

Article

Supplementary Materials: Circulating miRNAs in Women with Polycystic Ovary Syndrome: A Longitudinal Cohort Study

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Supplementary Table S1: List of microRNAs, Taqman, miRNA mature sequences tested on the 96-miR custom card and phenotype associations previously reported

Mature miRNA	TaqMan ID	Sequence	Phenotype association	Pubmed reference ID
ath-miR-159a	338	uuuggauugaagggagcucua	-	
cel-miR-39-3p	200	ucaccggguguaaaucagcuug	-	
dme-miR-7-5p	268	uggaagacuagugauuuuguugu	-	
U6	1973	-	-	
miR-133a-3p	2246	uuugguccccuuaaccagcug	PCOS (FF)	PMID: 23666971
miR-625-3p	2431	gacuaauagaacuuuuuuuuu	Associated with cholesterol levels	PMID: 24784704
mmu-miR-140-5p	1187	cagugguuuuuacccuauugguag	Associated with granulosa cell proliferation	PMID: 32024547
miR-1225-3p	2766	ugagccccugugccgccccag	Associated with inflammation	PMID: 29496996
miR-519b-3p	2384	aaagugcauccuuuuuagagguu	Associated with inflammation	PMID: 32146774
miR-221-3p	524	agcuacauugucugggguuuc	Associated with inflammation	PMID: 35120179
miR-107	443	agcagcauuguacagggcuauca	Associated with insulin resistance	PMID: 21654750
miR-664a-3p	2897	uauucauuuuauccccagccuaca	Associated with insulin resistance	PMID: 29495974
miR-363-3p	1271	aaugcacgguaucacugua	Associated with insulin resistance	PMID: 32104036
miR-886-3p	2194	cgcgggugcuuacugacccuu	Associated with metabolic syndrome	PMID: 34294131
miR-122-5p	2245	uggagugugacaugguguuug	Associated with metabolic syndrome and Type 2 diabetes	PMID: 27899485
miR-30b-5p	602	uguaaacauccuacacucagcu	Associated with NAFLD	PMID: 28119530

miR-190b	2263	ugauauguuugauauuggguu	Associated with NAFLD	PMID: 29575055
miR-629-5p	2436	uggguuuacguugggagaacu	Biomarker of ovarian cancer	PMID: 28972400
miR-193b-3p	2367	aacuggcccucaaaguccgcgu	Circulating biomarker for impaired glucose tolerance in PCOS	PMID: 26860517
miR-194-5p	493	uguaacagcaacuccaugugga	Circulating biomarker for impaired glucose tolerance in PCOS	PMID: 26860517
miR-19b-3p	396	ugugcaaaucgaugcaaacuga	Circulating biomarker for PCOS	PMID: 32411089
miR-17-5p	2308	caaagugcuuacagucagguag	Circulating biomarker of metabolic syndrome	PMID: 23032062
miR-195-5p	494	uagcagcacagaaauauuggc	Circulating biomarker of metabolic syndrome	PMID: 23032062
miR-324-3p	2161	acugccccaggugcugcugg	Circulating biomarker of metabolic syndrome	PMID: 32353552
miR-148a-3p	470	ucagugcacuacagaacuuugu	Circulating biomarker of metabolic syndrome	PMID: 34954485
miR-215-5p	518	augaccuaugaauugacagac	Circulating biomarker of obesity	PMID: 30651891
miR-532-5p	1518	caugccuugaguguaggaccgu	Circulating biomarker of ovarian cancer	PMID: 26873729
miR-376a-3p	565	aucauagaggaaaauccacgu	Circulating biomarker of ovarian cancer	PMID: 27979415
miR-518b	1156	caaagcgcuccccuuuagaggu	Circulating biomarker of ovarian cancer	PMID: 32083575
miR-1305	2867	uuuuaacucuaaugggagaga	Circulating biomarker of ovarian cancer	PMID: 32854743
miR-766-3p	1986	acuccagccccacagccucagc	Circulating biomarker of ovarian cancer	PMID: 32854743
miR-1233-3p	2768	ugagcccuguccccgcag	Circulating biomarker of ovarian cancer	PMID: 34899827
miR-222-3p	2276	agcuacaucuggcuacugggu	PCOS (circulating)	PMID: 24802714
miR-30c-5p	419	uguaaacauccuacacucucagc	PCOS (circulating)	PMID: 24802714
miR-223-3p	2295	ugucaguuugucaaaauaccca	PCOS (circulating)	PMID: 26582398

