



Prolonged interval before conception following aglepristone-induced abortion in albino rats

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Abstract

For many years now, the several drugs used as abortifacients in animals have often been associated with severe adverse effects on the general and reproductive health of treated animals. Aglepristone is a recent antiprogesterin (progesterone-receptor antagonist) used for the induction of abortion in animals. The goal of this study was to investigate the effect on further fertility after mid-gestation termination of pregnancy with aglepristone in albino rats. Fifteen healthy female, 12-14 weeks old, albino rats (*Rattus norvegicus*) were used. Group I (n = 7) comprised rats whose pregnancies were terminated mid-gestation (day 11) with a subcutaneous injection of 10 mg/kg b.w. aglepristone. Group II (n = 8) comprised rats which were treated mid-gestation with sterile water and had normal, spontaneous gestation and parturition. The further fertility of the groups was compared by monitoring the following parameters for two subsequent pregnancies: interval before pregnancy, gestation length, litter size, litter weight at birth, fetal weight at birth and percentage of fetuses alive at birth. A subcutaneous injection of aglepristone caused abortion in 100% of rats in group I. There was a longer ($P < 0.05$) interval between abortion and pregnancy in the aglepristone-treated group (23.7 ± 6.6 days) than in group II (12.5 ± 2.7 days). However, there were no significant differences ($P > 0.05$) between the groups when gestation length, litter size, litter weight, fetal weight and percentage of live fetuses were compared. In the subsequent pregnancy, both groups displayed no differences ($P > 0.05$) in all the reproductive parameters compared. Aglepristone can be used to induce a single instance of mid-gestation abortion without any severe or prolonged adverse effects on the fertility of treated animals. It will be interesting, however, to evaluate the effects on reproduction following prolonged use or repeated induction of abortion with aglepristone.

Keywords: abortion, aglepristone, fertility, rat.

Introduction

Prevention of pregnancy or premature termination of pregnancy in animals is often required, particularly in the occurrence of mismating/misalliance, failure of contraception or in cases of fetal abnormalities

(Noakes, 2001; Schäfer-Somi *et al.*, 2007). For many decades, contraception and termination of unwanted pregnancy performed with varying regimens of estrogens, progestins, prostaglandins, antiprolactins and other abortifacients have been associated with undesirable and often severe side effects (Bowen *et al.*, 1985; Feldman *et al.*, 1993; Fieni *et al.*, 2006).

Progesterone, a natural steroid hormone secreted by the corpus luteum in the ovary, plays a crucial role in establishing and maintaining pregnancy in all mammals by promoting endometrial differentiation and embryonic implantation, cervical closure and uterine quiescence (Pineda, 2003). These biological effects of progesterone are mediated by the progesterone receptor (PR), a member of the nuclear/intracellular receptor superfamily of ligand-dependent transcription factors (Leonhardt *et al.*, 2003).

Antiprogesterins (progesterone antagonists) are synthetic steroids that bind to the progesterone receptor but fail to initiate the activities normally initiated by progesterone, and by occupying the receptors they prevent the actions of endogenous progesterone (Wanke *et al.*, 2002). Aglepristone is an antiprogesterin recently developed by Roussel Uclaf veterinary research specifically for animal use (Fieni *et al.*, 2001).

Aglepristone has been applied most prominently in the prevention or termination of pregnancy in bitches (Galac *et al.*, 2000; Schäfer-Somi *et al.*, 2007) and queens (Georgiev and Wehrend, 2006; Goericke-Pesch *et al.*, 2010). Recently, aglepristone was used in rabbits to prevent implantation (Özalp *et al.*, 2010) and to induce abortion (Özalp *et al.*, 2008).

Although antiprogesterins showed useful potential in female reproduction, further clinical applications of these agents were suggested to be marred by several undesirable effects on the endometrium (Chwalisz *et al.*, 2005). Furthermore, irregular menstrual cycles, alteration of hepatic enzymes, and hypoadrenalism following the use of antiprogesterins have been reported (Spitz, 2003). In addition, there is a poor understanding of the divergent antiprogesterin effects on the endometrium, and a potential risk of altered estrogen action as the result of prolonged use (Chwalisz *et al.*, 1998; Gopalkrishnan *et al.*, 2003).

There are few reports concerning the effects of aglepristone on the fertility of bitches (Schäfer-Somi *et al.*, 2007) and rabbit does (Özalp *et al.*, 2008). To our

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knowledge, there is a paucity of information concerning the effects of aglepristone on reproduction in female albino rats. Thus, the objective of this study was to investigate the effect on further fertility after mid-gestation termination of pregnancy with aglepristone in albino rats.

