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# Initiation Timing Cannot Predict Success of Superovulation in Rhesus Monkeys (*Macaca mulatta*)

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**Abstract:** Current protocols for Superovulation (SO) of rhesus monkeys are not optimized and outcomes vary considerably. There has been little attempt to predict SO outcome according to the stimulation starting time point (Day of cycle) of the candidate oocyte donors. In the present study, researchers focus on the SO initiation timing and we hypothesized that this factor could be used to select optimal oocyte donor monkeys. Superovulation was initiated on menstrual cycle days 1-4 or 5 of 107 adult female rhesus monkeys then 81 animals were identified as good responders by ultrasonography after 6 days of treatment. Oocytes Pick Up (OPU) was performed by laparoscopic follicular aspiration. The numbers of total oocytes and metaphase II (MII) oocytes from each retrieval were recorded. The results showed that SO begun on days 1-4 or 5 of the menstrual cycle produced similar outcomes indicating that SO initiation in rhesus monkeys can be on days 1-5 of menses. These data showed that SO initiation timing can not be used to predict the SO outcome in rhesus monkeys.

**Key words:** Superovulation, initiation timing, rhesus monkey, ultrasonography, China

## INTRODUCTION

The rhesus monkey is one of the most widely used laboratory animals in medical and biological research due to its close relationship to humans in anatomy, genetics and physiology. Rhesus monkeys have similar characteristic of the menstrual cycle and pregnancy to humans so it is an ideal model to study the biology of human reproduction.

Collection of oocytes from rhesus monkeys is a basic procedure for *in vitro* embryology research. In order to obtain ample numbers of good quality oocytes, animals are given exogenous gonadotropins to cause Superovulation (SO) (Bavister *et al*., 1986; Wolf *et al*., 1989; Zelinski-Wooten *et al*., 1995). Researchers and others have paid great attention to SO in rhesus monkeys for improving the yield of retrieved oocytes. These studies revealed numerous factors affecting SO outcome, including age (Hull *et al*., 1996; Nichols *et al*., 2010; Wolf *et al*., 1989; Yang *et al*., 2009), regimen and time interval (Yang *et al*., 2008), dose of gonadotropins (Yang *et al*., 2007a), physiological condition of donors and repeated ovarian stimulation (Bavister *et al*., 1986; Ottobre and Stouffer, 1985; Yang *et al*., 2008), etc. In the most common SO procedure for rhesus monkeys, administration of recombinant human Follicle-Stimulating Hormone (rhFSH) is begun on days 1-3 of the menstrual cycle. But in the experience, the duration of menses in some monkeys lasts up to 5 days and there is no information about starting SO on days 4 or 5 of the menstrual cycle. Therefore, we hypothesized that changing initiation timing to include days 4-5 would affect SO outcomes. We hoped these data could be useful parameters for selecting optimal rhesus monkey oocyte donors.

## MATERIALS AND METHODS

**Animals and reagents:** All animal procedures were approved in advance by the Institutional Animal Care and Use Committee of the Kunming Biomed International (KBI) and Kunming Institute of Zoology, Chinese

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Academy of Sciences and were performed in accordance with the experimental animal ethical guidelines. Healthy female adult rhesus monkeys aged 5-15 years old with regular menstrual cycle were selected as oocyte donors from the KPRC colony. All of these animals were caged individually in a controlled environment (Temperature 20-24°C, humidity 40-60%) and exposed to a 08:00-20:00 light cycle.

Vaginal bleeding was monitored twice daily to detect the onset of menses. All experiments were performed during the rhesus monkey breeding season (October to March). Unless stated otherwise, all reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

**Superovulation (SO) and Ovum Pick Up (OPU):** Adult female rhesus monkeys were given rhFSH (Gonal F, Laboratories Serono SA, Aubonne, Switzerland) for ovarian stimulation by intramuscular injection during the physiological breeding seasons as described (Yang *et al*., 2007b). Treatment was initiated on days 1-5 of menses. For the first injection, 37.5 IU of rhFSH was given followed by 18 IU of rhFSH twice daily for 8 days. On day 9th, 1000 IU of rhCG (Recombinant Human Chorionic Gonadotropin alfa for Injection, Merck Serono) was injected.

