Available online at www.sciencedirect.com
ScienceDirect
Theriogenology 67 (2007) 1194–1201
Theriogenology
www.theriojournal.com

Effects of rhFSH dose on ovarian follicular response, oocyte recovery and embryo development in rhesus monkeys

S. Yang [a], X. He [a], T.B. Hildebrandt [b], K. Jewgenow [b], F. Goeritz [b], X. Tang [a], Q. Zhou [c,**], W. Ji [a,*]

[a] *Kunming Primate Research Center and Kunming Institute of Zoology, Chinese Academy of Sciences, Yunnan Key Laboratory for Animal Reproduction, Kunming, Yunnan 650223, PR China*
[b] *Institute for Zoo and Wildlife Research, PF 601103, 10252 Berlin, Germany*
[c] *State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100080, PR China*

Received 21 September 2006; accepted 30 October 2006

Abstract

The objective was to study the effects of dose of recombinant human follicular stimulating hormone (rhFSH) for ovarian stimulation in rhesus monkeys. Nineteen pubertal and 109 adult female rhesus monkeys were given 37.5, 18, or 9 IU of rhFSH twice-daily for 8 days (total of 600, 300, or 150 IU of rhFSH per cycle, respectively; designated Regimens 1, 2 and 3). Ovarian responses were assessed with ultrasonography, serum concentrations of E2 and FSH, and by in vitro developmental potential (following IVF) of retrieved oocytes. Regimen 1 had more monkeys with very large follicles (diameter > 8 mm) than Regimen 2 ($P < 0.05$), which impaired development potential. However, there were no differences between Regimens 1 and 2 in oocyte recovery, whereas Regimen 3 did not elicit superovulation. The developmental potential of embryos obtained from Regimen 2 was higher than that of Regimen 1, as determined by culture to the blastocyst stage in vitro (proportion of blastocysts relative to collected MII oocytes was 55.8% versus 36.8% in pubertal and 63.8% versus 44.2% in adult monkeys; $P < 0.05$ for each), and the results of embryo transfer from Regimen 2 were acceptable. In conclusion, we inferred that the optimal rhFSH dose for ovarian stimulation in rhesus monkeys was a total of 300 IU; this dose should be efficacious for ovarian stimulation as the quality or recovered oocytes was higher and the risk of overstimulation was reduced.

Keywords: Rhesus monkey; Ovarian follicle; Ovarian stimulation; rhFSH; Embryo development

1. Introduction

The rhesus monkey is considered a clinically relevant animal model for human physiology and disease and for determining the safety and efficacy of new therapies. Assisted reproductive technologies in nonhuman primates have the potential not only to promote animal propagation, but also to serve as a source of oocytes/embryos for monozygotic twinning, embryonic stem cell derivation and cloning [1]. However, due to the high cost and limited availability of these animals, it is essential that assisted reproductive

* Corresponding author at: Kunming Primate Research Center and Kunming Institute of Zoology, The Chinese Academy of Sciences, Kunming, Yunnan 650223, China.
** Corresponding author at: State Key Lab of Reproductive Biology, Institute of Zoology, The Chinese Academy of Sciences, Beijing 100860, China.
E-mail addresses: yangsh@mail.kiz.ac.cn (S. Yang), qzhou@ioz.ac.cn (Q. Zhou), wji@mail.kiz.ac.cn (W. Ji).

0093-691X/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2006.10.021

technologies procedures be optimized, thereby facilitating research endeavors using nonhuman primates as model systems for studies of human reproduction and fertility [2,3].

Substantial progress in the application of the assisted reproductive technologies in nonhuman primates has been made over the last 20 year, resulting in the routine production of in vitro-derived embryos and pregnancy establishment following embryo transfer [1,4]. Concurrently, protocols for ovarian stimulation in rhesus monkeys have received attention in terms of the gonadotropin preparation and the timing and dosage of administration. Originally, gonadotropins extracted from animal or human urine and/or pituitaries were employed, e.g. PMSG [5–9], porcine FSH [10,11], human FSH [12–14], and hMG [8,9,15] with limited success, undoubtedly reflecting variable amounts of LH activity, degraded or inactive gonadotropin remnants, and contaminating proteins that elicited an immune response and limited the number and quality of stimulations [7,12,16]. Recently, recombinant human gonadotropins have been employed in the rhesus monkey with improved reliability and results [17–19], although repeated exposure of animals to rhFSH and rhCG is still limited to approximately four cycles [20]. However, the optimal dose of recombinant gonadotropins for ovarian stimulation in monkeys has not been defined.

