Text S1: Hormone assay

Oestradiol ELISA

Oestradiol E_2 was assessed using the Demeditec Estradiol ELISA kit (DE2693; Demeditec Diagnostics GmbH, Germany), following the manufacturer's guidelines and analysing samples at a x2 dilution. Absorbances were quantified on an LT-4500 Microplate Absorbance Reader (LabTech, Version 7 2010, Tecan Group Ltd., Switzerland), and ELISA readings were analysed by four Parametric Logistic Curve (4PLC) nonlinear regression models on SoftMaxPro Software (Version 7.1, Molecular Devices, California, USA). The inter- and intra-assay coefficients of variation (CV) were 4.87% and 16.21%, respectively. The limit of quantification was 0.0212 ng/mL.

Testosterone ELISA

Testosterone (T) was measured by an 'in-house' developed ELISA as described by (Wilson et al., in press). Briefly, ELISA plates (96 well; Greiner Bio-One, GmbH, Germany) were coated overnight with 7.6 µg/mL donkey anti-rabbit serum IgG (Scottish Antibody Production Unit, UK) at 4 °C in 100 mM sodium bicarbonate buffer (100 µL). Plates were washed twice with wash buffer (300 µL; Tris Buffered Saline (TBS) with 0.05% Tween) and blocked (220 µL 0.5% Bovine Serum Albumin (BSA) Phosphate Buffered Saline (PBS), 1 hour, room temperature). Samples were tested at a x2 dilution in 0.1% BSA PBS. In samples which were too high at this dilution, additional x5, x10, x50 and x100 dilutions were tested. Standards (powdered T (Sigma-Aldrich, UK) diluted in 0.5% BSA PBS), quality controls (Lyphochek® Fertility Controls, Bio-Rad) and samples were added in duplicate (16 µL) with testosterone-Horse Radish Peroxidase (T-HRP, 84 µL of 1:20,000 diluted in androgen/progestogen assay buffer (0.1% BSA, 250 ng/mL cortisol PBS); #12-03, Astra Biotech, Germany). Testosterone antibody (T-Ab, 50 µL of 1/200,000 diluted in androgen/progestogen assay buffer; #R3S07-259, Meridian Life Science Inc., USA) was added, and the plates were incubated (2 hours, 28 °C, with shaking). Plates were washed four times, and 3,3',5,5'-tetramethylbenzidine substrate (TMB; 120 µL; Millipore, UK) was added (10 minutes, shaking, room temperature in the dark). The reaction was stopped (80 µL; 1N sulphuric acid), the absorbances quantified, and the readings analysed as described for E_2. This ELISA has inter- and intra-assay CVs of 16.5% and 4.3%, respectively. The limit of quantification was 0.3 ng/mL.

Progesterone ELISA

Progesterone (P_4) was measured by an 'in-house' developed ELISA, following a protocol close to that of T with the following changes. Plates were coated with 5 µg/mL goat anti-mouse IgG (A008, Arbor Assays, USA). Samples were tested at a x2 dilution in 0.1% BSA PBS for analysis. Standards (0.25 to 16 ng/mL, powdered P_4 (Sigma-Aldrich, UK)), quality controls (Lyphochek® Fertility Controls, Bio-Rad) and samples were added in duplicate (20 µL) with progesterone-HRP (80 µL of 1:10,000 diluted in androgen/progestogen assay buffer; #12-02, Astra Biotech, Germany). P_4-Ab (50 µL of 24 ng/mL diluted in androgen/progestogen buffer; #10-04, Astra Biotech, Germany) was added, and the plates were incubated (2 hours, 28 °C, with shaking). Plate washing and analysis was carried out as described for T. This ELISA has inter- and intra-assay CVs of 10.0% and 4.7%, respectively. The limit of quantification was 0.2 ng/mL.

Table S1: Average Quality Control values for the ELISA of each hormone.

	QC1	QC2	QC3
Estradiol (pg/mL)	116.5	413.2	-
Expected Estradiol Range	67.1-177.0	217.0-571.0	-
Testosterone (ng/mL)	0.38	1.91	2.7
Progesterone (ng/mL)	2.46	5.92	13.07

Table S2: Metadata, hormone levels, and ultrasound image measurements for female skate in this study.

