

1 **Running title:**

2 MiRNAs in human oocytes and blastocysts

3 **Title:**

4 **Investigation of microRNA expression and DNA repair gene transcripts in human oocytes and**
5 **blastocysts**

6 Tulay, P., Ph.D.^{a,b}, Naja, RP., Ph.D.^b, Cascales-Roman, O., M.Sc.^b, Doshi, A., MSc.^c, Serhal, P.,
7 M.D.^c, SenGupta, SB., Ph.D.^b

8 **Where the work was done:** UCL Centre for PGD, Institute for Women's Health, University College
9 London, UK

10 **Departmental affiliations:**

11 ^a Near East University, Faculty of Medicine, Medical Genetics Department, Yakin Dogu Bulvari,
12 Nicosia, Cyprus

13 ^b UCL Centre for PGD, Institute for Women's Health, University College London, UK

14 ^cThe Centre for Reproductive and Genetic Health, University College Hospital, The New Wing
15 Eastman Dental Hospital, London, UK

16 **Corresponding author:**

17 Pinar Tulay, Ph.D.

18 Near East University, Faculty of Medicine, Medical Genetics Department, Yakin Dogu Bulvari,
19 Nicosia, Cyprus

20 Telephone: (0090) 392 444 0938

21 Email: pintulay@gmail.com

22

23 **Capsule Summary**

24 Detection of DNA damage and activation of correct DNA repair mechanisms play a crucial role
25 during preimplantation embryo development. If the repair mechanisms are unable to repair the
26 damage, apoptosis of an embryonic cell can be detrimental to the early developing embryo. Therefore
27 correct activation of genes and proteins is critical for the survival of a preimplantation embryo.

28 The level of gene expression is affected by many factors and one of the regulatory factors is through
29 microRNA (miRNA) regulation. Several studies have shown that miRNAs regulate their target genes
30 in cancer cells and cell lines. In this study, we have shown that the miRNAs expressed in human
31 oocytes and blastocysts are associated with their target genes that are involved in DNA repair.

32

33 **Abstract**

34 Purpose: The aim of the study is to investigate the regulation of DNA repair genes by microRNAs
35 (miRNAs). MiRNAs are short non-coding RNAs that regulate transcriptional and post-transcriptional
36 gene silencing. Several miRNAs that are expressed during preimplantation embryo development have
37 been shown or are predicted to target genes that regulate cell cycle checkpoints and DNA repair in
38 response to DNA damage.

39 Methods: This study compares the expression level of 20 miRNAs and nine target transcripts involved
40 in DNA repair. The statistical significance of differential miRNA expression between oocytes and
41 blastocysts was determined by T-test analysis using GraphPad prism. The possible regulatory roles of
42 miRNAs on their target mRNAs were analysed by a Pearson correlation test.

43 Results: This study shows for the first time that several miRNAs are expressed in human oocytes and
44 blastocysts that target key genes involved in DNA repair and cell cycle checkpoints. Blastocysts
45 exhibited statistically significant lower expression levels for the majority of miRNAs compared to
46 oocytes ($p < 0.05$). Correlation analyses showed that there were both inverse and direct association
47 between miRNAs and their target mRNAs.

48 Conclusions: MiRNAs target many mRNAs including ones involved in DNA repair mechanisms. This
49 study suggests that miRNAs and their target mRNAs involved in DNA repair are expressed in
50 preimplantation embryos. Similar to the miRNAs expressed in adult tissues, these miRNAs seems to
51 have regulatory roles on their target DNA repair mRNAs during preimplantation embryo development.

52

53 **Keywords:** microRNA, expression, DNA repair, human oocyte and blastocyst, preimplantation
54 embryo development

55 INTRODUCTION

56 Coordination of the cell cycle and activation of DNA repair mechanisms upon DNA damage are
57 crucial in the early developing embryo to preserve the genomic integrity. If the repair mechanisms are
58 unable to repair the damage, apoptosis of an embryonic cell can be detrimental to the early developing
59 embryo [1]. Therefore correct activation of genes and proteins is critical for the survival of a
60 preimplantation embryo.

61 The regulation of transcripts is controlled by complex mechanisms. Recent studies proposed that more
62 than half of the human transcriptome is regulated by microRNAs (miRNAs) [2, 3]. Several miRNAs
63 have been shown to regulate and be regulated by genes functioning at cell cycle checkpoints [4-10]
64 and in different repair mechanisms; nucleotide excision repair [11], mismatch repair [12-15] and
65 double strand break repair [16-21] in cancer cells and cell lines.

66 MiRNAs are small non-coding RNAs of 17-25 nucleotide in length which either degrade or inhibit the
67 expression and therefore function of their target genes [22-29]. More recently studies suggested that
68 these non-coding RNAs may stabilise the expression of their target mRNAs [27, 30-36].

