RESEARCH PAPER

Polymorphism of miRNA and its Impact on IVF Failure

Rande Khasro Dizay¹, Suhad Asaad Mustafa²

¹Department of Biology, College of Sciences, Salahaddin University-Erbil, Kurdistan Region, Iraq.
²Scientific Research Center, Salhaddin Uivnesity-Erbil, Erbil, Kurdistan Region, Iraq.

ABSTRACT:

In vitro fertilization (IVF) failure is not only the cause of despair among couples and individuals undergoing the treatment, it’s also been contributing to the impediment of assistive reproductive technologies (ART) development, miRNAs have been linked to significant events in the reproduction course and the identification of miRNA polymorphisms may provide a good lead for the potential of diagnosis and treatment of unidentified IVF failure causes, the aim of the present study was to explore the association between the miRNA polymorphism (mir-125a T>Crs12975333) and IVF failure. Our case-control study consisted of 100 Kurdish women in total, 50 belong to the case group that underwent IVF failure and the other 50 belong to the control group who have had at least two successful pregnancies and no history of pregnancy loss, we used tetra amplification refractory mutation system to identify the polymorphisms within the groups, the TT genotype was found more frequently in IVF failure patients when compared to the healthy women (OR: 5.268, CI: 1.07-25.7, P=0.025) and T allele was more present in the case group (OR:1.9, CI:1.06-3.41, P=0.028). The difference in genotype and allele frequencies of mir-125a of the two groups may indicate that it has an effect on the target mRNAs and alter the implantation of embryo during IVF cycles.

KEY WORDS: IVF; miRNA; Infertility; mir-125a; SNP.
DOI: http://dx.doi.org/10.21271/ZJPAS.31.6.9
ZJPAS (2019) , 31(6);84-91

1. INTRODUCTION:

MicroRNAs (miRNAs) are a class of small, single stranded, non-coding RNAs, first discovered in 1993 in c. Elegans (Lee et al., 1993), miRNA’s main function is regulation of gene expression at the post-transcriptional level, they do so by degrading or blocking the translation of mRNA (messenger RNA) mostly by interacting with the 3’ untranslated region of the mRNA(Pogribny, 2018, O’Brien et al., 2018). MiRNAs are widely conserved across many species thus making them a sought after topic of research. Since each miRNA usually targets hundreds of different mRNAs thus they act as the main controller of gene expression, the bulk of mammalian protein coding genes seem to be regulated under miRNA control(Bitetti et al., 2018), and the key biological processes; cell differentiation, proliferation, apoptosis, embryo implantation and its development are regulated by these molecules(Kim et al., 2019). MiRNAs have been associated with many female related disorders including ovarian cancer (Nakamura et al., 2016) breast cancer (Wang and Luo, 2015) endometriosis (Nothnick et al., 2015) and polycystic ovarian syndrome(Sørensen et al., 2014). The possibility of miRNAs affecting implantation in animal model was suggested by chakrabarty et al where they showed the effect of miRNA on certain genes that are involved in implantation(Chakrabarty et al., 2007). Another
study that was also based on animal model showed the differences in miRNA levels in IVF and in vivo embryos of porcine, where the changes were related to different stages of development and culture conditions (Stowe et al., 2012). Li et al. showed that alterations in miRNA subsets affect the expression of certain genes that are crucial for implantation and hence decrease the embryo receptivity in women undergoing IVF treatment (Li et al., 2011). The relationship between mmu-mir-367a and its mRNA target (pcna) was the first example of miRNA regulating primordial follicle assembly in mice (Zhang et al., 2014).

Louise Brown was the first child born using in vitro fertilization back in July 15th 1978. This event became a beacon of hope for couples suffering from long or short term infertility. IVF has since then undergone many modifications and advancements and in 2014 it was reported that more than 5 million babies had been born with the aid of IVF (Kovacs, 2014), however like most other procedures it is yet to be perfected and the success rates are still relatively low. In humans, about 40% of unsuccessful IVF treatments are due to implantation failure (Dentillo et al., 2007), this failure can be related to the embryo transfer technique, embryo quality and endometrial receptivity (O’Flynn, 2014), the latter two are regulated by a cascade of events including miRNAs effect on different genes (Kropp and Khatib, 2015, Shi et al., 2017).

