Randomized comparison of different ovarian stimulation regimens for assisted reproductive technology in baboons (*Papio anubis*)

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Objective: To compare different methods of ovarian stimulation (OS) for assisted reproductive technology in baboons.
Design: Prospective randomized study.
Setting: Institute of primate research.
Animal(s): Baboons (n = 10) were randomized into two groups (of five animals each) during three different cycles to compare six protocols of OS.
Intervention(s): Cycle 1: clomiphene citrate (CC) alone (group CC) versus CC and GnRH agonist (group CC-Ag); cycle 2: recombinant gonadotropins (GON) without GnRH agonist (group GON) versus GON and depot GnRH agonist (group GON-AgDepo-1); cycle 3: GON and depot GnRH agonist (group GON-AgDepo-2) versus GON and daily GnRH agonist in a classic long protocol (group GON-Ag). Oocyte aspiration was performed 34–36 hours after injecting 5,000 IU rhCG, followed by fertilization via intracytoplasmic sperm injection (ICSI).
Main Outcome Measure(s): Number and quality of oocytes retrieved and their fertilization rate.
Result(s): More metaphase II (MII) oocytes were retrieved using the GON-AgDepo-1 (n = 12; 64% MII), GON-AgDepo-2 (n = 9; 79% MII), GON-Ag (n = 16; 88% MII), and GON (n = 6; 59% MII) protocols compared with the CC (n = 9; 15% MII) and CC-Ag (n = 14; 20% MII) protocols. Fertilization by ICSI varied between 43% and 71%.
Conclusion(s): In baboons, long and depot protocols yield similar numbers of MII oocytes; however, depot protocol may be preferable because only one injection of GnRH agonist is needed. (Fertil Steril® 2011;95:1354–9. ©2011 by American Society for Reproductive Medicine.)

Key Words: Ovarian stimulation, baboon, ICSI, IVF, recombinant gonadotropins, agonist, antagonist, clomiphene citrate

In view of phylogenetic closeness and similarity of reproductive endocrinology, physiology, and anatomy between nonhuman primates (NHPs) and humans, baboons and rhesus monkeys are considered to be appropriate preclinical animal models for research in reproductive biology, including gamete biology, in vitro fertilization (IVF), embryo development and implantation, embryo transfer, and embryonic stem cell development (1–8). In rhesus monkeys but not in baboons, ovarian stimulation (OS), IVF, intracytoplasmic sperm injection (ICSI), embryo culture, and embryonic stem cell derivation has been well established (5–7). The baboon represents an attractive NHP model to supplement rhesus monkeys for reproductive studies for the following reasons. First, baboons have a well characterized reproductive anatomy and endocrinology very similar to humans (2, 9). Second, relatively easy transcervical access to the uterine cavity is possible in baboons but not in rhesus monkeys, allowing endometrial biopsy, embryo transfer, preimplantation embryo flushing, and hysteroscopy (2–4). Third, it is possible to follow the baboon menstrual cycle by perineal sex skin follow-up (9). And fourth, in contrast with the seasonal breeding of rhesus monkeys, baboons breed continuously without interruption in captivity and in the wild.

However, an optimal method of OS for assisted reproductive technology (ART) studies in baboons has not yet been established, as reviewed recently (3). In unrandomized trials, OS with clomiphene citrate (CC) or gonadotropins (GON) with or without GnRH agonist has been evaluated in olive baboons or chacma baboons (10–12). In a recent randomized trial, we have demonstrated that a classic long OS protocol with GON is superior to a short OS protocol with GON, to a GnRH antagonist OS protocol with GON, and to OS with CC with or without GnRH antagonist in olive baboons (13). The aim of the present study was to test other OS protocols in the baboon in a randomized way. First, we tested the hypothesis that OS with a high dose of CC (150 mg) would result in a higher number of retrieved eggs compared with OS with a low dose (50–100 mg) used in previous studies by us and other investigators (10, 11, 13) and that OS using 150 mg CC can be optimized by adding a GnRH agonist to achieve maximal endogenous release of FSH and LH. Second, we tested the hypothesis that a GnRH agonist is needed to prevent premature ovulation during OS with GON for ART, because a previous study in baboons demonstrated that a high number of mature eggs can be obtained after OS in baboons without GnRH agonist down-regulation (10). Third, we tested the hypothesis that OS with GON using a depot GnRH agonist protocol