69 Expression of miRNAs has been observed in murine, bovine and human gametes and preimplantation
70 embryos [22, 25, 37-40]. Similar expression profiles of miRNAs in mature mouse oocytes and in the
71 early developing embryos were observed that may be due to the maternally inherited miRNAs in the
72 early embryo [38]. Expression of miRNAs was also detected in the sperm and about 20% of these
73 miRNAs are present within the nuclear or perinuclear part of the sperm that are transferred to the
74 zygote [41]. However, these miRNAs are thought to not play a significant role since these miRNAs
75 are already present in the oocytes [42]. Expression levels of these miRNAs vary during cleavage stage
76 divisions, such that in murine embryos when the maternally inherited miRNAs are being degraded, a
77 60% decrease in the miRNA expression was observed [38, 39]. In this study, we investigate the
78 expression of specific miRNAs that are known to target mRNA transcripts associated with DNA
79 repair in human oocytes and blastocysts to correlate the expression profiles of these mRNA and

80 miRNAs and to elucidate the potential regulatory role and activity of the repair mechanisms in human
81 oocytes and preimplantation embryos.

82 **MATERIALS and METHODS**

83 This work was licensed by the Human Fertilisation and Embryology Authority (HFEA project
84 reference: RO113) and ethical approval was granted by the National Research Ethics Service (NRES),
85 Research Ethics Committee (REC reference number: 10/H0709/26).

86 **Sample collection and processing**

87 Immature oocytes and surplus cryopreserved blastocysts were collected from patients who had given
88 an informed consent following *in vitro* fertilisation (IVF) treatment. The zona pellucida of oocytes
89 and blastocysts was removed following brief exposure to acidified Tyrode's solution (Medi-Cult,
90 Surrey, UK). The samples were then washed in phosphate buffered saline with 0.1% polyvinyl
91 alcohol solution (PBS/PVA, Sigma, USA) and 0.3U/μl RNasin plus RNase inhibitor (Promega, UK)
92 to prevent RNA degradation. Single oocytes and single blastocysts were transferred in a minimum
93 volume of PBS/PVA with RNasin solution to an empty 0.2ml MicroAmp reaction tube.

94 **Selection of mRNA and miRNA for analysis**

95 An expression profile of DNA repair genes in human oocytes and blastocysts was previously
96 established using microarrays [43, 44]. This study was used as a guideline to identify mRNAs that
97 were differentially expressed in human oocytes relative to blastocysts. In order to deduce the activity
98 of different DNA repair pathways one sensor gene or a gene functioning at the earlier stages of the
99 particular repair pathway and one gene functioning at later stages of the repair pathway were selected.
100 In addition to this group of repair genes, the expression of one miRNA processing gene and one house
101 keeping gene was analysed as control genes. MiRNAs targeting the selected DNA repair genes were
102 identified using miRNA databases (<http://www.microrna.org/microrna/home.do>,
103 <http://www.targetscan.org/> and <http://mirdb.org/miRDB/>) and previously published articles [22, 38,

104 40, 43, 45, 46]. A further literature search was conducted to select from these miRNAs that were
105 shown to be expressed in human, murine and bovine embryos. Two miRNAs, namely hsa-miR-15a
106 and hsa-miR-212, that were previously shown to be expressed in human oocytes and two miRNAs,
107 hsa-let-7a, hsa-miR-21, that were reported to be expressed in human blastocysts were analysed as
108 controls. An additional 16 miRNAs expressed in murine and bovine oocytes and blastocysts and
109 embryonic stem cells were selected from the previous publications.

110 Oocytes and blastocysts were grouped according to morphological grade and maternal age. For each
111 mRNA and miRNA, expression analyses were performed in six repeat samples (six individual oocytes
112 and six individual blastocysts) to eliminate the sample variation with at least two replicates.

113 **mRNA and miRNA expression**

114 mRNA expression was analysed from nine oocytes and ten blastocysts using the TaqMan® gene
115 expression cells-to-CT™ kit (Ambion, Life Technologies, UK). MiRNA expression was analysed
116 using the TaqMan® microRNA cells-to-CT™ kit (Ambion, Life Technologies, UK) in a further 22
117 oocytes and 23 blastocysts. Both mRNA and miRNA quantification involved three main steps; lysis,
118 reverse transcription and real time quantitative PCR (qPCR). Cell lysis was performed in 25µl lysis
119 solution with 0.25µl DNase I at room temperature (RT) for 8 minutes. The lysis was stopped by
120 addition of the 2.5µl stop solution and incubation at RT for 2 minutes.

121 Reverse transcription and qPCR for both mRNA and miRNA was performed following the respective
122 manufacturer's protocol. For each reaction, negative controls in the absence of embryo lysates and
123 cDNA products were performed. Overall 10 mRNAs and 20 miRNAs were analysed. Real time qPCR
124 for each sample was carried out in duplicates and the expression level of each mRNA or miRNA was
125 determined in a minimum of six different oocytes and six different blastocysts. All the samples that
126 were used for mRNA expression analysis were also tested for *ACTB* expression. Similarly all samples
127 that were used for miRNA expression analysis were tested for *RNU48*. *ACTB* and *RNU48* were

128 selected for endogenous reference genes since both were shown to be expressed at a constant level in
129 human oocytes and preimplantation embryos.