There are approximately 240 Single nucleotide polymorphisms (SNP) and rare mutations have been reported in miRNAs and their association with IVF outcome has been established (Krolczewski et al., 2018, Lee et al., 2019, Cho et al., 2016). The appearance of a SNP in a miRNA’s mature sequence may have different but certain consequences on the miRNA either by loss of binding, change in the binding efficacy or recognizing different mRNA targets than their own (Brennecke et al., 2005, Agarwal et al., 2015). Changes can also happen to miRNA biogenesis if the SNPs were introduced at the pre-or pri-miRNA sequences (Sood et al., 2006). Even though the particular SNP has been studied to evaluate their relationship with other disease such as gastric cardia adenocarcinoma (Singh et al., 2017), Breast cancer (Peterlongo et al., 2011) autoimmune thyroid disease (Latini et al., 2017).

To our knowledge there is no previous publications to study the relation of rs12975333 to IVF failure, however mir-125a has been known to be a factor in many reproductive disorders in females such as primary ovarian failure in mice (Wang et al., 2016), Placenta Accreta (Gu et al., 2019), repeated pregnancy loss (Hu et al., 2014) unexplained recurrent spontaneous abortions (Li and Li, 2016) early pregnancy loss (Hosseini et al., 2018). In this present study we used Tetra ARMS (Amplification Refractory Mutation System) to detect SNPs of mir-125a G>T (rs12975333) in Kurdish women to evaluate their relation with IVF failure.

2. MATERIALS AND METHODS

2.1 Study Design

In total, 100 subjects were recruited for this study with ages ranging from (17-40) years, 50 female patients who have undergone failed IVF cycles were recruited from Hawler Maternal Hospital, Iraqi Kurdistan Region. The patients who had partners that were infertile (male factor infertility) and/or had any successful pregnancies were excluded. The control group also consisted of 50 subjects that were recruited in an outpatient clinic; these were healthy women who have had at least two successful pregnancies and had no history of miscarriages or any form of implantation failure. All subjects from both groups had their height and weight measured. Smokers and women above the age of 40 were excluded in this case-control study, the practical part of this study was conducted at the Scientific Research Center, Erbil, Iraq.

2.2 Biochemical Analysis and Genotyping

Venous blood (5mL) was drawn from all subjects, 3mL of which was placed into a vacuumed EDTA tube for DNA extraction, while the rest was placed into a vacuumed Gel tube, they were then spun for 15 minutes at 10,000 rpm, the separated serum was then transferred to an Eppendorf tube for Biochemical analysis, all the tubes were frozen at -20° C for further analysis.
The hormonal assays were analyzed using cobas e411 (Roche Ltd, Switzerland).

DNA was extracted using GeNet Bio DNA extraction kit for whole blood and then tested for concentration and purity using Nanodrop (Thermo Scientific, UK), the samples appeared to have a concentration range between (22.13-91.68 ng/µL) and were all within the normal range of purity. After optimization all samples were prepared for the PCR, In 0.2 mL PCR tubes containing 10µ of Master mix (Primer Taq Premix 2X, GeNet Bio) and the tetra primers each of 25 pmol concentration were added along with DNA and RNA free distilled water and DNA (50 ng). Then the PCR tubes were placed into the PCR wells (Techne TC-512, UK). DNA was visualized post PCR using a Gel Electrophoresis system (Cleaver, UK), the gel had a 2% agarose concentration and was stained with Safe Dye (Prime safe Dye, Genet Bio) prior to casting onto the tray. The DNA bands were then visualized using a Gel documentation system (Proxima 2500, India). Finally, the results were statistically analyzed using IBM SPSS Statistics v22.

### 2.3 Primer Design

The primers used in this research were designed by a web-based primer designing tool (Primer1) available at [http://primer1.soton.ac.uk/primer1.html](http://primer1.soton.ac.uk/primer1.html) (Collins and Ke, 2012). For checking the primer specificity we used another web-based tool (PrimerBLAST) available at [https://blast.ncbi.nlm.nih.gov/Blast.cgi](https://blast.ncbi.nlm.nih.gov/Blast.cgi), the designed primers are shown in Table 1.

