

Ultrasound-guided induced fetal death, an alternative method for induction of abortion in the bitch

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Abstract

This study was performed to achieve abortion in a short time and with minimum side effects in a bitch, as an alternative method for termination of unwanted pregnancy. The experimentation was performed on 10 privately owned crossbred pregnant bitches, in their late second trimester of pregnancy, having a variable number of fetuses (3 to 9). Fetal death was induced by transabdominal intracardiac injection of potassium chloride (KCl) into the fetal heart under ultrasonographic guidance. Pregnancy was terminated within 36 to 72 hr (51.60 ± 16.04 hr) and none of the patients experienced any side effects or clinical complications. Data presented in this report provided evidence for the possible use of this technique to selectively reduce the number of canine fetuses without terminating the whole pregnancy. Ultrasound-guided induced fetal death is a safe procedure and a viable method for the induction of abortion in a short time and with no apparent side effects.

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Introduction

Pregnancy termination due to mismatch is one of the most common "reproductive" requests from dog and cat owners. Where the animal is a potential breeder, drugs are available that can prevent or terminate the pregnancy.¹ The most common therapies to terminate pregnancy rely on luteal demise, because the bitch requires an ovarian luteal progesterone source to maintain pregnancy. Plasma progesterone concentrations also is diminished in response to fetal death. When serum progesterone drops below 2 ng mL^{-1} for 24 hr, abortion occurs.²

Prostaglandin F_{2α} (PGF_{2α}) and its synthetic analogs have luteolytic activities and abortive effects. Because of these pharmacological properties, these substances have been used for termination of canine pregnancy in many experimental studies.²⁻⁷ However, subcutaneous or intramuscular doses of these drugs need to be administered for several days to ensure luteolysis and usually cause undesirable side effects such as vomiting, salivation, defecation, dyspnea and hyperventilation, shock and in some cases death.⁸ Other drugs that cause serum progesterone to decline are the dopaminergic drugs that

are prolactin inhibitors. Prolactin is luteotropic and is required to maintain luteal function during the second half of gestation.⁹ Using prostaglandins, the bitch should be examined and confirmed at least 30 days pregnant before initiating a prolactin inhibitor treatment protocol.¹ Bromocriptine for six days intramuscularly induced abortion in all bitches greater than 42 days of pregnancy, however, it did not induce abortion in bitches less than 23 days of gestation. Side effects of bromocriptine treatments included vomiting, diarrhea, and listlessness 1 - 3 hr after treatment.¹⁰ Cabergoline injected subcutaneously for five or six days at 25 to 40 days after the first mating resulted in abortion for all bitches greater than 40 days of gestation, however, only 50.00% of those less than 40 days.¹¹ To reduce the side effects and increase the efficacy of prostaglandins and prolactin inhibitors, a combination of the two drugs has been used. Alphaprostol or cloprostenol was injected subcutaneously in combination with cabergoline daily for five days from midgestation. Efficacy for abortion was 100% and side effects were less severe with cloprostenol than with alphaprostol. In the group treated with the low dose of cloprostenol, no adverse side effects were noted.¹² Other drugs such as mifepristone and

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aglepristone which are progesterone blockers and compete with progesterone for binding to its receptor are also effective, however, very expensive.¹ Mifepristone, more commonly known for its use in preventing human pregnancies, works by preventing the progesterone binding to uterine progesterone receptors and therefore preventing the action of progesterone from occurring. Oral administration of mifepristone twice daily for 4.50 days starting at the thirty-second day of gestation resulted in 100% of the bitches having a decline in progesterone two days after treatment started and pregnancy loss with no side effects around three days after treatment initiation.¹³ Two doses of aglepristone given subcutaneously 24 hr apart on days 0 to 25 or day 26 to 45 after mating caused uncomplicated abortions within seven days in all bitches.¹⁴ If the bitch was in mid-pregnancy, a brown mucoid vaginal discharge was seen 24 hr before fetal expulsion. Other side effects included slight depression, transitory anorexia, and mammary gland congestion.¹⁵

