Characterization of the time course of 17 different steroids in serum from cows with elevated versus normal body condition score

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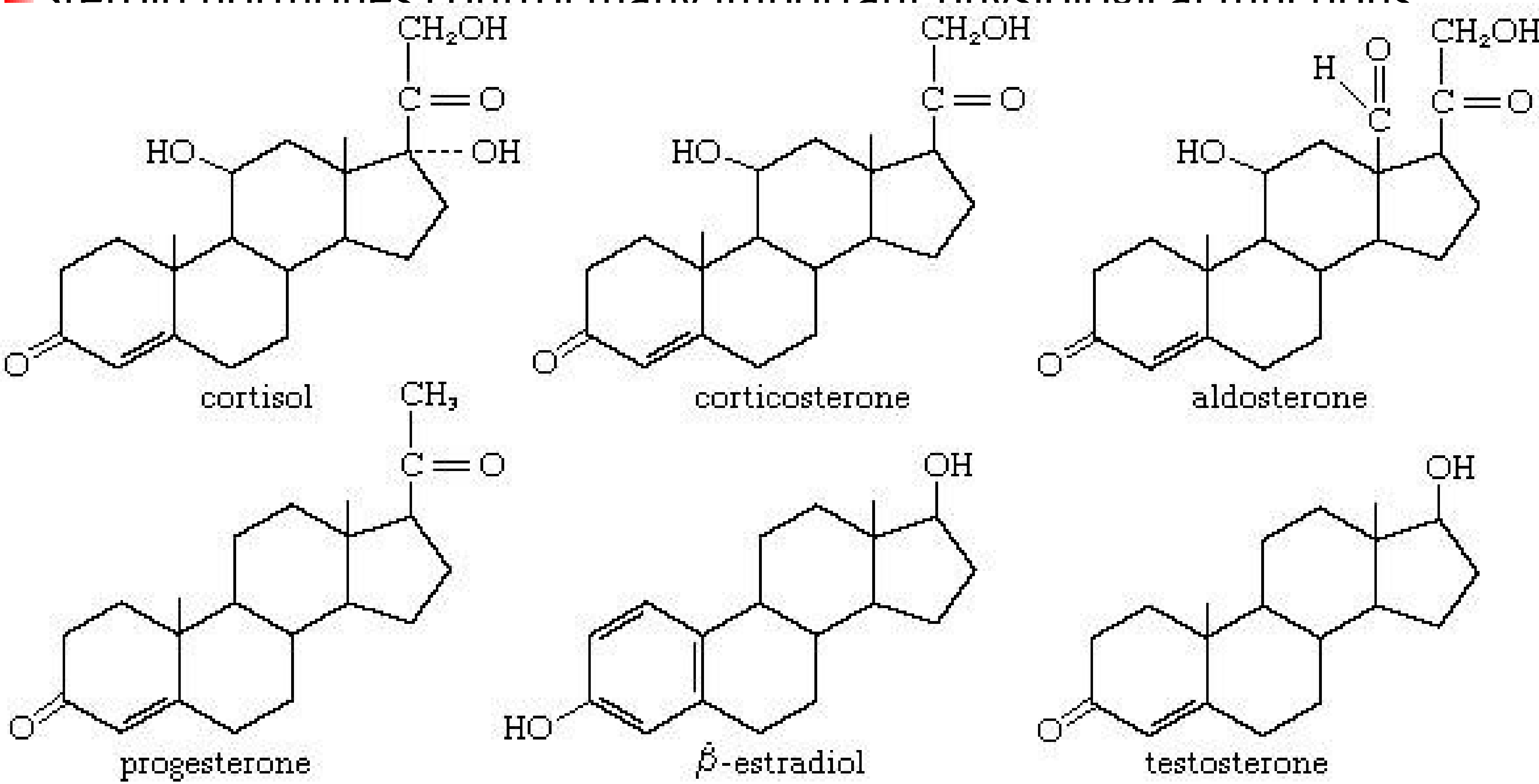
Background

- Steroid hormones control many important physiological functions



concentrations and thus interact with classical endocrine regulation, e.g., of metabolism or reproduction

Background

- With increasing lipid mobilization, steroid release from AT into the circulation likely changes as well:

"Body weight loss was associated with increased circulating concentrations of Progesterone in non-lactating, ovariectomized dairy cows; this was mainly attributed to fat mobilization and consequent release of Progesterone stored in adipose tissues"

(Rodrigues et al., 2011, Theriogenology 75:131-7.)

- The peripartal period is characterized by massive changes in the concentrations of steroid hormones from the adrenal gland, ovary and placenta, but also by mobilization of fat reserves

Objectives

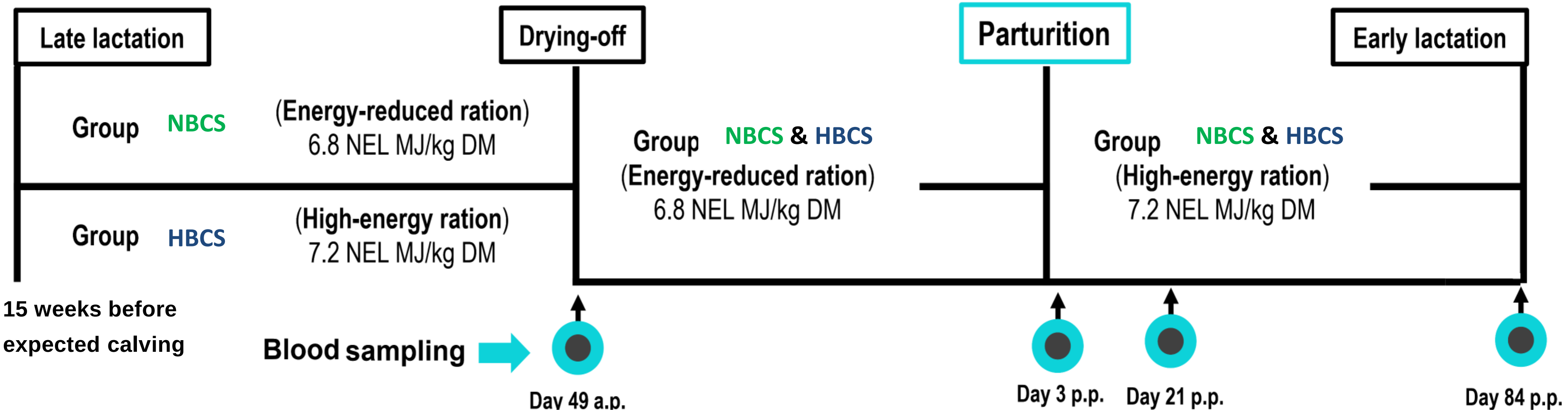
- Investigating the changes in circulating steroids (glucocorticoids, **mineralocorticoids**, gestagenes, estrogens and **androgens***) during the transiton from late pregnancy to lactation in dairy cows

- Comparing these changes between dairy cows of high and of normal body condition

- Analytical approach: Steroid-OMICs spectrometry for quantifying multiple steroid hormones in a single run with a higher accuracy than conventional assays

*Androgens are not well characterized in dairy cows, but studies in humans and laboratory animals point to hyperandrogenemia being related to obesity, diabetes (type 2), and to infertility

Animal model

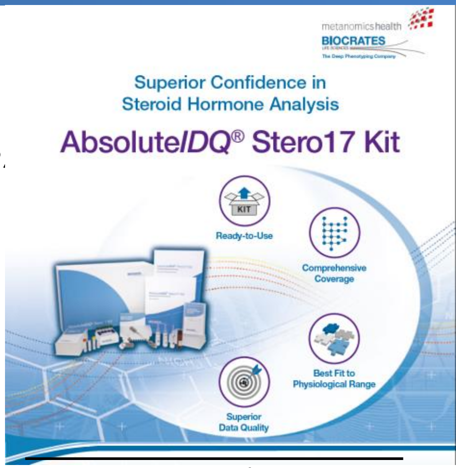


The targeted body condition score (BCS) and backfat thickness (BFT) at dry-off:

HBCS: BCS >3.75 and BFT >1.4 cm

NBCS: BCS <3.5 and < BFT1.2 cm

Steroid-OMICs



Quantitative profiling: up to 17 steroid hormones; 5 classes: Progestagens, Glucocorticoids, Mineralocorticoids, Androgens, and Estrogens

Separation of steroids: HPLC column for Absolute*IDQ*™ Stero17 Kit combined with the precolumn SecurityGuard Cartridge after solid phase extraction of serum samples.

Mass spectrometric analysis: QTRAP 5500 triple quadrupole system equipped with a 1260 Series HPLC and a HTC PAL auto sampler controlled by the software Analyst 1.6.2.

Data evaluation for quantification and quality assessment: MultiQuant and the Met*IDQ*™ software.

Calculation of steroid concentrations: based on internal standards and reported in nM or ng/mL.

- 11-Deoxycortisol
- 11-Deoxycorticosterone
- 17α-Hydroxyprogesterone
- Aldosterone
- Androstenedione
- Androsterone
- Corticosterone
- Cortisol
- Cortisone
- Dehydroepiandrosterone
- Dihydrotestosterone
- Estrone
- Estradiol
- Etiocholanolone
- Pregnanediol*
- Pregnenolone*
- Progesterone
- Testosterone

Statistics

Linear mixed model with repeated measurements (log10 transformed data)

Mixed models, fixed effects of
-treatment (group: HBCS or NBCS),
-time (weeks relative to calving),
-Interaction treatment x time,
-Random factor: individual "cow"
- Parity class and it´s interaction with time was also considered as a fixed effect, but when insignificant it was excluded from the model.

P < 0.05: significant
0.05 < P ≤ 0.10: trend

For all graphs, non-transformed data (means ± SEM) were used..