#### Table 1. List of Primers used

| Gene   | Primer | Sequence 5’-3’ | length | Tm1 | Tam2 | Expected amplicon size |
|--------|--------|----------------|--------|-----|------|------------------------|
| mir-125a rs12975333 | FO    | CTATACGGGCCTCCTAGCTTTCCTCCCAG | 26     | 71.0 | 58.9 | 673 (Non-Specific)     |
|        | RO    | CCAGGGAGAAGCTAGTAACCTTTATGA | 28     | 68.7 |      |                        |
|        | FI (T)| CATGGTGCAGTCTCAGGTGCTAAT   | 27     | 68.3 |      | 263 (T-allele)         |
|        | RI (G)| TGGATGTTCCTACAGGTTAAGGGATC | 30     | 66.3 |      | 463 (G-allele)         |

1: melting temperature, 2: annealing temperature, both measured in Celsius.

### 2.4 Tetra ARMS PCR Optimization

Optimization for *mir-125a* started by testing the primers at different concentrations (10, 25, 35 and 50 pmol) at an estimated annealing temperature, the optimum primer concentration was set at 25 pmol. Later we tested different annealing temperatures using Gradient PCR for each of the Forward Outer (FO) & Reverse Inner (RI), Forward Inner (FI) & Reverse Outer (RO), and FO & RO.

### 3. RESULTS

The first allele to be optimized was T-allele shown in Figure (1) which is an agarose gel image of a Gradient PCR for T-allele (FI & RO) at temperatures (57.1°-58.0°-58.9°-59.6°-61.3°-62.1°) we see the T-allele whose expected band size is at 263 bp shows up at all the temperatures with the thickest band at 58.9, as for G-allele whose expected band is at 463 bp and Non-specific whose expected band is at 673 bp are shown in Figures (2 and 3), respectively, they
both seem to demonstrate extra undesirable bands at temperatures lower than 59.6, as for all the tetra primers together we tested at the temperature range (57.1°-58.0°-58.9°-59.6°-61.3°-62.1°-63.0°) shown in Figure (4). The three expected bands show clearly at 58.9 however it fades in the following higher temperatures and doesn’t show at lower temperatures, based on the information shown in these figures and other trials we concluded that the optimum PCR conditions for Tetra ARMS for mir-125a is 25 pmol primer concentration at 58.9° annealing temperature.

The subjects’ clinical and anthropometric characteristics including; Luteinizing hormone (LH), Estradiol, Progesterone, Prolactin, Thyroid Stimulating Hormone (TSH), Anti-Mullerian Hormone (AMH), Follicle Stimulating Hormone (FSH) and Body Mass Index (BMI), the data is presented in Table (2) in the form of mean± Standard deviation and P-value. There was no significant difference between the groups in all the criteria except for (Age) where women of the Case group had higher age mean (P=0.008) and (Family History) with regards to sibling history of infertility there was a significant difference (P=0.027) when the Case group was compared to the Control group, the genotypes for both case and control groups were detected using Tetra ARMS PCR analysis, the genotypes are as follows TT, GG and TG and they are shown in Figure 5. The statistical analysis of the genotype and allele frequencies is shown in Table 3, the genotype frequencies for the case group were 27, 14 and 9 for TG, GG and TT genotypes , respectively, while for the control group it were 26, 22 and 2 for TG, GG and TT genotypes , respectively, the TT Genotype was significantly higher in the IVF failure women (OR: 5.26, CI:1.07-25.7, P=0.025) as for the alleles GG and TG no significant difference was found between the two groups, Also T-allele was found more in the patient group with a significant difference( OR:1.9, CI:1.06-3.14, P=0.028).
Figure 4. Image of tetra ARMS optimization A: DNA marker, B:57.1°, C: 58.0°, D:58.9°, E:59.6°, F:61.3°, G:62.1°, H: 63°. 2% Gel.

Figure 5. Image of gel showing all three genotypes A: DNA marker, B:GG, C: TT, D:TG, E:TG, F:GG, G:GG. 2% Gel.

