Supplemental Table S1: Extraction methods as mentioned in the reviewed scientific literature (1963-2020) regarding steroid hormones and chondrichthyan reproduction.

Data includes tissues (S: semen; P: plasma; Se: serum; G: gonad; Y: yolk; M: muscle; H: histotroph); reference; and analysed hormones (E2: 17β-oestradiol; T: testosterone; P4: progesterone; DHT: 5α-dihydrotestosterone).

Tissue	Reference	Extraction method
P, M	Barnett *et al*., 2008	Ether extractions were conducted by adding diethyl ether (2 ml) to each borosilicate vial and vortexing for 4 min on a multi-tube vortexer. The ether and aqueous phases were allowed to separate for 3 min, and the aqueous phase was then fast-frozen in a methanol-dry ice bath for 2 min before decanting the ether phase into a new 12 X 75-mm borosilicate vial.
P	Awruch *et al*., 2014	Plasma samples (200 µl) were extracted twice with ethyl acetate (1 ml), and 100 µl aliquots were transferred to assay tubes and evaporated before addition of an assay buffer.
P	Sueiro *et al*., 2019	Plasma samples (200 µl) were extracted twice with ethyl acetate (1 ml).
P	Tosti *et al*., 2006	Plasma samples from females, were extracted with ether.
P	Tsang and Callard *et al*., 1987	Each sample was extracted twice with 10 vol of diethyl ether from a freshly opened can (Doe and Ingalls, anesthesia grade) and centrifuged at 500g for 10 min.
P	Bubley *et al*., 2013	Extracted from plasma of mature individuals following protocols described in Tsang and Callard (1987) and Sulikowski et al. (2005).
M, G, P	Prohaska *et al*., 2013a	Samples were extracted with 10 volumes (5 mL) of 2:1 chloroform: methanol (ACS grade; histology grade), and vortexed for 1 min before snap-freezing in a dry ice acetone bath. The liquid phase was removed by piercing the frozen homogenate layer with a pasture pipette and transfer- ring it to a new test tube. The liquid phase was then evaporated at 37◦C in a heat block under a stream of nitrogen. One mL of 70% methanol (histology grade) was added to the dry extract, and the samples were stored at –20◦C for 24 h.
P, M	Prohaska *et al*., 2013b	Briefly, each plasma sample was extracted twice with 10 volumes (5 ml) of ethyl ether (ACS grade), and the organic phase was evaporated at 37 °C under a stream of nitrogen. Extracts were reconstituted in phosphate-buffered saline (PBS) containing 0.1% gelatin.
P	Prohaska *et al*., 2018	Briefly, each plasma sample was extracted twice with 10 volumes (5 ml) of ethyl ether (ACS grade) and the organic phase was evaporated at 37 °C under a stream of nitrogen. Extracts were reconstituted in phosphate buffered saline containing 0.1% gelatin (PBSG). Prior to extraction, each sample was spiked with 1,000 counts min−1 of tritiated P4, T, or E2 (Perkin Elmer, Waltham, MA, USA) to account for procedural loss.
P	Nau *et al*., 2018	Aliquots (0.2 to 1.0 ml) of each plasma sample were extracted twice with solvent (estradiol: diethyl ether; testosterone: benzene–hexane; progesterone: petroleum ether) at an extraction ratio of 1:5 (plasma/solvent, v/v) and then reconstituted in 0.5 to 1.5 ml of PBS-Gel (0.1% v/v, pH 7.0). Aliquots of 0.1 to 0.2 ml of the reconstituted plasma extracts were then used in the three assays.
P	Heupel *et al*., 1999	Not mentioned
P	Sulikowski *et al*., 2012	Plasma sample was extracted twice with 10 volumes of diethyl ether, which was evaporated under a stream of nitrogen before the dried extracts were reconstituted in phosphate-buffered saline with 0.1% gelatin.
Se	Henningsen *et al*., 2008	Blood was allowed to coagulate at 21–24°C for 30 min. It was then spun at 1,2869g for 3–5 min and the resultant serum stored at -80°C until analysed. Radioimmunoassays (RIA) for 17b-estradiol (E2), progesterone (P4), testosterone (T), and 5a-dihydro-testosterone (DHT) were conducted as described in Rasmussen and Gruber (1990), Rasmussen and Murru (1992), Manire et al. (1995), and Manire and Rasmussen (1997).
