Effect of estrus induction on pregnancy rates in domestic bitches and queens
Michelle A. Kutzler
Department of Animal Science-Companion Animal Industries, Oregon State University, Corvallis, OR

Abstract
Reported methods for estrus induction in bitches and queens include the use of synthetic estrogens (diethylstilbestrol), dopamine agonists (bromocriptine and cabergoline), gonadotropin releasing hormone agonists (lutrelin, buserelin, fertirelin, deslorelin, and leuprolide), exogenous gonadotropins (luteinizing hormone, follicle stimulating hormone, human chorionic gonadotropin, pregnant mare serum gonadotropin, and human menopausal gonadotropin) and opiate antagonists (naloxone). These methods vary widely in efficacy of inducing estrus as well as in the pregnancy rates following the induced estrus. The applicability of some of these methods for clinical practice is questionable. This review will summarize published reports on estrus induction protocols in domestic bitches and queens for which pregnancy rates are known. Only a brief overview of normal female canine and feline estrous cycle physiology will be discussed and non-medical methods of estrus induction (e.g. dormitory effect in the bitch and light therapy in the queen) will be excluded.

Keywords: Cat, dog, estrus induction, fertility, pregnancy rate

Introduction
Indications for estrus induction in the dog and cat include potential missed breeding opportunities or conception failure and the treatment of primary or secondary anestrus. Estrus induction improves the management of breeding colonies (number of litters per year), especially in establishments where a continuous supply of pups is required (e.g. service dog industry). In the research laboratory, it is often desirable to control the timing of pregnancy so that parturition occurs at the same time. In addition, reliable synchronous estrus induction is a necessity for synchronization of ovulation for embryo transfer programs. Estrus induction is also an effective tool for teaching veterinary reproduction.

Reported methods for estrus induction in bitches and queens include the use of synthetic estrogens (diethylstilbestrol), dopamine agonists (bromocriptine and cabergoline), gonadotropin-releasing hormone (GnRH) agonists (lutrelin, buserelin, fertirelin, deslorelin, and leuprolide), exogenous gonadotropins (luteinizing hormone [LH], follicle-stimulating hormone [FSH], human chorionic gonadotropin [hCG], pregnant mare serum gonadotropin [PMSG], and human menopausal gonadotropin [hMG]) and opiate antagonists (naloxone). These methods vary widely in efficacy of inducing estrus as well as in the pregnancy rates following the induced estrus. The applicability of some of these methods for clinical practice is questionable.

This review will summarize published reports on estrus induction protocols in domestic bitches and queens for which pregnancy rates are known. Only a brief overview of normal female canine and feline estrous cycle physiology will be discussed and non-medical methods of estrus induction (e.g. dormitory effect in the bitch and light therapy in the queen) will be left out.

Canine reproductive physiology
Canine reproductive physiology has unique characteristics that make extrapolation from farm animals unsuccessful in this species. Domestic bitches are nonseasonally monoestrous. As a result of this unique reproductive physiology, bitches spontaneously ovulate only once or twice per year and ovulation can occur at any time of the year. However, there are few exceptions, such as the Tibetan Mastiff and the Basenji. The interestrous interval is the time from the onset of proestrus to the subsequent onset of proestrus, and includes proestrus, estrus, diestrus and obligate anestrus periods. Proestrus is diagnosed clinically by the onset of vulvar edema and/or serosanguinous discharge, whereas estrus is defined as the onset of either behavioral signs (willingness to allow mating) or vaginal epithelial exfoliative cytology (>90% cornification). In some bitches, estrus is much less frequent or outward (visible) signs of estrus are minimal to non-existent, giving the appearance of a prolonged interestrous interval (persistent anestrus). The interestrous interval averages 31 weeks with a typical range of 16 to 56 weeks. Bitches with longer than average interestrous intervals have reduced opportunities of becoming pregnant. The variation in interestrous interval length owes itself to differences in the duration of anestrus. The duration of anestrus differs between and within dog breeds indicating a genetic basis for anestrus length. It is important to mention that neither the ovary nor the pituitary are quiescent during anestrus.

Factors that terminate anestrus and trigger the onset of an ensuing estrous cycle are not fully understood. In the bitch, progression from early to late anestrus is characterized by a higher amplitude and larger number of hypothalamic GnRH pulses, an increase in pituitary sensitivity to GnRH, and an increase in ovarian responsiveness.
to LH and FSH. Serum FSH concentrations are increased throughout much of canine anestrus while LH concentrations are low except near the end of anestrus. An increase in basal plasma FSH concentration is critical for initiation of folliculogenesis in dogs. Follicle-stimulating hormone induces expression of LH receptors in the ovarian granulosa cells. Following initial follicle recruitment, LH is progressively able to replace FSH in the support of follicular maturation. In fact, supraphysiological doses of LH alone administered to bitches in anestrus will induce follicle growth and proestrus. It is also important to note that factors causing a decrease in opioidergic activity promote LH release and the termination of anestrus.9

**Effect of estrus induction on pregnancy rates in bitches**

Estrus induction is most successful in normal females. Its efficacy in bitches with reproductive disorders is unknown. It is important to mention that histological changes similar to involution in the bitch’s endometrium are not complete until 135 days after the most recent estrous, regardless of whether the bitch was pregnant or not. Chakraborty, et al. demonstrated that induction of fertile estrous cycles is diminished when induction occurs less than 4 months following the onset of the last proestrus. Therefore, induction of estrus before this time may result in reduced pregnancy rates.

Gonadotropins

Both LH and FSH appear to be follicotropic in the dog as administration of pharmacologic doses of either LH or FSH alone induces estrus (Table 1). An estrus induction protocol was established with combined dosages of FSH and LH designed to resemble the gradual increase of endogenous FSH coincidentally with the LH increase during proestrus. However, this protocol was not successful (Table 1). The LH potency within purified or partially purified FSH products used may interfere with endogenous LH release. Bouchard, et al. demonstrated that LH cross-reactivity from contamination of porcine-derived FSH lasts 48 hours following administration. In addition, acute allergic reactions have been reported following intravenous administration of LH (5 mg) in two bitches.

In addition to exogenous pituitary gonadotropins, PMSG and HMG have been used for estrus induction in bitches. The most widely studied gonadotropin for estrus induction in the dog is PMSG, with protocols ranging from daily to weekly injections using either subcutaneous or intramuscular routes of administration (Table 1). Studies using PMSG have generally been more successful for estrus induction in bitches than those using FSH. Pregnant mare serum gonadotropin is not commercially available within the U.S. except in combination with hCG (PG600®, Intervet/Schering Plough Animal Health, Millsboro, DE). This product contains 80 IU PMSG and 40 IU hCG per ml. Nickson, et al. demonstrated that a single 5-ml injection of PG600® was highly effective at inducing proestrus in bitches (17 of 19). Unfortunately, the ovulation rate was poor (8 of 19), superovulation may have occurred and pregnancy rates were not reported. However, others have reported 50-84% whelping rates when PMSG and hCG are given in combination to induce estrus in bitches.

