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ScienceDirect
Theriogenology 72 (2009) 243–250
Theriogenology
www.theriojournal.com

Ovarian response to gonadotropin stimulation in juvenile rhesus monkeys

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Received 1 November 2008; received in revised form 23 February 2009; accepted 26 February 2009

Abstract

The objective of this study was to investigate juvenile rhesus monkeys responding to various gonadotropin regimen stimulations. Thirty-two prepubertal rhesus monkeys were randomly allocated into five groups for ovarian stimulation as follows: Groups I, II, and III were given 35, 18, and 9 IU recombinant human follicle-stimulating hormone (rhFSH), respectively, twice daily for 8 d; Group IV was given 18 IU rhFSH twice daily until the appearance of maximal increase in sex skin during the breeding season; and Group V was treated identically to Group II but during the nonbreeding season. In addition, nine menarchial monkeys (Group VI) were treated identically to Group II. Menarchial monkeys yielded two- to fivefold the numbers of MII oocytes (24.1) and almost twice the development potential of in vitro–fertilized oocytes (blastocyst rate: 50.0%) compared with those of the other groups. Moreover, prepubertal monkeys in Group V had approximately double the numbers of MII oocytes and in Groups IV and V twice the development potential compared with those of Groups I and II, whereas Group III did not respond to stimulation. The most prominent sex skin swelling was in association with peak serum estradiol concentrations, and good responses to stimulation were associated with reduced body temperatures. All stimulated monkeys had normal reproductive performance at adulthood, except those in Group I. In conclusion, gonadotropin stimulation of menarchial monkeys could be appropriate for addressing the high cost and limited availability of rhesus monkeys in studying reproductive biology in primates.

Keywords: Breeding season; Gonadotropin; Juvenile rhesus monkey; Ovarian stimulation; rhFSH

1. Introduction

Substantial progress in the application of assisted reproductive technology (ART) to non-human primates over the past two decades has resulted in the routine production of in vitro–derived embryos and the use of embryo transfers to establish pregnancy and produce offspring [1,2]. These advances were followed by the introduction of somatic cell nuclear transfer research and the isolation of blastocyst-derived embryonic stem cell lines [3–5]. However, all these achievements are all dependent on large numbers of oocytes being retrieved

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0093-691X/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2009.02.019

in ovarian stimulation cycle. Protocols for ovarian stimulation of adult rhesus monkeys have been developed that first used gonadotropins extracted from animal or human blood serum, urine, and/or pituitaries [6–8] and more recently used human recombinant gonadotropins [9–11]. Nevertheless, the availability of adult rhesus monkey oocytes is impeded by the high cost of ovarian stimulation, limited numbers of caged animals, and the availability of adult rhesus monkeys for only approximately half of the year due to their natural breeding season. As an alternative, it was reported in many species that peripubertal animals could be used as oocyte donors without loss of reproductive performance when they matured to adulthood compared with age-matched controls [12–14]. Moreover, using juvenile animals as sources of oocytes for embryo production can shorten the generation interval, thereby accelerating the rate of genetic advance achievable by either natural selection or the introduction of new genes by transgenesis [15]. Whether ovarian stimulation of peripubertal monkeys could yield the quality and quantity of oocytes required for reproductive research is still unclear. A previous study showed that adjusting the dose of exogenous follicle-stimulating hormone (FSH) can be beneficial for the quality and quantity of oocytes collected, but this has not been evaluated in prepubertal or menarchial monkeys [9]; in that regard, the standard daily FSH dose for adult rhesus monkeys might not be suitable for these juvenile animals.

The primary objective of this study was to evaluate the effectiveness of three gonadotropin doses on ovarian stimulation and to compare the feasibility of this technology in prepubertal and menarchial rhesus monkeys in terms of their ovarian response and the developmental competence of retrieved oocytes after in vitro fertilization (IVF). We also examined the long-term effects of peripubertal ovarian stimulation on the subsequent reproductive ability of animals. Additionally, changes in perineal sex skin and body temperature were recorded to assist in evaluating responses to FSH and timing of human chorionic gonadotropin (hCG) administration.

