

Efficient production of cynomolgus monkeys with a toolbox of enhanced assisted reproductive technologies

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Parameter	Pregnant monkeys just before ET			Non-pregnant monkeys just before ET		
	Max	Min	Means $\pm$ s.e.m.	Max	Min	Means $\pm$ s.e.m.
Body weight (kg)	11.0	8.0	9.1 $\pm$ 0.3	10.0	8.0	8.9 $\pm$ 0.3
Age (year)	8.0	4.7	6.0 $\pm$ 0.5	8.0	5.8	6.9 $\pm$ 0.4
Volume ovary (cm ³)	9.9	3.7	6.7 $\pm$ 1.1	9.6	2.7	5.5 $\pm$ 1.1
Menstrual cycle (day)	15.0	11.0	13.1 $\pm$ 0.7	15.0	11.0	14.4 $\pm$ 0.6
Dominant follicle / Freshly ovulated follicle	1 / 9			1 / 9		
Embryos transferred	5.0	3.0	4.1 $\pm$ 0.2	5.0	3.0	3.7 $\pm$ 0.2
E2 (pg/mL)	646.0	31.5	157.9 $\pm$ 122.1	653.5	32.8	177.8 $\pm$ 119.1
P4 (ng/mL)	1.5	0.9	1.6 $\pm$ 0.5	3.0	0.4	1.8 $\pm$ 0.5
Uterus length (mm)	26.9	23.7	20.9 $\pm$ 0.4	28.0	23.0	24.9 $\pm$ 0.6
Endometrium length (mm)	21.8	11.5	19.2 $\pm$ 1.0	21.2	12.5	18.9 $\pm$ 1.1
Uterus height (mm)	25.7	13.8	21.1 $\pm$ 1.3	23.3	13.4	18.9 $\pm$ 1.1
Endometrium thickness (mm)	17.2	8.4	14.1 $\pm$ 1.0	10.7	5.0	7.5 $\pm$ 0.6**
Myometrium thickness (mm)	4.8	2.7	3.5 $\pm$ 0.2	8.0	2.1	4.5 $\pm$ 0.6
E/M	5.3	3.1	4.1 $\pm$ 0.3	3.5	0.6	1.9 $\pm$ 0.3**

Table S1. Parameters of Uterus and Ovaries at Embryo Transfer of Pregnant and Non-Pregnant Rhesus Recipients Received Embryos from the

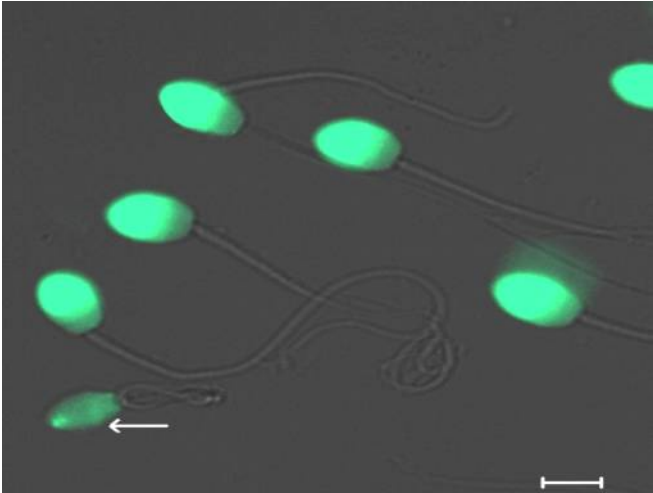
Same Fertilization Trial. Data are means $\pm$ s.e.m. with n=10 per group for the same experimental condition. P-values were analyzed with Student's t-test

(nonparametric test, two tailed), ** $P < 0.01$. Expect dominant follicle / freshly ovulated follicle with Fisher's exact test. Max, maximum value; Min,

minimum value; ET, embryo transfer; E2, estradiol; P4, progesterone; E/M = endometrial to myometrial thicknesses.

