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ScienceDirect
Theriogenology 70 (2008) 108–114
Theriogenology
www.theriojournal.com

Effects of rhFSH regimen and time interval on ovarian responses to repeated stimulation cycles in rhesus monkeys during a physiologic breeding season

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Received 12 December 2007; received in revised form 11 February 2008; accepted 12 March 2008

Abstract

We studied the effects of repeated stimulation by recombinant human FSH (rhFSH) at various time intervals during a physiologic breeding season in rhesus monkeys. Ovarian recovery and responses were assessed by ultrasonography, serum steroid concentrations, number of oocytes retrieved, and in vitro blastocyst development following IVF. One group underwent a single stimulation regimen with 18 IU rhFSH i.m., followed by 1000 IU hCG, and serum steroid concentrations and ovarian status were determined in the following three menses. Another group was stimulated as before and then allocated into three subgroups; each subgroup was re-stimulated once at the beginning of the ensuing first, second, or third menses. In the final experiment, one group was stimulated with 37.5 IU rhFSH, whereas another group received 18 IU rhFSH. In subsequent cycles, all were re-stimulated twice with 18 IU rhFSH at time intervals of two menstrual cycles (MCs). At the first menses after stimulation, serum progesterone concentrations were significantly higher and the ovaries larger than before stimulation. Monkeys that were re-stimulated at the first menses responded poorly; at the second menses, progesterone concentrations and ovarian size recovered, but the number of oocytes retrieved from re-stimulated monkeys was still significantly reduced. However, animals that were re-stimulated in two MCs later responded well (i.e., percentage of the animals responding, oocytes recovered, and potential for fertilization and blastocyst formation). In conclusion, rhesus monkeys were likely to have similar ovarian responses to repeated stimulation with the same regimen spaced at least two MCs apart.

Keywords: Rhesus monkey; Ovarian recovery; Repeated ovarian stimulation; rhFSH; Ultrasonography

1. Introduction

Substantial progress in the application of assisted reproductive technologies in non-human primates over the last two decades has resulted in the routine

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doi:10.1016/j.theriogenology.2008.03.012

production of in vitro-derived embryos and the use of embryo transfer to establish pregnancy [1–3]. These successes were followed by the development of somatic cell nuclear transfer research [4–6], which depends upon a large number of oocytes being retrieved by ovarian stimulation. However, the availability rhesus monkey oocytes is impeded by the high cost of ovarian stimulation, limited numbers of animals, and the availability of rhesus monkeys for only approximately half of the year, due to their physiologic breeding season [2]. Protocols for ovarian stimulation of the rhesus monkey have been developed that use gonadotropins extracted from animal or human urine and/or pituitaries [7–16]. Recently, recombinant human FSH (rhFSH) used for ovarian stimulation improved the reliability and results [17–19]. In previous studies, we reported that lower dosages of rhFSH not only reduced the cost of ovarian stimulation, but also improved results, as the number of oocytes retrieved increased and the developmental potential with improved IVF [20,21]. However, the effects of repetitive treatments with low doses of rhFSH and the optimal time interval for subsequent treatments in the same breeding season are unknown. Therefore, the objective of this study was to assess rhesus ovarian responses to repeated treatment cycles using various time intervals and protocols.

2. Materials and methods

2.1. Animals and chemicals

All animal procedures were approved in advance by the Institutional Animal Care and Use Committee of Kunming Primate Research Center. Adult female rhesus monkeys were housed in individual cages in a controlled environment (20–24 °C, humidity 40–60%) and exposed to a 08:00–20:00 h light cycle. Vaginal bleeding was monitored daily to detect the menstrual cycle (MC) and the onset of menses. Unless stated otherwise, all chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Ovarian stimulation and oocyte recovery

During the September to March physiologic breeding season, 73 adult females (mean age: 6.4 years; mean weight: 5.6 kg) were subjected to ovarian stimulation. Treatment with rhFSH (Gonal F, Laboratories Serono SA, Aubonne, Switzerland) was initiated 1–3 days after the onset of menses. Two regimens of rhFSH were used. Regimen I consisted of 37.5 IU of rhFSH given i.m. twice daily, 10–12 h apart, for 8 days [19,22]. Regimen II consisted of 18 IU of rhFSH, also given twice daily for 8 days [20]. Animals that exhibited increases of serum estrogen and more than five follicles (at least 3 mm diameter) combined for both ovaries were defined as ''responders'' and were given 1000 IU hCG (i.m.) at 21:00 h of Day 9 [20]; otherwise, they were characterized as ''poor responders'' and removed from the study. Ovaries were imaged with a Diasus^{TM} ultrasound system (Dynamic Imaging Ltd., Livingston, Scotland, UK) with 10–22 MHz linear-array transducers, and mean ovarian diameter was calculated by averaging the maximum length and width. Oocyte collection was performed through laparoscopic follicular aspiration [20].

There were three experiments in this study. In the first experiment, we assessed ovarian recovery after stimulation by Regimen II. Serum steroid concentrations and ovarian size were monitored in monkeys ($n = 26$) during the following three menses after stimulation, with the aim of understanding the potential for repeated ovarian stimulation.

The second experiment evaluated the effect of time interval between cycles on the ovarian responses to repeated stimulation. Monkeys ($n = 22$) were first stimulated by Regimen II and then divided into three subgroups for repeated treatments. The first subgroup ($n = 6$) was re-stimulated with Regimen II at the beginning of the first menstrual cycle after initial stimulation. The second ($n = 6$) and third ($n = 10$) subgroups were re-stimulated with the same regimen at the beginning of the second and the third menses, respectively, during the same physiologic breeding season.

The third experiment investigated the consistency of ovarian responses to three consecutive ovarian stimulation cycles and the effect of high dose and reduced dose rhFSH regimen treatments on subsequent repeated treatments. Monkeys were stimulated with Regimen I ($n = 15$) or with Regimen II ($n = 10$) for the first stimulation cycle. Each group then received another two stimulation cycles with Regimen II, with time intervals of two MCs (Fig. 1).

2.3. IVF and embryo culture

To assess developmental competence, freshly collected mature oocytes were inseminated as in our previous reports [20,23]. Briefly, hyperactivated sperm and mature oocytes (MII) from the collection were co-incubated for 12–16 h at 37 °C in a humidified atmosphere of 5% CO_2. Fertilized oocytes were cultured for embryonic development under mineral