

Manuscript Title: Murine Oviductosomes (OVS) microRNA profiling during the estrous cycle: Delivery of OVS-borne microRNAs to sperm where *miR-34c-5p* localizes at the centrosome

Zeinab Fereshteh¹, Skye A. Schmidt², Amal A. Al-Dossary^{1,3}, Monica Accerbi², Cecilia Arighi⁴, Julie Cowart⁴, Jia L. Song¹, Pamela J. Green², Kyungmin Choi⁵, Soonmoon Yoo⁵ and Patricia A. Martin-DeLeon¹

¹Department of Biological Sciences, University of Delaware, Newark, DE 19716, USA

²Department of Plant and Soil Sciences, Delaware Biotechnology Institute, University of Delaware, Newark, DE 19711, USA

³Present Address: Department of Biology, College of Medicine, Imam Abdulrahman Bin Faisal University, P.O. Box 1982, Dammam 31441, Saudi Arabia

⁴Center for Bioinformatics and Computational Biology, University of Delaware, Newark, DE 19711, USA

⁵A.I. DuPont Hospital for Children, 1600 Rockland Rd, Wilmington, Delaware 19803, USA

- Corresponding author: 219 McKinly Lab, Univ. Delaware, Newark, DE 19716, USA, Tel: 302- 831-2249: Fax: 302-831-2281, pdeleon@udel.edu

-

Running Title: The microRNA repertoire of oviductosomes includes microRNAs present in sperm

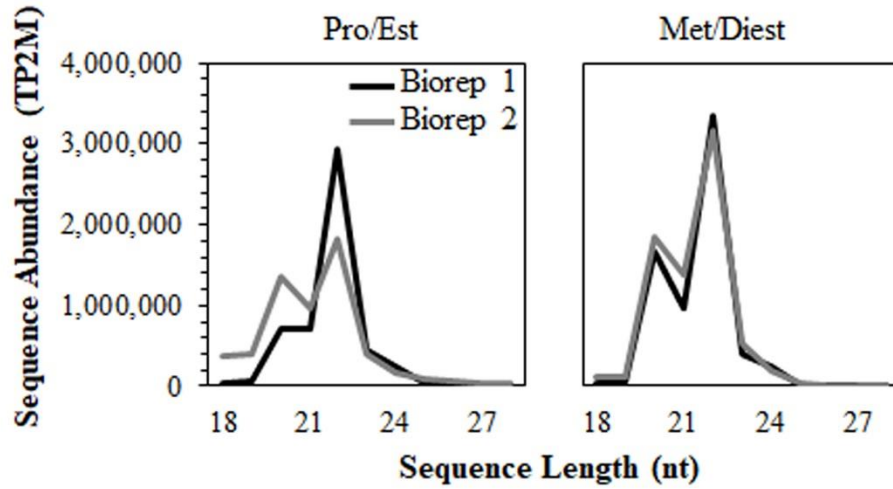
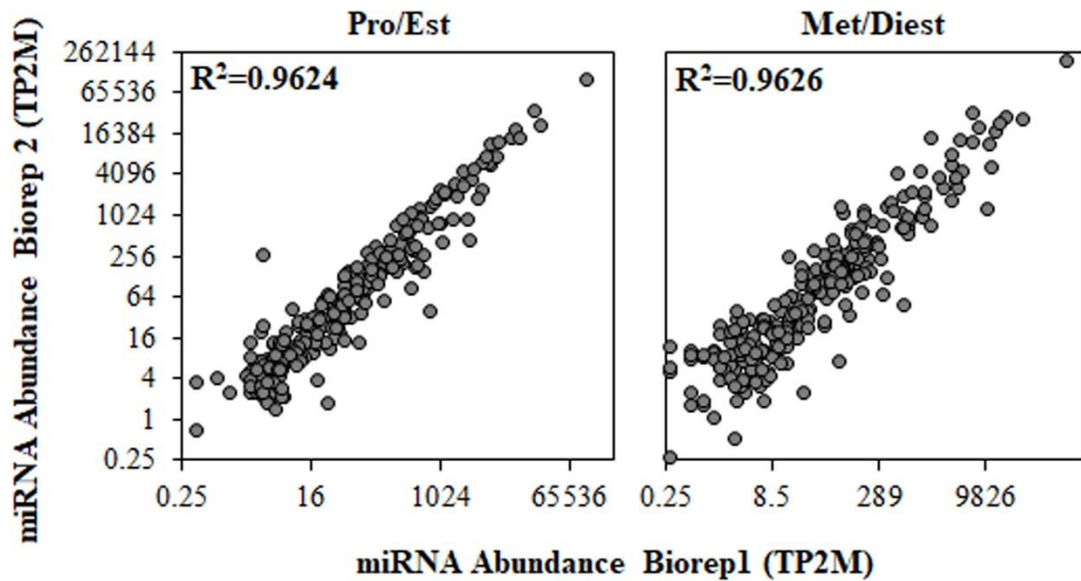
A**B**

Fig. S1. Evaluation of the sequencing data after normalization shows good agreement between biological replicates for **A)** the sequence size profile and **B)** miRNA sequence abundance, for Pro/Est and Met/Diest samples. A strong positive correlation is shown in B.