For oocyte retrieval, animals were anesthetized with ketamine (10-12 mg $kg^{-1}$) given intramuscularly. Oocytes were collected by laparoscopic follicular aspiration (STORZ, Germany) 32-35 h after rhCG administration. Follicular contents were collected into HEPES-buffered TALP (Modified Tyrode's solution with albumin, lactate and pyruvate) medium (Bavister *et al*., 1983a, b) containing 0.3% bovine serum albumin (BSA, Sigma) at 37°C.

Oocytes were stripped of cumulus cells by mechanical pipetting after brief exposure (<1 min) to hyaluronidase (0.5 mg $mL^{-1}$, Sigma), to allow classification of nuclear maturity as Prophase I (PI; intact germinal vesicle), Metaphase I (MI; no germinal vesicle, no polar body) or metaphase II (MII; one polar body).

**Ovarian development monitoring:** Ovarian development was monitored on day 6 of SO by ultrasonography. If there were more than 5 obvious well-proportioned follicles on each ovary, the animal was defined as a good responder and treatment was continued. Otherwise, if there were no or only 1-3 large follicles on each ovary, the animal was defined as a poor responder and dropped as an oocyte donor.

**Statistical analysis:** The total numbers of oocytes and of MII oocytes were recorded. The SO efficiency was defined according to [(No. of OPU/No. of SO)×100%]. Data were expressed as the Mean±SEM (Standard Error of the Mean) and were analyzed with SPSS software. Statistical analysis was performed using the least significant difference test. Significant difference was defined as $p<0.05$.

## RESULTS

The SO-OPU outcomes of animals that began stimulation on days 1-5 of menses is shown in Table 1. The efficiency of SO was measured by ultrasonography on day 6 of SO and responders or poor responders was differentiated according ovarian development. Figure 1 shows ovaries of a typical good responder rhesus monkey and a poor responder.

A total of 107 adult female monkeys were divided into 5 groups according to the initiation timing of SO and 81 of these animals were good responders and OPU was performed on day 11. The SO efficiency of animals initiated on days 1-5 was 81.82, 70.37, 68.57, 85.00 and 85.71%, respectively.

The mean number of oocytes was 42.00±6.14, 46.53±4.26, 51.79±7.35, 49.71±7.57 and 37.67±5.98, respectively while the mean number of MII oocytes was 22.78±3.20, 21.05±2.21, 25.13±4.53, 25.65±3.55 and 19.58±2.56, respectively. There was no significant difference among the 5 groups. These data showed that SO initiated on any day from 1-5 of menses produced similar outcomes.

Table 1: Efficiency and outcomes of SO initiation on different days of menses in rhesus monkeys

| Initiation time relative to start of menses | No. of SO procedures | No. of good responders | Efficiency (%)* | Mean no. of oocyte | No. of MII |
|---|---|---|---|---|---|
| Day 1 | 11 | 9 | 81.82 | 42.00±6.14 | 22.78±3.20 |
| Day 2 | 27 | 19 | 70.37 | 46.53±4.26 | 21.05±2.21 |
| Day 3 | 35 | 24 | 68.57 | 51.79±7.35 | 25.13±4.53 |
| Day 4 | 20 | 17 | 85.00 | 49.71±7.57 | 25.65±3.55 |
| Day 5 | 14 | 12 | 85.71 | 37.67±5.98 | 19.58±2.56 |