Table 2. Clinical and Anthropometric Characteristics of both case and control groups

| Characteristic                        | Case (n.50)       | Control (n.50) | P value |
|--------------------------------------|-------------------|----------------|---------|
| Luteinizing hormone                  | 6.617±3.652\(^a\) | 7.045±4.552\(^b\) | 0.602   |
| Estradiol                            | 190.2±626.5       | 34.89±19.83    | 0.083   |
| Progesterone                         | 0.644±0.483       | 0.792±0.577    | 0.167   |
| Prolactin                            | 21.07±9.44        | 17.59±17.61    | 0.221   |
| Thyroid Stimulating Hormone          | 2.505±1.523       | 2.227±1.833    | 0.310   |
| Anti-Mullerian Hormone               | 2.883±2.07        | 2.503±1.042    | 0.249   |
| Follicle Stimulating Hormone         | 6.951±2.952       | 6.579±1.822    | 0.450   |
| Body Mass Index                      | 29.0± 5.1         | 27.8±4.33      | 0.230   |
| Age                                  | 33.6± 4.19        | 31.3± 4.57     | 0.008   |
| Family History (Siblings) (n)% YES   | 7 (14)            | 1(2)           | 0.027   |
| Family History (Relatives) (n)% YES  | 10 (20)           | 8(16)          | 0.62    |

\(^a\): Mean value, \(^b\): Standard deviation
embryos they eliminated that possibility since among other of mir-125a exhibited no difference in expression levels (Borges et al.), bioinformatics study shows that mir-125a a member of the mir-125 family is involved in preimplantation embryo development by control sebox maternal effect gene which is known to pay role in very early embryonic development (Kim et al., 2016) the expression levels of miR-125a in mural and cumulus granulosa cells which are involved in folliculogenesis in healthy women who are undergoing IVF treatment (Andrei et al., 2019)lin-28, a controller of pluripotency of embryonic stem cells has been shown to be downregulated by mir-125a which further demonstrate the effect of this miRNA on embryonic development in mice (Potenza and Russo, 2013, Kim et al., 2016) Hu et al also studied mutations in the pri-region of mir-125a genes affect functions endometrium including cell proliferation, migration, proliferation and embryonic development in women with repeated pregnancy loss(Hu et al., 2014) the above mentioned research further validate our results.

5. CONCLUSIONS

In conclusion our study found a significant association the TT genotype of mir-125a G>T polymorphism and IVF failure. Although the exact roles of the genotype require further exploratory experiments, their discovery may lead to an insight into understanding the complex reasoning behind IVF failure. We recommend other researchers to go into expressional studies and the genotypes effect on its target genes.

Conflict of Interest

The authors declare no conflict of interest.
REFERENCES

AGARWAL, V., BELL, G. W., NAM, J. W. & BARTEL, D. P. 2015. Predicting effective microRNA target sites in mammalian mRNAs. Elife, 4.

ANDREI, D., NAGY, R. A., VAN MONTFOORT, A., TIEGTE, U., TERPSTRA, M., KOK, K., VAN DEN BERG, A., HOEK, A., KLUIVER, J. & DONKER, R. 2019. Differential miRNA Expression Profiles in Cumulus and Mural Granulosa Cells from Human Pre-ovulatory Follicles. Micron, 8, 61-67.

BITETTI, A., MALLORY, A. C., GOLONI, E., CARRIERI, C., CARRERO GUTIÉRREZ, H., PERLAS, E., PEREZ-RICO, Y. A., TOCCCHINI-VALENTINI, G. P., ENRIGHT, A. J., NORTON, W. H. J., MANDILLO, S., O’CARROLL, D. & SHKUMATAVA, A. 2018. MicroRNA degradation by a conserved target RNA regulates animal behavior. Nature Structural & Molecular Biology, 25, 244-251.

BORGES, E., JR., SETTI, A. S., BRAGA, D. P. A. F., GERALDO, M. V., FIGUEIRA, R. D. C. S. & IACONELLI, A. JR. miR-142-3p as a biomarker of blastocyst implantation failure - A pilot study. JBRA assisted reproduction, 20, 200-205.

BRENNECKE, J., STARK, A., RUSSELL, R. B. & COHEN, S. M. 2005. Principles of MicroRNA–Target Recognition. PLOS Biology, 3, e85.

CHAKRABARTY, A., TRANGUCH, S., DAIKOKU, T., JENSEN, K., FURNEAUX, H. & DEY, S. K. 2007. MicroRNA regulation of cyclooxygenase-2 during embryo implantation. Proc Natl Acad Sci U S A, 104, 15144-9.