Currently, twice-daily injections of $\geq$30 IU of rFSH are given to induce synchronous growth of ovarian follicles in rhesus monkeys [13,18–22], baboons [23], and cynomolgus monkeys [24,25]. Nevertheless, not all oocytes collected were healthy and fertilizable [26]. The amount of FSH given to rhesus monkeys (i.e. the standard ''one ampoule per day'' regimen) is 2–10 times the dose administered to women on a body weight basis, suggesting that reduced doses might be more appropriate for ovarian stimulation of monkeys [3]. The objective of the present study was to determine the effects of dose rhFSH for ovarian stimulation in rhesus monkeys.

2. Materials and methods

2.1. Animals and chemicals

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

2.2. Ovarian stimulation, oocyte recovery and maturation

During the physiologic breeding season (November–February), 19 pubertal females (3.5–4.5-year-old) and 109 adult females (5–10-year-old) were identified for incorporation into this study. Treatment with rhFSH (Gonal F, Laboratories Serono SA, Aubonne, Switzerland) was initiated 1–3 days after the onset of menses. Three doses of rhFSH were used. Regimen 1 (total of 600 IU) was 37.5 IU of rhFSH given twice-daily for 8 days intramuscularly (im), as previously described [17–19]. For Regimens 2 and 3, 18 and 9 IU were given twice-daily for 8 days (total of 300 and 150 IU, respectively). On Day 9, monkeys with multiple increases in serum estrogen concentrations above baseline and more than five follicles (>3 mm in diameter) on both ovaries were defined as responders and were given 1000 IU of hCG im (Laboratories Serono SA), whereas monkeys with multiple increases in estrogen concentrations, but <5 follicles (>3 mm diameter) on both ovaries were characterized as poor responders. Ovaries were imaged with a 5.5 MHz (SSD-500 V, Aloka Co. Ltd., Tokyo, Japan) or a Diasus^{TM} ultrasound system (Dynamic Imaging Ltd., Livingston, Scotland, UK) with 10–22 MHz linear-array transducers. Animals were anesthetized with ketamine (10–12 mg/kg) given im and cumulus–oocyte complexes were collected by laparoscopic follicular aspiration, 32–35 h post-hCG injection. Follicular contents were placed in Hepes-buffered TALP (modified Tyrode solution with albumin, lactate and pyruvate) medium [5] containing 0.3% BSA at 37 °C. Oocytes were stripped of cumulus cells by pipetting after brief exposure (<1 min) to hyaluronidase (0.5 mg/mL) and classified as metaphase I (MI; no germinal vesicle, no first polar body) or metaphase II (MII; PB1). The MI oocytes were cultured in a 50 μL drop of CMRL-1066 medium containing 10% FBS, 10 IU/mL porcine FSH, 10 IU/mL ovine LH at 37 °C in humidified 5% CO_2 in air for up to 10 h [22]. Mature oocytes (MII) were placed in HECM-10 medium [27] at 37 °C in humidified 5% CO_2 in air until IVF.

2.3. IVF and embryo culture

To assess developmental competence, oocytes were inseminated as described previously [5]. Briefly,

20×10^6 mL^{-1} washed fresh motile spermatozoa from two male rhesus monkeys previously successfully used for IVF were incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air for 4–6 h, followed by an additional 1–1.5 h in the presence of 1.0 mM each of caffeine and dibutyryl cAMP to induce hyperactivation. Hyperactivated sperms were diluted into 100 μL drops (2×10^5 mL^{-1} final sperm concentration) of TALP medium supplemented with 2% newborn calf serum (NCS, Hyclone) containing mature oocytes. The gametes were co-incubated for 12–16 h at 37 °C in a humidified atmosphere of 5% CO_2 in air before ova were examined for evidence of fertilization. Fertilized oocytes were cultured for embryo development in 50 μL drops of HECM-10 containing 10% fetal bovine serum for up to 7 days at 37 °C in a humidified 5% CO_2 in air under the mineral oil, with a change in culture medium every second day. The progression of embryo growth was monitored daily.

2.4. Embryo transfer and pregnancy

Multiparous females with normal menstrual cycles were used as embryo recipients. The embryos were transferred into the oviduct of synchronized recipients via a laparoscopic technique using a fixed polythene catheter (with outside and inside diameters of 1.09 and 0.38 mm, respectively), threaded through a 25-gauge hypodermic needle [28]. Briefly, morphologically normal embryos were transferred, depending on developmental stage (i.e. 2–4- or 8-cells) into the oviduct of recipients 1 or 2 days after spontaneous ovulation, respectively, as determined by serum concentrations of estradiol (E2) and progesterone (P4), as well as ultrasonography [6,12]. Pregnancy was confirmed ultrasonographically approximately 35 days after transfer (presence of a viable gestational sac and fetal heart activity).