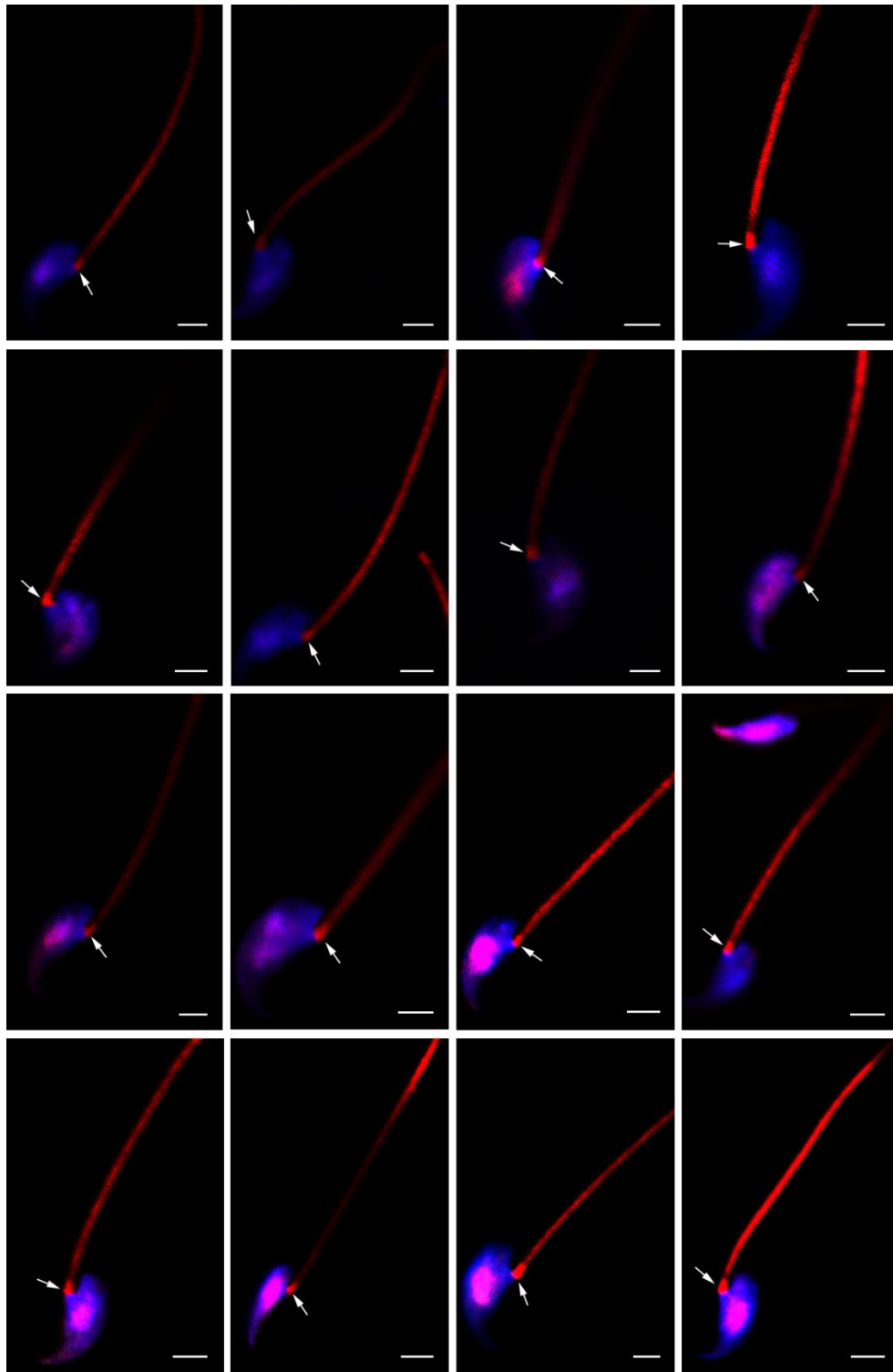


Fig. S2- Snapshot of 12 sperm after co-incubation with labeled *miR-34c-5p*. All show the red label localized at a high intensity at/near the centrosome (arrowed) after the miRNA internalization. Scale bars = 2 μ m

Fig. S3. Videos showing the internalization of the labeled miRNAs in 3D reconstructions

Video S3a: 3D volume rendering of a series of maximum intensity projections of a sperm co-incubated with OVS loaded with labeled *miR-143-3p* and *miR-34c-5p*

Video S3b: 3D volume rendering of a series of maximum intensity projections of a control sperm co-incubated with medium with free labeled miRNAs.

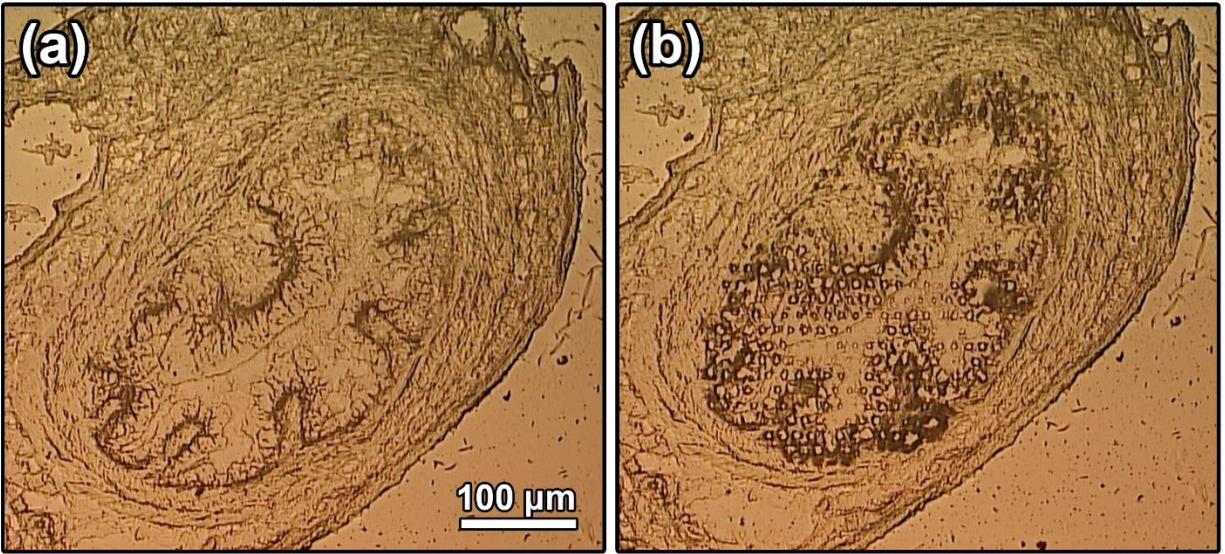


Fig. S4- LM-Mediated isolation of epithelial cells from 20 μm thick sections of the oviductal tissue. a) shows a cross-section of the oviductal tissue before and **b)** after microdissected.

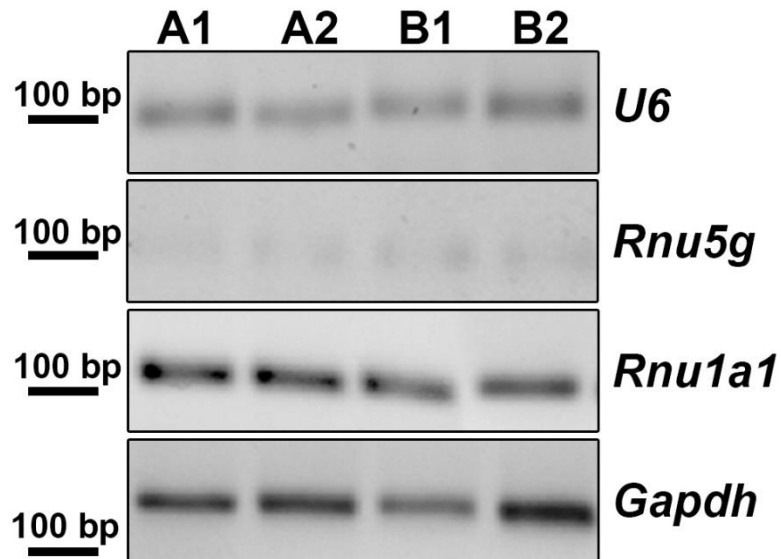


Fig. S5- Selecting an endogenous control from four RNAs. Four endogenous transcripts, *U6 snRNA*, *Rnu1a1A1*, *Rnu5g*, and *Gapdh*, were used to find the best internal control for the samples. There were two bioreplicates of purified OVS from pro/estrus (A) and met/diestrus (B). Gel electrophoresis of the RT-PCR products of ~ 100 bp are seen on 2% agarose gel stained with ethidium bromide. *U6 snRNA* was selected to normalize all samples, due to being consistent with all samples.

