Theriogenology 83 (2015) 1254–1263

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Critical estradiol dose optimization for oocyte *in vitro* maturation in the common marmoset

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ARTICLE INFO

Article history:
Received 25 February 2013
Received in revised form 8 January 2015
Accepted 9 January 2015

Keywords:
Marmoset monkey
Estradiol
IVM
Cumulus expansion
Embryo

ABSTRACT

The aim of the present study was to critically evaluate the effect of different concentrations of estradiol (E_2) during IVM of common marmoset (*Callithrix jacchus*) oocytes from antral follicles. The doses tested were 0, 0.1, 1, or 10 μg/mL E_2 (referred to as 0 E_2, 0.1 E_2, 1 E_2, and 10 E_2 groups). After a preincubation, the concentration of E_2 in IVM drops under oil was approximately 20% of the amount added (0.02; 0.2 and 1.9 μg/mL, respectively) because of absorption into the oil. Oocyte progression to metaphase II was significantly higher in the 0.1 E_2 group than that in the absence of E_2. With progressively higher doses, the maturation rate tended to decrease suggesting an overdose effect. Furthermore, the total first cleavage rate was significantly higher in the 0.1 E_2 group than that in the 0 E_2 group and decreased progressively with further increases in E_2 concentration, with the 10 E_2 group showing the same low rate as without E_2. The oocytes which failed to cleave, after maturation in 10 E_2, showed obvious signs of overdose with the highest rates of degeneration and abnormal spindle form, and an absence of embryo progression. In contrast to these obvious negative effects on the oocyte, 10 E_2 was the only group in which a significant increase in radial cumulus expansion was observed. The concentration 0.1 E_2, which is 10 times lower than the most commonly used E_2 dose, produced the best results in all oocyte factors evaluated. These results represent the first study for a primate species showing a strong positive effect of E_2 on oocyte maturation and embryo development, but only at the optimal concentration, and emphasize the critical limits of the optimal concentration range.

1. Introduction

The common marmoset monkey (*Callithrix jacchus*) is a nonendangered New World primate species. Due to its small size, high reproductive capacity, and relatively easy handling, it is often used in reproductive biology research as a model for its closely related endangered primates and humans. However, basic knowledge for this species is still being accumulated, and many necessary reproductive biology techniques are still in the process of being optimized. Our laboratory has been one of the leading in the world involved with the development of this field for the common marmoset. Reproductive technologies including oocyte IVM, IVF, and embryo culture for this species are

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http://dx.doi.org/10.1016/j.theriogenology.2015.01.012

a major aspect of our work [1–7]. One of the important findings of the recently published study on marmoset *in vitro* oocyte maturation is that the effects of epidermal growth factor were dependant on gonadotropin concentration [6].

To date, critical estradiol (E_2) dose–response studies have not been carried out for primates or humans. In the present study, we investigated the effect of E_2 concentration during IVM for marmoset monkey oocytes under the conditions, which produced the best results in our previous study [6]. Estradiol is almost universally included in oocyte IVM media, but its effects appear to differ with doses, species, and culture conditions [8–10]. Surprisingly, in the oocyte maturation studies, a strong positive effect is seldom reported [11] and some studies report negative effects [10,12–17]. The most common dose used in the literature is 1 μg/mL, which we also used for our previous studies with marmoset oocyte maturation [1,2,4,5,18], and published concentrations for rhesus and human oocyte IVM vary from 0.1 to 1 μg/mL [10,16,19–21]. In the present study, effects of no E_2 and 10 μg/mL E_2 were also tested, to create a dose-response series, each step 10 times more than the previous.

However, little attention has been paid to the importance of dose optimization in the literature. Because of this, results from the other species can not be simply applied to a new species especially when the culture conditions differ. This is an important issue because it is unknown how broad the dose tolerance is for E_2. Further, there is a possibility that oocytes from small antral follicles, which are usual material for IVM, may be more sensitive to the negative effects of high doses of E_2 than late-stage follicles and that high levels of estrogen may be part of the mechanism for inducing atresia in small follicles [22,23].

The aim of the present study was therefore to critically examine the effects of E_2, in a dose-response study, on marmoset oocyte maturation and fertilization, and the resulting embryo development under the *in vitro* conditions was found so far optimal for this species, to provide the first basic information for the marmoset.

2. Materials and methods

Methods used in the present study have been previously published in full in Tkachenko et al. [6] and will be presented here in brief in relation to the present experiment. Methods never before published by us will be described in detail.

2.1. Animals

All procedures with animals were carried out according to German Animal Experimentation Law (Animal Experiment Permission #33.42502/08–01.03). Marmosets were housed according to standard German Primate Center practice for *C jacchus* [3,4,6,7,24].

Six female marmosets aged from 20 to 38 months (mean age, 30 ± 7 [standard deviation, SD] months) and with a body weight range from 360 to 540 g (mean, 409 ± 68 [SD] g) were used for the experiment. The females were housed with castrated partners and allowed to cycle naturally.

In total, 211 culturable cumulus–oocyte complexes (COCs) were obtained from six animals. In the study, material from five of six animals has been used (184 COCs). Data from one animal were excluded from the statistics as the oocytes from this animal have shown extremely low metaphase II (MII) progression rates and failed to fertilize using sperm from a proven fertile male. Material from each animal has been cultured individually, and the COCs obtained were distributed randomly among the treatment groups.

2.2. Cycle monitoring and control

Ovarian cycles were monitored by plasma progesterone levels [25] and luteolysis was induced by an intramuscular injection of PGF2α analogue (Estrumate; Essex Tierarznei, Munich, Germany) on the cycle of the ovariectomy to time the start of the last cycle [6]. The mean length of the follicular phase was calculated from several cycles before the beginning of an experiment. Depending on the stability of the follicular phase length, the operation was planned from 1 to 3 days before the most probable day of ovulation.

Mean ovary volume, calculated as a product of height, width, and length, was 0.15 ± 0.042 (SD) cm^3. Only follicles with a diameter above 1000 μm were used for dissection. A minimum of 23 to a maximum of 49 COCs ($\bar{x}$ = 37 ± 10 [SD]) suitable for culture (based on the criteria given in Section 2.5) were recovered from one full + 2/3 ovaries. From each animal, one third of one ovary in the pair was always fixed for histology for another study.

2.3. Preparation of media and culture plates

All chemicals, unless otherwise stated, were obtained from Sigma–Aldrich Chemie GmbH, Munich, Germany. Osmolarity of all media was adjusted to 280 mOsm. One batch of fetal bovine serum (FBS, cat. no. S 3113; Biochrom AG, Berlin, Germany) was used throughout the whole experiment to eliminate potential background variability. The FBS used was charcoal stripped by the manufacturer and is hormone free. Therefore, the steroid hormone content was treated as zero. Additionally, the media assayed for E_2 confirm this: no E_2 was detected in the culture drops where none was added (see results). Fetal bovine serum was 20% v:v of total medium components.

All culture plates (for short-term storage and IVM; for IVF; for embryo culture) with drops of medium under light mineral oil were prepared each time the day before use and stored overnight in the incubator with gas conditions according to the developmental stage. Each four-well culture plate (NUNC, Thermo Fisher Scientific, Roskilde, Denmark) had one drop of medium per well, with 50-μL volume in first three drops used for washing and 25 μL of medium in the last (culture) drop.

2.4. Evaluation of E2 concentration in medium drops under oil

Published data report E_2 absorption by the mineral oil surrounding a culture drop [10,26]. Therefore, we have conducted a separate experiment to evaluate this effect under our conditions and establish the exact value of E_2