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Comparison of performance and metabolism from late pregnancy to early lactation in dairy cows with elevated *v.* normal body condition at dry-off

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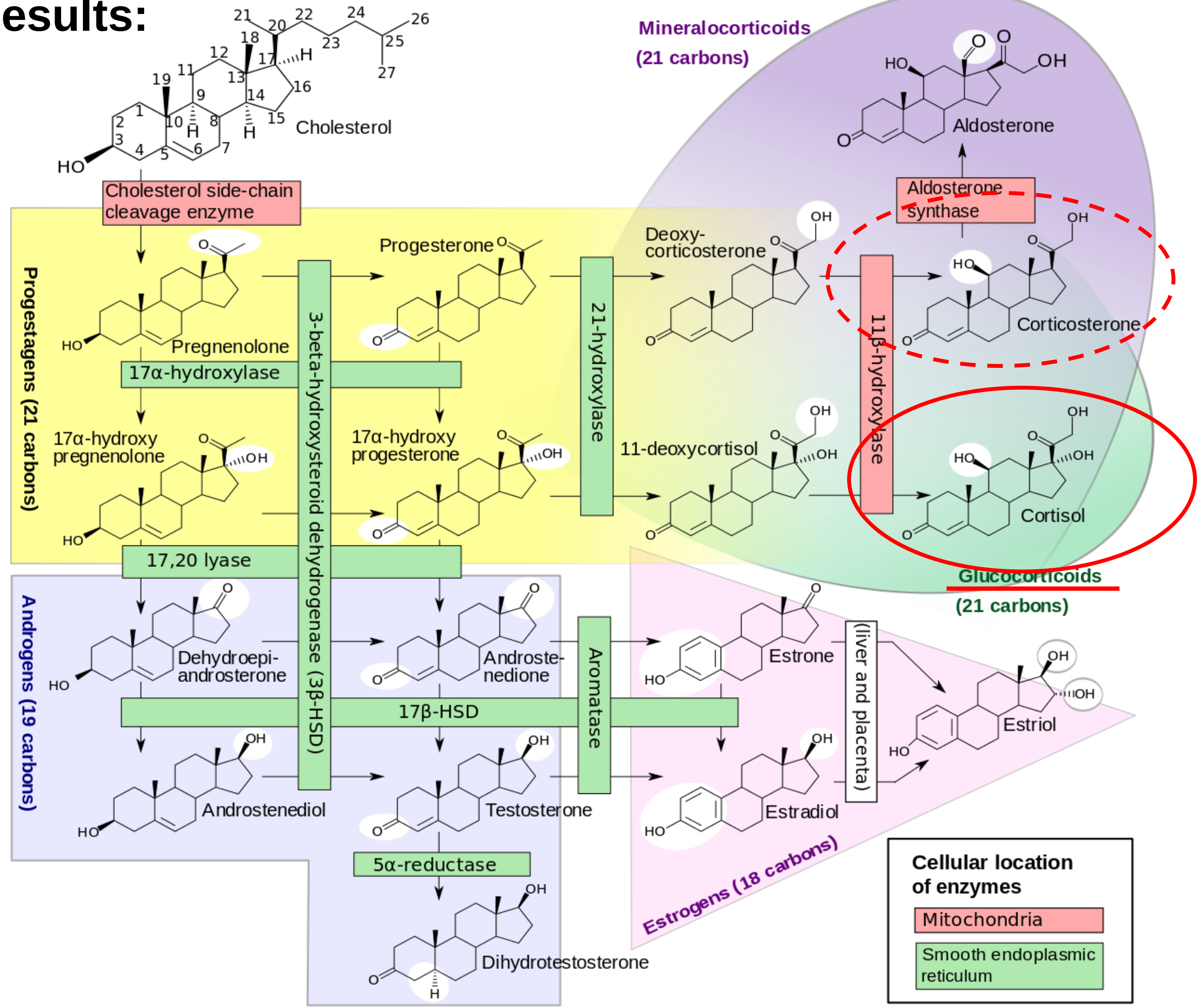
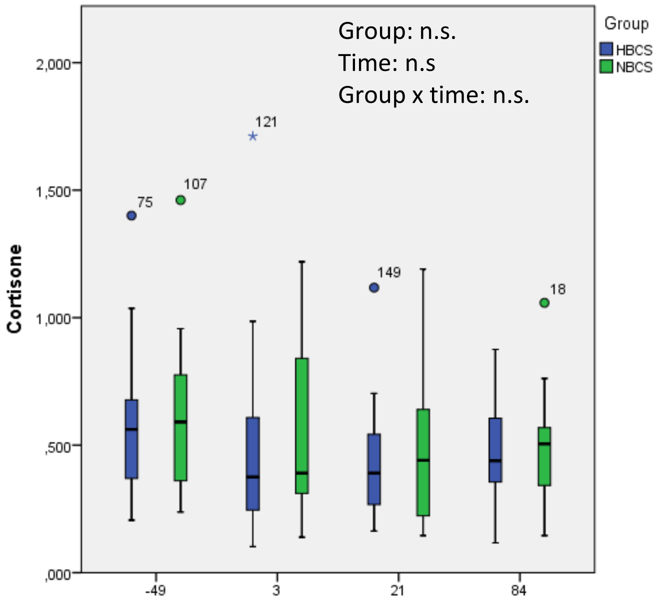
(Received 24 January 2018; Accepted 20 November 2018)

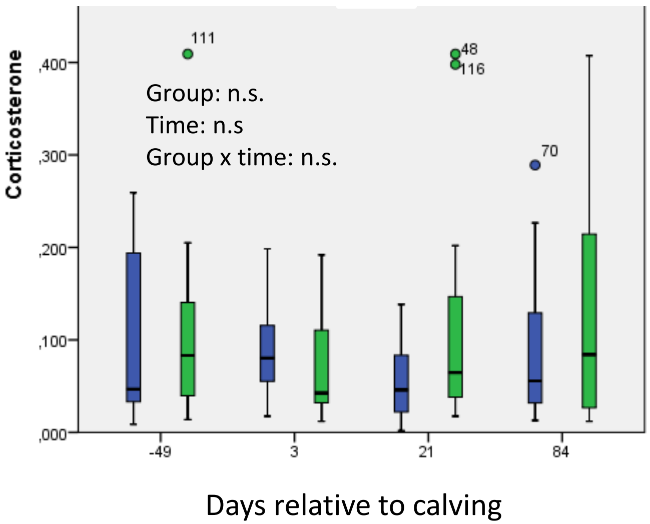
Results:

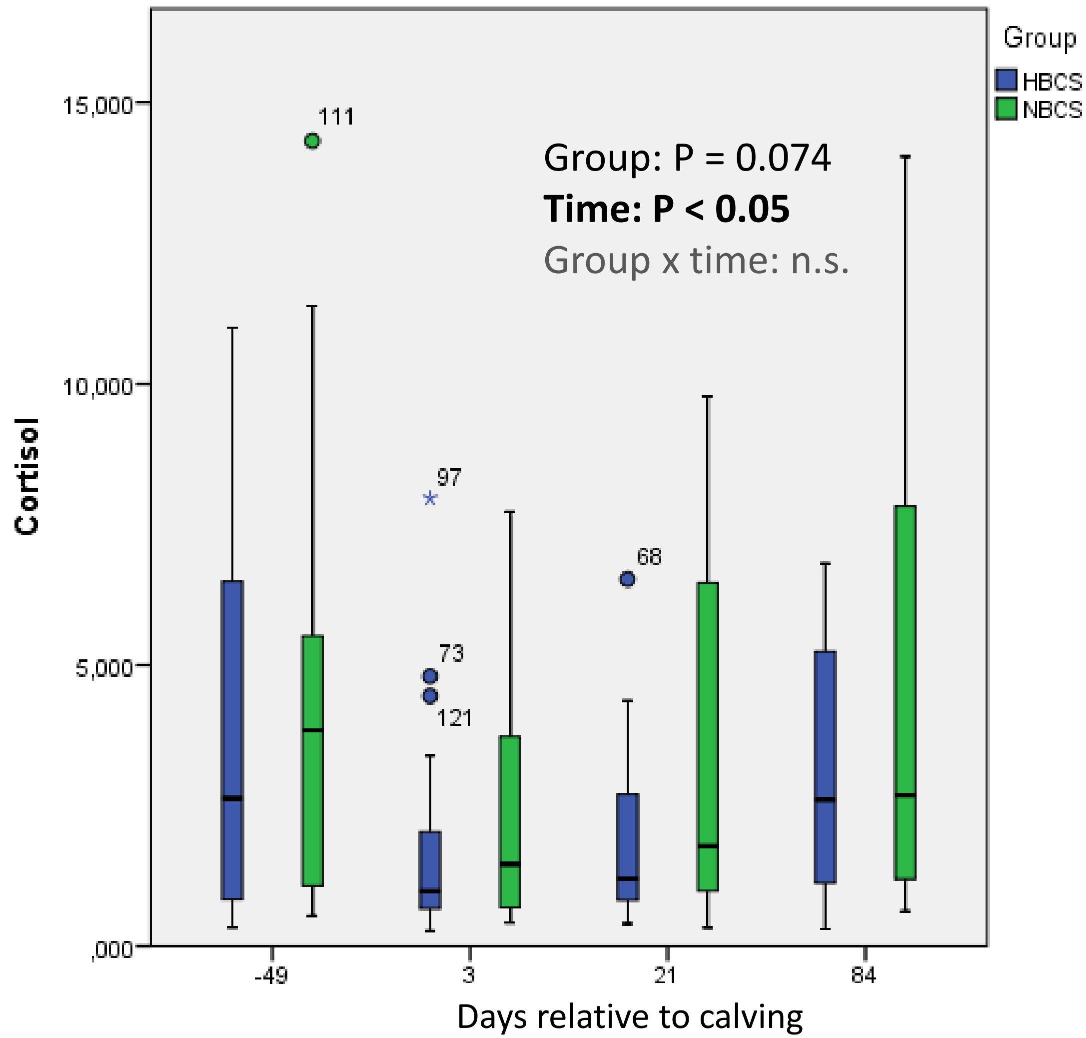


Cholesterol
HO
Cholesterol side-chain cleavage enzyme
Progestagens (21 carbons)
Pregnenolone
17α-hydroxylase
17α-hydroxy pregnenolone
17,20 lyase
Progesterone
17α-hydroxy progesterone
3-beta-hydroxysteroid dehydrogenase (3β-HSD)
21-hydroxylase
Deoxy-corticosterone
11-deoxycortisol
11β-hydroxylase
Mineralocorticoids (21 carbons)
Aldosterone
Aldosterone synthase
Corticosterone
Cortisol
Glucocorticoids (21 carbons)
Androgens (19 carbons)
Dehydroepi-androsterone
Androste-nedione
Androstenediol
Testosterone
17β-HSD
5α-reductase
Dihydrotestosterone
Aromatase
Estrone
Estradiol
(liver and placenta)
Estriol
Estrogens (18 carbons)
Cellular location of enzymes
Mitochondria
Smooth endoplasmic reticulum

