RESEARCH ARTICLE

Evaluation of the Vervet (C. aethiops) as a Model for the Assisted Reproductive Technologies

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The vervet monkey was evaluated as a primate model for use in assisted reproductive technologies (ARTs). Eight adult female vervets were hormonally monitored for their potential use as egg donors and those six females displaying regular menstrual cycles were subjected to controlled ovarian stimulation with recombinant human gonadotropins. Three animals failed to respond while laparoscopic follicular aspiration was performed on the other three females at 27–30 h post-human chorionic gonadotropin administration. A total of 62, 40, and 18 oocytes was recovered from these three animals of which 30, 20, and 4, respectively, matured to the metaphase II stage and were subsequently inseminated using intracytoplasmic sperm injection. An average of 40 ± 15% (SEM) of the inseminated oocytes were fertilized based on pronucleus formation and timely cleavage. One embryo from each of the two stimulated females developed into expanded blastocysts. Two adult male vervets were assessed as sperm donors. Neither adjusted well to the restraint and collection procedure required for penile electroejaculation. Samples collected via rectal electroejaculation were very low in sperm motility and concentration; however, cauda epididymal aspirations from one male yielded an adequate concentration of motile sperm. These results emphasize the need to establish species-specific ovarian stimulation protocols and semen collection techniques if vervets are to be considered for basic and applied (ARTs) research on primate gametes or embryos. Am. J. Primatol. 69:1–13, 2007. © 2007 Wiley-Liss, Inc.

Key words: vervet; ovarian stimulation; embryo

Contract grant sponsor: Oregon National Primate Research Center (ONPRC); Contract grant number: RR00163.

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Received 27 April 2006; revised 24 October 2006; revision accepted 7 November 2006

DOI 10.1002/ajp.20413
Published online in Wiley InterScience (www.interscience.wiley.com).

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INTRODUCTION

The vervet (*C. aethiops*) has the potential to be a valuable research model for studying the assisted reproductive technologies (ARTs). Unlike the commonly studied rhesus macaques, vervet females are believed to maintain a menstrual cycle year-round and have a straight cervix. Vervet males have been reported to possess human-like sperm characteristics (e.g. concentration, motility, pH of seminal fluid, acrosomal integrity) [Hess et al., 1979; Van Der Horst et al., 1999]. The rhesus macaque has been used extensively as a model for early human development and for fertilization and embryonic development in primates. Over the past 20 years, remarkable advancements in the ARTs have been achieved using the rhesus monkey model. Several of these accomplishments, including multiple follicle stimulation, oocyte fertilization by intracytoplasmic sperm injection (ICSI), and oocyte/embryo manipulation, were recently described [Wolf, 2004]. For a particular species such as the vervet to serve as an alternative model for studying the ARTs, the ability to obtain a high number of healthy, fertilizable oocytes, collect and cryopreserve fertile spermatozoa, as well as cryopreserve and transfer the resulting embryos when a recipient is available would be essential.

Initial attempts to stimulate ovaries of vervet females required repeated administrations of equine and hCG [Sankai et al., 1997]. In these studies, conventional in vitro insemination of meiotically mature, vervet oocytes led to a high incidence of polyspermy. We report on the recovery of mature oocytes following controlled ovarian stimulation (COS) of vervets with recombinant human gonadotropins, fertilization by ICSI, and oocyte/embryo manipulation, using assisted reproductive techniques developed for rhesus macaques.

MATERIALS AND METHODS

Animals

Two adult vervet males, weighing 7.0 and 8.8 kg, and eight adult vervet females, weighing 3.1–4.9 kg, were studied at the Oregon National Primate Research Center (ONPRC). All animals were acquired from the Behavioural Science Foundation at Basseterre, St. Kitts, Eastern Caribbean. The general care and housing of vervet monkeys by the Division of Animal Resources, ONPRC, was described previously [Molskness et al., 2007]. Animal protocols in this study were reviewed and approved by the ONPRC IACUC.