130 The comparative $\Delta\Delta Cq$ method was used to examine the expression levels of miRNAs and mRNAs
131 [40]. Determination of Cq values was performed using the LightCycler Nano software (Roche, UK)
132 and ΔCq values were determined as follows after normalisation with the endogenous reference gene
133 *ACTB* for mRNA and RNU48 for miRNA, respectively, for each oocyte and blastocyst sample:

$$134 \quad \Delta Cq = Cq_{\text{target gene}} - Cq_{\text{ACTB}}$$

$$135 \quad \Delta Cq = Cq_{\text{target gene}} - Cq_{\text{RNU48}}$$

136 The fold change was analysed by the relative quantification method, $2^{-\Delta\Delta Cq}$:

$$137 \quad \Delta\Delta Cq = (Cq_{\text{target}} - Cq_{\text{ACTB}})_{\text{blastocyst}} - (Cq_{\text{target}} - Cq_{\text{ACTB}})_{\text{oocyte}}$$

$$138 \quad \Delta\Delta Cq = (Cq_{\text{target}} - Cq_{\text{RNU48}})_{\text{blastocyst}} - (Cq_{\text{target}} - Cq_{\text{RNU48}})_{\text{oocyte}}$$

139 **Statistical analyses**

140 All the statistical analyses were carried out using the GraphPad prism v6 software. The level of
141 expression of each mRNA and miRNA in oocytes relative to blastocysts was examined by applying
142 an unpaired two-tailed student's T-test with the Welch correction, respectively. The correlation
143 between each miRNA and its target mRNA was investigated by the Pearson correlation test. An
144 inverse correlation was defined as $r=-1$ and a direct correlation as $r=+1$ following Pearson correlation
145 test. Similarly, a perfect correlation was defined as Pearson's r^2 coefficient equal to 1. For all the
146 statistical analysis, $p<0.05$ indicated a statistical significance.

147 **RESULTS**

148 A total of 31 immature oocytes and 33 surplus cryopreserved blastocysts were collected from twenty
149 women (maternal age; 35 ± 5) and sixteen couples (maternal age; 38 ± 7), respectively. mRNA

150 expression of nine transcripts involved in DNA repair and one involved in miRNA processing was
151 investigated in a total of nine oocytes and ten blastocysts. Differentially expressed DNA repair genes
152 in human oocytes and blastocysts were identified previously [43]. A literature search to identify
153 miRNAs expressed in human oocytes and blastocysts was performed. This search was narrowed down
154 on the miRNAs that were shown or predicted to regulate these differentially expressed DNA repair
155 genes. From these mRNA and miRNA profiles, repair genes such as miRNAs targeting an important
156 cell cycle checkpoint gene, sensor genes from each pathway and genes involved in different repair
157 pathways, were selected to be analysed in this study (Table 1 and figure 1). MiRNAs were selected to
158 target more than one gene that is involved in different repair pathways to be able to have a general
159 idea of the possible regulatory roles of miRNAs in DNA repair pathways. The expression of 20
160 miRNAs targeting these repair genes was analysed in 22 oocytes and 23 blastocysts, respectively. The
161 expression level of each mRNA or miRNA was determined in a minimum of six different oocytes and
162 six different blastocysts. Each analysis was carried out in duplicate.

163 **mRNA and miRNA expression**

164 Quantification for each mRNA and miRNA was performed after normalisation with endogenous
165 control genes, *ACTB* and *RNU48*, respectively. All ten mRNAs analysed (*PARP1*, *BRCA1*, *RAD50*,
166 *MSH2*, *MSH3*, *GTF2H2*, *ERCC3*, *DCLRE1A*, *RBI* and *DICER*) were expressed both in oocytes and
167 blastocysts. Of the twenty miRNAs analysed, eleven (*hsa-let-7a*, *hsa-miR-16*, *hsa-miR-21*, *hsa-miR-*
168 *31*, *hsa-miR-101*, *hsa-miR-145*, *hsa-miR-182*, *hsa-miR-192*, *hsa-miR-194*, *hsa-miR-210* and *hsa-miR-*
169 *212*) were detected in oocytes and eighteen (*hsa-let-7a*, *hsa-miR-7-2*, *hsa-miR-15a*, *hsa-miR-16*, *hsa-*
170 *miR-21*, *hsa-miR-23b*, *hsa-miR-31*, *hsa-miR-34c*, *hsa-miR-101*, *hsa-miR-128*, *hsa-miR-130*, *hsa-*
171 *miR-145*, *hsa-miR-155*, *hsa-miR-182*, *hsa-miR-192*, *hsa-miR-194*, *hsa-miR-210* and *hsa-miR-212*) in
172 blastocysts, respectively. Two miRNAs analysed (*hsa-miR-181c* and *hsa-miR-196*) were neither
173 detected in oocytes nor in blastocysts, whereas seven were only detected in blastocysts (*hsa-miR-7-2*,
174 *hsa-miR-15a*, *hsa-miR-23*, *hsa-miR-34*, *hsa-miR-128*, *hsa-miR-130* and *hsa-miR-155*). All miRNAs

175 detected in oocytes were also detected in blastocysts. The fold differences of these mRNAs and
176 miRNAs between oocytes and blastocysts are listed in the supplementary table 2.

