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Efficiency of PMSG +HCG Hormones in improvement Pregnancy Rate and Reproductive Performance in Anestrus Domestic Cats (*Felis catus*)

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Abstract

This study was designed to investigate the role of PMSG+ HCG hormone to improve ovulation in cats after natural breeding during out-of season and their effect to some reproductive parameters in domestic queen.-Twelve domesticated cats of known origin, age between 1-3 years old, addition to three a fertile male, cats were divided randomly into three equal groups; the first group of 4 queans was control (negative control) , the second group 4 queens treated with 200 IU PMSG hormone (positive control) to induction estrus cycle because the cats ~~are~~ were out of the breeding season, the third group 4 queens treated with 200 IU PMSG and 150 IU HCG. All females' three groups ~~are~~ were released with males during estrus to natural breeding. Blood collecting from jugular vein (2ml) before treatment in day zero and after treatment in day 14 to study the effect of treatment on level of progesterone hormone value. The male copulation and making a swab from the vagina to confirm estrus diagnosis. Also, in days 25 ultrasound techniques used to detect pregnancy then the animals follow up until parturition to detect gestation period, number of kitten birth and viability of kitten. The result of the current study records the control group not treated with PMSG did not show signs of estrus during the treatment period, while the two group when injected with PMSG showed estrus except one cat in one of the groups did not show estrus. The result of duration of response shows high significant effect in control group in $p \leq 0.05$ and showed the signs of estrus after 27± 7.00 days in two queens than treatment group (4.50± 0.86 and 3.67 ±0.88) in (PMSG and PMSG+HCG) groups respectively, also non-significant effect between two treatment groups in duration response. Also, the

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duration estrus (estrus phase) was 4.5 - 7 days did not have a significant difference between the two-treatment group (7.00±0.5 and 6.33±1.68) in (PMSG and HCG + PMSG) respectively than control group (4.50 ±.050). The result of present study we show the lowest pregnancy rate was in the control group and the PMSG group, followed by the HCG group high value increasingly. Also, the percentage of pregnancy rate high mathematically in PMSG +HCG group (**66.7 %**) than control, PMSG groups when the pregnancy rate percent was (50 %, 50%) respectively. The results of the current study show no significant effect in the pregnancy period, litter size, and viability rate in control group than two treatment group. Regarding the effect of treatment with HCG hormone to induced ovulation to progesterone hormonal value, we show increased the progesterone hormone after days 14 because induced ovulation and increased the number corpus luteal after used this program. Conclusion used of HCG to induce ovulation in domestic queens has no significant effect when the male is present with the females throughout the estrous phase but it lead to increase the pregnancy rate and progesterone value mathematically.

Key word: HCG, pregnancy, induce ovulation, estrus synchronization, female queens, progesterone hormone

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Introduction:

Domestic cats seasonally polyestrous, have induced ovulation and require external stimulation (natural breeding, for example) to trigger pituitary LH release and mature follicle ovulation (**Al-Zubaidi *et al*., 2024, Anas *et al.*,2025**). A female may release LH in response to one or several mating regimens, albeit not all mated queens will always exhibit this behavior (**Jeon *et al*., 2014; Hassan and Saleh, 2022).** Shorter duration or a decrease in amplitude of LH are only two of the many possible physiological reasons that might explain for ovulation failure in certain cats, even those with verified males that have copulated several times (**Shille *et al*., 1983**). But before ovulation can happen, queens are frequently sexually receptive so, breeding too early in estrus, that is, before the third or fourth day of estrus, can cause ovulatory failure and reduced LH production (**Goodrowe *et al*., 1989**). It may take many mating to elicit a maximum rise in LH, even if a single mating can trigger an LH response strong enough to trigger ovulation (**Wildt *et al*., 1980).** When Human chorionic gonadotropin (HCG) is used during queen breeding, fewer matings are required, and the gestation periods are predictable, in domestic cats, dosages of hCG used to induce ovulation vary from 25 to 500 IU (**Imad, 2025**). Using one dosage of hCG (250 IU) or two doses (100 IU each) to trigger ovulation, it was discovered that providing 100 IU HCG on the third day of estrus also produced ovulation rates that were adequate (**Donoghue *et al*., 1993**). Human chorionic gonadotropin and usually referred to as LH-releasing hormone (LH-RH) is commercially available

