Original Article

# The Effect of Endometrial Cell Culture on α*3* and *β1 integrin* Genes and Protein Expression in Type 2 Diabetic Rats at The Time of Implantation

**Fatemah Sadat Mostafavi, M.D, Ph.D.[1], Abbas Bakhteyari, Ph.D.[1], Parvaneh Nikpour, Ph.D.[2, 3], Nahid Eskandari, Ph.D.[4], Roshanak Aboutorabi, Ph.D.[1*]**

**1. Department of Anatomical Sciences, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran**
**2. Department of Genetics and Molecular Biology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran**
**3. Child Growth and Development Research Center, Research Institute for Primordial Prevention of Non-Communicable Disease, Isfahan University of Medical Sciences, Isfahan, Iran**
**4. Department of Immunology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran**

***Corresponding Address: P.O.Box: 8174673461, Department of Anatomical Sciences, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran***
***Email: aboutorabi.r@gmail.com***

**Received: 18/March/2020, Accepted: 19/July/2020**

Abstract

**Objective:** Given the prevalence of fertility problems in couples and the defect in embryo implantation as well as the low success rate of assisted reproductive techniques, it is necessary to investigate the causes of this phenomenon. Type 2 diabetes mellitus (T2DM) is a metabolic disease with multiple effects on various organs as well as the endometrium. In this study, the effects of endometrial cell culture on the expression of *α3* and *β1 integrin* genes and protein in type 2 diabetic rats were investigated.

**Materials and Methods:** In this experimental study, 35 female rats were divided into five groups: control, sham, diabetic, Pioglitazone-treated and Metformin-treated groups. First, rats were maintained in diabetic condition for 4 weeks. Then, treatment was performed for the next four weeks. Four weeks after induction of diabetes, rats were sacrificed at the time of embryo implantation. The uterus was removed. Endometrial cells were isolated and cultured for 7 days. Immunocytochemistry staining was used to confirm endometrial cells. Expression of *α3* and *β1 integrin* genes was determined by real-time polymerase chain reaction (PCR) technique and the α3β1 protein content measured using Western blot both before and after endometrial cell culture.

**Result:** The expression level of *α3 integrin* gene in the Pioglitazone-treated group compared with metformin-treated group was significantly decreased (P<0.001). The same result was observed in *β1 integrin* gene expression (P=0.004). Also, the α3β1 protein level increased in all diabetic groups, but its reduction was significantly greater in pioglitazone-treated group (P=0.004).

**Conclusion:** T2DM altered the expression of *α3* and *β1 integrin* genes and related proteins, which endometrial cell culture regulated this disorder. According to these results, may be the endometrial cell culture can reduce the adverse effects of diabetes on *α3* and *β1 integrin* expression at the level of gene and protein, in endometrial cells.

**Keywords:** Cell Culture, Diabetes Mellitus, Implantation, Integrin Alpha1, Integrin Beta3

Cell Journal(Yakhteh), Vol 24, No 1, January 2022, Pages: 22-27

**Citation:** Mostafavi FS, Bakhteyari A, Nikpour P, Eskandari N, Aboutorabi R. The effect of endometrial cell culture on α3 and β1 integrin genes and protein expression in type 2 diabetic rats at the time of implantation. Cell J. 2022; 24(1): 22-27. doi: 10.22074/cellj.2022.7445.


## Introduction

Diabetes mellitus can be associated with infertility problems so that fertility rates in women with diabetes are lower than in healthy women (1). In diabetic rats, the uterus is become atrophied, sexual activity reduced and the function of hypothalamic-pituitary-ovarian axis weakened. In addition, ovarian function is impaired and ovulation rates decreased (2). According to statistics, the rate of abortion and fetal loss at the window of embryo implantation in diabetic women is 9 times higher than healthy and non-diabetic women (3).