*Efficiency was calculated according to formula (No. of OPU/No. of SO)×100%

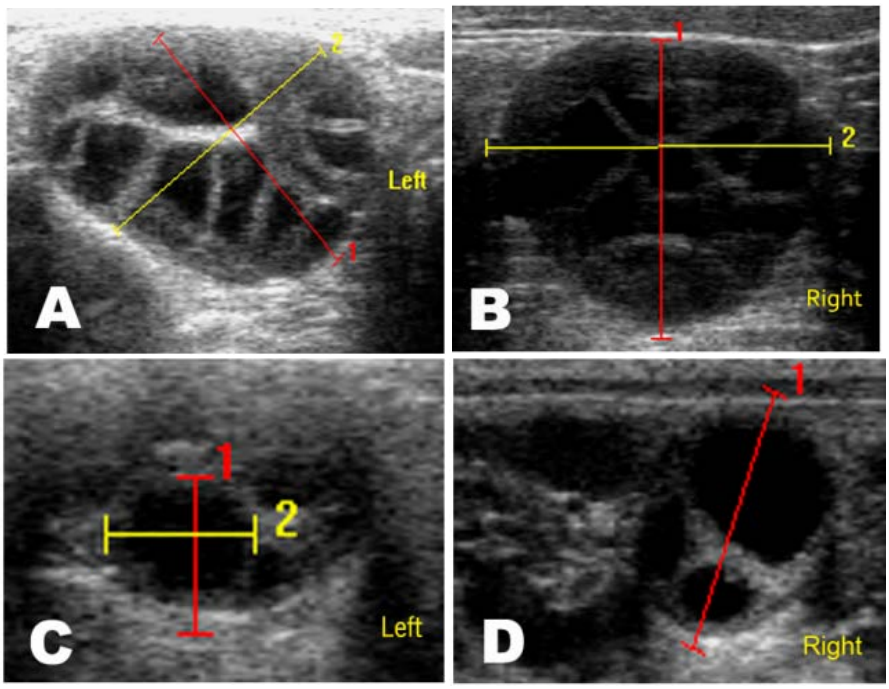


Fig. 1: Ultrasonographic images of ovaries of good vs. poor responder rhesus monkeys. A and B, left and right ovaries of a good responder rhesus monkey, monitored on 6th day of SO. There are multiple large-sized and well-proportioned follicles on both ovaries of this oocyte donor. C and D, left and right ovaries of a poor responder rhesus monkey, monitored on 6th day of SO. There are few or only 2-3 large follicles on ovaries of this animal which could not be an oocyte donor

## DISCUSSION

Superovulation in primates is usually started on days 1-3 of the menstrual cycle (Bavister *et al*., 1986; Schramm and Paprocki, 2000; Wolf *et al*., 1989), largely because during menses, serum hormones such as Luteinizing Hormone (LH) and FSH are at a low level (Muasher *et al*., 1988; Noci *et al*., 1998, 2000; Toner *et al*., 1991). In this condition, exogenous gonadotropins like FSH are able to stimulate multiple antral follicles that otherwise might become atretic to develop normally while rhCG or LH are helpful for oocyte maturation. Empirically, we find that some monkeys menstruate for up to 5 days. In order to verify whether the initiation time point affects SO efficiency, 107 female rhesus monkeys with 3-5 days menses records randomly began to receive stimulation on days 1-5. Surprisingly, there were no obvious differences in the yield of total oocytes and MII oocytes as well as SO efficiency among the groups, regardless of the initiation time indicating that initiation of SO in rhesus monkeys could extend from days 1-5. This finding has potential practical application value for flexible arrangement of SO according to the reproductive or experimental plan.

## CONCLUSION

In this study, we stimulated rhesus monkeys with recombinant gonadotropins for superovulation initiated on days 1-5. The results showed no obvious relationship between initiation timing and SO-OPU outcomes. These data indicate that initiation timing cannot predict the success of superovulation in rhesus monkeys.

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## REFERENCES


Bavister, B.D., C. Dees and R.D. Schultz, 1986. Refractoriness of rhesus monkeys to repeated ovarian stimulation by exogenous gonadotropins is caused by nonprecipitating antibodies. Am. J. Reprod. Immunol. Microbiol., 11: 11-16.

Bavister, B.D., D.E. Boatman, L. Leibfried, M. Loose and M.W. Vernon, 1983b. Fertilization and cleavage of rhesus monkey oocytes *in vitro*. Biol. Reprod., 28: 983-999.