Semen Collection and Cryopreservation

Fresh semen was collected from two adult male vervet monkeys. The fertility of both males was unknown before this study. Efforts to harvest progressively motile sperm involved the use of penile electroejaculation [Lanzendorf et al., 1990], rectal electroejaculation, and cauda epidydimal aspiration on anesthetized males. It should be noted that the epidydimal aspiration technique was performed only as a final attempt to recover a viable sperm sample from each male for our fertilization efforts. Electroejaculated semen samples were allowed to liquefy for 15–30 min before the liquefaction of the coagulated ejaculate was removed [Mitalipov et al., 2002]. Aspirated epidydimal tissue was immediately placed into Hepes-buffered Talp (modified Tyrode solution with albumin, lactate, and pyruvate) medium [Bavister et al., 1983] containing 0.3% bovine serum albumin (TH3) and transported to the laboratory for processing. All semen samples were...
washed two times in TH3 medium by centrifugation at 1,400 RPM for 8 min [Sanchez-Partida et al., 2000]. Sperm motility and concentration were examined in each sample. Sperm samples were either used immediately for in vitro fertilization of oocytes or cryopreserved and stored in liquid nitrogen.

For cryopreservation, the washed sperm pellet was resuspended in a TES-Tris buffer solution containing 30% egg yolk, 20% skim milk, 3% glycerol, and 0.06 M glucose and cooled to 4°C for 90 min [Tollner et al., 1990]. This sperm suspension was deposited as 20–50 μl drops into depressions on the surface of dry ice. The frozen drops were transferred into cryovials and stored on canes in liquid nitrogen. For thawing, a sperm pellet was transferred into a dry test tube and together were partially submerged into a 37°C water bath (<1 min). The thawed sample was washed in TH3 medium and evaluated as described above.

### Detection of Menstruation and Hormone Measurement

Females were vaginally swabbed on a daily basis to detect the onset of menstruation. The first day of menstruation was considered day 1 of the menstrual cycle. On a weekly basis, blood samples were collected from each female and analyzed for serum hormone levels by the Endocrine Services Core Laboratory, ONPRC. Serum estradiol (E2) and progesterone (P4) levels were measured using electrochemoluminescent assays [Young & Stouffer, 2004] and a Roche Elecsys 2010 analyzer (Roche Diagnostics, Indianapolis, IN). The detection of menses and the serum hormone levels were used to determine the regularity of monthly menstrual cycles [Eley et al., 1989; Molskness et al., 2007]. Serum luteinizing hormone (LH) levels were also measured by the Endocrine Services Core Laboratory in samples from two females during ovarian stimulation protocols when a spontaneous LH surge was suspected. The Core personnel stimulated mouse interstitial cell production of testosterone in vitro to determine serum LH [Yeoman et al., 1988].

### Stimulation of Ovaries

Multiple follicular development in the ovaries was induced in females, exhibiting regular menstrual cycles, using exogenous gonadotropins. A COS protocol, designed and used previously in rhesus macaques to generate numerous mature oocytes for in vitro fertilization, was employed in this study [Meng & Wolf, 1997; Wolf et al., 1990, 2004; Zelinski-Wooten et al., 1995]. Starting at menses (days 1–4 of the menstrual cycle), females received daily injections of a GnRH antagonist (Antide; Ares Serono, Aubonne, Switzerland; 0.5 mg/kg body weight, subcutaneous) to prevent an endogenous LH surge, plus recombinant human follicle stimulating hormone (r-hFSH; Gonal F; Ares Serono; 30 IU, intramuscular, twice daily) for 8 days. Recombinant human LH (r-hLH; Ares Serono; 30 IU, intramuscular, twice daily) was administered along with r-hFSH and GnRH antagonist on days 7 and 8 of the stimulation. All other reagents used in this study were from Sigma-Aldrich Co. (St. Louis, MO) unless otherwise indicated. Ultrasonography was performed starting on day 7 to assess ovarian response. Females with enlarged ovarian follicles 3–4 mm in diameter received hCG (Serono, Randolph, MA; 1,000 IU, intramuscular) to induce the resumption of oocyte maturation [Zelinski-Wooten et al., 1994]. Blood samples were collected daily during the stimulation and analyzed for E2 and P4 levels, as described earlier.
Oocyte Collection and In Vitro Fertilization