Figure S1

A



B

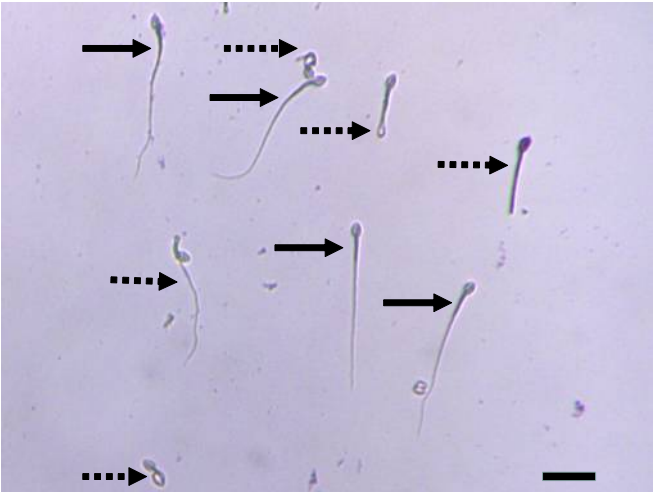


Figure S2

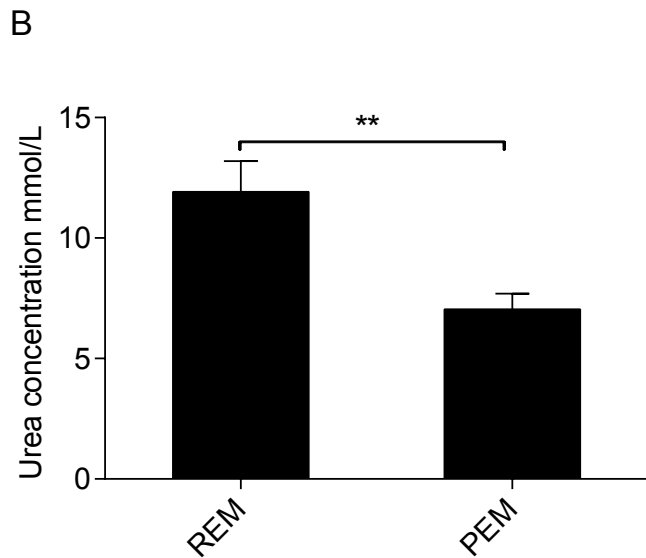
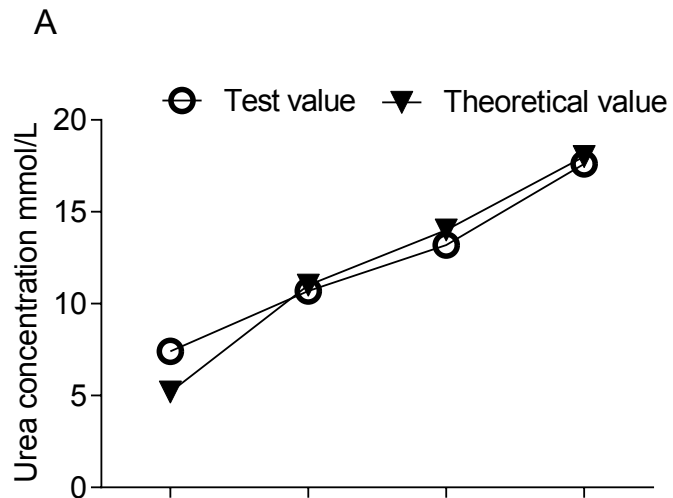