177 The expression levels of all mRNAs were significantly higher in oocytes compared to blastocysts ($p <$
178 0.05 , figure 2). Six miRNAs (hsa-miR- 31, hsa-miR-16, let-miR-7a, hsa- miR-145, hsa-miR-210 and
179 hsa-miR-212) were expressed at significantly higher levels in oocytes compared to blastocysts (Figure
180 3) whereas five miRNAs (hsa-miR-21, hsa-miR-101, hsa-miR-182, hsa-miR-192 and hsa-miR-194)
181 were expressed at similar levels in oocytes and blastocysts.

182 **Correlation between the expression of mRNA and miRNA**

183 Two miRNAs, hsa-miR-181c and hsa-miR-196, that were neither expressed in oocytes nor in
184 blastocysts were excluded from the correlation analysis. Pearson correlation test showed that there
185 was a trend for both negative and positive correlations between miRNAs and their target mRNAs.
186 Inverse associations were observed in blastocysts ($p < 0.05$) for hsa-miR-23b and its target gene
187 *GTF2H2*, hsa-miR-128 and *DCLRE1A* and hsa-miR-128 and *RAD50* and direct association for hsa-
188 miR-34c, and *RBI* that may indicate a possible stabilisation effect of miRNAs on their target
189 mRNAs.

190 **DISCUSSION**

191 The level of gene expression is affected by many factors that may influence the DNA repair capacity.
192 In the recent years, one of the regulatory mechanisms of transcripts was suggested to be through
193 miRNA control. Although many studies have shown that several repair gene transcripts are regulated
194 or regulate several miRNAs in cancer cells and cell lines, no studies were performed to analyse the
195 association between miRNAs and their target mRNAs involved in DNA repair in human oocytes and
196 embryos. The aim of this study was to analyse this association by correlating the expression levels of
197 a selection of miRNAs and their target mRNAs.

198 In this study, the expression of 10 mRNAs and 20 miRNAs were analysed in human oocytes and
199 blastocysts. The expression of all the mRNAs tested and 11 miRNAs were detected in both oocytes
200 and blastocysts. Higher mRNA expression levels were detected in oocytes relative to the blastocysts
201 (Figure 2). This is not surprising since the oocytes are required to be packed with mRNAs and passed
202 on the early embryo to support itself until the embryonic genome activation and the maternal mRNAs
203 are expected to be degraded post embryonic genome activation. Similar to the repair genes, majority
204 of the miRNAs showed a higher expression level in oocytes relative to blastocysts. It has been well
205 established that miRNAs silence many genes for translational inhibition, cleavage, degradation or
206 destabilisation [23-28]. Therefore, the higher expression of miRNAs in oocytes may be required to
207 degrade the maternally inherited mRNAs in the early developing embryos as also reported in
208 zebrafish [47, 48] and in rainbow trout [49].

209 More recently studies have proposed that miRNAs may stabilise their target mRNAs as well [27, 30-
210 36]. This study further investigated the possibility of the stabilisation effect of mRNAs on their target
211 mRNAs by analysing whether individual samples with higher miRNA expression levels might tend to
212 have higher or consistent expression levels of their target mRNAs. Alternatively a correlation in the
213 other direction was questioned in order to understand the possible down-regulatory roles of miRNAs
214 on their targets.

215 A trend for both direct and inverse relationships was observed between miRNAs and their target
216 mRNAs. It is possible that these inverse and direct relationships are miRNA specific and each
217 miRNA regulates each target differently. It may also depend on the embryonic development stage or
218 it is possible that these observations do not hold a biological significance. A direct association was
219 observed between the expression of miRNAs and *RBI*, active at G1/S transition, in oocytes and
220 blastocysts. This direct relationship was also observed in oocytes among the miRNAs and mismatch
221 repair genes, *MSH2* and *MSH3*, functioning at G2 cell cycle checkpoint. This association may be
222 necessary to stabilise the target mRNAs in the early developing embryos to direct DNA repair until
223 the embryonic genome activation. However both direct and indirect relationships were observed for

224 the nucleotide excision repair genes as also reported previously for miR-192 and *ERCC3* [11], double
225 strand break repair and interstrand crosslink repair genes. The down-regulatory effect of some
226 miRNAs on *BRCA1* and *PARP1* was also reported previously [50, 51]. The inverse relationships
227 between miRNAs and their target mRNAs functioning at the G1/S phase of cell cycle may impair the
228 detection and repair capacity of DNA damage. This may be the reason for oocytes to continue the
229 meiotic divisions in the presence of DNA damage as reported previously by Marangos and Carroll
230 (2012) [52].