2.5. Hormone assays

To quantify serum concentrations of FSH, E2, and P4, blood samples were drawn daily (∼09:00 h) from conscious adult animals by saphenous venipuncture. Serum concentrations of E2 and P4 were determined by RIA [12] (intra- and inter-assay coefficients of variation were all <10%). Serum FSH concentrations were determined with anFSH Immunoradiometric assay kit (Larwen Group, Shenzhen, China) validated for rhesus serum [29]; intra- and inter-assay coefficients of variation were 8.8% ($n = 6$) and 9.4% ($n = 6$).

2.6. Statistical analysis

Results are presented as the mean ± S.D. unless stated otherwise. The proportion of well-responders was analyzed within Regimens 1, 2 and 3 by Fisher's exact test. Prior to ANOVA and comparisons between Regimens 1 and 2 by unpaired Student's *t*-test, the numbers of oocytes recovered were transformed by square root and the proportion of oocytes at various stages of nuclear maturity and embryo development rates were transformed by arcsine of square root. Serum concentrations of steroids and FSH collected at various time points, transformed by logarithm prior to statistical analysis, were compared within three regimen groups with the values at the same time point by repeated measures ANOVA, followed by the *t*-test with Bonferroni method, whereas serum concentrations of E2 and P4 in blood collected at the same time points during the stimulation were compared between two follicle groups by unpaired Student's *t*-test, following transformation by logarithm. Values with $P < 0.05$ were considered different.

3. Results

3.1. rhFSH dose dependency in ovarian response and oocyte quality

3.1.1. Ovarian response

Allocation to treatment groups and ovarian responses are shown in Table 1. Nineteen pubertal and 109 adult monkeys were randomly allocated into three groups for the treatments. All pubertal monkeys and approximately 88% of adult monkeys in Regimens 1 and 2 had

Table 1
Ovarian responses to 600, 300, or 150 IU of rhFSH (Regimens 1, 2, and 3, respectively) in pubertal and adult rhesus monkeys

Status	Regimen (*n*)	No. responding (%)*	No. responding with large (>8 mm) follicles (%)‡
Pubertal	1 (8)	8 (100)[a]	7 (87.5)[a]
	2 (8)	8 (100)[a]	2 (25.0)[b]
	3 (3)	0 (0)[b]	–
Adult	1 (40)	35 (87.5)[a]	18 (51.4)[a]
	2 (45)	40 (88.9)[a]	6 (15.0)[b]
	3 (5)	0 (0)[b]	–

Within a category (pubertal and adult), means without a common superscript (a and b) differ ($P < 0.05$).

* Responders had at least five ovarian follicles >3 mm in diameter at the conclusion of rhFSH treatment.

‡ Responders had one or two very large follicles (>8 mm) at the conclusion of rhFSH treatment.

Table 2
Mean (±S.D.) oocytes recovered (per responding animal) following treatment with 600 or 300 IU of rhFSH (Regimens 1 and 2, respectively) in pubertal and adult rhesus monkeys (no significant differences between the two regimens)

Status	Regimen (*n*)	Oocytes recovered[a]			
		Total	MII (%)	MI (%)	MI to MII (%)
Pubertal	1 (8)	27.4 ± 16.5	19.6 ± 15.0 (71.4)	7.9 ± 6.6 (28.4)	71.1
	2 (8)	32.3 ± 17.0	23.6 ± 15.9 (73.3)	8.6 ± 2.3 (26.7)	77.9
Adult	1 (35)	40.5 ± 20.9	28.9 ± 14.9 (71.9)	11.7 ± 11.1 (28.1)	68.5
	2 (40)	32.6 ± 12.7	24.7 ± 11.8 (75.9)	8.0 ± 5.1 (24.1)	70.0

[a] Total includes both MII and MI oocytes; MII represents oocytes that were mature at collection, whereas MI refers to oocytes that were immature at collection. The percentages of immature oocytes that matured within 10 h of culture are expressed in the MI to MII column.

an ovarian follicular response that justified treatment with hCG; however, all of the monkeys given in Regimen 3 were classified as poor responders and were not subjected to further studies. The proportion of responders with large follicles (>8 mm) was approximately 3.5-fold higher in Regimen 1 versus Regimen 2.

