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Zoo clinical challenge - gorilla infertility

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Abstract

Reproductive monitoring and intervention are challenging in nondomestic species where manual restraint is often not possible or potentially dangerous for animals and the clinician. As a result, performing even simple procedures (e.g. acquiring blood or vaginal smears or using ultrasonography) on such species may not be possible without full anesthesia, as is the case for gorilla. Therefore, whenever possible, noninvasive techniques are preferred as tools for reproductive monitoring. Yet, for a large proportion of nondomestic species, many basic parameters (e.g. cycle length, duration of follicular and luteal phases, or ovulatory mechanism) are not known, so diagnosis and treatment of reproductive abnormalities are even more challenging. Similarly in males, many needed diagnostic procedures require either noninvasive techniques and/or anesthesia. An example of the clinical approach and challenges to breeding problems in gorillas in zoos is presented.

Keywords: Endometrial biopsy, gorilla, great apes, infertility, semen, vaginoscopy, zoo

Introduction

All gorilla species and subspecies (*Gorilla* spp) are either endangered or critically endangered (International Union for Conservation of Nature [IUCN] primate specialist group). Of the 2 species with 2 subspecies each (total of 4 subspecies), the only subspecies managed in North American zoos are Western Lowland gorillas (*G. gorilla gorilla*). As of 2019 there were ~ 354 individuals in 48 facilities accredited by the Association of Zoos and Aquariums (AZA).[1] These animals are managed collaboratively with oversight by the Gorilla Species Survival Plan (SSP), led by a group of individuals with expertise in gorillas that works to minimize inbreeding and maximize genetic diversity. The Gorilla SSP makes recommendations for breeding specific animals, taking into account their relatedness to the rest of the population, location, individual health, personality, genetic value, and available space in zoos.[1]

In the wild, Western Lowland gorillas live in tight-knit family groups averaging 10 individuals and consisting of an adult breeding male (silverback), a group of females, and their offspring.[2] Occasionally, the groups have multiple males. Reproduction is relatively slow, with inter-birth intervals of 4 - 6 years, low birth rates (< 0.2 offspring per female per year) and high juvenile mortality rates (up to 65% by 3 years of age).[2] Reproduction in zoos is usually better than in the wild, in part due to better nutrition and markedly lower infant mortality rates; however, inter-birth intervals are similar, ~ 4 years.[3]

Despite reproduction rates in zoos being slightly higher than in the wild, zoo clinicians are confronted with unique challenges when working on reproductive issues with this species. Some of these challenges are technical in nature, whereas others are secondary to a lack of baseline data. This report describes the clinical evaluation of a pair recommended to breed and the findings that may have contributed to the female's subfertility/infertility.

Case history

This case describes an adult female Western Lowland gorilla (27 years) at the Denver Zoo recommended to breed with a proven adult male (26 years) also housed at the Denver Zoo. Timeline of events is provided (Figure 1). The case female was considered to be overweight (body condition score 7/9), during both anesthetic events detailed here.

The female in question had given birth to 1 live offspring (at a previous institution), and was on oral contraception [ethynodiol diacetate (1 mg) and ethinyl estradiol (50 µg) Zovia 1/50E (NURK™), Mayne Pharma US, Raleigh, NC]. Treatment was discontinued when breeding between her and the male was attempted.