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 the Kunming Primate Research Center (KPRC). Prepubertal and menarchial rhesus monkeys were selected from the KPRC and caged individually in a controlled environment (20 to 24 °C, humidity 40% to 60%) and exposed to an 0800 to 2000 light cycle. Vaginal bleeding was monitored daily to detect 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

Thirty-two prepubertal females (mean weight, 2.7 kg) that had never showed menses and nine menarchial females (mean weight, 3.6 kg) in which the first menses had just been observed were identified for incorporation into this study. The prepubertal females were randomly allocated into five groups for ovarian stimulation. Treatment with recombinant human FSH (rhFSH; Gonal F; Laboratories Serono SA, Aubonne, Switzerland) was initiated on a random day for prepubertal monkeys and 1 to 3 d after the onset of the first menses for menarchial monkeys. We evaluated three dose levels in this study. During the breeding season (November to February), Group I (n = 4, prepubertal monkeys), Group II (n = 9, prepubertal monkeys), and Group III (n = 4, prepubertal monkeys) received intramuscular (im) treatments of 35, 18, or 9 IU rhFSH, respectively, twice daily, 10 to 12 h apart for 8 d, as described previously [9,11,16,17]. Group IV (n = 6, prepubertal monkeys) received 18 IU rhFSH im twice daily until appearance of the maximal change in sex skin (color and swelling) during the breeding season. Group V (n = 9, prepubertal monkeys) received the same hormone regimen as that of Group II but in the nonbreeding season (May to July). In addition, Group VI (n = 9, menarchial monkeys) was treated with 18 IU rhFSH as in Group II and in the breeding season. On Day 9, animals in Groups I, II, III, V, and VI that had multiple increases in serum estrogen concentrations above baseline, and those with more than five follicles (≥3 mm in diameter) combined for both ovaries were defined as good responders [9] and were given 500 IU hCG im (Serono Laboratories, SA) at 2100 on the same day, whereas other animals were characterized as ‘‘poor responders’’ and were not given any additional treatment. Animals in Group IV received rhFSH for up to 10 d until appearance of the maximal change of sex skin; the next day, 500 IU hCG im was given for oocyte nuclear maturation.

To assess follicular development in response to FSH and to give hCG for nuclear maturity, animals were scanned by ultrasound on Day 9 of ovarian stimulation as follows. Animals were anesthetized with ketamine

(10 to 12 mg/kg) given intramuscularly and shaved in the lower abdominal and inguinal regions when they were laid on an operating table. Ovaries were imaged transcutaneously using a Diasus ultrasound system (Dynamic Imaging Ltd., Livingston, Scotland, UK), equipped with a 10- to 22-MHz linear-array transducer. To help locate the ovaries, the middle finger of the operator, wearing a sterile glove, was inserted into the animal's rectum after flushing out feces with warm water.

For oocyte retrieval, animals were anesthetized with ketamine (10 to 12 mg/kg) given intramuscularly, and cumulus-oocyte complexes (COCs) were collected by laparoscopic follicular aspiration 32 to 35 h after hCG administration. Follicular contents were placed into HEPES-buffered TALP (modified Tyrode solution with albumin, lactate, and pyruvate) medium [18] containing 0.3% bovine serum albumin (BSA) at 37 °C. Oocytes were stripped of cumulus cells by mechanical pipetting after brief exposure (<1 min) to hyaluronidase (0.5 mg/mL) to allow classification of nuclear maturity as metaphase I (MI; no germinal vesicle, no polar body) and metaphase II (MII; one polar body). Mature oocytes (MII) were placed in hamster embryo culture medium-10 (HECM-10) medium [19] at 37 °C in humidified 5% CO_2 in air until IVF.