3.1.2. Oocyte recovery

There was no significant difference between Regimens 1 and 2 in the number of oocytes recovered in responding females (Table 2); the average number of MII and MI oocytes in pubertal females was 19.6 and 7.9 in Regimen 1 and 23.6 and 8.6 in Regimen 2, respectively. The corresponding numbers in adult females were 28.9 and 11.7 in Regimen 1 and 24.7 and 8.0 in the Regimen 2. The ability of MI oocytes to progress (within 10 h of culture in vitro) to the MII stage was not significantly different between Regimens 1 and 2 (Table 2).

3.1.3. Developmental potential

In order to minimize oocyte factors in this comparison, approximately five mature oocytes available at the time of collection from each animal and three to seven IVM-MII oocytes per animal (total of 241 and 268 mature oocytes from Regimens 1 and 2, respectively) were subjected to IVF (Table 3). There were no differences between the two regimens in fertilization rates of MII oocytes for either pubertal or adult females. However, the developmental potential was significantly higher in adult females in Regimen 2 (rates of 80.9% for morula and 64.3% for blastocysts; Table 3). In pubertal females, the same trend was apparent with the (morula rate, 71.8% and blastocyst rate, 53.8% for oocytes/embryos from Regimen 2). Furthermore, MI oocytes matured in vitro to the MII stage from Regimen 2 adult females also had higher development competence compared to those from Regimen 1 (47.6% versus 24.4%, $P < 0.05$).

Table 3
Mean (±S.D.) in vitro developmental competence of mature oocytes collected from pubertal and adult rhesus monkeys following ovarian stimulation with 600 or 300 IU of rhFSH (Regimens 1 and 2, respectively)

Source of oocytes		Regimen	MII oocytes	Developmental stage (%)			
				Fertilized	8-cell	Morula	Blastocyst
Pubertal	Fresh MII	1	44	93.3 ± 12.8	75.4 ± 17.6	56.4 ± 15.0[a]	36.8 ± 27.1[a]
		2	47	83.5 ± 15.7	88.8 ± 16.8	74.6 ± 18.7[b]	55.8 ± 22.2[b]
	IVM-MII	1	21	90.2 ± 12.2	78.3 ± 15.8	35.0 ± 22.0	26.7 ± 28.3
		2	13	84.9 ± 10.8	46.8 ± 17.1	36.2 ± 10.4	27.7 ± 20.8
Adult	Fresh MII	1	130	91.8 ± 9.9	95.0 ± 8.0	67.7 ± 16.0[a]	44.2 ± 20.8[c]
		2	134	87.9 ± 9.8	94.1 ± 8.0	79.4 ± 9.8[b]	63.8 ± 11.4[d]
	IVM-MII	1	50	90.2 ± 10.2	95.8 ± 7.2	47.1 ± 20.1	25.4 ± 15.8[a]
		2	75	83.5 ± 18.7	88.4 ± 13.9	59.5 ± 26.8	52.1 ± 26.6[b]

Superscripts (a–d) within a category (pubertal and adult with fresh or IVM-MII oocytes), difference between Regimens 1 and 2: [a,b]($P < 0.05$); [c,d]($P < 0.01$). Fresh refers to oocytes that were mature at collection. IVM-MII indicates that immature oocytes were cultured in vitro within 10 h before maturing. Fertilized: fertilized (ova exhibiting two pronuclei, referenced as diploid)/MII oocytes; 8-cell: 8-cell stage oocytes/fertilized (diploid) oocytes; morula: morula/fertilized (diploid) oocytes; blastocyst: blastocysts/fertilized (diploid) oocytes.

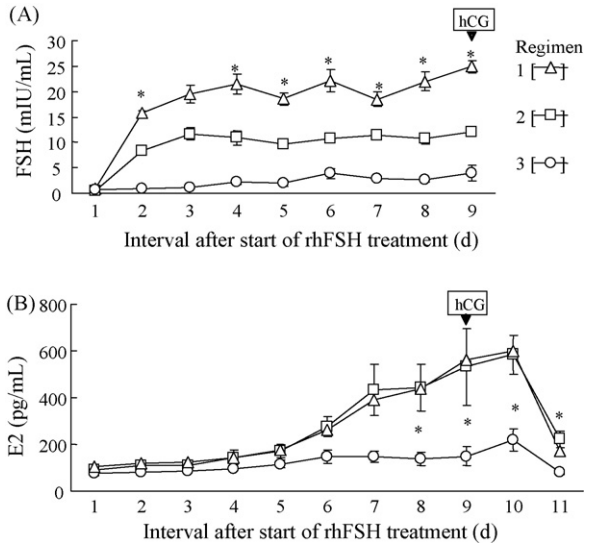

Fig. 1. (A and B) Serum concentrations of FSH and estradiol (E2) in adult female rhesus monkeys subjected to ovarian stimulation with a high-, medium-, or low-dose of rhFSH (Regimens 1, 2, and 3 with $n = 8$, 6, and 3, respectively). Results are plotted relative to the day of the first rhFSH treatment (Day 1). Human chorionic gonadotropin (hCG) was given on Day 9 to induce nuclear maturation. *Difference among the three regimens (FSH) or between Regimen 1 vs. Regimens 2 and 3 on these days ($P < 0.05$).