Se	Rasmussen and Muru, 1992	Were isolated from each serum sample and assayed. techniques were modified from Resko et al. (1975, 1980) and are described in detail in Rasmussen and Gruber (1990)
Se	Rasmussen and Muru, 1992	Were isolated from each serum sample and assayed. techniques were modified from Resko et al. (1975, 1980) and are described in detail in Rasmussen and Gruber (1990)

P	Sulikowski *et al*., 2016	A 500 µl aliquot of each plasma sample was extracted twice for each hormone with 10 volumes of ethyl ether (ACS grade), and the liquid phase was evaporated at 37°C in a heat block under a stream of nitrogen.
Se	Rasmussen and Muru, 1992	Purification procedures and radioimmunoassay techniques were modified from Resko et al. (1975,1980) and are described in detail in Rasmussen & Gruber (1990).
Se	Rasmussen and Gruber, 1993	Purification procedures and radioimmunoassay techniques were modified from Resko et al. (1975,1980) and are described in detail in Rasmussen & Gruber (1990).
P	Waltrick *et al*., 2014	Plasma aliquots of 100 ml were extracted with ethyl acetate (1 ml) and 100 ml aliquots were then analysed
M, G, P	Prohaska *et al*., 2013a	Samples were extracted with 10 volumes (5 mL) of 2:1 chloroform: methanol (ACS grade; histology grade), and vortexed for 1 min before snap-freezing in a dry ice acetone bath.
P, M	Prohaska *et al*., 2013b	each plasma sample was extracted twice with 10 volumes (5 ml) of ethyl ether (ACS grade), and the organic phase was evaporated at 37°C under a stream of nitrogen. Extracts were reconstituted in phosphate-buffered saline (PBS) containing 0.1% gelatin.
P	Prohaska *et al*., 2018	Briefly, each plasma sample was extracted twice with 10 volumes (5 ml) of ethyl ether (ACS grade) and the organic phase was evaporated at 37°C under a stream of nitrogen. Extracts were reconstituted in phosphate buffered saline containing 0.1% gelatin (PBSG). Prior to extraction, each sample was spiked with 1,000 counts min−1 of tritiated P4, T, or E2 (Perkin Elmer, Waltham, MA, USA) to account for procedural loss.
P	Awruch *et al*., 2008a	Plasma samples (200 µl) were extracted twice with ethyl acetate (1 ml) and 100 µl aliquots were transferred to assay tubes for evaporation prior to addition of an assay buffer
P	Awruch *et al*., 2008b	Plasma samples (200mL) were extracted twice with ethyl acetate (1mL), and 100 µl aliquots were transferred to assay tubes for evaporation before the addition of an assay buffer.
P	Awruch *et al*., 2009	Plasma samples (200 µL) were extracted twice with ethyl acetate (1 mL) and 100-µL aliquots were transferred to assay tubes for evaporation before addition of an assay buffer.
P	Sumpter and Dodd, 1979	Extraction of 50~1 aliquots of plasma with 4 ml light petroleum (60-80" C): diethyl ether (4 : 1, *V N)*
P, G	Garnier *et al*., 1999	Estradiol-17β, estrone, and 17α- hydroxy,20β-dihydroprogesterone were extracted from plasma or gonads with dichloromethane, whereas 11-ketotestosterone was extracted with ethyl acetate/ cyclohexane (1/1, vol/vol). The other steroids (testosterone, Δ4-androstenedione, dihydrotestosterone, 5α- androstane 3α, 17β-diol, progesterone) were extracted with the same ethyl acetate/cyclohexane preparation as above and were separated on a celite column using an isoctane–benzene mixture as the eluant (Garnier, 1985).
Se	Manire *et al*., 1995	Serum aliquots of 500 ul were extracted by shaking for 5 min with 5 ml of freshly opened or redistilled diethyl ether, the organic phase was decanted after freezing the aqueous phase in an ethanol/dry ice bath and the ether dried under a stream of air.
Se	Manire *et al*., 1997	The procedure is fully described by Manire et al. (1995)
Se	Manire *et al*., 1999	Samples (50 µl) were extracted twice with 4 ml of diethyl ether prior to analysis.
Se	Gelsleichter *et al*., 2002	Serum aliquots of 500 µl were extracted with 5 ml of freshly opened diethyl ether. The organic phase was decanted after freezing the aqueous phase in an ethanol/dry ice bath and the ether dried under a stream of air.
Se, Y	Manire *et al*., 2004	Serum aliquots of 500 µl were extracted by shaking for 5 min with 5 ml of freshly opened or redistilled diethyl ether. The organic phase was decanted, after freezing the aqueous phase in an ethanol/dry ice bath, and the ether dried under a stream of air.