2.3. Measurements of sex skin and body temperature

In an attempt to establish a noninvasive method for monitoring follicular development by using the correlation between the maximal changes in sex skin and body temperature curves with estradiol (E2) profiles, the sex skin around the perineum and anus [20] were monitored daily in Group II, and rectal temperatures were measured in four prepubertal rhesus monkeys of Group II. During ovarian stimulation, rectal temperatures were measured by gently inserting the probe of an electronic thermometer approximately 3 cm into the rectum of conscious animals to measure body temperatures in the morning (0800), and assessment of the sex skin around the perineum and anus was also done in the morning. The hardness of the sex skin (determined by hand) and the reddening of the sex skin were recorded.

2.4. IVF and embryo culture

To assess developmental competence, freshly collected mature oocytes were inseminated as in our previous reports [9]. Briefly, hyperactivated spermatozoa and mature oocytes (MII) were coincubated for 12 to 16 h at 37 °C in a humidified atmosphere of 5% CO_2. Fertilized oocytes exhibiting two pronuclei were cultured for embryonic development in 50-μL drops of HECM-10 containing 10% fetal bovine serum for up to 7 d at 37 °C in humidified 5% CO_2 in air under mineral oil, with a change in culture medium every second day. Progress of embryo growth was monitored daily using Nomarski optics (×200 to ×400 magnification) on a Nikon (Japan) Diaphot TMD microscope.

2.5. Hormone assays

To measure serum concentrations of E2, blood samples were drawn daily (0900) from conscious animals by saphenous venipuncture in Group II to determine the administration of hCG. Serum E2 concentration was determined by radioimmunoassay (RIA) [16,21]. The intra-assay and interassay coefficients of variation were all <10%.

2.6. Reproductive performance of prepubertal and menarchial monkeys subsequent to ovarian stimulation

To address the possibility that treatment of juvenile animals with gonadotropins might affect their future reproductive performance, which would diminish the usefulness of this protocol, prepubertal and menarchial monkeys after reaching puberty at the age of 5 yr (∼60 mo of age) were mated with fertile males, and thereafter pregnancy rates were recorded and compared among groups.

2.7. Statistical analysis

Results obtained are presented as the mean ± SD unless stated otherwise. The proportions of good responders and of pregnancy were analyzed by Fisher's exact test. For statistical purposes, 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 [16] prior to ANOVA and comparisons among the five groups, which responded to stimulation, by post hoc with least significant difference (LSD). Values with $P < 0.05$ were considered different.

The daily changes of E2 concentrations and the ranges of sex skin changes were calculated by $[b - a]/a$, where a = value of one day and b = value of the next day (for each parameter).

3. Results

3.1. Ovarian responses and oocyte development in stimulated prepubertal and menarchial monkeys

Ovarian responses and development of retrieved oocytes are shown in Table 1. Although the mean age was significantly different between the prepubertal monkey groups and the menarchial monkey group, there were no differences in the proportion of good responders among the five groups (Groups I, II, IV, V, VI), except that Group III had no ovarian responses and was dropped from further study. However, there were significant differences in the numbers of oocytes recovered from responding monkeys among the remaining five groups. The mean numbers of MII oocytes collected in menarchial monkeys in Group VI (24.1) were two- to fivefold higher than that in the prepubertal monkey groups ($P < 0.05$). The mean number of MII oocytes in Group V (9.3) was higher than that in Group I (4.9), Group II (5.9), and Group IV (4.7), respectively ($P < 0.05$), whereas the average numbers of MI oocytes were not significantly different among the five groups. Recovered MII oocytes had the same ability to undergo fertilization among the five groups, however the ability of IVF oocytes to develop into blastocysts was at least twofold higher in menarchial monkeys in Group VI (50.0% blastocysts) compared with that in all of the stimulated prepubertal monkeys ($P < 0.05$). Moreover, in prepubertal monkey groups, IVF oocytes in Groups IV and V had at least twofold higher development potential than that in Groups I and II (blastocyst rate: 27.8% in Group IV and 25.0% in Group V versus 0 in Group I and 10.5% in Group II, respectively, $P < 0.05$).

