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Medical termination of pregnancy in cynomolgus macaques

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Abstract

Background—Although pregnancy is expected during studies of novel contraceptives in nonhuman primates, gestation, delivery and lactation remove females from groups for prolonged intervals. Since the macaque cervix does not facilitate transcervical surgical termination of pregnancy, we sought to establish a medical termination protocol.

Methods—A descriptive case-series of outcomes of medical termination of pregnancy up to 32 days gestation in cynomolgus monkeys. Efficacy and time to uterine resolution were determined according to medication, dose, and route of administration.

Results—37 macaques underwent 65 medical terminations. Over 80% of animals terminated after initial treatment with mifepristone 20 mg intramuscularly (IM). Intrafetal methotrexate was effective for salvage treatment. Medical termination regimens were less effective for animals receiving investigational contraceptive agents.

Conclusions—Medical termination for macaques is safe and effective. We recommend a protocol with mifepristone 20 mg IM and misoprostol 200 mcg buccally as initial treatment.

Keywords

Medical termination of pregnancy; contraception; mifepristone; methotrexate; misoprostol

Introduction

Macaques are commonly used laboratory animals in reproductive research, including studies investigating novel contraceptive agents. Cynomolgus macaques (Macaca fascicularis) are particularly well suited for proof-of-concept studies of novel contraceptives [1]. Their relatively small size, social behavior, and non-seasonal breeding allow the establishment of breeding groups of females. Introduction of a single fertile male cynomolgus monkey into these groups will result in high rates of pregnancy [2]. During a contraceptive trial, 80–90% of control animals will become pregnant within five cycles [1]. Pregnancies may also occur in animals receiving contraceptive agents as a result of failure of the method. Continuing a pregnancy will remove the animal from research participation for over a year to complete gestation, give birth, and then wean the infant [3]. Therefore from a husbandry standpoint, a systematic way to terminate unwanted pregnancies to enable return of females to study groups is needed.

Routine surgical termination of pregnancy via transcervical uterine aspiration is not feasible due to the cervical anatomy of the macaque [4] (Figure 1). Medical termination represents

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an alternative means of managing unnecessary pregnancies in research animals. Although literature has established the safety and efficacy of medical termination in humans [5], few studies have investigated the utility of these regimens in nonhuman primates.

Like humans, macaques exhibit regular, approximately 28-day menstrual cycles [6]. In the first half of the cycle, estrogen is produced during follicle maturation and stimulates proliferation of the endometrium. The midcycle luteinizing hormone surge triggers ovulation and the transformation of the follicle into the progesterone-secreting corpus luteum. Progesterone transforms the endometrium to a secretory state receptive to implantation of the embryo. In the absence of pregnancy, the corpus luteum undergoes regression after about 14 days. The resulting drop in serum progesterone triggers an irreversible cascade of signaling events that culminate in endometrial destabilization and menstruation. If pregnancy occurs, human (or macaque) chorionic gonadotropin (hCG) produced by the syncytiotrophoblast cell layer of the embryo prevents regression of the corpus luteum and maintains progesterone production. The most widely used medical termination strategy for women exploits this pathway by using mifepristone (RU-486), a potent selective competitive inhibitor of the progesterone receptor [7]. In an established early pregnancy, treatment with mifepristone results in endometrial epithelial, stromal, and vascular changes that compromise trophoblastic proliferation, resulting in a reduction in hCG production, which in turn reduces progesterone production by the corpus luteum and further exaggerates the inhibition. The destabilization of the endometrium increases the production of prostaglandins that soften the cervix and stimulate myometrial contractions [8]. These effects are also observed after progesterone withdrawal preceding normal labor [9]. In human medical termination protocols, misoprostol, an orally active prostaglandin E1 analogue, is dosed 24–48 hours after mifepristone to improve expulsion rates [10]. In women, mifepristone has good bioavailability when dosed orally [11]. Misoprostol can be dosed orally, buccally, sublingually, or vaginally [12].

Methotrexate is a chemotherapeutic agent which inhibits dihydrofolate reductase, thereby halting DNA synthesis and preventing cell division in the placental and fetal tissues [13]. It is generally given as an intramuscular (IM) injection, though oral administration has been described in some studies [14]. This medication is used in medical termination regimens in humans when mifepristone is unavailable [15] and for the treatment of ectopic pregnancy. Like mifepristone, it is generally administered with misoprostol, but a longer delay (7 days) is recommended [10]. Methotrexate can cause systemic adverse effects in humans and in research animals at therapeutic doses. Toxicity is generally manifested as diarrhea leading to dehydration, or as anemia. To counteract overdose of methotrexate or to prevent systemic effects, leucovorin, or citrovorum factor, a chemically reduced derivative of folic acid is frequently used [13]. This medication enters cells and participates in DNA synthesis as a reduced folate.

