Supporting Online Material for

A Bruce Effect in Wild Geladas

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Other Supporting Online Material for this manuscript includes the following:
(available at www.sciencemag.org/cgi/content/full/science.1213600/DC1)

Data files 1 to 3 as Excel files: Data_Fig1, Data_Fig2, Data_Fig3
Materials and Methods

Hormone collection, extraction, and analysis

Fecal samples were collected within minutes after deposition from positively-identified individuals. Hormones were then extracted from feces using a method described previously (34). Specifically, the entire fecal sample was mixed thoroughly with a wooden spatula, and an aliquot of the mixed sample (~ 0.1 g wet feces) was placed in 3 ml of a MeOH/acetone solution (4:1). The solution was immediately homogenized for 1 min using a battery-powered vortexer (BioVortexer, BioSpec Products, Inc., Bartlesville, OK). Approximately 6-8 hours later, 2.5 ml of the fecal homogenate was filtered through a 0.2 μm polytetrafluoroethylene (PTFE) syringeless filter (Whatman, Florham Park, NJ), and the filter was subsequently washed with an additional 1 ml of MeOH/acetone (4:1). We then added 7 ml of distilled water to the filtered homogenate, capped and mixed the solution, and loaded it onto a reverse-phase C_{18} solid-phase extraction cartridge (Sep-Pak Plus, Waters Corporation, Milford, MA). Prior to loading, Sep-Pak cartridges were prepped according the manufacturer’s instructions (with 2 ml MeOH followed by 5 ml filtered water). After the sample was loaded, the cartridge was washed with 1 ml of a sodium azide solution (0.1%). All samples were stored on cartridges in separate sealed bags containing ~2 g of silica beads. Cartridges were stored at ambient temperatures for one week (to ensure adequate drying) after which all samples were stored at subzero temperatures (-10°C) until transported to the University of Michigan for analysis. In the laboratory, steroids were eluted from cartridges with 2.5 ml 100% MeOH and subsequently stored at -20°C until the time of assay.
All samples were assayed for 17β-estradiol (E2) using a radioimmunoassay (RIA) kit produced by MP Biomedicals. Because commercially-available antibodies have been validated only for a specific taxonomic group (i.e., humans for the antibody used here) and for a specific substrate (serum), adapting the antibody for use on gelada fecal hormones requires analytical validation (i.e., establishing parallelism, accuracy, sensitivity, and precision). Our validation demonstrated that the antibody was (1) parallel (a dose-response curve using serially-dilated samples was parallel to the standard curve), (2) accurate (accuracy tests where we spiked standards with a low sample found a mean recovery of 100.38% (N=6, y=1.36x-2.44, R²=0.99)), (3) sensitive (lowest detection for our samples was 0.125 pg/tube (or 5 pg/ml)), and (4) precise (inter- and intra-assay CVs for a low and high sample were 15.42% and 14.24% (N=34) and 8.74% and 14.73% (N=10), respectively).

Prior to RIA, all samples were incubated at room temperature for one hour. Then, an aliquot of each sample was evaporated to dryness under nitrogen. Sample aliquots were determined such that hormone metabolite values were within the range of optimal precision of the assay. Kit protocols were followed except that all reagents were halved from the amount suggested by the manufacturer (a common technique employed by researchers measuring fecal steroids to maximize the use of each kit). Internal controls were run in every assay and consisted of a high (binding at 20%) and a low (binding at 80%) “pool” (a composite of many fecal samples). All standards were run in triplicate, all controls and samples were run in duplicate, and mean concentrations are expressed as ng per dry gram of fecal material (ng/g). The MP Biomedicals E2 antibody is known to have
minor cross-reactivities with other estrogen metabolites (estrone: 20%; estriol: 1.5%;
estriol-17α: 0.7%).
Table S1.
Females with hormonally-determined pregnancies at the time of male replacement, pregnancy outcome, and female demographics

<table>
<thead>
<tr>
<th>Female ID</th>
<th>Pregnancy outcome</th>
<th>Trimester¹</th>
<th>Observed signs²</th>
<th>Group Size³</th>
<th>Rank⁴</th>
<th>Parity⁵</th>
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<td>primiparous</td>
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<tr>
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<tr>
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<tr>
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<tr>
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<td>multiparous</td>
</tr>
</tbody>
</table>

¹ Trimester the female was in when dominant male was replaced by a new male
² Any visual signs recorded by observers that indicate fetal loss
³ Number of adult (i.e., post-menarche) females in the group
⁴ Females in each group were split evenly into thirds and assigned high, mid, and low categories
⁵ We do not have known ages for these females, so parity is presented as an age estimate
References