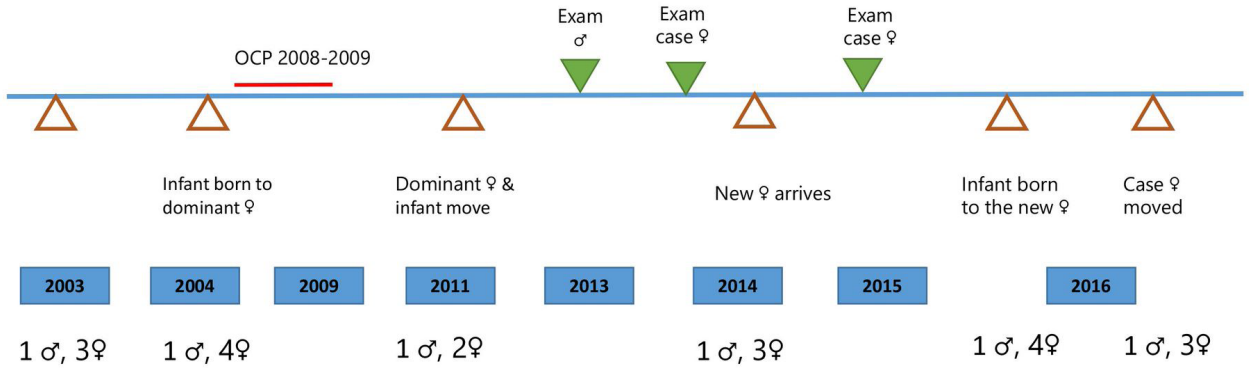

Figure 1. Timeline of the social changes in the gorilla group. OCP – oral contraceptive pill

Behavioral management

First attempts to encourage reproduction focused on husbandry and behavioral management as these techniques are the most natural, noninvasive and usually very low cost. Mating was occasionally observed and regularly suspected by animal care staff during the initial period of introductions, but did not result in pregnancy. Over the following several years, the male became increasingly aggressive to this female; although this was thought largely due to redirected aggression as the dominant female did not like the male to breed with other females, interfered with breeding attempts and would start fights over food that caused the male to aggress on this female. These led to occasional (1 or 2 per year) mild to moderate injuries to the case female and ultimately led to the social group being restructured (Figure 1).

Sometimes when pairs are kept together for extended intervals sexual activity decreases; therefore, a technique that can be employed to increase interest is to separate the male from the female and reintroduce them at estrus. However, accurately knowing when the female is receptive so that introductions can be appropriately timed is a key factor in the success of this approach. Some primates advertise the period around ovulation by developing large perineal (sexual) swellings.[4] Gorillas, however, do not exhibit obvious sexual skin swelling associated with fertile periods in their estrous cycle and visually determining the period of estrus is more challenging than in primates with perineal swellings.[5] With the goal of separating this female from the male and reintroducing them at the time of ovulation, steps were taken to pinpoint the day of ovulation in this female.

Monitoring for ovulation

In humans, over-the-counter urine dipstick tests for ovulation may be used to determine the time of ovulation. These tests qualitatively measure human luteinizing hormone (LH) concentrations in urine; human LH and LH in great apes is similar enough that these tests are effective,[6] and have been used to detect ovulation in gorillas.[7] However, this testing was considered cost-prohibitive as it would require testing the female at least every other day for a minimum of 2 cycles in order to obtain a baseline cycle and then a comparison cycle when introductions would be attempted based on predicted time of ovulation.

Ovulation generally occurs midway between menses; therefore, a second option to predict ovulation was to monitor menses. In gorillas, menses is subtle but can be confirmed with urine reagent strips (Multistix®, Siemens, Munich, Germany) to detect blood in the morning urine. This method is easy and economical. Urine was sampled every other day over the course of 16 months. With a few exceptions, menses occurred at relatively regular intervals from March 2013 until September 2013 (Cycles 1-5), and menstrual cycles became more irregular after that (September 2013 - October 2014; from Cycle 6 onwards) (Figure 2).

A third option for detecting ovulation was to monitor ovarian hormone metabolites in feces over the course of several ovarian cycles. This requires fecal sample collection, storage, shipping, and laboratory analysis, so there is a lag between endocrine events and when results are obtained. Although there were substantial costs associated with this monitoring method, fecal hormone analysis was performed in parallel to the urine Multistix® reagent strips (to monitor blood in the urine as an indicator of menses) so that the accuracy of reagent strips as a proxy for fecal hormones could be assessed. Fecal samples were collected 3 times weekly and stored frozen (– 80 °C) until analysis. Fecal samples were lyophilized and pulverized; ~ 0.5 gram was first extracted with 5 ml of double deionized water, vortexed and centrifuged and 3 ml of supernatant was removed and frozen (– 20 °C). Furthermore, 500 µl of supernatant were vortexed with 5 ml of petroleum ether, and snap frozen in a

