# A unique mechanism of successful fertilization in a domestic bird

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**Supplementary Information**

**Table S1. Free monosaccharide composition from water soluble extracts of the cloacal gland and testis.**

| Gland | Sugar content | | | | | |
|---|---|---|---|---|---|---|
| | Gal | Fru | Glc | Gal | Fru | Glc |
| | (μg/g) | | | (μg/gland) | | |
| Cloacal gland | 115 | 43 | 85 | 75 | 28 | 55 |
| Testis | 45 | 49 | 0* | 136 | 148 | 0* |

Free fructose (Fru) content in the extracts was determined by an HPLC system equipped with a pulsed amperometric detector, and free galactose (Gal) and glucose (Glc) were reduced and acetylated to alditol acetate form and were determined by GLC using inositol as an internal standard. *, Free glucose (Glc) in the testis was not detected by gas chromatography.

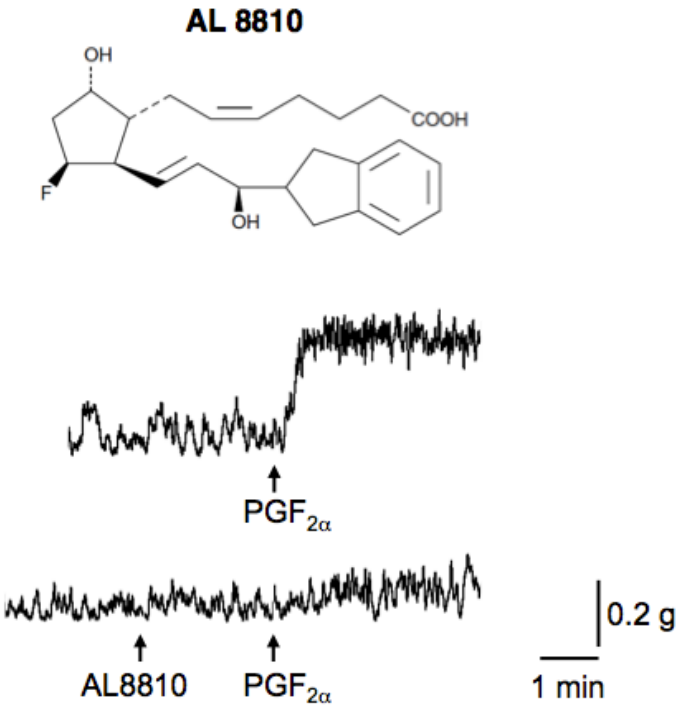


**Figure S1 | Effects of AL 8110 on the $PGF_2$ –induced spontaneous contractions of the isolated vagina.** The upward arrow indicated application of each chemical.