Oocyte collection followed previously described protocols for the rhesus macaque [Wolf et al., 2004; Zelinski-Wooten et al., 1995]. Laparoscopic follicular aspiration was performed on anesthetized monkeys 27–30 h post-hCG injection [Wolf et al., 1996] by the Department of Surgery, Division of Animal Resources, ONPRC. Tubes containing follicular aspirates were transported to the laboratory in a portable incubator (Minitube, Verona, WI) at 37°C. The aspirates were sifted through a cell strainer (Becton-Dickonson, Franklin Lakes, NJ; Falcon, 70 um pore size) and then immediately backwashed with Hepes-buffered Talp (modified Tyrode solution with albumin, lactate, and pyruvate) medium [Bavister et al., 1983] containing 0.3% bovine serum albumin (TH3) to recover the oocytes. Oocytes were stripped of cumulus cells by mechanical pipetting and a brief (30 s) exposure to hyaluronidase (0.5 mg/ml), examined for determination of developmental stage (germinal vesicle-intact: GV; metaphase I: MI; metaphase II: MII; degenerate), morphology, color of the cytoplasm, granularity, and then cultured in HECM-9 medium [McKiernan & Bavister, 2000; VandeVoort et al., 2003; Wolf et al., 2004] overlaid with paraffin oil (Ovoil, Zander IVF [in vitro fertilization], Vero Beach, FL) at 37°C in 5% CO₂ until fertilization. Oocyte maturity (progression from MI- to MII-stage) was evaluated and recorded approximately every 2 h following collection until fertilization.

On the day of collection (day 0; d0), meiotically mature MII oocytes were fertilized with a single vervet spermatozoan using ICSI [Meng & Wolf, 1997; Mitalipov et al., 2001; Nusser et al., 2001]. Injections were performed at 37°C in 30 µl drops of TH3 medium in the lid of a 60 mm tissue culture dish, covered with paraffin oil, using an Olympus inverted microscope (Melville, New York, USA) equipped with micromanipulators (Narishige, Tokyo, Japan). Sperms were placed in a 4 µl drop of 10% polyvinylpyrrolidone (Irvine Scientific, Santa Ana, CA), immobilized, aspirated tail first into a microinjection needle (Humagen, Charlottesville, VA), and injected into the cytoplasm of each oocyte. Following ICSI, all oocytes were returned to a 4-well dish (Nalge Nunc International Co., Naperville, IL) containing HECM-9 and cultured overnight at 37°C in 6% CO₂, 5% O₂, balance N₂.

Embryo Culture and Cryopreservation

Successful fertilization, determined by the presence of two pronuclei, was assessed 14–16 h post-injection. Cleavage-stage embryos were cultured in 4-well dishes in HECM-9 medium containing 5% fetal bovine serum (Hyclone, Logan, UT) overlaid with paraffin oil at 37°C in 6% CO₂, 5% O₂, balance N₂. The progression of embryonic growth was assessed daily and culture medium was changed every other day. Embryos were either cultured under these conditions to the blastocyst stage or cryopreserved on day 3 for future thaw and transfer into a surrogate female. Embryos designated for cryopreservation were placed in increasing concentrations of the cryoprotectant propanediol [Wolf et al., 1989], transferred to a cryovial, and cooled at a rate of −2°C/min to a seeding temperature of −7°C in a BioCool control rate freezer (FTS Systems, New York, USA). Once ice crystal formation was manually initiated, the freezer temperature was dropped at −0.3°C/min to −30°C. The cryovial containing embryos was then plunged into liquid nitrogen for storage. Thawing was performed initially at 37°C followed by the dilution of the propanediol in a step-wise manner at room temperature [Wolf et al., 1989].
Embryo Transfer