Adhesion molecules play an important role in the embryo implantation process in the endometrium (4). The expression of endometrial integrins at the level of gene and protein is varied in different species of animals, and not even one species itself have the same cycle at different times. The expression of these heterodimeric glycoproteins is closely related to the tissue maturation (5). For example, in humans and primates, it has been shown that the expression of integrin genes such as *α3β1*, *αvβ3*, *α4β1* and *α1β1* during the reproductive cycle is affected by ovarian hormones (6). In patients with infertility problems integrins genes and proteins expression significantly changed during implantation (7). Lacking β1 integrins in mice lead to implantation failure in the uterine wall and the blastocysts cannot attach to the uterine endometrial cells and penetrate the underlying tissue (8). Despite advances in various techniques of infertility treatment in recent years, the rate of implantation and pregnancy following assisted reproductive technique (ART) is very low (9). The co-culture method is one of the most important techniques for optimizing the conditions of culture in order to obtain embryos with better quality

(10). There is ample evidence to suggest that the use of co-culture system versus conventional and traditional embryo culture systems results in the production of high quality embryos as well as higher pregnancy rates (11). Various cells, such as oviduct, endometrial and granulosa cells have been used to co-culture with the embryo (12). Several studies have pointed to the efficacy of employing one's own endometrial cells in the co-culture system improves fetal quality as well as increases implantation rates in individuals with recurrent failure after *in vitro* fertilization (IVF) techniques (13).

Among different blood glucose regulatory drugs that are used in Type 2 diabetes mellitus (T2DM) treatment, Metformin is the most frequent. The primary function of this drug is reduction of hepatic glucose production (14). Also, Pioglitazone is an insulin-sensitizing agent regulator which can reduce insulin resistance in liver, muscle and adipose tissue and improve glucose and lipid metabolism (15).

The result of our previous work revealed that α3 and β1 integrin protein will be changed in diabetic condition which could affect the fertility status. Treatment with Pioglitazone and Metformin improved the level of α3 and β1 integrin protein while Pioglitazone was more effective (16).

The hypothesis is this: in women with recurrent IVF failure and diabetic condition, cytokines such as tumour necrosis factor α (TNFα), various molecules including adhesion proteins and the large family of integrins were affected (17, 18). Follow our previous work, we investigated the impression of diabetes on the fetal implantation in uterine endometrium. In this study, we evaluated genes and protein expression of intergrin α3β1 and also, the effect of two common drugs in the treatment of diabetes (Metformin and Pioglitazone) in this regard.

## Material and Methods

This experimental study was performed at the Central Laboratory of Isfahan University of Medical Sciences under Ethical Committee permission (IR.MUI.REC.1394.1.184). In this study, 35 adult female Wistar rats weighing 175 to 225 g and 6 to 8 weeks old were randomly divided into five groups of 7 rats in each group). The first group was the control group, with no intervention, the second group was the sham group which received only drugs solvent (normal saline) by orogastric gavage, the third group was type 2 diabetic model rats (T2DM rats), the fourth group was Metformin-treated (100 mg/kg/day by orogastric gavage) T2DM rats and the fifth group was Pioglitazone-treated (20 mg/kg/day by orogastric gavage T2DM rats.

T2DM was induced by nicotine amide (NA) at a dose of 200 mg/kg and streptozotocin (STZ, Sigma-Aldrich, Germany) at a dose of 65 mg/kg. Three days later, fasting blood sugar (FBS) was measured using a glucometer (HemoCue Glucose 201+, Sweden). Also, blood glucose levels above 250 mg/dl were considered as a T2DM model. The animals in all groups were kept in diabetic condition for four weeks. Treatment begun during the next 4 weeks. On 3rd day of the eighth week, animals were mated with male rats. In the next morning all rats were checked. Observation of sperm in the vaginal smears was the indication for the first gestational day and 4 days later it was implantation time (19), in which the endometrial biopsy was also performed (Fig.1).

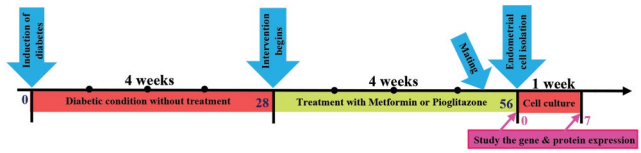


**Fig. 1:** This study performance: from diabetes induction till tissue sampling and cell culture. The total study time was 9 weeks: diabetic condition=4 weeks, drugs treatment=4 weeks and cell culture=one week. Genes and protein expression was studied at days zero and 7 of the cell culture.