Table S1

miRNA	A Bio1 Abundance (TP2M)	A Bio2 Abundance (TP2M)	B Bio1 Abundance (TP2M)	B Bio2 Abundance (TP2M)	Fold Change Bio1 (A/B)	Fold Change Bio2 (A/B)	Log2 Fold Change Bio1	Log2 Fold Change Bio2
mmu-miR-451a	3.4416	271.2108	0.2952	11.8492	11.6585	22.8885	3.5433	4.5166
mmu-miR-1940	3.4416	2.0916	0.2952	0.2693	11.6585	7.7668	3.5433	2.9573
mmu-miR-488-3p	2.2944	2.4402	2.3616	0.5386	0.9715	4.5306	-0.0416	2.1797
mmu-miR-3107-5p	425.6112	298.4016	319.9968	69.2101	1.3300	4.3115	0.4115	2.1082
mmu-miR-1843a-3p	8.7952	11.1552	5.0184	3.5009	1.7526	3.1864	0.8095	1.6719
mmu-miR-541-5p	57.36	32.7684	10.9224	11.3106	5.2516	2.8971	2.3928	1.5346
mmu-miR-350-3p	52.7712	68.6742	1.476	24.237	35.7528	2.8334	5.1600	1.5026
mmu-miR-30c-2-3p	4.5888	12.8982	4.428	4.5781	1.0363	2.8174	0.0515	1.4943
mmu-miR-15b-5p	8.4128	42.1806	1.476	17.7738	5.6997	2.3732	2.5109	1.2468
mmu-miR-185-5p	3.824	2.4402	1.1808	1.0772	3.2385	2.2653	1.6953	1.1797
mmu-miR-31-3p	45.888	36.603	16.8264	16.6966	2.7271	2.1922	1.4474	1.1324
mmu-miR-125a-3p	4.2064	3.486	0.5904	1.6158	7.1247	2.1574	2.8328	1.1093
mmu-miR-339-5p	2.2944	4.8804	0.5904	2.4237	3.8862	2.0136	1.9584	1.0098
mmu-miR-32-5p	2.6768	3.1374	0.8856	1.6158	3.0226	1.9417	1.5958	0.9573
mmu-miR-148b-5p	8.0304	7.3206	4.1328	3.7702	1.9431	1.9417	0.9584	0.9573
mmu-miR-34c-3p	11.472	13.944	2.3616	7.2711	4.8577	1.9177	2.2803	0.9394
mmu-miR-141-3p	71.8912	13.2468	76.4568	7.2711	0.9403	1.8218	-0.0888	0.8654
mmu-miR-125a-5p	48.9472	133.8624	31.2912	73.7882	1.5642	1.8141	0.6455	0.8593
mmu-miR-301b-3p	5.3536	5.5776	2.6568	3.2316	2.0151	1.7260	1.0108	0.7874
mmu-miR-92a-3p	135.3696	162.099	94.464	93.9857	1.4330	1.7247	0.5191	0.7864
mmu-miR-92b-3p	18.7376	18.1272	9.7416	10.772	1.9235	1.6828	0.9437	0.7509
mmu-miR-30b-5p	728.8544	1358.4942	229.3704	826.751	3.1776	1.6432	1.6680	0.7165
mmu-miR-872-5p	47.4176	38.346	49.0032	23.4291	0.9676	1.6367	-0.0475	0.7108
mmu-miR-191-5p	5069.8592	5414.1066	3666.384	3367.8658	1.3828	1.6076	0.4676	0.6849
mmu-miR-425-5p	3.824	7.6692	1.7712	4.8474	2.1590	1.5821	1.1104	0.6619
mmu-miR-154-5p	6.1184	11.5038	5.0184	7.5404	1.2192	1.5256	0.2859	0.6094
mmu-miR-511-3p	5.736	12.201	0.8856	8.079	6.4770	1.5102	2.6953	0.5948