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medications most frequently used for inducing ovulation in cats (**Pelican *et al.*, 2006; Almeeni *et al.*, 2024**). This hormone acts directly on ovarian vesicular follicles (**Schmidt, 1986**). Ovarian stimulation procedures for felid assisted reproduction employ combination regimens of human chorionic gonadotrophin and equine chorionic gonadotrophin (eCG) (**Swanson *et al.*, 1995**). **La Polt *et al*, (1990**) found that treatment of eCG is used to stimulate ovarian follicular growth, and hCG is administered several days later to induce final follicular maturation and ovulation (**Brown *et al.*, 2002; Alabodi *et al.* ,2024**). The current study was conducted for the purpose of the study the ability of PMSG –HCG to induce fertile estrus in domestic queen.

Materials and Methods:

Ethics

Ethics Approval was granted through the local Animal Care Committee and used at the College of Veterinary Medicine within the University of Baghdad (Approval Number :P. G/2299 on 3/12/2024).

Experimental Animals

The 12 females queens will be randomly divided into three treatment groups: Group One (G1) without any treatment to keep as the control group. Group two (G2) was treatment with 200IU PMSG to control positive group, and group three (G3) was treatment with 200IU PMSG+150 IU HCG. The three males were used to estrus detection of the queen when they became in the estrus phase and natural breeding the queens. The current investigation was carried out in the animal house of the University of Baghdad's Faculty of Veterinary Medicine during out of breeding season between December 2023 and March 3, 2024. Three months for the experiment and one month for adaption.

Experimental design

-The queens will be observed daily for behavioral signs of estrus according **(Younis and Akram, 2023), (Alabodi and Almeeni ,2024) by** observation estrus clinical signs**. The** 12 females queens will be randomly divided into three treatment groups:

1-G1(4 cats without treatment) control negative group.

2-G2 (4 cats' treatment with 200 IU PMSG + natural mating) to control positive group.one dose

3- G3 (4 cats' treatment with 200 IU PMSG + 150IU HCG+ natural mating) to treatment group.one dose

All females' three groups are released with males during estrus to natural breeding. Blood collecting from jugular vein (2ml) before treatment in day zero and after treatment in day 14 to study the effect of treatment on level of progesterone hormone value.

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Results:

Effect of PMSG, PMSG + HCG on duration of response and estrus length in domestic queen cat

This study was conducted outside the breeding season for cats in November, which required the induction of estrus using the PMSG hormone. Therefore, two control groups were required (the first control group to confirm that the experimental cats were out of breading season and the second control group (PMSG group) to confirm the role of the hormones HCG in inducing ovulation after inducing estrus using the PMSG hormone).

Table (1) Effectiveness of application different hormones on response of duration and estrus phase in domestic queen cat

Groups	No.	Duration	Estrous phase
Control	2	27.00±7.00a	4.50±0.50a
PMSG	4	4.50±0.86b	7.00±0.91a
HCG +PMSG	3	3.67±0.88b	6.33±1.86a
LSD		7.11	4.33

Means with a different small letter in the same column are significantly different (P<0.05)

Effect of PMSG and PMSG+ HCG on pregnancy rate in domestic queens cat:

Table (2) Effectiveness of application different hormones on pregnancy rate in domesticated queens cat

Groups	N.equeen in estrus	N.queen pregnant	Pregnancy rate %	X^2value	P-value
Control	2	1	50 %	0.67	0.87NS
PMSG	**4**	**2**	**50 %**		
PMSG + HCG	**3**	**2**	**66.7 %**		

Effect of PMSG and PMSG +HCG on pregnancy period in domestic queen cat:

Table (3) Effectiveness of application different hormonal regimes on gestation period in domestic queen cat.