3.1.4. Serum hormone concentrations

Serum concentrations of E2 and FSH in responding adult females were monitored in Regimens 1, 2, and 3 ($n = 8$, 8, and 3, respectively) during ovarian stimulation (Fig. 1). Maximal concentrations of FSH were proportional to the dosage administered with significant differences among Regimens 1, 2, and 3 ($P < 0.05$; Fig. 1A). Maximal serum E2 concentrations were similar between Regimens 1 and 2, with no significant elevation relative to baseline concentrations in Regimen 3 (Fig. 1B); 9–11 day after the start of stimulation, mean concentrations in Regimens 1 and 2 were higher ($P < 0.05$) than those in Regimen 3.

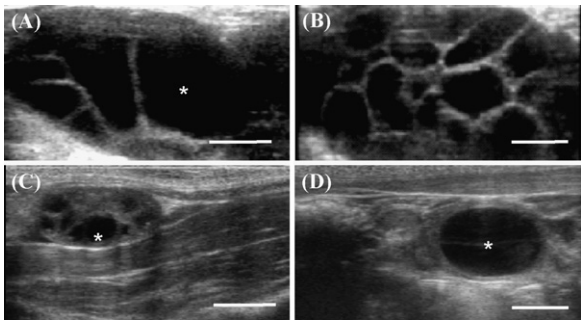

Fig. 2. Ultrasonograms of ovaries in rhesus monkeys subjected to ovarian stimulation. (A) An ovary ($L = 19.0$ mm, $W = 11.8$ mm) with a very large follicle (*9.4 mm in diameter) just before oocyte collection and representative of the large follicles group. (B) An ovary ($L = 27.4$ mm, $W = 19.5$ mm) with relatively uniform follicles 4–7 mm in diameter, representative of the normal follicles group. (C) An ovary at Day 9 of stimulation (ovary: $L = 12.1$ mm, $W = 5.8$ mm) with the largest follicle; *only 2.8 mm in diameter, characteristic of a poor responder. (D) An ovary (ovary: $L = 11.3$ mm, $W = 7.4$ mm) containing a large follicle (*approximately 8.3 mm) on Day 9 of ovarian stimulation using low dose rhFSH (Regimen 3). Bars represent 5 mm.

3.1.5. Pregnancy outcome after embryo transfer

To assess in vivo developmental potential of embryos derived from the reduced of gonadotropins (Regimen 2), 18 IVF-produced embryos (2–16-cell stage) from this regimen were transferred to 6 surrogate monkeys. Two monkeys established pregnancies (based on serum E2 and P4 profiles and confirmed ultrasonographically) and two healthy babies were delivered after 142 and 151 days gestation, respectively.

3.2. Large follicles and oocyte development

In an effort to evaluate the effect of very large follicles (8–17 mm in diameter) on oocyte quality, 19 adult females stimulated with Regimen 1 were allocated into two groups according to follicular size. Nine

Table 4
Mean (±S.D.) oocytes recovered and in vitro developmental potential in rhesus monkeys stimulated with a high-dose rhFSH (Regimen 1), designated into two groups according to large- or normal-sized follicles at the conclusion of stimulation

Follicle group	Oocyte recovered*				Development stage (%)§		
	Total	MII	MI	IVM-MII (%)	Fertilization	8-cell	Blastocyst
Large	19.9 ± 9.4^{a}	16.2 ± 6.8^{a}	3.7 ± 3.4^{c}	2.3 ± 1.6 (69.7)^{a}	79.4 ± 18.2	81.5 ± 20.2	24.1 ± 24.6^{a}
Normal	38.8 ± 12.9^{b}	30.6 ± 9.8^{b}	8.2 ± 5.9^{d}	6.3 ± 3.1 (76.8)^{b}	94.4 ± 14.6	89.6 ± 17.9	52.2 ± 22.4^{b}

Superscripts (a–d) within a column, differences between groups: $^{a,b}P < 0.05$; $^{c,d}P < 0.01$.

* Total includes both MII and MI oocytes; MII represents oocytes that were mature at collection, whereas MI refers to oocytes that were immature at collection. The percentages of immature oocytes that matured within 10 h of culture are expressed in the MI to MII column.