mmu-miR-93-5p	1090	caaagugcuguucgugcagguag	PCOS (circulating)	PMID: 26582398
miR-4522	462414_mat	ugacucugccuguaggccggu	PCOS (circulating)	PMID: 27677182
miR-6767-5p	466470_mat	ucgcagacaggacacauggaga	PCOS (circulating)	PMID: 27677182
miR-23a-3p	399	aucacauugccagggauuucc	PCOS (circulating)	PMID: 28193283
miR-23b-3p	400	aucacauugccagggauuacc	PCOS (circulating)	PMID: 28193283
miR-9-5p	583	ucuuugguuaucuagcuguauga	PCOS (circulating)	PMID: 30794820
mmu-miR-451a	1141	aaaccguuaccauuacugaguu	PCOS (circulating)	PMID: 31730174
miR-139-5p	2289	ucuacagugcacguguccagu	PCOS (circulating)	PMID: 31737638
miR-143-3p	2249	ugagaugaagcacuguagcuc	PCOS (circulating)	PMID: 31737638
miR-21-5p	397	uagcuuauacagacugauguuga	PCOS (circulating)	PMID: 31737638
miR-34a-5p	426	uggcagugucuauagcugguugu	PCOS (circulating)	PMID: 31737638
miR-34b-3p	2102	caaucacuaacuccacugccau	PCOS (circulating)	PMID: 31737638
miR-361-5p	554	uuaucagaauuccaggguac	PCOS (circulating)	PMID: 31737638
miR-433-3p	1028	aucaugaugggcuccucggugu	PCOS (circulating)	PMID: 31737638
miR-485-3p	1277	gucauacacggcuccucucu	PCOS (circulating)	PMID: 31737638
miR-572	1614	guccgcucggcguggccca	PCOS (circulating)	PMID: 31737638
miR-27b-3p	409	uucacaguggcuaaguucugc	PCOS (circulating)	PMID: 32848854

miR-106a-5p	2169	aaaagugcuuacagugcagguag	PCOS (circulating)	PMID: 33041053
miR-126-3p	2228	ucguaccgugaguaaauaugcg	PCOS (circulating)	PMID: 33041053
miR-146a-5p	468	ugagaacugaaauccauggguu	PCOS (circulating)	PMID: 33041053
miR-18b-5p	2217	uaaggugcaucuagugcaguuag	PCOS (circulating)	PMID: 33041053
miR-20a-5p	580	uaaagugcuauagugcagguag	PCOS (circulating)	PMID: 33041053
miR-16-5p	391	uagcagcacguaauauuggcg	PCOS (circulating)	PMID: 33148068
miR-145-5p	2278	guccaguuuuccaggaaucccu	PCOS (circulating)	PMID: 33763302
miR-192-5p	491	cugaccuaugaauugacagcc	PCOS (circulating)	PMID: 33763302
miR-103a-3p	439	agcagcauuguacagggcuauga	PCOS (circulating)	PMID: 34403358
miR-342-3p	2260	ucucacacagaaucgcacccgu	Insulin resistance (circulating)	PMID: 24784704
miR-331-3p	545	gccccugggccuauccuagaa	Fatty acids (circulating)	PMID: 24784704
miR-330-3p	544	gcaaagcacacggccugcagaga	Circulating levels regulated by fatty acids	PMID: 26092372
miR-769-5p	1998	ugagaccucuggguucugagcu	Circulating levels regulated by fatty acids	PMID: 26092372
miR-155-5p	2623	uuaaugcuaaucgugauaggggu	Circulating biomarker of metabolic syndrome and inflammation	PMID: 26421388
miR-130a-3p	454	cagugcaaugaugaagggcrau	Controls theca cell androgen production	PMID: 31184707
miR-142-3p	464	uguaguguuuccuacuuuaugga	Dysregulated in PCOS granulosa cells	PMID: 30865275