Groups	Gestation period
Control	67.00±0.00
PMSG	65.50±1.50
PMSG +HCG	64.00±1.00
LSD	2.61 NS

NS=non-significant

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Effect of the treatment of PMSG and PMSG+ HCG on litter size in domestic queen cat:

Table (4) Effectiveness of application different hormonal on litter size in domestic queen cat.

Groups	N. queen born	N.of kittens	Litter size
Control	1	4	4.00±0.00
PMSG	2	9	4.50±0.50
PMSG + HCG	2	9	4.50±0.50
LSD			2.71 NS

NS=non-significant

Effect of the treatment of PMSG and PMSG+ HCG on viability rate in domestic queen cat:

After the end of the pregnancy period and immediately during birth, live and stillborn births were recorded to determine the effect of inducing estrus and ovulation in the two treatment groups (PMSG and PMSG+ HCG) on live and dead (stillbirth) in domestic queens table 5. The result of present study not found significant effect in viability rate between the control group when the live kitten (75%) than treatment group (PMSG and PMSG+ HCG) when the number of live kitten (77.8 and 88.9) respectively also the stillbirth not found significant effect in control group (25%) than treatment groups (PMSG and PMSG+ HCG) when the stillbirth was (22.2 and 11.1) respectively. This result confirmed not effect of treatment in (PMSG and PMSG+ HCG) hormone to induction estrus and ovulation on viability rate this result agreement with **Socha *et al*. (2019)** Where he record the kittens, 12.5% were stillborn, and the kitten mortality rate increased when the time between expulsions exceeded 2 hours or when birth weight lower in female kitten. But my result disagrees with **Romagnoli *et al*., (2019**) when record stillbirths averaged 11.8% across the four breeds the number of stillborn kittens was positively correlated with litter size and this different result explain different breed and environment during parturition

Table (5) Effectiveness of application different hormonal on viability rate of new born kitten in domestic queen cat.

Groups	N. Kitten	N. alive %	N. stillbirth%	X2 Value	P-value
Control	4	2(50%)	2(50%)	8.18	0.04
PMSG	9	7(77.8%)	2(22.2%)		
PMSG +HCG	9	8 (88.9%)	1(11.1%)		
		N.S.	N.S.		

NS=non-significant

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Effect of the treatment of PMSG and PMSG+ HCG on progesterone hormone in domestic queen cat:

Table (6) Effectiveness of application different hormonal on progesterone hormone in domestic queen cat.

Groups	P4value before treatment Ng/ml	P4 value after treatment Ng/ml
Control	A 0.53±0.15 a	A 3.32±1.79 b
PMSG	A 0.57±0.16 a	A 4.32±1.79 b
PMSG + HCG	B 0.39±0.11 a	A 8.70±4.81 ab
LSD	4.11	7.11

Means with a different small letter in the same column are significantly different (P<0.05)
Means with a different capital letter in the same row are significantly different (P<0.05)

Discussion

In this study used PMSG hormone to induced estrus in tow group (PMSG group and PMSG+ HCG) to study queens' response to PMSG and duration response in table (1). The result of the current study is that the control group not treated with PMSG did not show signs of estrus during the treatment period, while the two groups when injected with PMSG showed estrus except for one cat in one of the groups that did not show estrus. The result of duration response we show high significant effect in control group in $p \leq 0.05$ showed the signs of estrus after 27± 7.00 days than in two queens than treatment group (4.50± 0.86 and 3.67 ±0.88) in (PMSG and PMSG+HCG) group respectively , also non-significant effect between two treatment groups in duration response , as two cats in the control group showed estrus after 27 days, indicating that the cats are out of the breeding season, as the control group enter estrus after approach the breeding season in January , while the two groups responded to the treatment and showed estrus after (4.50- 6.33) days . This is consistent with Swanson *et al.*,(1997) when study the pharmacokinetics and ovarian-stimulatory effects of equine and human chorionic gonadotropins administered singly and in combination in the domestic cat , when he used PMSG hormone to induce estrus in cats, he noted that 80% entered estrus at 3 -4 days after treatment this result agreement with **Yu *et al.*,(2010)** and **Nawaf and Ibrahim .,(2019)** when study effects of various dosages of equine chorionic gonadotropin (eCG) on superovulation induction for in vivo and in vitro embryo production.