§ Fertilized: fertilized (ova exhibiting two pronuclei, referenced as diploid)/MII oocytes; 8-cell: 8-cell stage oocytes/fertilized (diploid) oocytes; morula: morula/fertilized (diploid) oocytes; blastocyst: blastocysts/fertilized (diploid) oocytes. All mature oocytes by IVF were from MII oocytes at collection.

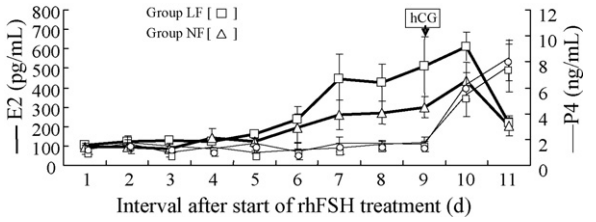

Fig. 3. Serum concentrations of estradiol (E2) and progesterone (P4) from adult female rhesus monkeys exposed to ovarian stimulation with high-dose rhFSH (Regimen 1). Results are plotted separately for monkeys with large follicles (LF, diameter >8 mm, range 8–17; $n = 9$) and normal follicles (NF, 4–7 mm in diameter; $n = 10$) groups. To induce ovulation, hCG was given on Day 9.

animals had very large follicles (large follicles group; diameter >8 mm, range 8–17 mm; Fig. 2A), and 10 did not (normal follicles group; diameter 4–7 mm; Fig. 2B). The average numbers of total and MII oocytes recovered in the group with large follicles was markedly less than those in the normal follicles group (19.9 versus 38.3 in total and 16.2 versus 30.6 in MII oocytes, respectively, $P < 0.01$; Table 4). Oocytes obtained from the normal follicles group had higher developmental potential than those for the large follicles group (52.2% versus 24.1% blastocyst rate following IVF, $P < 0.05$; Table 4). However, serum E2 and P4 concentrations were not significantly different between the two groups (Fig. 3).

4. Discussion

To our knowledge, this is the first report to assess the effects of rhFSH dose on rhesus monkey ovarian stimulation in terms of oocyte recovery, in vitro developmental potential of IVF-produced embryos, and embryo quality based on embryo transfer and pregnancy. Clearly, a regimen that used approximately half of the standard dose of rhFSH yielded superior results.

There are currently two main regimens for ovarian stimulation in rhesus monkeys: (1) 30 IU rhFSH twice-daily for 6 days and 30 IU rhFSH plus 30 IU LH twice-daily for another 3 days; and (2) 30–35 IU rhFSH per se twice-daily for 8 or 9 days. Rhesus monkeys given exclusively FSH during the preovulatory interval produced oocytes that developed to embryos either in vitro or in vivo [17]; the efficacy of this regimen was comparable to a combination of rhFSH and rhLH [19,21,30]. Similarly, the reduced dose of rhFSH per se in the present study resulted in adequate ovarian stimulation in mature rhesus monkeys, comparable to previous reports [19,21,26,30,33]. Although the addition of LH to the treatment regimen may improve embryo viability and the rate of development [18,31], the role of LH in ovarian stimulation in woman remains unclear [32].

Remarkably, the developmental potential of oocytes from animals given 70 IU rhFSH per day (Regimen 1) was poorer than those treated with 35 IU rhFSH per day (Regimen 2), indicating that the follicular development was impaired due to excessive stimulation by the higher dose [3]. The embryo transfer study using oocytes derived from Regimen 2 clearly demonstrated the in vivo development potential of these oocytes, consistent with previous studies [33].

In previous studies, follicle diameter was positively associated with rates of fertilization and cleavage, but not pregnancy, in humans [34,35]; large dominant follicles seemed to have reduced capability of yielding oocytes that resulted in successful pregnancies [36]. In the present study, Regimen 1 resulted in 51.4–87.5% of females with large follicles (>8 mm), whereas, the incidence was only 15.0–25.0% in females subjected to Regimen 2. Impairments in follicular and oocyte of development may have been due to the inhibin–activin–follistatin system [37]. However, the serum E2 profile and maximal concentrations were different neither between regimens nor between monkeys with normal versus large follicles.

In conclusion, a reduced dose of rhFSH (300 IU total/cycle versus 600 IU total/cycle) provided a low-risk, cost-effective protocol for ovarian stimulation in pubertal or adult rhesus monkeys, whereas a further reduction in dose (150 IU total/cycle) was ineffective.