The most frequent problems encountered with PMSG arise from the unpredictability of an individual bitch’s response both in the number of follicles that develop and in the potential for allergic reaction and premature luteal failure. There has been one report of an immune-mediated reaction in a Boxer bitch that received a second PMSG injection for estrus induction and another case of a bitch that died unexpectedly following treatment with PMSG (2000 IU). Premature luteal failure with subsequent shortening of diestrus and pregnancy loss is a frustrating sequelae of PMSG use in canids. In one study, treatment with PMSG was followed by a progressive decline in progesterone concentrations below 1 ng/ml between 38-40 days post-estrus. Histologically, luteal cells from corpora lutea formed in bitches following PMSG treatment have reticulated and vacuolated cytoplasm compared to luteal cells from corpora lutea of normal, non-fertile estrous cycles that have compact and granulated cytoplasm.

Premature luteolysis of induced corpora lutea have also been reported in ewes, beef cows and dairy cows. In ruminants, pretreatment with a progestin prior to ovulation induction increases luteal weight and secretion of progesterone. However, bitches pretreated with megestrol acetate (2.2 mg/kg orally once daily for 8 days) before undergoing an estrus induction with PMSG (44 IU/kg intramuscularly once daily for 9 days) were not prevented from undergoing premature luteolysis as progesterone values were <1 ng/ml by 50 days post-estrus in all PMSG-treated bitches. It is of interest to note that such premature luteal regression appears to be independent of the presence or absence of the uterus. This was demonstrated following hysterectomy of normal, nonpregnant bitches on day 4 of estrus during a non-induced cycle. Hysterectomy resulted in premature regression of the corpora lutea. The authors speculated that a luteotrophic factor of uterine origin, which may be active in the normal cycle of the bitch at 24 days of diestrus may be involved in luteal maintenance.
Administration of an ovulation induction agent in bitches as part of an estrus induction protocol is controversial since bitches are spontaneous ovulators and such a treatment would be unnecessary. Administration of hCG has no positive effects on ovulation rates, pregnancy rates or number of offspring per pregnancy when administered at the onset of or during estrus. In fact, treatment with hCG on the first and third days of estrus significantly prolongs behavioral estrus and lowers serum progesterone concentration of day 5 of estrus. Volkmann, et al. found similar results when hCG was administered to bitches after day 40 of gestation; in that following an initial increase in serum progesterone concentrations, hCG dramatically suppressed progesterone secretion. Nevertheless, Wright reported that ovulation in the bitch occurs 26-30 hours following the administration of hCG and many protocols for estrus induction in bitches include its use. It should be noted that intravenous dosages of 5-10 mg LH have also been recommended to induce ovulation in bitches.

Estrogens

In anestrous bitches, treatment with estradiol 17-β has been shown to increase the concentration of GnRH in the hypothalamus. An estrogen peak occurring approximately 30 days before the onset of estrus is believed to be required to prime the hypothalamus-pituitary-ovarian axis, causing pulsatile release of LH. In addition, levels of mRNA encoding estrogen receptors α and β in the hypothalamus, pituitary and ovaries increase from late anestrus to proestrus in bitches. Different approaches have been investigated to induce the release of LH and the formation of LH receptors in preovulatory follicles using estrogenic compounds.

Protocols using estrogens for estrus induction in bitches typically also include FSH or PMSG for folliculogenesis and hCG or LH for induction of ovulation (Table 2). Successful induction of fertile estrus in bitches has been accomplished with diethylstilbestrol (DES) in various doses with or without FSH and LH. Using a combination of DES and FSH, pregnancy rates of 33% could be obtained immediately following parturition or following prostaglandin F2α (PGF) termination of diestrus. It is important to note that successful induction of fertile estrus in bitches can also be accomplished with DES alone (Table 2). In addition, substances with estrogenic properties (such as bis(p-acetoxyphenyl)cyclohexlidenemethane) have been used to successfully induce fertile estrus in bitches. While short-term (seven days or less) oral treatment with estradiol 17-β or DES reportedly does not produce any side effects, long-term oral DES therapy used to treat urinary incontinence may result in alopecia and bone marrow suppression.

GnRH and GnRH analogs

In the bitch, progression from early to late anestrus is characterized by a higher amplitude and larger number of GnRH pulses, an increase in pituitary sensitivity GnRH. Different approaches have been investigated to directly stimulate the activity of the pituitary with GnRH and GnRH analogs to induce estrus (Table 3). Pulsatile administration of GnRH at doses of 0.2-0.4 μg/kg at 90 min intervals is sufficient to obtain increases in LH similar to the endogenous pulses that normally occur at the end of proestrus. However, estrus induction protocols using short-acting native GnRH or GnRH agonists are not clinically applicable due to the expense of pulsatile infusion pumps or need for hospitalization during continuous intravenous infusion.

It is important to note that increases in GnRH do not need to be pulsatile to induce estrus. Constant infusion or release of a GnRH analog via a subcutaneous osmotic mini pump or implant resulted in similar estrus induction and pregnancy rates as GnRH pulsatile infusion, provided that the GnRH agonist therapy is discontinued. Premature luteal failure resulting in a shortened diestrus with subsequent pregnancy loss has been reported with GnRH agonist therapy for estrus induction.

