

Efficacy of Cloprostenol or Aglepristone at 21–22 and 35–38 Days of Gestation for Pregnancy Termination in Queens

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Contents

The objective of this study was to evaluate the efficacy of cloprostenol (CLO) or aglepristone (ALI) for pregnancy termination in queens at 21–22 and 35–38 days of gestation. Two experiments (EXP) were carried out to accomplish this aim. Thirty-seven 12- to 14-month-old mixed breed queens were used in a randomized design. At oestrus, queens were housed for mating with a tom, and pregnancy was confirmed by transabdominal ultrasonographic examination (US). On days 21–22 of pregnancy (EXP1) or 35–38 of pregnancy (EXP2), queens were divided into three groups (G). Queens in G1 received ALI (10 mg/kg, sc; EXP1, n = 6; EXP2, n = 6) on two consecutive days. Queens in G2 received CLO (5 µg/kg, sc; EXP1, n = 6; EXP2 = 7) on three consecutive days. Queens in G3 received 1 ml of saline solution (PLA, sc; EXP1, n = 6; EXP2 = 6). After treatment, females were monitored daily by US during for 10 days and weekly until the end of gestation. In EXP1, pregnancy was terminated in (6/6, 100%), (0/6, 0%) and (0/6, 0%), for the ALI, CLO and PLA groups, respectively ($p < 0.001$). In EXP2, pregnancy was terminated in (6/6, 100%), (1/7, 14%) and (0/6, 0%) for the ALI, CLO and PLA groups, respectively ($p < 0.001$). In both EXP, after CLO administration, animals vomited and were depressed for 30 min; but no side effects were observed in the animals in the ALI group. In conclusion, the results from this study indicate that three injections of CLO are not effective, but two injections of ALI are effective to induce abortion in queens at 21–22 or 35–38 days of pregnancy.

Introduction

Termination of unwanted pregnancies in the queen can be achieved using cabergoline alone or in combination with prostaglandin $F_{2\alpha}$ (PGF; Onclin and Verstegen 1997; Erünal-Maral et al. 2004). Previous studies have shown that multiple doses of PGF in cats from day 21 to day 25 of pseudopregnancy depressed luteal function, but did not produced complete luteolysis of the corpus luteum (Shille and Stabenfeldt 1979). Prostaglandins have been proved effective to induce abortion in queens with more than 40 days of gestation, but failed to induce abortion in queens with <40 days of pregnancy (Nachreiner and Marple 1974). It is not clear whether the effect of PGF in these animals was the result of direct abortifacient action on the uterus or because of luteolysis of the corpora lutea of pregnancy. It is possible that abortions occurred because of a direct contractile effect on the uterus in the presence of depressed luteal function, but complete luteolysis did not happen (Shille and Stabenfeldt 1979). Furthermore, when PGF was administered at 45 days of gestation, a higher percentage of abortion was obtained compared when it was used at day 30 of gestation. All cats had decreasing P_4 concentrations during treatment, but only

queens with abortion showed P_4 levels <1 ng/ml (Baldwin et al. 2000). In addition, PGF administration at day 33 of pregnancy induces luteolysis and pregnancy termination with expulsion of foetuses (Verstegen et al. 1993). Furthermore, PGF and cloprostenol (CLO) are used to induce luteolysis in several species in which the unique source of P_4 is the corpus luteum; and it is known that synthetic prostaglandins are more potent than natural prostaglandins. The use of dopamine agonist in combination with PGF analogue such as CLO or alfaprostol has been effective in cats to induce abortion (Onclin and Verstegen 1997; Erünal-Maral et al. 2004). However, the efficacy of CLO without dopamine agonist has not been studied yet. Therefore, CLO has the potential to be effective to induce luteolysis in queens at 21–22 days of pregnancy.