miR-423-5p	2340	ugaggggcagagagcgagacuuu	Dysregulated in PCOS granulosa cells	PMID: 30865275
miR-135a-5p	460	uauggcuuuuuauuccuauuguga	Dysregulated in PCOS granulosa cells	PMID: 31173821
miR-429	1024	uaauacugucugguaaaaccgu	Dysregulated in PCOS granulosa cells	PMID: 32759991
miR-224-5p	2099	caagucacuagugguuccguu	Granulosa cell proliferation	PMID: 20118412
miR-28-3p	2446	cacuagauugugagcuccugga	Granulosa cell proliferation	PMID: 31322191
miR-200b-3p	2251	uaauacugccugguaaugauga	Granulosa cell proliferation	PMID: 31421682
let-7d-5p	2283	agagguaguagguugcauaguu	Granulosa cell proliferation	PMID: 34246727
miR-448	1029	uugcauauaguaggauugccau	Involved in ovarian cancer growth	PMID: 26103953
miR-520c-3p	2400	aaagugcuuccuuuuagagggu	PCOS (FF)	PMID: 23666971
miR-383-5p	573	agaucagaaggugauuguggcu	PCOS (FF)	PMID: 24828505
miR-132-3p	457	uaacagucuacagccauggucg	PCOS (FF)	PMID: 26771704
miR-151-3p	2254	cuagacugaagcuccuugagg	PCOS (FF)	PMID: 26771704
miR-151-5p	2642	ucgaggagcucacagucuagu	PCOS (FF)	PMID: 26771704
miR-24-3p	402	uggcucaguucagcaggaacag	PCOS (FF)	PMID: 26771704
miR-29a-3p	2112	uagcaccaucugaaaucgguua	PCOS (FF)	PMID: 26771704
miR-320a	2277	aaaagcuggguugagagggcga	PCOS (FF)	PMID: 26771704
miR-518f-3p	2388	gaaagcgcuucucuuuagagg	PCOS (FF)	PMID: 26771704
miR-574-3p	2349	cacgcuaugcacacaccaca	PCOS (FF)	PMID: 26771704
miR-720	2895	ucucgcuggggccucca	PCOS (FF)	PMID: 26771704
miR-127-3p	452	ucggauccgucugagcuuggcu	PCOS (FF)	PMID: 31705013
miR-199b-5p	500	cccaguguuuagacuaucuguuc	PCOS (FF)	PMID: 31705013

miR-484	1821	ucaggcucaguccccuccgau	Possible reference miR	PMID: 31737638
miR-1267	2885	ccuguugaaguguaaucccca	Preliminary inhouse data	-
miR-618	1593	aaacucuacuuguccuucugagu	Preliminary inhouse data	-
miR-636	2088	ugugcuugcuguccgcccgca	Preliminary inhouse data	-
miR-138-5p	2284	agcugguguugugaaucaggccg	Regulated by estradiol	PMID: 24205079
mmu-miR-96-5p	186	uuuggcacuagcacauuuuugcu	Regulated by estradiol	PMID: 24205079
miR-10b-5p	2218	uaccuguagaaccgaauuugug	Regulated by estradiol	PMID: 33982889