Oral dexamethasone appears to be a safe, efficacious abortifacient in the bitch. The most effective dosage regimen was an administration of oral dexamethasone using a 9.50-day dosage schedule which is more effective than a shorter 7.50-day dosage regimen in terminating pregnancies. Because dexamethasone is so readily available, inexpensive, with relatively minor side effects reported, does not require hospitalization, and is effective, it is the method recommended. Although not the ideal drug, it offers the most advantages of the ideal mismatch drug when compared to all the other products available. No drug, however, meets all the following criteria of a perfect mismatch drug including no restriction for the time of administration (can be given at any stage of estrus or pregnancy), being 100% effective, causing no vaginal discharge with no side effects or lasting damage which may impair future fertility, inexpensive and readily available.¹

Apart from the use of systemic drugs, the administration of chemicals directly to the fetus has also been practiced to terminate the pregnancy or to reduce the number of fetuses. In a recent study, it has been demonstrated that the intra-vesicle administration of a single low dose of d-cloprostenol is an effective and safe technique for the induction of abortion in the mid-gestation in bitches within five days.¹⁶ In human medicine, multifetal pregnancy reduction technique (MFPR) is used to decrease the risk of complications associated with multiple gestations.¹⁷ The most common method of reduction is the transabdominal injection of potassium chloride into the fetal cardiac region, usually performed at or after the ninth week of gestation. A 22-gauge needle is inserted through the uterine wall, then into the amniotic cavity, and finally pushed into the fetal thorax. Then, potassium chloride solution (amount ranging from 0.50 to 5.00 mL) is injected, leading to the cessation of heartbeat.

It is believed that a significantly higher risk of pregnancy loss is associated with a fetal reduction in humans after the first trimester.¹⁸ In the present study, utilizing this technique, we attempted to induce fetal death and eventual abortion in the bitches. The goal was to decrease the period required for abortion to occur and to prevent the incidence of adverse effects, compared to methods in which prostaglandin or other drugs are administered. We did not reduce all of the fetuses at once so that there would be an opportunity to also determine if it is possible to decrease the number of fetuses in dogs as is the case in human medicine.

Materials and Methods

Eighteen bitches were referred to the Small Animal Hospital, Faculty of Veterinary Medicine, University of Tehran, to have their pregnancies terminated after unplanned breeding. Ten dogs were confirmed to be pregnant by abdominal ultrasonography and were used for the investigation described below. Ethical approval was obtained from the local Research Ethics Committee, and legislation on animal protection was applied. All the animals enrolled in the study were crossbred bitches of different ages (1.00 to 8.00 years) and weighted 12.00 to 31.00 kg. Clinical examinations were performed and blood samples were collected for routine hematologic studies before the procedure. Estimation of gestational age was determined by measuring the gestational sac diameter,¹⁹ up until about day 40 when it reaches approximately 3.00 cm in diameter. Head diameter was measured as the most accurate predictor of gestational age when gestational sac was greater than 3.00 cm in diameter and the skull bones could be identified.¹⁹

The animals were fasted for 24 hr and were given intravenous prophylactic antibiotic (Cefazolin, 20 mg kg⁻¹; Daana Pharma Co., Tabriz, Iran) before the treatment. The bitches were anesthetized using 10 mg kg⁻¹ ketamine hydrochloride (Alfasan, Woerden, Netherlands) and 0.20 mg kg⁻¹ diazepam (Mylan, California, USA), intravenously. The abdominal wall was shaved and aseptically prepared. The head of the transducer was covered with a sterile surgical glove containing a small amount of coupling gel. The operator wore surgical gloves and the sterile gel was used to maintain proper contact between the transducer and the skin. All the procedures were performed using B-mode ultrasonography (Voluson 730 PRO; General Electric Co., New York, USA) and using multifrequency (5.00 - 8.00 MHz) convex transducer. Using a trans-abdominal approach, with the transducer held by the operator and under direct ultrasonic visualization, a 22-gauge disposable spinal needle was introduced into the thorax of the most accessible fetuses with their heart closest to the probe (Fig. 1). Those fetuses farther away from the abdominal wall or being underneath the other fetuses