mmu-miR-106b-5p	53.1536	150.9438	32.7672	101.5261	1.6222	1.4867	0.6979	0.5722
mmu-miR-574-3p	306.6848	204.9768	81.18	140.8439	3.7778	1.4553	1.9176	0.5414
mmu-miR-376b-3p	4.5888	13.2468	2.952	9.1562	1.5545	1.4468	0.6364	0.5328
mmu-miR-200b-5p	14.5312	19.5216	10.0368	13.7343	1.4478	1.4214	0.5339	0.5073
mmu-miR-200b-3p	185.0816	179.8776	64.6488	126.8403	2.8629	1.4181	1.5175	0.5040
mmu-miR-375-3p	119.3088	176.043	79.1136	127.3789	1.5081	1.3820	0.5927	0.4668
mmu-miR-3068-5p	3.824	5.5776	1.7712	4.0395	2.1590	1.3808	1.1104	0.4655
mmu-miR-31-5p	258.8848	307.8138	200.4408	224.3269	1.2916	1.3722	0.3691	0.4565
mmu-miR-450b-5p	6.5008	12.8982	1.1808	9.4255	5.5054	1.3684	2.4609	0.4525
mmu-miR-30c-5p	1528.4528	901.4796	777.2616	662.2087	1.9665	1.3613	0.9756	0.4450
mmu-miR-200a-3p	185.464	299.796	113.9472	222.9804	1.6276	1.3445	0.7028	0.4271
mmu-miR-376c-3p	2.2944	13.5954	1.1808	10.2334	1.9431	1.3285	0.9584	0.4098
mmu-miR-34b-3p	36.7104	31.0254	21.5496	23.4291	1.7035	1.3242	0.7685	0.4051
mmu-miR-192-5p	2779.2832	3423.9492	3860.6256	2586.8958	0.7199	1.3236	-0.4741	0.4044
mmu-miR-3068-3p	2.6768	2.4402	0.8856	1.8851	3.0226	1.2945	1.5958	0.3724
mmu-miR-221-5p	8.4128	8.0178	3.8376	6.1939	2.1922	1.2945	1.1324	0.3724
mmu-miR-411-5p	126.5744	344.7654	120.7368	268.4921	1.0483	1.2841	0.0681	0.3607
mmu-miR-34c-5p	3885.5664	2426.9532	1324.8576	1893.4483	2.9328	1.2818	1.5523	0.3581
mmu-miR-429-3p	331.9232	393.2208	175.644	308.8871	1.8897	1.2730	0.9182	0.3483
mmu-miR-351-5p	7.2656	19.8702	3.5424	15.6194	2.0510	1.2721	1.0364	0.3473
mmu-miR-582-3p	6.5008	14.2926	14.1696	11.5799	0.4588	1.2343	-1.1241	0.3036
mmu-miR-16-5p	1014.5072	2352.7014	647.6688	1907.9905	1.5664	1.2331	0.6475	0.3023
mmu-miR-30e-3p	163.2848	240.8826	138.1536	195.5118	1.1819	1.2321	0.2411	0.3011
mmu-miR-125b-2-3p	5.736	12.8982	5.0184	10.5027	1.1430	1.2281	0.1928	0.2964
mmu-miR-19a-3p	31.3568	34.5114	14.76	28.2765	2.1244	1.2205	1.0871	0.2875
mmu-let-7g-5p	818.336	1559.6364	10722.2544	1280.2522	0.0763	1.2182	-3.7118	0.2848
mmu-miR-204-5p	592.3376	151.2924	368.4096	124.4166	1.6078	1.2160	0.6851	0.2822
mmu-miR-138-5p	23.3264	57.8676	11.808	47.6661	1.9755	1.2140	0.9822	0.2798
mmu-miR-200c-3p	391.5776	381.0198	117.1944	314.0038	3.3413	1.2134	1.7404	0.2791
mmu-miR-301a-3p	279.5344	390.0834	143.172	322.6214	1.9524	1.2091	0.9653	0.2739
mmu-miR-421-3p	3.0592	4.5318	1.476	3.7702	2.0726	1.2020	1.0515	0.2654

mmu-miR-182-5p	607.6336	270.8622	301.104	226.212	2.0180	1.1974	1.0129	0.2599
mmu-miR-30a-3p	136.8992	198.0048	104.5008	167.5046	1.3100	1.1821	0.3896	0.2413
mmu-miR-423-3p	96.3648	87.4986	39.5568	74.3268	2.4361	1.1772	1.2846	0.2354
mmu-miR-299a-5p	0.3824	3.486	5.6088	2.9623	0.0682	1.1768	-3.8745	0.2349
mmu-miR-96-5p	39.0048	17.7786	11.2176	15.3501	3.4771	1.1582	1.7979	0.2119
mmu-miR-125b-5p	302.8608	717.4188	114.5376	622.8909	2.6442	1.1518	1.4028	0.2038
mmu-miR-425-3p	3.824	2.7888	3.5424	2.4237	1.0795	1.1506	0.1104	0.2024
mmu-miR-15a-5p	19.8848	34.86	2.6568	30.4309	7.4845	1.1455	2.9039	0.1960
mmu-miR-423-5p	24.0912	29.9796	7.6752	26.6607	3.1388	1.1245	1.6502	0.1693
mmu-miR-3102-3p	9.1776	11.5038	2.952	10.2334	3.1089	1.1241	1.6364	0.1688
mmu-miR-383-5p	5.736	4.5318	3.8376	4.0395	1.4947	1.1219	0.5798	0.1659
mmu-miR-10a-5p	4635.0704	6098.757	3266.388	5478.1006	1.4190	1.1133	0.5049	0.1548
mmu-miR-495-3p	6.5008	2.0916	2.6568	1.8851	2.4469	1.1095	1.2909	0.1500
mmu-miR-674-3p	4.5888	8.3664	2.3616	7.5404	1.9431	1.1095	0.9584	0.1500
mmu-miR-298-5p	4.2064	2.0916	6.4944	1.8851	0.6477	1.1095	-0.6266	0.1500
mmu-miR-130b-3p	8.4128	8.0178	3.2472	7.2711	2.5908	1.1027	1.3734	0.1410
mmu-miR-1843b-5p	5.736	13.5954	4.7232	12.3878	1.2144	1.0975	0.2803	0.1342
mmu-miR-126a-3p	253.9136	736.5918	149.3712	674.8658	1.6999	1.0915	0.7654	0.1263
mmu-miR-150-5p	48.1824	112.9464	38.0808	103.6805	1.2653	1.0894	0.3394	0.1235
mmu-miR-26a-5p	19975.4288	35930.5506	6204.2184	33019.6809	3.2197	1.0882	1.6869	0.1219
mmu-miR-152-3p	444.3488	168.7224	93.5784	155.3861	4.7484	1.0858	2.2474	0.1188
mmu-miR-361-5p	44.3584	42.5292	13.8744	39.3178	3.1971	1.0817	1.6768	0.1133
mmu-miR-142-3p	27.5328	67.977	15.6456	63.2855	1.7598	1.0741	0.8154	0.1032
mmu-let-7a-1-3p	18.3552	24.7506	5.6088	23.1598	3.2726	1.0687	1.7104	0.0958
mmu-miR-1249-3p	11.0896	14.6412	2.952	13.7343	3.7566	1.0660	1.9094	0.0923
mmu-miR-135a-5p	42.064	42.1806	11.5128	39.5871	3.6537	1.0655	1.8693	0.0915
mmu-miR-326-3p	12.6192	30.3282	3.8376	28.5458	3.2883	1.0624	1.7173	0.0874
mmu-miR-148a-5p	62.7136	104.58	50.184	98.5638	1.2497	1.0610	0.3216	0.0855
mmu-miR-374b-5p	99.424	281.3202	47.5272	268.2228	2.0919	1.0488	1.0648	0.0688
mmu-miR-744-3p	3.0592	7.3206	1.476	7.0018	2.0726	1.0455	1.0515	0.0642
mmu-miR-1247-5p	8.4128	9.7608	1.1808	9.4255	7.1247	1.0356	2.8328	0.0504