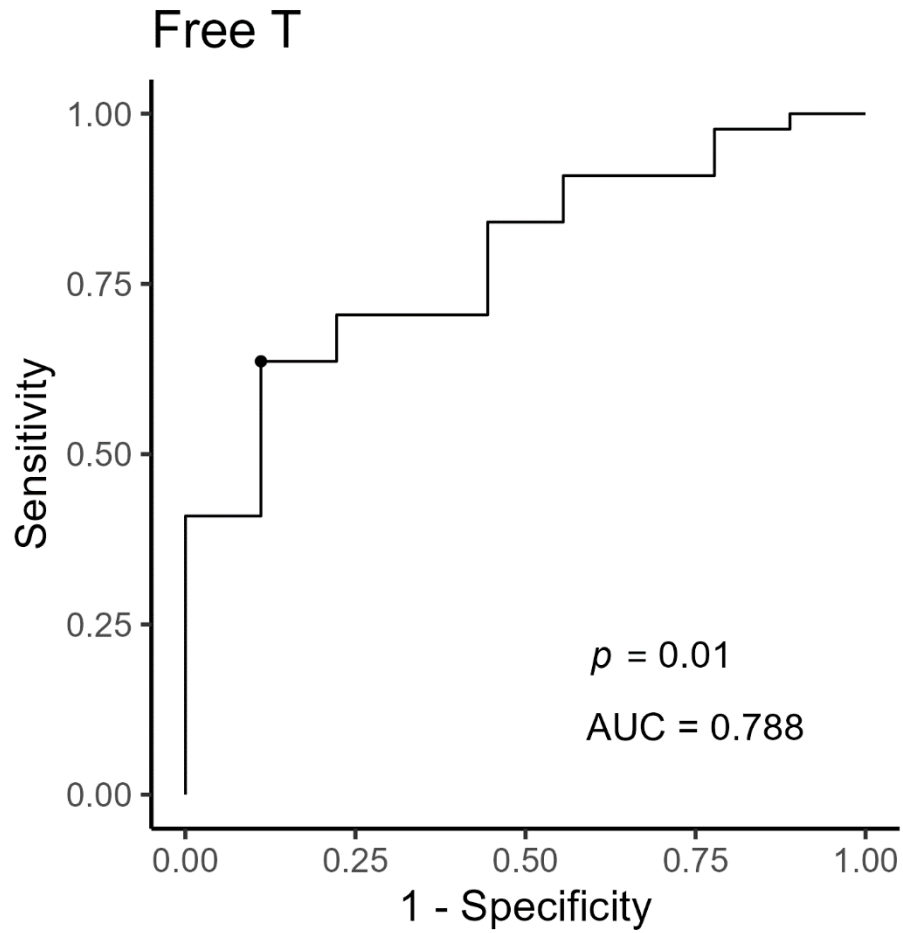
MicroRNAs are human microRNAs (*hsa-miR*) unless otherwise specified. Some assays were designed for murine miRNAs, but in these cases the miRNA was completely homologous. PCOS (FF): Levels in follicular fluid associated with PCOS, PCOS (circulating): Levels in circulation associated with PCOS. NAFLD: Non-alcoholic fatty liver disease.

Supplementary Table S2: Change in miRNA levels in women without PCOS from BL to FU.

miRNA Control	Reported association	Difference in dCt (\pm SD)	Direction	n	p	p _{adj}
miR-451a	PCOS (circulating)	6.5 \pm 3.5	Decreased	7	0.003	0.29
miR-16-5p	PCOS (circulating)	5.1 \pm 4.7	Decreased	6	0.044	1.0
miR-223-3p	PCOS (circulating)	4.4 \pm 3.8	Decreased	8	0.013	1.0
miR-532-5p	Ovarian cancer (circulating)	4.4 \pm 1.1	Decreased	3	0.021	1.0
miR-1305	Ovarian cancer (circulating)	4.1 \pm 2.1	Decreased	4	0.03	1.0
miR-24-3p	PCOS (FF)	4.1 \pm 3.9	Decreased	7	0.032	1.0
miR-342-3p	Circulating levels associated with insulin resistance	3.8 \pm 3.1	Decreased	7	0.017	1.0
miR-720	PCOS (FF)	3.4 \pm 2.8	Decreased	8	0.01	0.96
miR-145-5p	PCOS (circulating)	3.3 \pm 1.8	Decreased	7	0.003	0.29
miR-127-3p	PCOS (FF)	3.1 \pm 0.7	Decreased	3	0.017	1.0
miR-151-3p	PCOS (FF)	3.1 \pm 2	Decreased	7	0.006	0.58
miR-146a-5p	PCOS (circulating)	3 \pm 2.7	Decreased	6	0.042	1.0
miR-130a-3p	Controls theca cell androgen production	3 \pm 0.4	Decreased	3	0.005	0.48
miR-320a-3p	PCOS (FF)	2.6 \pm 1	Decreased	7	0.001	0.10
miR-423-5p	Dysregulated in PCOS granulosa cells	2.3 \pm 0.8	Decreased	4	0.01	0.96
miR-886-3p	Associated with metabolic syndrome	1.8 \pm 1	Decreased	5	0.018	1.0
miR-518f-3p	PCOS (FF)	7.4 \pm 6.9	Increased	6	0.047	1.0
miR-520c-3p	PCOS (FF)	10.6 \pm 7.5	Increased	6	0.018	1.0
miR-618	-	11.1 \pm 9	Increased	6	0.03	1.0
miR-636	-	11.7 \pm 7	Increased	4	0.044	1.0
miR-518b	Ovarian cancer (circulating)	15.9 \pm 8.9	Increased	5	0.017	1.0