Although effects of mifepristone, misoprostol, and methotrexate have been studied in macaques, there are no published protocols for medical termination in research animals [16–21]. Breeding groups of cynomolgus macaques have been established at the Oregon National Primate Research Center (ONPRC) to support contraceptive projects. The purpose of this study was to review the safety and efficacy of different medical termination regimens utilized at our center for macaques in contraceptive studies for the purpose of returning macaques in control groups (i.e., not requiring completion of pregnancy) back to research groups.

Materials and Methods

Data was extracted from records of all macaques that underwent medical termination procedures between March 2007 and August 2011 at ONPRC in Beaverton, Oregon in support of contraceptive research studies. All protocols were approved by the Institutional Animal Care and Use Committee.

The general approach to animal care during the contraceptive studies has been described [1]. Females are examined daily for evidence of menstruation and mating via vaginal swabs, and undergo routine weekly monitoring of progesterone levels. If the serum progesterone remains elevated (> 1 ng/mL) for three consecutive weeks, the animal is examined with abdominal ultrasound under ketamine restraint (10 mg/kg). Pregnancy is diagnosed by the appearance of a gestational sac within the uterine cavity. To confirm viability, pregnancies may be followed by ultrasound on a weekly basis (Figure 2).

Recorded data for each confirmed pregnancy in this analysis included date of last menses, ultrasound information (crown-rump length, presence of cardiac activity, size of uterine opening (e.g. gestational sac and extraembryonic space, or retained tissue), medical termination regimen (drug type, dose, route of administration, number of doses), and amount of bleeding noted. Once fetal demise was noted, animals were followed with ultrasound examinations every two weeks and then monthly until there was no evidence of retained fluid or tissue and the uterine cavity appeared completely normal (uterine resolution) (Figure 3). If expelled tissue was recovered, it was submitted to the Pathology Services Unit at ONPRC. For each attempted medical termination, selection of primary treatment and subsequent outcome were assessed, as well as any secondary, or salvage, treatments used and outcomes.

Insemination date was estimated by weekly serum steroid profiles suggestive of ovulation (estradiol rise preceding an elevation in progesterone) and the presence of sperm in the vagina assessed via microscope evaluation of vaginal swabs. Gestational age was calculated as six days after insemination date. Each female was removed from the social group and placed in individual housing for the duration of the medical termination protocol. Clinical veterinary staff closely monitored the health and wellbeing of each animal.

Preparation and administration of medications

Mifepristone—Bulk drug was obtained from Danco Corporation (New York, NY). Oral dosing was prepared by mixing dry powder with a food treat (sweet dough). If administration as a food treat was not possible, oral dosing was accomplished under sedation by placing the tablet on the tongue with a small amount of water. When indicated, animals were sedated with ketamine 10 mg/kg. For IM injection, mifepristone was dissolved in Captex oil (ABITEC Corporation, Janesville, WI) (20 mg/0.25 ml) or saline: polyethylene glycol (PEG) 1:1 (100 mg/2 ml).

Methotrexate—A dose of 50 mg/m^2 was prepared using the standard 25 mg/ml stock solution of methotrexate (GeneraMedix Inc., Liberty Corner, NJ). Surface area was calculated using weight and length: square root ([length(cm)*weight(kg)]/3600). Length was measured from the top of the head to the base of the tail. The calculated dose was delivered either IM or by direct intrafetal injection. Intrafetal injection was performed using sterile technique under ultrasound guidance, with a 22 gauge needle.

Misoprostol—Misoprostol (Greenstone LLC, Peapack, NJ) 200 mcg tablets were administered orally, vaginally, or buccally, based on pharmacokinetic studies in humans demonstrating comparable efficacy [12]. Doses were given under sedation (ketamine 10 mg/

ml) for all routes of administration. For oral administration, crushed tablets were placed on the tongue with small amounts of water. Buccal dosing was provided by placement of crushed tablets into the cheek pouch. Vaginal administration was accomplished by placement of whole tablets just inside the vaginal opening. Misoprostol was dosed either immediately or delayed 24–48 hours after mifepristone, and 7 days after methotrexate.

Development of the protocols

We based our initial regimens on established human protocols [5]. The standard approach in the United States for medical termination in women is a 200 mg oral dose of mifepristone followed 24–48 hours later by buccal misoprostol 800 mcg. However, since immediate dosing with misoprostol at the same time as mifepristone shows similar efficacy [22], and we wished to establish both high efficacy and minimal stress, pain, and handling of the animals, our initial approach was to administer oral mifepristone 20 mg concurrently with misoprostol 100 mcg vaginally at the time of pregnancy confirmation. Our first animal treated with this protocol failed to terminate even after additional oral mifepristone and misoprostol, and ultimately underwent surgical termination by hysterotomy two weeks later for a continuing pregnancy.