231 One of the main limitations of this study was the practical inability of following the expression of
232 miRNA and mRNA through different developmental stages. We have analysed the expression level of
233 miRNAs and mRNAs in immature oocytes and at blastocyst stage of embryonic development.
234 Previously published studies in humans have shown that 2% of the miRNAs analysed showed
235 different expression levels between GV and MII oocytes [40]. Similarly, bovine studies have shown
236 that miRNA expression varies during preimplantation embryo development and embryonic genome
237 activation [38, 39]. However, in our study due to the scarcity of human samples, we could only
238 analyse the expression of a limited number of miRNA and mRNA in limited sample types. Although
239 the investigation of the miRNA and the target mRNA expression within the same group of oocytes
240 and blastocysts would provide a more applicable analysis, due to technical reasons and scarcity of the
241 samples, the analysis was performed on a separate group of oocytes and blastocysts obtained from
242 different women and couples. While use of oocytes and embryos from different women and couples is
243 a weakness of our study, it could also be considered as strength since it eliminates any possible
244 hierarchal conclusions. Furthermore, even though there may be some differential miRNA expression
245 among different oocytes and embryos, the difference is not expected to be significant since none of
246 the patients were diagnosed with complications, such as polycystic ovarian syndrome or
247 endometriosis that may have caused differences in miRNA expression [53, 54]. With the
248 improvements in the amplification techniques and lowering the bias introduced, future experiments
249 may be performed on amplified human samples enabling expression studies on the same set of
250 oocytes and blastocysts. The amplification has the benefit of analysis many more miRNAs and

251 mRNAs from the same set of oocytes and blastocysts. Further studies focusing on expression analysis
252 of more miRNAs and target mRNAs will provide better understanding the association between
253 miRNAs and their target mRNAs. However, the expression analyses alone would still not be
254 sufficient to prove a true regulation and functional studies are crucial for a definite conclusion.

255 **CONCLUSIONS**

256 Regulation of mRNAs involves complex mechanisms, amongst which are included the action of
257 miRNAs. It is well established that miRNAs target many mRNAs including ones involved in DNA
258 repair mechanisms. This study showed that miRNAs and their target mRNAs involved in DNA repair
259 are expressed in preimplantation embryos. Similar to the miRNAs expressed in different tissue types,
260 the miRNAs expressed in embryos seem to have regulatory roles on their target DNA repair mRNAs
261 during preimplantation embryo development.

262

- 264 1. Lukas, J., C. Lukas, and J. Bartek, *Mammalian cell cycle checkpoints: signalling pathways and*
 265 *their organization in space and time*. DNA Repair (Amst), 2004. **3**(8-9): p. 997-1007.
- 266 2. Bartel, D.P., *MicroRNAs: target recognition and regulatory functions*. Cell, 2009. **136**(2): p.
 267 215-33.
- 268 3. Pasquinelli, A.E., *MicroRNAs and their targets: recognition, regulation and an emerging*
 269 *reciprocal relationship*. Nat Rev Genet, 2012. **13**(4): p. 271-82.
- 270 4. Liu, Y., et al., *miR-375 targets the p53 gene to regulate cellular response to ionizing radiation*
 271 *and etoposide in gastric cancer cells*. DNA Repair (Amst), 2013. **12**(9): p. 741-50.
- 272 5. Pok, S., et al., *Cyclin E facilitates dysplastic hepatocytes to bypass G1/S checkpoint in*
 273 *hepatocarcinogenesis*. J Gastroenterol Hepatol, 2013. **28**(9): p. 1545-54.
- 274 6. Noto, J.M. and R.M. Peek, *The Role of microRNAs in Helicobacter pylori Pathogenesis and*
 275 *Gastric Carcinogenesis*. Front Cell Infect Microbiol, 2012. **1**: p. 21.
- 276 7. Aqeilan, R.I., G.A. Calin, and C.M. Croce, *miR-15a and miR-16-1 in cancer: discovery, function*
 277 *and future perspectives*. Cell Death Differ, 2009. **17**(2): p. 215-220.
- 278 8. Krichevsky, A.M. and G. Gabriely, *miR-21: a small multi-faceted RNA*. Journal of Cellular and
 279 Molecular Medicine, 2009. **13**(1): p. 39-53.
- 280 9. Jurado, J.O., et al., *IL-17 and IFN-gamma expression in lymphocytes from patients with active*
 281 *tuberculosis correlates with the severity of the disease*. J Leukoc Biol, 2012. **91**(6): p. 991-
 282 1002.
- 283 10. Massirer, K.B., et al., *The miR-35-41 family of microRNAs regulates RNAi sensitivity in*
 284 *Caenorhabditis elegans*. PLoS Genet, 2012. **8**(3): p. e1002536.
- 285 11. Xie, Q.H., et al., *MiR-192 inhibits nucleotide excision repair by targeting ERCC3 and ERCC4 in*
 286 *HepG2.2.15 cells*. Biochem Biophys Res Commun, 2011. **410**(3): p. 440-5.
- 287 12. Svrcek, M., et al., *Overexpression of microRNAs-155 and 21 targeting mismatch repair*
 288 *proteins in inflammatory bowel diseases*. Carcinogenesis, 2013. **34**(4): p. 828-34.
- 289 13. Mao, G., et al., *Modulation of microRNA processing by mismatch repair protein MutLalpha*.
 290 Cell Res, 2012. **22**(6): p. 973-85.
- 291 14. He, Y.Q., et al., *Estradiol regulates miR-135b and mismatch repair gene expressions via*
 292 *estrogen receptor-beta in colorectal cells*. Exp Mol Med, 2012. **44**(12): p. 723-32.
- 293 15. Valeri, N., et al., *Modulation of mismatch repair and genomic stability by miR-155*.
 294 Proceedings of the National Academy of Sciences, 2010. **107**(15): p. 6982-6987.
- 295 16. Hoffman, A.E., et al., *microRNA miR-196a-2 and breast cancer: a genetic and epigenetic*
 296 *association study and functional analysis*. Cancer Res, 2009. **69**(14): p. 5970-7.
- 297 17. Neijenhuis, S., et al., *Identification of miRNA modulators to PARP inhibitor response*. DNA
 298 Repair (Amst), 2013. **12**(6): p. 394-402.
- 299 18. Krishnan, K., et al., *MicroRNA-182-5p targets a network of genes involved in DNA repair*.
 300 RNA, 2013. **19**(2): p. 230-42.
- 301 19. Jiang, Y., et al., *Genetic variation in a hsa-let-7 binding site in RAD52 is associated with breast*
 302 *cancer susceptibility*. Carcinogenesis, 2013. **34**(3): p. 689-93.
- 303 20. Mansour, W.Y., et al., *Aberrant overexpression of miR-421 downregulates ATM and leads to*
 304 *a pronounced DSB repair defect and clinical hypersensitivity in SKX squamous cell carcinoma*.
 305 Radiother Oncol, 2012. **106**(1): p. 147-54.
- 306 21. Wu, C.W., et al., *MicroRNA-18a attenuates DNA damage repair through suppressing the*
 307 *expression of ataxia telangiectasia mutated in colorectal cancer*. PLoS One, 2013. **8**(2): p.
 308 e57036.
- 309 22. McCallie, B., W.B. Schoolcraft, and M.G. Katz-Jaffe, *Aberration of blastocyst microRNA*
 310 *expression is associated with human infertility*. Fertil Steril, 2010.