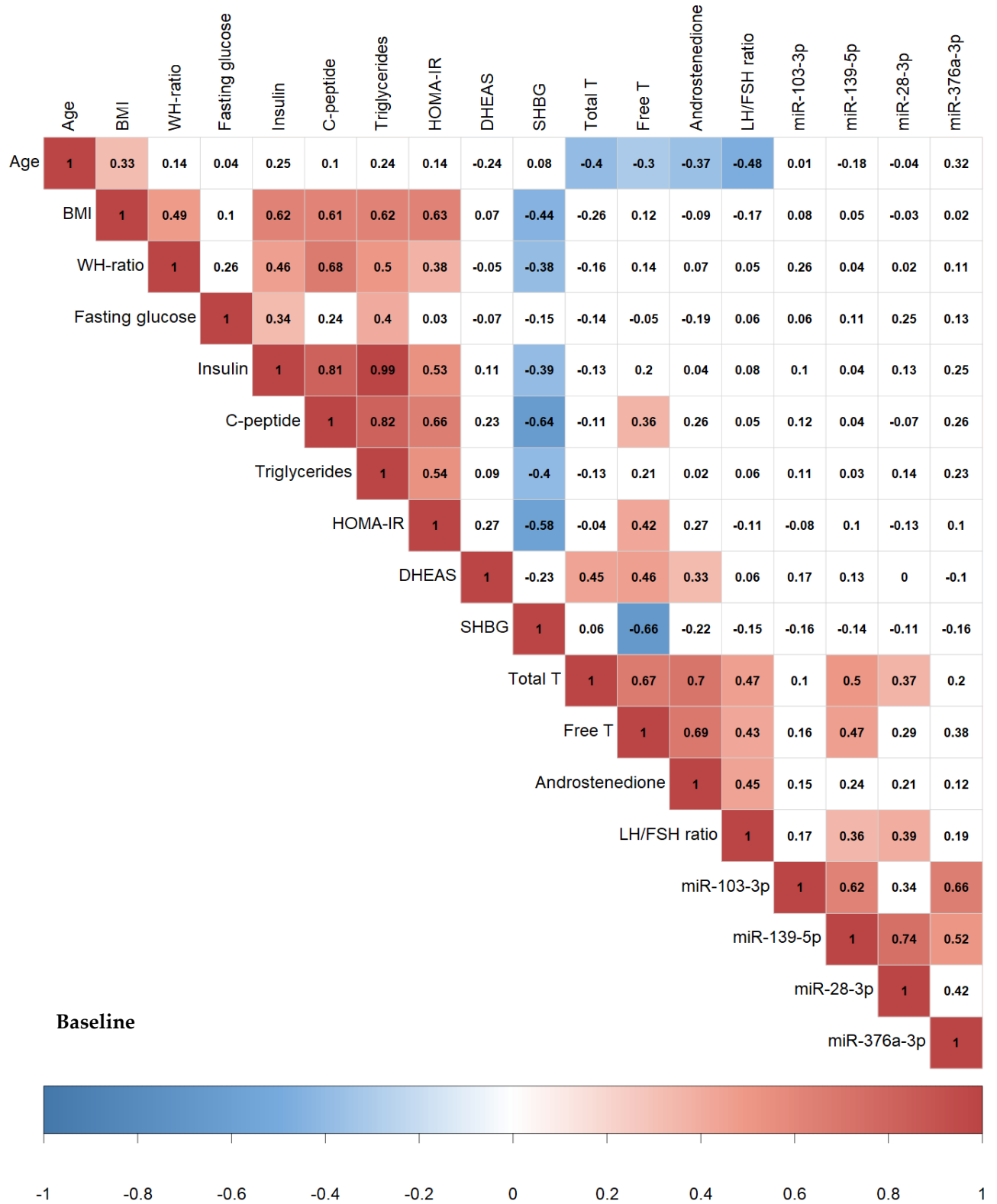
Supplementary Table S2: Changes in miRNA levels from BL to FU in control women. Shown are the mean fold change \pm standard deviation as well as the number of women included. Changes in expression levels were evaluated with a paired student t-test. *) also reported in PCOS women in the current study. P values were corrected for multiple testing using Bonferroni corrections. PCOS (FF): Levels in follicular fluid associated with PCOS, PCOS (circulating): Levels in circulation associated with PCOS. Ovarian cancer (circulating): Circulating biomarker of ovarian cancer.