Skate ID	Date	Sex	TL (cm)	Oestradiol ng/ml	Testosterone ng/ml	Progesterone ng/ml	Imaged	Max number of follicles counted in an image	Max follicle area (cm2)	Egg present	yolk max area (cm2)
2972	18/08/2018	F	224	0.25264	3.12	0	N	-	-	-	-
2935	21/08/2018	F	226	0.17665	4.12	0.29	Y	8	0.94	No	
3338	21/08/2018	F	221	0.24146	4.11	0.2	Y	9	2.2	No	
2092	22/08/2018	F	218	0.06319	1.58	0	N	-	-	-	-
2902	23/08/2018	F	218	0.06357	2.59	0.3	Y	7	0.82	No	
3576	02/04/2019	F	137	0	0	0	N	-	-	-	-
1467_1	02/04/2019	F	229	2.03683	25.91	1.83	Y	5	1.29	No	
1486	04/04/2019	F	211	1.37707	19.58	0.57	N	-	-	-	-
3330	04/04/2019	F	183	0.04043	0.43	0.33	N	-	-	-	-
1524	06/08/2019	F	152	0.04992	0.36	0	N	-	-	-	-
2877	06/08/2019	F	155	0	1.49	0	N	-	-	-	-
2910	06/08/2019	F	124	0.02266	1.17	0	N	-	-	-	-
3977	06/08/2019	F	208	0.26702	4.3	0	N	-	-	-	-
2316	07/08/2019	F	203	0.37958	0.95	0	Y	11	3.24	No	
2433	07/08/2019	F	155	0.05537	0	0	N	-	-	-	-
2920	07/08/2019	F	140	0.03388	0	0	N	-	-	-	-
2980	08/08/2019	F	216	3.34036	2.44	0	Y	6	4.42	No	
1473	13/08/2019	F	212	0.92744	1.02	0.28	Y	10	2.8	No	
3367	13/08/2019	F	220	1.54334	1.06	0	Y	8	3.54	No	
1363_1	13/08/2019	F	152	0.02296	0	0	N	-	-	-	-
1491	14/08/2019	F	118	0.02926	0	0	N	-	-	-	-
1334	18/11/2019	F	217	3.81202	4.96	0	Y	5	1.08	No	
1513	04/03/2020	F	222	1.30618	19.75	0	Y	5	4.46	Yes	8.12
3185	06/03/2020	F	213	2.54538	25.2	0	Y	-	-	Yes	9.8
3370	06/03/2020	F	132	0.03729	0	0	N	-	-	-	-
1467_2	06/03/2020	F	229	1.96573	20.99	0.39	N	-	-	-	-
4522_1	06/03/2020	F	179	0.05884	0	0	Y	6	0.98	No	-
2215	19/03/2020	F	172	0.02455	1.04	0	Y	9	1.45	No	-
9256	19/03/2020	F	213	0.86852	78.42	0.53	Y	5	1.43	No	-
9293	20/03/2020	F	130	0.02227	2.75	0	N	-	-	-	-
9492	20/03/2020	F	112	0	0	0	N	-	-	-	-
9172	29/06/2021	F	150	0.02632	0.45	0	N	-	-	-	-
3033	01/07/2021	F	211	0.15344	6.25	0	Y	7	1.79	No	
9205	01/07/2021	F	180	0.07915	0	0	N	-	-	-	-
1363_2	02/07/2021	F	170	0.02986	1.26	0	N	-	-	-	-
4522_2	02/07/2021	F	185	0.07625	1.76	0	N	-	-	-	-
22090	14/09/2021	F	212	-	-	-	Y	6	5.36	No	
21580	15/09/2021	F	210	-	-	-	Y	10	5.3	Yes	6.99
22150	15/09/2021	F	220	-	-	-	Y	1	1.83	No	
22160	15/09/2021	F	196	-	-	-	Y	19	3.48	No	
31780	16/09/2021	F	200	-	-	-	Y	17	3.78	No	
3329	05/10/2021	F	214	-	-	-	Y	-	-	Yes	10.65