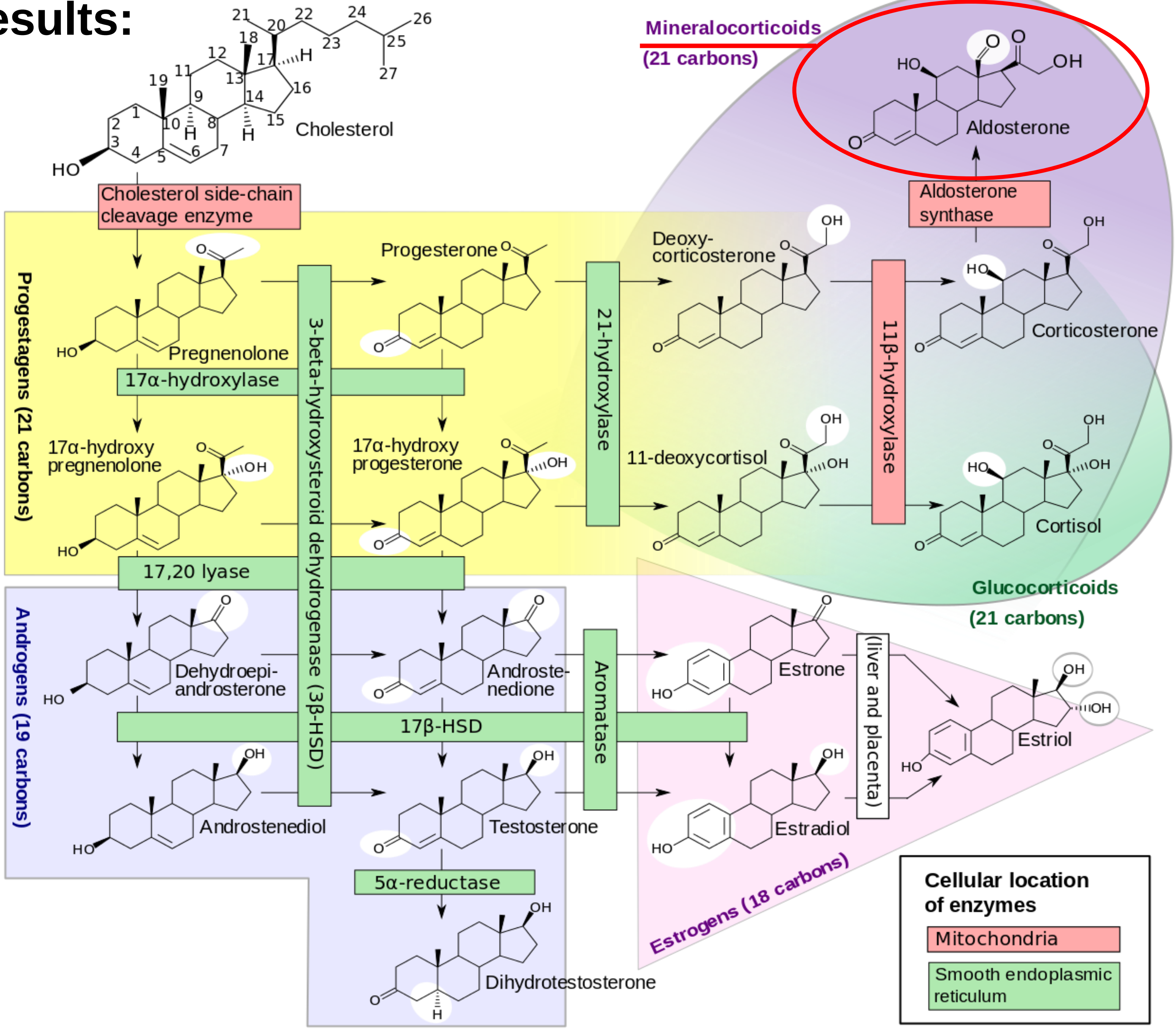
Results: Glucocorticoids







Results:



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Testosterone
Estradiol
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5α-reductase
Dihydrotestosterone
Cellular location of enzymes
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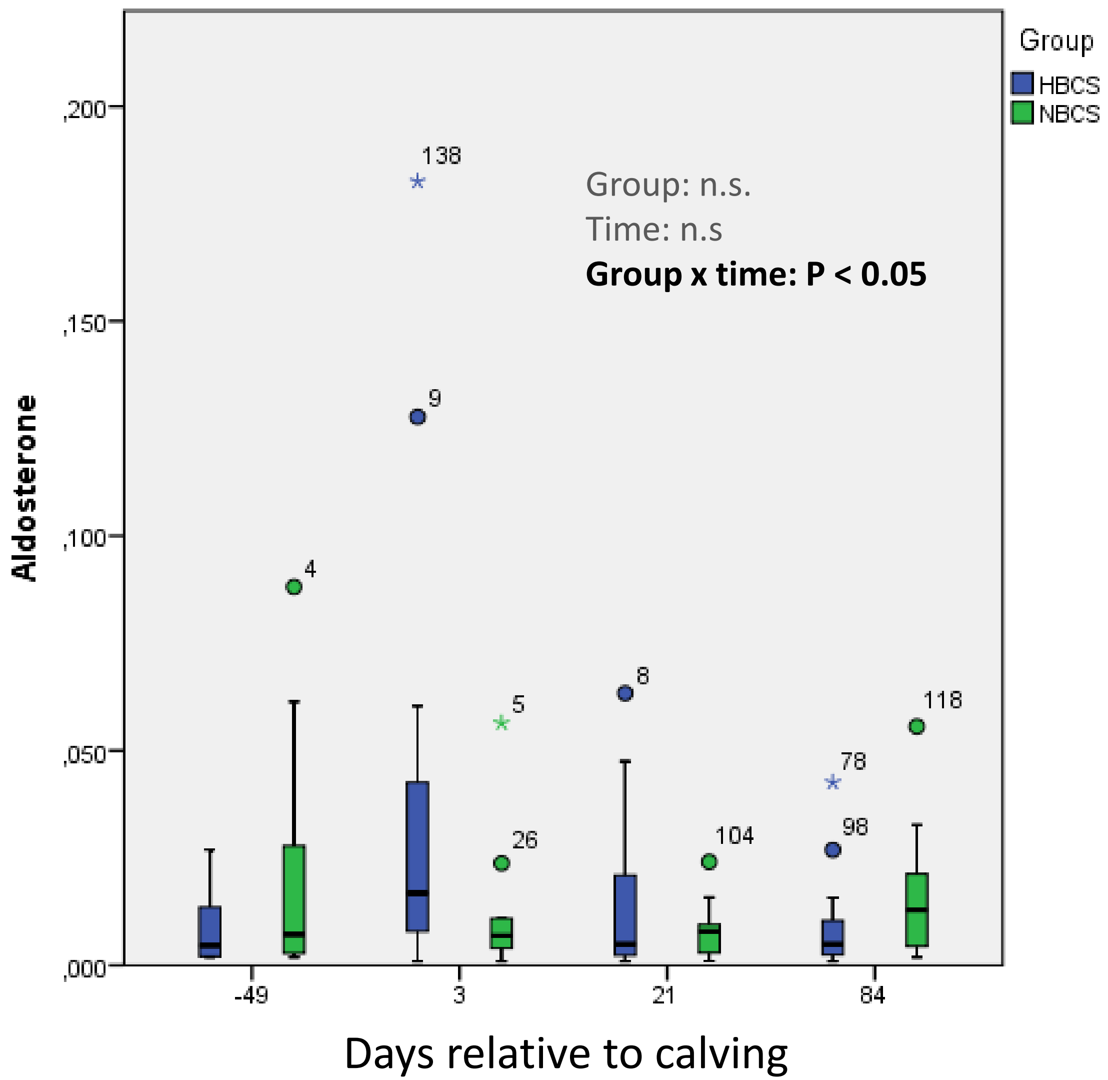
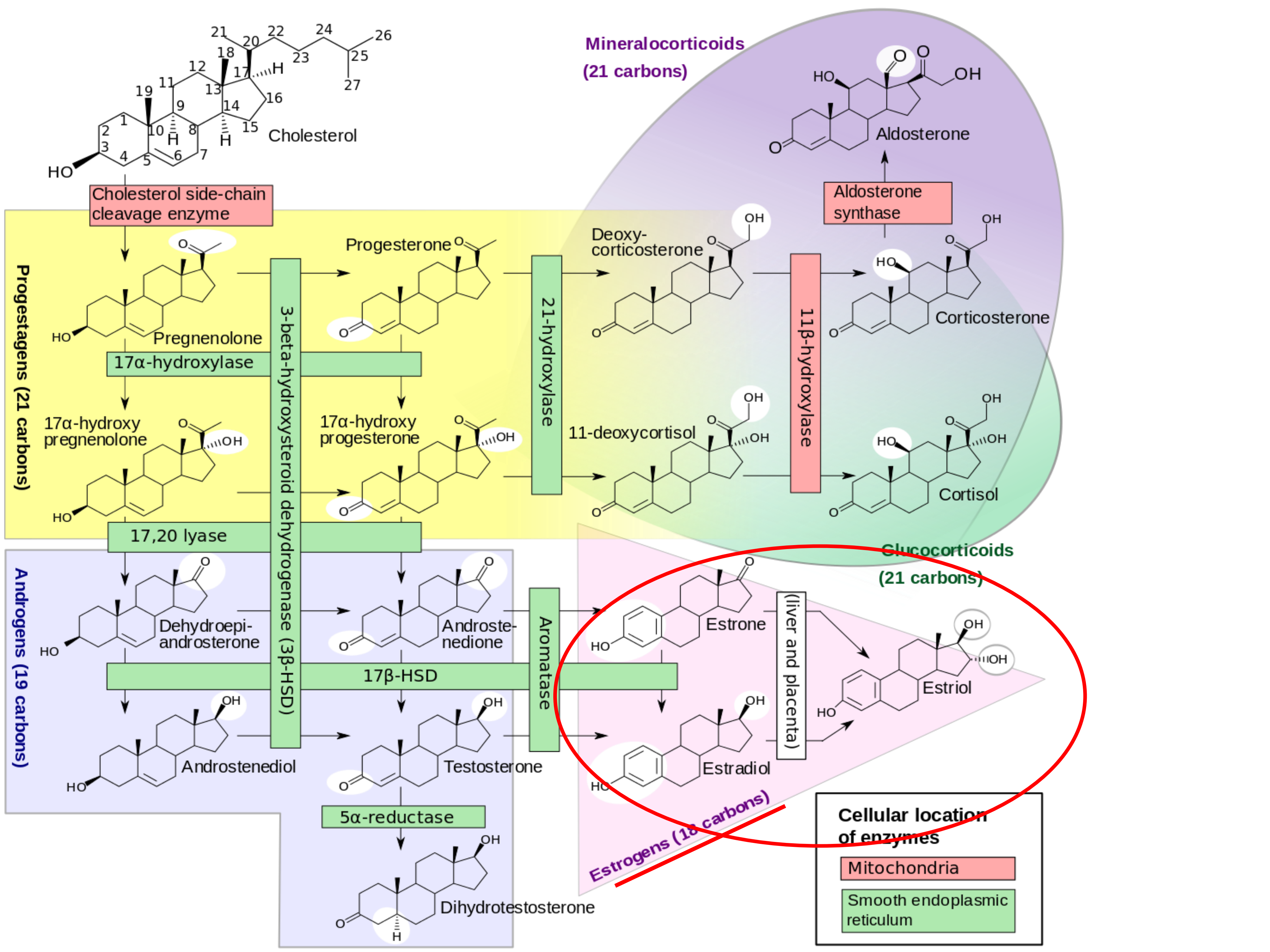
Smooth endoplasmic reticulum