Analogs of GnRH are used in human and veterinary medicine to stimulate (upregulate) as well as downregulate LH and FSH within the pituitary gland. By making molecular changes to native GnRH, more than 700 GnRH analogs have been synthesized that have an increased receptor affinity and enhanced stability. High rates of fertile estrus induction required GnRH agonist administration for >8 days. However, reduced efficacy of GnRH agonists occurs at high doses due to a failed or insufficient LH surge at the end of proestrus at doses of 24-48 μg/kg/day. In humans, intranasal administration of a GnRH agonist is used as a painless, simple and practical method for several gynecologic conditions. This intranasal spray (Leupron Depot, Takeda Chemical Industries, Osaka, Japan) was administered to 14 anestrous beagle bitches and produced no negative clinical effects and appeared to cause little stress to the animals. Deslorelin is a D-Trp6-Pro9-des-Gly10GnRH analog with two amino acid substitutions. Veterinary clinical applications of deslorelin in bitches were first introduced by Trigg, et al. during an investigation for a novel contraceptive, which is now commercially available in Australia (Suprelorin®, Peptech, North Ryde, NSW, Australia). Preliminary investigations with this product demonstrated that it induced estrus in all anestrous bitches treated initially, which was followed by prolonged estrus suppression. Deslorelin implants (Ovuplant®, Wyeth Animal Health, Guelph, ON, Canada) is a biodegradable, sustained release, subdermal
implant containing 2.1 mg of deslorelin, licensed for use in horses. According to label claims, Ovuplant® induces ovulation in mares within 48 h. Previous studies in dogs with this product demonstrate its reliability for inducing a rapid and synchronous estrus.\textsuperscript{56,57,71} Also, in diestrous bitches (n=15), synchronous estrus could be induced following termination of diestrus using PGF with either a whole (2.1 mg) or a half (1.05 mg) of a deslorelin implant.\textsuperscript{72} Treatment with PGF included starting with a low dose (50 μg/kg subcutaneously twice daily on the first day, followed by 100 μg/kg twice daily on the second day) and then the full dose (250 μg/kg subcutaneously twice daily) for 5 days.\textsuperscript{72}

Dopamine agonists

Dopamine agonists successfully induce fertile estrus in most bitches (Table 4). Dopaminergic agonists are ergot derivatives that inhibit prolactin secretion by stimulating secretion of dopamine or suppressing secretion of serotonin.\textsuperscript{73} Prolactin appears to play a part in canine interestrus intervals, possibly by affecting gonadotropin secretion and/or ovarian responsiveness to gonadotropins. Administration of dopamine agonists shortens the duration of anestrus\textsuperscript{74,75} or induces estrus in cases of prolonged anestrus.\textsuperscript{73-76} However, prolactin inhibition alone is not sufficient to terminate anestrus in bitches. This was demonstrated by treating bitches with low doses of a serotonin receptor antagonist (metergoline). Low doses of metergoline lower the plasma prolactin concentration via a serotonin-antagonistic pathway, while higher dosages also result in a dopamine-agonistic effect.\textsuperscript{77-79} At low dosages, metergoline suppresses prolactin concentrations similar to concentrations observed with dopamine agonists (bromocriptine and cabergoline), but does not induce estrus.\textsuperscript{78} However, at higher dosages (12.5 mg intramuscularly every three days until onset of proestrus), metergoline administration will result in estrus induction.\textsuperscript{80} These observations indicate that the induction of the follicular phase is not initiated by only the suppression of prolactin secretion but by other dopaminergic effects.\textsuperscript{81}

It was previously believed that prolactin inhibition was necessary for estrus induction to occur using dopamine agonists. Bitches that did not respond to dopamine agonist therapy (e.g. proestrus was not initiated) did not have a decrease in prolactin concentrations.\textsuperscript{9} These observations suggested that an inhibition of prolactin secretion may regulate the initiation of proestrus. However, in normal cycling bitches, prolactin concentrations during late anestrus do not change prior to the onset of proestrus.\textsuperscript{82} Then, in 2003, Beijerink, et al. demonstrated that bromocriptine shortens the interestrus interval in the bitch even when the dose is so low that it does not lower plasma prolactin concentration.\textsuperscript{83} This suggests that dopamine agonists induce estrus with another mechanism other than via lowering plasma prolactin concentration. Kooistra, et al. reported that follicle development and resulting estrus induction with bromocriptine was associated with an increase in plasma FSH concentration without a concomitant increase in plasma LH concentration.\textsuperscript{84} The dopamine agonist induced rise in the basal plasma FSH concentration was similar to what is observed during physiologic late anestrus.\textsuperscript{5} In rats, the dopamine agonists and antagonists affect ovarian steroidogenesis.\textsuperscript{85} Interestingly, in the mare, both dopamine receptors are present in the ovary\textsuperscript{86} indicating a possible direct role for dopamine agonists. However, data are not available for dogs. It should be noted that prolonged cabergoline administration during proestrus and estrus does not affect follicular development.\textsuperscript{87}

The effectiveness of dopamine agonists for estrus induction depends on dose, treatment duration and stage of anestrus. Beijerink, et al. demonstrated that the extent of shortening the interestrus interval by bromocriptine is dose dependent.\textsuperscript{88} Jöchle, et al. treated 28 beagle bitches that were 4-6 months after the last estrus with cabergoline (0.005 mg/kg/day for 14 days orally) and found no difference between controls in the interestrus interval.\textsuperscript{73} In addition, this method of estrus induction may require longer than 30 days of treatment before the onset of proestrus occurs, which is dependent upon the stage of anestrus (early versus late anestrus).\textsuperscript{88} In contrast to this report, Cirit, et al. found no correlation between the stage of anestrus and treatment duration when cabergoline was used for estrus induction.\textsuperscript{88}

Administration of dopamine agonists can be cost prohibitive in the United States, where these drugs are not readily available for veterinary use. Cabergoline (0.5 mg/tablet) is available as Dostinex® (Pfizer, New York, NY) and in a generic form (Par Pharmaceutical, Inc., Woodcliff Lake, NJ), but remains relatively expensive (approximately $20.00/tablet) and difficult to dose accurately in small dogs.\textsuperscript{89} For accurate dosing in dogs, tablets can either be compounded into the appropriate-strength capsules, or a portion of the tablet can be crushed and diluted with fluid just before dosing.\textsuperscript{89} Cirit, et al. observed that Dostinex® tablets totally and easily dissolve in distilled water at room temperature (10 μg cabergoline/ml).\textsuperscript{88} It had previously been reported by Persiani, et al. that the relative bioavailability of tablets versus aqueous solution of cabergoline was 99% and the pharmacodynamics and relative bioavailability was not influenced by formulation (tablet versus solution).\textsuperscript{90} Cabergoline is inactivated over time in aqueous solutions containing water, such that the cabergoline solutions should be prepared fresh daily.
and used within 15 minutes of preparation. However, McLean, et al. also report that cabergoline is stable for 28 days if compounded in acidic fluids (1% acetic acid solution).

There are two prominent side effects with dopamine agonists: coat color changes and vomiting. Approximately 25% of bitches that received cabergoline for 14-45 days developed coat color changes beginning the second week of administration and lasting until the next coat shedding. Of these, fawn-colored bitches developed a yellowish coat color while Argentine boarhounds became black spotted, mainly on their extremities. In previous untreated estrous periods, these bitches had shown no coat color changes. These authors postulated that a color shift in certain hair coats of particular breeds could be mediated through inhibition of melanocyte-stimulating hormone secretion. Transient coat color changes should be considered a possible side effect when planning long-term treatment with dopaminergic agonists in dogs.