- 311 23. Bagga, S., et al., *Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation.* Cell, 2005. **122**(4): p. 553-63.
- 312
- 313 24. Hayashi, K., et al., *MicroRNA biogenesis is required for mouse primordial germ cell*
- 314 *development and spermatogenesis.* PLoS One, 2008. **3**(3): p. e1738.
- 315 25. Tesfaye, D., et al., *Identification and expression profiling of microRNAs during bovine oocyte*
- 316 *maturation using heterologous approach.* Mol Reprod Dev, 2009. **76**(7): p. 665-77.
- 317 26. Bartel, D.P., *MicroRNAs: genomics, biogenesis, mechanism, and function.* Cell, 2004. **116**(2):
- 318 p. 281-97.
- 319 27. Bartel, D.P. and C.Z. Chen, *Micromanagers of gene expression: the potentially widespread*
- 320 *influence of metazoan microRNAs.* Nat Rev Genet, 2004. **5**(5): p. 396-400.
- 321 28. Miranda, K.C., et al., *A pattern-based method for the identification of MicroRNA binding sites*
- 322 *and their corresponding heteroduplexes.* Cell, 2006. **126**(6): p. 1203-17.
- 323 29. Place, R.F., et al., *MicroRNA-373 induces expression of genes with complementary promoter*
- 324 *sequences.* Proc Natl Acad Sci U S A, 2008. **105**(5): p. 1608-13.
- 325 30. Salmena, L., et al., *A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language?* Cell,
- 326 2011. **146**(3): p. 353-8.
- 327 31. Seitz, H., *Redefining microRNA targets.* Curr Biol, 2009. **19**(10): p. 870-3.
- 328 32. Arvey, A., et al., *Target mRNA abundance dilutes microRNA and siRNA activity.* Mol Syst Biol,
- 329 2010. **6**: p. 363.
- 330 33. Lee, D.Y., et al., *A 3'-untranslated region (3'UTR) induces organ adhesion by regulating miR-*
- 331 *199a* functions.* PLoS One, 2009. **4**(2): p. e4527.
- 332 34. Poliseno, L., et al., *A coding-independent function of gene and pseudogene mRNAs regulates*
- 333 *tumour biology.* Nature, 2010. **465**(7301): p. 1033-8.
- 334 35. Franco-Zorrilla, J.M., et al., *Target mimicry provides a new mechanism for regulation of*
- 335 *microRNA activity.* Nat Genet, 2007. **39**(8): p. 1033-7.
- 336 36. Cazalla, D., T. Yario, and J.A. Steitz, *Down-regulation of a host microRNA by a Herpesvirus*
- 337 *saimiri noncoding RNA.* Science, 2010. **328**(5985): p. 1563-6.
- 338 37. Rosenbluth, E.M., et al., *MicroRNA expression in the human blastocyst.* Fertil Steril, 2012.
- 339 **99**(3): p. 855-861.
- 340 38. Tang, F., et al., *Maternal microRNAs are essential for mouse zygotic development.* Genes
- 341 Dev, 2007. **21**(6): p. 644-8.
- 342 39. Yang, Y., et al., *Determination of microRNAs in mouse preimplantation embryos by*
- 343 *microarray.* Dev Dyn, 2008. **237**(9): p. 2315-27.
- 344 40. Xu, Y.W., et al., *Differentially expressed microRNAs in human oocytes.* J Assist Reprod Genet,
- 345 2011. **28**(6): p. 559-66.
- 346 41. Liu, J., et al., *MicroRNA-dependent localization of targeted mRNAs to mammalian P-bodies.*
- 347 Nat Cell Biol, 2005. **7**(7): p. 719-23.
- 348 42. Amanai, M., M. Brahmajoyula, and A.C. Perry, *A restricted role for sperm-borne microRNAs*
- 349 *in mammalian fertilization.* Biol Reprod, 2006. **75**(6): p. 877-84.
- 350 43. Jaroudi, S., et al., *Expression profiling of DNA repair genes in human oocytes and blastocysts*
- 351 *using microarrays.* Hum Reprod, 2009. **24**(10): p. 2649-55.
- 352 44. Kakourou, G., et al., *Investigation of gene expression profiles before and after embryonic*
- 353 *genome activation and assessment of functional pathways at the human metaphase II*
- 354 *oocyte and blastocyst stage.* Fertil Steril, 2012. **99**(3): p. 803-814 e23.
- 355 45. Yang, G.H., et al., *MicroRNAs are involved in erythroid differentiation control.* J Cell Biochem,
- 356 2009. **107**(3): p. 548-56.
- 357 46. Mondou, E., et al., *Analysis of microRNAs and their precursors in bovine early embryonic*
- 358 *development.* Mol Hum Reprod, 2012.
- 359 47. Chen, P.Y., et al., *The developmental miRNA profiles of zebrafish as determined by small RNA*
- 360 *cloning.* Genes Dev, 2005. **19**(11): p. 1288-93.