We treated our next several animals with an oral dose of mifepristone 100 mg co-administered with oral misoprostol 200 mcg, followed by vaginal or oral misoprostol 400 mcg every 24 hours for the next two days. Continuing pregnancies were managed with either IM methotrexate, followed seven days later by oral misoprostol 400 mcg, or IM mifepristone 20 mg and oral misoprostol.

Observations from these treatments led to the selection of IM mifepristone 20 mg as the primary therapy. While a dose of up to 100 mg of mifepristone was administered IM in some animals without any adverse effects, this did not improve outcomes over the 20 mg dose. Oral or buccal misoprostol 200 or 400 mcg was given 48 hours later and repeated if there was no evidence of vaginal bleeding.

Animals that failed to terminate with the IM mifepristone regimen received a secondary treatment with methotrexate. To maximize exposure to the fetus and placenta, and minimize systemic exposure, the administration of methotrexate (50 mg/m^2) was switched from IM to intrafetal injection under ultrasound guidance.

Assessment and treatment of adverse effects

Animals were closely monitored by veterinary staff during treatment protocols. Behavior indicative of pain included decreased appetite, lethargy, hunched sitting position, decreased vocalizations and decreased interaction. Pain was treated with buprenorphine 0.01–0.03 mg/kg IM or hydromorphone 0.25 ml (0.5 mg) IM every 4–6 hours. Vomiting was treated with chlorpromazine 1 mg/kg orally or IM if recurrent. Diarrhea and low appetite were treated with sucralfate 0.5 g orally twice a day and famotidine 0.5 mg/kg orally or IM twice a day, and affected animals were provided favored foods such as fruit and vegetables. Sunken eyes and decreased urine production suggested dehydration, which was treated with subcutaneous or intravenous fluid therapy. All treatments were at the discretion of veterinary staff. Non-steroidal anti-inflammatory drugs (NSAIDs) were avoided to reduce gastrointestinal side effects.

Data analysis

The primary outcome was success of the termination treatment, defined as fetal demise and expulsion or absorption of the pregnancy without need for surgical intervention. Failure of treatment was defined as continuation of pregnancy. We also tracked time to uterine

resolution, defined as a closed uterus, i.e. devoid of all fetal tissues and fluid, visualized on ultrasound, as this is a clinically important endpoint that allows females to re-enter the contraceptive group and be receptive to another pregnancy. This study represents a clinical case series; a formal statistical plan was not developed and power analysis was not performed.

Results

A total of 37 macaques underwent 65 medical termination procedures. Most animals (89%) were enrolled in contraceptive studies. 31 procedures (48%) were carried out animals receiving contraceptive study agents, 27 (42%) were in control animals, and 7 (11%) were for ONPRC nonhuman primate core husbandry purposes. Due to potential drug interactions between contraceptive agents and medications used in termination protocols, results for controls (including those not part of a study) and active arm animals (concurrently receiving investigational contraceptive agents including selective estrogen receptor modulators or cyclooxygenase inhibitors) are presented separately.

Route of administration of mifepristone

Table 1 presents the efficacy rates achieved with mifepristone according to route of administration and dose. The rate of successful termination was low with oral administration. Of the six macaques treated with oral mifepristone, only one animal successfully terminated. In contrast, IM injection of 20 mg mifepristone resulted in a higher rate of success (83%, 43/52). Compared to controls, efficacy was lower for animals receiving contraceptive agents (87% for controls, versus 76% for those receiving contraceptive agents), suggesting potential interactions between the investigational drugs and mifepristone. Notably, 7 pregnant animals in the active drug arm of one contraceptive study received 100 mg mifepristone IM, but only one had a successful termination of pregnancy (data not shown).

Salvage therapy after failed mifepristone treatment

Animals that failed to terminate after receiving IM mifepristone received salvage medical therapy. Most had received an active contraceptive study agent. These animals were given additional IM mifepristone, IM methotrexate, or intrafetal methotrexate (Table 2). The highest efficacy (100% for both controls and those receiving contraceptive agents) was achieved with intrafetal methotrexate. The two animals that were treated with additional doses of IM mifepristone after failed initial treatment both resulted in continued pregnancy (data not shown); each subsequently received methotrexate.

Misoprostol dosing and route of administration

Oral or buccal doses of misoprostol 200 or 400 mcg were standard for most treatment regimens. The number of doses in successful primary treatments ranged from one to three compared with a range of 1 to 11 in animals receiving salvage treatments. There was no association between number of misoprostol doses and successful expulsion for active contraceptive treatment or control animals.

The rate of successful fetal demise with mifepristone did not differ if misoprostol was delivered via the buccal or oral route. However, the buccal route was associated with lower rates of retained tissue (Figure 4) during development of the termination protocol. Retained tissue was also seen after intrafetal methotrexate treatment in three monkeys receiving oral misoprostol. For this reason, buccal misoprostol was selected as the primary route in our medical termination protocol at ONPRC.