Materials and Methods

Animals

Experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals by the Institute of Laboratory Animal Research – ILAR (National Research Council – NRC, 1996) as specified by the University of Nigeria. Healthy albino rats (*Rattus norvegicus*) of the Sprague-Dawley strain comprising 15 females (12–14 weeks old) and 7 males (15 weeks old) were used for the study. The animals were housed in metal cages at room temperature (28–32°C) in the Laboratory Animal House of the Department of Veterinary Obstetrics and Reproductive Diseases, University of Nigeria Nsukka. Commercial feed (Vital® Growers feed, GCOML, Jos, Nigeria) and water were provided *ad libitum* for the duration of the study.

Determination of mating

The vaginal plug method as described by Ochiogu *et al.* (2006) was used to determine successful mating in the female rats. Following the introduction of groups of two female rats to a male of proven fertility, vaginal wet smears were grossly examined every 12 h for the presence of protein coagulates (remnants of the copulatory plug) as evidence of successful mating. The day of observation of vaginal coagulates was designated day 1 of pregnancy (Orihuela *et al.*, 2009). The body weights of the female rats were determined on day 1 of pregnancy, and subsequently at four day intervals.

Induction of abortion with aglepristone

Following confirmation of mating, a simple randomization procedure was used to allocate the female rats to either the test group (I) or control group (II). Aglepristone (Alizin®, Virbac, Suffolk, United Kingdom) was administered to group I ($n = 7$) subcutaneously below the loose skin over the neck at a dose of 10 mg/kg b.w. on days 10 and 11 of gestation (24 h apart). Rats in group II ($n = 8$) were injected with an equivalent volume (0.33 ml/kg) of sterile water for injection (Dana®, Nigeria) via the same route and on the same days as indicated for the test group. The site of subcutaneous injection was gently massaged for a few sec. The time of treatment was recorded and all the animals were placed on close observation.

Abortion was determined in group I by physical observation of bloody vaginal discharges following

aglepristone treatment. The females in group II were observed to have a normal duration of pregnancy but the pups were quickly separated from their mothers following parturition to preclude suckling.

Fertility assessment

Four days after the onset of abortion (group I) or spontaneous parturition (group II) the females were introduced to male rats of proven fertility for further breeding. The further fertility of the groups was compared by monitoring the following parameters: interval before pregnancy, gestation length, litter size and litter weight at birth, weight per fetus at birth and percentage of fetuses alive at birth. No treatment was administered prior to the subsequent breeding to observe for any carryover effect from the initial treatments.

Statistical analyses

Statistical analyses were performed using SPSS software® (Version 15.0 for Windows, SPSS Inc., Chicago, IL, USA). Means and standard deviations (SD) were calculated for data groups. The reproductive parameters of both groups were compared using the Student's *t*-test (two-tailed). Values are expressed as mean \pm SD. Results were considered significant when $P < 0.05$.

Results

Aglepristone-induced abortion

Pregnancy was terminated by aglepristone ranging from 21 h to 3.5 days after initiation of treatment in all animals in group I. Vaginal bleeding and discharge started 21 h after the first injection of aglepristone in two rats, at 24 h in two rats and after 24 h in the others (mean: 24.2 ± 3.3 h). The mean duration of bloody vaginal discharge was 51.3 ± 8.6 h. No vaginal discharges were observed in the control group following treatment with the placebo. Group II had an average gestation length of 21.8 ± 0.8 days. A group mean litter size of 7.7 ± 2.0 with a litter weight of 44.0 ± 6.8 g was delivered. The average weight of the fetuses at birth was 5.9 ± 0.7 g.

Effects on further fertility

Following mid-gestation termination of pregnancy with aglepristone, the further fertility of the animals post-treatment was verified (Table 1). The results showed a longer ($P < 0.05$) interval before pregnancy in the aglepristone-treated group (23.7 ± 6.6 days) than in group II (12.5 ± 2.7 days). However, there were no differences ($P > 0.05$) between the groups when gestation length, litter size, litter weight, fetal weight and percentage of live fetuses were compared.



Table 1. Reproductive indices following mid-gestation pregnancy termination with aglepristone in albino rats.

| Groups | Interval from parturition or abortion to next pregnancy (days) | Gestation length (days) | Litter size | Litter weight (g) | Weight per fetus (g) | Fetuses alive at birth (%) |
|------------|--|-------------------------|-------------|-------------------|----------------------|----------------------------|
| I (n = 7) | 23.7 ± 6.6 ^a | 21.1 ± 1.3 | 8.9 ± 1.8 | 48.1 ± 4.3 | 5.5 ± 0.6 | 91.1 ± 9.5 |
| II (n = 8) | 12.5 ± 2.7 ^b | 21.8 ± 1.3 | 8.5 ± 2.2 | 44.2 ± 7.1 | 5.3 ± 0.5 | 92.3 ± 8.6 |

^{ab} Within columns, different superscripts indicate significant differences ($P < 0.05$).