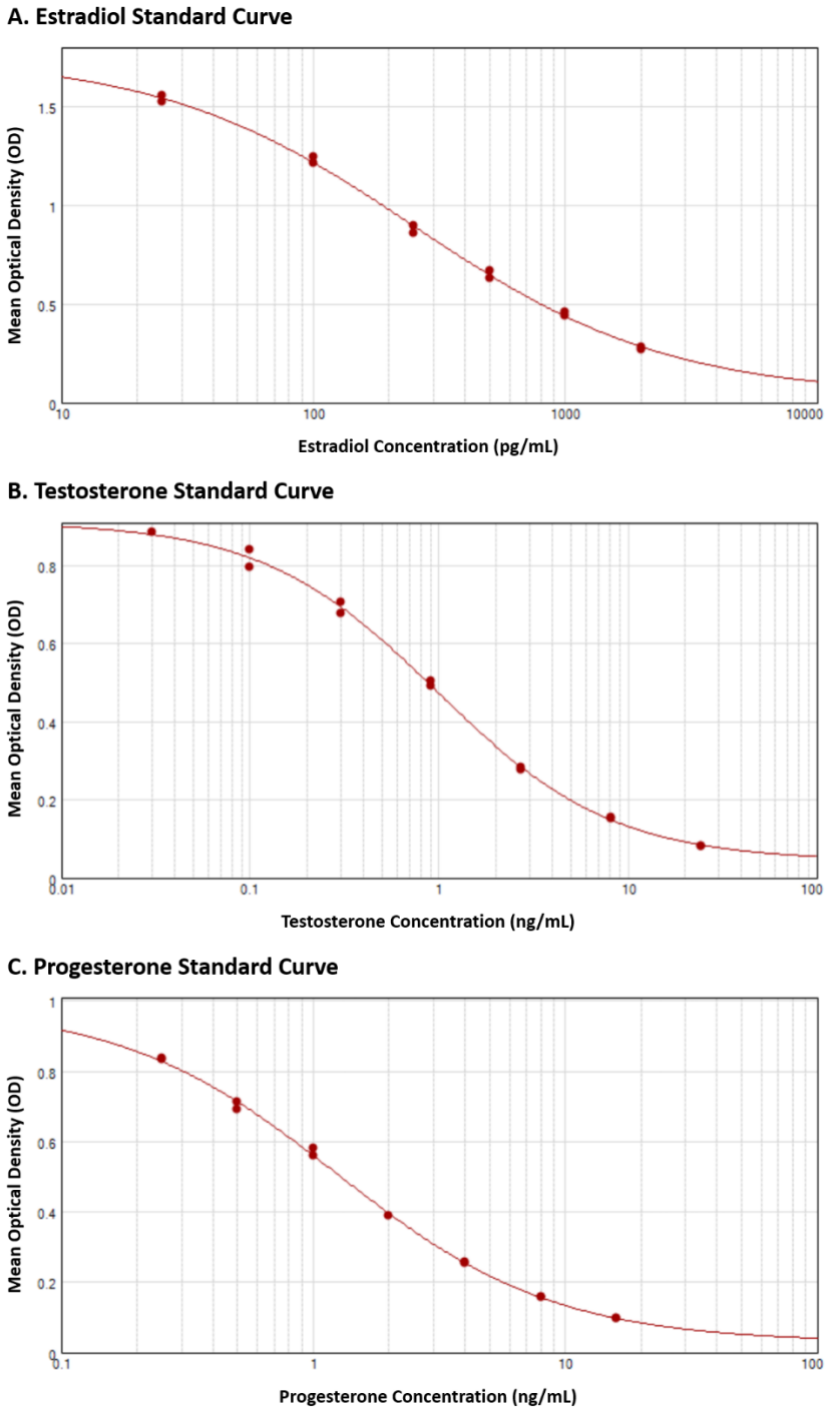

Figure S1: ELISA standard curves for each hormone.

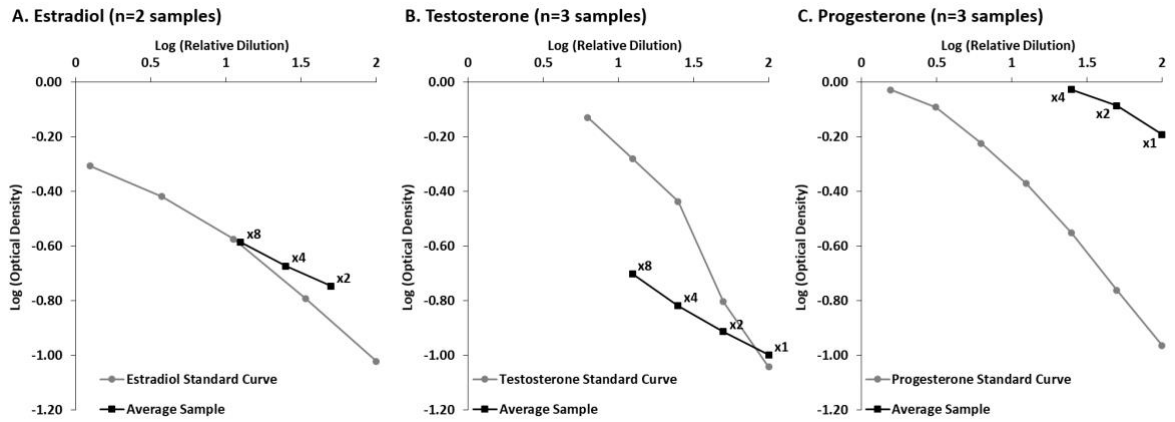

Figure S2: Parallelism results. The grey line on each is the standard. The black line is the average of the samples tested on each dilution. The number of samples is indicated for each graph (n=X). Oestradiol was only tested using 2 samples due to the availability of samples during testing. Oestradiol was tested at x2, x4, and x8 dilutions; Testosterone was tested at x1, x2, x4, and x8 dilutions; Progesterone was tested at x1, x2, x4, x8, and x16; however, the x8 and x16 results are not included as these did not result in a parallel line likely due to progesterone concentrations were generally quite low in all of the samples, and it is unlikely this high dilution will be required.