To our knowledge, there are no reports using CLO for terminating unwanted feline pregnancies. Although the source of P_4 before days 25–30 of pregnancy in the queen appears to be solely from the corpora lutea, there is still debate whether the feline placenta is a P_4 source during the second half of pregnancy (Onclin and Verstegen 1997). Whereas some authors claim that the feline placenta essentially contributes to P_4 secretion (Malassine and Ferre 1979), others claim that luteal P_4 seems to be necessary to maintain pregnancy until days 45–50 (Scott 1970). Furthermore, it has been reported that bilateral ovariectomy performed before day 50 of pregnancy in the queen resulted in abortion (Verstegen et al. 1993). Collectively, these data are conflicting in terms of the potential efficacy of CLO to terminate pregnancy at 35–38 days of gestation in the queen. Additionally, to our knowledge, there are no reports of the efficacy of CLO for terminating unwanted pregnancies at 35–38 days of gestation.

Therefore, the aim of the study was to assess the efficacy of using CLO or aglepristone (ALI) at 21–22 and 35–38 days of pregnancy for pregnancy termination in queens. Two experiments (EXP) were carried out to accomplish this objective. The hypothesis was that CLO or ALI when administered in three or two times, respectively, could effectively terminate 21–22 days (EXP1) and 35–38 days (EXP2) pregnancies in queens.

Materials and Methods

Mixed breed queens (n = 37), aged between 12 and 14 months and weighing between 2 and 4 kg, were used in a randomized design. In addition, two 3-year-old intact tom cats were used for breeding. The queens were housed alone in stainless steel cages and were fed commercial cat food (Fit 32[®]; Royal Canin, Buenos

Aires, Argentina) and water *ad libitum*. The toms were housed separately and fed the same diet. All animals were maintained in a controlled environment with artificial incandescent illumination (14 h of daily bright light). Animal care, housing and experimentation complied with the International Guiding Principles for Biomedical Research Involving Animals (1985). Queens were observed on a daily basis to detect behavioural oestrus and receptivity to the male and vaginal cytology were performed to detect cytologic oestrus. When queens showed behavioural and cytologic oestrus, each queen was placed with the tom for 48 h. First mating was documented, and pregnancies were confirmed by an ultrasonographic examination using an ultrasound scanner equipped with a 5–7.5–10 MHz linear transducer (Mindray™, DP-6600 Vet, Nanshan, China) 21 days after the first mating.

On days 21–22 of pregnancy (EXP1) or 35–38 of pregnancy (EXP2), queens were divided into three groups (G). In G1, animals received 10 mg/kg ALI sc (Alizin®, Virbac, Germany, ALI; EXP1, n = 6; EXP2, n = 6) on two consecutive days. In G2, animals received 5 µg/kg CLO sc (Ciclar, p.a.®, Zoovet, Argentina, CLO; EXP1, n = 6; EXP2 = 7) on three consecutive days. In G3, animals received 1 ml of saline solution sc (PLA; EXP1, n = 6; EXP2 = 6). After treatment, females were daily monitored by ultrasonography for 10 days and weekly until the end of gestation to obtain gestational sac (GS) and foetal measurements. During each ultrasound examination, the length (LEN, mm), anterior–posterior (AP, mm) and width (WID, mm) of each GS were measured. The GS volume (GSV, mm³) was calculated using the ellipsoid shape formula ($4/3 \pi * [GSLEN/2] * [GSAP/2] * [GSWID/2]$), and GS diameter (GSD, mm) was calculated as the mean of the three measurements of the GS. In addition, the crown-rump length (CRL, mm), the biparietal diameter (BD, mm) and the thoracic diameter (TD, mm) were measured. The number of foetuses (NFET), days of gestation (DG), days from treatment to termination of pregnancy (DTP), days from treatment to the presence of vulvar discharge (DTV) and the GS measurements were analysed by the GLM and MIXED procedures of SAS; while the success or failure to terminate pregnancy (0–1), by the GENMOD procedure of SAS; (10). Data are presented as LSM ± SEM. Significance was defined as $p < 0.05$.