Lastly, centrally acting dopamine agonists (e.g. bromocriptine) commonly induce vomiting. Vomiting was a frequent side effect (3-25% of cases) with bromocriptine or cabergoline occurring within one hour after the first treatment. Vomiting tends to be a less common side effect following cabergoline when compared to bromocriptine, probably because cabergoline binds more specifically to dopamine type-2 receptors in the hypothalamus and pituitary gland. It is important to note that Gunay, et al. did not observe any side effects of vomiting when they administered cabergoline to German Shepherds using a much higher dose of cabergoline (6 mg/kg) than the optimal effective dose (5 μg/kg/day once daily orally) as determined by dose response. Concomitant treatment with metoclopramide (0.5 mg/kg) relieved the vomiting and did not change the effect of the bromocriptine. Habitation to bromocriptine, beginning with lower doses initially, is reported to almost completely eliminate emesis as a side effect of treatment. It should be noted that even very high doses of metergoline do not induce vomiting in bitches.

In contrast to bitches, cabergoline has not been shown to be effective in domestic cats for estrus induction. However, administration of 25 μg/day for 5 days or 50/μg/day for 3 days of cabergoline is effective at pregnancy termination.

Normal feline reproductive physiology

Domestic cats are seasonally polyestrous. Queens are long-day breeders such that a prolonged anestrus occurs during short-day length (September–January in the Northern Hemisphere) but may cycle throughout the year in regions where natural day length exceeds 12 hours per day yearlong (e.g. Bangkok). Feline proestrus (0.5 - 2 days) is not commonly observed in queens. Estrus is defined by estrous (receptive) behavior or changes in cornified vaginal cytology secondary to increased circulating estradiol 17-β concentrations and last for a week on average. It is important to note that some queens do not display obvious signs of sexual receptivity. The first estrous cycle in cats is at 6 to 12 months of age on average, but can be as early as 4 months. Cats are induced (reflex) ovulators following adequate stimulation during mating. However, as many as 60% of unpaired, unmated and unstimulated female domestic cats ovulate without external provocation. If the queen does not ovulate, an interestrus period follows and the cycle repeats until the daylight length is shorter than 8 hours. Average length of the interestrous interval is 9.0 ± 7.6 days. If the queen ovulates but does not become pregnant, diestrus is shortened. Reported lengths of the interestrous interval in bred queens that ovulated but did not become pregnant are 45.0 ± 10.3 days, 50.3 ± 2.7 days and 61.5 ± 14.5 days.

The frequent natural estrous cycles in the nonbred queen make the utilization of estrus induction in clinical practice less likely. However, follicular development is readily induced during the non-breeding season. In fact, estrus induction response rates in queens are better during the non-breeding season. Tsutsui, et al. reported that quiescent feline ovaries during the non-breeding season respond more predictably to gonadotropin stimulation compared to during the breeding season when the onset of estrus is irregular in queens and hormone administration cannot be initiated on the same day for embryo transfer synchronization. The ability to suppress cyclic activity prior to ovarian stimulation has improved assisted reproductive success in the domestic cat. In part, this is because a quiescent ovary is more likely to respond to estrus induction treatments with a predictable number of ovulations and a lower incidence of ovarian hyperstimulation.

Effect of estrus induction on pregnancy rates in queens

Follicle-stimulating hormone

In domestic queens, estrus can be induced with FSH administered at a dose of 2 mg IM daily until the onset of estrus, which is typically in 3 to 7 days (Table 5). However, inconsistent results are observed with FSH in queens due to the variability observed among batches of partially purified FSH that has LH contamination. Excessive follicle number (pronounced ovarian hyperstimulation) can also result from FSH treatment, which may be
dose dependent. Approximately 10 ova were ovulated when partially purified porcine FSH was administered (6
mg total). Ovarian hyperstimulation reduces fertility in queens. Ovarian hyperstimulation is associated with
increased embryo degeneration in vitro, and possibly increased embryo degeneration in vivo resulting in reduced
litter sizes and pregnancy rates. Embryo degeneration may result from abnormal tubal transport or excessive
amounts of endogenous estrogen resulting from excessive numbers of follicles.

Pregnant mare serum gonadotropin

Pregnant mare serum gonadotropin has interesting effects as it exhibits both LH and FSH activity and has a
long half-life. In contrast to partially purified FSH, PMSG persists in circulation for at least 120 hours. As a
result, only one injection of PMSG is needed to induce follicular growth. This is in contrast to estrus induction with
FSH that requires daily or twice daily injections for up to a week. The feline ovary is very sensitive to PMSG.
However, season, age (prepubertal compared to postpubertal), and individual threshold all contribute to variation in
dosage needed to induce estrus. Colby found that PMSG doses of <100 IU in domestic cats would not induce
estrus. However, ovarian hyperstimulation was induced when a PMSG dose of 200 IU was administered to
domestic queens, resulting in an average of 39.1 ova ovulated per cat. Cline, et al. reported that the ovarian
hyperstimulation response produced by a PMSG dose of 300-500 IU resulted in reduced pregnancy rates (Table
5).

La Polt, et al. described an increase in ovarian LH receptors resulting from PMSG administration. This
may explain the reports of a high percentage of follicular cysts, prematurely luteinized follicles, unovulated follicles,
or follicles ovulated prior to breeding following PMSG estrus induction. Another disadvantage of PMSG is the
production of anti-gonadotropin antibodies and a secondary decrease in ovarian responsiveness to stimulation if
PMSG is administered too frequently. However, queens will repeatedly respond to PMSG with follicle
development as long as the duration between consecutive PMSG treatments is at least 6 months.

Naloxone

Endogenous opioids (e.g. β-endorphins) act on μ-receptors within the hypothalamus, which inhibit GnRH,
and subsequently LH, secretion. Administration of naloxone, an opioid antagonist, inhibits endogenous
opioidergic tone and induces estrus in queens. Gonadotropin-releasing hormone and LH release is also calcium
dependent. Binding of GnRH to receptors within the pituitary produces a rapid and temporary increase in
intracellular calcium ions, which results in LH secretion. Naloxone also modulates calcium entry through L-
type calcium channels. In patients with high endorphin concentrations, naloxone treatment induces a rapid
increase in intracellular calcium, resulting in LH secretion. However, pretreatment with hCG, which increases the
number of LH receptors, is necessary as administration of either hCG or naloxone alone does not induce estrus in
queens. Following pretreatment with hCG, 0.04 mg/kg of naloxone in a 20% calcium gluconate solution
administered intramuscularly once daily for 4 days beginning on the day of hCG administration resulted in an 88%
and 67% ovulation and pregnancy rate, respectively, with no undesirable side effects.