### Endometrial cell isolation

Validated method was used for endometrial cells isolation of rat uterus (20). At the end of the eighth week (56th day) of the study and 4 days after the observation of sperm in the vaginal smears, rats in all groups were sacrificed by intraperitoneal injection of ketamine hydrochloride (50 mg/kg, Alfasan, Woerden, Netherlands) and xylazine hydrochloride (Alfasan, Woerden, Netherlands, 7 mg/kg, Fig.1). Their uterine horns were dissected and the endometrium was removed in a sterile condition. The endometrial samples were washed with Hanks' balanced salt solution (Sigma-Aldrich, St. Louis, MO, USA), then chopped into several fragments and kept in the sterile micro tubes. The endometrium of one uterine horn was used directly for molecular experiments, and another was used for cell isolation and culture, followed by molecular experiments on day 7.

The isolated cells in the all groups were maintained in DMEM F-12 (1:1, Sigma-Aldrich, St. Louis, MO, USA) containing fetal calf serum (FCS, 10%, Sigma-Aldrich, St. Louis, MO, USA), penicillin (-Aldrich, St. Louis, MO, USA) and streptomycin (Sigma-Aldrich, St. Louis, MO, USA). The cells were then transferred to special culture flasks. Initial incubation was carried out for 2 hours at 37˚C with 5% carbon dioxide concentration. Then, every three days, the cell culture medium changed.

### Immunocytochemistry assay

Using immunocytochemistry staining, isolated epithelial cells were identified based on response to the Cytokeratin-19 antigen, a marker of epithelial cells. After centrifugation and removing the culture medium, paraformaldehyde 4% (Sigma-Aldrich, St. Louis, MO, USA) was used to fix the cells and then, kept at room temperature for 30-60 minutes. Primary antibody, anti-Cytokeratin-19 (1:300; Abcam, Cambridge, MA, USA) was added to the cell (1: 300), incubated for 2 hours at

4˚C and then, at room temperature overnight. Secondary antibody FITC-conjugated (1:500; Abcam, Cambridge, MA, USA) was added to the cells at a concentration of 1: 1000 and kept at room temperature for 2 hours. DAPI (1:1000, Sigma, USA), at a concentration of 1: 1000 for 2 min, was used to stain the nuclei. Finally, the cells were examined by fluorescent microscopy.

### Real-time polymerase chain reaction technique

Gene expression was evaluated on days 0 and 7th of the cell culture. RNA extraction from endometrial cells was performed according to the manufacturer's guideline of RNX solution (Qiagen, Germany) (17). NCBI and Ensembl sites and Gene Runner software (http://www.generunner.net) were used to design primers (Table 1). These primers were then blasted to ensure their specificity. Finally, expression of the target genes was measured using real time polymerase chain reaction (PCR) technique. The expression level of each target gene was calculated as $2^{-\Delta\Delta Ct}$, as previously described.

**Table 1:** Sequences of the real time-polymerase chain reaction primer sets

| Gene | Primer sequence (5′-3′) |
|---|---|
| *β-actin* | F: GCCTTCCTTCCTGGGTATG |
| | R: AGGAGCCAGGGCAGTAATC |
| *Itg α3* | F: AGCAGCCTCAGCAGATAATC |
| | R: GGAGGATATTGATGACAGGTC |
| *Itg β1* | F: TACTTCAGACTTCCGCATTG |
| | R: GCTGCTGACCAACAAGTTC |

### Western blot

The western blot technique was performed to measure the amount of α3 and β1 integrin proteins. After electrophoresis, the separated proteins were blotted onto a nitrocellulose membrane (AR0135-02, Boster, USA) Incubation was then performed with a primary antibody solution against rabbit anti-rat α3 and β1 integrin antibody (Bs-1057R, Bioss, London) in appropriate dilution for 2 hours. at room temperature with gentle shaking. Then, incubation in the secondary antibody solution against the primary antibody (mouse anti-rabbit IgG -HRP secondary antibody) (P1308; Applygen Technologies, China) with 1:1000 dilution for 1.5 hours at room temperature with gentle shaking was done.

According to the Bio-Rad kit protocol (170-5060, Bio Rad, USA), the nitrocellulose membrane was coated with an Emission Chemo luminescence (ECL) solution (solution 1 and 2 at a ratio of 1:1) (sc-2048, Santa Cruz Biotechnology, USA), which is actually a substrate of horseradish peroxidase (HRP) enzyme (P8375, Sigma-Aldrich, Germany) for 1 minute. Finally, the western blot bands were evaluated and analyzed by device software.