Effect of gestational age

Most of the macaques that received 20 mg IM mifepristone in this series (88%, 46/52) were at gestational ages of 25 days or less upon initiation of the termination protocol. Macaques at gestational ages greater than 25 days had an 83% success rate for termination, similar to the rate for earlier gestations. Animals with successful termination had gestational ages ranging from 11 to 32 days; those that failed to terminate ranged from 18 to 27 days.

Time to uterine resolution

Time to uterine resolution was influenced by the termination protocol and contraceptive study treatment. Following successful fetal demise with 20 mg IM mifepristone as primary treatment, among animals with complete expulsion of the fetus after treatment, uterine resolution occurred at a median of 14 days (range 7 to 73 days) for controls, and 21 days (range 7 to 21 days) for animals receiving contraceptive agents (Table 3). Retained fetal tissue (Figure 4) was observed in 30% (13/43) of the animals with successful fetal demise after primary treatment with 20 mg IM mifepristone. This tissue was absorbed after three weeks in all but one female from the control group, but frequently persisted for 3 to 4 months among the females that received a contraceptive agent. Several animals were noted to have delayed uterine resolution despite apparent fetal expulsion. In these cases, a persistent opening (but no tissue) was observed in the uterine cavity (Figure 5).

The three animals with successful pregnancy termination after receiving IM methotrexate all failed to expel the fetal tissue and ultimately underwent surgical evacuation of the uterus. Similarly, while intrafetal methotrexate resulted in successful fetal demise in all cases, fetal tissue was generally retained (93%, 14/15) and time to uterine resolution was highly variable (16–300 days)(Table 4). In two cases, retained tissue was present on ultrasound for over four months post treatment despite up to eleven doses of misoprostol. Both animals had a crown-rump length of approximately 4.5 mm at initial treatment and received 20 mg mifepristone. One was receiving a contraceptive agent and one was part of a control group.

Due to the significant delay to uterine resolution in some cases, treatment with a prostaglandin F2α analogue (carboprost tromethamine, Hemabate, Pharmacia & Upjohn, New York, NY) was attempted in a small number of animals. In one animal, carboprost was administered at an increasing dose regime (up to 500 mcg) initially via intramuscular and then intrauterine injection. While some vaginal bleeding was observed at the higher doses, expulsion of tissue did not occur. Two additional females received intrauterine injections of carboprost 250 mcg and failed to expel the retained tissue.

Side effects

Frequencies of observed side effects with mifepristone are shown in Table 5. Pain, vomiting, diarrhea, decreased appetite, and dehydration were the most commonly observed side effects of these medical termination treatments. Decreased appetite was the most common symptom, occurring in 32%. Behaviors indicative of pain were common with all termination regimens, and were generally noted following the administration of misoprostol. Pain and other effects were immediately mitigated by veterinary staff using the treatments described above. Two animals required treatment for dehydration. There were no other serious adverse events and no deaths related to termination procedures.

Methotrexate was associated with a higher incidence of side effects compared to mifepristone (Table 6). A large percentage of animals receiving methotrexate treatment subsequently developed diarrhea. To prevent diarrhea, leucovorin (Bedford Laboratories, Bedford, OH) was administered to three animals in this series. Two animals received a regimen of two doses of 20 mg/m^2 every 12 hours, beginning 24 hours after methotrexate

injection. One animal received three doses at the 12 hour interval due to persistent loose stool. Only a small number of animals received methotrexate IM, thus is difficult to determine whether this route of administration was associated with increased side effects compared to intrafetal injection. Of the four animals who received an IM dose, two were noted to have pain, diarrhea, and low appetite, and two did not have any side effects.

Discussion

This is the largest reported series of medical terminations in cynomolgus macaques. Our experience suggests that medical termination for macaques in reproductive studies is feasible, safe, and effective, and enables return to fertility and inclusion in further studies. Regimens with mifepristone, misoprostol, or methotrexate are well established in humans [5, 10], but little information is available describing the use of these medications for the indication of pregnancy termination in macaques.

While human termination protocols use oral dosing of mifepristone, this route failed to reliably result in termination of pregnancy in macaques. This is consistent with results of previous studies of mifepristone in macaques [19, 23], and suggests important species differences in pharmacokinetics and oral bioavailability. Importantly, bypassing the oral route in macaques using IM treatment produced efficacy approaching that of oral mifepristone in human studies. Intrafetal injection of methotrexate was a feasible and well tolerated technique that produced reliable termination of pregnancy in the event of failure with mifepristone. However, persistent retained tissue was common after methotrexate.

