Cyclosporine A exposure during pregnancy in mice: effects on reproductive performance in mothers and offspring

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BACKGROUND: Pregnancies after organ transplantation and under immunosuppressive treatment are associated with slightly elevated risks for obstetric and post-natal complications but can usually be managed well. However, little is known about the effects of intrauterine exposure (IUE) to immunosuppressants in the growing and adult offspring. One major issue is the potentially negative effects of immunosuppressive medication on reproduction. This study investigates the effect of exposure during pregnancy to the most commonly used immunosuppressant in organ transplantation, cyclosporine A (CsA), on the reproductive outcome in mothers and offspring.

METHODS: Female C57CBA-F1 mice received 0, 10, 20 or 30 mg/kg bodyweight of CsA daily by subcutaneous mini-osmotic pumps during mating and pregnancy. Blood concentrations of CsA, implantation rates, resorption rates and fetal weights were analysed. In addition, female and male mice exposed to CsA in utero were mated to unexposed partners and pregnancy outcomes were analysed.

RESULTS: Direct maternal exposure to CsA at high doses reduced implantation rates and fetal survival. IUE to CsA reduced adolescent growth but did not affect fertility, although a reduction in birthweight was seen in offspring of females exposed to CsA in utero.

CONCLUSIONS: CsA exposure during pregnancy correlates with impaired reproductive outcome, but offspring fertility is not affected. The cause of reduction in adolescent weight gain and low birthweight in offspring of females exposed to CsA in utero need further investigation.

Key words: cyclosporine A / mouse / offspring / pregnancy / uterus transplantation

Introduction

The improved survival of transplant recipients has led to increased attention on quality-of-life issues, including fertility and having children. Today, many females who receive organ transplants are children or women of fertile age, and a proportion of these may consider pregnancy after transplantation in spite of being on immunosuppressive medication. In the Scandinavian countries, with a total population of 25.1 million, the number of women who received organ transplants during the time period 1995–2008 was 266, 305, 617 and 1091 in the age groups 0–9, 10–19, 20–29 and 30–39, respectively (Scandinavian transplant, Frank Pedersen, personal communication). The first post-transplant pregnancy with a live birth in humans was reported more than 50 years ago (Murray et al., 1963), and today, three major registries (the European Dialysis and Transplantation Association Registry, the UK Transplant Pregnancy Registry and the National Transplantation Pregnancy Registry in the USA) collect data about pregnancies in transplanted women. These registries have together accumulated data from more than 14,000 births among female solid organ transplant recipients (McKay et al., 2006).

Even though pregnancies in transplanted women under immunosuppressive therapy usually can be managed with a positive outcome (EBPG, 2002; Yassaee and Moshiri, 2007; Kim et al., 2008), these pregnancies are associated with an increased risk of hypertension, pre-eclampsia, miscarriage and pre-term delivery of babies that are small for gestational age (Armenti et al., 2002; Kallen et al., 2005; Sibanda et al., 2007), and there are several concerns regarding specific effects of immunosuppressants on fetal and postnatal development (Cleary and Kallen, 2009). For example, studies in rodents have shown that intrauterine exposure (IUE) to the most
commonly used immunosuppressant, cyclosporine A (CsA), at different developmental stages can induce permanent renal nephron deficiency in the offspring (Brown et al., 1985; Fein et al., 1989; Tendron-Franzin et al., 2004). The severity of these effects seems to differ between species and no study so far has identified similar effects in humans (Al-Khader et al., 2004; Cochat et al., 2004). However, studies both in animals and humans have shown persistent alterations in the immune cell repertoire after in utero exposure to CsA (Hussain et al., 2005) as well as an increased risk for induction of auto-antibodies (Classen and Shevach, 1991; Pilarski et al., 1994; Cimaz et al., 2004; Biggiogero et al., 2007).