361 48. Giraldez, A.J., et al., *Zebrafish MiR-430 promotes deadenylation and clearance of maternal*
362 *mRNAs*. Science, 2006. **312**(5770): p. 75-9.

363 49. Ramachandra, R.K., et al., *Cloning and characterization of microRNAs from rainbow trout*
364 *(Oncorhynchus mykiss): their expression during early embryonic development*. BMC Dev Biol,
365 2008. **8**: p. 41.

366 50. Moskwa, P., et al., *miR-182-Mediated Downregulation of BRCA1 Impacts DNA Repair and*
367 *Sensitivity to PARP Inhibitors*. Molecular cell, 2010. **41**(2): p. 210-220.

368 51. Volinia, S., et al., *Breast cancer signatures for invasiveness and prognosis defined by deep*
369 *sequencing of microRNA*. Proc Natl Acad Sci U S A, 2012. **109**(8): p. 3024-9.

370 52. Marangos, P. and J. Carroll, *Oocytes progress beyond prophase in the presence of DNA*
371 *damage*. Curr Biol, 2012. **22**(11): p. 989-94.

372 53. Sorensen, A.E., et al., *MicroRNAs Related to Polycystic Ovary Syndrome (PCOS)*. Genes
373 (Basel), 2014. **5**(3): p. 684-708.

374 54. Yang, L. and H.Y. Liu, *Small RNA molecules in endometriosis: pathogenesis and therapeutic*
375 *aspects*. Eur J Obstet Gynecol Reprod Biol, 2014. **183**: p. 83-8.

376 55. McPhee, F., et al., *Resistance Analysis of the Hepatitis C Virus NS3 Protease Inhibitor*
377 *Asunaprevir*. Antimicrob Agents Chemother, 2012.

378 56. Valeria, N., et al., *MicroRNA-21 induces resistance to 5-fluorouracil by down-regulating*
379 *human DNA MutS homolog 2 (hMSH2)*. PNAS, 2010. **107**: p. 21098-21103.

380 57. Lee, R.C., R.L. Feinbaum, and V. Ambros, *The C. elegans heterochronic gene lin-4 encodes*
381 *small RNAs with antisense complementarity to lin-14*. Cell, 1993. **75**(5): p. 843-54.

382 58. Mueller, A.C., D. Sun, and A. Dutta, *The miR-99 family regulates the DNA damage response*
383 *through its target SNF2H*. Oncogene, 2012.

384

385

386

FIGURE LEGENDS

Figure 1 Schematic diagram of the complex association of miRNAs in DNA repair pathways.