Results

EXP1

While all queens treated with ALI terminated pregnancy (6/6, 100%), none of those treated with CLO (0/6, 0%) or with PLA (0/6, 0%) terminated pregnancy ($p < 0.001$). The interval from treatment to first signs of pregnancy termination in the ALI group was significantly shorter than the interval from treatment to the end of a normal pregnancy in the CLO- and PLA-treated queens (4.1 ± 0.6 vs 42.58 days, $p < 0.001$). The average days of gestation for queens with a normal pregnancy were similar in PLA and CLO groups (63.8 ± 0.6 days, $p > 0.20$). The number of GS at the

beginning of the study was similar among queens in all treatment groups (3.0 ± 0.5 , $p > 0.70$). The number of kittens born from normal pregnancies was similar in the CLO- and PLA-treated group (3.4 ± 0.4 , $p > 0.41$). The interval from the beginning of treatment to presence of vulvar discharge in the queen that aborted in the ALI group was 3.6 ± 0.9 days. The return to oestrus after pregnancy termination or after normal pregnancy was similar among all groups (64.0 ± 10.5 , $p > 0.57$). At the start of the study, all GS measurements were similar for all treatment groups ($p > 0.39$). The GS length, anterior–posterior and width dimensions and the GS volume and diameter increased at a different rate among treatments across days of gestation (interaction of treatment by day of gestation; $p < 0.01$; Fig. 1). In the group of queens that pregnancy was terminated (ALI), all GS and foetal measurements did not increase after treatment; where in the groups with normal pregnancy (PLA and CLO), GS and foetal measurements continued to increase during the next 10 days after treatment ($p < 0.01$; Fig. 1). After CLO administration, all animals vomited and were depressed for 30 min. No side effects were seen with ALI-treated animals.

EXP2

While all queens treated with ALI terminated pregnancy (6/6, 100%), only one treated with CLO (1/7, 14%) aborted 5 days after the beginning of the treatment, and none of PLA (0/6, 0%) showed abortion ($p < 0.001$). The interval from treatment to pregnancy termination was similar between ALI- and CLO-treated queens (4.4 ± 1.6 days, $p > 0.39$). The average days of gestation for queens with a normal pregnancy were similar in PLA and CLO groups (65.1 ± 0.3 days, $p > 0.54$). The NFET at the beginning of the study and the interval from parturition or termination of pregnancy to the next oestrus were similar among queens in all treatment groups (3.4 ± 0.5 , $p > 0.74$; 39.4 ± 7.8 days, $p > 0.89$; respectively). The interval from the beginning of

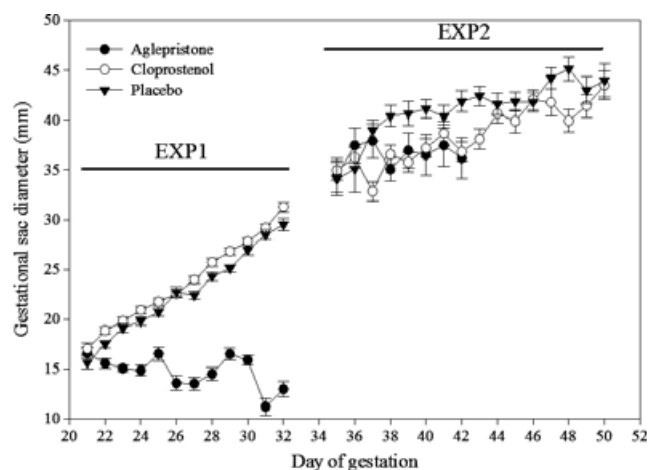


Fig. 1. Least squares means and SE of the gestational sac diameter (mm) during pregnancy after treatment with placebo, cloprostenol (5 µg/kg, three consecutive days) or aglepristone (10 mg/kg, two consecutive days) at 21–22 days (EXP1) or 35–38 days (EXP2) of gestation for pregnancy termination in queens

