegen *et al.* (2001b) and Romagnoli and Concannon (2003) have emphasized the inconsistent hormonal responses among individual bitches subjected to induction protocols, with some failing to exhibit predictable changes in progesterone levels. Similarly, England and Concannon (2002c) noted a lack of direct correlation between hormonal profiles and behavioral signs of estrus, highlighting that estrous behavior may occur even in the absence of a notable rise in progesterone.

Such observations underscore the limitations of relying exclusively on progesterone measurements to define the stages of the estrous cycle. Additionally, Kirk and Bistner (2000) documented cases of silent heats and subclinical ovarian activity, where estrus occurred without significant hormonal elevation.

In summary, despite the significant intergroup differences in progesterone concentrations observed in this study, the data suggest that serum progesterone levels alone are insufficient to precisely determine the end of the estrus phase. This reinforces the need for a more comprehensive approach that considers both hormonal and clinical indicators to accurately assess reproductive status in anestrous bitches.

The current findings are consistent with those reported by England and Concannon (2002a), Kustritz (2005), and Concannon (2009), who documented that the typical duration of the proestrus phase in cycling bitches ranges from 6 to 11 days, while the estrus phase generally lasts between 5 and 9 days.

In contrast, other studies have described a broader variation in estrus duration. Concannon (2011) reported an average estrus length of 9 days in domestic dogs, with a range extending from 3 to 21 days. Similarly, Johnston *et al.* (2001) and Pretzer (2008) found that estrus in clinically normal bitches often persists for no fewer than 7 days. These authors also highlighted the limitations of relying exclusively on vaginal cytology, due to the inherent physiological overlap between the proestrus and estrus stages.

Furthermore, England and Concannon (2002b) emphasized the need for concurrent hormonal assessment, noting that vaginal cytology alone may not provide sufficient accuracy in determining the precise onset and duration of estrus.

The observed discrepancies among studies may be attributed to differences in methodology, breed-specific reproductive patterns, and the inherent limitations of cytological evaluation when used in isolation from other diagnostic tools.

CONCLUSIONS

The findings of this study indicate that both Cabergoline and Buserelin acetate are effective in inducing estrus in anestrous bitches. Cabergoline demonstrated higher overall effectiveness in achieving successful estrus induction, making it a more reliable option in clinical settings. On the other hand, Buserelin acetate led to an earlier onset of estrus-related signs, suggesting its potential use when a more rapid response is desired. The choice between the two protocols may depend on specific clinical goals whether prioritizing success rate or time to onset.

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NOVELTY STATEMENT

This study introduces a novel approach by comparative between use of Buserelin, Cabergoline and control group in induce estrus suggesting their potential utility in practical breeding practices.

AUTHOR'S CONTRIBUTION

Hani Muneeb Alrawi conceptualized and designed the experiments. Amir Ali Hardan carried out the experimental work. All authors reviewed and approved the final version

of the manuscript.

Generative AI and AI-assisted technology statement

Artificial intelligence tools were not used.

Conflict of interest

The authors have declared no conflict of interest.

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