When the first, and so far only, clinical transplantation of a uterus was performed in Saudi Arabia 9 years ago (Fageeh et al., 2002), it was an attempt to realize an idea that has attracted the attention of several researchers and clinicians over the years (Barron-Kamren and Wraning, 2007). In this case, the uterus had to be removed after 3 months due to necrosis of the organ (Fageeh et al., 2002) but nevertheless it stimulated research in this field. Since then, animal models for uterus transplantation have been developed in rodents (Racho El-Akouri et al., 2002; Jiga et al., 2003; Wraning et al., 2008) and large animals (Seunarine et al., 2005; Wraning et al., 2006; Dahm-Kahler et al., 2008; Ramirez et al., 2008; Avison et al., 2009) for studies of different aspects such as rejection (Jiga et al., 2003; El-Akouri et al., 2006), effects of cold ischaemia (Racho El-Akouri et al., 2003a; Wraning et al., 2005, 2008a) and reproductive outcome after a non-rejecting uterus transplantation (Racho El-Akouri et al., 2003b). The collective experience from these as well as from older studies shows that the surgical intervention of transplantation of the uterus can restore normal function and result in normal pregnancies with healthy offspring, at least in the absence of allogeneic reactions. However, there are still several issues that need attention before any further attempts to transplant a uterus in a woman are performed and one concern is the effect of immunosuppression on reproductive outcome and long-term health of the offspring.

As a first step to study the effects of immunosuppression during pregnancy in this context, the present study investigates the effect of continuous administration of CsA on mating and reproductive outcome in mice and their offspring.

Materials and Methods

Study design

To study the direct effect of CsA exposure on reproductive measures, female mice were exposed to CsA from 1 week before mating and throughout pregnancy [maternal exposure (ME); vehicle (ME 0, n = 10), 10 mg/kg/day (ME 10, n = 10), 20 mg/kg/day (ME 20, n = 10) and 30 mg/kg/day (ME 30, n = 3)] before mating. Pregnancy was followed to Day 18 of estimated pregnancy duration (21 days) when the animals were euthanized. CsA blood concentrations (before and during pregnancy), mating frequency, implantation rate, intrauterine deaths of fetuses and fetal weights on Day 18 of pregnancy were recorded.

To study the effects of IUE to CsA on reproduction on male and female mice, offspring of CsA-exposed mothers (IUE) were mated and reproductive measures were analysed. To produce this group, C57BL/6 females of proven fertility were exposed to CsA and mated to CBA/ca males, also of proven fertility. The females were exposed to CsA at doses of 0 (n = 6), 10 (n = 6) and 20 mg/kg/day (n = 6) from 8 days before mating and throughout pregnancy. CsA was withdrawn on Day 21 of pregnancy (expected partus). Pregnancy frequency and litter size were recorded. Due to the severe feto-toxicity of 30 mg/kg/day in the ME experiment, this dose was excluded when producing the IUE animals.

Male and female offspring of these CsA-treated mothers [maternal dose of 0 (IUE 0 female, n = 5; IUE 0 male, n = 5) and 20 (IUE 20 female, n = 6 and IUE 20 male, n = 6) mg/kg/day CsA] were followed to adulthood and mated with unexposed partners. In these animals, growth trajectory, mating frequency, implantation rate (for exposed females) or live birth rate (mating partners of exposed males) and fetal/newborn pup weight were analysed. Also, serum creatinine concentrations and adult kidney morphology after intrauterine CsA exposure was analysed in pregnant females.

Animals

C57BL6-F1 hybrid mice (cross between C57BL6 females and CBA/ca males) 8–16 weeks of age were used. Animals used in the ME group and intact mating partners for IUE group were produced by Harlan Laboratories, Horst, The Netherlands. Animals in the IUE groups were produced by in-house (Institute for Experimental Biomedicine, University of Gothenburg, Sweden) mating intact CBA/ca males and CsA-treated C57BL/6 females (Harlan Laboratories). Untreated mating partners (males for ME group and males and females for IUE group) and parental animals for production of IUE group animals were all of proven fertility. The animals were housed in controlled conditions (21–23°C, relative humidity of 50–60%, illumination between 07.00 and 19.00 h) and had free access to water and pellet food. The study was approved by the Animal Ethics Committee in Gothenburg, Sweden, and was carried out according to the principles and procedures outlined in the ‘Guide for the Care and Use of Laboratory Animals’ (National Institute of Health, USA).

