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A retrospective analysis of adverse effects of an *in vivo* fluoroquinolone antibiotic enrofloxacin treatment on oocyte quality in the common marmoset☆

O.Y. Tkachenko[a,b,*], J.U. Scheerer-Bernhard[a,c], S. Delimitreva[a,d], E. Wedi[a,e], R.R. Valle[a,f], M. Heistermann[g], P.L. Nayudu[a]

[a] *Reproductive Biomedicine Group, Reproductive Biology Unit, German Primate Centre, Leibniz Institute for Primate Research, Kellnerweg 4, Goettingen, 37077 Germany*
[b] *Division of Reproductive and Developmental Sciences, Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, OR, 97006, USA*
[c] *Department of Internal Medicine, Roskilde Hospital, Roskilde, 2100, Denmark*
[d] *Department of Biology, Medical University of Sofia, 1 St. Georgi Sofiyski Street, Sofia, 1431, Bulgaria*
[e] *Gastroenterologie und GI Onkologie, Universitätsmedizin Göttingen, Robert-Koch-Straße 40, 37073 Göttingen, Germany*
[f] *Instituto de Ciências da Saúde, Universidade Paulista, Institute of Health Science, Paulista University, Rua Dr. Bacelar, 1212, São Paulo, SP, Brazil*
[g] *Endocrinology Laboratory, German Primate Centre, Leibniz Institute for Primate Research, Göttingen, Germany*

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ABSTRACT

Here we report a retrospective analysis of negative effects of routine enrofloxacin treatment of recurrent diarrhea on the ovary and the developing oocytes of the common marmoset, a small New World primate. The most deleterious effect on oocytes was observed about two months post treatment suggesting that the enrofloxacin effect is on early growing follicles. Manifestations of toxicity included decreased numbers of growing follicles and recovered culturable oocytes, as well as signs of early atresia of granulosa cells. In addition, increased amounts of holed stroma after treatment strongly suggested increased death of the early growing follicles. Of the oocytes judged to be of adequate quality for culture, maturation rates were not affected but fertilization of *in vitro* matured MII oocytes and subsequent cleavage rates were severely reduced in the enrofloxacin treated animals. Further, the arrested oocytes, which failed to mature or fertilize, showed obvious meiotic spindle abnormalities.

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1. Introduction

Callithrix jacchus (the common marmoset) is a valuable primate model in studies of reproductive biomedicine due to ease of handling, high fecundity and applicability of the research results to human [1,2]. As a general rule, captive management of any species used for scientific research must be critically evaluated and optimized to ensure animal health and to minimize the risk of background variables interfering and reducing the validity of experimental results. Achieving this status is critical and difficult for species which are essentially wild and outbred, such as the common marmoset, which is a tree living small primate with a specialized diet. Captive conditions have usually been developed through practical experience in each colony, essentially independently. But in all colonies, animals are maintained in conditions that dramatically differ from their native habitat. This includes, for example, indoor housing in relatively small cages and feeding a commercial pellet-based diet developed from experience with domestic animals [3–5], which deviates from the tree-gum, fruit and insect feeding which constitutes a major part of the diet in animals living in their natural habitat.

Critical testing of the effects of specific management practices for the marmoset, to our knowledge, have not been carried out despite the reports of the common occurrences of several chronic disease conditions, such as recurring diarrhea, gut pathology, kidney disease, and diabetes [6–9] all suggesting the need for wide ranging optimization and controlled studies of causal factors of such diseases. Important general issues here may involve inappro-

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* Corresponding author at: Division of Reproductive and Developmental Sciences, Oregon National Primate Research Center, Oregon Health & Science University, Beaverton, OR 97006, USA.
E-mail addresses: tkacheno@ohsu.edu, olenatkachenko@googlemail.com (O.Y. Tkachenko).

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priate diet or even cleaning practices. For example, our group has found that strong cleaning chemicals have been associated with an increased rate of chromosomal abnormalities in marmosets [10].

The repeated outbreaks of specific diseases, such as diarrhea associated with haemolytic *E.coli*, can also implicate a serious underlying management problem, possibly involving diet and or crowding. The standard treatment of this condition is with enrofloxacin (an antibacterial drug sold by the Bayer Corporation under trade name Baytril®). However, no studies have been carried out to determine if there are any negative side effects associated with this treatment. Of particular interest in this respect are potential side effects on the functioning of the female reproductive system which is one of the major areas of research in which the marmoset monkey is used [1]. Here, we report potential reproductive effects (i.e. influences on ovarian physiology and histology) of enrofloxacin treatment in females.

In the course of one of our studies involving *in vitro* maturation of oocytes and embryo culture, negative changes in outcome of the experiments became apparent in animals which were treated with enrofloxacin prior to their use in these experiments, compared with results from untreated females. All animals in the study group were treated with enrofloxacin and changes in outcome were independent of whether the animals had shown symptoms of diarrhea before treatment or not, and therefore, were not disease-related. This led us to the idea to carry out a retrospective analysis comparing the *in vitro* performance of the oocytes from treated and untreated females and to evaluate the ovaries histologically, as previously reported in [11]. By this, we hoped to provide information on specific effects that enrofloxacin treatment exerts on ovarian physiology and histology in a primate model that is widely used in reproductive biomedical research.