mmu-miR-30d-5p	159.0784	247.1574	97.1208	239.1384	1.6379	1.0335	0.7119	0.0476
mmu-miR-186-5p	385.0768	328.0326	141.696	318.3126	2.7176	1.0305	1.4423	0.0434
mmu-miR-652-3p	43.2112	44.6208	28.6344	43.3573	1.5091	1.0291	0.5937	0.0414
mmu-miR-205-5p	19.8848	34.86	112.4712	33.9318	0.1768	1.0274	-2.4998	0.0389
mmu-let-7f-2-3p	3.0592	6.6234	1.476	6.4632	2.0726	1.0248	1.0515	0.0353
mmu-miR-18a-5p	37.4752	21.9618	2.952	21.544	12.6949	1.0194	3.6662	0.0277
mmu-miR-30d-3p	10.3248	24.7506	13.8744	24.5063	0.7442	1.0100	-0.4263	0.0143
mmu-let-7i-3p	10.3248	28.5852	5.904	28.8151	1.7488	0.9920	0.8063	-0.0116
mmu-miR-17-5p	60.4192	93.7734	48.4128	95.6015	1.2480	0.9809	0.3196	-0.0279
mmu-miR-141-5p	16.8256	9.7608	6.1992	9.9641	2.7142	0.9796	1.4405	-0.0297
mmu-miR-329-3p	3.0592	6.972	1.7712	7.2711	1.7272	0.9589	0.7884	-0.0606
mmu-miR-194-5p	97.8944	130.0278	29.8152	136.2658	3.2834	0.9542	1.7152	-0.0676
mmu-miR-455-3p	8.4128	9.7608	0.5904	10.2334	14.2493	0.9538	3.8328	-0.0682
mmu-miR-100-5p	260.7968	220.6638	144.3528	232.4059	1.8067	0.9495	0.8533	-0.0748
mmu-miR-139-5p	7.648	7.6692	1.476	8.079	5.1816	0.9493	2.3734	-0.0751
mmu-miR-181a-5p	1659.2336	2402.2026	2419.4592	2553.7719	0.6858	0.9406	-0.5442	-0.0883
mmu-miR-148b-3p	469.5872	182.3178	73.2096	195.5118	6.4143	0.9325	2.6813	-0.1008
mmu-miR-23b-3p	184.6992	180.9234	85.608	195.2425	2.1575	0.9267	1.1094	-0.1099
mmu-miR-142-5p	511.6512	1236.4842	378.7416	1342.9991	1.3509	0.9207	0.4339	-0.1192
mmu-miR-20a-5p	69.5968	140.4858	66.7152	152.9624	1.0432	0.9184	0.0610	-0.1228
mmu-let-7d-3p	94.4528	109.4604	59.04	120.3771	1.5998	0.9093	0.6779	-0.1372
mmu-miR-136-5p	2.2944	4.8804	2.3616	5.386	0.9715	0.9061	-0.0416	-0.1422
mmu-miR-99a-3p	7.648	8.0178	2.952	8.8869	2.5908	0.9022	1.3734	-0.1485
mmu-miR-615-3p	6.1184	5.5776	2.0664	6.1939	2.9609	0.9005	1.5660	-0.1512
mmu-miR-23a-3p	161.7552	199.3992	85.0176	221.6339	1.9026	0.8997	0.9280	-0.1525
mmu-miR-223-3p	88.7168	147.1092	36.3096	164.0037	2.4433	0.8970	1.2889	-0.1568
mmu-miR-151-3p	499.032	973.9884	597.78	1092.2808	0.8348	0.8917	-0.2605	-0.1654
mmu-miR-382-5p	9.9424	7.6692	3.8376	8.6176	2.5908	0.8899	1.3734	-0.1682
mmu-miR-344-3p	13.7664	18.8244	7.38	21.2747	1.8654	0.8848	0.8995	-0.1765
mmu-miR-434-5p	4.2064	5.229	1.476	5.9246	2.8499	0.8826	1.5109	-0.1802
mmu-miR-195a-5p	523.888	980.6118	91.512	1111.4011	5.7248	0.8823	2.5172	-0.1806

mmu-miR-378a-3p	358.3088	568.5666	622.2816	646.8586	0.5758	0.8790	-0.7964	-0.1861
mmu-let-7a-5p	2054.6352	4470.0978	11931.984	5087.077	0.1722	0.8787	-2.5379	-0.1865
mmu-miR-296-5p	7.2656	8.0178	2.0664	9.1562	3.5161	0.8757	1.8140	-0.1915
mmu-miR-181a-1-3p	43.2112	52.29	51.9552	59.7846	0.8317	0.8746	-0.2659	-0.1932
mmu-miR-676-3p	205.3488	305.7222	181.548	349.8207	1.1311	0.8739	0.1777	-0.1944
mmu-miR-148a-3p	5049.2096	11374.818	4342.392	13037.8902	1.1628	0.8724	0.2176	-0.1969
mmu-miR-24-2-5p	85.6576	131.4222	49.5936	152.9624	1.7272	0.8592	0.7884	-0.2190
mmu-miR-25-3p	1765.9232	1919.7402	1305.3744	2238.9602	1.3528	0.8574	0.4360	-0.2219
mmu-miR-155-5p	14.1488	24.402	6.1992	28.5458	2.2824	0.8548	1.1905	-0.2263
mmu-miR-210-3p	186.2288	224.4984	92.1024	263.6447	2.0220	0.8515	1.0158	-0.2319
mmu-let-7e-5p	403.8144	769.3602	790.8408	907.541	0.5106	0.8477	-0.9697	-0.2383
mmu-miR-669c-5p	5.3536	5.229	5.3136	6.1939	1.0075	0.8442	0.0108	-0.2443
mmu-miR-212-5p	1.912	4.5318	4.1328	5.386	0.4626	0.8414	-1.1120	-0.2491
mmu-let-7d-5p	555.6272	900.4338	1185.5232	1071.0061	0.4687	0.8407	-1.0933	-0.2503
mmu-miR-484	26.0032	50.8956	19.188	60.8618	1.3552	0.8362	0.4385	-0.2580
mmu-miR-434-3p	48.1824	141.5316	22.14	169.659	2.1763	0.8342	1.1219	-0.2615
mmu-miR-214-3p	69.5968	82.9668	22.14	99.9103	3.1435	0.8304	1.6524	-0.2681
mmu-miR-28a-5p	82.5984	60.6564	42.2136	73.5189	1.9567	0.8250	0.9684	-0.2775
mmu-miR-337-3p	4.5888	2.4402	2.952	2.9623	1.5545	0.8238	0.6364	-0.2797
mmu-let-7b-5p	906.288	1761.4758	850.4712	2149.2833	1.0656	0.8196	0.0917	-0.2871
mmu-miR-127-3p	997.2992	754.0218	1194.6744	920.1981	0.8348	0.8194	-0.2605	-0.2873
mmu-miR-222-3p	73.8032	81.5724	60.2208	99.9103	1.2255	0.8165	0.2934	-0.2926
mmu-miR-151-5p	204.2016	434.3556	120.1464	533.4833	1.6996	0.8142	0.7652	-0.2966
mmu-miR-331-3p	3.4416	6.2748	1.1808	7.8097	2.9146	0.8035	1.5433	-0.3157
mmu-miR-320-3p	92.9232	105.9744	92.6928	133.0342	1.0025	0.7966	0.0036	-0.3281
mmu-miR-200a-5p	45.888	31.7226	27.7488	39.8564	1.6537	0.7959	0.7257	-0.3293
mmu-miR-101b-3p	47.0352	118.1754	53.7264	148.9229	0.8755	0.7935	-0.1919	-0.3336
mmu-miR-99b-3p	8.4128	7.6692	6.4944	9.6948	1.2954	0.7911	0.3734	-0.3381
mmu-miR-152-5p	40.5344	48.1068	26.8632	60.8618	1.5089	0.7904	0.5935	-0.3393
mmu-miR-29b-3p	125.4272	254.8266	65.8296	323.16	1.9053	0.7885	0.9300	-0.3427
mmu-miR-183-5p	724.648	38.346	673.9416	48.7433	1.0752	0.7867	0.1047	-0.3461