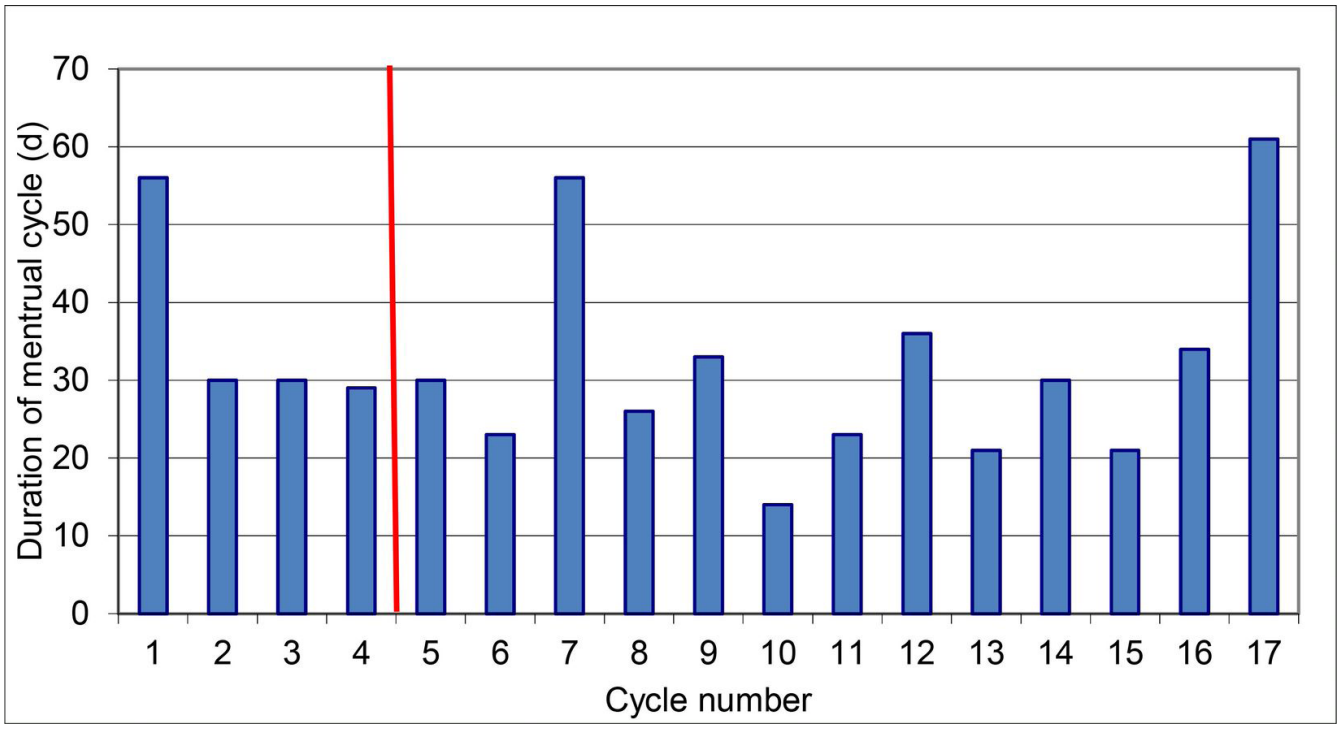

Figure 2. Female gorilla was monitored for menses between March 2013 and October 2014. Duration of menstrual cycles (from first day of menses). With 1 exception, menstrual cycles from March 2013 through September 2013 (Cycles # 1-5) were regular. Cycles became irregular September 2013 through October 2014 (Cycles # 6 - 17). The vertical line indicates the shift from regular to irregular cycles.

methanol dry ice bath. The organic phase was poured into a 16 x 150 mm tube containing 500 µl deionized H_2O. The second tube was then vortexed for 2 minutes, snap frozen in a dry ice methanol bath and the washed organic phase was poured into a 12 x 75 mm glass tube, which was placed into a heating block and the petroleum ether evaporated under a stream of nitrogen. A second extraction of the original 500 µl aliquot was performed using the same protocol to yield the final washed extract. The dried extract was reconstituted in 0.5 ml phosphate-buffered saline containing 0.1% gelatin and progesterone quantified by radioimmunoassay.