P	Manire *et al*., 2007	Two different volumes of serum (25 µl and 75 µl) were extracted with diethyl ether and then analysed by RIA directly (using the antiserum reported in Gruenewald et al., 1992).
P	Elisio *et al*., 2019	Briefly, steroids from the plasma samples (500–1000 µl) were extracted twice with diethyl ether in a 1:5 ratio. The mixtures were vortexed for 1 min and the allowed for the two phases (aqueous below and organic above) to separate perfectly. In each extraction, the aqueous phase was frozen at −80 °C and transferred the organic phase onto a 15 ml glass tube, which was placed in an immersion bath at 45 °C, under an extraction fume hood, for solvent evaporation
P	Guida *et al*., 2017	Plasma samples (200 µl) were extracted twice with ethyl acetate (1 ml), and two 100 µl aliquots were transferred to assay tubes. Samples were then evaporated by air and 100 µl of PBS assay buffer was added to reconstitute each extract.

P	Sulikowski *et al*., 2006	Each sample was extracted twice with 10 volumes of diethyl ether (anhydrous), which was evaporated under a stream of nitrogen before the dried extracts were reconstituted in phosphate-buffered saline with 0·1% gelatine.
P	Kneebone *et al*., 2007	Each sample was extracted twice with 10 volumes of diethyl ether (T and E) or petroleum ether (P4) before snap freezing in an acetone/dry ice bath.
P	Sulikowski *et al*., 2007	The full procedural details for plasma steroid extraction and radioimmunoassay are found in Tsang and Callard (1987) and Sulikowski et al. (2004).
P	Koob *et al*., 1986	Plasma aliquots of 0.5 ml were extracted twice with 10 vol of diethyl ether
P, M	Prohaska *et al*., 2013b	Briefly, each plasma sample was extracted twice with 10 volumes (5 ml) of ethyl ether (ACS grade), and the organic phase was evaporated at 37°C under a stream of nitrogen. Extracts were reconstituted in phosphate-buffered saline (PBS) containing 0.1% gelatin.
P	Williams et al., 2013	Each plasma sample was extracted twice using 10 volumes of diethyl ether. Following each extraction, the aqueous phase was snap frozen in a dry ice and acetone bath. The ether phases were decanted into the same test tube and evaporated under a stream of nitrogen. The dried extracts were then reconstituted in phosphate-buffered saline with 0.1 % gelatin (PBSG) and stored at -20 °C until assay.
P	Sulikowski *et al*., 2004	Each sample was extracted twice with 10 vol of diethyl ether (anesthesia grade) before snap freezing in an acetone/dry ice bath. The ether was then evaporated to dryness under a stream of nitrogen and reconstituted in phosphate-buffered saline with 0.1% gelatin (PBSG)
P	Sulikowski *et al*., 2005	We extracted each sample twice with 10 volumes of diethyl ether (anesthesia grade), which was evaporated under a stream of nitrogen before the dried extracts were reconstituted in phosphate- buffered saline with 0.1% gelatin (PBSG)
P	Sulikowski *et al*., 2007	Each skate plasma sample was extracted twice with 10 volumes of diethyl ether (anhydrous), which was evaporated under a stream of nitrogen before the dried extracts were reconstituted in phosphate-buffered saline with 0.1% gelatin (PBSG) and stored at -20 °C until assay.
P	Kneebone *et al*., 2007	Each sample was extracted twice with 10 volumes of diethyl ether (T and E) or petroleum ether (P4) before snap freezing in an acetone dry ice bath.
P	Rasmussen *et al*., 1999	Serum aliquots of 500 µl were extracted by shaking for 5 min with 5 ml of freshly opened or redistilled diethyl ether. After freezing the aqueous phase in an ethanol/ dry ice bath, the organic phase was decanted and brought to dryness under a stream of air.
P, G, H	Fasano *et al*., 1992	Aliquots of tissue extracts were pipetted into 16x 150-mm glass tubes and extracted twice (2 x 7 ml with diethyl ether (anesthesia grade). The aqueous layer was snap-frozen in an acetone/dry ice bath, and the organic layer was decanted into tubes maintained at 36°C under a stream of nitrogen. The evaporated extract was reconstituted in phosphate
P	Snelson *et al*., 1997	Serum aliquots (500 µl) were extracted with redistilled ether and the extract was sequentially chromatographed on two different Sephadex LH-20 chromatographic columns prior to analysis
P	Tricas *et al*., 2000	Serum aliquots of 500 µl were extracted by shaking for 5 min with 5 ml of freshly opened or redistilled diethyl ether. After freezing the aqueous phase in an ethanol/dry ice bath, the organic phase was decanted and brought to dryness under a stream of air.