The response to misoprostol represents another important species difference. Although buccal misoprostol typically resulted in vaginal bleeding, the activity was reduced compared to women. As seen in a previous study [19], large doses of misoprostol given orally, buccally, or vaginally to macaques generally do not result in expulsion of fetal tissue. In human efficacy studies, 800 mcg misoprostol resulted in expulsion within 4 hours in 49% and within 24 hours in 75% of women [8]. Since the addition of misoprostol to human medical termination protocols greatly improves efficacy [10], it is not surprising that our results with medical termination are inferior to those reported in women. Decreased sensitivity of macaques to other prostaglandins has also been observed [20, 21]. Notably, direct intrauterine injection of carboprost at doses up to 500 mcg in our series failed to result in expulsion of retained pregnancy tissue.

Based on our results, the current macaque medical termination protocol at ONPRC consists of 20 mg mifepristone suspended in Captex oil via IM injection as soon as possible after fetal cardiac activity is visualized on ultrasound. Misoprostol 200 mcg is crushed and placed in the cheek pouch (buccal administration) 48 hours later. If the fetus is not viable on ultrasound one week later, the female can be returned to her breeding group, with ultrasounds every two weeks and then monthly until the uterus is completely closed (no fluid or retained tissue observed in the endometrial cavity). If the fetus is still viable on ultrasound one week after mifepristone, methotrexate (50 mg/m^2) is injected directly into the fetus under ultrasound guidance. Ultrasound is performed one week later to confirm fetal demise. The female then has monthly follow up ultrasounds until the uterus is completely closed.

Nearly all animals in this series had very early pregnancies of 25 days gestation or less and we did not observe increased efficacy at lower gestational ages. In some instances, the IM mifepristone regimen failed in pregnancies of 11 days gestation. However, based on human studies, the medical termination protocol is likely more effective at earlier gestations.

The medical termination protocol includes routine provision of medications to prevent common side effects. Pain-associated behaviors are frequently noted following the administration of misoprostol. Astandard one-time dose of buprenorphine 0.01–0.03 mg/kg IM is now given at the time of misoprostol administration. This supplemental pain medication has reduced the overall occurrence of pain-related behaviors. Leucovorin (20 mg/m^2 every 12 hours for two doses) is routinely provided to all animals 24 hours after receiving methotrexate treatment, as this is a safe and low cost intervention that may reduce the occurrence of diarrhea. A multivitamin with iron is also provided daily to all animals for the duration of the termination protocol.

Medical termination has been initiated in two animals with abnormal pregnancies (data not shown), diagnosed by persistently elevated progesterone levels but lack of expected development on serial ultrasounds. These pregnancies are referred to as “biomedical” pregnancies at ONPRC. Treatment was successful in both cases.

Retained tissue after medical termination is common in macaques, particularly in those receiving intrafetal methotrexate. Most animals will eventually reabsorb the tissue, but in some cases this can take months. Since uterine aspiration is impractical and risky, further research must be directed towards improved expulsion rates.

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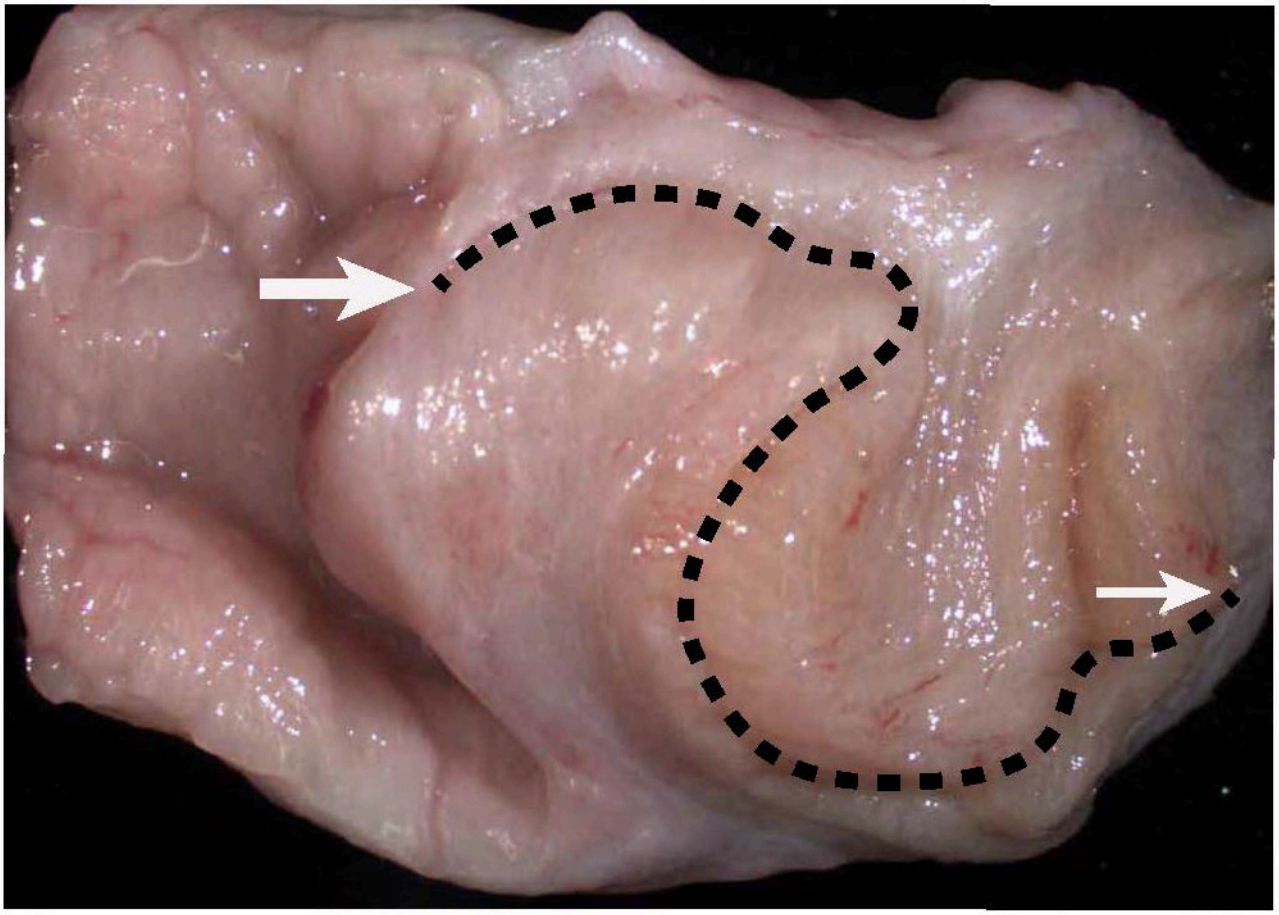