were avoided. The needle tip was placed directly into the fetal heart and 200.00 μL of potassium chloride (KCL; 2.00 mEq mL^{-1} , Institute Pasteur, Tehran, Iran) was inoculated to cease the cardiac activity. The dosage of injected potassium chloride was simulated by human fields based on previously published articles.²⁰⁻²² When the cardiac arrest was observed for 60 sec, the needle was withdrawn. The procedure was repeated for two additional fetuses. To have an opportunity to simultaneously assess the possibility of a selective fetal reduction in the bitches, no more than three fetuses were reduced. After the procedure, all patients underwent a repeat scanning. If the cardiac activity was observed in a fetus subjected to a termination procedure, the procedure was repeated. If any of the animals did not abort and live fetuses were observed on ultrasound scans after 72 hr, the procedure was repeated on more fetuses.



Fig. 1. A) Voluson 730 PRO, GE unit with a multifrequency (5.00-8.00 MHz) convex transducer. **B)** Transabdominal approach, an external guide was attached to the transducer and held by the operator. Under direct visualization, a spinal needle (22-gauge) was introduced into the thorax of the most accessible fetus.

To determine if potassium chloride had any effects on the electrical activity of the maternal heart, simultaneous electrocardiograms of the bitches were recorded before, during and 5 min after the procedure. Furthermore, each 12 hr, the bitches were monitored by ultrasonographic and radiographic studies from the day of treatment until the completion of abortion for any evidence of uterine contractions, vaginal bleeding, leakage of amniotic fluid and other fetuses' growth condition. Any change in temperature, behavior, appetite, nature of feces, presence, and nature of vaginal discharge were documented as well.

Results

All animals were proved to be clinically healthy and their routine blood measures were within normal limits. The number of fetuses that could be distinguished among individual bitches varied from three to seven. Animals were on different days of pregnancy ranging as early as 35 and as late as 40 days. The period which took for each operation to be performed was not more than 15 min. In all patients when the needle was accurately placed into the fetal heart, and blood was seen within the hub of the needle, there was no sign of cardiac arrest but a few seconds later when a very little amount of potassium chloride was injected, immediate cessation of the heartbeats occurred. However, in some other instances, the cardiac arrest did not happen instantaneous to the injection and it was after a few seconds of bradycardia that contractions finally faded away. All animals recovered from anesthesia quickly, without any complications or any signs of pain or discomfort. The dark vulval discharge was observed in four of the bitches, beginning just a few hours before initiation of fetal expulsion. After abortion, ultrasound examinations were performed. Even though no evidence of retained fetal or placental masses was seen in any of the cases, the expulsion of fetuses was documented in only eight bitches. No changes in behavior, appetite, or general health were observed in any of the treated animals. Complete termination of pregnancy, defined as expulsion of all fetuses, occurred 36 to 72 hr after initiation of the procedure (Table 1). Three of the bitches (numbers 6, 7, and 8) did not abort after the first attempt. Following 72 hr from the treatment all three cases underwent ultrasonographic exams and reduced fetuses were detected with no heartbeats, collapsed posture, and hypoechoic internal organs. At the same time, other viable fetuses were grown and had healthy heart rates. The experiment was repeated on three more fetuses in each animal. Following 72 hr, pregnancy was terminated in the bitch (number 8) which had all of its fetuses reduced. The treatment was repeated for case numbers 6 and 7 for the third time and it was within the next 36 hr (180 hr from the first treatment) that total abortion took place for both bitches when all of their fetuses were reduced (Fig. 2).

