Supplementary Table 1

Antibodies and protocol for multiplex immunohistochemistry

Cycle	Marker ENSG	Marker Gene	Marker protein name	Marker antibody ID	Dilution	OPAL fluorophore	OPAL dilution
Cycle 1	ENSG00000171345	KRT19	Keratin 19	HPA002465	1 : 600	OPAL 690	1 : 500
Cycle 2	ENSG00000150093	ITGB1 / CD29	Integrin subunit beta 1	HPA059297	1 : 40	OPAL 620	1 : 500
Cycle 3	ENSG00000143178	TPIT	T-box transcription factor 19	HPA072686	1 : 500	OPAL 520	1 : 500
Cycle 4	ENSG00000181449	SOX2	SRY-box transcription factor 2	HPA045725	1 : 500	OPAL 570	1 : 500
Cycle 5	ENSG00000136931	SF1 / NR5A1	Steroidogenic factor 1	EPR19744 (Abcam, Cambridge, United Kingdom)	1 : 200	OPAL 480	1 : 500
Cycle 6	ENSG00000125398	SOX9	SRY-box transcription factor 9	CAB068240	1 : 4500	TSA + digoxigenin (DIG)	1 : 500
Post-cycle						Anti-DIG OPAL 780 antibody	1 : 125
						DAPI	1 : 1000

Cycle protocol

Steps	Duration (min)
Blocking	10
Primary antibody	30
Wash	2
Enhancer	20
Wash	2
Horseradish peroxidase (HRP)	10
Wash	2
OPAL Fluorophore	10
Wash	2
Elution in 90°C	20

Post-cycle steps	Duration (min)
Anti-DIG OPAL 780 antibody	60
DAPI	5
TBS wash	5
TBS wash	5
Deionized water wash	2
Mount	

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Supplementary Table 2

	SOX2 neg	SOX2 pos	P-value	SOX9 neg	SOX9 pos	P-value	PROP1 neg	PROP1 pos	P-value
Female / Male	25 / 43 (63 %)	9 / 24 (73 %)	0.34	11 / 6 (60 %)	23 / 51 (69 %)	0.36	27 / 53 (66 %)	7 / 14 (67 %)	0.97
Age	62 (51 - 72)	57 (47 - 73)	0.29	60 (50 - 71)	61 (50 - 72)	0.91	61 (51 - 72)	55 (44 - 70)	0.16
Reintervention	27 (41.5 %)	6 (19.4 %)	0.03	12 (46.2 %)	21 (30 %)	0.14	25 (33 %)	8 (38.1 %)	0.69
Invasiveness	12 (46 %)	6 (30 %)	0.27	5 (63 %)	13 (34 %)	0.14	16 (42 %)	2 (25 %)	0.37
Tumor volume mm^3	7115 (3823 - 10098)	6340 (4329 - 13865)	0.64	7223 (4030 - 10400)	6480 (4054 - 11267)	0.91	6653 (3855 - 10581)	6340 (5065 - 14610)	0.60
IHC FSHβ	1 (0 - 2)	3 (1 - 4)	< 0.001	1 (0 - 2)	2 (1 - 3)	0.079	1 (0 - 2)	3 (1.5 - 4)	< 0.001
IHC LHβ	1 (1 - 2)	2 (0 - 3)	0.936	1 (1 - 2.5)	1 (1 - 3)	0.693	1 (1 - 2)	1 (0 - 3)	0.877
IHC ERα	1 (0 - 3)	3 (1.5 - 4)	0.001	0.5 (0 - 2)	2 (0 - 4)	0.034	1 (0 - 3)	3 (1 - 4)	0.049
ΔCT SF1	0.06 (0.05 - 0.10)	0.07 (0.05 - 0.13)	0.765	0.09 (0.06 - 0.10)	0.06 (0.05 - 0.10)	0.238	0.06 (0.05 - 0.10)	0.07 (0.05 - 0.12)	0.501
ΔCT FSH	0.13 (0.02 - 0.25)	0.82 (0.23 - 1.63)	0.001	0.06 (0.01 - 0.22)	0.24 (0.06 - 1.28)	0.009	0.11 (0.02 - 0.25)	1.19 (0.70 - 1.68)	< 0.001
ΔCT LH	0.05 (0.01 - 0.19)	0.14 (0.03 - 0.24)	0.936	0.05 (0.01 - 0.17)	0.08 (0.01 - 0.21)	0.463	0.05 (0.01 - 0.23)	0.11 (0.03 - 0.20)	0.615
ΔCT GnRHR	0.06 (0.03 - 0.23)	0.27 (0.12 - 0.45)	0.002	0.03 (0.02 - 0.13)	0.18 (0.07 - 0.35)	< 0.001	0.10 (0.03 - 0.24)	0.27 (0.16 - 0.35)	0.014
ΔCT ESR1	0.26 (0.17 - 1.13)	1.56 (0.78 - 2.42)	< 0.001	0.23 (0.17 - 0.44)	0.98 (0.28 - 2.11)	0.004	0.35 (0.18 - 1.36)	1.97 (0.87 - 4.56)	< 0.001
Total	**68**	**33**		**27**	**74**		**80**	**21**	

Supplementary Table 2: Distribution of SOX2, SOX9 and PROP1 in non-functioning gonadotroph tumours. A staining score of ≥ 1 was considered as a positive staining score. Invasiveness was defined as Knosp score ≥ 3. The Immunohistochemical (IHC) staining for FSHβ and LHβ was graded from 0-4 based on the percentage of positive cells. The IHC staining for Estrogen Receptor α (ERα) was based on the immunoreactive score (IRS). Staining for FSHβ, LHβ and ERα was lacking in 2 patients. Frozen tissue for RT-qPCR was available in 57 patients that also had staining for SOX2, SOX9 and PROP1. Five patients with gonadotroph tumours had less than 12 months follow-up and were therefore not included in the analyses concerning reintervention. Numbers and percentages are given for categorical variables; median and interquartile range (parenthesis) are given for continuous and ordinal variables.

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