DERIVATION OF RUSSIAN REFERENCE INTERVALS FOR ANTI-MÜLLERIAN HORMONE (AMH) USING ACCESS AMH ASSAY

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BACKGROUND

A study was organized to determine age-specific Russian reference intervals (RIs) for AMH in women and men and to compare the newly derived RIs with the published literature for other populations and by the assay manufacturer.

VOLUNTEERS

Following CLSI EP 28AC[1], volunteers aged 18-60 years were recruited: 436 women and 24 men; women were further grouped by age (Table 1).
Due to a small number of volunteers aged 18-25, the common group 18-30 was created.

Table 1 N of volunteers grouped by age

Age	Females	Males
18-25	20	
26-30	120	
31-35	120	
36-40	120	
41-45	28	
>46	28	
All ages	436	24

METHODS

AMH was measured on the Access 2 analyzer (Beckman Coulter, Inc.) at CIR Laboratories in serum collected without regard to menstrual cycle day. AFCs were assessed in women on days 1–4 of their menstrual cycles using transvaginal ultrasound (TVUS, Medison Ultrasound Systems).

AFCs were defined as the sum of follicles 2–10 mm in diameter in both ovaries. The women were divided into groups with non-detectable, low (<10) and normal (11-20) AFCs by TVUS. A non-parametric analysis of RIs was carried out using Stata 11 statistical analysis software. Pregnant women, women with polycystic ovary syndrome (PCOS) and women undergoing ovarian surgery were excluded from the study.

RESULTS 1

Final RIs for females by age and males; comparison with other studies

In the Russian population, women aged 26-30 and 31-35 years had median and upper limit (UL) values for AMH that were significantly higher than those provided by the manufacturer (median 3.38 vs. 2.27, 2.85 vs. 1.88; UL 11.03 vs. 7.37, 11.61 vs. 7.35, respectively, $p<0.05$). In comparison to a Brazilian study[2], the median and UL in the Russian population were lower for women aged 18-30 years group (median 3.35 vs. 3.7, respectively). In women aged 41-45, >46 and men, RIs were consistent with those provided by the manufacturer (Table 2).

Table 2 RIs for women grouped by age and men: comparison with other investigations

N	Units	Russian study, 95% CI					Instruction for use Access AMH[3], 95% CI					Brazilian study, 95% CI				
				Reference interval					Reference interval					Reference interval		
		Age	N	LL	Me	UL	Age	N	LL	Me	UL	Age	N	LL	Me	UL
Females																
1	ng/ml	18-30	140	0.47	3.35	11.17	18-25	80	0.96	3.71	13.34					
2	ng/ml	26-30	120	0.52	3.38	11.03	26-30	82	0.17	2.27	7.37	18-30	50	0.6	3.7	12.5
3	ng/ml	31-35	120	0.05	2.85	11.61	31-35	80	0.07	1.88	7.35					
4	ng/ml	36-40	120	0.03	1.59	6.60	36-40	80	0.03	1.62	7.15	31-40	37	0.50	2.40	9.60
5	ng/ml	41-45	28	RIs were verified*			3.27	79	0.00	0.29	3.27					
6	ng/ml	46+	28	RIs were verified*			1.15	82	0.00	0.01	1.15	41-50	13	<0.2	0.80	1.40
Males																
1	ng/ml	>18	24	RIs were verified*			>18	83	0.73	4.87	16.05					
2	ng/ml	18-30										18-30	63	1.90	6.30	20.50
3	ng/ml	31-40										31-40	29	2.70	6.0	14.80
4	ng/ml	41-50										41-50	12	2.00	4.70	16.20