Although initially menses appeared very regular (Figure 2), menstrual intervals became irregular during the observation period. Fecal progesterone metabolites indicated the female was undergoing repeated luteinization and luteolysis (Figure 3). Dates with positive results from urine reagent strips frequently followed progesterone peaks as would be expected, and menses occurred at the end of the luteal phase. However, over the period monitored, progesterone did not increase to peak concentrations during several cycles and menses were not detected (e.g. February, April, and June 2014). Differential diagnoses for the irregular bleeding included breakthrough bleeding, occasional anovulatory cycles (obesity or premenopausal transition), inflammatory/infectious processes, and malignancy. Although the level of blood in urine did not occur in a predictable time

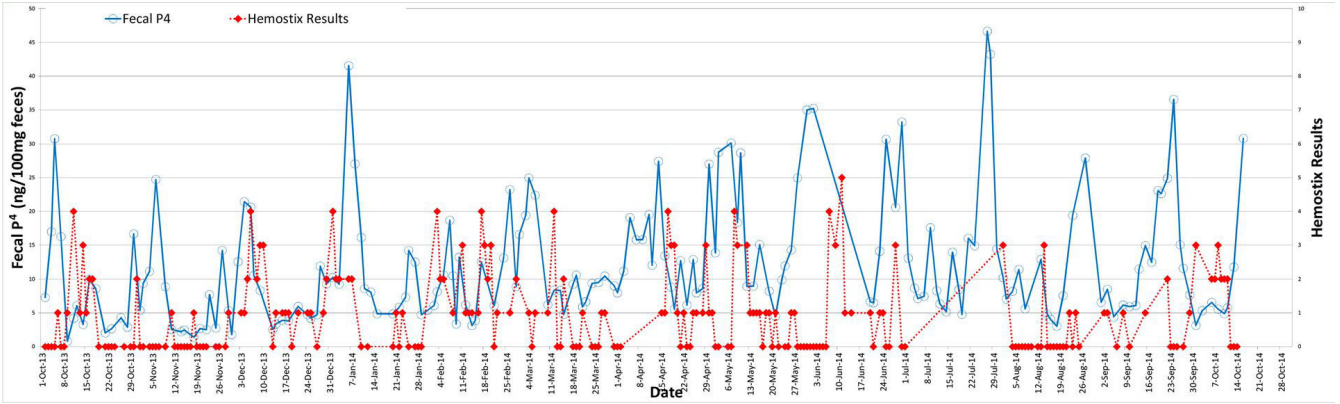

Figure 3. Fecal progesterone (P_4) metabolites (open circles/solid line) and hematuria score on urine reagent strips (solid diamonds/dashed line) in a female gorilla with failure to conceive

relative to the progesterone peaks, lack of blood in the urine (extended menstrual cycles) did correspond to extended interluteal phases as measured via fecal progesterone metabolites.

Male reproductive examination

Because further evaluation of the female would be more invasive, it was chosen to perform a full reproductive evaluation of the male. The male was examined under full anesthesia (induction: 220 mg tiletamine/zolazepam via intramuscular hand-injection (Telazol®, Zoetis, Kalamazoo, MI) and 7 mg medetomidine (Wildlife Pharmaceuticals, Windsor, CO); maintenance: isoflurane (1 - 2%) via endotracheal tube (VetOne, MWI, Boise, ID). During the physical examination, there were no notable abnormal findings, including in the external genitalia. Ultrasonography of the external genitalia was not performed due to time constraints of anesthesia. Testes were normal when palpated, testicular length and width were determined using calipers, and testicular volume was calculated (total combined volume = 27.5 cc). The prostate felt soft and enlarged (70 x 40 mm) compared to a previous report (15 x 7 mm).[8,9] After the urinary bladder was manually expressed to the extent possible, semen was collected via electroejaculation using a rectal probe (31 mm diameter) and giving stimuli at low voltage (2 - 4 v, 40 -100 mA). Progressive sperm motility was assessed by examining semen at 37°C. Semen pH (7.4 - 8.3) was determined using pH strips (EM Science, Gibbstown, NJ) and volume determined using an adjustable Eppendorf pipettor (Brinkman Instruments, Westbury, NY). Sperm concentration was determined using a hemocytometer and an aliquot of raw semen fixed in 0.3% glutaraldehyde was used to determine the percentage of morphologically normal sperm, assessed under a phase contrast microscope at x1000.