### Statistical analysis of data

Statistical analysis was carried out with SPSS 20 software (University of Stanford, USA). Using one-way analysis of variance (ANOVA), the results were analyzed. And also, the Bonferroni post hoc test was applied for multiple comparisons. The results were presented as mean ± SEM and the level less than 0.05 was considered significant.

## Results

### Immunocytochemistry assay

More than 80% of the isolated cells were endometrial cells (Fig.2).

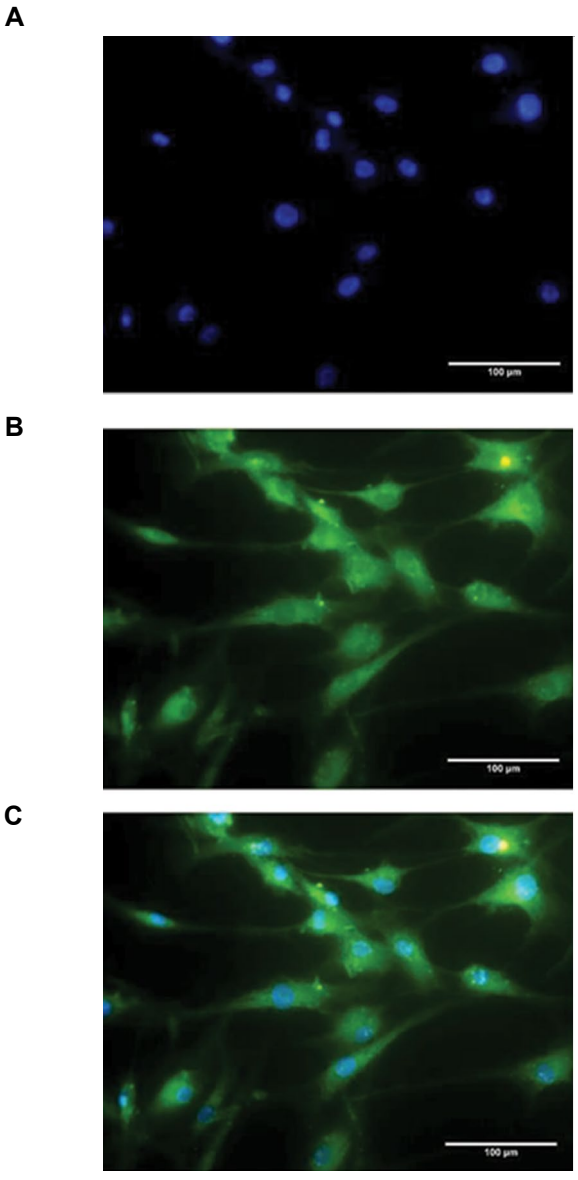


**Fig.2:** Immunocytochemistry before treatment. **A.** Cell nuclei that are blue with DAPI. **B.** The cell wall appears bright green by antibody conjugated with FITC. **C.** The merged images of A and B represent cells expressing the cytokeratin 19 marker (scale bar: 100 µm).

### *α3 integrin* gene expression

On day zero (Fig.3, black columns) the rate of the

expression of the *α3 integrin* gene in the diabetic group showed a significant increase in comparison with the control group (P<0.001). After treatment with Metformin, *α3 integrin* gene's expression increased and was significant compared to the diabetic group (P=0.009). However, the expression of this gene decreased significantly after treatment with Pioglitazone compared to the diabetic group (P<0.001) and also Metformin treated group (P<0.001). Also, *α3 integrin* gene expression on day 7 after endometrial culture (Fig.3, white columns) had a similar pattern to the day zero although, it was so close. And also, there was no significant difference between any of the groups (P>0.05). In comparing α3 gene expression between days zero and seven, it was observed that diabetic group (P=0.006) and Metformin-treated group (P<0.001) had a significant decrease on day 7 while, Pioglitazone-treated group showed a significant increase (P=0.022).