Figure S3

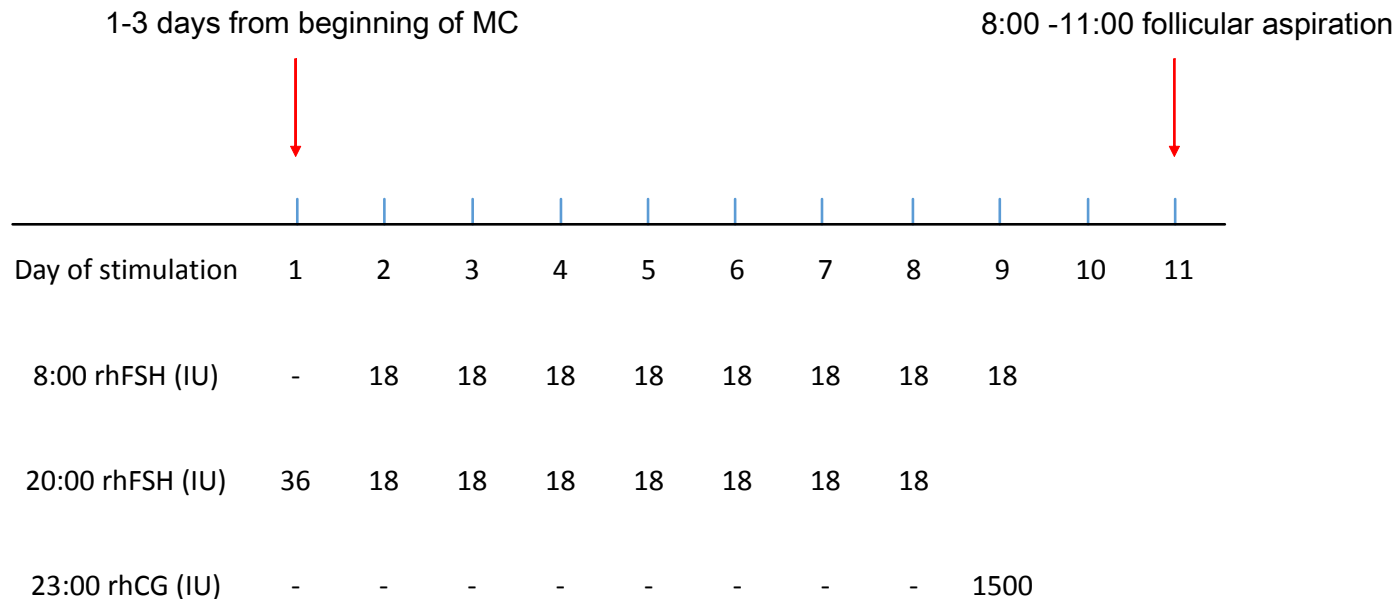


Figure S4

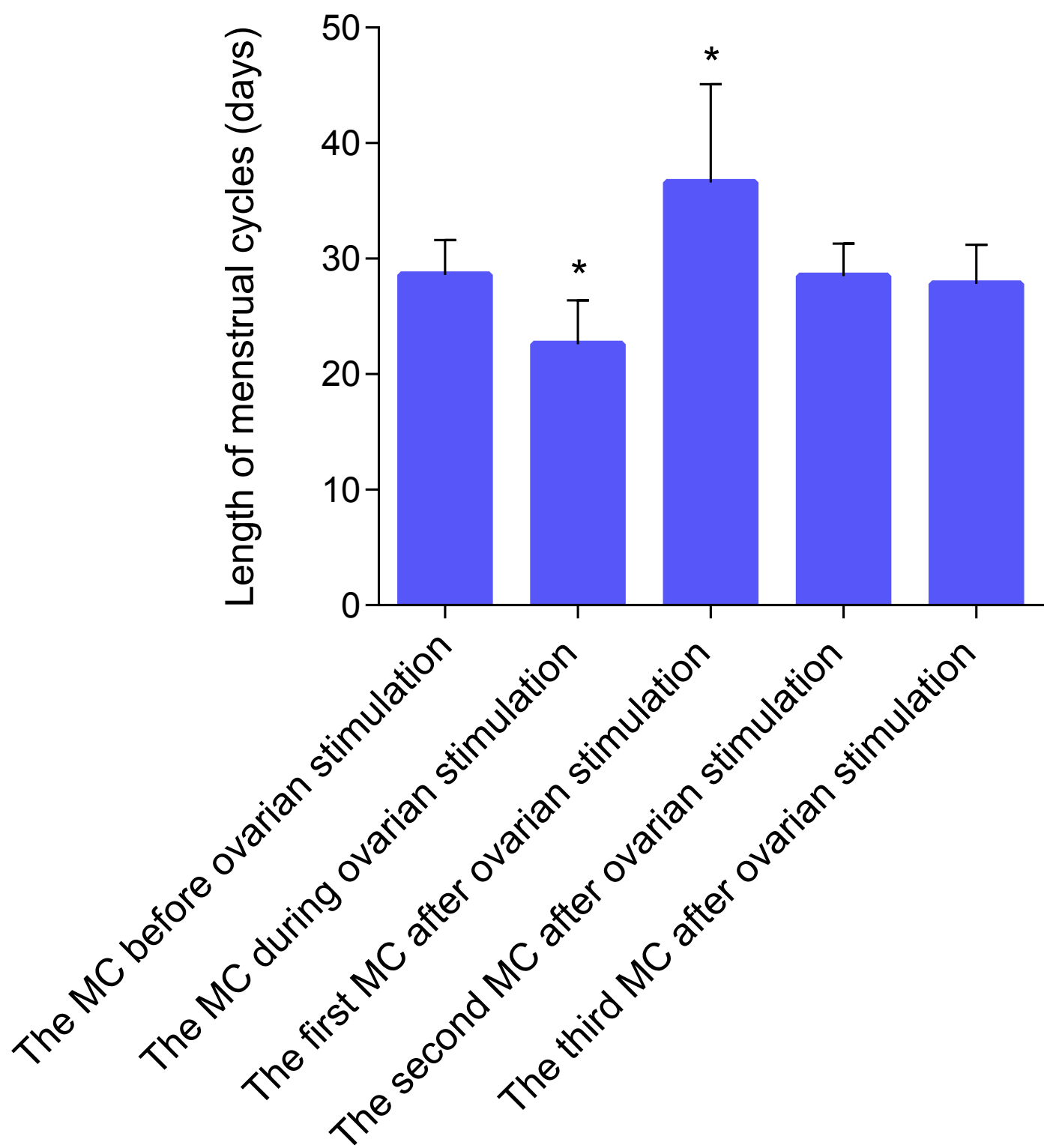


Figure S5

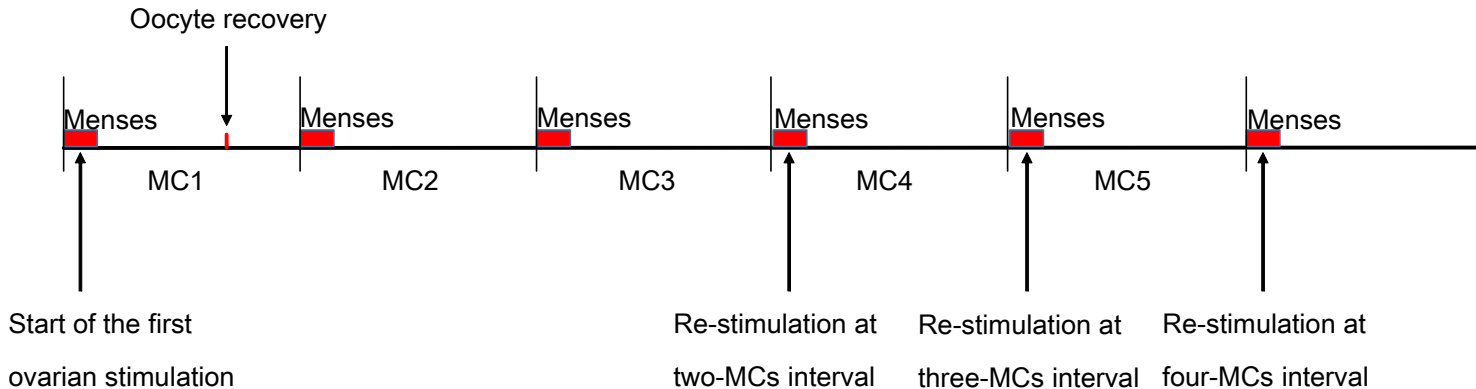


Figure S6

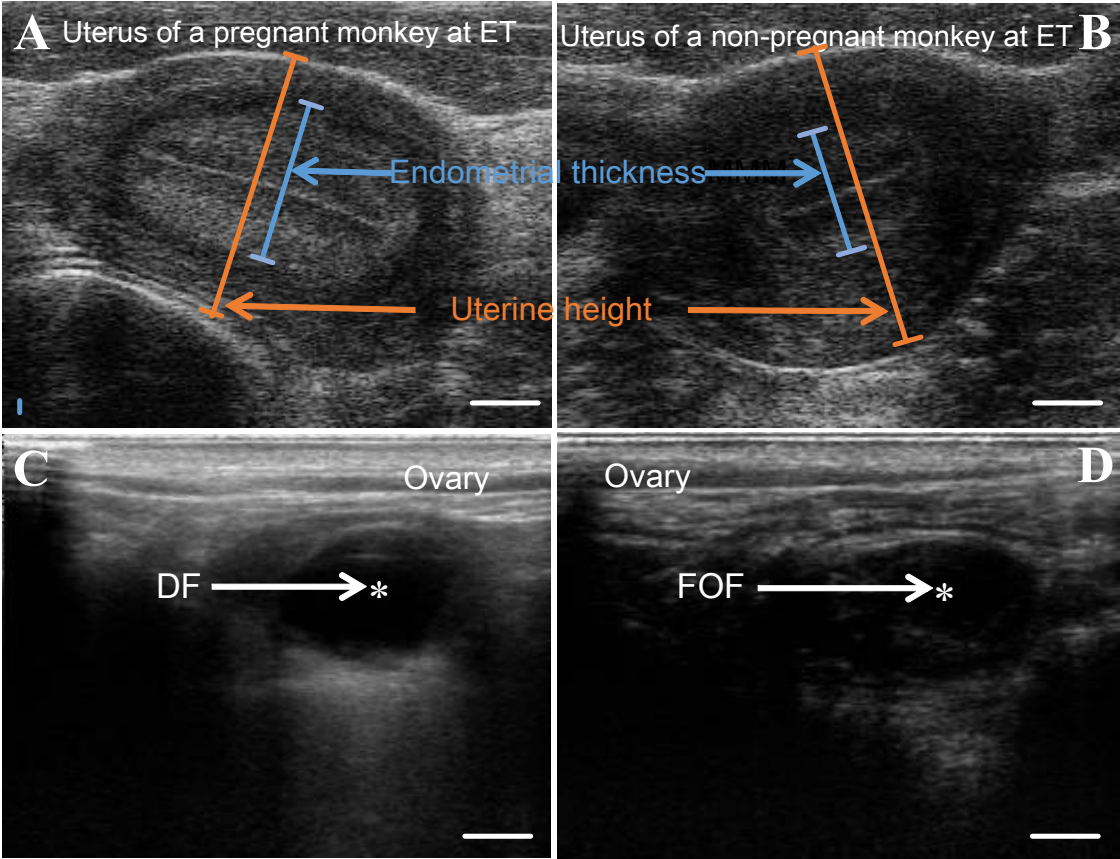


Figure S1. Cynomolgus Monkey Sperm. (A) Acrosome images, broken acrosomes marked with solid arrows, magnified at $\times 400$ with an inverted microscope. (Scale bar, 5 μm). (B) Image of sperm morphology magnified at $\times 200$ under an optical microscope. Normal sperm marked with solid arrow, sperm without tail or head, or short tail, and even sperm with sharp tail bend marked with dotted arrow. (Scale bar, 20 μm).