3.2. Changes of sex skin and body temperature associated with profile of plasma E2 in stimulated prepubertal monkeys

In all of the responding monkeys in Group II, regardless of the initial degree of reddening of the sex skin, the swelling increased after stimulation and reached a maximum when oocytes were retrieved. The maximal changes in sex skin and the peak level of E2 were two- to threefold higher than those of the previous day. The day of maximal increase in sex skin corresponded with the peak level of plasma E2. In Group II, one animal on Day 6, two animals on Day 7, three animals on Day 8, and one animal on Day 10 simultaneously had maximal increases in sex skin appearance and E2 peak level, as shown in Figs. 1 and 2,

Table 1
Numbers of responding animals and retrieved oocytes and subsequent development after IVF in prepubertal and menarchial rhesus monkeys stimulated with various gonadotropin regimens. *

Group (n)	Animals	Mean age, yr	The dose of rhFSH/d given and period	Number of responders (%)	Mean number of retrieved oocytes per animal			Development	
					Total	MII	MI	Fertilized, n (%)	Blast, n (%)
I (4)	Prepubertal	2.5 ± 0.1^{a}	70 IU, Nov. to Feb.	3 (75)[a]	11.9 ± 8.6^{a}	4.9 ± 2.5^{a}	7.0 ± 8.9^{a}	10/15 (66.7)[a]	0/10 (0)[a]
II (9)	Prepubertal	2.6 ± 0.3^{a}	36 IU, Nov. to Feb.	7 (78)[a]	9.9 ± 3.8^{a}	5.9 ± 2.7^{a}	4.0 ± 5.2^{a}	19/28 (67.9)[a]	2/19 (10.5)[b]
III (4)	Prepubertal	2.6 ± 0.3^{a}	18 IU, Nov. to Feb.	0[b]	–	–	–	–	–
IV (6)	Prepubertal	2.5 ± 0.2^{a}	36 IU, until the appearance of maximal changes of sex skin, Nov. to Feb.	5 (83)[a]	8.8 ± 3.2^{a}	4.7 ± 2.6^{a}	4.1 ± 6.2^{a}	18/25 (72)[a]	5/18 (27.8)[c]
V (9)	Prepubertal	2.9 ± 0.3^{a}	36 IU, May to July	9 (100)[a]	15.0 ± 6.7^{a}	9.3 ± 6.2^{b}	5.7 ± 5.6^{a}	16/20 (80.0)[a]	4/16 (25)[c]
VI (9)	Menarchial	3.7 ± 0.3^{b}	36 IU, Nov. to Feb.	9 (100)[a]	30.9 ± 18.5^{b}	24.1 ± 16.6^{c}	6.7 ± 6.2^{a}	20/23(87.0)[a]	10/20 (50.0)[d]

[a–d] Among treatment categories, means without a common superscript differ ($P < 0.05$).
* Responders had at least five ovarian follicles ≥ 3 mm in diameter at the end of rhFSH treatment. MII represents oocytes that were mature at collection, whereas MI refers to oocytes that were immature at collection. % Fertilized = (ova exhibiting two pronuclei/MII oocytes) × 100. Blast = (blastocysts/fertilized oocytes) × 100.

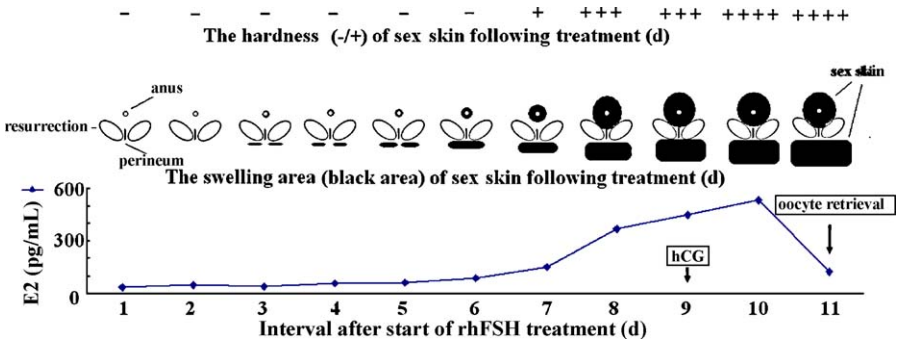

Fig. 1. Diagram showing changes of hardness and area of sex skin and its association with estradiol (E2) concentrations in one Group II good responder during ovarian stimulation in a rhesus monkey.