Figure 1. Longitudinal section through the macaque cervix demonstrating the tortuous canal
The vagina is on the left, the external cervical os indicated with the thick arrow, and the internal os indicated with the thin arrow. The dotted line shows the path of the cervical canal.

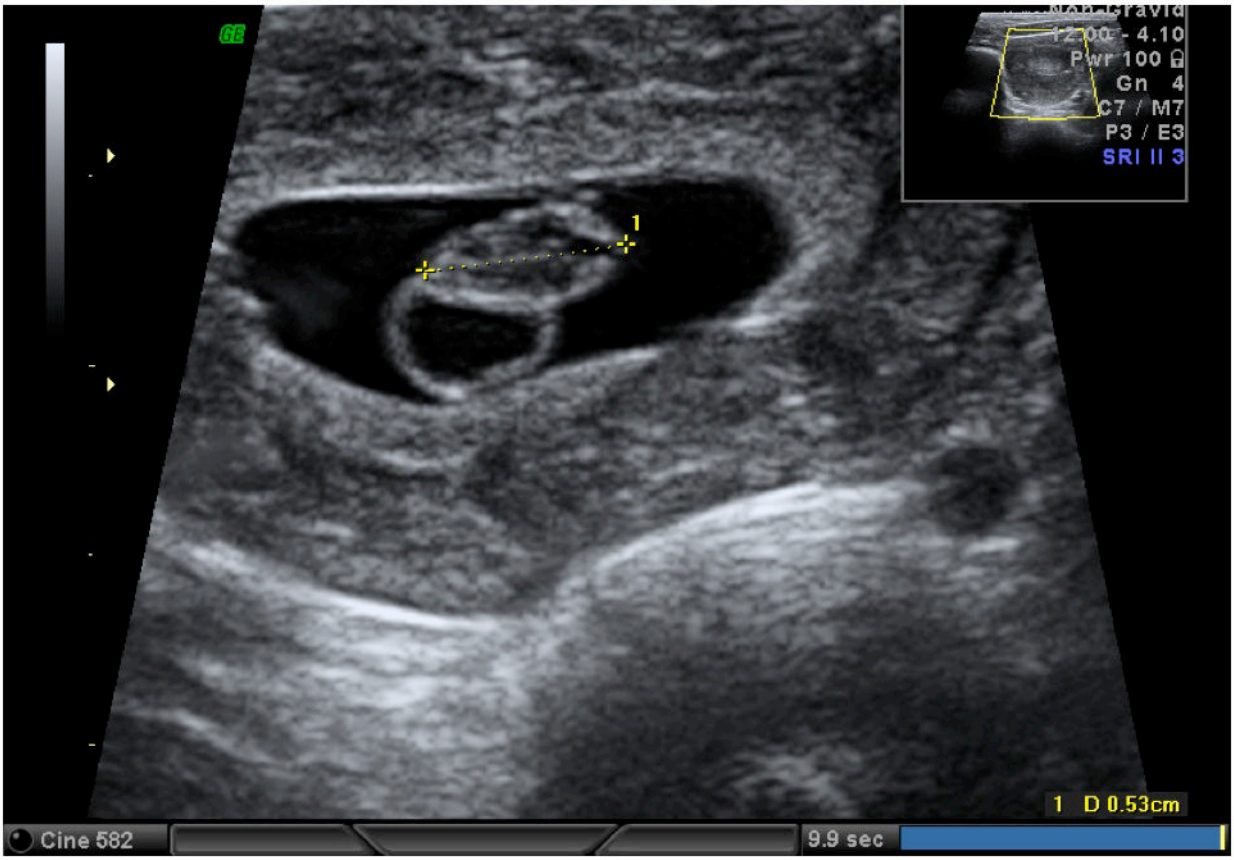

Figure 2. Normal appearing pregnancy on ultrasound

Intrauterine gestational sac containing yolk sac and 0.53 cm fetal pole. Fetal cardiac activity was noted.

