# Ovarian primordial and primary follicle density and serum AMH concentration – an analysis based on >1000 ovarian tissue and serum samples cryopreserved before gonadotoxic therapy

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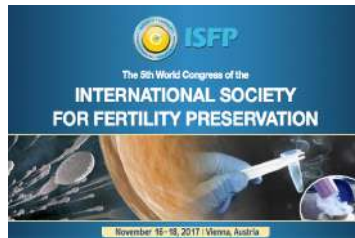
Theoretical Background

- Ovarian reserve – mainly determined through AMH – is predictive for infertility treatment and for fertility protection – for estimating the risk to get a chemotherapy induced POI (Broughham et al., 2012).

- Nevertheless, the reliability may be limited as a prognostic factor for the ovarian response (Broer et al., 2013, Kelsey et al., 2011).

- This questions if AMH reflects the real follicular reserve (primordial/primary follicles).

Study question

Does serum AMH correlate with the number of primary/primordial follicles?
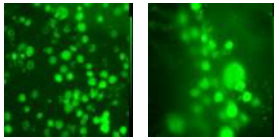
Material & Methods

- 1.068 patients underwent ovarian tissue freezing before gonadotoxic treatment from 03/11 to 09/2016.

- Three standardized 2-mm biopsies were taken from the tissue before freezing (n=1068) and follicular density (FD) was analysed by tissue digestion, calcein staining and mircoscopically counting.

- Concentration of AMH was measured (n=880) in serum, taken at the the time of tissue removal (ng/ml, Gen II Elisa Kit, Beckman Coulter GmbH).

- Normograms of AMH concentration and follicular density were drawn in relation to women's age.

Characteristics of trial participants, included in the analysis

Age (years, mean±SD, range)   28.11 (±6.92, 3-45 y)

AMH (ng/mL, mean±SD, range)   3.09 (±2.89, 0.2-23.1)
FD (mean, mean±SD, range)   163.81 (±98.71, 2-1751)

Diseases (n/total)
Breast cancer   546/1.068
Hodgkin's Lymphoma   193/1.068
Non-Hodgkin's Lymphoma   51/1.068
Leukemia   18/1.068
Sarcoma   53/1.068
Cerebral cancer   20/1068
Gastrointestinal cancer   26/1.068
Gynecological cancer   28/1.068
Other types of malignancies   20/1.068
Benign disease   48/1.068
Unknown diseases   65/1.068

FertiPROTEKT

Results Ia
Normogram FD

FertiPROTEKT
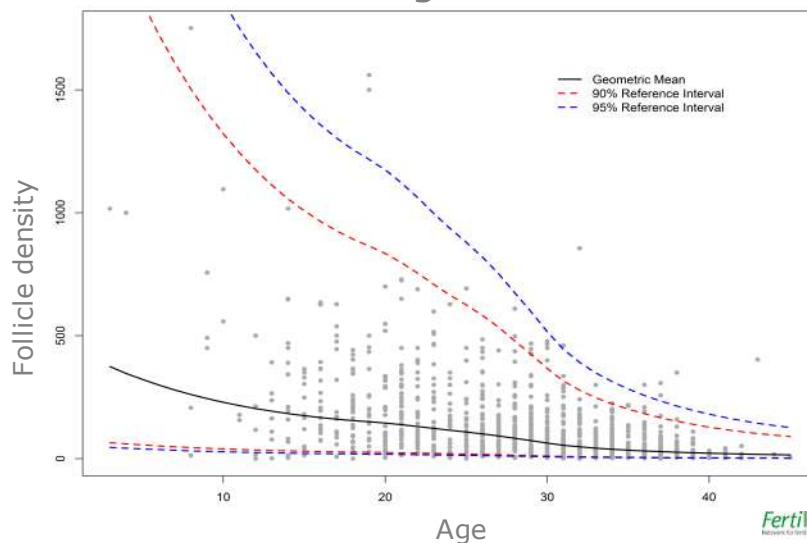
Results Ib
Normogram AMH
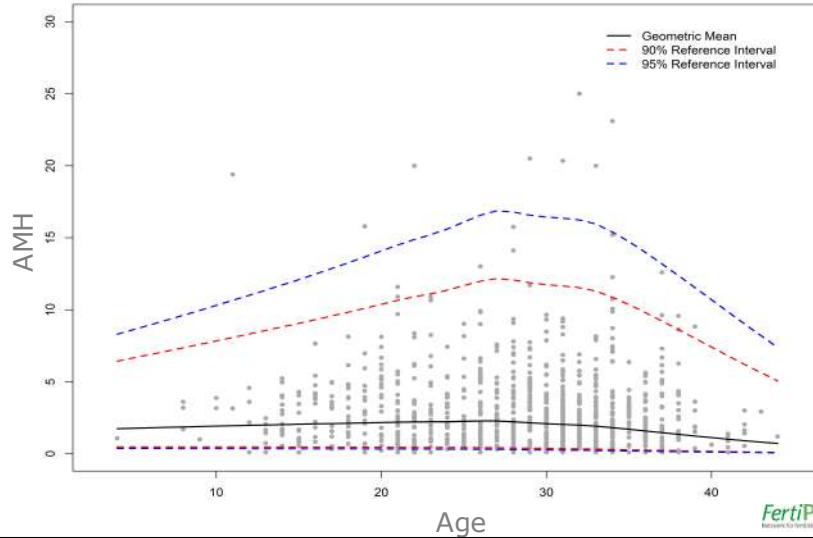
Results Ia+b
Data Normogram FD/AMH