Embryo transfer procedures were similar to those described for rhesus macaques [Nusser et al., 2001; Wolf et al., 2004]. Blood samples were collected daily starting on day 8 of the menstrual cycle and E2 levels were determined by radioimmunoassay. The day following the E2 peak is commonly referred to as day 0 [Wolf et al., 2004]. This information was used to determine the timing of the embryo transfer. On day 1, 2 days after the estradiol peak the embryos were thawed and placed in culture the afternoon preceding the scheduled day of transfer. On day 2, embryo survival was assessed based on the following criteria: presence of the zona pellucida, recovery of >50% of the blastomeres, and overall embryo morphology and quality [Wolf et al., 2004]. Embryos that survived were placed in TH3 medium and immediately transported to the operating room at ONPRC. A surgical embryo transfer [Nusser et al., 2001; Wolf et al., 2004] was performed, with the assistance of the Department of Surgery at ONPRC, by transferring two frozen–thawed vervet embryos into an oviduct of an anesthetized recipient vervet female (3–4-day old embryos into a day 2 oviduct). The embryos were deposited approximately 2 cm into the oviduct via the fimbria on the side with an ovulation site. Blood samples were collected daily and analyzed for serum E2 and P4 levels and ultrasonography was performed on day 35 post-transfer to determine implantation and pregnancy.

RESULTS
Response to Controlled Ovarian Stimulation

Eight females were screened as potential egg donors, with six maintaining regular menstrual cycles. Multiple follicular development was successfully induced in three of six regularly cycling monkeys, with exogenous gonadotropins as determined by ultrasonography (Fig. 1a and b; female 1). Figure 2a and b display the E2 and P4 levels, respectively, measured during the COS cycle. Three females that responded to the stimulation (successful COS) had E2 levels exceeding 4,000 pg/ml between days 10 and 12 of the menstrual cycle. A peak E2 level of 6,270 ± 1,223 pg/ml (mean ± SEM) was detected on day 11, whereas a P4 peak of 52 ± 7 ng/ml (mean ± SEM) was measured on day 18. These peak estradiol and P4 levels were ten-fold higher than levels observed in the two females displaying what seemed like an abbreviated response (E2: 568 pg/ml; P4: 4.3 ng/ml; mean). This abbreviated-like response was not associated with a spontaneous LH surge during hormonal treatment (based on LH bioassay results). Additionally, one female did not respond to the exogenous r-hFSH treatment and the protocol was terminated on day 8 of treatment. Menstruation was detected in all three successful responders 17–20 days post-hCG.

Spermatozoa Motility and Concentration

Table I depicts the results of semen analysis on samples recovered by various collection methods from two male vervet monkeys. Multiple attempts to collect semen samples by penile electroejaculation were not successful as neither male adjusted to the restraint apparatus and collection procedures. Most samples obtained by rectal electroejaculation had few or no motile spermatozoa, except one ejaculate that yielded approximately 0.2 x 10^6 sperms/ml with 28% motility. In a final attempt to recover spermatozoa for in vitro fertilization, a biopsy of epidydimal tissue was surgically taken from each male. An adequate concentration of motile spermatozoa was obtained from the epidydimal tissue of one of the
two males. The two samples with measurable sperm populations were used for ICSI (both samples) and to evaluate sperm survival post-thaw (only one sample). Inadequate motility (low percentage: 32%; minimal progressive movement,

Fig. 1. Ultrasonographic images depicting large multiple follicles in ovaries of one successful controlled ovarian stimulation animal (female 1; day 9 of treatment). (A) Left ovary is depicted by LO in the top image. (B) Right ovary is depicted by RO in the lower image; both from the same vervet female. Numerical values on the ovary represent the diameter of each follicle (in millimeters). The numerical value next to the RO and LO text represents the measured diameter of each ovary (in millimeter).
twitching) and abnormal morphology (bent tails: 42%; broken tails: 39%) of frozen/thawed spermatozoa were observed.

**In vitro Fertilization, Embryonic Development, Embryo Transfer**

Table II summarizes the results of oocyte collection, fertilization by ICSI, and subsequent embryonic development. Meiotically active (MI, MII) oocytes were recovered from large (3–4 mm diameter) follicles of all three vervets successfully.
stimulated with r-hFSH, r-hLH, Antide, and hCG. A total of 46% of the oocytes from two of the three females (1 and 2) matured to the metaphase II stage of meiosis on the day of aspiration (d0) and 55% within 24 h of oocyte collection (d1). An average rate of 55% fertilization by ICSI (Fig. 3a) was achieved in all injected oocytes (d0 and d1) from two females (1 and 3) as evident by the presence of two pronuclei within 14–16 h of ICSI (Fig. 3b). In female 1, 80% (13/21) of oocytes matured at d0 were fertilized, whereas fertilization was 55% (5/9) with oocytes matured at d1. The poor fertilization rate (10%) in female 2 was likely attributed to the inability to collect a fresh sperm sample from either male on the day of oocyte retrieval and the use of frozen-thawed sperm. Total loss of sperm viability was observed after 3 h post-thaw. A delayed rate of cleavage was detected as early as day 3 when the embryos were at the 4–6 cell stage and a high rate of developmental arrest (80%) occurred at the 6–8 cell stage (days 3–4) in subsequent vervet embryos. One embryo from each of two females developed to the morula stage on day 6, compact morula stage on day 7, and blastocyst stage between days 9–11 of culture (Fig. 3c). Only one of the two blastocysts hatched from the zona pellucida by day 12 of culture. On day 3, the highest scoring