Table 1. Termination of pregnancy in the bitch by ultrasound-guided injection of potassium chloride. The Table shows the information on the day of pregnancy in which operation was performed, the total number of fetuses, number of fetuses which underwent reduction and the time it took for abortion to take place. To assess the possibility of fetal reduction, for bitches numbers 6, 7, and 8, the procedure was deliberately repeated once or twice on the remaining live fetuses 72 hr after 1st, 2nd, and 3rd attempts.

Bitch No.	Day of Pregnancy	Total number of fetuses	Number of reduced fetuses			Time of pregnancy termination
			1 st	2 nd	3 rd	
1	41	6	3	-	-	Within 72 hr
2	39	7	2	-	-	Within 48 hr
3	40	7	3	2	2	Within 36 hr after 3 rd attempt
4	37	7	2	2	3	Within 36 hr after 3 rd attempt
5	38	5	3	2	-	Within 36 hr after 2 nd attempt
6	35	6	3	-	-	Within 72 hr
7	34	5	3	-	-	Within 72 hr

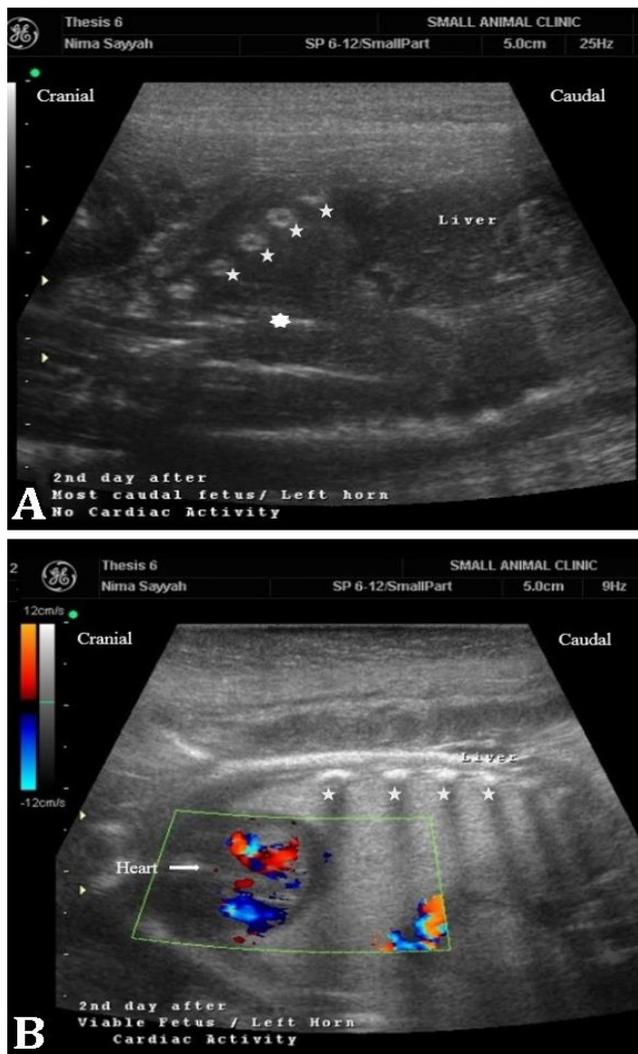


Fig. 2. A) Sagittal plane of the dead fetus without cardiac activity. Internal organs of the thoracic cavity were hypo-echoic with a minimum distinction. **B)** Color Doppler image of the viable fetus with normal cardiac activity. Both images were obtained from the same depth and with the same machine settings. ★ indicates Abnormal ribs with decreased density without acoustic shadowing (A), normal ribs with proper density and acoustic shadowing (B), and * indicates Location of collapsed heart in the thoracic cavity of a dead fetus.

Comparing electrocardiograms recorded before the procedure and those immediately after the last injection, no obvious changes were detected that could be correlated with the effects of potassium chloride on the maternal cardiac activity.

Discussion

This is the first report of termination of unwanted pregnancy using ultrasound-guided induced fetal death in the bitch. The outcome of the current investigation suggested that ultrasound-guided injection of potassium chloride into the heart of the fetuses was an effective technique that can offer a rapid and side effect free alternative to induce abortion in dogs.