Acknowledgements

This study was supported by: Chinese Academy of Sciences, KSCX1-05-02 and Joint Scholar Plan for the Development of Western China; Major State Basic Development Program, 2004CCA01300, 2005AA 219010 and 2006CB701500; and China National Science Foundation 30370166. We thank the German Max-Planck-Society for its financial support of the bilateral researchers exchange program. The authors also gratefully acknowledge that Dr. Don Wolf from the ONPRC revised the manuscript.

References

[1] Wolf DP. Assisted reproductive technologies in rhesus macaques. Reprod Biol Endocrinol 2004;2:37.

[2] Abbott DH, Foong SC, Barnett DK, Dumesic DA. Nonhuman primates contribute unique understanding to anovulatory infertility in women. ILAR J 2004;45:116–31.
[3] Bavister BD. ARTs in action in nonhuman primates: symposium summary—advances and remaining issues. Reprod Biol Endocrin 2004;2:43.
[4] Bavister BD. The role of animal studies in supporting human assisted reproductive technology. Reprod Fertil Dev 2004;16: 719–28.
[5] Bavister BD, Boatman DE, Leibfried L, Loose M, Vernon MW. Fertilization and cleavage of rhesus monkey oocytes in vitro. Biol Reprod 1983;28:983–99.
[6] Bavister BD, Boatman DE, Collins K, Dierschke DJ, Eisele SG. Birth of rhesus monkey infant after in vitro fertilization and nonsurgical embryo transfer. Proc Natl Acad Sci USA 1984;81:2218–22.
[7] Bavister BD, Dees HC, Schultz RD. Refractoriness of rhesus monkeys to repeated ovarian stimulation by exogenous gonadotropins is caused by non-precipitating antibodies. Am J Reprod Immunol Microbiol 1986;11:11–6.
[8] Morgan PM, Boatman DE, Bavister BD. Relationships between follicular fluid steroid hormone concentrations, oocyte maturity, in vitro fertilization and embryonic development in the rhesus monkey. Mol Reprod Dev 1990;27:145–51.
[9] Johnson LD, Mattson BA, Albertini DF, Sehgal PK, Becker RA, Biggers JD. Quality of oocytes from superovulated rhesus monkeys. Hum Reprod 1991;6:623–31.
[10] Schramm RD, Bavister BD. Follicle-stimulating hormone priming of rhesus monkeys enhances meiotic and developmental competence of oocytes matured in vitro. Biol Reprod 1994;51: 904–12.
[11] Schramm RD, Bavister BD. Use of purified porcine follicle-stimulating hormone for ovarian stimulation of macaque monkeys. Theriogenology 1996;45:727–32.
[12] Wolf DP, Vandevoort CA, Meyer-Haas GR, Zelinski-Wooten MB, Hess DL, Baughman WL, et al. In vitro fertilization and embryo transfer in the rhesus monkey. Biol Reprod 1989;41: 335–46.
[13] Vandevoort CA, Baughman WL, Stouffer RL. Comparison of different regimens of human gonadotropins for superovulation of rhesus monkeys: ovulatory response and subsequent luteal function. J In Vitro Fert Embryo Transf 1989;6:85–91.
[14] Lanzendorf SE, Zelinski-Wooten MB, Stouffer RL, Wolf DP. Maturity at collection and the developmental potential of rhesus monkey oocytes. Biol Reprod 1990;42:703–11.
[15] Abbasi R, Kenigsberg D, Danforth D, Falk RJ, Hodgen GD. Cumulative ovulation rate in human menopausal/human chorionic gonadotropin-treated monkeys: ''step-up'' versus ''step-down'' dose regimens. Fertil Steril 1987;47:1019–24.
[16] Stouffer RL, Zenlinski-Wooten MB, Chandrasekher YA. Stimulation of follicle and oocyte development in macaques for IVF procedures. In: Wolf DP, Stouffer RL, Brenner RM, editors. In vitro fertilization and embryo transfer in primates. New York: Springer-Verlag; 1983. p. 124–38.
[17] Zelinski-Wooten MB, Hutchison JS, Hess DL, Wolf DP, Stouffer RL. Follicle stimulating hormone alone supports follicle growth and oocyte development in gonadotrophin-releasing hormone antagonist-treated monkeys. Hum Reprod 1995;10:1658–66.
[18] Weston AM, Zelinski-Wooten MB, Hutchison JS, Stouffer RL, Wolf DP. Developmental potential of embryos produced by in-vitro fertilization from gonadotrophin-releasing hormone antagonist-treated macaques stimulated with recombinant human follicle stimulating hormone alone or in combination with luteinizing hormone. Hum Reprod 1996;11:608–13.