Despite efforts to evacuate the bladder, the ejaculate was contaminated with urine and no motile sperm were observed. Total sperm (10×10^6 sperm) were within the range considered fertile.[9] Observed morphological abnormalities were consistent with urine contamination and precluded assessment of percent normal morphology. However, most sperm heads were morphologically normal and the ejaculate was considered fertile. However, serum testosterone concentration (959 pg/ml) was below published concentrations for fertile male gorillas (4,137 pg/ml ± 2,191),[9] and may have reduced libido.

Female reproductive examination

Because noninvasive diagnostics and the examination of the male did not yield an explanation for the failure to conceive, a comprehensive reproductive examination on the female was performed under full anesthesia. The examination included external examination, vaginoscopy, vaginal swab culture, ultrasonography, and transcervical uterine biopsy. Additionally, a blood sample was collected to determine serum concentrations of the following hormones: estradiol, progesterone, antiMüllerian hormone [AMH], follicle stimulating hormone [FSH], and luteinizing hormone [LH]).

Anesthesia was induced via intramuscular hand injection with 115 mg tiletamine/intramuscular zolazepam (Telazol®, Zoetis) and 3.34 mg medetomidine (Wildlife Pharmaceuticals). Anesthesia was maintained with isoflurane (1.5 - 2%) via endotracheal tube. General physical examination findings were normal except that the animal continued to be over-conditioned. Vaginoscopy revealed a moderate amount of foreign material (straw, sticks/woody material, and hair). After removing the organic material, superficial ulcerations on the cervical mucosa were visible, although the remaining mucosa was normal (pink and without abrasions). No sample for culture was taken due to the contamination.

Vaginal cytology sample was of moderate cellularity, with epithelial cells as the predominant cell type. Epithelial cells were characterized by distinct, round, basophilic nuclei, large cytoplasm, angular margins, and were fairly uniform in appearance. There were few to moderate numbers of red blood cells, rare leukocytes, and large amount of mixed population bacteria. The vaginal vault was cleaned and rinsed with a dilute betadine solution.

Ultrasonography was performed with a transvaginal transducer (4 - 9 MHz; Fazone CB, FujiFilm, Valhalla, NY). The uterus was in axial position, had a normal appearing myometrium, but irregular endometrial lining with a possible soft tissue mass arising from the dorsal wall of the uterus. The right ovary appeared normal with a structure consistent with an ovarian follicle (nearing ovulation). However, the left ovary had a complex cyst with solid and cystic components (Figure 4), the solid component protruded into the cyst (possibly pedunculated). Based on the female gorilla's last date of menses, she was expected to be in the late follicular phase at the time of the examination, consistent with the large follicle on the right ovary. Uterus and ovary sizes were similar to published dimensions for gorillas.[10] Structures on the left ovary were considered potentially malignant.

After the external cervical os was cleaned with betadine solution, a Pederson's speculum was placed to facilitate introduction of an endometrial suction curette (Pipelle® CooperSurgical, Trumbull, CT) through the cervix. This device was used to obtain multiple endometrial biopsies; histopathology revealed a marked, subacute to chronic lymphoplasmacytic and neutrophilic erosive endometritis. Based on culture and sensitivity results, a course of azithromycin (500 mg for 1 day and 250 mg for 5 days, Tagi Pharma, Inc., South Beloit, IL) was prescribed. A follow-up examination was scheduled to perform surgery to remove the left ovary.

At the follow-up examination (~ 17 months after the initial examination), vaginal evaluation revealed minimal foreign material and cytology was consistent with vaginitis (mixed bacterial population with rare yeast and suppurative inflammation). Transvaginal ultrasonography revealed a normal right