Results: Mineralocorticoids





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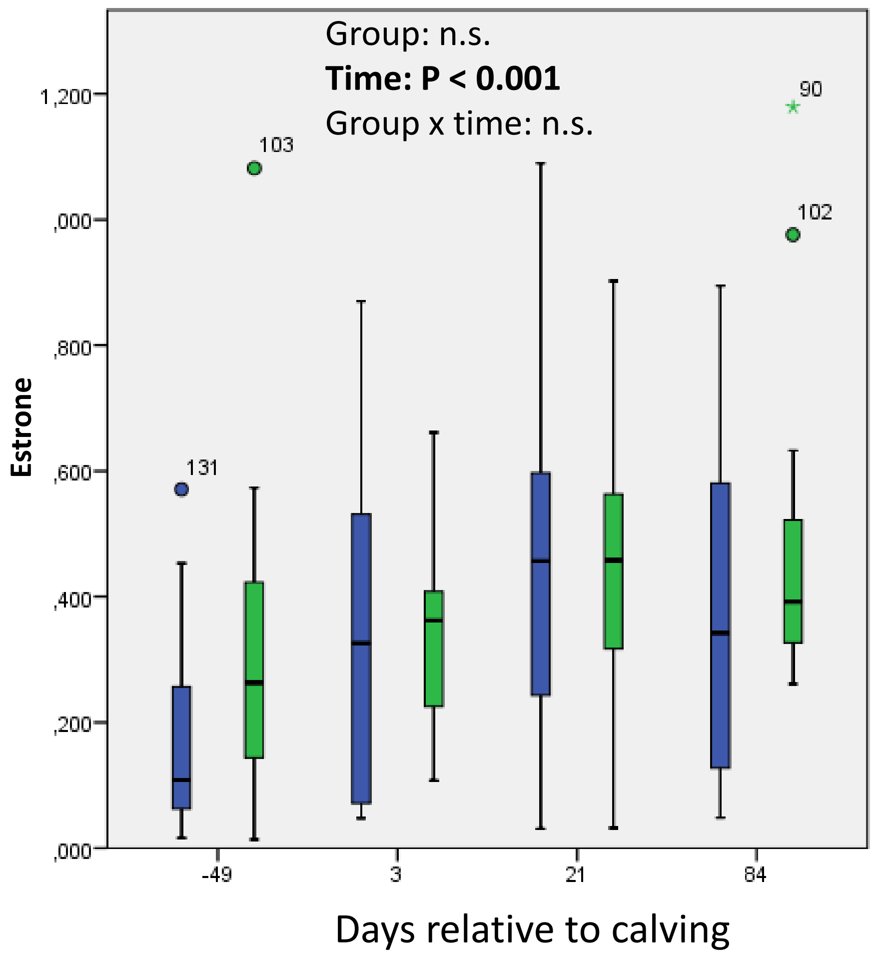
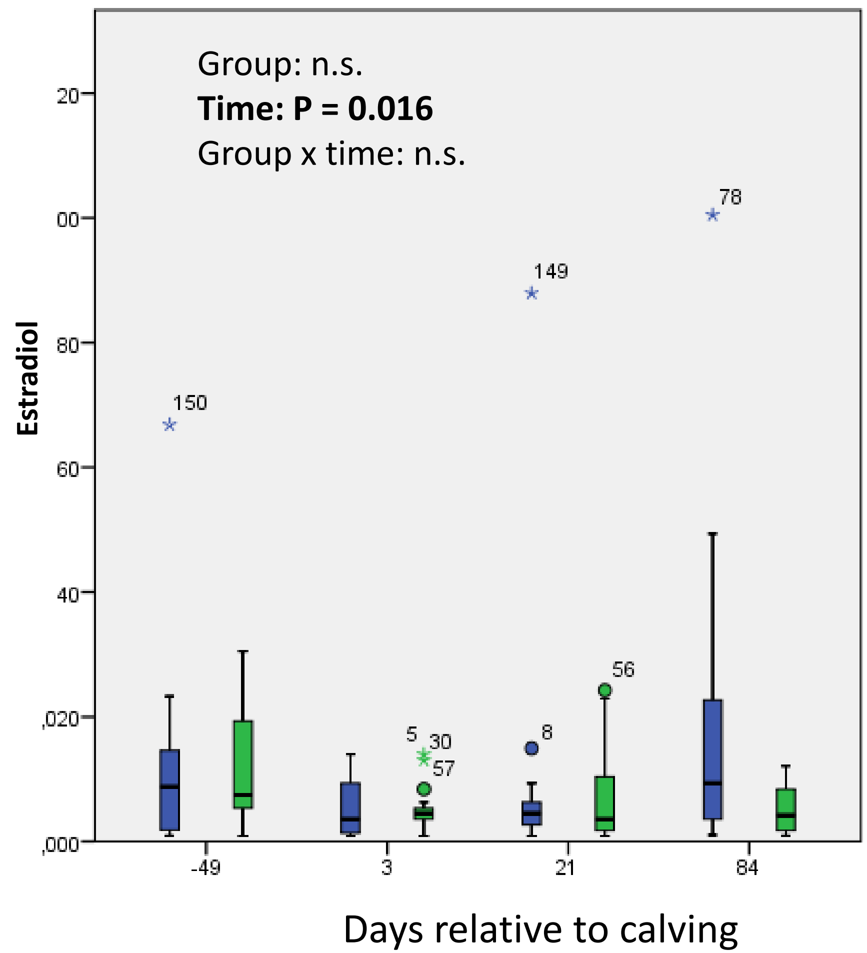
Testosterone
Estradiol
Estrogens (18 carbons)
5α-reductase
Dihydrotestosterone
Cellular location of enzymes
Mitochondria
Smooth endoplasmic reticulum