FD

Alters klasse	n	Min	emp. 5%	est. 5%	emp. 10%	est. 10%	Mean	geom. MW	Median	emp. 90%	est. 90%	emp. 95%	est. 95%	Max
0-5	1	1000	1000	.	1000	.	6.90875	1000.00	1000.0	1000	.	1000	.	1000
6-10	5	13	13	4.5811	13	15.5668	5.59395	267.79	491.0	1751	4360.16	1751	12944.47	1751
11-15	35	0	1	5.7464	5	12.0657	4.81979	122.94	194.0	647	1174.66	1017	2275.88	1096
16-20	83	0	29	31.6997	59	47.3386	5.23685	187.08	206.0	500	730.77	627	1080.74	1560
21-25	137	4	18	23.6548	29	34.7228	4.87230	129.62	138.0	450	476.62	598	691.03	729
26-30	208	3	14	16.4988	25	24.0679	4.48223	87.43	95.5	309	310.96	426	445.89	610
31-35	283	1	6	6.8804	11	10.6021	3.80984	44.14	45.0	177	174.65	218	257.60	856
36-40	114	0	3	3.6846	6	6.0702	3.39238	28.74	30.5	126	124.07	191	187.76	350
41-45	14	2	2	1.0353	2	2.4428	2.92295	17.60	18.0	52	99.45	403	168.91	403

AMH

Alters klasse	n	Min	emp. 5%	est. 5%	emp. 10%	est. 10%	Mean	geom. MW	Median	emp. 90%	est. 90%	emp. 95%	est. 95%	Max
0-5	1	1.08	1.08	.	1.08	.	0.07696	1.08000	1.080	1.08	.	1.08	.	1.08
6-10	5	1.00	1.00	0.68708	1.00	0.97397	0.86728	2.38043	3.200	3.88	5.81788	3.88	8.2471	3.88
11-15	35	0.20	0.20	0.35033	0.44	0.51336	0.63381	1.88478	2.180	4.58	6.91992	5.23	10.1402	19.40
16-20	83	0.22	0.52	0.58537	0.67	0.79788	0.85077	2.34146	2.880	5.42	6.87120	7.65	9.3657	15.80
21-25	137	0.10	0.33	0.44068	0.53	0.62970	0.78540	2.19328	2.490	7.42	7.63935	9.03	10.9160	20.00
26-30	208	0.20	0.23	0.38421	0.43	0.56502	0.78164	2.18505	2.765	7.15	8.45011	9.00	12.4267	20.50
31-35	283	0.18	0.36	0.44174	0.56	0.62574	0.75431	2.12615	2.540	6.44	7.22423	8.48	10.2335	23.10
36-40	114	0.20	0.20	0.24308	0.20	0.36530	0.41460	1.51376	1.765	5.50	6.27288	7.31	9.4270	12.60
41-45	14	0.20	0.20	0.20368	0.20	0.29342	-0.05465	0.94682	1.200	2.93	3.05520	3.00	4.4014	3.00