mmu-miR-93-5p	322.7456	553.5768	327.0816	705.8353	0.9867	0.7843	-0.0193	-0.3505
mmu-miR-744-5p	23.7088	23.3562	14.4648	29.8923	1.6391	0.7813	0.7129	-0.3560
mmu-miR-133a-3p	147.9888	142.926	72.6192	183.3933	2.0379	0.7793	1.0271	-0.3597
mmu-miR-381-3p	60.8016	126.1932	89.4456	162.9265	0.6798	0.7745	-0.5569	-0.3686
mmu-miR-146b-5p	159.4608	56.8218	166.788	73.5189	0.9561	0.7729	-0.0648	-0.3717
mmu-miR-218-5p	45.1232	90.2874	70.5528	116.8762	0.6396	0.7725	-0.6448	-0.3724
mmu-miR-872-3p	113.1904	232.1676	34.8336	300.8081	3.2495	0.7718	1.7002	-0.3737
mmu-miR-140-5p	21.7968	49.1526	15.3504	64.0934	1.4199	0.7669	0.5058	-0.3829
mmu-miR-328-3p	75.7152	58.2162	63.7632	75.9426	1.1874	0.7666	0.2479	-0.3835
mmu-miR-1843a-5p	29.0624	65.8854	34.5384	86.4453	0.8415	0.7622	-0.2490	-0.3918
mmu-miR-503-3p	6.1184	6.972	0.8856	9.1562	6.9088	0.7615	2.7884	-0.3932
mmu-miR-532-5p	147.224	129.3306	183.6144	169.9283	0.8018	0.7611	-0.3187	-0.3939
mmu-miR-365-3p	46.2704	128.6334	50.184	169.1204	0.9220	0.7606	-0.1171	-0.3948
mmu-let-7e-3p	5.736	5.9262	4.1328	7.8097	1.3879	0.7588	0.4729	-0.3982
mmu-miR-126a-5p	3952.4864	6138.846	3109.932	8093.0036	1.2709	0.7585	0.3459	-0.3987
mmu-miR-29a-3p	560.9808	912.2862	176.8248	1203.5017	3.1725	0.7580	1.6656	-0.3997
mmu-miR-467d-3p	1.1472	2.4402	2.3616	3.2316	0.4858	0.7551	-1.0416	-0.4052
mmu-miR-149-5p	77.6272	36.9516	97.7112	49.0126	0.7945	0.7539	-0.3320	-0.4075
mmu-miR-676-5p	29.8272	22.659	28.044	30.1616	1.0636	0.7513	0.0889	-0.4126
mmu-miR-672-5p	90.6288	179.8776	113.3568	239.9463	0.7995	0.7497	-0.3228	-0.4157
mmu-miR-21a-5p	25239.5472	21749.154	20055.888	29025.154	1.2585	0.7493	0.3317	-0.4163
mmu-miR-10b-5p	10203.1968	14401.7118	8150.472	19249.2947	1.2519	0.7482	0.3241	-0.4186
mmu-miR-127-5p	4.9712	5.5776	0.5904	7.5404	8.4201	0.7397	3.0738	-0.4350
mmu-miR-450a-5p	18.7376	12.5496	4.428	16.9659	4.2316	0.7397	2.0812	-0.4350
mmu-miR-190a-3p	4.5888	5.9262	8.5608	8.079	0.5360	0.7335	-0.8996	-0.4471
mmu-miR-101a-3p	396.1664	1127.0238	436.6008	1545.782	0.9074	0.7291	-0.1402	-0.4558
mmu-miR-455-5p	17.208	31.7226	21.2544	43.8959	0.8096	0.7227	-0.3047	-0.4686
mmu-miR-501-3p	26.3856	1.743	23.3208	2.4237	1.1314	0.7191	0.1781	-0.4756
mmu-miR-379-5p	28.68	11.1552	18.8928	15.6194	1.5180	0.7142	0.6022	-0.4856
mmu-miR-34b-5p	85.6576	85.0584	63.1728	119.2999	1.3559	0.7130	0.4393	-0.4881
mmu-miR-299a-3p	2.2944	3.8346	1.7712	5.386	1.2954	0.7120	0.3734	-0.4901