CsA administration

CsA was administered by subcutaneously placed mini-osmotic pumps (model 2001 and 2002, Alzet Osmotic Pumps, Cupertino, CA, USA) that continuously deliver the agent it is filled with. In both the ME and IUE groups, pump models lasting for 2 weeks (model 2002) were used at mating and during the first 3–7 days of pregnancy. Thereafter, 1-week pumps (model 2001) were used and changed accordingly. All animals (CsA-treated and vehicle) went through the same procedures at placement and change of pumps. Before placement of the pump, the mice were weighed and CsA concentrations for each dose group were calculated for 5 g intervals of mouse weight and rounded up to compensate for growth gain during the experiment. CsA (Sandimmun, Novartis Pharma AG, Basel, Switzerland; 50 mg/ml in ethanol and macroglucglycerinoleate) was diluted in 90% propylene glycol in saline (Fluka, Buchs, Switzerland) to desired concentrations and mini-osmotic pumps were loaded with either CsA solution or 90% propylene glycol (vehicle group) and placed in warm (37°C) physiological saline overnight for activation. At placement and change of mini-osmotic pumps, the animals were anaesthetized (isoﬂurane, 5% induction, 2% maintenance on 500 ml/min air and 500 ml/min O2). The neck was shaved and local analgesia (bupivacain; Marcan, Astra Zeneca, Södertälje, Sweden; 2.5 mg/ml, s.c. injection of 0.3 ml) was applied to the shaven area. A small skin incision (~10 mm) was cut on the lateral side of the neck, just cranial of the scapulae and a subcutaneous pocket (~10 × 25 mm) was created in a medial-caudal direction from the incision by gentle separation of the membranes overlying the muscle fascia. The pump was placed in this pocket and the incision was closed with three interrupted 6-0 polyglactin sutures or metal clips.
Mating

Females under CsA exposure were placed with unexposed males 3 (parental females for production of IUE group animals) or 8 (ME group) days after placement of mini-osmotic pumps. In the ME group, females were randomly allocated two and two to each male. Parental females for production of IUE group animals and in utero-exposed females in IUE group were randomly allocated to one male each. Unexposed females were also randomly allocated to one in utero-exposed (IUE group) male each. The females were checked every morning for appearance of vaginal semen plug and if one was found, the female was considered mated (pregnancy day 0) and separated from the male. Females not displaying a vaginal plug for 5 consecutive days were removed from the male and treated as if mated for the duration of a theoretical pregnancy, calculated from the day of removal from male.

Assessment of implantation rate and fetal development

Females exposed to CsA during pregnancy (ME group) and females exposed to CsA in utero (IUE group) were anaesthetized (isoflurane, 5% induction, 2% maintenance on 500 ml/min air, 500 ml/min O2) on Day 18 or 14 of pregnancy, respectively. A midline laparotomy was performed and the uterus was exposed. Viability of existing fetuses was judged from movements, colouration and size as well as bleeding from the umbilical cord when cut. The number of viable and resorbed fetuses was counted. Darkened remnants of fetuses and placentas and non-viable fetuses of approximately the same size and appearance as viable fetuses were considered as resorbed pregnancies. Each viable fetus was carefully freed from its placenta and amniotic sac and weighed before both fetus and mother were euthanized.

Pregnant female mating partners of in utero-exposed males in the IUE group were checked for partus every morning from Days 18 to 22 of pregnancy. Newborn pups were counted and weighed on Day 1 after birth and mothers and pups were then euthanized.

Analysis of CsA blood levels, serum creatinine and kidney morphology in exposed animals

To establish baseline data on CsA blood concentrations, serum creatinine and kidney morphology after CsA exposure in this particular setting, a pilot study was performed. A smaller number of animals was exposed to CsA (0, 10, 20 and 30 mg/kg/day, n = 3 in each dose group) for 7 weeks. At the time of euthanasia, blood and serum samples as well as the right kidney were collected for analysis.

In the ME group, blood samples for determination of CsA blood concentrations were taken after 3 days of treatment (before mating) and on Day 12 of pregnancy (in conjunction with change of mini-osmotic pump). Venous blood from the tail (~200 μl) was sampled into an EDTA-coated micro-tube (Microtainer, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and kept in +8°C until analysis the next day.

Analysis of circulating CsA concentrations was performed on whole blood by enzyme immunochemistry using a CyA-specific assay (Emit® 2000, Dade Behring, Milton Keynes, UK) according to the manufacturer’s instructions. Where appropriate, the samples were diluted before analysis and results were expressed as nanograms per millilitre. The lower detection limit of the assay was 30 ng/ml and the inter-assay coefficient of variation was below 9% for the targeted concentration at analysis (200–400 ng/ml).