treatment to the presence of vulvar discharge was similar between queens that aborted in the ALI and CLO groups (3.1 ± 0.7 days, $p > 0.57$). On the day of treatment initiation, there were no significant differences in GS and foetal measurements among treatments ($p > 0.05$). The GS length, antero-posterior and width, the GS volume and diameter, and the CRL, biparietal diameter and thoracic diameter increased at a different rate among treatments across days of gestation (interaction of treatment by day of gestation; $p < 0.05$; Fig. 1). Additionally, PLA-treated animals had a greater increase of GS and foetal measurements compared to ALI- and CLO-treated animals ($p < 0.05$). Conversely, although in ALI and CLO groups, GS and foetal measurements increased after treatment ($p < 0.05$), there were no significant differences between GS and foetal measurements between ALI group and the queen that aborted in CLO group ($p > 0.05$). Furthermore, in the PLA group, the GS and foetal measurements increased at a higher rate compared to the CLO group ($p < 0.05$). After CLO administration, animals vomited and were depressed for 30 min. No side effects were observed in the animals in the ALI group.

Discussion

Contradictory to our hypothesis, CLO was not effective to induce abortion in queen at days 21–22 of pregnancy. On the contrary, ALI was effective at inducing abortion in queens at days 21–22 of pregnancy. Whereas previous reports have shown that ALI was effective in the queen to terminate pregnancy on days 25–26 without side effects (Georgiev and Wehrend 2006, 2008), to our knowledge, this is the first study to report the lack of efficacy of CLO to induce abortion in the queen. Previous studies using of PGF in cat at 21–25 days of a pseudopregnancy depressed luteal function, but did not induce complete luteolysis (Shille and Stabenfeldt 1979). Others investigations using CLO in combination with cabergoline were successful to induce abortion by day 30 of pregnancy (Onclin and Verstegen 1997). Because there were no studies about the efficacy of CLO without dopamine agonist, we hypothesized that CLO could be useful to induce luteolysis in queens at 21–22 days of pregnancy. However, our result showed CLO did not induce abortion on days 21–22 of pregnancy in the queen. This lack of efficacy could be explained by having used a dose not high enough to induce luteolysis

or by an earlier luteotrophic support of prolactin to the corpora lutea rescuing the corpora lutea from the luteolytic doses of CLO.

Prostaglandins are used to induce luteolysis in several species in which the unique source of progesterone is the corpus luteum. Prostaglandin $F_{2\alpha}$ administration at days 33 of pregnancy induces luteolysis and pregnancy termination with expulsion of foetuses (Verstegen et al. 1993). It is known that synthetic prostaglandin is more potent than natural prostaglandin. The use of oral dopamine agonist administration in combination with PGF analogues such as CLO or alfaprostol has been effective in cats to induce abortion (Onclin and Verstegen 1997; Erüinal-Maral et al. 2004). Greater than or equal to 40 days of pregnancy appears to be critical times for effectiveness of PGF (Nachreiner and Marple 1974; Baldwin et al. 2000). It is not clear whether the effect of PGF in these animals was the result of direct abortifacient action on the uterus or because of luteolysis of the corpora lutea of pregnancy. It is possible that abortions occurred because of a direct contractile effect on the uterus in the presence of depressed luteal function, but complete luteolysis did not happen (Shille and Stabenfeldt 1979).

In conclusion, the results from this study indicate that CLO is not effective, but ALI is effective to induce abortion in queens at 21–22 or 35–38 days of pregnancy. This seems to be in agreement with other studies showing that prostaglandins are not effective at inducing luteolysis before day 40.

Acknowledgement

This study was supported in part by UNLP grant V11/162 and V11/200 to RL de la Sota and MA Stornelli. In addition, MC Garcia Mitacek was supported with a scholarship from CONICET, and R Praderio with a scholarship from CIC.

Conflicts of interest

None of the authors have any conflicts of interest to declare.

Author contributions

Garcia Mitacek MC helped to design, conduct the experiment and process the samples, analysed data and drafted manuscript. Stornelli MC and Praderio R helped to conduct the experiment and process the samples. De la Sota RL and Stornelli MA helped to design and conduct the experiment and critically revised the manuscript.

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- Submitted: 29 May 2012; Accepted: 24 Jul 2012**
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