Fig. 2. Sex skin photos from one rhesus monkey in Group II. Day 1 shows the sex skin at the first rhFSH treatment, Day 7 shows the maximal increase of sex skin swelling, and Day 11 shows the maximal extent of the sex skin just before oocyte retrieval.

whereas the other two animals that were poor responders did not show any changes in these aspects. Moreover, according to the maximal changes of sex skin as above, prepubertal monkeys in Group IV received hCG on Days 7, 8, 9, and 11 of stimulation, and subsequently, the development potential of IVF oocytes retrieved were higher than that in Group II, although the number of oocytes retrieved was not different between the two groups (Table 1). Additionally, lowered body temperatures were associated with good responses to stimulation. Three of the four prepubertal monkeys in Group II, who responded well to stimulation, lowered

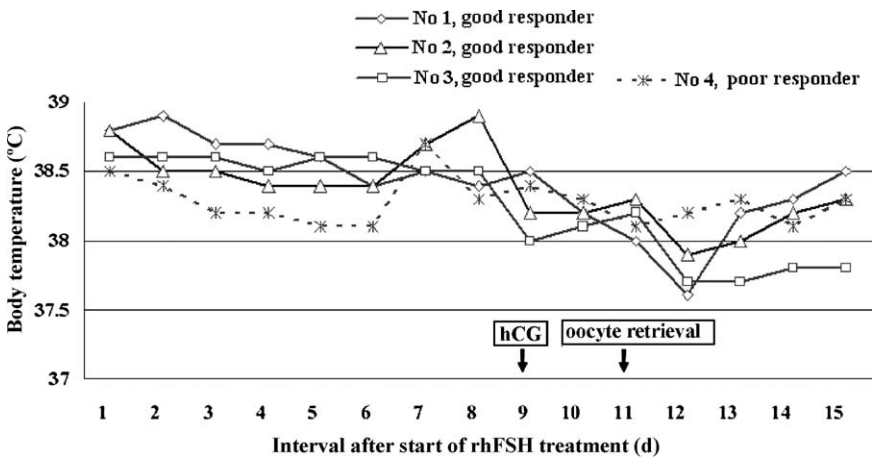

Fig. 3. Body temperature curve of prepubertal rhesus monkeys in three good responders and one poor responder in Group II during and after ovarian stimulation.

Table 2
Reproductive ability of stimulated prepubertal and menarchial rhesus monkeys during reproductive ages.

Group (n)	Animals, dose of rhFSH/d, and period	Pregnancy*	
		Mean pregnancy rate per animal, %	Pregnancy/reproductive years recorded (%)
I (4)	Prepubertal, 70 IU, Nov. to Feb.	10 ± 20	2/12 (17)[a]
II (9)	Prepubertal, 36 IU, Nov. to Feb.	75 ± 16	22/30 (73)[b]
III (4)	Prepubertal, 18 IU, Nov. to Feb.	79 ± 25	6/8 (75)[b]
IV (6)	Prepubertal, 36 IU, until the appearance of maximal changes of sex skin, Nov. to Feb.	68 ± 37	14/19 (74)[b]
V (9)	Prepubertal, 36 IU, May to July	70 ± 17	17/25 (68)[b]
VI (9)	Menarchial, 36 IU, Nov. to Feb.	81 ± 13	37/46 (80)[b]

[a,b] Among treatment categories, means without common superscript differ ($P < 0.05$).
* Pregnancies were recorded after the animal was 5 yr old and kept together with a fertile male all year round until separated.

their rectal temperature from more than 38.5 °C to <38 °C, whereas such a temperature decrease was not detected in the poor-responding female (Fig. 3).