CHO, S. H., CHUNG, K. W., KIM, J. O., JANG, H., YOO, J. K., CHOI, Y., KO, J. J., KIM, J. H., NISHI, Y., YANASE, T., LEE, W. S. & KIM, N. K. 2016. Association of miR-146aC>G, miR-196a2T>C, and miR-499A>G polymorphisms with risk of recurrent implantation failure in Korean women. Eur J Obstet Gynecol Reprod Biol, 202, 14-9.

COLLINS, A. & KE, X. 2012. Primer3: primer design web service for tetra-primer ARMS-PCR. The Open Bioinformatics Journal, 6.

DENTILLO, D. B., SOUZA, F. R., MEOLA, J., VIEIRA, G. S., YAZLLE, M. E., GOULART, L. R. & MARTELLI, L. 2007. No evidence of association of MUC-1 genetic polymorphism with embryo implantation failure. Braz J Med Biol Res, 40, 793-7.

GU, Y., MENG, J., ZUO, C., WANG, S., LI, H., ZHAO, S., HUANG, T., WANG, X. & YAN, J. 2019. Downregulation of MicroRNA-125a in Placenta Accreta Spectrum Disorders Contributes Antioptosis of Implantation Site Intermediate Trophoblasts by Targeting MCL1. Reproductive Sciences, 1937319119828040.

HOSSEINI, M. K., GUNEL, T., GUMUSOGLU, E., BENIAN, A. & AYDINLI, K. 2018. MicroRNA expression profiling in placenta and maternal plasma in early pregnancy loss. Mol Med Rep, 17, 4941-4952.

HU, Y., HUO, Z.-H., LIU, C.-M., LIU, S.-G., ZHANG, N., YIN, K.-L., QI, L., MA, X. & XIA, H.-F. 2014. Functional study of one nucleotide mutation in primary miR-125a coding region which related to recurrent pregnancy loss. PloS one, 9, e114781-e114781.

HU, Y., LIU, C.-M., QI, L., HE, T.-Z., SHI-GUO, L., HAO, C.-J., CUI, Y., ZHANG, N., XIA, H.-F. & MA, X. 2011. Two common SNPs in pri-miR-125a alter the mature miRNA expression and associate with recurrent pregnancy loss in a Han-Chinese population. RNA biology, 8, 861-872.

KIM, J., LEE, J. & JUN, J. H. 2019. Identification of differentially expressed microRNAs in outgrowth embryos compared with blastocysts and non-outgrowth embryos in mice. Reprod Fertil Dev, 31, 645-657.

KIM, K.-H., SEO, Y.-M., KIM, E.-Y., LEE, S.-Y., KWON, J., KO, J.-J. & LEE, K.-A. 2016. The miR-125 family is an important regulator of the expression and maintenance of maternal effect genes during preimplantational embryo development. Open biology, 6, 160181.

KOVACS, P. 2014. Embryo selection: the role of time-lapse monitoring. Reprod Biol Endocrinol, 12, 124.

KROLICZEWSKI, J., SOBOLEWSKA, A., LEJNOWSKI, D., COLLAWN, J. F. & BARTOSZEWSKI, R. 2018. microRNA single nucleotide polymorphism influences on microRNA biogenesis and miRNA target specificity. Gene, 640, 66-72.

KROPP, J. & KHTAB, H. 2015. Characterization of microRNA in bovine in vitro culture media associated with embryo quality and development. J Dairy Sci, 98, 6552-63.

LATINI, A., CICCACCI, C., NOVEGLI, G. & BORGIANI, P. 2017. Polymorphisms in miRNA genes and their involvement in autoimmune diseases susceptibility. Immunologic research, 65, 811-827.

LEE, H. A., AHN, E. H., JANG, H. G., KIM, J. O., KIM, J. H., LEE, Y. B., LEE, W. S. & KIM, N. K. 2019. Association Between miR-605A>G, miR-608G>C, miR-631I>D, miR-938C>T, and miR-1302-3C>T Polymorphisms and Risk of Recurrent Implantation Failure. Reprod Sci, 26, 409-475.

LEE, R. C., FEINBAUM, R. L. & AMBROS, V. 1993. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell, 75, 843-54.

LI, D. & LI, J. 2016. Association of miR-34a-3p/5p, miR-141-3p/5p, and miR-24 in decidual natural killer cells with unexplained recurrent spontaneous abortion. Medical science monitor: international medical journal of experimental and clinical research, 22, 922.