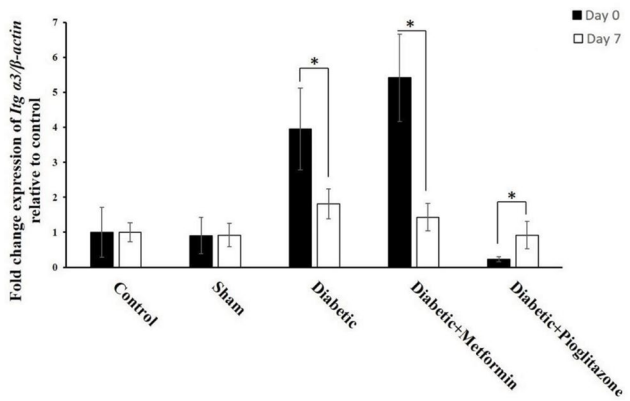


**Fig.3:** The level of *α3 integrin* gene expression in the isolated endometrial cells on days 0 and 7th after cell culture at the time of embryo implantation. *; P<0.05.

### *β*1 *integrin* gene expression

The expression of *β1 integrin* gene on the day zero (Fig.4, black columns) showed a significant increase rate in the diabetic group in comparison with the control group (P<0.001). The expression of this gene decreased significantly after treatment with Metformin (P=0.008) and Pioglitazone (P<0.001) compared to the diabetic group. Moreover, the reduction rate was significantly higher in the Pioglitazone-treated group than in the Metformin-treated group (P=0.004). *β1 integrin* gene expression on the day 7 after culture (Fig.4, white columns) had a similar pattern to the day zero. There was no significant difference among all groups (P>0.05) except the diabetic group, that this group had a significant difference with the control (P<0.001), Metformin-treated (P=0.008) and Pioglitazone-treated (P<0.001) groups. In comparing *β1* gene expression between days zero and seven, the diabetic group (P=0.006), Metformin-treated group (P<0.001) and Pioglitazone-treated group had a significant decrease on day 7.

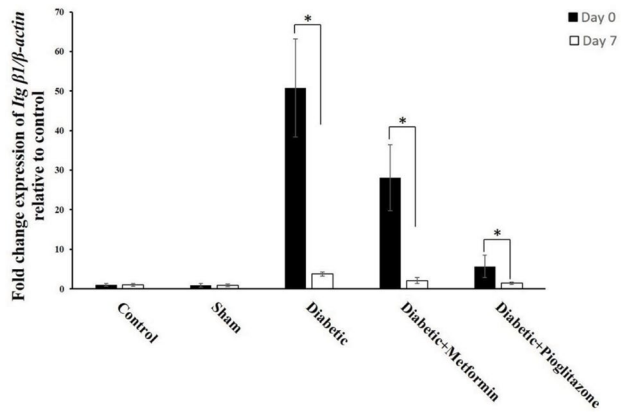


**Fig.4:** The level of *β1 integrin* gene expression in the isolated endometrial cells on days 0 and 7 after cell culture at the time of embryo implantation. *; P<0.05.

### α3 and β1 integrin protein expression

At the time of the implantation (day zero) (Fig.5, black columns), the amount of α3β1 integrin protein in the diabetes group increased significantly compared with the control group (P<0.001). However, the amount of this protein in the treatment groups with Metformin (P=0.026) and Pioglitazone (P<0.001) has been significantly reduced. In addition, there was a significant difference between the two treatment groups (P=0.033) and the protein content of α3 and β1 integrin in the Pioglitazone treatment group was lower than in the Metformin treatment group. After 7 days endometrial culture (Fig.5, white columns), there was no significant difference between any of the groups (P>0.05) in α3 and β1 integrin protein expression, but, protein expression in the different groups, including diabetic (P<0.001), Metformin-treated (P=0.003) and Pioglitazone-treated (P=0.024) was significantly decreased in comparison with the day of implantation.