Also, the duration estrus (estrus phase) was 4.5 - 7 days did not have a significant difference between the two treatment groups (7.00±0.5 and 6.33±1.68) in (PMSG and HCG + PMSG) respectively than control group (4.50 ±.050). This result confirms that induction ovulation by HCG in cats shortens the estrus period than PMSG, but without a significant difference. This my results agrees with **Yoshimura *et al.*,(2021)** he confirmed the combination of HCG injection with PMSG

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enhanced the ovulation rate of follicles without effect on estrus length also agreement with Kanca *et al*., (2014) and **A Abid *at el*., (2011**) he concluded the duration of behavioral estrus in non-ovulating (6.65 days) and ovulating (4.71) days in queens without significant effect also **Kutzler, (2007)** confirmed the induction of ovulation during estrus when used human chorionic gonadotropin (250 IU/cat, IM) the induction of ovulation will not shorten the length of that estrus period.

The queen induction estrus and ovulation by PMSG and PMSG+ hCG then natural breading queen by tom during estrous, after 25 days of treatment, the pregnancy was diagnosed by ultrasound to detection pregnancy rate in table 2. The result of present study we show the lowest pregnancy rate was in the control group and the PMSG group, followed by the HCG group increasingly, and the highest pregnancy rate was in the HCG group. Also, the percentage of pregnancy rate high mathematically in PMSG +HCG group (**66.7 %**) than control, PMSG groups when the pregnancy rate percent was (50 %, 50%) respectively. This is the first study that relies on evaluating ovulation hormones HCG on the pregnancy rate. All previous studies relied on evaluating ovulation hormones on the percentage of corpus luteum numbers, or the number of non-ovulatory follicles, or the number and quality of embryos collection from donor cats during their embryo transfer to recipient cats during assisting reproduction. This result agreement with Yoshimura ***et al*., (2021**) and Ibrahim and Nawaf, (2015) when enhances ovulation of queen ovaries treated using a combination of eCG and hCG, he records eCG treatment increased the number of follicles, regardless of the specific follicle size, hCG injection induced ovulation of developed follicles, and some follicles have regressed without ovulation. **Also, Swanson *et al*., (1997)** explain low fecundity after exogenous gonadotropin treatment possibly is related to a maternal environment unsuitable for supporting embryo development. It is well known that queens treated with eCG/hCG combinations typically develop ancillary ovarian follicles several days after induced ovulation and these follicles subsequently form secondary corpora lutea (CL), possibly disrupting the maternal environment.

After calculating the pregnancy rate by ultrasound, the birth stages were waited for to calculate the pregnancy period and investigation the effect of inducing estrus and ovulation by hormone (PMSG and PMSG +HCG) on the pregnancy period was known. The pregnancy period was calculated from the beginning of pregnancy (after mating) to the day birth in table **3** .The results of the current study show no significant effect in the pregnancy period in control group (67.00± 0.00) than treatment group (PMSG and PMSG +HCG) when the pregnancy period was (65.50± 1.5 and 64.00±1.00) but there was no significant difference between the groups, as the longest pregnancy period was 67 days in the control group and the shortest pregnancy period was 64 days in the HCG group. This explains that hormonal treatment led to early ovulation and decreased the pregnancy period in days, but this increase was not significant, and this is consistent with **Socha *et al*.,(2019)** and **Alabodi and Almeeni, (2024 b)** as the pregnancy period was ranging from 60 to 72 days, and 95% of the deliveries occurred between gestation day 62 and 68 and mean length of gestation was 65.5±1.32 days **,**where they confirmed that gestation length was influenced by litter size and gestation was shorter in queens carrying larger litters (seven or more kittens). Also, the result