Results II

Correlation analysis FD/AMH

P < 0.001
R = 0.2795
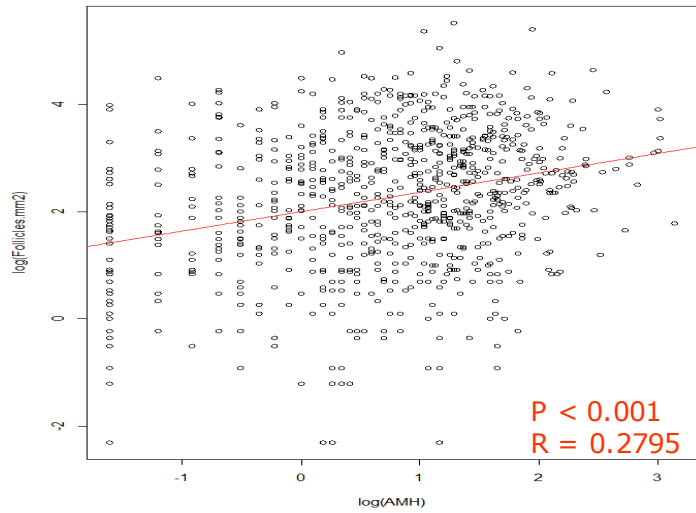
Results III

Correlation analysis FD/AMH in different age groups
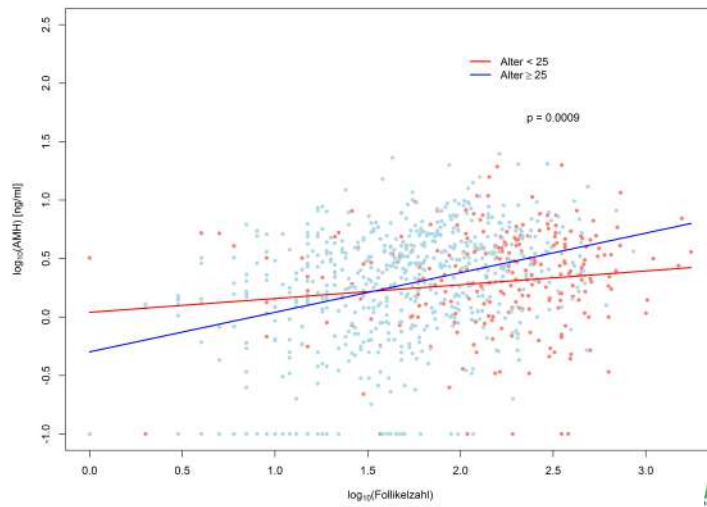
Results IV
Evaluation in relation to different diseases

Anamnese	Anzahl Beob	Etikett	N	Mittelwert	Std.abweichung	Std.fehler
A	454	Follikelanzahl	454	90.8193833	109.2151732	5.1257221
		FollAnzahl mm2	454	12.8463145	15.4507864	0.7251413
		AMH	454	3.2185683	2.9697055	0.1393752
B	161	Follikelanzahl	161	204.3975155	188.0262043	14.8195408
		FollAnzahl mm2	161	28.9163333	26.6002665	2.0963947
		AMH	161	2.5768385	2.2872887	0.1802636
C	40	Follikelanzahl	40	117.4750000	120.0042700	18.9743412
		FollAnzahl mm2	40	16.6193128	16.9771215	2.6843202
		AMH	40	2.7910000	2.7666615	0.4370219
D	15	Follikelanzahl	15	124.7333333	166.9464056	42.8471777
		FollAnzahl mm2	15	17.6461569	23.4766140	6.0616357
		AMH	15	1.8906667	1.5018059	0.3877646
E	43	Follikelanzahl	43	207.8372093	349.0682107	53.2324031
		FollAnzahl mm2	43	40.7206353	49.3830500	7.5308445
		AMH	43	3.5820930	2.6478674	0.4037990
F	17	Follikelanzahl	17	257.0588235	255.6477259	62.0621881
		FollAnzahl mm2	17	36.3663844	36.1960491	8.7784659
		AMH	17	3.9000000	3.1881029	0.7732285
G	22	Follikelanzahl	22	118.7727273	174.1351232	37.1257330
		FollAnzahl mm2	22	16.8026937	24.6350806	5.2522168
		AMH	22	4.0277273	3.5165553	0.7497321
H	20	Follikelanzahl	20	103.5000000	108.2290456	24.2007503
		FollAnzahl mm2	20	14.6422548	15.3112779	3.4237058
		AMH	20	3.3785000	2.7235470	0.6090036
I	12	Follikelanzahl	12	213.4166667	240.7833648	69.5081703
		FollAnzahl mm2	12	30.1922822	34.0638780	9.8333946
		AMH	12	2.4533333	1.9797628	0.5715083
J	44	Follikelanzahl	44	199.6363636	225.2652277	33.9600109
		FollAnzahl mm2	44	26.2427681	31.8685107	4.8043588
		AMH	44	2.9168182	4.0575498	0.6116987
K	52	Follikelanzahl	52	84.3076923	81.5724238	11.3120599
		FollAnzahl mm2	52	11.9270986	11.5401373	1.6003291
		AMH	52	3.2023077	3.0092567	0.4173088

Sarcoma, n=43

19.7y ± 5.1

AMH 3.6 ng/ml

40.7 follicles per mm^2

Gastrointest. cancer, n=22

32.1y ± 4.3

AMH 4.0 ng/ml

16.8 follicles per mm^2

Summary

I. The profile of the normograms are different for follicle density and AMH.

II. The correlation of follicle density and AMH is very low (R=0.28).

III. The correlation is especially low in younger women.

IV. AMH does not reflect the real follicle density.

Discussion

- Is AMH a good prognostic factor for the ovarian reserve?

- Is AMH a good prognostic factor for estimating the risk of infertility of women receiving gonadotoxic therapy?

- Should we rather use the follicle density to calculate the amout of ovarian tissue to be transplanted?

THANK YOU FOR YOUR ATTENTION!