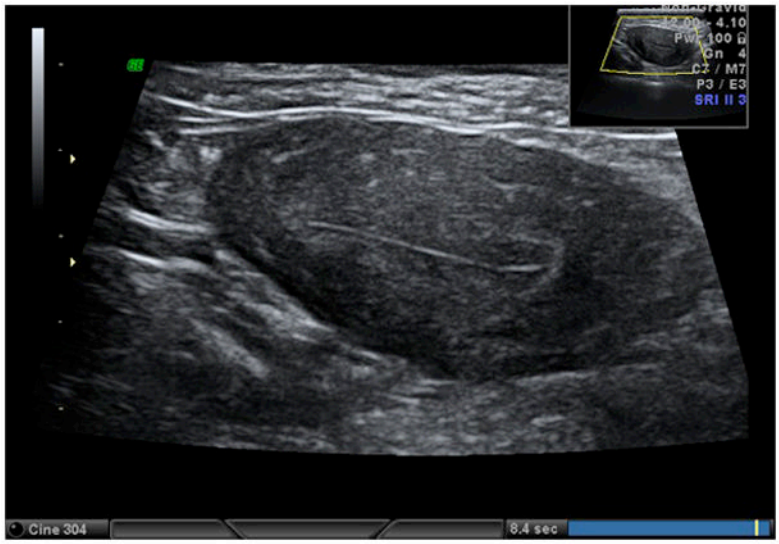

Figure 3. Uterine resolution one week after mifepristone 20 mg IM primary treatment

Complete uterine resolution determined by completely closed uterus with no retained fluid or fetal tissue.

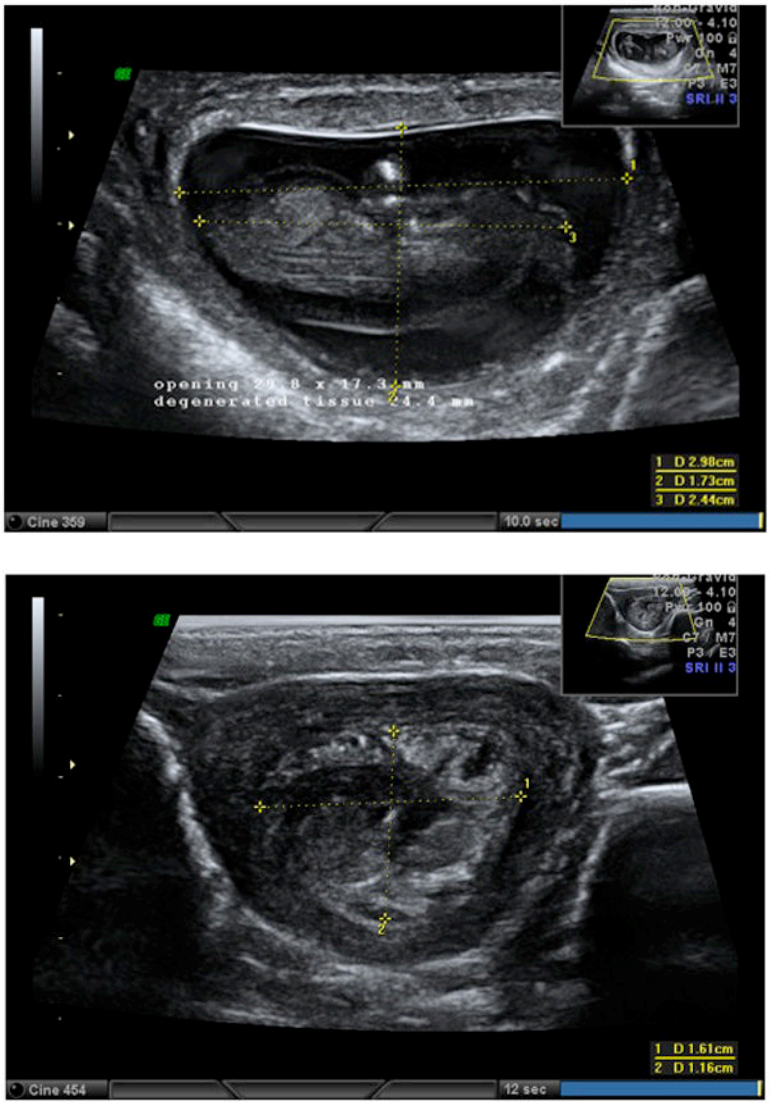

Figure 4. Retained fetal tissue after fetal demise following treatment with intrafetal methotrexate
a. Longitudinal view
b. Transverse view