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agreement with **Thakur *et al*., (2015)** when confirmed the mean gestational length in Persian cats was 64.92 ± 0.56 days with the range of 62 to 69 days and the correlation of the gestation length with the actual litter size gave a negative correlation value

After the birth, the number of kitten birth in queen during parturition was record to calculate the litter size in table 4 .The results of the current study showed that there was no significant difference in value levels of litter size in the control group was (4.00±0.00) than in treatment group (PMSG and PMSG +HCG) when the litter size was(3.5± 0.50 and 4.50± 0.50) respectively .The result shows the litter size ranged between(3.50 -5.67) within the normal range in the control group, and this is consistent with **Socha *et al*.,(2019)** when record the mean litter size was 5.3±2.3 kittens, his indicates that litter size is not affected by the induction of ovulation using hormones record from. Also, **Romagnoli *et al* (2019)** he records the mean litter size for Bengal and Norwegian Forest Cats was 4.2 ± 1.8 kittens, while the mean litter size of Maine Coons (5.5 ± 2.3). Other study **Sparkes *et al*., (2006)** were found to have a mean litter size of 4.0 close to their overall mean of 4.4, although found no evidence of an effect of age on litter size.

In this study, progesterone was measured before hormone therapy and 14 days after treatment to confirm ovulation in table 6 .The result of current study shown on significant effect before treatment between control(0.53±0.15) than treatment group (**PMSG and PMSG + HCG**) the progesterone value was (0.57±0.16 and 0.39±0.11) respectively but 14 days after treatment we show high significant p≤ 0.05 in PMSG + HCG when the progesterone record (8.70±4.81) than other group (**control, PMSG)** the progesterone value was **(3.32±1.79 ,** 4.32±1.79) respectively, Also with groups we shoe high significant effect after treatment in (**PMSG+HCG)** when the progesterone value was **(**8.70±4.81) than before treatment when the progesterone value was **(**0.39±0.11) but not found significant effect in progesterone value after treatment in control and PMSG group (3.32±1.79 , 4.32±1.79) than value record before treatment (53±0.15 , 0.57±0.16) respectively . This result explains increase the progesterone value after 14 days in increase the follicle ovulation by HCG and increase the corpora lutea, this organ secretion the progesterone hormone, this result agreement with **Rohlertz *et al*., (2012)** when used HCG and GnRH to induced ovulation, he explains the high-level progesterone after treatment the queen in the luteal phase and ovulation occur. Also, **Johnson, (2022)** he records the progesterone value in pregnancy, progesterone has been shown to peak between days 13 and 21 and then gradually decline, reaching baseline by 65 days after mating, the serum progesterone 5–7 days after mating will also determine if the queen has ovulated. Progesterone will be low (<2.0 ng/ml) if the queen failed to ovulate, but elevated (>2.0 ng/ml) if the queen did ovulate. The concentration of progesterone in serum rises after either spontaneous or induced ovulation or it must be present throughout gestation for pregnancy to be maintained **(Kustritz, 2006).**

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Conclusion the rate of pregnancy increased when PMSG and HCG hormones were used to induce ovulation when compared to the control group but the administration of HCG had no effect on improving some aspects of reproductive performance, such as the pregnancy rate, gestation time, proportion of living and dead fetuses, but lead to increasr the progesterone hormone value after pregnanc

Recommendation

The best way to induce estrus in cats outside the breeding season is to use the hormones hCG and PMSG, which is a preferred method to increase the pregnancy rate in cats, and we recommend using it. Using different concentrations of the hormones HCG and PMSG to induce ovulation in queens. Using other non-hormonal methods to induce ovulation in cats, such as vaginal stimulation and back caressing after natural insemination to stimulate ovulation.

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