3.3. Reproductive performance of prepubertal and menarchial monkeys subsequent to ovarian stimulation

When prepubertal and menarchial animals that were stimulated at young ages subsequently reached 5 yr of age, they were mated with fertile males until they were removed from the mating for other aims (the range of reproductive interval was from 5 to 6 yr to 5 to 10 yr). Pregnancies in stimulated monkeys are recorded in Table 2. Clearly, monkeys in Group I that had received the highest dose of rhFSH during prepuberty had a very low rate of pregnancy compared with that of other groups (17% in Group I vs. 73%, 75%, 74%, 68%, and 80% in Groups II to VI, respectively, $P < 0.05$). Monkeys stimulated with the reduced rhFSH regimen of 18 IU or 9 IU per injection, regardless of being prepubertal or menarchial, during the nonbreeding or breeding seasons, had similar pregnancy potentials after they reached reproductive ages.

4. Discussion

This report assessed the effects of rhFSH dose on prepubertal and menarchial monkeys in terms of ovarian responses, oocyte recovery, and in vitro development of IVF-produced embryos, as well as the subsequent fertility of stimulated monkeys. Clearly, the highest dose regimen of rhFSH (70 IU/d) imposed on prepubertal monkeys impaired the developmental capacity of retrieved oocytes and the reproductive performance of these animals after they reached reproductive adulthood. In this study, the menarchial monkey was an effective oocyte donor for reproductive biology research, but the prepubertal monkeys did not respond to the treatment protocol used.

The peripubertal period is a turning point for the potential improvement of oocyte development after aging [22]. Menarche could be a critical turning point for reproductive maturation, as demonstrated in the current study in rhesus monkeys. It may simply reflect an initial maturation of the hypothalamic-pituitary-gonadal axis, because beyond this point, all facets of the axis are functionally capable [23]. The current study revealing the differences between poor-responding prepubertal monkeys (20% to 25%) and menarchial monkeys, all of which responded, illustrates the improvement in the efficacy of stimulation with age. Another consideration is that the onset of puberty is closely related to the attainment of a crucial percentage of body fat. The increased percentage of body fat is a crucial factor in the timing of sexual maturation, which is expressed in the establishment of hypothalamic pulsatile release of gonadotropin-releasing hormone (GnRH) and in the activation of the gonadotropin-ovarian axis and the inauguration of menstrual cyclicity [24]. The explanation for the improved efficacy of ovarian stimulation in prepubertal monkeys treated during the nonbreeding season, compared with that during the breeding season, could be that the animals were nearer to menarche, attributable to more than 3 to 6 mo of additional growth and increased body fat. Additionally, the breeding season factor needs to be further studied in prepubertal monkeys to understand the apparent differences [13].

Menarchial monkeys in the current study and pubertal monkeys [9] can produce numerous high-quality oocytes after ovarian stimulation without impairing their subsequent reproductive performance, but prepubertal monkeys cannot yield the same results. Probably, the

rhFSH/hCG protocol in this study was not ideal for supporting oocyte development in the prepubertal monkeys and needs to be optimized in future studies.

That mammalian females are endowed with a finite and nonrenewing germ-cell reserve during the prenatal period is dogma that has persisted for greater than 50 yr. Recently, this dogma was challenged from a report that mammalian female germ-line stem cells could regenerate the follicle reserve in adult life [25]. Regardless, the fate of most ovarian follicles is atresia, especially before puberty in rhesus monkeys [26,27]. However, during the preovulatory period in mature females, many of these oocytes can be rescued by supplementation with exogenous gonadotropins during the breeding season, indicating that some of the rescued oocytes are capable of developing to term [9,11,21,28–34]. In non-primate species, many studies have shown that some oocytes also can be rescued from this fate in juveniles, and even though the average quality of these oocytes was relatively low, nevertheless many valuable embryos could be produced [12–15,35,36].