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TABLE I. Analysis of Vervet Spermatozoa Recovered by Various Semen Collection Techniques: Penile Electroejaculation, Rectal Electroejaculation, or Epidydimal Aspiration

<table>
<thead>
<tr>
<th>Male no.</th>
<th>Sperm collection technique</th>
<th>Total sperm count (million/ml)</th>
<th>Sperm motility</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Penile electroejaculation</td>
<td>No sperm recovered</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Rectal Electroejaculation</td>
<td>Too few to accurately assess</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Cauda epidydimal aspiration</td>
<td>230.7 x 10⁶/ml</td>
<td>32%</td>
</tr>
<tr>
<td>2</td>
<td>Penile electroejaculation</td>
<td>No sperm recovered</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Rectal electroejaculation</td>
<td>0.2 x 10⁶/ml</td>
<td>28%</td>
</tr>
<tr>
<td></td>
<td>Cauda epidydimal aspiration</td>
<td>No sperm recovered</td>
<td>NA</td>
</tr>
</tbody>
</table>

Sperm count, displayed as total million sperm (included non-motile sperm) per ml of TH3 media, and sperm motility expressed as total percent motile, were determined immediately following the recovery of the sperm sample. Note that the use of penile electroejaculation, commonly used in rhesus macaques, did not produce a sperm sample in both males. NA, not assessed, because of the lack of sperm recovered.

TABLE II. Summary of Vervet Oocyte Recovery, Fertilization, and Subsequent Development to the Blastocyst Stage In Vitro

<table>
<thead>
<tr>
<th>Female no.</th>
<th>Total no. oocytes</th>
<th>Oocytes: degen</th>
<th>Oocytes: GV</th>
<th>Oocytes: MI</th>
<th>Oocytes: MII day 0¹</th>
<th>Oocytes: MII day 1³</th>
<th>No. (%) Fertilized</th>
<th>No. (%) Blastocysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>62</td>
<td>20</td>
<td>7</td>
<td>30</td>
<td>21</td>
<td>32</td>
<td>18 (60%)</td>
<td>1 (9%)</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>4</td>
<td>10</td>
<td>22</td>
<td>23</td>
<td>23</td>
<td>2 (10%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>5</td>
<td>9</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>2 (50%)</td>
<td>1 (50%)</td>
</tr>
</tbody>
</table>

Oocytes were recovered from three females (day 0) and assessed immediately for total number and developmental stages. Degen, degenerated; GV, germinal vesicle intact; MI, metaphase I; MII, Metaphase II. Oocyte maturation from MI to MII stage was monitored on days 0 and 1. MII stage oocytes were inseminated by intracytoplasmic sperm injection (ICSI) on days 1 and 2, and fertilization status (based on the presence of two pronuclei within 14–16 h of ICSI) and subsequent embryonic development to the blastocyst stage were recorded every day. The number fertilized is also expressed as a percent of the MII oocytes that had two pronuclei following ICSI. The number of blastocysts is also expressed as the percent of fertilized embryos that developed to the blastocyst stage. Note that seven of the 18 embryos from female 1 were cryopreserved and not used to evaluate progression to the blastocyst stage.

¹Indicates the day of in vitro culture. d0, day of oocyte recovery.