To detect any nephrotoxic effects of CsA in this setting, serum from animals in the pilot study as well as from pregnant females in the IUE group was analysed for concentration of creatinine using enzymatic conversion of creatinine to quinon-imin, for which absorbance of light at 546 nm wavelength is proportional to the concentration of creatinine. The analysis was performed using a spectrophotometer (Modular P, Roche Diagnostics, GmbH, Mannheim, Germany) and a reagent kit (CREAplus R1, R2, Roche Diagnostics, GmbH) according to the manufacturer’s instructions. The lower detection limit of the assay was 2.7 μmol/l and the inter-assay coefficient of variation was 4% for concentrations <85 μmol/l.

At the time of euthanasia of animals in the pilot study (7-week exposure to 0, 10, 20 or 30 mg/kg/day, non-pregnant animals, n = 3 in each group) and IUE group (n = 5 and n = 6 of the 0 and 20 mg/kg/day, respectively, pregnant on Day 14), the right kidney was harvested, bisected longitudinally and put in formaldehyde (4% in phosphate buffer) for histology analysis. After fixation, the kidneys were dehydrated and embedded in paraffin. Tissue sections (4–6 μm thick) were mounted on glass slides, stained with haematoxylin and eosin and examined for morphological changes related to CsA treatment, such as tubular cell necrosis. Since the animals of the pilot study were not pregnant and the IUE animals were pregnant, these two arms were not compared.

Statistics

Correlation between maternal CsA dose and number of implantations, live fetuses, resorbed fetuses and fetal weight (ME group) was performed using Spearman’s rank correlation test. Offspring differences in body weight at different time points (IUE group), number of implantations (IUE group), number of resorbed fetuses (IUE group), number of live born pups (unexposed female mating partners to males in IUE group), fetal/newborn pup weight (exposed females in IUE group and unexposed female mating partners of exposed males in IUE group) and CsA dose and serum creatinine concentrations (IUE females) were analysed using the Student’s t-test for independent samples. The relationship between CsA dose and maternal CsA blood concentrations (ME group) was evaluated by regression analysis.

Results

Pilot study

Continuous exposure of female mice to CsA in doses of 0, 10, 20 and 30 mg/kg/day for 7 weeks resulted in CsA blood concentrations of 0, 785–1245, 1500–2000 and 2800–3900 ng/ml, respectively. All animals were weighed weekly, and during the 7 weeks of exposure, their weight increased by 7.2–9.5 g (vehicle), 7.3–9.6 g (10 mg/kg/day), 8.0–4.1 g (20 mg/kg/day) and 0.2–4.3 g (30 mg/kg/day). After 7 weeks of exposure, serum creatinine concentrations ranged from 10 to 25 (vehicle), 8 to 10 (10 mg/kg/day), 10 to 11 (20 mg/kg/day) and 10 to 12 (30 mg/kg/day) μmol/L and kidney morphology was similar between animals from either dose group (data not shown).

Direct exposure to CsA during pregnancy

In the ME experiment, CsA dose correlated negatively with the number of implantations (correlation coefficient = −0.520, P < 0.01) and positively with the number of resorbed fetuses (correlation coefficient = 0.367, P < 0.05) found on Day 18 after vaginal plug (Fig. 1). There were no differences in the number of females displaying a vaginal plug between the vehicle, ME 10 and ME 20 groups (9, 10 and 10 out of 10) and even though in the ME 30 group only 6 out of
9 females displayed a vaginal plug, this was not significantly lower than in the other groups. In the ME 0, ME 10 and ME 20 groups, all animals with a confirmed vaginal plug were pregnant on examination, while in the ME 30 group only four of six animals with confirmed vaginal plug were pregnant. The average weight of viable fetuses at Day 18 of pregnancy was not different between dose groups [ME 0: 1.2 g ± 0.17, ME 10: 1.15 ± 0.22, ME 20: 1.15 ± 0.22, ME 30: 1.15 ± 0.22 (mean ± SD)]. The concentration of CsA in maternal blood at a specific dose decreased significantly (P < 0.01) during pregnancy and the level of this reduction differed significantly between dose groups (P < 0.01) (Fig. 2).