Effect of ovulation induction on pregnancy rates

As stated previously, queens are induced ovulators, requiring external stimulation (such as natural
breeding) to stimulate the release of pituitary LH and ovulation of mature follicles. However, queens are
often sexually receptive prior to the time when ovulation can occur. Breeding too early in estrus (prior to the third
or fourth day of estrus) can result in an attenuated LH secretion and ovulation failure. In addition, while the LH
response sufficient to induce ovulation can occur after a single mating, repeated matings may be needed to produce a
maximal rise in LH. In one report, 21% of queens ovulated after a single mating, whereas 83% of queens ovulated
after multiple matings. For this reason, allowing a queen to mate three times per day at 4 hour intervals
troughout estrus is recommended.

Use of an ovulation inducing agent in conjunction with estrus induction in queens reduces the number of
matings necessary and results in predictable gestation lengths. Once follicles are mature, ovulation can be induced
via exogenous administration of GnRH or hCG. Protocols using GnRH and hCG vary by frequency and route of
administration as well as timing of administration relative to the onset of estrus or initiation of estrus induction
treatment.

Single or multiple injections of GnRH have been reported to induce ovulation in queens. Although a
single injection of 5 to 25 μg/cat of GnRH increases serum LH concentrations in estrual domestic queens,
Chakraborty, et al. reported that only the 25 μg/cat dosage consistently resulted in ovulation. This is in contrast to
Swanson, et al. who found that domestic queens required two injections of GnRH 12 hours apart on fourth day of
estrus reliably induce ovulation.
Single or multiple injections of hCG will also induce ovulation in queens.\textsuperscript{119,120,126,127,129,146,147} Dosages of hCG for inducing ovulation in domestic cats range from 25 IU to 500 IU.\textsuperscript{119,120,126,127,129,146,147} Tsutsui, et al. used a single (250 IU) or two doses (100 IU each) of hCG to induce ovulation.\textsuperscript{148} Donoghue, et al. found that 100 IU hCG given on the third day of estrus also yielded satisfactory ovulation rates.\textsuperscript{149} Wildt, et al. reported that administration of 250 IU or 500 IU of hCG twice during estrus resulted in a significantly higher ovulation rates than 50 IU hCG twice during estrus.\textsuperscript{150} However, higher doses of hCG have a detrimental effect on oocyte quality.\textsuperscript{151,152} There was no significant difference on pregnancy rates when an hCG dose of 100 IU was compared to 200 IU.\textsuperscript{151} The hCG can be administered intramuscularly or intravenously. A 95% ovulation rate was reported following a single intramuscular dose of hCG (100 IU) on the third day of estrus.\textsuperscript{153} Tanaka, et al. reported a 100% ovulation rate with intravenous administration of two doses of hCG (100 IU) at 24 hour intervals between the second and fourth day of estrus.\textsuperscript{146} The same authors reported an ovulation rate of 91.7\% in queens given a single intravenous dose of hCG (250 IU).\textsuperscript{146} Queens that ovulate have higher estradiol concentrations during estrus than non-ovulating queens.\textsuperscript{154} The time of ovulation following hCG administration in domestic cats is reported to be 25-27 hours.\textsuperscript{155,156} However, Tsutsui, et al. reported ovulation occurring in 40\% of cats between 15 and 20 hours after hCG administration.\textsuperscript{155} Pregnancy rates (~33\%) following intravaginal artificial insemination with fresh semen at the time of hCG administration compared to 28 hours after hCG administration were not significantly different, although the results were slightly better when the AI was done 28 h after hCG treatment.\textsuperscript{155} Fertilization has also resulted after natural breeding or artificial insemination as late as 41 to 49 hours after hCG administration.\textsuperscript{125,157} The duration of sperm survival in the genital tract of the queen is not known but the observation that queens inseminated at the time of hCG administration provides evidence that fresh ejaculated cat sperm can survive in the female reproductive tract for at least 38 hours.\textsuperscript{153} Chatdarong, et al. also provided evidence that frozen-thawed cat semen can survive in the female reproductive tract for several hours after intrauterine insemination as 45\% of queens became pregnant when inseminated at the time of hCG administration.\textsuperscript{153} Artificial insemination more than 49 hours after hCG treatment does not result in fertilization presumably due to oocyte degeneration after this time.\textsuperscript{153,157} The timing of administration of an ovulation inducing agent relative to the administration of FSH or PMSG can affect pregnancy rates.\textsuperscript{129,154} By increasing the time after PMSG from 80 hours compared to 72 hours, pregnancy rates are increased from 35\% to 45\%.\textsuperscript{151} More recent protocols recommend extending the interval between PMSG and hCG to 84 hours.\textsuperscript{158} It is important to note that given alone to anestrous queens, hCG is highly folliculogenic.\textsuperscript{159} When hCG is given during estrus, it will often induce secondary follicular growth,\textsuperscript{124,129} which can result in embryo degeneration due to impaired oviductal transport secondary to abnormally elevated estradiol concentrations\textsuperscript{160,161} and reduce pregnancy rates.\textsuperscript{131,162} Also, because hCG persists in circulation for at least 96 hours\textsuperscript{124} it can stimulate the production of anti-gonadotropin antibodies, similar to the administration of repeated injections of PMSG.\textsuperscript{131} Therefore, administration of hCG more frequently than every six months is not recommended.

**Conclusion**

While many methods of estrus induction exist for both canids and felids, success (induction of estrus, ovulation, pregnancy and delivery of offspring) rates vary between and within various protocols. It is important to note that the results that have been reported here are for research animals. Long-acting preparations (placental gonadotropins, GnRH analog implants) are convenient for the owner and less stressful for the patient but are associated with premature luteal failure and subsequent reduced pregnancy rates. Because none of the agents discussed above are labeled for use in the United States, cabergoline, which is licensed in Europe for estrus induction in bitches, may be the preferred agent for canine patients. Although generic tablets are available in the United States, costs remain high. Gonadotropins (FSH, PMSG) can be used successfully in both dogs and cats, whereas dopamine agonists are only effective in bitches. Before administration, clients should be counseled on the fact that these are not approved agents in the United States and their use is off-label. Knowledge of the strengths and weakness of each regimen will assist the veterinarian in making a selection that will be best suited for each owner and patient. Owner consent must be obtained prior to using any estrus induction method in client-owned animals.

**Acknowledgements**

I thank Susan Craig for support in manuscript preparation.