In the subsequent pregnancy (Table 2), both groups had no significant differences in the interval before pregnancy, gestation length and other reproductive indices. Although the group values for litter size, litter

weight, weight per fetus and percentage of live fetuses were higher than observed for the pregnancy immediately after aglepristone administration, these increases were not significant ($P > 0.05$).

Table 2. Reproductive indices in the subsequent pregnancy in albino rats (to observe for carryover effects following initial treatment).

| Groups | Interval from parturition to next pregnancy (days) | Gestation length (days) | Litter size | Litter weight (g) | Weight per fetus (g) | Fetuses alive at birth (%) |
|------------|--|-------------------------|-------------|-------------------|----------------------|----------------------------|
| I (n = 7) | 13.6 ± 2.8 | 21.6 ± 1.6 | 9.3 ± 2.3 | 52.6 ± 8.0 | 5.8 ± 0.8 | 93.6 ± 8.4 |
| II (n = 8) | 15.3 ± 3.6 | 21.1 ± 1.3 | 9.9 ± 2.0 | 55.8 ± 6.3 | 5.8 ± 0.6 | 97.3 ± 4.6 |

No difference has been detected between groups for any end point.

Discussion

The dose of 10 mg/kg aglepristone was selected based on published reports about the use of the antiprogestin in the guinea pig (Baron von Engelhardt, 2006) and rabbit (Özalp *et al.*, 2008), and also on the successful use of the same dose in rats in our previous studies (unpublished data). Although the antiprogestin was generally well tolerated, a slight local irritation was observed following s.c. injection of aglepristone in one rat (6.7%). This manifested as restlessness and an attempt to scratch the scruff of the neck but did not persist for more than 10 min following treatment. This observation has been reported in 1.8% of cats (Fieni *et al.*, 2006) and 9% of dogs (Pettersson and Tidholm, 2009) and is suggested to be a transient side effect of the oily-alcohol solution of Alizin[®] (Fieni *et al.*, 2001).

The results of this study demonstrate the efficacy of aglepristone in the termination of pregnancy in rats. A subcutaneous injection of aglepristone caused abortion in 100% of treated rats which is in agreement with reports in dogs (Galac *et al.*, 2000; Schäfer-Somi *et al.*, 2007) and rabbits (Özalp *et al.*, 2008). Other studies in cats reported lower efficacy rates of 88.5% (Fieni *et al.*, 2006) and 87% (Georgiev and Wehrend, 2006).

Clinical applications of antiprogestins in humans and animals appear to be generally limited because of potential reproductive hazards (Sitruk-Ware and Spitz, 2003; Chwalisz *et al.*, 2005). Although aglepristone has been applied clinically in different animals, there are few reports concerning its effects on the further fertility of treated animals. Schäfer-Somi *et al.* (2007) conducted two subsequent inductions of abortion in bitches with aglepristone and a combination of aglepristone and cabergoline and concluded that

subsequent cycles were normal and fertile in the majority of treated bitches. In addition, rabbit does whose pregnancies were terminated mid-gestation with aglepristone were reported to have normal fertility post abortion (Özalp *et al.*, 2008). In this study, pregnant rats that aborted from aglepristone treatment were subsequently bred twice to observe any long term effects of aglepristone. The short generation time and the multiple-litter bearing nature of the rat allowed us to investigate subtle negative effects on reproduction which otherwise may be difficult to observe in the monotocous species. In this study, longer interval between abortion and subsequent pregnancy was observed, which is in agreement with the findings of Özalp *et al.* (2008) in which rabbits treated with aglepristone became non-pregnant for a relatively long interval (46-63 days). Following daily examination for evidence of mating post-abortion, the aglepristone-treated rats revealed a prolonged period of sexual non-receptivity. Özalp *et al.* (2008) made a similar observation and suggested that the prolonged period of sexual non-receptivity might explain the extended non-pregnant period following abortion. Apart from this delay in conception post-abortion, no other negative effects were observed on fertility, thus corroborating the reports in bitches (Schäfer-Somi *et al.*, 2007) and rabbits (Özalp *et al.*, 2008). Following abortion, the pregnancy rate in rats was 100%, whereas a lower rate of 80% was reported in dogs (Schäfer-Somi *et al.*, 2007) and rabbits (Özalp *et al.*, 2008). On further breeding, the aglepristone-treated rats had 100% fertility. However, unlike this study, Schäfer-Somi *et al.* (2007) induced abortion two subsequent times and reported a pregnancy rate of 80% following the second abortion.

In summary, aglepristone can be used to induce



a single instance of mid-gestation abortion without any severe or prolonged adverse effects on the fertility of treated animals. It is recommended, however, that further investigations be carried out on the effects of prolonged use of aglepristone on reproduction in animals.

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