mmu-let-7c-5p	6786.4528	11957.3286	14089.896	16862.2195	0.4817	0.7091	-1.0539	-0.4959
mmu-miR-342-3p	398.4608	85.407	128.7072	121.4543	3.0959	0.7032	1.6303	-0.5080
mmu-miR-322-5p	148.3712	294.9156	61.6968	422.5317	2.4048	0.6980	1.2659	-0.5188
mmu-miR-29c-3p	28.68	65.5368	30.7008	93.9857	0.9342	0.6973	-0.0982	-0.5201
mmu-miR-497-5p	68.4496	172.2084	15.0552	249.6411	4.5466	0.6898	2.1848	-0.5357
mmu-miR-1839-5p	104.7776	106.6716	213.7248	154.8475	0.4902	0.6889	-1.0284	-0.5377
mmu-miR-19b-3p	112.808	128.2848	67.0104	186.8942	1.6834	0.6864	0.7514	-0.5429
mmu-miR-184-3p	33.2688	15.3384	27.4536	22.3519	1.2118	0.6862	0.2772	-0.5432
mmu-miR-103-3p	684.8784	656.4138	773.1288	961.9396	0.8859	0.6824	-0.1749	-0.5513
mmu-miR-195a-3p	14.5312	23.7048	15.9408	34.7397	0.9116	0.6824	-0.1336	-0.5514
mmu-miR-99a-5p	460.0272	356.2692	761.0256	524.3271	0.6045	0.6795	-0.7262	-0.5575
mmu-miR-190a-5p	4.9712	9.0636	2.0664	13.465	2.4057	0.6731	1.2665	-0.5711
mmu-miR-582-5p	3.0592	18.8244	3.2472	28.0072	0.9421	0.6721	-0.0860	-0.5732
mmu-miR-27b-5p	13.7664	25.4478	21.5496	37.9713	0.6388	0.6702	-0.6465	-0.5774
mmu-miR-22-3p	5982.648	7482.0018	10954.5768	11216.0757	0.5461	0.6671	-0.8727	-0.5841
mmu-miR-335-5p	6.1184	12.5496	2.0664	18.851	2.9609	0.6657	1.5660	-0.5870
mmu-miR-199a-3p	1659.9984	2924.0568	1147.1472	4397.3997	1.4471	0.6650	0.5331	-0.5887
mmu-let-7f-5p	11215.0272	18096.8718	32925.7224	27305.6735	0.3406	0.6628	-1.5538	-0.5935
mmu-miR-500-3p	13.0016	8.0178	10.6272	12.1185	1.2234	0.6616	0.2909	-0.5959
mmu-miR-362-3p	33.2688	36.603	12.3984	55.7451	2.6833	0.6566	1.4240	-0.6069
mmu-miR-130a-3p	107.8368	159.6588	70.5528	243.9858	1.5285	0.6544	0.6121	-0.6118
mmu-miR-503-5p	2.6768	5.5776	0.5904	8.6176	4.5339	0.6472	2.1807	-0.6276
mmu-miR-140-3p	2.2944	8.3664	3.8376	12.9264	0.5979	0.6472	-0.7421	-0.6276
mmu-miR-1983	3.0592	2.4402	6.7896	3.7702	0.4506	0.6472	-1.1502	-0.6276
mmu-miR-181c-3p	64.2432	66.5826	100.0728	103.4112	0.6420	0.6439	-0.6394	-0.6352
mmu-miR-1198-5p	25.6208	13.5954	7.38	21.544	3.4717	0.6311	1.7956	-0.6642
mmu-miR-199b-5p	294.0656	880.5636	84.132	1395.5126	3.4953	0.6310	1.8054	-0.6643
mmu-miR-146a-5p	1086.7808	417.2742	648.5544	663.5552	1.6757	0.6288	0.7448	-0.6692
mmu-miR-22-5p	3.4416	23.7048	2.6568	37.9713	1.2954	0.6243	0.3734	-0.6797
mmu-miR-26b-5p	949.1168	797.5968	1326.3336	1279.7136	0.7156	0.6233	-0.4828	-0.6821
mmu-miR-27b-3p	12894.528	14036.0304	16209.1368	22528.8301	0.7955	0.6230	-0.3300	-0.6826

mmu-let-7i-5p	4287.8512	7342.2132	6255.288	11801.2646	0.6855	0.6222	-0.5448	-0.6847
mmu-miR-28a-3p	236.7056	244.3686	275.4216	393.7166	0.8594	0.6207	-0.2185	-0.6881
mmu-miR-340-5p	2152.1472	2762.3064	4417.668	4484.9222	0.4872	0.6159	-1.0375	-0.6992
mmu-miR-128-3p	17.9728	13.2468	13.8744	21.544	1.2954	0.6149	0.3734	-0.7016
mmu-miR-130a-5p	5.3536	3.1374	0.2952	5.1167	18.1355	0.6132	4.1807	-0.7056
mmu-miR-9-5p	2.2944	3.1374	7.38	5.1167	0.3109	0.6132	-1.6855	-0.7056
mmu-miR-410-3p	68.4496	91.3332	87.3792	150.808	0.7834	0.6056	-0.3522	-0.7235
mmu-miR-30a-5p	494.0608	708.3552	474.0912	1170.6471	1.0421	0.6051	0.0595	-0.7248
mmu-miR-99b-5p	2536.4592	429.8238	1636.2936	712.5678	1.5501	0.6032	0.6324	-0.7293
mmu-miR-324-5p	6.5008	14.2926	6.4944	23.6984	1.0010	0.6031	0.0014	-0.7295
mmu-miR-378a-5p	20.6496	29.9796	9.1512	49.8205	2.2565	0.6018	1.1741	-0.7328
mmu-miR-181d-5p	275.328	234.9564	243.2448	390.485	1.1319	0.6017	0.1787	-0.7329
mmu-miR-181b-5p	171.6976	247.8546	133.4304	414.9913	1.2868	0.5973	0.3638	-0.7436
mmu-miR-221-3p	262.3264	257.964	188.928	432.2265	1.3885	0.5968	0.4735	-0.7446
mmu-miR-1247-3p	4.2064	5.9262	5.904	9.9641	0.7125	0.5948	-0.4891	-0.7496
mmu-miR-30e-5p	68.4496	107.0202	62.5824	181.7775	1.0938	0.5887	0.1293	-0.7643
mmu-miR-29a-5p	4.5888	3.486	1.476	5.9246	3.1089	0.5884	1.6364	-0.7651
mmu-miR-132-3p	12.6192	13.944	9.7416	23.9677	1.2954	0.5818	0.3734	-0.7814
mmu-miR-199a-5p	341.8656	573.7956	182.4336	991.024	1.8739	0.5790	0.9061	-0.7884
mmu-miR-181c-5p	1173.5856	2036.5212	2130.4584	3525.6756	0.5509	0.5776	-0.8602	-0.7918
mmu-miR-341-3p	10.3248	11.5038	13.8744	19.9282	0.7442	0.5773	-0.4263	-0.7927
mmu-miR-669a-5p	19.8848	3.8346	13.8744	6.7325	1.4332	0.5696	0.5192	-0.8121
mmu-miR-125b-1-3p	4.2064	5.9262	2.952	10.5027	1.4249	0.5643	0.5109	-0.8256
mmu-miR-98-5p	1186.9696	2292.045	516.8952	4070.4695	2.2963	0.5631	1.1993	-0.8286
mmu-miR-24-3p	47.0352	71.1144	22.4352	127.1096	2.0965	0.5595	1.0680	-0.8379
mmu-miR-27a-3p	2416.3856	884.7468	3305.0592	1623.6097	0.7311	0.5449	-0.4518	-0.8759
mmu-miR-449a-5p	47.0352	14.6412	48.1176	26.93	0.9775	0.5437	-0.0328	-0.8792
mmu-miR-196b-3p	3.0592	3.1374	0.2952	5.9246	10.3631	0.5296	3.3734	-0.9171
mmu-miR-708-3p	42.8288	50.1984	33.6528	95.0629	1.2727	0.5281	0.3479	-0.9212
mmu-miR-202-5p	134.9872	95.5164	69.372	181.2389	1.9458	0.5270	0.9604	-0.9241
mmu-miR-143-3p	111893.6816	102954.4782	141006.1176	195677.1502	0.7935	0.5261	-0.3336	-0.9265