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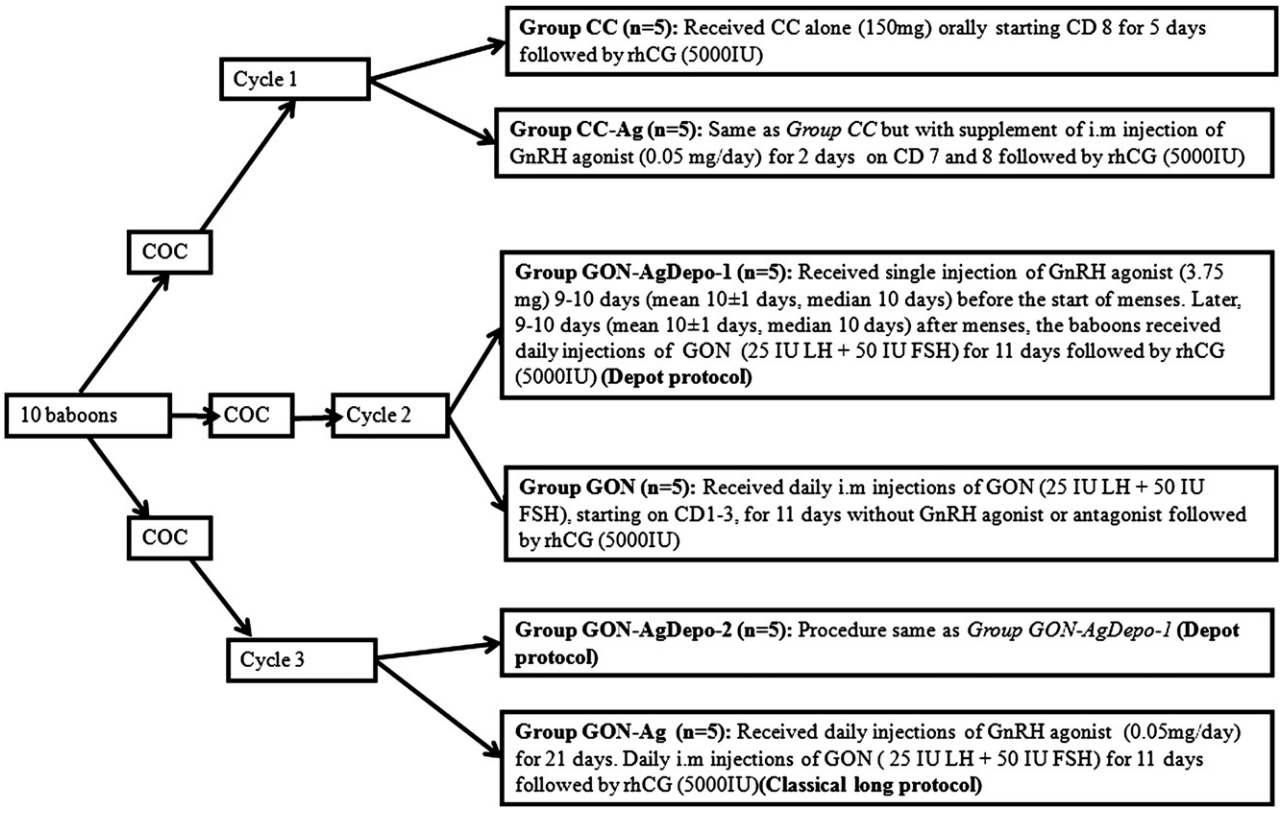
FIGURE 1

Experimental design for randomized study of various ovarian stimulation protocols. CC = clomiphene citrate; CD = cycle day; COC = combined oral contraceptive; GON = gonadotropins.



Nyachieo. Ovarian stimulation in baboons. Fertil Steril 2011.

(14) is similar to OS with GON using daily injection of GnRH agonist (classic long protocol) regarding number and maturity of oocytes and their fertilization rate.

MATERIALS AND METHODS

Animals

The study protocol was approved by the Institutional Review Board of the Institute of Primate Research (IPR), Nairobi, Kenya. Twelve healthy adult female olive baboons (*Papio anubis*; 12–15 kg), maintained in captivity at IPR, without previous surgeries or diseases, and with regular menstrual cycles (31 ± 2 days), were selected for this study. The animals were fed on commercial monkey chow with fruit and vegetable supplementation three times a week; water was provided ad libitum.

Ovarian stimulation

Ovarian stimulation was conducted in 10 baboons during three cycles as shown in Figure 1. Because two baboons were nonresponders in cycle 2, they were replaced by two other female baboons for cycle 3.

In every cycle, two different controlled OS methods were compared in two groups of five baboons each (Fig. 1). The GnRH agonist depot used in cycles 2 and 3 is a known long-acting agonist, as demonstrated previously in baboons (14). The time interval between each cycle of OS was at least 3 months. Before each cycle of OS, the menstrual cycle of the ten baboons were synchronized using an oral contraceptive (Mercilon; Organon, Dublin, Ireland) as described previously (13), to plan OS in such a way that two egg retrievals and laboratory ICSI procedures could be planned per day during 5–7 consecutive days for logistic reasons (shipment of culture medium to Kenya, availability of trained lab and clinical faculty during a limited period of time). Transabdominal ultrasound was performed to assess follicular diameter 2 days before rhCG injection and on the day of rhCG injection by using a vaginal ultrasound probe (Basi Unit; Pie Medical Equipment, Maastricht, The Netherlands). Oocyte retrieval was performed 34–36 hours after the injection of rhCG as described previously (13, 15). Because OS was carried out during a fixed period in all protocols, results of the ultrasound follicular measurements did not influence the preplanned time of rhCG injection but were used for retrospective analysis. Similarly, hormonal analysis of collected serum samples was done retrospectively.

The source for the drugs used were as follows: CC (Clomid; Aventis Pharma, Brussels, Belgium); rhCG (Ovitrelle; Serono, Bari, Italy); GnRH agonist triptorelin (Decapeptyl; Ipsen, Merelbeke, Belgium); and GON including 25 IU LH (Luveris; Serono, Rome, Italy) and 50 IU FSH (Gonal-F; Serono, Rome, Italy).

Laparoscopic egg retrieval

Laparoscopic transabdominal follicular aspiration was performed 34–36 hours after injection of rhCG as described previously (13, 15).

Perineal staging

The perineal staging method, based on daily external observation of the baboon perineal sex skin (9), was used to assess the effect of the oral contraceptive and of OS on the menstrual cycle, as described elsewhere (13).