[19] Schramm RD, Paprocki AM, Bavister BD. Features associated with reproductive ageing in female rhesus monkeys. Hum Reprod 2002;17:1597–603.
[20] VandeVoort CA, Tarantal AF. Recombinant human gonadotropins for macaque superovulation: repeated stimulations and post-treatment pregnancies. J Med Primatol 2001;30: 304–7.
[21] Zelinski-Wooten MB, Hutchison JS, Hess DL, Wolf DP, Stouffer RL. Initiation of periovulatory events in gonadotrophin-stimulated macaques with varying doses of recombinant human chorionic gonadotrophin. Hum Reprod 1997;12:1877–85.
[22] Zheng P, Wang H, Bavister BD, Ji W. Maturation of rhesus monkey oocytes in chemically defined culture media and their functional assessment by IVF and embryo development. Hum Reprod 2001;16:300–5.
[23] Cseh S, Corselli J, Chan P, Bailey L. Controlled ovarian stimulation and ultrasound guided follicular aspiration in the baboon (*Papio cynocephalus anubis*). Reprod Nutr Dev 2001;41:531–4.
[24] Balmaceda JP, Pool TB, Arana JB, Heitman TS, Asch RH. Successful in vitro fertilization and embryo transfer in cynomolgus monkeys. Fertil Steril 1984;42:791–5.
[25] Ng SC, Martelli P, Liow SL, Herbert S, Oh SH. Intracytoplasmic injection of frozen–thawed epididymal spermatozoa in a nonhuman primate model, the cynomolgus monkey (*Macaca fascicularis*). Theriogenology 2002;58:1385–97.
[26] Wolf DP, Thormahlen S, Ramsey C, Yeoman RR, Fanton J, Mitalipov S. Use of assisted reproductive technologies in the propagation of rhesus macaque offspring. Biol Reprod 2004;71: 486–93.
[27] McKiernan SH, Bavister BD. Culture of one-cell hamster embryos with water soluble vitamins: pantothenate stimulates blastocyst production. Hum Reprod 2000;15:157–64.
[28] Ng SC, Chen N, Yip WY, Liow SL, Tong GQ, Martelli B, et al. The first cell cycle after transfer of somatic cell nuclei in a non-human primate. Development 2004;131:2475–84.
[29] Dumesic DA, Schramm RD, Peterson E, Paprocki AM, Zhou R, Abbott DH. Impaired developmental competence of oocytes in adult prenatally androgenized female rhesus monkeys undergoing gonadotropin stimulation for in vitro fertilization. J Clin Endocrinol Metab 2002;87:1111–9.
[30] Mitalipov SM, Yeoman RR, Kuo H, Wolf DP. Monozygotic twinning in rhesus monkeys by manipulation of in vitro-derived embryos. Biol Reprod 2002;66:1449–55.
[31] Karnitis VJ, Townson DH, Friedman CI, Danforth DR. Recombinant human follicle-stimulating hormone stimulates multiple follicular growth, but minimal estrogen production in gonadotropin-releasing hormone antagonist-treated monkeys: examining the role of luteinizing hormone (LH) in follicular development and steroidogenesis. J Clin Endocrinol Metab 1994;79:91–7.
[32] Griesinger G, Diedrich K. Role of LH in ovarian stimulation: considerations. Reprod Biomed Online 2006;12:404–6.
[33] Nusser KD, Mitalipov S, Widmann A, Gerami-Naini B, Yeoman RR, Wolf DP. Developmental competence of oocytes after ICSI in the rhesus monkey. Hum Reprod 2001;16:130–7.
[34] Wittmaack FM, Kreger DO, Blasco L, Tureck RW, Mastroianni Jr L, Lessey BA. Effect of follicular size on oocyte retrieval, fertilization, cleavage, and embryo quality in in vitro fertilization cycle: a 6-year data collection. Fertil Steril 1994;62:1205–10.

[35] Dubey AK, Wang HA, Duffy P, Penzias AS. The correlation between follicular measurements, oocyte morphology, and fertilization rates in an in vitro fertilization program. Fertil Steril 1995;64:787–90.
[36] Pohl M, Hohlagschwandtner M, Obruca A, Poschalko G, Weigert M, Feichtinger W. Number and size of antral follicles as predictive factors in vitro fertilization and embryo transfer. J Assist Reprod Gene 2000;17:315–8.
[37] Welt C, Sidis Y, Keutmann H, Schneyer AL. Activins, inhibins, and follistatins: from endocrinology to signalling. A paradigm for the new millennium. Exp Biol Med (Maywood) 2002;27:724–52.