LI, R., QIAO, J., WANG, L., LI, L., ZHEN, X., LIU, P. & ZHENG, X. 2011. MicroRNA array and microarray evaluation of endometrial receptivity in patients with high serum progesterone levels on the day of hCG administration. Reproductive biology and endocrinology : RB&E, 9, 29-29.

NAKAMURA, K., SAWADA, K., YOSHIMURA, A., KINSE, Y., NAKATSUKA, E. & KIMURA, T. 2016. Clinical relevance of circulating cell-free
microRNAs in ovarian cancer. *Molecular cancer*, 15, 48-48.

NOTHICK, W. B., AL-HENDY, A. & LUE, J. R. 2015. Circulating Micro-RNAs as Diagnostic Biomarkers for Endometriosis: Privation and Promise. *Journal of minimally invasive gynecology*, 22, 719-726.

O’BRIEN, J., HAYDER, H., ZAYED, Y. & PENG, C. 2018. Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. *Frontiers in Endocrinology*, 9.

O’FLYNN, N. 2014. Assessment and treatment for people with fertility problems: NICE guideline. *Br J Gen Pract*, 64, 50-51.

PETERLONGO, P., CALECA, L., CATTANEIO, E., RAVAGNANI, F., BIANCHI, T., GALASTRI, L., BERNARD, L., FICARAZZI, F., DALL’OLIO, V. & MARME, F. 2011. The rs1297533 variant in the miR-125a and breast cancer risk in Germany, Italy, Australia and Spain. *Journal of medical genetics*, 48, 703-704.

POGRIBNY, I. P. 2018. MicroRNAs as biomarkers for clinical studies. *Exp Biol Med (Maywood)*, 243, 283-290.

POTENZA, N. & RUSSO, A. 2013. Biogenesis, evolution and functional targets of microRNA-125a. *Molecular genetics and genomics*, 288, 381-389.

SHI, C., SHEN, H., FAN, L.-J., GUAN, J., ZHENG, X.-B., CHEN, X., LIANG, R., ZHANG, X.-W., CUI, Q.-H., SUN, K.-K., ZHAO, Z.-R. & HAN, H.-J. 2017. Endometrial MicroRNA Signature during the Window of Implantation Changed in Patients with Repeated Implantation Failure. *Chinese medical journal*, 130, 566-573.

SINGH, D. K., ZHANG, W., XU, Y., YIN, J., GU, H. & JIANG, P. 2017. Hsa-miR-34b/c rs4938723 T> C, pri-miR-124-1 rs531564 C> G, pre-miR-125a rs12975333 G> T and hsa-miR-423 rs6505162 C> A polymorphisms and the risk of gastric cardia adenocarcinoma. *Int J Clin Exp Med*, 10, 14919-14926.

SOOD, P., KREK, A., ZAVOLAN, M., MACINO, G. & RAJEWSKY, N. 2006. Cell-type-specific signatures of microRNAs on target mRNA expression. *Proc Natl Acad Sci U S A*, 103, 2746-51.

SØRENSEN, A., WISSING, M., SALÖ, S., ENGLUND, A. & DALGAARD, L. 2014. MicroRNAs related to polycystic ovary syndrome (PCOS). *Genes*, 5, 684-708.

STOWE, H. M., CURRY, E., CALCATERA, S. M., KRISHER, R. L., PACZKOWSKI, M. & PRATT, S. L. 2012. Cloning and expression of porcine Dicer and the impact of developmental stage and culture conditions on MicroRNA expression in porcine embryos. *Gene*, 501, 198-205.

WANG, C., LI, D., ZHANG, S., XING, Y., GAO, Y. & WU, J. 2016. MicroRNA-125a-5p induces mouse granulosa cell apoptosis by targeting signal transducer and activator of transcription 3. *Menopause*, 23, 100-107.

WANG, W. & LUO, Y.-P. 2015. MicroRNAs in breast cancer: oncogene and tumor suppressors with clinical potential. *Journal of Zhejiang University. Science. B*, 16, 18-31.

ZHANG, H., JIANG, X., ZHANG, Y., XU, B., HUA, J., MA, T., ZHENG, W., SUN, R., SHEN, W., COOKE, H. J., HAO, Q., QIAO, J. & SHI, Q. 2014. microRNA 376a regulates follicle assembly by targeting Pcna in fetal and neonatal mouse ovaries. *Reproduction*, 148, 43-54.