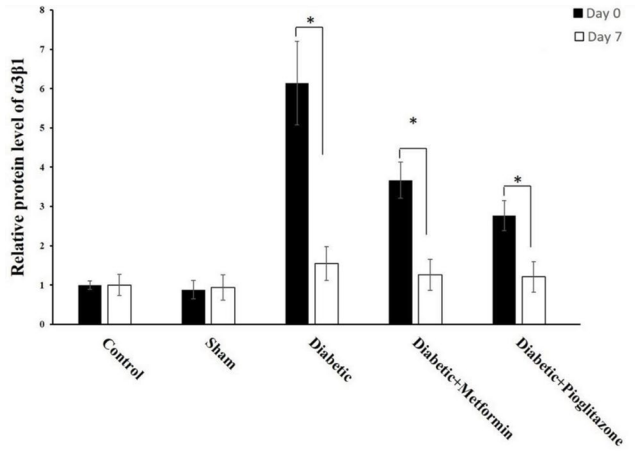


**Fig.5:** Comparison of α3 and β1 integrin protein levels in isolated endometrial cells on days 0 and 7 after cell culture at the time of embryo implantation. *; P<0.05.

## Discussion

Nowadays, in developed countries, 7% of all births are due to assisted reproductive methods (21). Despite

advances in the modern infertility therapies, their success rate is still low. Also, in some patients the infertility cause is unknown (22). Increased blood glucose level (hyperglycemia) could have a detrimental effect on oocyte, embryo and the mother's endometrium at the time of embryo implantation. The complications of type 2 diabetes on the fetus are similar to the complications of type 1 diabetes, which can increase the rate of miscarriage and the rate of fetal abnormalities (23). Maternal hyperglycemia affects the integrins molecules that are involved in the process of apoptosis, proliferation, migration, differentiation of embryonic cells and leads to several complications, although the exact mechanism of hyperglycemic effect on the embryo at implantation is still unclear (24).

In the present study, we worked on the effect of diabetes on the expression of genes and protein of α3β1 integrin. Our results revealed that the expression rate increased significantly in diabetic rats. After 7 days of endometrial cell culture in the laboratory, we encountered with a decrease expression even in the diabetic group. The significant reduction was observed in the treated groups, Metformin and Pioglitazone, in comparison with the control group. Briefly, this rate was higher in the Pioglitazone treated group.

Adhesion molecules, specially the integrin family, play an important role in the endometrial receptivity and pregnancy onset (25). Alteration in the endometrium expression of some integrins, were observed by Bakhteyari et al. (17) in the implantation failure cases. Bakhteyari et al. (18) showed that diabetes mellitus may alter integrin gene expression in the many tissues and organs such as reproductive system. Here, our study showed diabetes associated with an increase in the expression of *α3* and *β1 integrin* genes and protein in endometrial cells.

Due to the location of α3 and β1 integrin protein in the basolateral part of the endometrial cells, it is likely that increasing the expression will increase the connection of these cells to each other, which can prevent the embryo from penetrating and implanting in the uterine endometrium. In addition, increased expression of integrins in other cells, such as platelets, causes plaque buildup and clots at the site of embryo implantation, which can impede embryo implantation and prevent blood flow between the placenta and mother tissue, leading to disruption in implantation process (16).

In a study in 2000 scientist showed that integrins expression increases during the endometrial receptivity phase (26). They proved that the pattern of endometrial integrins expression, indicates their hormonal regulation (27). Also, they revealed integrins in the endometrium play an essential role in the process of pregnancy and implantation and are known as a means of attaching the fetus to the mother tissue (28). Numerous studies of integrins in various tissues have shown that diabetes and hyperglycemia alter the expression of integrins (28) which in the present study it was proved too. Also, Rutherford et al. (29) showed that changes in the integrins expression can reduce the female fertility rate, which in various ways probably result from a decrease in the uterine endometrium ability to accept blastocyst. The results of the both studies are in line with our study that diabetes causes significant changes in the expression of α3 and β1 integrin protein and genes. Moreover, by keeping endometrial cells for 7 days in an environment outside the body of diabetic animals, we observed that even in rats who did not receive drug treatment, the expression of these integrins decreased significantly.

So far, little research has been done on the diabetes effects on the integrins expression in the endometrial tissue. On the other hand, there is no study about the effects of anti-diabetic drugs, such Metformin, and specially Pioglitazone. Although, there are studies on organs other than the reproductive system, particularly, endometrium (30, 31). Also, there is no study on diabetes and endometrial cell culture.