Am. J. Primatol. DOI 10.1002/ajp
Fig. 3. (A) Micrograph of a metaphase II-stage vervet oocyte during intracytoplasmic sperm injection (ICSI). Note the presence of the polar body at the 6 o'clock position and the sperm located in the ICSI pipette on the right side of the image. (B) Micrograph of a fertilized vervet oocyte containing two pronuclei. Fertilization status was assessed 14–16h following intracytoplasmic sperm injection (ICSI) and was based on the presence of two pronuclei in the cytoplasm. (C) Micrograph of a vervet blastocyst. This embryo was derived from a vervet oocyte fertilized by ICSI and developed to the blastocyst stage after extended culture in HECM-9 media containing 5% fetal bovine serum.

Am. J. Primatol. DOI 10.1002/ajp
embryos (determined by overall morphology; $n = 7$) from one female were cryopreserved and were not used to assess embryonic development to the blastocyst stage. Upon thawing, three of the seven cryopreserved embryos survived, but only two, based on morphological evaluation, were transferred into a recipient vervet monkey. No pregnancy was established.

DISCUSSION

In this report, a sequential treatment of recombinant human gonadotropins (r-hFSH, r-hLH, Antide, and hCG) was administered to induce controlled follicular stimulation in vervet females at doses commonly used for rhesus macaques [Wolf et al., 1990, 2004]. The only reported COS protocol in vervets employed equine chorionic gonadotropin (CG) for follicular development and human CG for ovulatory events [Sankai et al., 1997]. FSH is considered the predominant gonadotropin needed for follicular growth and development. When FSH is administered along with LH on days 7 and 8 of the stimulation in rhesus macaques, this combination results in large preovulatory follicles, 3–4 mm in diameter [Wolf et al., 1989]. After an injection of hCG for ovulation induction, a cohort of oocytes can be recovered suitable for fertilization and embryo production. Non-primate gonadotropin preparations, such as eCG, are available however use of these hormones for macaques results in the production of antibodies even after one COS cycle [Bavister et al., 1986]. The use of recombinant human gonadotropins (r-hFSH, r-hLH, and r-hCG) apparently delays the production of antibodies allowing at least three COS cycles per animal [Stouffer & Zelinski-Wooten, 2004]. Our ability to recover mature oocytes from vervet monkeys using the follicular stimulation protocol established for rhesus monkeys is encouraging. In 2001, Nusser et al. reported that an average of 30 oocytes was recovered per stimulated rhesus monkey. In this study, two of the three vervet females (1 and 2) provided 40 or more oocytes per animal with over 50% of the total number of oocytes matured to the MII stage of meiosis.

Despite our ability to recover oocytes from three vervet females, two of the six females displayed what initially seemed like abbreviated responses to the sequential gonadotropin treatment and one female failed to respond at all to the exogenous gonadotropins. Since we did not detect an LH surge in the two females with abbreviated-like responses, it is unlikely that the GnRH-antagonist (Antide) failed to suppress the animal’s endogenous hormones. This suggests that three of the six females with regular menstrual cycles failed to fully respond to the hormonal treatment. It is noteworthy to add that the two abbreviated-like response females started receiving r-hFSH on day 4 of their menstrual cycle. In rhesus macaques, we have reduced our stimulation cancellation rate by initiating all stimulations on days 1–3 of the cycle (unpublished result). A typical cancellation rate for rhesus macaque stimulations is 22% [Wolf et al., 2004].

To our knowledge, this study is the first to report successful ICSI with vervet spermatozoa and oocytes. In rhesus monkeys, this insemination technique eliminates the need to chemically capacitate sperm, minimizes the chance of polyspermy since only one sperm is injected, and typically results in a high level of oocyte fertilization and subsequent live offspring following transfer of ICSI-derived embryos [Hewitson et al., 1998, 1999; Meng & Wolf, 1997; Nusser et al., 2001]. Sankai et al. [1997] reported on the first successful in vitro fertilization (66%) of C. aethiops oocytes using hyperactivated spermatozoa. Of the fertilized oocytes, over one-third (37%) were polyspermic. Recently, Wolf et al. [2004] achieved over 80% fertilization rates following ICSI of rhesus oocytes with fresh spermatozoa.
or frozen-thawed spermatozoa. We used ICSI in this study because of its success in the rhesus macaque and the lack of fresh semen samples with adequate sperm concentration and motility, needed for IVF. Although a 50–60% fertilization rate of MII oocytes from two vervets is below 80%, these initial ICSI attempts are encouraging.