**References**


202
Table 1. Published pregnancy rates following protocols using gonadotropins for estrus induction in the bitch.

<table>
<thead>
<tr>
<th>Reference</th>
<th>N</th>
<th>Estrus induction protocol</th>
<th>Pregnancy rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>[5]</td>
<td>5</td>
<td>FSH 0.77-1.1 mg IM once</td>
<td>20%</td>
</tr>
<tr>
<td>[5]</td>
<td>4</td>
<td>FSH 0.077-0.11 mg to 1.23-1.78 mg IM every 2 d; double dose of FSH every 2 d; repeat low dose once then double dose of FSH every 2 d; repeat low dose four times then double dose of LH for 2 d; injections given on 1st, 3rd, 5th, 7th, 9th, 11th d</td>
<td>0%</td>
</tr>
<tr>
<td>[5]</td>
<td>5</td>
<td>FSH 0.077-0.11 mg to 1.23-1.78 mg IM every 48 hours; repeat low dose once then double dose of FSH every 2 d; LH 0.077-0.11 mg to 0.38-0.55 mg IM every 48 hours; repeat low dose four times then double dose of LH for 2 d; injections given on 1st, 3rd, 5th, 7th, 9th, 11th d</td>
<td>0%</td>
</tr>
<tr>
<td>[13]</td>
<td>16</td>
<td>LH 0.1 IU/kg TID for 7 d</td>
<td>37.5%</td>
</tr>
<tr>
<td>[19]</td>
<td>18</td>
<td>PMSG 187 MU IM once</td>
<td>50%</td>
</tr>
<tr>
<td>[24]</td>
<td>11</td>
<td>PMSG 44 IU/kg SID IM for 9 d</td>
<td>13%</td>
</tr>
<tr>
<td>[25]</td>
<td>14</td>
<td>PMSG 20 IU/kg SID IM for 5 d</td>
<td>43%</td>
</tr>
<tr>
<td>[48]</td>
<td>9</td>
<td>PMSG 200-300 IU SQ once or 100 IU SQ every 4 d for a total of 200-300 IU</td>
<td>11.1%</td>
</tr>
<tr>
<td>[80]</td>
<td>10</td>
<td>PMSG 33.3-71.4 IU/kg SID IM until proestrus or up to 9 d</td>
<td>0%</td>
</tr>
<tr>
<td>[163]</td>
<td>6</td>
<td>PMSG 15.6-35.7 IU/kg SID SC for 10 d</td>
<td>50%</td>
</tr>
<tr>
<td>[163]</td>
<td>7</td>
<td>PMSG 15.6-35/7 IU/kg SID SC for 10 d</td>
<td>57%</td>
</tr>
<tr>
<td>[164]</td>
<td>8</td>
<td>PMSG 44 IU/kg SID IM for 9 d</td>
<td>60%</td>
</tr>
<tr>
<td>[164]</td>
<td>5</td>
<td>PMSG 44 IU/kg SID SQ for 9 d</td>
<td>60%</td>
</tr>
<tr>
<td>[165]</td>
<td>15</td>
<td>PMSG 27.8-41.6 IU/kg SID IM for 10 d</td>
<td>20%</td>
</tr>
<tr>
<td>[165]</td>
<td>5</td>
<td>PMSG 27.8-41.6 IU/kg SID IM for 10 d</td>
<td>0%</td>
</tr>
<tr>
<td>[166]</td>
<td>17</td>
<td>PMSG 20 IU/kg SID IM for 10 d</td>
<td>35%</td>
</tr>
<tr>
<td>[166]</td>
<td>6</td>
<td>PMSG 20 IU/kg SID IM for 5 d</td>
<td>50%</td>
</tr>
<tr>
<td>[167]</td>
<td>4</td>
<td>PMSG 21.2-26.9 IU SC every 48 hours for a total of 5 injections (1,250 IU total)</td>
<td>25%</td>
</tr>
<tr>
<td>[168]</td>
<td>3</td>
<td>PMSG 500 IU SID SC until onset of proestrus or up to 10 d</td>
<td>100%</td>
</tr>
<tr>
<td>[169]</td>
<td>10</td>
<td>hMG 1.7 U/kg SID IM for 9 d</td>
<td>40%</td>
</tr>
</tbody>
</table>

a Mouse units
b hCG 50 MU IM at time of PMSG injection
c hCG 500 IU IM or SQ on 10th d of treatment
d Premature luteal failure
e hCG 500 IU IM on 5th d of treatment
f hCG 500 IU IM on 2nd d of estrus
g Gonadoliberin 0.05 mg IM on 10th d of treatment
h hCG 200 IU IM on 5th d of treatment
i Pregnant with 17 fetuses at 36 d post mating
j All pregnancies resorbed
Table 2. Published pregnancy rates following protocols using estrogens for estrus induction in the bitch.

<table>
<thead>
<tr>
<th>Reference</th>
<th>N</th>
<th>Estrus induction protocol</th>
<th>Pregnancy rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>[16]</td>
<td>13</td>
<td>Diethylstilbestrol 5 mg(^a) PO SID until 2(^{nd}) d of proestrus; FSH 10 mg IM or 5(^{th}), 9(^{th}), and 11(^{th}) d from onset of proestrus</td>
<td>30.8%</td>
</tr>
<tr>
<td>[44]</td>
<td>5</td>
<td>Diethylstilbestrol 5 mg/dog(^a) PO SID for 6-9 d until onset of proestrus</td>
<td>100%</td>
</tr>
<tr>
<td>[46]</td>
<td>13</td>
<td>Diethylstilbestrol 0.1-0.2 mg/kg SID PO for 14 d; FSH 0.2-0.4 mg/kg IM on 5(^{th}), 9(^{th}), and 11(^{th}) d of treatment</td>
<td>31%</td>
</tr>
<tr>
<td>[48]</td>
<td>28</td>
<td>Diethylstilbestrol 0.5-2.0 mg(^b) every 4-5 d IM</td>
<td>62.5%</td>
</tr>
<tr>
<td>[20]</td>
<td>7</td>
<td>Estrone 100-600 μg(^d) IM every 24-48 hours (total dose 300-30,000 μg); PMSG 200-400 IU and hCG 1000 MU SQ at onset of proestrus(^e)</td>
<td>83.7%</td>
</tr>
<tr>
<td>[50]</td>
<td>7</td>
<td>Estradiol 17-β 0.5 mg/kg PO SID for 3 d; Leuprolide 0.0036 mg(^f) intranasal spray once daily following estradiol treatment until onset of proestrus or up to 14 d</td>
<td>71.4%</td>
</tr>
<tr>
<td>[132]</td>
<td>8</td>
<td>Bis(p-acetoxyphenyl)cyclohexylidene methans (P6066) 4 mg/kg PO SID until estrus</td>
<td>71.4%</td>
</tr>
</tbody>
</table>

\(^a\)Mongrel dogs were used but weight was not given;  
\(^b\)Airedale, foxhound and mongrel bitches, weights not given  
\(^c\)Results of breeding data only reported for 8 bitches  
\(^d\)Weight not given  
\(^e\)hCG 1000 MU and PMSG 200 IU given SQ at onset of estrus to induce ovulation  
\(^f\)Beagles were used but weight was not given;  
\(^g\)Only 7 bitches bred
Table 3. Published pregnancy rates following protocols using GnRH and GnRH analogs for estrus induction in the bitch.