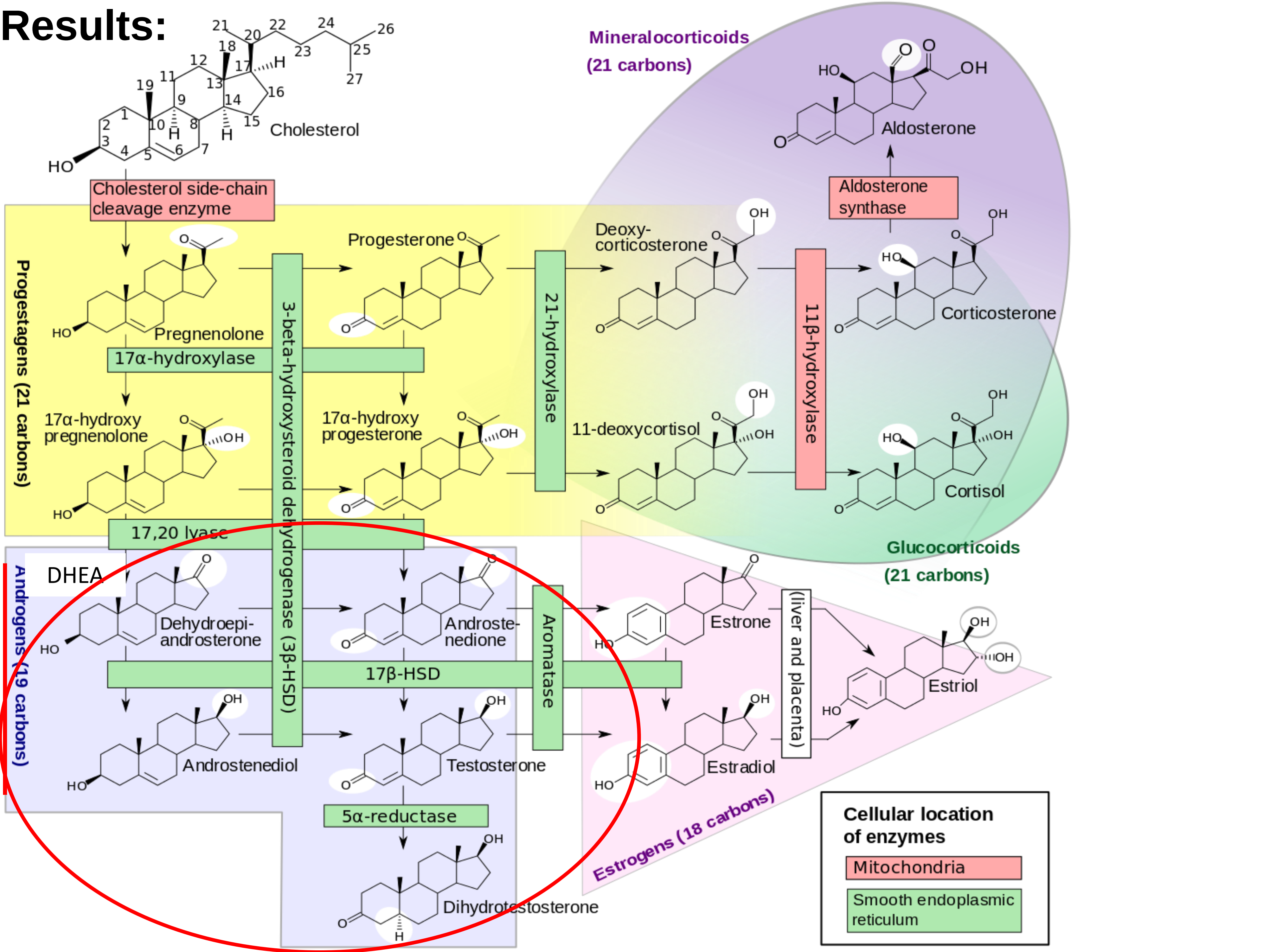
Results: Estrogens





HBCS **NBCS**

Results:



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11β-hydroxylase
17α-hydroxy pregnenolone
17α-hydroxy progesterone
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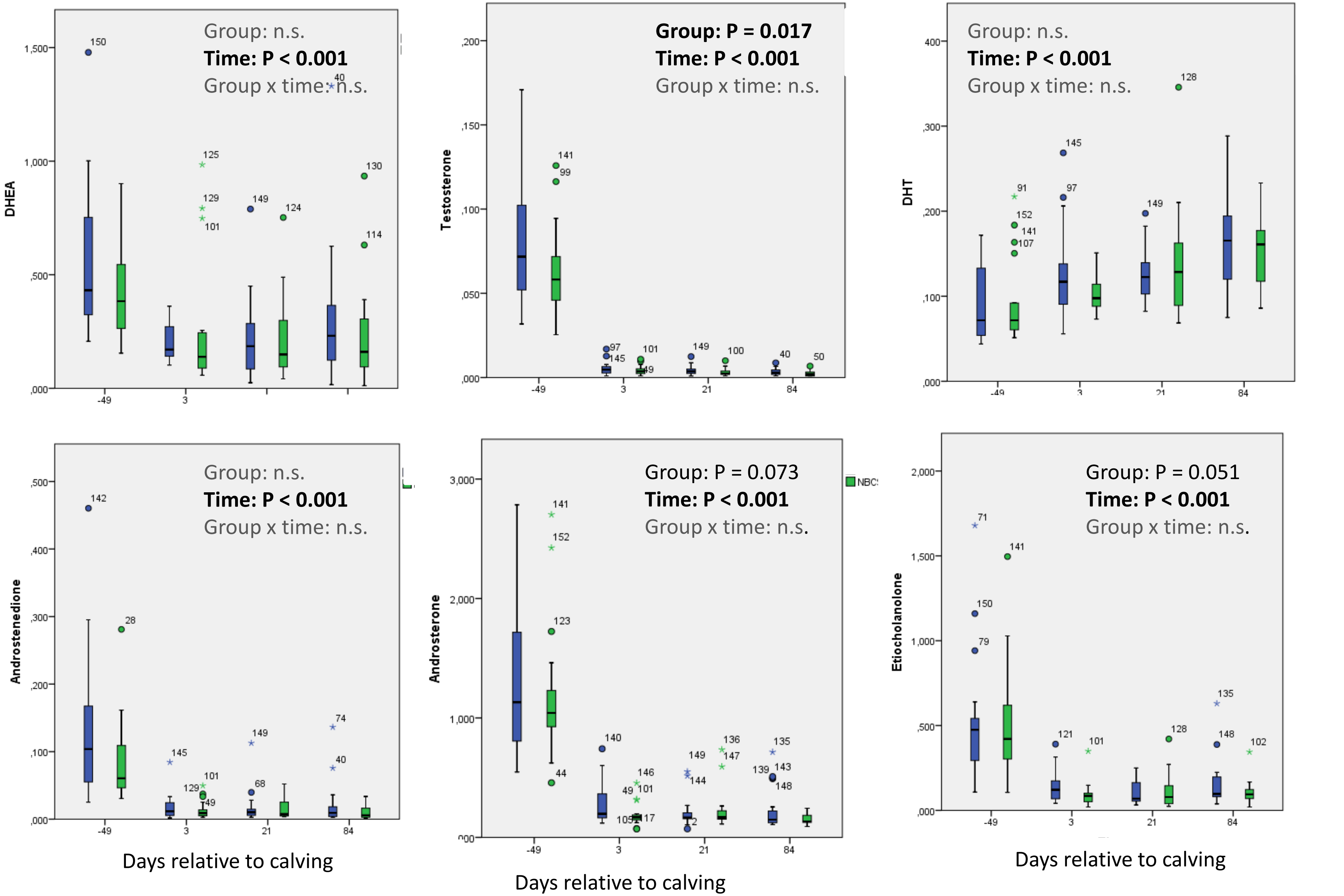
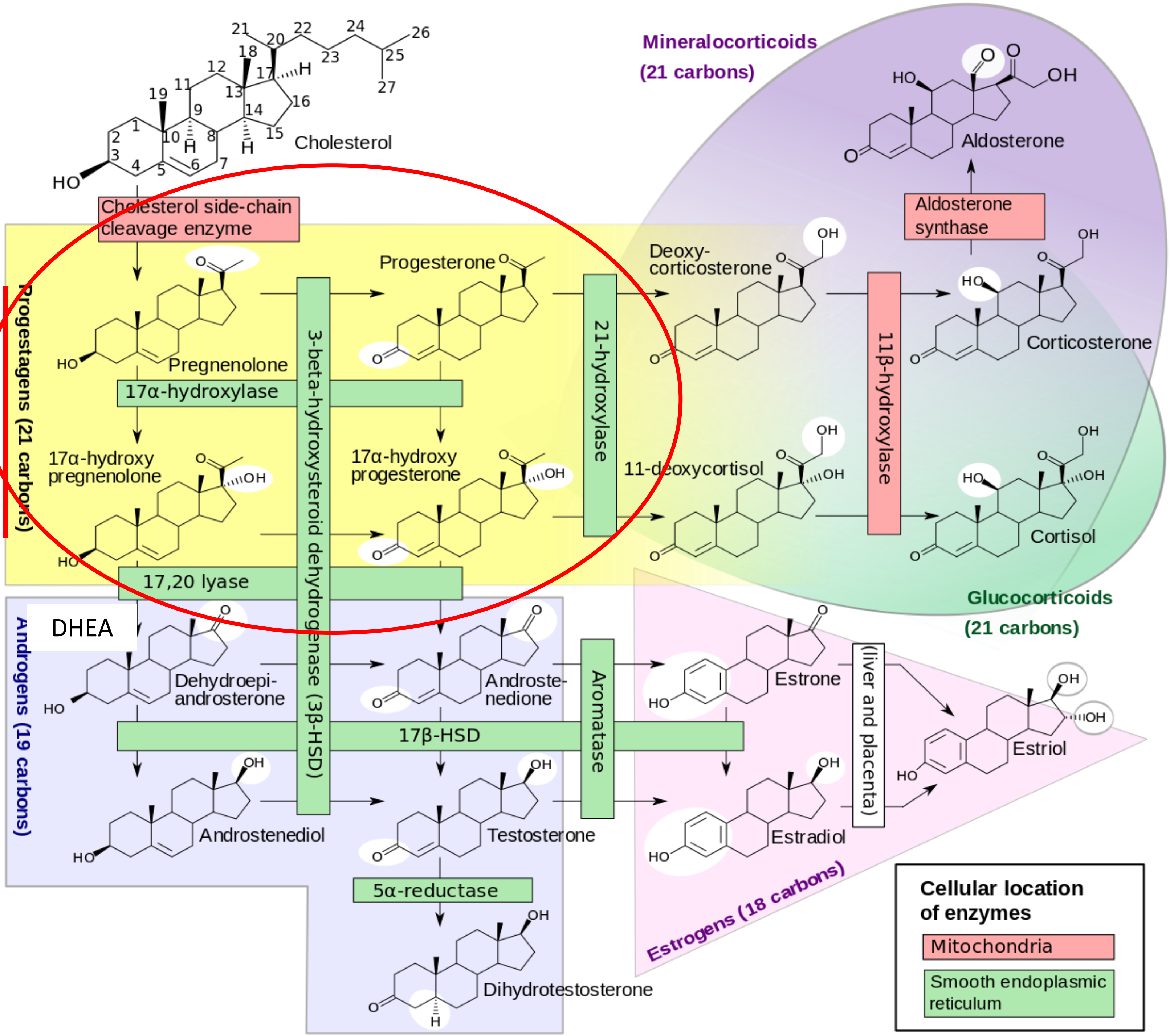
Cortisol
17,20 lyase
Glucocorticoids (21 carbons)
DHEA
Androgens (19 carbons)
Dehydroepi-androsterone
Androste-nedione
Aromatase
Estrone
(liver and placenta)
Estriol
17β-HSD
Androstenediol
Testosterone
Estradiol
5α-reductase
Dihydrotestosterone
Estrogens (18 carbons)
Cellular location of enzymes
Mitochondria
Smooth endoplasmic reticulum