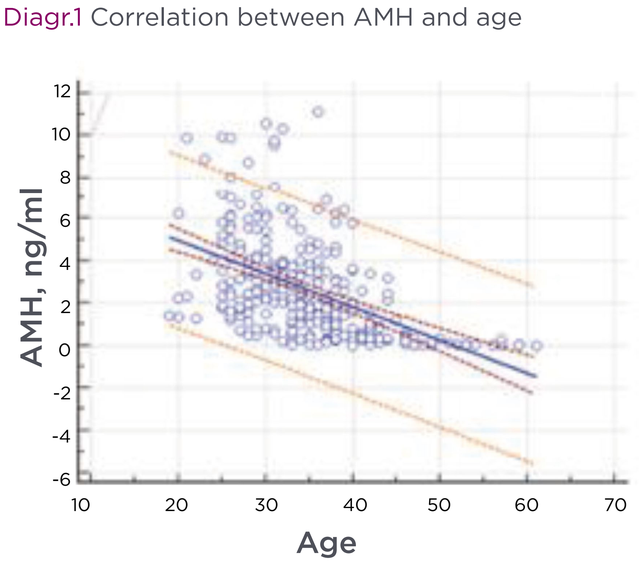
*LL – low limit; UL- upper limit; Me – Median, CI – confidence interval; *- RIs were verified to those provided in Instruction for use of Access AMH method.*

RESULTS 2

AMH decreases with age

Significant negative correlation between AMH and age was shown in women over 35 (r_p=-0.46; $p<0.05$) (Diagr.1)

Diagr.1 Correlation between AMH and age



RESULTS 3

AMH has significant positive correlation with antral follicle count

Positive correlation between AMH concentration and AFC in left and right ovaries was demonstrated (r_p=0.69; 0.64 respectively; $p<0.05$, Diagr.2). It was shown that in volunteers of the same age range, divided into groups with low and normal AFCs by TVUS, the average AMH level is significantly different: 2.6 ng/ml vs 1.15 ng/ml, respectively; $p<0.05$ (Table 3). AMH increases in groups with high AFC (Table 4)

Diagr.2 Correlation between AMH and AFC

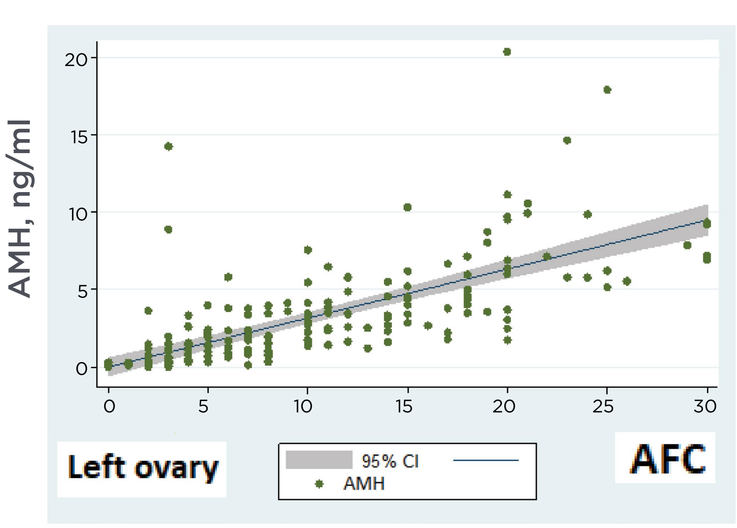


Table 3 Comparison of AMH mean values with AFC

	Normal AFC	Low AFC
N	20	20
Mean age	35.4	35.4
Mean AMH, ng/ml	2.6	1.15
95% CI	1.6-3.4	0.6-1.7

Table 4 AMH median values with different AFC

AFC	AMH ng/ml, 90% CI		
	LL	Median	UL
Non-detectable	0.01	0.01	0.03
Low	0.591	0.805	1.027
Normal	2.84	3.085	3.349

CONCLUSION

This study establishes Russian population-specific RIs for AMH using Beckman Coulter's Access AMH assay. These results reinforce the importance of obtaining population-specific reference intervals.

References
1) CLSI EP28-A3c document; Approved Guideline-Third Edition; 2010; 2) Woloszynek R.R. et al., Ann Clin Biochem. 2015; Jan;52 (Pt 1):67-75; 3) Access AMH, B13127, June 2017, Instruction for use