mmu-miR-196b-5p	3322.6736	1867.1016	3727.4904	3571.4566	0.8914	0.5228	-0.1659	-0.9357
mmu-miR-193b-3p	69.9792	78.7836	82.3608	151.6159	0.8497	0.5196	-0.2350	-0.9445
mmu-miR-26b-3p	5.3536	5.5776	3.5424	10.772	1.5113	0.5178	0.5958	-0.9496
mmu-miR-203-3p	44.7408	71.8116	180.3672	138.6895	0.2481	0.5178	-2.0113	-0.9496
mmu-miR-107-3p	11.0896	8.0178	14.1696	15.6194	0.7826	0.5133	-0.3536	-0.9621
mmu-miR-34a-5p	54.3008	54.3816	57.2688	106.3735	0.9482	0.5112	-0.0768	-0.9679
mmu-miR-21a-3p	4.2064	1.743	4.7232	3.5009	0.8906	0.4979	-0.1672	-1.0062
mmu-miR-129-2-3p	9.1776	10.1094	2.3616	20.4668	3.8862	0.4939	1.9584	-1.0176
mmu-miR-409-3p	19.5024	17.7786	18.5976	36.0862	1.0487	0.4927	0.0685	-1.0213
mmu-miR-30b-3p	6.1184	2.0916	7.9704	4.3088	0.7676	0.4854	-0.3815	-1.0427
mmu-miR-193a-3p	172.08	247.1574	129.5928	523.7885	1.3279	0.4719	0.4091	-1.0836
mmu-miR-300-3p	9.56	9.4122	7.6752	20.1975	1.2456	0.4660	0.3168	-1.1016
mmu-miR-3057-5p	3.824	1.743	3.2472	3.7702	1.1776	0.4623	0.2359	-1.1131
mmu-miR-135b-5p	3.4416	2.4402	5.904	5.386	0.5829	0.4531	-0.7786	-1.1422
mmu-miR-338-3p	0.7648	4.1832	5.0184	9.4255	0.1524	0.4438	-2.7141	-1.1720
mmu-miR-196a-5p	252.0016	152.6868	282.2112	346.0505	0.8930	0.4412	-0.1633	-1.1804
mmu-miR-708-5p	217.968	178.4832	178.596	409.0667	1.2205	0.4363	0.2874	-1.1965
mmu-miR-214-5p	87.5696	53.6844	145.8288	126.8403	0.6005	0.4232	-0.7358	-1.2404
mmu-miR-322-3p	3.824	3.486	1.7712	8.3483	2.1590	0.4176	1.1104	-1.2599
mmu-miR-106b-3p	6.1184	2.7888	11.2176	6.7325	0.5454	0.4142	-0.8745	-1.2715
mmu-miR-335-3p	8.0304	9.0636	6.4944	22.6212	1.2365	0.4007	0.3063	-1.3195
mmu-miR-542-3p	11.0896	6.972	8.5608	18.851	1.2954	0.3698	0.3734	-1.4350
mmu-miR-129-5p	9.9424	6.2748	8.2656	17.5045	1.2029	0.3585	0.2665	-1.4801
mmu-miR-145a-5p	3002.9872	4793.5986	1614.4488	13542.2891	1.8601	0.3540	0.8954	-1.4983
mmu-miR-700-5p	4.9712	1.3944	2.0664	4.0395	2.4057	0.3452	1.2665	-1.5345
mmu-miR-134-5p	5.736	5.229	10.332	19.1203	0.5552	0.2735	-0.8490	-1.8705
mmu-miR-377-3p	0.3824	0.6972	2.3616	2.9623	0.1619	0.2354	-2.6266	-2.0871
mmu-miR-143-5p	34.416	22.3104	84.7224	95.6015	0.4062	0.2334	-1.2997	-2.0993

Table S2. Rates of cytoplasmic droplets (CD) after sperm co-incubation with HTF (as control) and OVS for 30 min and 3h. Using χ^2 analysis, there is no significant ($P > 0.05$) difference between samples after co-incubation with OVS and HTF for different time periods.

Incubation time	Status	Number (%)	
		HTF	OVS
30 min	No CD	162	194
	CD	11 (6.4)	18 (8.5)
	Total	173	212
3 h	No CD	199	184
	CD	10 (4.8)	15 (7.5)
	Total	209	199
Grand Total	No CD	361	378
	CD	21 (5.8)	33 (8.7)
	Total	382	411

Table S3. Primers used for amplification of reverse-transcribed RNAs.

Assay Name	U6 as an endogenous control and selected miRNA Sequence (5' to 3')	Accession Number (Exiqon Inc.)
<i>U6 snRNA (hsa, mmu)</i>	GUGCUCGCUUCGGCAGCACAUUAUACUAAAAUUGGAA CGAUACAGAGAAGAUUAGCAUGGCCCCUGCGCAAGG AUGACACGCAAUUCGUGAAGCGUCCAUAUUUUU	203907
<i>miR-143-3p</i>	UGAGAUGAAGCACUGUAGCUC	205992
<i>miR-34c-5p</i>	AGGCAGUGUAGUUAGCUGAUUGC	205659
<i>miR-22-3p</i>	AAGCUGCCAGUUGAAGAACUGU	204606
<i>let-7a-5p</i>	UGAGGUAGUAGGUUGUAUAGUU	205727

Table S4. Primers for conventional RT-PCR.

Gene	Forward Primer Sequence	Reverse Primer Sequence	Product Size (kbp)
<i>U6 snRNA</i>	CGCTTCGGCAGCACATATAC	AAATATGGAACGCTTCACGA	100
<i>Rnu5g</i>	CTCTGGTTTCTCTTCAGATCGT	TTGTCAAGACAAGGCCTCAA	109
<i>Rnu1a1</i>	GATCACGAAGGTGGTTTTCC	CAGTCCCCCACTACCACAAA	119
<i>Gapdh</i>	CCGCATCTTCTTGTGCAGT	GAATTTGCCCGTGAGTGGAGT	204