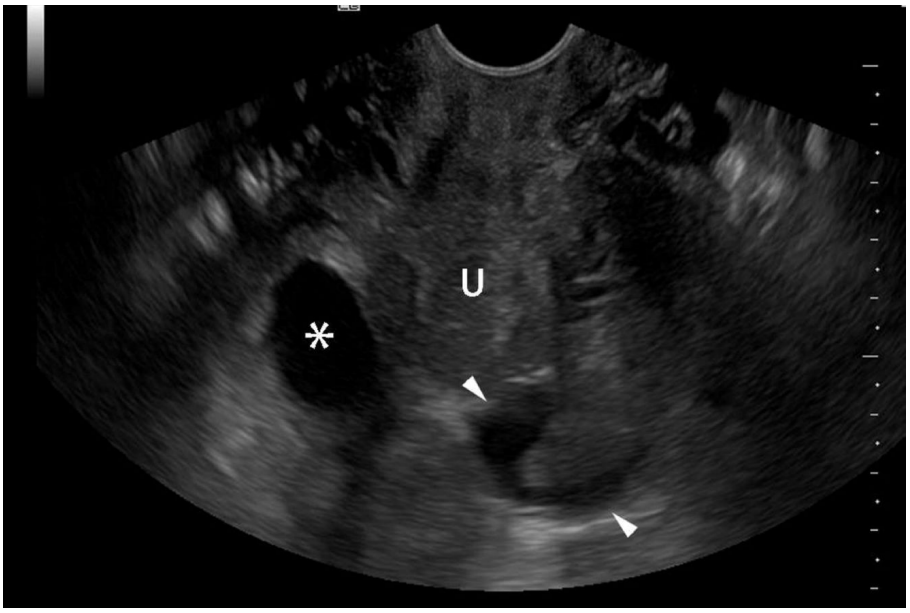

Figure 4. Transvaginal ultrasonogram from a gorilla with failure to conceive. U = uterus, * = ovarian follicle on the right ovary, arrowheads indicate a complex cyst in the left ovary

ovary with multiple small follicles and no visible luteal tissue, and a normal left ovary with no visible follicles. The abnormal structure on the left ovary could no longer be visualized and the surgery was cancelled, as the high risk of abdominal adhesions in gorillas outweighed the benefits of exploratory laparotomy. Additional transcervical suction endometrial biopsies were obtained and revealed minimal inflammation and no evidence of neoplasia.

Serum hormone concentrations ruled out perimenopausal transition and included: estradiol (45.7 pg/ml), progesterone (0.2 ng/ml), AMH (1.25 ng/ml), FSH (0.075 mlU/ml), and LH (< 0.1 mlU/ml). Estradiol and progesterone concentrations were compatible with a follicular phase. Serum AMH concentration in this female was similar to median concentrations in women 40 years old,12 and higher than for female gorillas 21- 35 years old (1.07 ng/ml, n = 8),13 and was interpreted as not compatible with ovarian senescence. During menopause in humans, concentrations of FSH rise substantially during transition into menopause; however, this change has not been studied in gorillas.[14] Similarly, FSH concentrations were not considered consistent with ovarian senescence in women.

Although the female remained without a specific diagnosis, she was transferred to a different facility to place her in a better social environment since that would be the first hurdle in solving breeding problems. She was well integrated in her new group, and matings were observed; however, no pregnancy has resulted from those matings.

Discussion

Although a final diagnosis was not reached, there were multiple factors potentially hindering reproduction. In women, obesity is associated with increased risk of anovulation (reviewed[15]). Obesity may have played a role initially; however, later the female was in better body condition. Although she was still relatively young, under human care, female gorillas tend to experience many more infertile cycles than in the wild and 'ovarian senescence' may occur at an earlier age. Endometrial biopsies revealed mild uterine pathology that may have negatively affected fertility, although at the follow-up this appeared to have resolved. In the end, the female was transferred to a different group with the goal of addressing mate incompatibility. However, that she has still not become pregnant in spite of favorable social dynamics indicates there may be additional factors contributing to infertility.

This case is an example of the challenges faced by zoo clinicians working with nondomestic species. General challenges for work with zoo animals include the inability to obtain blood samples without anesthesia, the lack of documented normal ranges for every species held in zoos, the need for nonstandard sizes of instruments and equipment, the need to plan involved procedures and then reassess due to new information. In gorillas in particular it also includes the fact that they are prone to abdominal adhesions and abscesses, changing the risk-to-benefit considerations of exploratory laparotomy.[11]

This case also illustrates solutions to some of these challenges such as animal care staff working to habituate animals to new situations and voluntarily administration of medication, the development of noninvasive monitoring methods, including low-tech methods as an alternative to more expensive methods. Lastly, it illustrates the collaboration needed to work up 1 animal: keepers, supervisors, veterinary staff, and national groups such as those advising on matches of genetic value (SSP).

Conflict of interest

There are no conflicts of interest to declare.

Acknowledgments

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