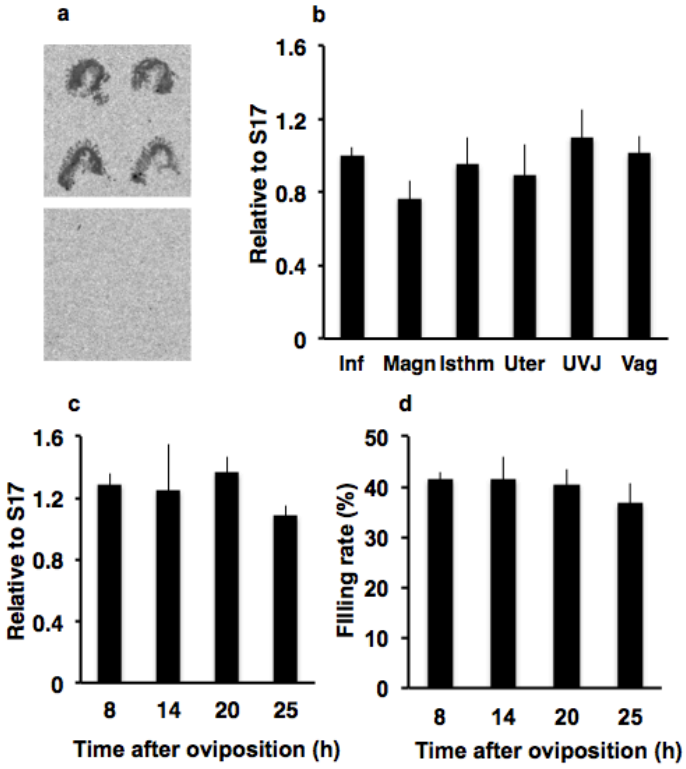


**Figure S2 | Expression of the receptor for $PGF_{2\alpha}$. a,** Autoradiograms of the UVJ sections after hybridization with $^{33}$P-labeled antisense probe specific for the receptor for $PGF_{2\alpha}$ (upper panel) or sense probe (lower panel) are shown. Representative results of two experiments are shown (n = 2). **b,** RT-PCR analysis of the receptor for $PGF_{2\alpha}$. The infundibulum (Inf), magnum (Magn), isthmus (Isthm), uterus (Uter), UVJ or vagina (Vag) isolated from females at 14 h after egg-laying were processed for RT-PCR analysis using gene-specific primers. Data were normalized with S17 ribosomal protein and expressed as the mean ± SEM of three independent experiments. **c,** RT-PCR analysis of the receptor for $PGF_{2\alpha}$ during ovulatory cycle. The UVJ isolated from females at 8, 14, 20 or 25 h after egg-laying were processed for RT-PCR analysis using gene-specific primers. Data were normalized with S17 ribosomal protein and expressed as the mean ± SEM of three independent experiments. **d,** Comparison of sperm filling rate during ovulatory cycle. Females were mated at 8, 14, 20 or 25 h after egg-laying. The utero-vaginal junction mucosa was isolated from the bird, and SST was observed under fluorescence microscope. Sperm filling rate was calculated and expressed as the mean ± SEM. 3–5 birds were used within each treatment.

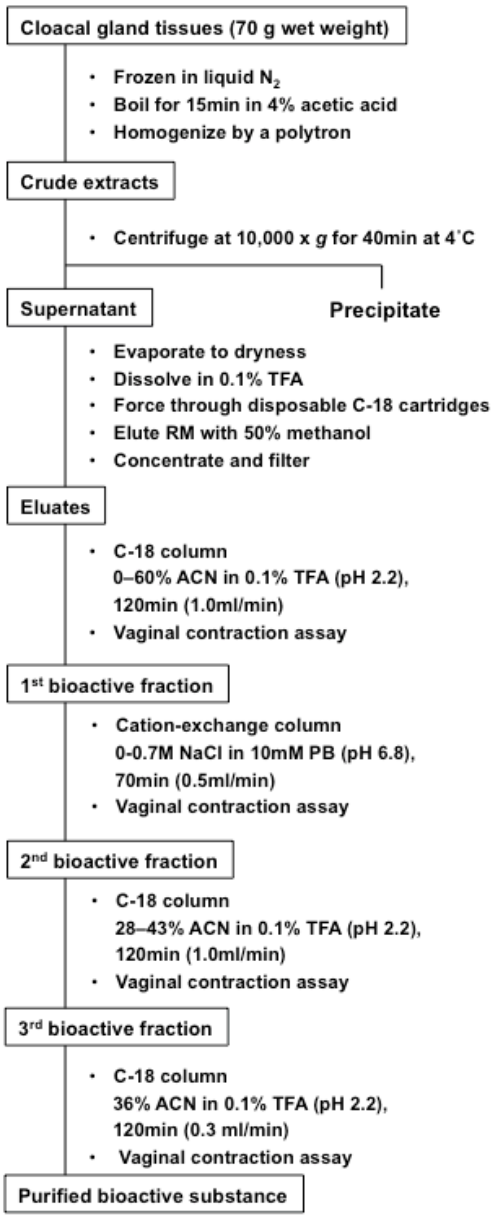


**Figure S3 | Purification scheme of bioactive substance from cloacal gland extracts.** TFA: trifluoroacetic acid , RM: retained materials, ACN: acetonitrile, PB: phosphate buffer.

**Caption to Movie S1**

**Appearance of cloacal gland secretion (CGS).**

The cloacal gland (CG) of male quail ejects CGS as a meringue-like foam by pushing lightly with the thumb and index finger.

**Data of structural analysis**

The MS, IR and 1H NMR spectra of the isolated bioactive substance were as follows: MS m/z 355 (M+1), 337 [(M-H2O)+1], 319 [(M-2H2O)+1] and 301 [(M-3H20)+1]; IR (Liq. film) υ 3400-3300 (OH) and 1708 cm-1 (COOH); 1H NMR δ 0.89 (3H, t, J=6.5 Hz, 20-H3), 4.02 (1H, m, 11-H), 4.22 (1H, q, J=6.2 Hz, 15-H), 4.25 (1H, dd, J=5.4 and 4.5 Hz, 9-H), 5.38 (1H, bq, J=7.5 Hz, 5-H), 5.53 (1H, bq, J=7.5 Hz, 6-H), 5.59 and 5.60 (2H, m, 13- and 14-H). The spectroscopic data of the isolated bioactive substance were found to be prostaglandin F2α (PGF2α) as compared with the spectra of authentic specimens, such as PGF2α, (15R)-PGF2α and (13Z, 15R)-PGF2α (Cayman Chemical).