<table>
<thead>
<tr>
<th>Reference</th>
<th>N</th>
<th>Estrus induction protocol</th>
<th>Pregnancy rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>[46]</td>
<td>36</td>
<td>GnRH 0.000015-0.000500 mg/kg IV every 90 min for 7-9 d</td>
<td>33%</td>
</tr>
<tr>
<td>[170]</td>
<td>8</td>
<td>GnRH 0.000096-0.000139 mg/kg IV every 90 minutes for 11-13 d</td>
<td>87.5%</td>
</tr>
<tr>
<td>[171]</td>
<td>8</td>
<td>GnRH 0.000040-0.000430 mg/kg IV every 87 minutes for 9 d</td>
<td>37.5%</td>
</tr>
<tr>
<td>[172]</td>
<td>10</td>
<td>Buserelin 0.0015 mg/kg SQ TID for 11 d and 0.00075 mg/kg SQ TID for 3 d</td>
<td>20%</td>
</tr>
<tr>
<td>[55]</td>
<td>24</td>
<td>Lutrelin 0.0017-0.0025 mg/kg/d SQ for 12-14 d</td>
<td>37.5%</td>
</tr>
<tr>
<td>[64]</td>
<td>6</td>
<td>Lutrelin 0.048 mg/kg/d SQ for 12-14 d</td>
<td>0%</td>
</tr>
<tr>
<td>[64]</td>
<td>6</td>
<td>Lutrelin 0.0024 mg/kg/d SQ for 12-14 d</td>
<td>33.3%</td>
</tr>
<tr>
<td>[64]</td>
<td>20</td>
<td>Lutrelin 0.0018 mg/kg/d for SQ 12-14 d</td>
<td>35%</td>
</tr>
<tr>
<td>[64]</td>
<td>6</td>
<td>Lutrelin 0.0012 mg/kg/d for SQ 12-14 d</td>
<td>33.3%</td>
</tr>
<tr>
<td>[64]</td>
<td>18</td>
<td>Lutrelin 0.0006-0.0024 mg/kg/d SQ for 12-14 d</td>
<td>88.9%</td>
</tr>
<tr>
<td>[64]</td>
<td>7</td>
<td>Lutrelin 0.0002 mg/kg/d SQ for 12-14 d</td>
<td>57.1%</td>
</tr>
<tr>
<td>[64]</td>
<td>24</td>
<td>Lutrelin 0.0006-0.0024 mg/kg/d SQ for 7-8 d</td>
<td>16.7%</td>
</tr>
<tr>
<td>[50]</td>
<td>7</td>
<td>Leuprolide 0.0036 mg/c intranasal spray once daily until onset of proestrus of up to 14 d</td>
<td>42.9%</td>
</tr>
<tr>
<td>[173]</td>
<td>18</td>
<td>Leuprolide 0.10 mg/kg SQ one</td>
<td>78%</td>
</tr>
<tr>
<td>[56,57]</td>
<td>7</td>
<td>Deslorelin 2.1 mg/d SQ one</td>
<td>43%</td>
</tr>
<tr>
<td>[71]</td>
<td>6</td>
<td>Deslorelin 2.1 mg vestibular submucosa one</td>
<td>67%</td>
</tr>
<tr>
<td>[71]</td>
<td>5</td>
<td>Deslorelin 2.1 mg vestibular submucosa one</td>
<td>40%</td>
</tr>
<tr>
<td>[72]</td>
<td>3</td>
<td>Deslorelin 2.1 mg vestibular submucosa once</td>
<td>67%</td>
</tr>
<tr>
<td>[72]</td>
<td>10</td>
<td>Deslorelin 1.05 mg vestibular submucosa once</td>
<td>70%</td>
</tr>
<tr>
<td>[72]</td>
<td>6</td>
<td>Deslorelin 2.1 mg vestibular submucosa once</td>
<td>16.7%</td>
</tr>
<tr>
<td>[72]</td>
<td>9</td>
<td>Deslorelin 2.1 mg vestibular submucosa once</td>
<td>11.1%</td>
</tr>
<tr>
<td>[174]</td>
<td>5</td>
<td>Deslorelin 1.5 mg IM once</td>
<td>60%</td>
</tr>
<tr>
<td>[175]</td>
<td>7</td>
<td>Deslorelin 2.1 mg vestibular submucosa once</td>
<td>42.9%</td>
</tr>
</tbody>
</table>

*aVia osmotic mini pump

*bPremature luteal failure

*cBeagles were used but weight was not given

*dFertirelin 0.003 mg/kg IM given on 1st d of estrus to induce ovulation

*eBeagle bitches weighing 5.4-13.6 kg

*fPregnancy resulted in complete resorption and 22% of bitches developed pyometra
Table 4. Published pregnancy rates following protocols using dopamine agonists (bromocriptine, cabergoline) or serotonin antagonists (metergoline) for estrus induction in the bitch.