Bavister, B.D., M.L. Leibfried and G. Lieberman, 1983a. Development of preimplantation embryos of the golden hamster in a defined culture medium. Biol. Reprod., 28: 235-247.

Hull, M.G., C.F. Fleming, A.O. Hughes and A. McDermott, 1996. The age-related decline in female fecundity: A quantitative controlled study of implanting capacity and survival of individual embryos after *in vitro* fertilization. Fertil. Steril., 65: 783-790.

Muasher, S.J., S. Oehninger, S. Simonetti, J. Matta and L.M. Ellis *et al*., 1988. The value of basal and/or stimulated serum gonadotropin levels in prediction of stimulation response and *in vitro* fertilization outcome. Fertil. Steril., 50: 298-307.

Nichols, S.M., L. Gierbolini, J.A. Gonzalez-Martinez and B.D. Bavister, 2010. Effects of *in vitro* maturation and age on oocyte quality in the rhesus macaque *Macaca mulatta*. Fertil. Steril., 93: 1591-1600.

Noci, I., M. Maggi, B. Fuzzi, R. Biagiotti, F. Ricci and M. Marchionni, 2000. Effects of low day 3 luteinizing hormone levels on *in vitro* fertilization treatment outcome. Gynecol. Endocrinol., 14: 321-326.

Noci, I., R. Biagiotti, M. Maggi, F. Ricci, A. Cinotti and G. Scarselli, 1998. Low day 3 luteinizing hormone values are predictive of reduced response to ovarian stimulation. Hum. Reprod., 13: 531-534.

Ottobre, J.S. and R.L. Stouffer, 1985. Antibody production in rhesus monkeys following prolonged administration of human chorionic gonadotropin. Fertil. Steril., 43: 122-128.

Schramm, R.D. and A.M. Paprocki, 2000. Birth of rhesus monkey infant after transfer of embryos derived from *in vitro* matured oocytes: Short communication. Hum. Reprod., 15: 2411-2414.

Toner, J.P., C.B. Philput, G.S. Jones and S.J. Muasher, 1991. Basal follicle-stimulating hormone level is a better predictor of *in vitro* fertilization performance than age. Fertil. Steril., 55: 784-791.

Wolf, D.P., C.A. Vandevoort, G.R. Meyer-Haas, M.B. Zelinski-Wooten, D.L. Hess, W.L. Baughman and R.L. Stouffer, 1989. *In vitro* fertilization and embryo transfer in the rhesus monkey. Biol. Reprod., 41: 335-346.

Yang, S., X. He, T.B. Hildebrandt, K. Jewgenow and F. Goeritz *et al*., 2007a. Effects of rhFSH dose on ovarian follicular response, oocyte recovery and embryo development in rhesus monkeys. Theriogenology, 67: 1194-1201.

Yang, S., X. He, T.B. Hildebrandt, Q. Zhou and W. Ji, 2007b. Superovulatory response to a low dose single-daily treatment of rhFSH dissolved in polyvinylpyrrolidone in rhesus monkeys. Am. J. Primatol., 69: 1278-1284.

Yang, S., X. He, Y. Niu, T.B. Hildebrandt, K. Jewgenow and F. Goeritz *et al*., 2009. Ovarian response to gonadotropin stimulation in juvenile rhesus monkeys. Theriogenology, 72: 243-250.

Yang, S., Y. Shen, Y. Niu, T.B. Hildebrandt and K. Jewgenow *et al*., 2008. Effects of rhFSH regimen and time interval on ovarian responses to repeated stimulation cycles in rhesus monkeys during a physiologic breeding season. Theriogenology, 70: 108-114.

Zelinski-Wooten, M.B., J.S. Hutchison, D.L. Hess, D.P. Wolf and R.L. Stouffer, 1995. Follicle stimulating hormone alone supports follicle growth and oocyte development in gonadotrophin-releasing hormone antagonist-treated monkeys. Hum. Reprod., 10: 1658-1666.