The commonly used procedures for monitoring follicular responses to gonadotropin stimulation (i.e., using ultrasound or blood samples to assess E2 concentrations) always cause capture panic and drug-induced trauma to animals. It is well known that the females of many Old World primate species during the time of ovulation develop a prominent reddening and swelling of the sex skin around their perineum related to peak serum E2 concentrations [20]. In the current study, using the changes of sex skin as an indicator of ovarian follicular development during ovarian stimulation was effective in establishing the optimal time for administering hCG, thereby enhancing the development potential of retrieved oocytes. In addition, changes in body temperature could be helpful along with the sex skin swelling for predicting the optimal timing of hCG injection. Thus, in the future, we may be able to use these less stressful measurements to indicate ovarian responsiveness and the optimal timing of hCG injection for peripubertal monkeys.

In conclusion, compared with prepubertal monkeys, peripubertal monkeys can yield high quality and quantity of oocytes; this could be a successful strategy for addressing the high cost and limited availability of rhesus monkey oocytes for research in reproductive biology in non-human primates. Also, the efficiency of using prepubertal and juvenile monkeys as oocyte donors might become higher when ovarian stimulation hormone regimens are improved, providing a cost-effective method for increasing the supply of materials for ART procedures and related research in non-human primates.

Acknowledgments

This work was supported by research grants from the Chinese Major State Research Development Program (20060102A1070), the Chinese National Basic Research Program (2007CB947701 and 2006CB701505), the Chinese Academy of Sciences (KSCX2-YWR-47 and KSCX2-YWR-89), and the Chinese National Science Foundation (30871232). We thank Dr. Barry Bavister for editing the manuscript.

References

[1] Wolf DP. Assisted reproductive technologies in rhesus macaques. Reprod Biol Endocrinol 2004;2:37.
[2] Bavister BD. The role of animal studies in supporting human assisted reproductive technology. Reprod Fertil Dev 2004;16:719–28.
[3] Zhou Q, Yang SH, Ding CH, He XC, Xie YH, Hildebrandt TB, et al. A comparative approach to somatic cell nuclear transfer in the rhesus monkey. Hum Reprod 2006;21:2564–71.
[4] 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.
[5] Byrne JA, Pedersen DA, Clepper LL, Nelson M, Sanger WG, Gokhale S, et al. Producing primate embryonic stem cells by somatic cell nuclear transfer. Nature 2007;450:497–502.
[6] Zelinski-Wooten M, 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.
[7] 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 U S A 1984;81:2218–22.
[8] Abbasi RKD, 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.
[9] Yang S, He X, Hildebrandt TB, Jewgenow K, Goeritz F, Tang X, et al. Effects of rhFSH dose on ovarian follicular response, oocyte recovery and embryo development in rhesus monkeys. Theriogenology 2007;67:1194–201.
[10] VandeVoort CA, Tarantal AF. Recombinant human gonadotropins for macaque superovulation: repeated stimulations and post-treatment pregnancies. J Med Primatol 2001;30:304–7.
[11] Schramm RD, Paprocki AM, Bavister BD. Features associated with reproductive ageing in female rhesus monkeys. Hum Reprod 2002;17:1597–603.
[12] Cognie Y, Baril G, Poulin N, Mermillod P. Current status of embryo technologies in sheep and goat. Theriogenology 2003;59:171–88.
[13] Gou KM, Guan H, Bai JH, Cui XH, Wu ZF, Yan FX, An XR. Field evaluation of juvenile in vitro embryo transfer (JIVET) in sheep. Anim Reprod Sci 2008.
[14] Kelly JM, Kleemann DO, Walker SK. Enhanced efficiency in the production of offspring from 4- to 8-week-old lambs. Theriogenology 2005;63:1876–90.