<table>
<thead>
<tr>
<th>Reference</th>
<th>N</th>
<th>Estrus induction protocol</th>
<th>Pregnancy rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>[96]</td>
<td>48</td>
<td>Bromocriptine 0.3 mg/bitch for 3 d, then 0.6-2.5 mg/bitch PO SID continued 3-6 d after onset of estrus</td>
<td>83%</td>
</tr>
<tr>
<td>[38]</td>
<td>10</td>
<td>Cabergoline 0.005 mg/kg PO SID until 2nd d of proestrus or d 42 of treatment</td>
<td>60%</td>
</tr>
<tr>
<td>[38]</td>
<td>19</td>
<td>Cabergoline 0.0006 mg/kg PO SID until 2nd d of proestrus or d 42 of treatment</td>
<td>57.9%</td>
</tr>
<tr>
<td>[38]</td>
<td>8</td>
<td>Cabergoline 0.0006 mg/kg PO SID until 2nd d of proestrus or d 42 of treatment</td>
<td>75%</td>
</tr>
<tr>
<td>[73]</td>
<td>28</td>
<td>Cabergoline 0.005 mg/kg PO SID for 7-10 d</td>
<td>93.3%</td>
</tr>
<tr>
<td>[81]</td>
<td>5</td>
<td>Cabergoline 6 mg/kg SID PO until 2nd d of proestrus</td>
<td>100%</td>
</tr>
<tr>
<td>[88]</td>
<td>5</td>
<td>Cabergoline 0.005 mg/kg PO SID until 3-8 d after onset of proestrus or 40 d</td>
<td>60%</td>
</tr>
<tr>
<td>[88]</td>
<td>5</td>
<td>Cabergoline 0.005 mg/kg PO SID until 3-8 d after onset of proestrus or 40 d</td>
<td>100%</td>
</tr>
<tr>
<td>[88]</td>
<td>5</td>
<td>Cabergoline 0.005 mg/kg PO SID until 3-8 d after onset of proestrus or 40 d</td>
<td>80%</td>
</tr>
<tr>
<td>[96]</td>
<td>13</td>
<td>Cabergoline 6 mg/kg SID PO until onset of estrus or up to 14 d</td>
<td>84.6%</td>
</tr>
<tr>
<td>[172]</td>
<td>12</td>
<td>Cabergoline 0.005 mg/kg PO SID until progression of proestrus to estrus</td>
<td>83%</td>
</tr>
<tr>
<td>[176]</td>
<td>5</td>
<td>Cabergoline 0.005 mg/kg PO SID from 30 d past the LH peak until onset of preestrus</td>
<td>0%</td>
</tr>
<tr>
<td>[80]</td>
<td>12</td>
<td>Metergoline 0.56-1.2 mg/kg IM every 3rd d until proestrus of d 40 of treatment</td>
<td>75%</td>
</tr>
<tr>
<td>[80]</td>
<td>8</td>
<td>Metergoline 0.56-1.2 mg/kg IM every 3rd d until proestrus of d 40 of treatment</td>
<td>50%</td>
</tr>
</tbody>
</table>

*aCG 500 IU administered IM on 1st and 3rd d of estrus to induce ovulation
*bCG 500 IU administered IM in late proestrus to induce ovulation
Table 5. Published pregnancy rates following protocols using gonadotropins for estrus induction in the queen.

<table>
<thead>
<tr>
<th>Reference</th>
<th>N</th>
<th>Estrus induction protocol</th>
<th>Pregnancy rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>[50]</td>
<td>10</td>
<td>FSH 2.0 mg IM, then 1.0 mg IM SID until onset of estrus or the day following onset of estrus (5-6 mg total)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50%&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>[98]</td>
<td>3</td>
<td>FSH 0.5 mg SID IM for 5 d, then ½ FSH dose on 6&lt;sup&gt;th&lt;/sup&gt; d&lt;sup&gt;c&lt;/sup&gt;</td>
<td>100%&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>[98]</td>
<td>3</td>
<td>FSH 0.5 mg SID IM for 5 d, then ½ FSH dose on 6&lt;sup&gt;th&lt;/sup&gt; d&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0%&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>[98]</td>
<td>3</td>
<td>FSH 0.5 mg SID IM for 5 d, then ½ FSH dose on 6&lt;sup&gt;th&lt;/sup&gt; d&lt;sup&gt;c&lt;/sup&gt;</td>
<td>100%&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>[98]</td>
<td>3</td>
<td>FSH 0.5 mg SID IM for 5 d, then ½ FSH dose on 6&lt;sup&gt;th&lt;/sup&gt; d&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0%&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>[98]</td>
<td>3</td>
<td>FSH 0.5 mg SID IM for 5 d, then ½ FSH dose on 6&lt;sup&gt;th&lt;/sup&gt; d&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0%&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>[98]</td>
<td>3</td>
<td>FSH 0.5 mg SID IM for 5 d, then ½ FSH dose on 6&lt;sup&gt;th&lt;/sup&gt; d&lt;sup&gt;c&lt;/sup&gt;</td>
<td>100%&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>[177]</td>
<td>7</td>
<td>FSH 2.0 mg in saline IM SID until onset of estrus&lt;sup&gt;d&lt;/sup&gt;</td>
<td>71.4%</td>
</tr>
<tr>
<td>[45]</td>
<td>9</td>
<td>PMSG 100 IU SQ once&lt;sup&gt;e&lt;/sup&gt;</td>
<td>78%</td>
</tr>
<tr>
<td>[45]</td>
<td>20</td>
<td>PMSG 100 IU SQ once&lt;sup&gt;f&lt;/sup&gt;</td>
<td>18%</td>
</tr>
<tr>
<td>[45]</td>
<td>18</td>
<td>PMSG 100 IU SQ once&lt;sup&gt;e&lt;/sup&gt;</td>
<td>19%</td>
</tr>
<tr>
<td>[46]</td>
<td>10</td>
<td>PMSG 100 IU IM 1&lt;sup&gt;st&lt;/sup&gt; d, then 50 IU SID for 2 d&lt;sup&gt;h&lt;/sup&gt;</td>
<td>100%&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>[58]</td>
<td>5</td>
<td>PMSG 100 IU IM 1&lt;sup&gt;st&lt;/sup&gt; d, then 50 IU IM on 2&lt;sup&gt;nd&lt;/sup&gt; and 3&lt;sup&gt;rd&lt;/sup&gt; d&lt;sup&gt;i&lt;/sup&gt;</td>
<td>100%&lt;sup&gt;k&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Bred to a fertile male  
<sup>b</sup>On the basis of embryos collected  
<sup>c</sup>hCG 250 IU IM on 6<sup>th</sup> and 7<sup>th</sup> treatment d  
<sup>d</sup>hCG 250 IU IM on 1<sup>st</sup> and 2<sup>nd</sup> of estrus  
<sup>e</sup>hCG 50 IU IM once 7 d after PMSG  
<sup>f</sup>hCG 50 IU IM at time of PMSG and each subsequent d for 4-5 d (300-350 IU total)  
<sup>g</sup>hCG 50 IU IM at time of PMSG and each subsequent d for 7-8 d (400-450 IU total)  
<sup>h</sup>hCG 250 IU twice at 16 h intervals 7-8 d after initial PMSG injection  
<sup>i</sup>26-65 embryos produced/queen  
<sup>j</sup>hCG 500 IU on 7<sup>th</sup> d  
<sup>k</sup>24-53 embryos recovered/queen