CsA exposure during pregnancy in C57BL/6 females (for production of the IUE group) resulted in a reduction of pregnancies and litter size [0 mg/kg/day: four (of six) females pregnant, six to nine pups/litter; 10 mg/kg/day: one (of six) pregnancies, five pups; 20 mg/kg/day: two (of six) pregnancies, five and seven pups per litter] compared with the F1-hybrid females in the ME group. Due to the small number of pregnant animals (one of six), pups of the female exposed to 10 mg CsA/kg/day were excluded from the study. At weaning, the vehicle-exposed pups were reduced to five animals per sex group by removal of the smallest and largest animals from each litter.

IUE to CsA

There was no difference in birthweight between the IUE 0 and the IUE 20 groups (both genders included, n = 31 and n = 12, respectively) (Fig. 3A). At 4 weeks of age, when the animals were sorted by gender and the vehicle group was reduced, female mice exposed in utero were of lower weight than females exposed to vehicle (P < 0.01), a difference that persisted throughout the study period (Week 5: P < 0.01, Week 6: NS and Week 7 P < 0.01) (Fig. 3B). Exposed males were also of significantly lower body weight than unexposed males at 4 and 5 weeks of age (Week 4: P < 0.05 and Week 5: P < 0.001), but not at later time points (Fig. 3C).

At mating, all IUE females displayed a vaginal plug within 5 days after introduction to the male and all were pregnant on examination. In female mating partners to IUE males, only one displayed a visible vaginal plug but since these males were young and inexperienced, the females were treated as if mated and showed 100% frequency of delivery. There was no significant difference in the numbers of foeti (IUE 0 females: median 8, range 7–9 fetuses/female; IUE 20 females: median 7, range 2–9 fetuses/female) or born pups (IUE 0 males: median 10, range 8–11 pups/female; IUE 20 males: median 9, range 7–10 pups/female) between exposed or non-exposed IUE animals and no increase in the number of resorbed fetuses in exposed IUE females (one resorption in each dose group). Fetuses of IUE 20 females were of lower weight on Day 14 of pregnancy than were fetuses of non-exposed females (P < 0.05) (Fig. 4). There was no difference in the birthweights of pups of untreated females mated with exposed or non-exposed IUE males (Fig. 4).

Serum creatinine concentrations in IUE females on Day 14 of pregnancy were not significantly different for the IUE 20 group compared with the vehicle group (IUE 0: median 43.5, range 24.0–52.0 μmol/l; IUE 20: median 25.0, range 16.0–41.0 μmol/l). Analysis of kidney histology did not show any structural differences between animals exposed to CsA and vehicle in utero (data not shown).
Discussion

Although several animal studies have investigated the effect of CsA on pregnancy and fetal development, there are very few reports on long-term effects on health and no studies on reproductive health after in utero exposure to immunosuppressants. In one case report on severe pregnancy complications in a daughter to a transplanted mother, concerns were raised regarding late onset autoimmunity possibly caused by in utero exposure to immunosuppressants (Scott et al., 2002). If transplantation is to be used as treatment of absolute uterine factor infertility in an otherwise healthy woman, such concerns need to be addressed by studies in animals before clinical trials commence. The present study is, to our knowledge, the first study on reproductive health in animals exposed to immunosuppressive drugs in utero.

Long-term treatment with daily CsA doses of 20 and 30 mg/kg (pilot group) reduced animal growth rate compared with animals given vehicle or 10 mg/kg. Others have reported similar findings in mice (Masri et al., 1988) where supra-therapeutic doses of CsA induced anorexia, and thereby weight loss, that correlated with renal nephron deficit. Also, in a report of small children treated with CsA for diabetes type 1, anorexia was one of several side effects of the treatment (Jenner et al., 1992). In the present study, there seemed to be no correlation between reduced weight gain and CsA nephrotoxicity, since kidney morphology and creatinine values were not affected. However, it cannot be ruled out that subtle functional renal changes influencing body growth were present.

In the present study, we found that direct CsA exposure during pregnancy (ME group) correlated with reduced implantation frequency and increased number of fetal deaths in a dose-dependent manner. This observation is in line with previous findings in the mouse (Fein et al., 1989) and the rat (Brown et al., 1985; Mason et al., 1985). The observed reduced implantation rate suggests that CsA, directly
or indirectly, affects the endometrium or decidua in a negative way. It has been demonstrated that CsA may interfere with ovarian function and the finely tuned steroid secretion pattern around and after ovulation, which may lead to disturbances in the transition of the endometrium into the secretory phase and, specifically, at the implantation window. Thus, CsA at higher doses suppresses progesterone secretion from rat granulosa cells in vitro (Gore-Langton, 1988) and a direct CsA effect on the ovarian level has been demonstrated in rats (Esquifino et al., 1995). The disturbance in endometrial receptivity by CsA was also indicated by reduced litter sizes when CsA was given to rats during the fertilization and implantation phases (Brown et al., 1985). The profound effects of CsA on T-cell (Tumlin et al., 2009) and NK cell (Wang et al., 2007) activation will of course interfere with the local cytokine networks that are needed for the intricate cross-talk between the endometrium and embryo at implantation (Saito, 2001).