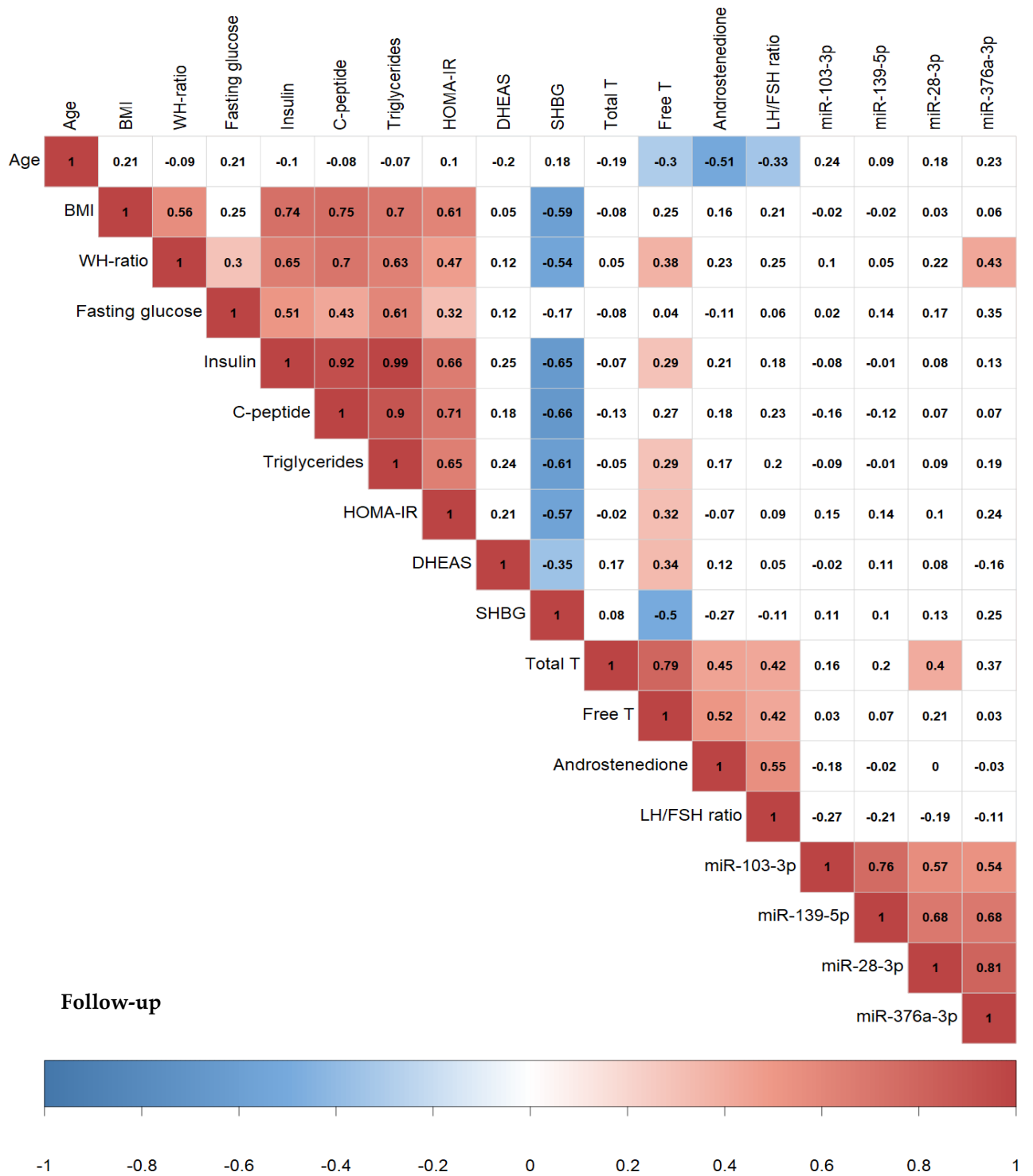
Supplementary Figure S1: ROC curve of free T



Supplementary Figure S1: ROC curve of free T in which age and BMI at BL was included in the logistic regression model. $n = 53$, sensitivity = 63% while specificity = 89%.

Supplementary Figure S2: Correlations between clinical and biochemical variables and miRNAs for women with PCOS, at BL or FU.





Supplementary Figure S2: Correlations between clinical and biochemical variables and the selected miRNAs, at either BL or FU, measured in women with PCOS. Colors represent significant ($p \leq 0.05$) correlations by Spearman rho with color intensity ranging from -1 to +1.