MiRNAs that were shown or predicted to target genes involved in repair were shown in a target map. MiRNAs that were associated with microsatellite instability (indicative of defective mismatch repair) were also shown in this diagram. MiRNAs were colour coded according to their target mRNAs. Double strand break repair genes (*DCLRE1A*, *PARP1*, *BRCA1* and *RAD50*) are shown in pink, mismatch repair genes (*MSH2* and *MSH3*) and microsatellite instability in green, nucleotide excision repair genes (*ERCC3* and *GTF2H2*) in purple and checkpoint genes (*RB1* and) in blue shades, respectively.

Figure 2 mRNA expression levels quantified by real time PCR after normalisation with the endogenous control, *ACTB*.

Quantification of target mRNAs compared to *GTF2H2*, which was shown to be expressed at the highest level in a) oocytes and b) blastocysts. ANOVA followed by Dunnett's post test was applied to all the mRNA normalised to *ACTB*; where **** $p < 0.0001$ indicates the most statistical significance, *** $0.0001 < p < 0.001$, ** $0.001 < p < 0.01$, * $0.01 < p < 0.05$ less statistical significance and $p \geq 0.05$ indicates no statistical significance. The x-axis represents all the miRNAs analysed in this study and the y-axis represents the ΔCq values. *GTF2H2* is shown in purple, *PARP1* in red, *BRCA1* in pink, *RAD50* in light pink, *MSH2* in light green, *MSH3* in dark green, *ERCC3* in purple, *DCLRE1A* in mid pink, *RBI* in aqua, and *DICER1* in black shades, respectively.

Figure 3 miRNA expression levels quantified by real time PCR after normalisation with the endogenous control, RNU48.

Quantification of target miRNAs compared to hsa-miR-16 a) in oocytes and b) in blastocysts. ANOVA followed by Dunnett's post test was applied to all miRNAs compared to hsa-miR-16. The x-axis represents all the miRNAs analysed in this study and the y-axis represents the ΔCq values. In oocytes the expression of hsa-miR-16 was at a higher expression level compared to the endogenous control RNU48 represented by a negative profile for this miRNA. MiRNAs were colour-coded according to their target genes as in figure 2, i.e. miRNAs targeting *PARP1* is shown in red, *BRCA1* in pink, *RAD50* in mid pink, *DCLRE1A* in light pink, *MSH2* in light green, *MSH3* in dark green, *ERCC3* in purple, *GTF2H2* in lilac and *RBI* in aqua shades, respectively. **** represents that the difference in level of expression of the miRNA from that of hsa-miR-16 is statistically significant ($p < 0.001$).

Tables

Table 1 List of miRNAs and their association with DNA repair.

Cell Cycle Checkpoint	Nucleotide Excision Repair		Base Excision Repair	Double Strand Break Repair				Mismatch Repair		
<i>RB1</i>	<i>GTF2H2</i>	<i>ERCC3</i>	<i>PARP1</i>	<i>DCLRE1A</i>	<i>PARP1</i>	<i>BRCA1</i>	<i>RAD50</i>	<i>MSH2</i>	<i>MSH3</i>	<i>MSI</i>
Let-7	miR-23b	miR-192	miR-7	miR-15a	miR-7	miR-7	miR-15a	miR-21	miR-7	Let-7a
miR-7	miR-101		miR-31	miR-16	miR-31	miR-145	miR-16	miR-145	miR-21	miR-31
miR-34c	miR-128		miR-130	miR-23b	miR-130	miR-155	miR-128	miR-155	miR-192	miR-101
miR-101	miR-181c		miR-182	miR-128	miR-182	miR-182	miR-155	miR-192		miR-145
miR-128	miR-192		miR-192	miR-145	miR-192	miR-196	miR-194			miR-155
miR-181c	miR-212		miR-196	miR-155	miR-196	miR-210	miR-212			miR-181c
miR-192			miR-210	miR-212	miR-210	miR-212				miR-192
miR-194										miR-196b
miR-212										miR-212

Genes involved in cell cycle checkpoint, nucleotide excision, base excision, double strand break and mismatch repair pathways and the miRNAs targeting these genes are listed. These associations were either published previously [11, 55-58] or bioinformatics studies showed that these miRNAs target the mRNAs (<http://www.targetscan.org/>, <http://www.microrna.org/microrna/home.do>, <http://mirdb.org/miRDB/>).

SUPPLEMENTAL TABLE LEGENDS

Supplemental Table 1 Δ Cq values and fold changes for mRNA expression in blastocysts compared with oocytes.

List of a) mRNAs and b) miRNAs, fold difference and log₂ of fold change in blastocyst compared with oocyte are shown. mRNAs and miRNAs and Δ Cq values in oocytes and blastocysts are listed. Average normalised Δ Cq values generated from six repeats with two replicates of each sample is shown. Standard deviation (SD) for each data point of Δ Cq is shown in parenthesis. The greater Cq values reflect lower expression. b) MiRNAs, fold change difference and log₂ of fold change in blastocyst compared with oocyte are listed. Fold change was calculated by relative quantification method ($2^{-\Delta\Delta Cq}$). The log₂ scale transformation of the fold change facilitates interpretation of the difference in expression between the two samples. The negative log₂ values indicated increased expression of the gene in the blastocyst compared with the oocyte. N/A stands for not applicable.