P	Gelsleichter *et al*., 2006	Serum aliquots of 500 µl were extracted with 5 ml of freshly opened diethyl ether. The organic phase was decanted after freezing the aqueous phase in an ethanol/dry ice bath and the ether dried under a stream of air.
P	Manire *et al*., 2007	Two different volumes of serum (25 µl and 75 µl) were extracted with diethyl ether and then analysed by RIA directly (using the antiserum reported in Gruenewald et al., 1992).
P	Sheldon *et al*., 2018	Extractions were performed only for the androstenedione assay per kit instructions.
P	Mull *et al*., 2008	A subset of samples was extracted using a triple diethyl ether extraction method (Laidley and Thomas, 1997) and compared to non-extracted samples; no differences were observed in measured T concentrations between preparation methods therefore non-extracted samples were used for analysis.
P	Mull *et al*., 2010	Plasma analysed for estradiol was prepared using a triple diethyl ether extraction (Laidley and Thomas, 1997) to account for potential assay interferences.
P	Nozu *et al*., 2018	Plasma steroids were extracted three times using 2.5 mL diethyl ether. The extracts were evaporated, and the residue was

		reconstituted with 3 times the original volume of assay buffer (0.05 M borate buffer, pH 7.8, containing 0.5% bovine serum albumin).
Se	Nozu *et al*., 2015	Serum steroids were extracted three times with 2.5 mL diethyl ether. The extracts were evaporated, and the residue was reconstituted with assay buffer (0.05M borate buffer, pH 7.8, containing 0.5% BSA).
P	Wyffles et al., 2019	Pre-coated, pre-blocked, 10 μg/ml goat anti-rabbit IgG (#A009-25MG; Arbor Assays, Ann Arbor, MI) plates were maintained until use at 4°C in zip lock bags with desiccant.
P	Fujinami et al., 2020	Not mentioned; TECAN Infinite M200 plate reader
P	Hoffmayer *et al*., 2010	Steroids were isolated from the plasma by ethyl acetate extraction. The extracts were dried overnight or placed under a nitrogen blower until the ethyl acetate completely evaporated.
P	Marina *et al*., 2008	Plasma samples (100 l) were vortexed with ethyl ether (1:10, v/v) for 5 min and centrifuged at 3000g for 10 min. The upper phase (ethyl ether) was transferred to a glass tube. Two extractions were performed. The pooled ether phases were left to evaporate on a hot plate at 40–50 °C under a hood. The residue was dissolved in a 0.5 ml sodium phosphate buffer 0.05 M, pH 7.5, containing BSA at a concentration of 10 mg/ml.
P	Mylniczenko et al., 2019	Plasma samples were first pre-treated with a commercially available dissociation reagent to provide a measure of the total steroid hormone concentration.
P	Nozu *et al*., 2017	Briefly, plasma steroids were extracted three times using 2.5 mL diethyl ether. The extracts were evaporated, and the residue was reconstituted with 2 times its volume of assay buffer (0.05 M borate buffer, pH 7.8, containing 0.5% bovine serum albumin).
P	Matsumoto et al., 2019	After thawing, the plasma was mixed with diethyl ether, and steroids were extracted by using a common procedure (Suzuki et al., 1998). The extract was diluted 2.5-fold with 0.05 M Tris-HCl (pH 7.75) buffer solutions, so that it could be used for measurements.
S	Simpson *et al*., 1963	Not mentioned.
S	Simpson *et al*., 1964	Not mentioned.
S	Gottfried and Chieffi, 1967	A steroid extract (chloroform: methanol,1:1) was prepared from the semen (19g.) of 5 sexually mature animals.
P	Di Prisco *et al*., 1967	The plasma was extracted three times with two volumes of ethyl acetate.
P, H	Lyons and Wynne-Edwards, 2018	A 50 μL sample of maternal plasma or histotroph was transferred into a 0.5 mL microcentrifuge tube, followed by 50 μL of protein precipitation solution ($ZnSO_4 \cdot 7H_2O$ at 9 mg/mL, in methanol spiked with deuterated internal standards [IS]). After 20 min of cold incubation (4 ℃), the mixture was vortexed for 15 s, centrifuged at 14,000 rpm for 15 mins, and the 50% water/50% methanol supernatant (75 μL) was submitted for LC-MS analysis.