**Title: Development of Non-Invasive Cervical Insemination in Rhesus Macaques**

Emily Mishler[1]; Jared Jensen[2]; Fernanda Burch[1]; Philberta Leung[1]; Carol Hanna[1]

1. Division of Reproductive and Developmental Science, Oregon National Primate Research Center, Oregon Health and Science University, Beaverton, Oregon, United States
2. College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado, United States

With the high demand of rhesus macaques for biomedical research, breeding programs are under pressure to increase reproductive efficiency while maintaining genetic diversity through intentional breeding strategies. One such strategy is artificial insemination (AI). Transcervical AI in the Rhesus macaque is challenging due to the tortuous path of the cervical canal. However, successful pregnancies have been previously reported by both direct intrauterine insemination (IUI) using animals pre-screened with less cervical impedance (57%) and by ultrasound-guided percutaneous insemination through the abdominal wall and directly into the uterine lumen (33%). Both methods are invasive and require veterinary support. As a refinement, we hypothesized that we could achieve similar pregnancy rates by depositing semen into the external os of the cervix. In this pilot study, regularly cycling adult rhesus females (n=10; average age 13.7 ± SEM 0.46 years) underwent a total of 17 insemination cycles. Animals were either inseminated once (n=5), or twice (n=12) during the periovulatory window. In dual inseminating cycles, semen from different males were used for each insemination to allow for genetic testing of the offspring to identify which insemination resulted in conception. Starting 7 days from the onset of frank menses, we collected blood daily to monitor estradiol (E2) over the periovulatory phase. Inseminations occurred between one day prior and 3 days post-E2 peak (average E2 peak = 360 ± SEM 19.1 pg/ml). Sedated animals were placed in dorsal recumbency with pelvis elevated and monitored by pulse oximetry. Perineum was cleaned of gross contaminants, and a lightly lubricated nasal speculum was inserted in the vaginal canal. Utilizing a surgical headlamp, the external cervical os was located and a Smith's Insemination Catheter was inserted with placement confirmed by ultrasonography. Following fresh semen collection, at least 50 million motile spermatozoa suspended in 0.5ml TALP-Hepes with BSA were infused into the external cervical os. The catheter was then removed and the pelvis remained elevated for 5 minutes while the animal recovered. Pregnancy was diagnosed approximately 30 days later by ultrasound assessment of the uterus. Data were interrogated by 2-way ANOVA with significance considered $p<0.05$. Of the 5 cycles where animals underwent a single insemination, no females conceived. From the 12 cycles involving dual insemination, 25% conceived and went on to produce healthy offspring. Genetic testing of the offspring confirmed conception occurred from inseminations performed on days 1-2 after the E2 peak. These data show that dual inseminations resulted in higher pregnancy rates than single inseminating cycles ($p<0.05$). Further, age had a significant impact on conception rates even though all animals were considered reproductively fecund. Those who conceived were on average younger than females who did not conceive (11.3 ± SEM 1.28 vs. 14.2 ± SEM 0.39 years, respectively; $p<0.05$). Overall, non-surgical cervical insemination resulted in a 25% pregnancy rate when using dual inseminations during the periovulatory window. Despite the lower pregnancy rate, when the invasiveness of both cervical cannulation for IUI and percutaneous intrauterine insemination are considered, cervical insemination offers an excellent alternative. This ongoing project will continue to test optimal insemination timepoints around E2 peak, methods to increase conception, and if utilizing cryopreserved sperm yields a similar outcome.