Pregnancy was terminated on an average of 51.60. ±16.04 hr after the procedure. Compared to other methods of pregnancy termination, the ultrasound-guided injection of potassium chloride into the heart of the fetuses significantly reduced the time before an abortion. Abortion is usually complete within nine days with systemic administration of natural PGF2 α ,² within 4 to 7 days with synthetic analogs such as d-cloprostenol,²³ and within eight days with progesterone receptor antagonists such as aglepristone.²⁴ Based on the time of pregnancy termination for those cases in which all of the fetuses were reduced, we postulated that abortion may be reached in a mean time closer to 36 hr if all of the fetuses were reduced altogether.

Pregnancy seemed to have continued in three bitches (numbers 6, 7, and 8) following 72 hr after the first attempt for fetal reduction. The procedure was repeated, however, only bitch number 8 in which no live fetuses were remaining, aborted in 36 hr. Cases number 6 and 7 aborted after the third attempt and they carried their pregnancy for approximately 7.50 days after the first procedure. It was observed that in such cases, if not subjected to next treatments, undeveloped reduced fetuses would be expelled at the time of labor with the remaining viable fetuses without compromising the healthy growth of other fetuses.

In the present study, fetus expulsion was documented in only one of the bitches. This could be suggestive that the

other two animals might have digested their fetuses as soon as they were expelled.

Nevertheless, there were bitches in similar stages of pregnancy, in which selective reduction could not be achieved and the experiment resulted in total pregnancy loss. It has been demonstrated that in humans, a significantly higher risk of pregnancy loss is associated with fetal reduction after the first trimester.¹⁸ Furthermore, it is suggested that total pregnancy loss may have resulted if the KCl solution accidentally reaches the amniotic fluid of the remaining fetuses.²⁵ We predicted that there would be a higher success rate for selective reduction of the fetuses in the bitches if the procedure was performed early in the second trimester, as soon as the heart was detectable on ultrasound examinations. The potentiality of selective fetal reduction in the bitches may find some application in researches or where medical issues necessitate reproductive interventions.

In one study Pasquini *et al.* found out that potassium chloride injected directly into the left ventricle induced immediate asystole, and it was a safe and effective method of termination of pregnancy.²¹ They also emphasized that no maternal or fetal complications were associated with the procedure in their series. However, it was important to follow a strict protocol to confirm fetal asystole as the consequences for failed feticide were significant.²¹

In conclusion, ultrasound-guided induced fetal death was a very effective technique for pregnancy termination. No immediate side effects were noted, there was no sign of a problem in future pregnancy as well. Ultrasound-guided intracardiac injection is reasonably feasible, quick and safe to perform. This precision, however, has the downside of requiring anesthesia, specialized equipment, and trained operators which might limit its applicability to veterinary medical centers. Ultrasound-guided intracardiac injection is feasible to perform, provided that adequate sedation is achieved so that respiratory movements do not interfere with accurate needle placement. Inhalation anesthesia would be appropriate if the procedure is going to be performed on all of the fetuses, more time is required, or when better respiratory management is preferred. Injecting potassium chloride into the fetal hearts did not seem to influence maternal cardiac activity, and there is no such report in the literature addressing KCl affecting the mother's health after MFPR. Although doses of potassium chloride used in this study were much lower those used in humans, more work is required to specifically show that injecting six or nine fetuses all at once is also safe for the bitch. Whilst transabdominal injection of KCL is the most common method of fetal reduction, several methods of MFPR are available in human medicine based on different combinations of three components, i.e., the use of an embryotoxic agent, the timing of the procedure, and the route of approach.²⁶⁻²⁸ Further studies will be required to specify when and which method is the best option in the

bitch either for a total termination of pregnancy or for selective fetal reduction.

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Conflict of interest

The authors declare that there is no conflict of interest.

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