The results of the present study showed that the separation and culture of endometrial cells in diabetic rats by itself could reduce the expression of genes and protein of α3 and β1 integrin, which had increased during diabetic condition. Therefore, there was no significant difference between the level of the expression in the diabetes group and the control group. So, if diabetic patients had several embryo implantation failures in the assisted reproductive techniques, it could be assumed this culturing procedure will improve the success rate of implantation and pregnancy. However, in order to graft these cells to the maternal endometrium, the endometrial cell culture needs biological scaffolds i.e., fibrin, alginate and etc. Eventually, the embryo along with the endometrial cells and scaffold will be located and grafted to the mother's uterus. During the present study, we have taken the first step in this direction. And certainly, obtain the favorable outcomes requires further studies and follow-up.

## Conclusion

Based on the results of this study, diabetes can increase the expression of genes and protein of α3 and β1 integrin during implantation of blastocyst in the uterus endometrial tissue. This protein can increase the connection among endometrial cells. This strong connection does not allow the complete and perfect blastocyst invasion into the endometrial tissue. Between two drugs which were tried in this study, Pioglitazone had a more beneficial and further regulating effect on the expression of α3 and β1 integrin genes and protein compared with Metformin.

## Acknowledgements

The authors would like to acknowledge the financial support of Isfahan University of Medical Sciences (project number 194184). The authors declare that they have no conflict of interest.

## Authors' Contributions

R.A.; Contributed to conception and design. P.N., N.E.; Contributed to data and statistical analysis, interpretation of data and manuscript revision. A.B.; Contributed to all experimental works. F.S.M.; Contributed to data collection and interpretation, and manuscript writing. All authors read and approved the final manuscript.