The observed increase in resorption rates on the other hand could be ascribed to direct CsA toxicity on the fetus or to the fact that the uterine milieu is suboptimal which will lead to fetal death during later stages of pregnancy, with the increasing demand on blood circulation by the placenta. Cyclosporine passes over the placenta in a dose-dependent manner and this is counteracted by the activity of P-glycoprotein in trophoblasts (Pavek et al., 2001). The results from the present study indicate a threshold for the CsA effect of fetal resorption at around 1000 mg/ml of maternal CsA blood concentrations.

The concentration of CsA in maternal blood was found to decrease during pregnancy and this decrease was also more pronounced for the higher dose groups. A similar decrease of CsA blood concentrations has also been observed in pregnant kidney transplanted women (Fischer et al., 2005). The mechanisms behind these observations are not entirely clear but possibly can be partly ascribed to an increased expression of CYP3A isoforms induced by both pregnancy (Zhang et al., 2008) and CsA (Nakamura et al., 1994; Lemahieu et al., 2004) that would accelerate the catabolism of CsA and thus reduce maternal blood concentrations of the drug.

Contrary to other studies in rodents (Brown et al., 1985; Tendron et al., 2003) and indications from registry data in humans (Armenti et al., 2002), CsA exposure during pregnancy did not result in reductions of fetal weight (ME group) or birthweight (IUE group) in the present study. However, at 4 weeks of age, a difference could be seen in exposed female mice (IUE group) that persisted for the rest of the study period. The principle immunosuppressive mechanism of CsA is its inhibition of the phosphatase activity of calcineurin that will lead to inhibition of T-cell production of IL-2 and thus reduce T-cell proliferation. However, calcineurin is a ubiquitous phosphatase involved in a multitude of other basal physiological functions and one can speculate that suppression of calcineurin activity by CsA during embryonic and fetal development might induce persisting changes. Others have also reported that calcineurin Aα and Aβ null mice show reduced adult body weight (Parsons et al., 2003) but the mechanisms by which calcineurin inhibition would affect post-natal weight gain are yet to be revealed.

Others have shown that CsA exposure during male rat adolescence induces chronic impairment of spermatogenesis (Srinivas et al., 1998), and in adult rats, CsA induced changes in sperm morphology (Masuda et al., 2003). However, this observed adverse effect of CsA on male reproduction does not seem to involve testis formation since the reproductive health of the male mice exposed to CsA in utero was not different from controls. Mating behaviour was intact, litter sizes and pup newborn body weight were similar to controls and no abnormal morphological features of pups could be seen.

Female mice exposed to CsA in utero also showed intact mating behaviour and receptivity and implantation rates were similar to controls. However, these females carried fetuses that were slightly but significantly smaller than those of vehicle-exposed females. Considering the well-documented nephrotoxic effect of CsA, also in mice (Masri et al., 1998), it could be suspected that physiological changes related to impairment of kidney function, such as hypertension, could be the underlying cause of the low birthweight. However, neither serum creatinine concentrations nor analysis of kidney morphology indicated CsA-induced impairment of kidney function. Although more subtle changes not detected by these measures cannot be ruled out, an alternative explanation of the observed reduced birthweight is that it is related to the smaller size of the females. In humans, birthweight is directly correlated to maternal pre-gestational body mass index (Frederick et al., 2008) and even though not shown for mice, this is a plausible explanation.

In conclusion, the results of the present study show that continuous administration of CsA throughout pregnancy negatively affect reproductive performance and pregnancy outcome in mice in dose-dependent manners. IUE to CsA reduced weight gain during adolescence but did not induce any changes in mating behaviour or implantation rate. A small but significant reduction of fetal body weight was seen in offspring to females exposed to CsA in utero, which could not be correlated to CsA nephrotoxicity.

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