Results: Androgens

HBCS NBCS





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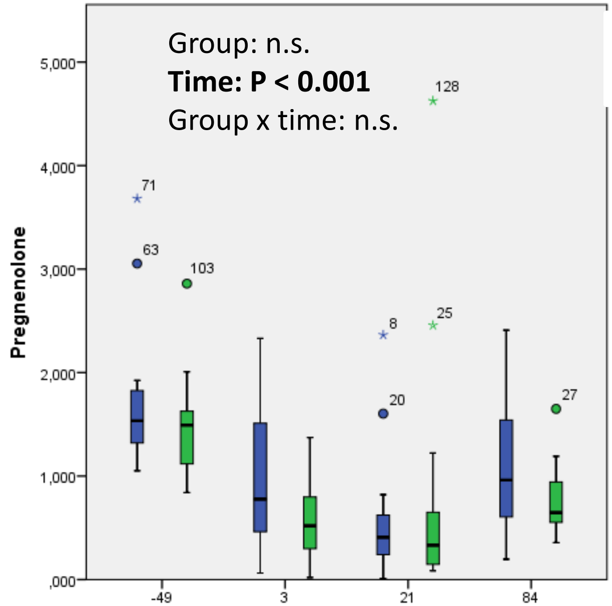
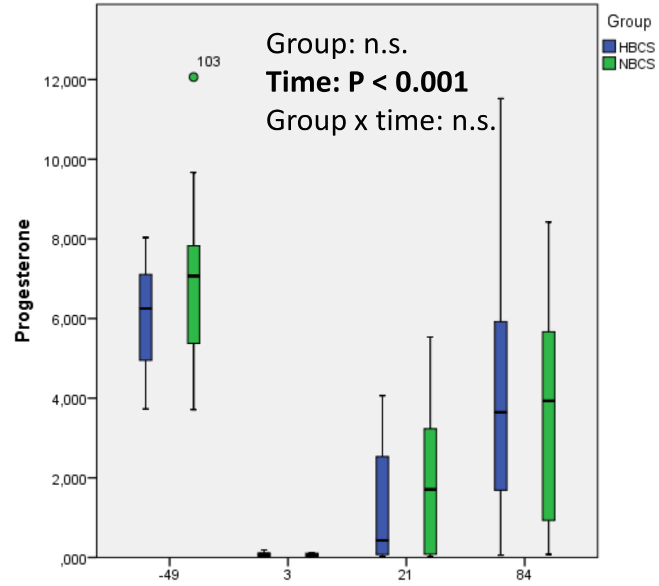
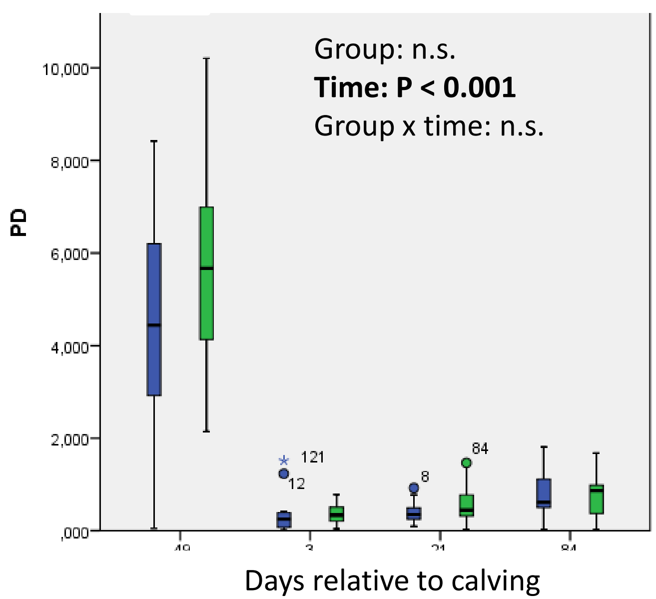
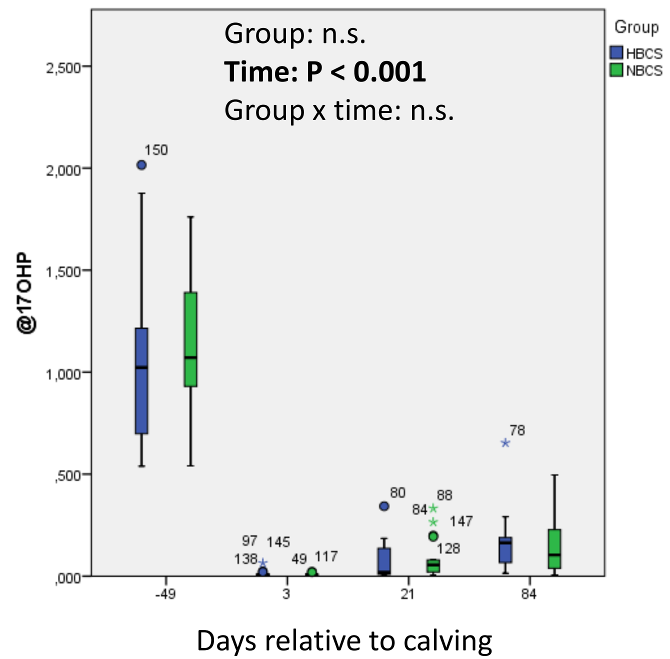
Results: Gestagens









Summary & Conclusions

- Quantification of 17 (19) different steroids in one run
- Expectedly, the concentrations messured in cattle were different from the patterns know from human serum samples (example: E2);
- Most hormones were affected by time (except Aldosterone, corticosterone, and cortisone)
- Group differences were limited to:
- - 1 glucocorticoid (Cortisol, P = 0.74),
- - 3 androgens: Etiocholanolone (P = 0.051), Androsterone (P = 0.073), and Testosterone (P = 0.017)
- Overconditioning was related to increased circulating concentrations of androgens pointing to dysbalances which may also compromise reproduction
- The lack of group effects on progesterone was confirmed in weekly blood samples assessed by ELISA; neither the concentration nor the time of returning into ovarian cyclicity seemed to be affected by overconditioning.

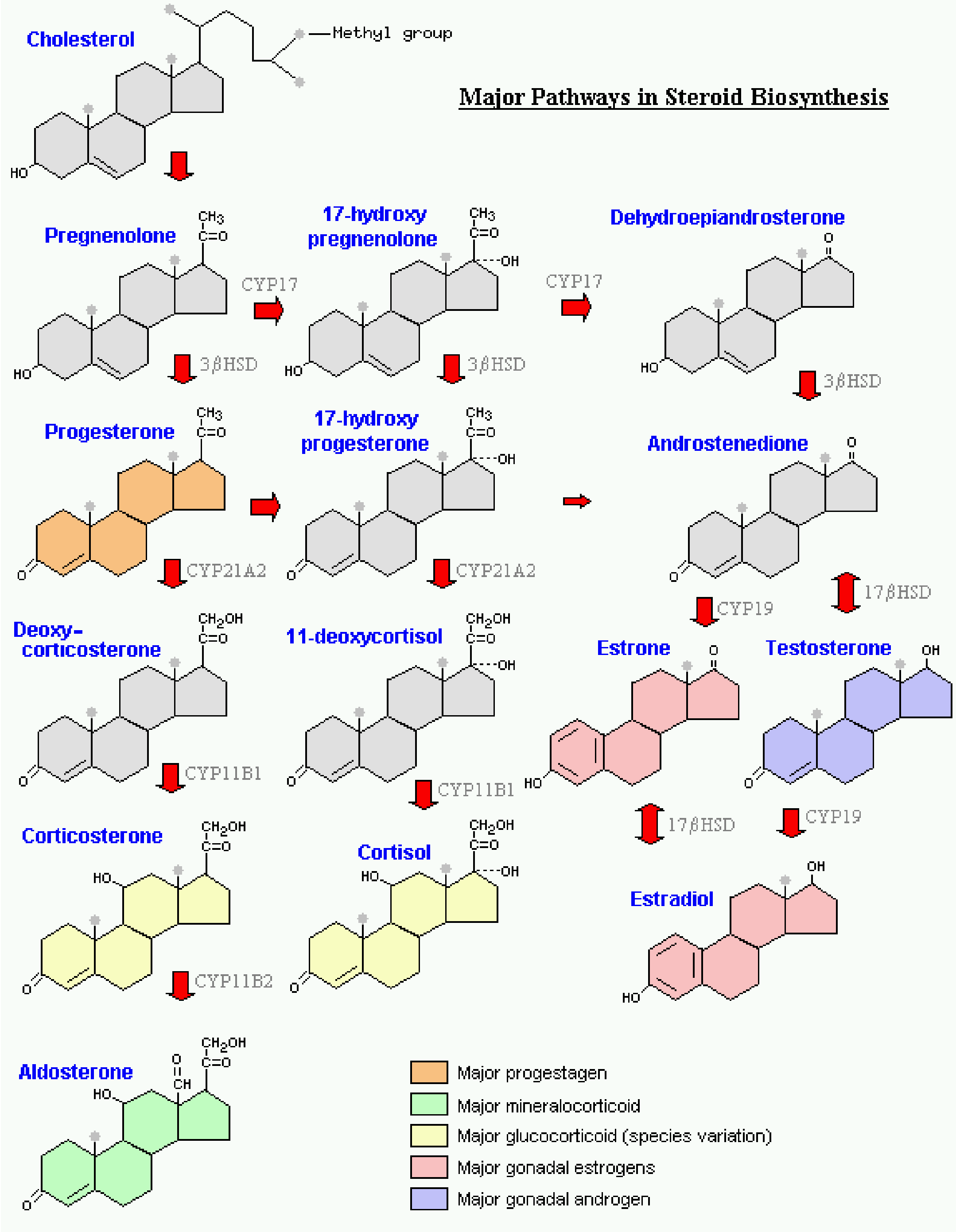
Thank you for your attention

…and mind the Physiology meeting
after this session

- The concentrations of some steroid hormones were affected by cows parity
- Mineral - & Glucocorticoide concentration was not affected by parity
- In most cases (except Progesterone) the cows in second parity (parity class 1) showed higher concentrations of steroid hormones compared to older cows