## References


1. Szaboova R, Devendra S. Infertility in a young woman with Type 2 diabetes. London J Prim Care (Abingdon). 2015; 7(3): 55-57.
2. Ge ZJ, Liang XW, Guo L, Liang QX, Luo SM, Wang YP, et al. Maternal diabetes causes alterations of DNA methylation statuses of some imprinted genes in murine oocytes. Biol Reprod. 2013; 88(5): 117.
3. Albaghdadi AJH, Kan FWK. Endometrial receptivity defects and impaired implantation in diabetic NOD mice. Biol Reprod. 2012; 87(2): 30.
4. Simopoulou M, Nikolopoulou E, Dimakakos A, Charalabopoulos K, Koutsilieris M. Cell adhesion molecules and in vitro fertilization. In Vivo. 2014; 28(5): 683-690.
5. Kim SM, Kim JS. A review of mechanisms of implantation. Dev Reprod. 2017; 21(4): 351-359.
6. Singh H, Aplin JD. Adhesion molecules in endometrial epithelium: tissue integrity and embryo implantation. J Anat. 2009; 215(1): 3-13.
7. Su R-W, Fazleabas AT. Implantation and establishment of pregnancy in human and nonhuman primates. Adv Anat Embryol Cell Biol. 2015; 216: 189-213.
8. Chen G, Xin A, Liu Y, Shi C, Chen J, Tang X, et al. Integrins β1 and β3 are biomarkers of uterine condition for embryo transfer. J Transl Med. 2016; 14(1): 303.
9. Jie Z, Yiling D, Ling Y. Association of assisted reproductive technology with adverse pregnancy outcomes. Iran J Reprod Med. 2015; 13(3): 169-180.
10. Swain JE, Carrell D, Cobo A, Meseguer M, Rubio C, Smith GD. Optimizing the culture environment and embryo manipulation to help maintain embryo developmental potential. Fertil Steril. 2016; 105(3): 571-587.
11. Dominguez F, Gadea B, Mercader A, Esteban FJ, Pellicer A, Simón C. Embryologic outcome and secretome profile of implanted blastocysts obtained after coculture in human endometrial epithelial cells versus the sequential system. Fertil Steril. 2010; 93(3): 774-782. e1.
12. Vithoulkas A, Levanduski M, Goudas VT, Illmensee K. Co-culture of human embryos with autologous cumulus cell clusters and its beneficial impact of secreted growth factors on preimplantation development as compared to standard embryo culture in assisted reproductive technologies (ART). Middle East Fertil Soc J. 2017; 22(4): 317-322.
13. Eyheremendy V, Raffo FGE, Papayannis M, Barnes J, Granados C, Blaquier J. Beneficial effect of autologous endometrial cell coculture in patients with repeated implantation failure. Fertil Steril. 2010; 93(3): 769-773.
14. He L, Wondisford FE. Metformin action: concentrations matter. Cell Metab. 2015; 21(2): 159-162.
15. Zou C, Hu H. Use of pioglitazone in the treatment of diabetes: effect on cardiovascular risk. Vasc Health Risk Manag. 2013; 9: 429-433.
16. Zarrin Y, Bakhteyari A, Nikpour P, Mostafavi FS, Eskandari N, Matinfar M, et al. A study on the presence of osteopontin and α3β1 integrin in the endometrium of diabetic rats at the time of embryo implantation. J Reprod Infertil. 2020; 21(2): 87-93.
17. Bakhteyari A, Zarrin Y, Nikpour P, Hosseiny ZS, Mostafavi FS, Eskandari N, et al. Diabetes mellitus increased integrins gene expression in rat endometrium at the time of embryo implantation. Int J Reprod Biomed. 2019; 17(6): 395-404.
18. Bakhteyari A, Nikpour P, Mostafavi FS, Eskandari N, Matinfar M, Soleimani Asl S, et al. Impact of metformin and pioglitazone on serum level of tumor necrosis factor-alpha and lipid profiles during implantation window in diabetic rats. Int J Fertil Steril. 2019; 13(2): 148-153.
19. Spósito DR, Santos AR. Histochemical study of early embryo implantation in rats. Int J Morphol. 2011; 29(1): 187-192.
20. Bläuer M, Heinonen PK, Martikainen PM, Tomas E, Ylikomi T. A novel organotypic culture model for normal human endometrium: regulation of epithelial cell proliferation by estradiol and medroxyprogesterone acetate. Hum Reprod. 2005; 20(4): 864-871.
21. Kushnir VA, Barad DH, Albertini DF, Darmon SK, Gleicher N. Systematic review of worldwide trends in assisted reproductive technology 2004-2013. Reprod Biol Endocrinol. 2017; 15(1): 6.
22. Pandian Z, Bhattacharya S, Nikolaou D, Vale L, Templeton A. In vitro fertilisation for unexplained subfertility. Cochrane Database Syst Rev. 2002; (2): CD003357.
23. Zhao J, Hakvoort TBM, Willemsen AM, Jongejan A, Sokolovic M, Bradley EJ, et al. Effect of hyperglycemia on gene expression during early organogenesis in mice. PLoS One. 2016; 11(7): e0158035.
24. Uhde K, van Tol HTA, Stout TAE, Roelen BAJ. Exposure to elevated glucose concentrations alters the metabolomic profile of bovine blastocysts. PLoS One. 2018; 13(6): e0199310.
25. van Mourik MSM, Macklon NS, Heijnen CJ. Embryonic implantation: cytokines, adhesion molecules, and immune cells in establishing an implantation environment. J Leukoc Biol. 2009; 85(1): 4-19.
26. Lessey BA, Castelbaum AJ, Wolf L, Greene W, Paulson M, Meyer WR, et al. Use of integrins to date the endometrium. Fertil Steril. 2000; 73(4): 779-787.
27. Yoshimura Y. Integrins: expression, modulation, and signaling in fertilization, embryogenesis and implantation. Keio J Med. 1997; 46(1): 16-24.
28. Koot YEM, Teklenburg G, Salker MS, Brosens JJ, Macklon NS. Molecular aspects of implantation failure. Biochim Biophys Acta. 2012; 1822(12): 1943-1950.
29. Rutherford EJ, Hill ADK, Hopkins AM. Adhesion in physiological, benign and malignant proliferative states of the endometrium: microenvironment and the clinical big picture. Cells. 2018; 7(5): 43.
30. Zhou X, Zhang J, Haimbach R, Zhu W, Mayer-Ezell R, Garcia-Calvo M, et al. An integrin antagonist (MK-0429) decreases proteinuria and renal fibrosis in the ZSF 1 rat diabetic nephropathy model. Pharmacol Res Perspect. 2017; 5(5): e00354.
31. Sawada K, Toyoda M, Kaneyama N, Shiraiwa S, Moriya H, Miyatake H, et al. Upregulation of α3β1-integrin in podocytes in early-stage diabetic nephropathy. J Diabetes Res. 2016; 2016: 9265074.