Figure S2. Measurement of Urea Concentration in Cynomolgus Monkey Semen. (A) Calibration curve of urea concentration in serum, the differences between test value and theoretical value is less than 15%. (B) Urea concentrations in fresh semen from REM and PEM. REM, rectal probe electrical stimulus method; PEM, penile probe electrical stimulation. Results are means $\pm$ s.e.m. Statistical analysis was performed with one-way analysis of variance. $**P < 0.01$.

Figure S3. The Regimen of Ovarian Stimulation in Cynomolgus Monkeys.

Figure S4. Length of MCs Before and After Ovarian Stimulation. Data are means $\pm$ s.e.m. with $n=8$. Statistical analysis was done among different MCs with a generalized linear model repeated-measures $*P < 0.05$.

Figure S5. Schematic of Repeated Ovarian Stimulation Separated at Intervals of Two, Three, or Four MCs after the Initial Stimulation with the Same Regimen. MCs, menstrual cycles.

Figure S6. Ultrasonic Images of Uterus and Ovary in Recipient Monkeys just before Embryo

Transfer. (A and B) Measurement of the endometrial thickness and uterine height (blue and red arrows, respectively) in a pregnant monkey and a non-pregnant monkey just before embryo transfer (Scale bar, 3 mm). (C and D) ovary state of recipient cynomolgus monkeys just before embryo transfer. A ovary with a dominant follicle (white arrow) and a ovary with a freshly ovulated follicle (white arrow) (Scale bar, 3 mm). ET, embryo transfer.