	Parity class[1]				
Steroid hormone	1	2	3	SEM	P-value
Pregnenolone	1.303	0.831	1.003	0.066	0.021
Progesterone	2.895	3.452	2.790	0.247	0.030
DHEA	0.452	0.264	0.288	0.021	0.024
DHT	0.144	0.133	0.118	0.004	0.003
Etiocholanolone	0.257	0.215	0.216	0.021	0.035
Estrone	0.597	0.454	0.276	0.019	< 0.001

[1] Parity classes: 1 = second parity, 2 = third parity, 3 = parity 4 or higher



Major Pathways in Steroid Biosynthesis
Cholesterol
Methyl group
HO
Pregnenolone
CYP17
17-hydroxy pregnenolone
Dehydroepiandrosterone
3βHSD
Progesterone
17-hydroxy progesterone
Androstenedione
CYP21A2
CYP19
17βHSD
Deoxy-corticosterone
11-deoxycortisol
Estrone
Testosterone
CYP11B1
Corticosterone
Cortisol
Estradiol
CYP11B2
Aldosterone
CH_3
C=O
CH_2OH
OH
O
CH
Major progestagen
Major mineralocorticoid
Major glucocorticoid (species variation)
Major gonadal estrogens
Major gonadal androgen

Key Features

Quantification of 17 Steroid Hormones from all Five Classes

Metabolite Coverage

Glucocorticoids
- Cortisol
- Cortisone
- 11-Deoxycortisol

Progestogens
- 17α-Hydroxyprogesterone (17α-OHP)
- Progesterone

Mineralocorticoids
- Aldosterone
- Corticosterone
- 11-Deoxycorticosterone (DOC)

Estrogens
- Estradiol (E2)
- Estrone (E1)

Androgens
- Androstenedione
- Androsterone
- Dehydroepiandrosterone (DHEA)
- Dehydroepiandrosterone sulfate (DHEA-S)
- Dihydrotestosterone (DHT)
- Etiocholanolone
- Testosterone

Best Fit to Physiological Range

Capture the Broadest Steroid Hormone Profile

The serum concentrations of steroid hormones vary considerably depending on age, gender, and health status. The Absolute*IDQ*® Stero17 Kit has been developed to provide **highly accurate results that fit the expected physiological ranges** (see table below). For more details, please contact us or refer to the respective client note.

Steroid Hormone		Calibration Standard							Calibration Range Cal 1-7 [ng/mL]
		Cal 1	Cal 2	Cal 3	Cal 4	Cal 5	Cal 6	Cal 7	
Gluco-corticoids	Cortisol								1.0 – 1000
	Cortisone								0.10 – 100
	11-Deoxycortisol								0.010 – 10
Mineralo-corticoids	Aldosterone	<							0.025 – 5.0
	Corticosterone								0.030 – 30
	DOC	<							0.0075 – 15
Progestogens	17α-OHP								0.050 – 50
	Progesterone								0.060 – 9.0
Estrogens	Estradiol	<							0.005 – 20
	Estrone	<							0.030 – 15
Androgens	Androstenedione								0.032 – 8.0
	Androsterone	<							0.050 – 5.0
	DHEA								0.12 – 30
	DHEA-S								32 – 8000
	DHT	<							0.012 – 30
	Etiocholanolone	<							0.060 – 6.0
	Testosterone								0.010 – 10

Physiological range in human serum, according to Human Metabolome Database (HMDB), May 2017

Physiological range in human serum, according to Biocrates in-house database, March 2017

< <

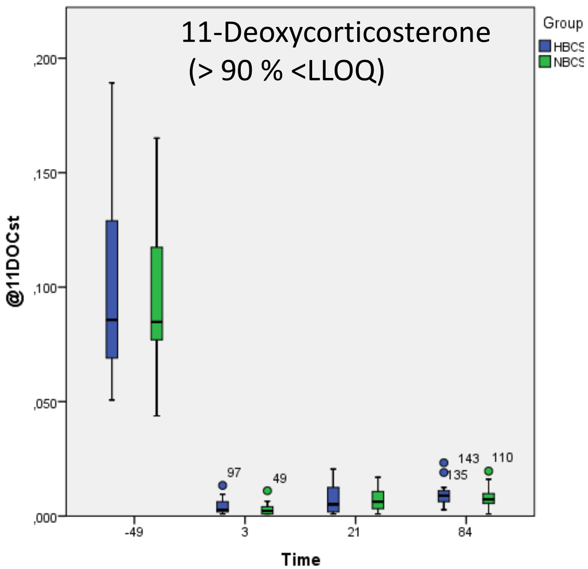
Physiological concentration in human serum may be below LLOQ, according to HMDB May 2017 or Biocrates in-house database, March 2017

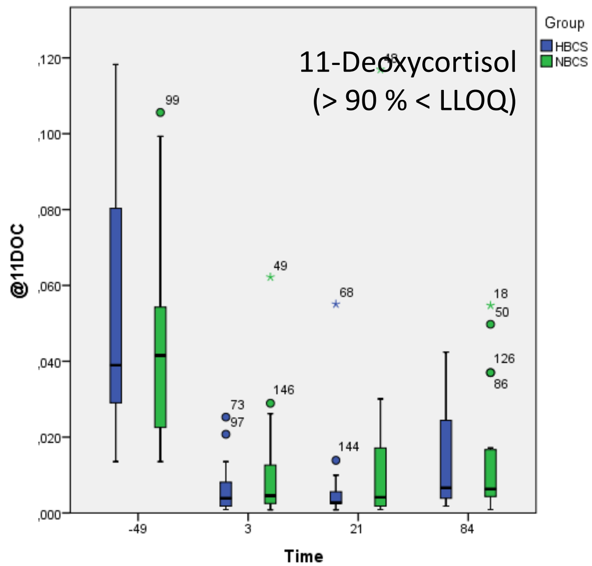
Concentration of steroids (ng/mL) in serum of cows with high (HBCS) or normal (NBCS) body condition

	Days relative to calving												
	49 a.p.		3 p.p.		21 p.p.		84 p.p.						
Metabolite, [ng/mL]	**HBCS**	**NBCS**	**HBCS**	**NBCS**	**HBCS**	**NBCS**	**HBCS**	**NBCS**	**SEM**	**Group**	**Time**	**Group* Time**	**Parity[1]**
11-Deoxycortisol	0.053	0.047	0.007	0.011	0.007	0.015	0.014	0.015	0.004	n.s.	**< 0.001**	n.s.	***0.092***
11-Deoxycorticosterone	0.101	0.096	0.004	0.003	0.007	0.007	0.009	0.008	0.002	n.s.	**< 0.001**	n.s.	n.s.
17α-Hydroxyprogesterone	1.069	1.136	0.011	0.008	0.075	0.083	0.161	0.150	0.040	n.s.	**< 0.001**	n.s.	n.s.
Aldosterone	0.009	0.019	**0.034**	**0.011**	0.014	0.008	0.009	0.016	0.002	n.s.	n.s.	**0.033**	n.s.
Androstenedione	0.128	0.086	0.018	0.014	0.017	0.015	0.021	0.010	0.005	n.s.	**< 0.001**	n.s.	***0.087***
Androsterone	1.304	1.205	**0.288**	**0.185**	0.207	0.227	0.233	0.154	0.045	***0.073***	**< 0.001**	n.s.	n.s.
Corticosterone	0.133	0.128	0.086	0.068	0.076	0.111	0.090	0.147	0.009	n.s.	n.s.	n.s.	n.s.
Cortisol	3.685	4.412	1.844	2.505	1.921	3.575	3.117	4.576	0.251	***0.074***	**0.014**	n.s.	n.s.
Cortisone	0.572	0.599	0.481	0.549	0.423	0.510	0.476	0.488	0.024	n.s.	n.s.	n.s.	n.s.
Dehydroepiandrosterone	0.556	0.418	0.206	0.248	0.208	0.220	0.299	0.239	0.021	n.s.	**< 0.001**	n.s.	**0.024**
Dihydrotestosterone	0.092	0.093	0.125	0.103	0.125	0.142	0.162	0.157	0.004	n.s.	**< 0.001**	n.s.	**0.003**
Estrone	0.187	0.315	0.346	0.337	0.442	0.446	0.352	0.474	0.019	n.s.	**< 0.001**	n.s.	**< 0.001**
Estradiol	0.012	0.013	0.006	0.005	0.010	0.007	0.018	0.005	0.001	n.s.	**0.016**	n.s.	***0.099***
Etiocholanolone	0.525	0.516	**0.142**	**0.092**	0.108	0.116	0.157	0.107	0.021	***0.051***	**< 0.001**	n.s.	**0.035**
Pregnanediol[2]	4.494	5.675	0.350	0.364	0.408	0.585	0.749	0.767	0.196	n.s.	**< 0.001**	n.s.	n.s.
Pregnenolone[3]	1.752	1.493	0.967	0.542	0.578	0.772	1.149	0.749	0.066	n.s.	**< 0.001**	n.s.	**0.021**
Progesterone	6.022	6.835	0.077	0.069	1.349	1.856	3.899	3.833	0.247	n.s.	**< 0.001**	n.s.	**0.030**
Testosterone	0.084	0.064	0.005	0.004	0.004	0.003	***0.004***	***0.002***	0.003	**0.017**	**< 0.001**	n.s.	n.s.