[15] Armstrong DT. Effect of maternal age on oocyte development competence. Theriogenology 2001;55:1303–22.
[16] Yang S, Shen Y, Niu Y, Hildebrandt TB, Jewgenow K, Goeritz F, He X, et al. Effects of rhFSH regimen and time interval on ovarian responses to repeated stimulation cycles in rhesus monkeys during a physiologic breeding season. Theriogenology 2008;70:108–14.
[17] Weston AM, Zelinski-Wooten MB, Hutchison JS, Stouffer RL, Wolf D. 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.
[18] 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.
[19] McKiernan SH, Bavister BD. Culture of one-cell hamster embryos with water soluble vitamins: pantothenate stimulates blastocyst production. Hum Reprod 2000;15:157–64.
[20] Czaja JA, Robinson JA, Eisele SG, Scheffler G, Goy RW. Relationship between sexual skin colour of female rhesus monkeys and midcycle plasma levels of oestradiol and progesterone. J Reprod Fertil 1977;49:147–50.
[21] Wolf DP, Vandevoort CA, Meyer-Haas GR, Zelinski-Wooten MB, Hess DL, Baughman WL, Stouffer RL. In vitro fertilization and embryo transfer in the rhesus monkey. Biol Reprod 1989;41:335–46.
[22] Steeves TE, Gardner DK, Zuelke KA, Squires TS, Fry RC. In vitro development and nutrient uptake by embryos derived from oocytes of pre-pubertal and adult cows. Mol Reprod Dev 1999;54:49–56.
[23] Kinder JE, Bergfeld EG, Wehrman ME, Peters KE, Kojima FN. Endocrine basis for puberty in heifers and ewes. J Reprod Fertil Suppl 1995;49:393–407.
[24] Maclure M, Travis LB, Willett W, MacMahon B. A prospective cohort study of nutrient intake and age at menarche. Am J Clin Nutr 1991;54:649–56.
[25] Johnson J, Canning J, Kaneko T, Pru JK, Tilly JL. Germline stem cells and follicular renewal in the postnatal mammalian ovary. Nature 2004;428:145–50.
[26] Baker TG. A quantitative and cytological study of oogenesis in the rhesus monkey. J Anat 1966;100:761–76.
[27] Vermande-Van Eck GJ. Neo-ovogenesis in the adult monkey; consequences of atresia of ovocytes. Anat Rec 1956;125: 207–24.
[28] Armstrong DT, Kotaras PJ, Earl CR. Advances in production of embryos in vitro from juvenile and prepubertal oocytes from the calf and lamb. Reprod Fertil Dev 1997;9:333–9.
[29] Bavister BD. ARTs in action in nonhuman primates: symposium summary-advances and remaining issues. Reprod Biol Endocrinol 2004;2:43.
[30] Meng LEJ, Stouffer RL, Wolf DP. Rhesus monkeys produced by nuclear transfer. Biol Reprod 1997;57:454–9.
[31] Wolf DP, Thomson JA, Zelinsk-Wooten MB, Stouffer RL. In vitro fertilization-embryo transfer in nonhuman primates: the technique and its application. Mol Reprod Dev 1990;27:261–80.
[32] Johnson LD, Mattson BA, Albertini DF, Sehgal PK, Becker RA, Avis J, Biggers JD. Quality of oocytes from superovulated rhesus monkeys. Hum Reprod 1991;6:623–31.
[33] 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.
[34] Yang S, He X, Hildebrandt TB, Zhou Q, Ji W. Superovulatory response to a low dose single-daily treatment of rhFSH dissolved in polyvinylpyrrolidone in rhesus monkeys. Am J Primatol 2007;69:1278–84.
[35] O'Brien JK, Catt SL, Ireland KA, Maxwell WM, Evans G. In vitro and in vivo developmental capacity of oocytes from prepubertal and adult sheep. Theriogenology 1997;47:1433–43.
[36] Izquierdo D, Villamediana P, Lopez-Bejar M, Paramio MT. Effect of in vitro and in vivo culture on embryo development from prepubertal goat IVM-IVF oocytes. Theriogenology 2002;57:1431–41.