In vitro derived rhesus monkey embryos fertilized by ICSI and cultured in HECM-9 develop to the compacting morula stage on day 3 of culture, blastocyst stage around day 5, and hatched blastocyst stage as early as day 8 [Wolf et al., 2004]. These in vitro rates of development are thought to be similar to in vivo rates of embryonic development [Wolf, 2004]. In comparison, cleavage and development rates in vitro to the blastocyst stage for vervet embryos were slower. Sankai et al. [1997] also reported delayed development among in vitro-derived vervet embryos (morula stage: day 5+ post-insemination; early blastocyst stage: day 7; expanded blastocyst stage: day 8+). Although it is difficult to pinpoint the cause(s), it is possible that delayed vervet embryonic development, compared with rhesus embryos, is a result of sub-optimal culture conditions and/or sublethal damage to the sperm during processing, cryopreservation, or insemination. The limited selection of motile vervet spermatozoa for ICSI may also have adversely affected the developmental competence of subsequent embryos. The slow progression to the blastocyst stage emphasizes the need to further understand embryonic development in this species and optimize culture conditions. Considering that post-thaw survival of rhesus embryos averages 66% (unpublished result), our initial success with the post-thaw survival of vervet embryos (43%) also supports a promising future for use of the vervet in the ARTs.

The ability to use the vervet in the ARTs was limited by our difficulty in recovering ejaculated sperm samples. In the rhesus electroejaculation program at ONPRC if a male proves uncooperative or an ejaculate is not obtained after several attempts, the animal may be released from the program. Further selection criterion requires that the males produce ejaculates with over $100 \times 10^6$ sperm per ejaculate of which over 70% are motile and morphologically normal [Wolf et al., 1990]. At the California National Primate Research Center (CNPRC), three males are typically trained to the electroejaculation procedure and the male with the best semen parameters is selected [VandeVoort et al., 2004]. Unfortunately we did not have more than two males to evaluate. The semen collection procedures performed on the vervets are the described techniques for single-caged rhesus males that regularly provide quality spermatozoa for in vitro fertilization and cryopreservation efforts [Wolf et al., 1990]. Application of these procedures to the vervet did not yield the progressively motile spermatozoa ($184 \times 10^6$/ml; 55.5% motility) recovered at other facilities [Seier et al., 1989]. Testing of additional males and species-specific semen collection protocols [Seier et al., 1989] may help generate ejaculates with normal semen characteristics. Although sperm motility and concentration from both males were inadequate for traditional in vitro fertilization, the use of ICSI overcame this limitation. Even frozen-thawed sperm, nearly immotile and morphologically abnormal post-thaw, could be used to produce viable embryos. Mdhluli et al. [2004] recently found similarities in sperm parameters (e.g. concentration, motility, acrosomal integrity) between vervet and man. These findings further support the potential value of the vervet as a model for human reproductive research.

With an increasing number of infertile patients using the ARTs, specifically ICSI, there is a greater focus now on optimizing these techniques to ensure patient safety. Today it is estimated that over 50% of all IVF cycles in women involve ICSI [Hewitson, 2004]. This fertilization technique has been associated
with an increased frequency of specific chromosomal abnormalities and imprinting defects [Bonduelle et al., 1996; Cox et al., 2002]. Non-human primates, including rhesus macaques and vervets, reach sexual maturity and produce offspring within a few years making them an ideal model for studying the long-term effects of ICSI and other ART procedures. Here, we show that vervet oocytes can be collected in large numbers following ovarian stimulation with exogenous human gonadotropins and fertilized by ICSI using procedures effective in the rhesus macaque. These results suggest that while modifications to these procedures are necessary for application in the vervet, the vervet monkey can be used as a potential model for studying the clinical and scientific application of the ARTs.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to the surgical staff at ONPRC for assistance with laparoscopic oocyte retrievals, semen collections, and the embryo transfer; Division of Animal Resources at ONPRC for hormone administration, blood draws, and semen collections; and Dr. David Hess and the Endocrine Services Core at ONPRC for hormone assays. The Serono Reproductive Biology Institute, a member of Serono International, generously donated the human recombinant hormones and Antide used in this study.

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