Numbers in bold type indicate significant ($P < 0.05$) changes between groups; numbers in italic and bold type indicate trends ($P < 0.10$);
[1] Parity was removed from the model when insignificant; [2,3]contamination of samples yielded no results for pregnanediol and pregnenolone in 6 out of 38 samples for timepoint -49 [2 NBCS, 4 HBCS], and 4 out of 38 samples for each following timepoint +3 [1 NBCS, 3 HBCS], +21 [2 NBCS, 2 HBCS], and +84[[4 HBCS].

Results: Gestagens





- Some steroid concentrations were below the LLOQ
- In most cases (except Aldosterone and Estradiol) the values below the LLOQ were found after calving

Steroid hormone	Samples below LLOQ
Aldosterone	95%
Estradiol	91%
11-Deoxycorticosterone	75%
Testosterone	72%
Androstenedione	67%
11-Deoxycortisol	53%
17α-Hydroxyprogesterone	45%
Cortisol	30%
Dehydroepiandrosterone	26%
Corticosterone	24%
Pregnanediol	24%
Etiocholanolone	23%
Progesterone	18%
Pregnenolone	14%
Estrone	3%

Quantitative profiling of up to 17 steroid hormones from five steroid hormone classes:

- Progestagens
- Glucocorticoids
- Mineralocorticoids
- Androgens
- Estrogens

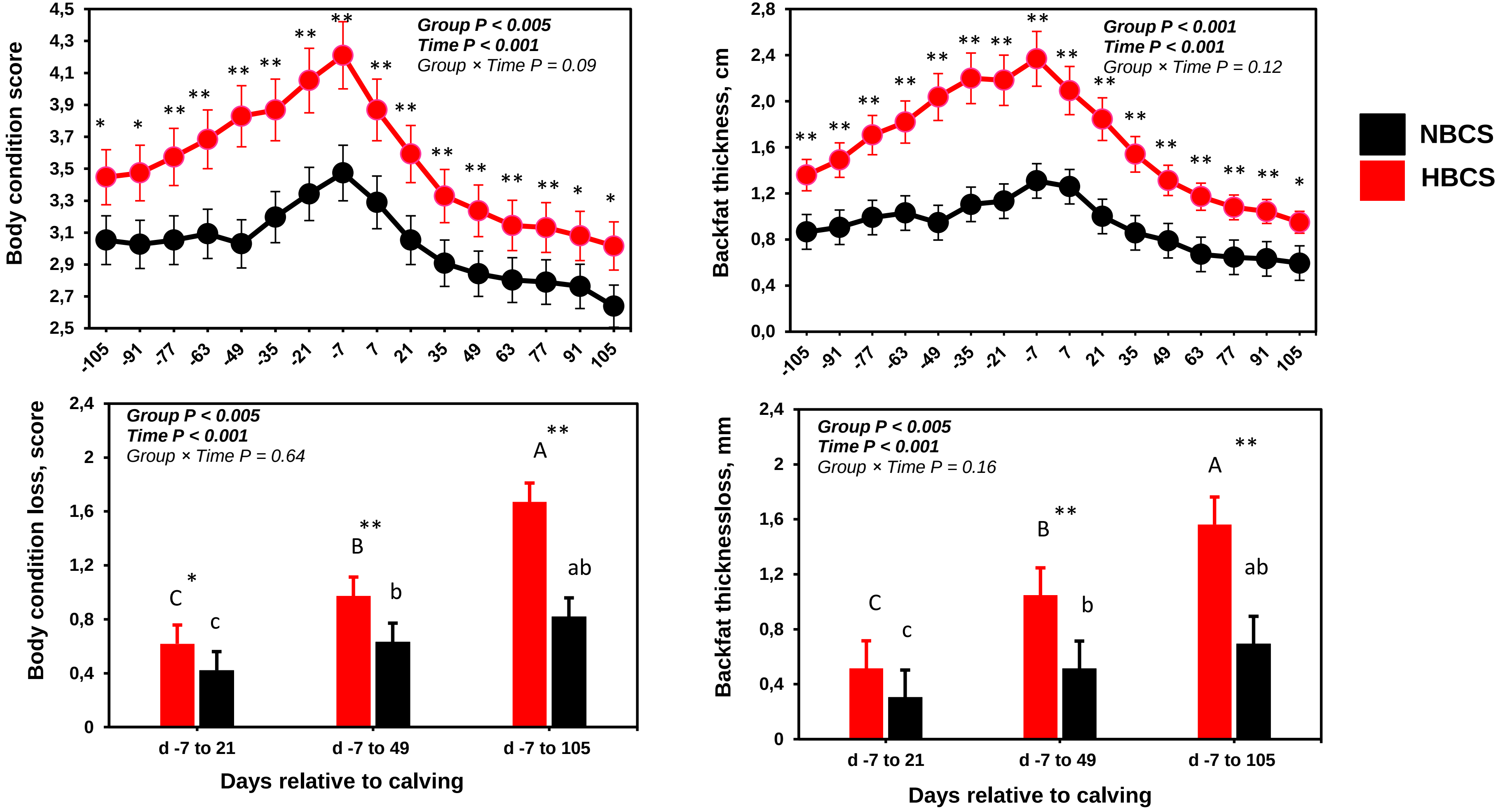
Proven to provide highly accurate results in ring trials by DGKL (German United Society for Clinical Chemistry and Laboratory Medicine).

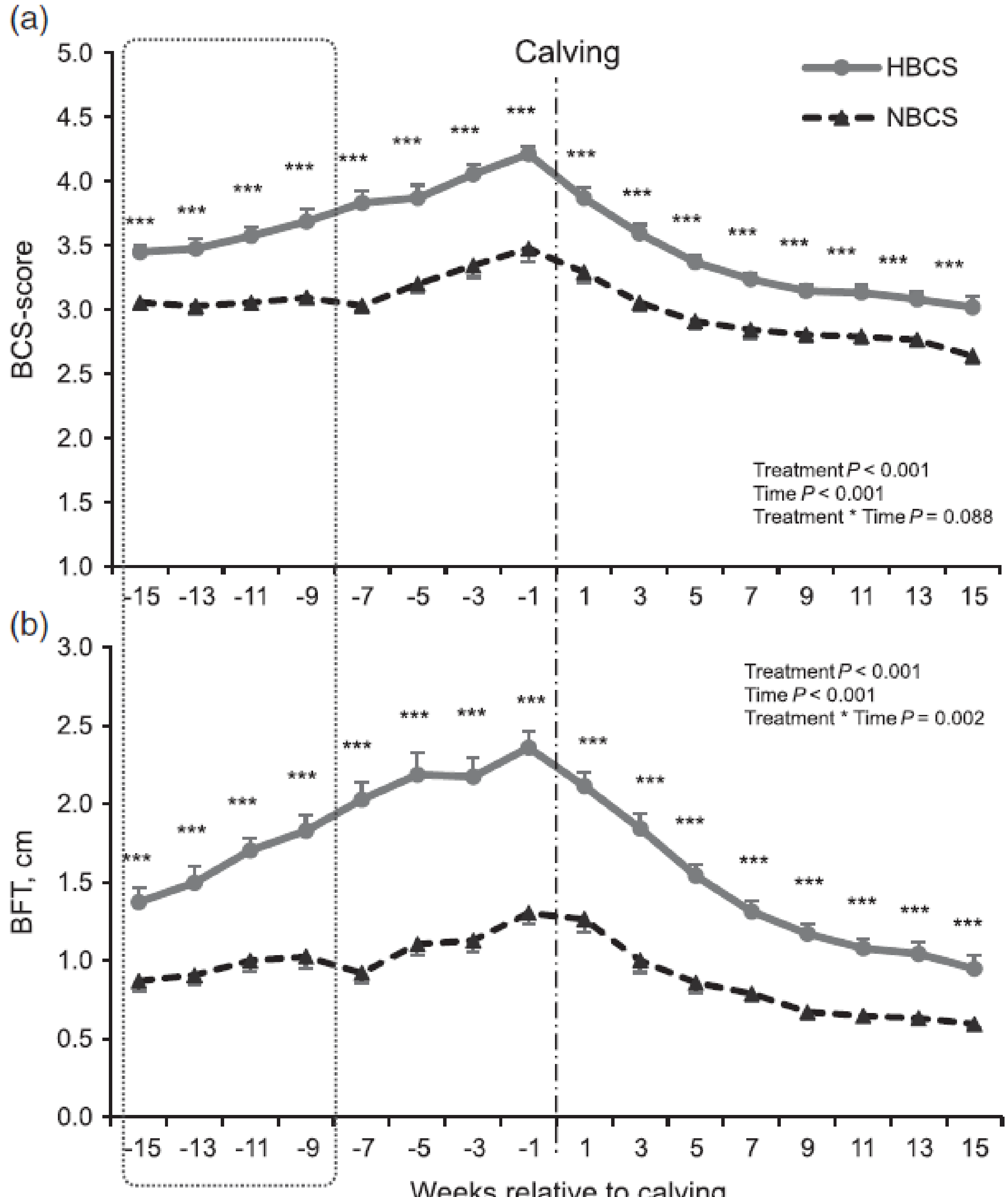
The kit includes standardized reagents, as well as validated protocols for sample preparation and mass spectrometric analysis.

As compared to other techniques, mass spectrometry offers the opportunity to quantify multiple steroid hormones in a single run with a **higher accuracy**.

11-Deoxycortisol
11-Deoxycorticosterone
17α-Hydroxyprogesterone
Aldosterone
Androstenedione
Androsterone
Corticosterone
Cortisol
Cortisone
Dehydroepiandrosterone
Dihydrotestosterone
Estrone
Estradiol
Etiocholanolone
Pregnanediol[2]
Pregnenolone[3]
Progesterone
Testosterone

Results: Animal model




(a)
Calving
HBCS
NBCS
BCS-score
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Treatment P < 0.001
Time P < 0.001
Treatment * Time P = 0.088
-15 -13 -11 -9 -7 -5 -3 -1 1 3 5 7 9 11 13 15
(b)
BFT, cm
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Treatment P < 0.001
Time P < 0.001
Treatment * Time P = 0.002
-15 -13 -11 -9 -7 -5 -3 -1 1 3 5 7 9 11 13 15
Weeks relative to calving