Enrofloxacin belongs to antibiotics of the fluoroquinolone class, which are the most active oral antibacterials used in the treatment of a wide variety of bacterial infections in both humans and other mammals. Enrofloxacin itself is not approved for use in humans due to its stimulatory effect on the central nervous system, possibly due to inhibition of gamma-aminobutyric acid (GABA) binding to the GABA receptor [12,13]. It is still widely used in veterinary practice though. Unfortunately, there is a complete lack of information about the effects which fluoroquinolones in general, and enrofloxacin specifically, have on the female reproductive system, in particular on folliculo- and gametogenesis in any species. The aim of the present analysis was therefore to examine the effect of the *in vivo* enrofloxacin treatment on marmoset ovary over time to evaluate short- and long-term effects, with a particular focus on the oocyte quality. Data of experimental outcomes were supported by histological observation of ovarian structure and hormonal measurements.

2. Materials and methods

2.1. Housing conditions

All procedures were carried out in accordance with the German Animal Experimentation Law (Animal Experiment Permission # 33.42502/08-01.03); animals were housed and surgery procedures carried out according to the standard German Primate Centre practice for Callithrix jacchus [14–16].

In brief, common marmosets in the German Primate Centre were housed in the rooms with either only artificial light or with artificial light together with daylight coming through glass windows. Artificial light was controlled by a timer and was on from 6:30 AM till 7 PM. Room temperature was 25 °C with air humidity of 60%. Animals in the experimental colonies were kept in metal cages (0.7 m × 0.5 m × 1.0 m). Cages were furnished with wood pieces and metal or wood sleep boxes. Experimental animals were kept in pairs, where one of the partners was castrated/ovariectomized, or in some cases in unisexual pairs.

Water and pellets (New-World primate pellets (ssniff®, ssniff Spezialdiäten GmbH, Germany) with the addition of cat pellets (domino®, Kofur Handelsgesellschaft mbH, Germany)) were always available *ad libitum*. Fresh food was supplied twice daily (first meal – fresh cheese, cooked baby cereal, yoghurt, dry bread, noodles and second meal – fresh fruits and vegetables supplemented by vitamins, proteins, minerals in a weekly rotation. Arabic gum was offered every day in slices prepared from air-dried Arabic gum syrup. Insects were offered twice weekly [17–19].

2.2. Cycle monitoring and ovarectomy

Cycle monitoring was performed by plasma progesterone (P4) levels to evaluate natural length of follicular (FP) and luteal phases (LP). Concentration of P4 below 10 ng/ml was considered to indicate FP. P4 levels during luteal phase were assessed quantitatively up to 48 ng/ml [20].

On the cycle of the ovariectomy luteolysis was artificially induced by intramuscular injection of prostaglandin F2α analogue (Estrumate, Essex Munich, Germany) to allow accurate timing of the ovariectomy. The injection has been performed between days 10 and 15 of LP. The dose of Estrumate (used in concentration 8 μg/ml) was adjusted according to the animal body weight, with 0.15 ml estrumate injected to animals with the weight below 450 g and 0.2 ml – to animals weighing above 450 g. The day after injection was counted as day 1 of FP.

For all animals both-sides ovariectomy was performed at the day of operation. Ovariectomy was performed on empty stomach (12 h starvation before operation) and animals were anesthetized with "Goettingen mixture II" (0.1 ml/100 g animal body weight) + diazepam (0.05 ml/animal). The composition of the "Goettingen mixture II" included ketamine (50 mg/ml), xylazine (10 mg/ml), and atropine sulfate (0.1 mg/ml) in NaCl. Amoxicillin was used post-surgery to prevent bacterial infection. In addition, animals received an analgetic for minimum 3 days post-surgery.

2.3. Study design

Data from oocytes obtained from 18 naturally cycling female marmosets were used for the present retrospective analysis. From these, 7 animals had never been treated with enrofloxacin and served here as a control group. The remaining 11 animals received Baytril®, a commercial solution of 2.5% enrofloxacin, in a standard therapeutic dose (0.1 ml = 2.5 mg/animal daily) orally with food during 5 days all at the same time as a group treatment, independent of whether they had diarrhea symptoms or not. This was a standard management strategy, applied without informing experimenters prior to the treatment.

There was no difference in the age and weight of the animals between untreated and treated animals. Untreated animals had mean body weight and age of 412 ± 62 g and 29 ± 7 months and treated animals had body weight of 402 ± 64 g and were 27 ± 8 months old, respectively. No treatment related weight change was observed during the study.

The central aim of the analysis was to evaluate the effects over time after the enrofloxacin treatment, in the ovary and on the oocytes collected from antral follicles for *in vitro* culture. Five groups have been formed according to the time after treatment: control (7 untreated animals); 5-week group (5W, 2 animals analysed 5 weeks post treatment (p.t.)), 7-week group (7W, 5 animals – 7–9 weeks p.t.); 15-week group (15W, 2 animals – 15 weeks p.t.), and a 17-week group (17W, 2 animals – 17 weeks p.t.).

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