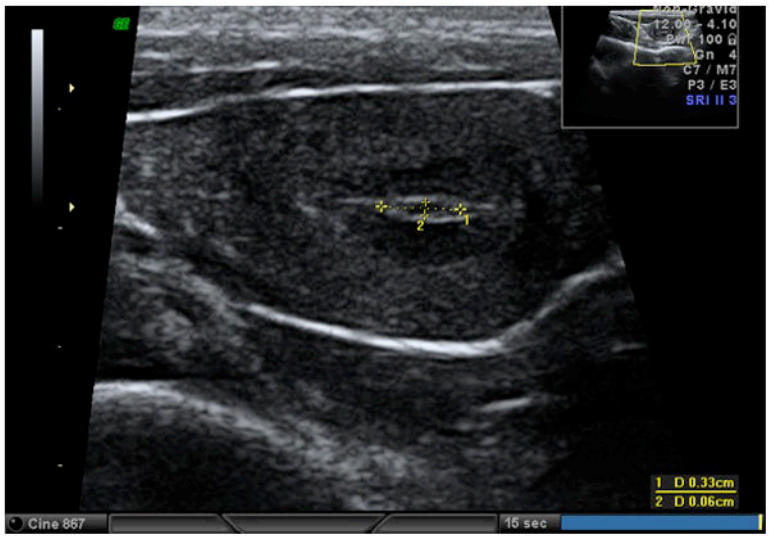

Figure 5.
Persistent uterine opening one week after initial treatment (delayed uterine resolution)

Table 1

Primary treatment with mifepristone: efficacy in pregnancy termination

Regimen	Overall Efficacy	Efficacy for controls	Efficacy for animals receiving contraceptive agents
Oral mifepristone	1/6 (17)	1/3 (33)	0/3 (0)
20 mg IM mifepristone	43/52 (83)	27/31 (87)	16/21 (76)

Given as n/N (%)

Table 2

Efficacy of secondary treatment with IM or intrafetal methotrexate after failure of initial treatment

Regimen	Overall Efficacy	Efficacy for controls	Efficacy for animals receiving contraceptive agents
IM methotrexate	3/4(75) *	1/2 (50)	2/2 (100)
Intrafetal methotrexate	15/15 (100)	4/4 (100)	11/11 (100)

Given as n/N (%)

* Two received IM mifepristone the following day

Table 3

Time to uterine resolution (Completely closed uterus documented on ultrasound) with successful primary treatment

Regimen	# Days to uterine resolution Median (range)	Controls	Contraceptive agents
20 mg IM mifepristone, fetal demise with retained fetal tissue, n=13(5controls, 8active)	22 (21–142)	22 (21–92)	49 (21–142)
20 mg IM mifepristone, fetal demise with complete expulsion, n=30(22controls, 8active)	14 (7–73)	14 (7–73)	21 (7–21)

Given as median days (range)

Table 4

Time to uterine resolution with successful salvage treatment

Regimen	# Days to uterine resolution Median (range)	Controls	Contraceptive agents
Intrafetal methotrexate, fetal demise with retained fetal tissue, n=14 (4controls, 10active)	98 (16–300)	70 (42–300)	109 (16–238)
Intrafetal methotrexate, no retained tissue, n=1 (1active)	17 (17)	-	17 (17)

Given as median days (range)

Table 5

Adverse effects of mifepristone

	Frequency of symptom n(%) (N=65)	Among animals with symptom, number receiving medication[a] n (%)
Pain	12 (18)	11 (92)
Diarrhea	8 (12)	3 (36)
Vomiting	8 (12)	0 (0)
Decreased appetite	21 (32)	3 (14)
Dehydration	2 (3)	2 (100)

[a]Pain was treated with buprenorphineor hydromorphone. Diarrhea and decreased appetite weretreated with sucralfate and famotidine. Vomiting was treated with chlorpromazine. Dehydration was treated with subcutaneous or intravenous fluid therapy.

Table 6

Adverse effects of methotrexate

	Frequency of symptom n(%) (N=19)	Among animals with symptom, number receiving medication[a] n(%)
Pain	7 (37)	5 (71)
Diarrhea	13 (68)	8 (62)
Vomiting	6 (32)	3 (50)
Decreased appetite	14 (74)	5 (36)
Dehydration	5 (26)	5 (100)

[a] See Table 5 for list of medications used to alleviate adverse effects.